PTO/SB/05 (09-04)

02570 U.S. PTO 031910

UTILITY PATENT APPLICATION TRANSMITTAL

Only for new nonprovisional applications under 37 CFR 1.53(b))

Attorney Docket No.	0800-0045.01
First Inventor	Wright et al.
Title	COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION
Express Mail Label No.	EV 514 190 691 US

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

1. ☒ **Fee Transmittal Form** (e.g., PTO/SB/17)
   (Submit an original and a duplicate for fee processing)
2. ☐ **Applicant claims small entity status.**
   See 37 CFR 1.27.
3. ☒ **Specification** [*Total Pages* 29]
   Both the claims and abstract must start on a new page
   (For information on the preferred arrangement, see MPEP 608.01(a))
4. ☒ **Drawing(s)** *(35 U.S.C.113)* [*Total Sheets* 3]
5. **Oath or Declaration** [*Total Sheets* 2]
   a. ☐ Newly executed (original or copy)
   b. ☒ A copy from a prior application (37 CFR 1.63 (d))
   (for a continuation/divisional with Box 18 completed)
   i. ☐ DELETION OF INVENTOR(S)
   Signed statement attached deleting inventor(s) named in the prior application, see 37 CFR 1.63(d)(2) and 1.33(b).
6. ☒ **Application Data Sheet.** See 37 CFR 1.76 (4 pages)
7. ☐ **CD-ROM** or **CD-R** in duplicate, large table or Computer Program *(Appendix)*
   ☐ Landscape Table on CD
8. **Nucleotide and/or Amino Acid Sequence Submission**
   (if applicable, items a. - c. are required)
   a. ☐ Computer Readable Form (CRF)
   b. Specification Sequence Listing on:
      i. ☐ CD-ROM or CD-R (2 copies); or
      ii. ☐ Paper
   c. ☐ Statements verifying identity of above copies

ADDRESS TO: **Commissioner for Patents**
P.O. Box 1450
Alexandria, VA 22313-1450

ACCOMPANYING APPLICATION PARTS

9. ☐ **Assignment Papers** (cover sheet & document(s))
   Name of Assignee _____
10. ☐ **37 CFR 3.73(b) Statement** *(when there is an assignee)* ☐ **Power of Attorney**
11. ☐ **English Translation Document** *(if applicable)*
12. ☐ **Information Disclosure Statement** (PTO/SB/08 or PTO-1449)
    ☐ Copies of citations attached
13. ☐ **Preliminary Amendment**
14. ☒ **Return Receipt Postcard** (MPEP 503) (1 page)
    (Should be specifically itemized)
15. ☐ **Certified Copy of Priority Document(s)**
    (if foreign priority is claimed)
16. ☐ **Nonpublication Request** under 35 U.S.C. 122 (b)(2)(B)(i).
    Applicant must attach form PTO/SB/35 or its equivalent.
17. ☒ Other: Check for $1090.00

18. If a CONTINUING APPLICATION, *check appropriate box, and supply the requisite information below and in the first sentence of the specification following the title, or in an Application Data Sheet under 37 CFR 1.76:*

☒ Continuation ☐ Divisional ☐ Continuation-in-part (CIP) of prior application No 11/141,996

Prior application information: *Examiner* S.K. Singh *Art Unit:* 1657

19. CORRESPONDENCE ADDRESS

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Address					
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Country		Telephone		Fax	

Signature	Dahna Pasternak	Date	3/19/10
Name (Print/Type)	Dahna S. Pasternak	Registration No. (Attorney/Agent)	41,411

Effective on 12/08/2004.
Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).

FEE TRANSMITTAL
For FY 2010

☐ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT	($)1090.00

Complete if Known	
Application Number	Not Asssigned
Filing Date	Herewith
First Named Inventor	Wright et al.
Examiner Name	Not Assigned
Art Unit	Not Assigned
Attorney Docket No.	0800-0045.01

METHOD OF PAYMENT (check all that apply)

☒ Check ☐ Credit Card ☐ Money Order ☐ None ☐ Other (please identify): _____

☒ Deposit Account Deposit Account Number: 18-1648 Deposit Account Name: Robins & Pasternak LLP

For the above-identified deposit account, the Director is hereby authorized to: (check all that apply)

☐ Charge fee(s) indicated below ☐ Charge fee(s) indicated below, **except for the filing fee**

☒ Charge any additional fee(s) or underpayments of fee(s) under 37 CFR 1.16 and 1.17 ☒ Credit any overpayments

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038

FEE CALCULATION

1. BASIC FILING, SEARCH, AND EXAMINATION FEES

Application Type	FILING FEES Fee ($)	FILING FEES Small Entity Fee ($)	SEARCH FEES Fee ($)	SEARCH FEES Small Entity Fee ($)	EXAMINATION FEES Fee ($)	EXAMINATION FEES Small Entity Fee ($)	Fees Paid ($)
Utility	330	165	540	270	220	110	1090
Design	220	110	100	50	140	70	
Plant	220	110	330	165	170	85	
Reissue	330	165	540	270	650	325	
Provisional	220	110	0	0	0	0	

2. EXCESS CLAIM FEES

Fee Description	Small Entity Fee ($)	Small Entity Fee ($)
Each claim over 20 or, for Reissues, each claim over 20 and more than in the original patent	52	26
Each independent claim over 3 or, for Reissues, each independent claim more than in the original patent	220	110
Multiple dependent claims	390	195

Total Claims	Extra Claims	Fee ($)	Fee Paid ($)
11	-20 or HP = 0	x 0	= 0

HP = highest number of total claims paid for, if greater than 20

Indep. Claims	Extra Claims	Fee ($)	Fee Paid ($)
2	-3 or HP = 0	x 0	=

HP = highest number of independent claims paid for, if greater than 3

Multiple Dependent Claims Fee ($)	Fee Paid ($)

3. APPLICATION SIZE FEE

If the specification and drawings exceed 100 sheets of paper, the application size fee due is $270 ($135 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

Total Sheets	Extra Sheets	Number of each additional 50 or fraction thereof	Fee ($)	Fee Paid ($)
32 - 100 =	0 / 50 =	(round up to a whole number)	x 0	=

4. OTHER FEE(S)0

	Fees Paid ($)
Non-English Specification, $130 fee (no small entity discount)	
Other:	

SUBMITTED BY

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Name (Print/Type)	Dahna S. Pasternak	41,411	Date 3/19/10

COPY

Attorney Docket No.: 0800-0045
Client Ref. No.:

PTO/SB/01A (09-04)

DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION

As the below named inventor(s), I/we declare that:

This declaration is directed to:

☐ The attached application, or

☒ Application No. 11/141,996, filed on June 1, 2005,

☐ as amended on _____ (if applicable);

I/we believe that I/we am/are the original and first inventor(s) of the subject matter which is claimed and for which a patent is sought;

I/we have reviewed and understand the contents of the above-identified application, including the claims, as amended by any amendment specifically referred to above;

I/we acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me/us to be material to patentability as defined in 37 CFR 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT International filing date of the continuation-in-part application.

All statements made herein of my/our own knowledge are true, all statements made herein on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001, and may jeopardize the validity of the application or any patent issuing thereon.

FULL NAME OF INVENTOR(S)

Inventor one: John Fraser Wright — Date: _____

Signature: _____ — Citizen of: Canada

Inventor two: Guang Qu — Date: 9/27/2005

Signature: [signature] — Citizen of: United States of America

Inventor three: _____ — Date: _____

Signature: _____ — Citizen of: _____

Inventor four: _____ — Date: _____

Signature: _____ — Citizen of: _____

☐ Additional inventors or a legal representative are being named on _____ additional form(s) attached hereto.

COPY

Attorney Docket No.: 0800-0045
Client Ref. No.:

PTO/SB/01A (09-04)

DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION

As the below named inventor(s), I/we declare that:

This declaration is directed to:

☐ The attached application, or

☒ Application No. 11/141,996, filed on June 1, 2005,

☐ as amended on _____ (if applicable);

I/we believe that I/we am/are the original and first inventor(s) of the subject matter which is claimed and for which a patent is sought;

I/we have reviewed and understand the contents of the above-identified application, including the claims, as amended by any amendment specifically referred to above;

I/we acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me/us to be material to patentability as defined in 37 CFR 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT International filing date of the continuation-in-part application.

All statements made herein of my/our own knowledge are true, all statements made herein on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001, and may jeopardize the validity of the application or any patent issuing thereon.

FULL NAME OF INVENTOR(S)

Inventor one: John Fraser Wright — Date: 04 Oct 05

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Inventor three: _____ — Date:

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Inventor four: _____ — Date:

Signature: _____ — Citizen of: _____

☐ Additional inventors or a legal representative are being named on _____ additional form(s) attached hereto.

Application Data Sheet

Application Information

Application Number::	
Filing Date::	
Application Type::	Regular
Subject Matter::	Utility
Suggested classification::	
Suggested Group Art Unit::	
CD-ROM or CD-R?::	None
Number of CD disks::	
Number of copies of CDs::	
Sequence submission?::	
Computer Readable Form (CRF)?::	No
Number of copies of CRF::	
Title::	COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION
Attorney Docket Number::	0800-0045.01
Request for Early Publication?::	No
Request for Non-Publication?::	No
Suggested Drawing Figure::	
Total Drawings Sheets::	3
Small Entity?::	No
Latin Name::	
Variety denomination name::	
Petition included?::	No
Petition Type::	
Licensed US Govt. Agency::	
Contract or Grant Numbers::	
Secrecy Order in Parent Appl.?::	No

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DOMESTIC PRIORITY INFORMATION

Application::	Continuity Type::	Parent Application::	Parent Filing Date::
This Application	Claims the benefit under 35 USC 120 of	11/141,996	June 1, 2005
and	Claims the benefit under 35 USC 119(e) of	60/575,997	June 1, 2004
and	Claims the benefit under 35 USC 119(e) of	60/639,222	December 22, 2004

FOREIGN PRIORITY INFORMATION

Country::	Application Number::	Filing Date::	Priority Claimed::

ASSIGNEE INFORMATION

Assignee name::

Street of mailing address::

City of mailing address::

State of mailing address::

Country of mailing address::

Zip Code of mailing address::

0800-0045.01
PATENT

UTILITY PATENT APPLICATION

COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION

Inventor: John Fraser Wright

Guang Qu

Entity: Large

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COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. application serial no. 11/141,996, from which application priority is claimed pursuant to 35 U.S.C. §120; which application claims the benefit under 35 U.S.C. § 119(e) of provisional applications 60/575,997 filed June 1, 2004 and 60/639,222 filed December 22, 2004. The foregoing applications are hereby incorporated by reference in their entireties.

FIELD OF THE INVENTION

The present invention relates to compositions and methods of preparing and storing AAV virions that prevent aggregation.

BACKGROUND

Recombinant adeno-associated virus (rAAV) is a promising vector for human gene transfer. Grimm, D., and Kleinschmidt, J. A. (1999) *Hum Gene Ther.* 10: 2445-2450; High, K. A. (2001) *Ann. N. Y. Acad. Sci.* 953: 64-67; Pfeifer, A., and Verma, I. M. (2001) *Ann. Rev. Genomics Hum. Genet.* **2**: 177-211. AAV is a member of the Dependovirus genus of the parvoviruses. AAV serotype 2 (AAV2) is composed of a single-strand DNA molecule of 4680 nucleotides encoding replication (*rep*) and encapsidation (*cap*) genes flanked by inverted terminal repeat (ITR) sequences. Berns, K. I. (1996) in *Fields Virology* (B. N. Fields *et. al.* Eds.), pp. 2173-2197. Lippincott-Raven Publishers, Philadelphia. The genome is packaged by three capsid proteins (VP1, VP2 and VP3), which are amino-terminal variants of the *cap* gene

product. The resulting icosahedral virus particle has a diameter of ~26 nm. A high resolution crystal structure of AAV2 has been reported. Xie, Q. *et al.* (2002) *Proc. Natl. Acad. Sci. U.S.A.* **99**: 10405-10410.

The solubility of purified AAV2 virus particles is limited, and aggregation of AAV2 particles has been described as a problem. Croyle, M. A. *et al.* (2001) *Gene Therapy* 8: 1281-1290; Huang, J. *et al.* (2000) *Mol. Therapy* 1: S286; Wright, J. F. *et al.* (2003) *Curr. Opin. Drug Disc. Dev.* 6: 174-178; Xie, Q. *et al.* (2004) *J. Virol. Methods* 122: 17-27. In commonly used buffered-saline solutions, significant aggregation occurs at concentrations of 10^{13} particles/mL, and aggregation increases at higher concentrations. Huang and co-workers reported that AAV vectors undergo concentration-dependent aggregation. Huang, J. *et al.* (2000) *Mol. Therapy* 1: S286. Xie and coworkers (Xie, Q. *et al.* (2004) *J. Virol. Methods* 122: 17-27) similarly reported that at concentrations exceeding 0.1mg/mL, AAV2 vectors require elevated concentrations of salt to prevent aggregation. Aggregation of AAV2 vectors occurs at particle concentrations exceeding 10^{13} particles/mL in commonly used neutral-buffered solutions such as phosphate- and Tris-buffered saline. This corresponds to a protein concentration of ~0.06 mg/mL, and emphasizes the low solubility of AAV2 under these conditions. The effective vector concentration limit may be even lower for vectors purified using column chromatography techniques because excess empty capsids are co-purified and contribute to particle concentration.

Particle aggregation is a significant and not fully resolved issue for adenovirus vectors as well. Stability of a recently established adenovirus reference material (ARM) was recently reported. Adadevoh, K. *et al.* (2002) *BioProcessing* 1(2): 62-69. Aggregation of the reference material, formulated in 20mM Tris, 25 mM NaCl, and 2.5% glycerol at pH 8.0, was assessed by dynamic light scattering, photon correlation spectroscopy and visual appearance. A variable

level of vector aggregation following either freeze-thaw cycling or non-frozen storage was observed, resulting in restrictive protocols for the use of the ARM.

Aggregation can lead to losses during purification and inconsistencies in testing of purified vector preparations. The *in vivo* administration of AAV2 vectors to certain sites, such as the central nervous system, may require small volumes of highly concentrated vector, and the maximum achievable dose may be limited by low vector solubility.

Vector aggregation is also likely to influence biodistribution following *in vivo* administration, and cause adverse immune responses to vectors following their administration. As has been reported for proteins (Braun, A. *et al.* (1997) *Pharm. Res.* 14: 1472-1478), aggregation of vector may increase immunogenicity by targeting the vector to antigen presenting cells, and inducing enhanced immune responses to the capsid proteins and transgene product. The reports of immune responses to AAV vectors in pre-clinical (Chenuaud, P. *et al.* (2004) *Blood* 103: 3303-3304; Flotte, T. R. (2004) *Human Gene Ther.* 15: 716-717; Gao, G. *et al.* (2004) *Blood* 103: 3300-3302) and clinical (High, K. A. *et al.* (2004) *Blood* 104: 121a) studies illustrate the need to address all factors that may contribute to vector immunogenicity.

Testing protocols to characterize purified vectors are also likely to be affected by vector aggregation. Determination of the infectivity titer of vector was reported to be highly sensitive to vector aggregation. Zhen, Z. *et al.* (2004) *Human Gene Ther.* 15: 709-715. An important concern is that vector aggregates may have deleterious consequences following their *in vivo* administration because their transduction efficiency, biodistribution and immunogenicity may differ from monomeric particles. For example, intravascular delivery of AAV vectors to hepatocytes requires that the vectors pass through the fenestrated endothelial cell lining of hepatic sinusoids. These fenestrations have a radius ranging from 50 to 150 nm (Meijer, K. D.

F., and Molema, G. (1995) *Sem. Liver Dis.* 15: 206) that is predicted to allow the passage of monomeric AAV vectors (diameter ~26 nm), but prevent the passage of larger vector aggregates. In biodistribution studies in mice, aggregated AAV2 vectors labeled with the fluorescent molecule Cy3 were sequestered in liver macrophages following vascular delivery. Huang, J. *et al.* (2000) *Mol. Therapy* 1: S286.

Formulation development for virus-based gene transfer vectors is a relatively recent area of investigation, and only a few studies have been reported describing systematic efforts to optimize AAV vector formulation and stability. Croyle, M. A. *et al.* (2001) *Gene Therapy* 8: 1281-1290; Wright, J. F. *et al.* (2003) *Curr. Opin. Drug Disc. Dev.* 6: 174-178; Xie, Q. *et al.* (2004) *J. Virol. Methods* 122: 17-27. Defining formulations compatible with pre-clinical and clinical applications that minimize changes in vector preparations is an important requirement to achieve consistently high vector safety and functional characteristics. As is well established for protein therapeutics (Chen, B. *et al.* (1994) *J. Pharm. Sci.* 83: 1657-1661; Shire, S. J. *et al.* (2004) *J. Pharm. Sci.* 93: 1390-1402; Wang, W. (1999) *Int. J. Pharm.* 185: 129-188; Won, C. M. *et al.* (1998) *Int. J. Pharm.* 167: 25-36), an important aspect of vector stability is solubility during preparation and storage, and vector aggregation is a problem that needs to be fully addressed. Vector aggregation leads to losses during vector purification, and while aggregates can be removed by filtration, the loss in yield results in higher costs and capacity limitations when producing vector for pre-clinical and clinical studies. Even after filtration to remove aggregates, new aggregates can form in concentrated preparations of AAV2 vector in buffered-saline solutions.

The need exists for improved formulations and methods for purification and storage of AAV vectors, such as rAAV2, that prevent aggregation of virus particles.

SUMMARY OF THE INVENTION

These and other needs in the art are met by the present invention, which provides high ionic strength solutions for use in preparing and storing AAV vectors that maintain high infectivity titer and transduction efficiency, even after freeze-thaw cycles.

In one aspect the invention relates to methods of preventing aggregation of virions in a preparation of virions by adding excipients to achieve an ionic strength high enough to prevent aggregation. In another aspect the invention relates to compositions of virions having an ionic strength high enough to prevent aggregation.

In some embodiments of the invention, the ionic strength is at least about 150mM, 200mM, 250mM, 300mM, 350mM, 400mM, 450mM, 500mM, 600mM, 700mM or more. In some embodiments this ionic strength is accomplished using excipients comprising one or more multivalent ions, for example citrate, sulfate, magnesium or phosphate.

In additional embodiments, the osmolarity of the preparation of virions is maintained at near isotonic levels, for example 200mOsm, 250mOsm, 280mOsm, 300mOsm, 350mOsm or 400mOsm, even though the ionic strength is high enough to prevent virion aggregation.

In some embodiments the virions are adeno-associated virus (AAV) virions, for example AAV-2.

In other embodiments of the methods of the present invention preparations of virions are treated with a nuclease, for example Benzonase®. In further embodiments, nuclease treatment is combined with addition of excipients that achieve an ionic strength high enough to prevent aggregation.

In some embodiments of the present invention, the surfactant Pluronic® F68 is added to a preparation of virions, for example to 0.001%. In one embodiment, the composition comprises purified virus particles, 10 mM Tris pH 8.0, 100mM sodium citrate and 0.001% Pluronic® F68.

In one embodiment, AAV vectors can be stored as compositions of the present invention at concentrations exceeding $1x10^{13}$ vg/mL, for example $2x10^{13}$, $3x10^{13}$, $4x10^{13}$, $5x10^{13}$ and up to $6.4x10^{13}$ vg/mL, without significant aggregation. In some embodiments, AAV vectors stored using the methods and compositions of the invention do not exhibit significant aggregation when stored at 4°C for five days. In other embodiments, AAV vectors that are stored as such compositions do not exhibit significant aggregation after one, five, ten or more freeze-thaw cycles at -20°C or at -80°C.

In some embodiments, preparations of virions stored according to the methods and compositions of the invention exhibit an average particle radius (*Rh*), as measured by dynamic light scattering, indicating that no significant aggregation of virions has taken place. In some embodiments, preparations of virions stored according to the methods and compositions of the invention exhibit an average particle radius (*Rh*) greater than about 15nm, 20nm, or 30nm.

In some embodiments, recovery of virions from preparations of virions stored according to the methods and compositions of the invention is greater than about 85%, 90% or 95% following filtration through a 0.22μm filter.

In yet another aspect, the invention relates to kits comprising the high ionic strength formulations of the invention. In one embodiment the kit comprises a pre-mixed solution of excipients. In another embodiment the kit comprises two or more separate components of a high ionic strength composition of the present invention to be mixed by a user. In some embodiments the kit comprises sodium citrate, Tris® and Pluronic® F68. In other embodiments, the kit further

comprises instructions for making a composition or performing a method of the present invention.

DESCRIPTION OF THE DRAWINGS

FIGS. 1A and 1B present data showing aggregation of AAV2-FIX particles as a function of osmolarity (FIG. 1A) or ionic strength (FIG. 1B) for various buffer compositions. AAV2-FIX vectors are prepared by Method 2 of Example 1. Average particle radius is measured by dynamic light scattering (DLS) following vector dilution in varying concentrations of excipients buffered with 10 mM sodium phosphate at pH 7.5. Excipients include sodium chloride (●), sodium citrate (○), sodium phosphate (■), sodium sulfate (□), magnesium sulfate (▲), and glycerol (Δ).

FIG. 2 presents data on AAV2-FIX aggregation as a function of the method of purification. The average particle radius is measured by DLS following vector dilution in varying concentrations of sodium chloride buffered with10mM sodium phosphate at pH 7.5. Vectors are purified by Method 1 (double CsCl gradient) (○); Method 2 (cation exchange chromatography) (□); Method 2 plus nuclease digestion (■); or Method 3 (chromatography plus one CsCl gradient) (Δ). Purification Methods 1-3 are described in Example 1.

FIG. 3 presents data on transgene expression from D7/4 cells transduced with rAAV2-AADC virions prepared and stored in high ionic strength formulation (□) or in a control formulation (●). The concentration of AADC was measured by ELISA (in triplicate for each data point) 72 hours post-transduction. Error bars represent standard deviations.

DETAILED DESCRIPTION OF THE INVENTION

AAV2 vector aggregation is frequently observed in concentrated preparations of vectors and can affect purification recovery, and *in vivo* potency and safety. Hence, an important objective for the development AAV2 vectors is to identify methods and formulations that prevent aggregation of vectors when concentrated stocks are prepared.

Unless otherwise indicated, the term "vector" as used herein refers to a recombinant AAV virion, or virus particle, regardless of the frequent use of "vector" to also refer to non-viral DNA molecules, such as plasmids, in other contexts.

The present invention is based in part on the observation that solution ionic strength is an important parameter in AAV vector aggregation, implicating the involvement of ionic interactions between virus particles in the aggregation process. The observation that elevated ionic strength increases AAV2 vector solubility regardless of the identity of the charged excipient supports the hypothesis that ionic strength of solution *per se*, rather than interactions involving a specific ionic species, is the relevant physico-chemical parameter. A threshold ionic strength of at least 200mM is required to prevent aggregation at vector particle concentrations examined herein.

Of practical concern, commonly used buffered saline solutions have insufficient ionic strength to prevent AAV2 vector aggregation at concentrations exceeding 10^{13} particles/mL. It is known that high salt concentrations increase AAV2 vector solubility (e.g. highly concentrated AAV2 vectors recovered from gradients generally remain soluble in concentrated CsCl). However, optimal formulations for pre-clinical and clinical studies should be close to isotonic (280-400 mOsm), especially for *in vivo* administration of vector to sites where dilution of hypertonic solutions may be slow. In embodiments of the present invention the exponential

relationship of ionic strength with charge valency is used to develop isotonic formulations with high ionic strengths. Salt species with multiple charge valencies (e.g. salts of sulfate, citrate, and phosphate) that are commonly used as excipients in human parenteral formulations can provide the level of ionic strength needed to prevent AAV2 vector aggregation when used at isotonic concentrations. While isotonic (150mM) sodium chloride has an ionic strength of 150mM, a value insufficient to maintain AAV2 solubility at high vector concentrations, isotonic sodium citrate, with an ionic strength of ~500mM, can support AAV2 vector concentrations of at least 6.4×10^{13} vg/mL without aggregation.

Without intending to be limited by theory, the low solubility of AAV2 particles may be caused by their highly symmetrical nature in conjunction with the stabilizing effect of complementary charged regions between neighbouring particles in aggregates. The surface charge density based on the crystal structure of AAV2 (Xie, Q. *et al.* (2002) *Proc. Natl. Acad. Sci. U.S.A.* 99: 10405-10410) reveals a pattern of positive and negative charges on the virus surface. Previous reports have shown that AAV2 vector aggregation is pH dependent, and hypothesized that amino acids with charged side groups are involved in inter-particle binding. Qu, G. *et al.* (2003) *Mol. Therapy* **7**: S238. These reports hypothesized that if charged amino acid side chains are involved in vector aggregation, high concentrations of free amino acids could block vector particle interactions. However, we have found that amino acids with charged side chains are not effective in preventing AAV2 vector aggregation beyond their contribution to ionic strength.

Vector aggregation at low ionic strength was also found to be reduced but not prevented by efficient nuclease treatment of purified vector particles. Digestion at an earlier stage of the purification process (clarified HEK cell lysate) did not reduce aggregation following vector

purification. It is likely that digestion of already purified virions is more efficient because of a higher enzyme to nucleic acid substrate ratio. One mechanism to explain these results is that residual nucleic acid impurities (e.g. host cell and plasmid DNA) bound to the vector surface can bridge to binding sites on neighbouring virus particles and thus cause aggregation. Purified AAV2 vectors (empty capsid free) have been reported to contain approximately 1% non-vector DNA. Smith, P. *et al.* (2003) *Mol. Therapy* 7: S348. While >50% of this non-vector DNA was reported to be nuclease resistant and was packaged within capsid particles, some impurity DNA was nuclease resistant and appeared to be associated with the surface of purified vector particles. The observation that efficient nuclease treatment can reduce vector aggregation suggests that nucleic acids associated with the vector surface at an average level not greater than ~25 nucleotides per vector particle can contribute to AAV vector aggregation.

In summary, the use of high ionic strength solutions during AAV2 vector purification and final formulation, and efficient removal of residual vector surface DNA are two effective strategies to achieve highly concentrated solutions of AAV2 vectors for use in pre-clinical and clinical studies. High ionic strength solutions and nuclease treatment can be used in combination or separately. Although data were obtained using AAV2 vectors, the composition and methods of the present invention may also be useful with other AAV serotypes / variants, or other viral vectors such as adenoviruses, lentiviruses and retroviruses.

AAV Aggregation as a Function of Excipient Concentration

Initial screening experiments are performed to elucidate the mechanism of AAV vector aggregation and to identify classes of excipients that can reduce / prevent aggregation. Vector aggregation can be caused by dilution (5-fold) of vector in neutral-buffered saline with low

concentration buffer (20mM sodium phosphate, pH 7.2). Excipients are screened using this "dilution-stress" method to identify excipients that are able to prevent vector aggregation when included in the diluent. For screening, aggregation is measured by dynamic light scattering (DLS). Classes of excipients examined included selected inorganic salts, amino acids, uncharged carbohydrates, and surfactants. Results are presented in Table 1.

TABLE 1

SCREENING FOR EXCIPIENTS THAT PREVENT AAV2 VECTOR AGGREGATION USING DILUTION-STRESS METHOD

Excipient	Osm required to prevent aggregation (max tested)
Magnesium sulfate	180 mOsm
Sodium citrate	220 mOsm
Sodium chloride	320 mOsm
Sodium phosphate	220 mOsm
Sodium sulfate	220 mOsm
Arginine	NIA (200 mOsm)
Aspartic acid	320 mOsm
Glutamic acid	320 mOsm
Glycine	NIA (200 mOsm)
Histidine	NIA (200 mOsm)
Lysine	300 mOsm
Glycerol	NIA (5% w/v, 543 mOsm)
Iodixanol	NIA (5% w/v, 32 mOsm)
Mannitol	NIA (5% w/v, 275 mOsm)
Sorbitol	NIA (5% w/v, 275 mOsm)
Sucrose	NIA (5% w/v, 146 mOsm)
Trehalose	NIA (5% w/v, 146 mOsm)
Pluronic® F68	NIA (10% w/v, 12 mOsm)
Polysorbate 80	NIA (1% w/v)

NIA: No inhibition of aggregation

As illustrated in Table 1, charged excipients (inorganic salts and amino acids) prevent aggregation when present at sufficient concentrations. However, salt concentrations required to prevent vector aggregation vary, ranging from 180 mOsm for magnesium sulfate, to 320 mOsm for sodium chloride. The amino acids arginine, aspartic acid, glutamic acid, glycine, histidine, and lysine do not prevent aggregation at 200 mOsm, but lysine, aspartic acid, and glutamic acid prevent aggregation at 300-320 mOsm. Arginine, glycine and histidine were not tested at concentrations other than 200 mOsm. Selected carbohydrates have no effect on vector particle aggregation when present at concentrations up to 5% w/v. For example, 5% w/v glycerol (543 mOsm) does not prevent aggregation. The surfactants Polysorbate80 (1% w/v) and Pluronic® F68 (10% w/v) similarly have no effect on aggregation using the "dilution-stress" method.

AAV Aggregation as a Function of Osmolarity and Ionic Strength

FIGS. 1A and 1B show the results of a more detailed analysis of vector aggregation as a function of the concentration of various salts. FIG. 1A shows vector aggregation as a function of the osmolarity of selected excipients. For charged species a concentration-dependent inhibition of AAV2 vector aggregation is observed. Salts with multivalent ions achieve a similar degree of inhibition of aggregation at lower concentrations than monovalent sodium chloride. For example, magnesium sulfate prevents aggregation at $\geq$ 200 mOsm whereas sodium chloride requires $\geq$ 350 mOsm to achieve a similar effect. Sodium citrate, sodium sulfate, and sodium phosphate are intermediate in their potency to prevent vector aggregation.

Although the results in FIG. 1A and Table 1 show no effect of glycerol and certain sugars at concentrations up to 5% on AAV2 vector aggregation induced by low ionic strength, the data

cannot rule out improvement of AAV2 solubility at glycerol concentrations above 5%. For example, Xie and co-workers reported that 25% (w/v) glycerol enabled concentration of AAV2 to very high concentrations (4.4 to 18 x 10^{14} particles/ml) in low ionic strength solutions. Xie, Q. *et al.* (2004) *J. Virol. Methods* 122: 17-27.

FIG. 1B shows the data of FIG. 1A plotted as a function of the calculated ionic strength, rather than osmolarity, for each excipient. FIG. 1B demonstrates that vector aggregation is prevented when ionic strength is ~200 mM or greater regardless of which salt is used. These data suggested that the ionic strength (μ) of a solution, a parameter that depends on both solute concentration and charge valency, is the primary factor affecting aggregation.

Ionic strengths useful to prevent aggregation in embodiments of the present invention include, for example, 250 mM, 300 mM, 350 mM, 400 mM, 450 mM, 500 mM, 600 mM, 700 mM or higher ionic strengths. Multivalent ions are preferred to achieve these ionic strengths in methods and formulations of the present invention, such as divalent, trivalent, tetravalent, pentavalent ions and ions of even higher valency. The pH buffer in solutions and formulations of the present invention may be phosphate, Tris, or HEPES (or other Good's buffers), but any other suitable pH buffer may be used. In preferred embodiments, the multivalent ions and buffer are selected to be compatible with the target tissue for the vector being prepared.

Use of multivalent ions in the methods and compositions of the invention makes it possible to create compositions of high ionic strength but relatively low osmolarity. High ionic strength compositions of the present invention may be nearly isotonic, and may be, for example, about 200mOsm, 250mOsm, 280mOsm, 300mOsm, 350mOsm or 400mOsm, although other osmolarities may be acceptable for some uses of the compositions.

AAV Aggregation as a Function of the Method of AAV Purification

Recombinant AAV2 purified using different methods (e.g. density gradient purification versus ion-exchange chromatography) would be expected to have different impurity profiles. FIG. 2 shows vector aggregation as a function of ionic strength for several preparations of AAV differing in the purification method. Purification methods are described in Example 1. Sodium chloride is used to vary the ionic strength. AAV2-FIX vectors purified by double cesium chloride gradient ultracentrifugation (Method 1), by cation exchange column chromatography (Method 2), or by combined column and cesium chloride gradient ultracentrifugation (Method 3) each demonstrate similar aggregation responses as ionic strength is decreased. In contrast, AAV2-FIX purified by the column method and then subjected to a nuclease digestion step (Method 2 + nuclease) shows reduced aggregation at low ionic strength.

AAV Aggregation at Preparative Scale

The data in Table 1 and FIGS. 1A, 1B and 2 involve vector aggregation *at an analytical scale*, employing DLS to measure aggregation. Table 2, in contrast, shows the effects of elevated ionic strength and nuclease treatment on AAV2 vector aggregation at a larger scale, using methods to induce and quantify vector aggregation that are relevant to *preparative scale* vector purification. Experimental details are provided in Example 2. Purified AAV vectors are diafiltered into solutions of various ionic strengths, the volume is reduced to achieve high vector concentrations, and aggregation is then assessed by measuring vector recovery after filtration through a 0.22μm filter. Aliquots from a single pool of AAV2-AADC vector purified by Method 1 through the second CsCl gradient centrifugation step ($1.8x10^{15}$ vg in 91mL, $1.8x10^{13}$ vg/mL, in ~3M CsCl) are used as starting material in the diafiltration experiments. Tangential

flow filtration using hollow fibers is used for diafiltration because it is scalable and yet it still enables preparation of volumes (min. 1.4mL), and thus AAV concentrations, at which aggregation would be expected in neutral buffered saline.

In Experiment 1, three hollow fiber units are used to diafilter AAV2-AADC vector in formulations CF, TF1, or TF2, and the volume is reduced to a target of $2.5x10^{13}$ vg/mL. *See* Example 2. The samples are then filtered through a 0.22μm filter. Results are shown in Table 2. Vector recovery ("Yield %") for both elevated ionic strength formulations TF1 (95 ± 7.4%) and TF2 (93 ± 7.4%) are significantly higher than the recovery using the control formulation CF (77 ± 6.6%).

TABLE 2
AAV VECTOR RECOVERY AT PROCESS SCALE

Experiment	Formulation	μ (mM)	Target (vg/mL)	Actual (vg/mL)	Yield % (RSD)
1	CF	160	2.5E13	1.93E13	77 (6.6)
1	TF1	310	2.5E13	2.38E13	95 (7.4)
1	TF2	510	2.5E13	2.33E13	93 (7.4)
2	CF	160	6.7E13	3.98E13	59 (6.0)
2	TF2	510	6.7E13	6.42E13	96 (4.4)
3	CF (-Bz)	160	3.6E13	2.46E13	68 (11)
3	CF (+Bz)	160	3.6E13	3.29E13	91 (12)

In Experiment 2, AAV2-AADC is concentrated to a higher target value ($6.7x10^{13}$ vg/mL) in CF or TF2. Vector recovery using TF2 (96 ± 4.4%) is again significantly higher than recovery using CF (59 ± 6.0%). Within the variability of the assays used, vector was recovered fully at both target concentrations using TF2, indicating that aggregation was prevented. In contrast, significant aggregation was observed at both target concentrations using CF, and the extent of

aggregation (i.e. loss following 0.22μm filtration) was higher at the higher target vector concentration. In an additional experiment (not shown), 50 μL samples of AAV2 vector are taken following concentration but prior to the 0.22μm filtration step of Experiment 2, and examined by light microscopy. Vector concentrated in CF contains obvious amounts of visible material (not shown), while no such material is seen in vector concentrated in TF2.

Experiment 3 examines the effect of prior nuclease digestion of purified vector on aggregation. In the absence of nuclease digestion recovery of AAV2-AADC in CF is 68 ± 11%, similar to the recoveries in Experiments 1 and 2. In contrast, purified vector treated with nuclease and then concentrated in CF gives higher recovery (91 ± 12%). These prep scale results reflect the same effect of nuclease digestion shown in FIG. 2 using the "dilution-stress" (analytical scale) method.

The results presented in Table 2 demonstrate that the methods and compositions of the present invention increase the recovery of AAV vector recovery. For example, in various embodiments of the present invention, recovery is improved from less than about 80% to at least about 85%, 90%, 95% or more.

AAV Stability and Activity Following Storage or Freeze-Thaw Cycling

Croyle and coworkers reported a significant loss of titer of AAV and adenovirus following multiple freeze-thaw cycling in sodium phosphate buffer, and demonstrated that the better pH buffering provided by potassium phosphate during freeze-thaw cycling prevented titer loss. Croyle, M. A. *et al.* (2001) *Gene Therapy* 8: 1281-1290. Results of our freeze-thaw stability study using sodium phosphate support these findings. We find that while 150mM sodium phosphate provides sufficient ionic strength to prevent aggregation during preparation

and non-frozen storage of concentrated AAV2-AADC vector, even a single freeze-thaw cycle at -20 or -80 °C results in aggregation.

AAV stability after storage or freeze-thaw (F/T) cycling is assessed in buffers of the present invention as follows. The concentrated vectors prepared in CF, TF1, and TF2 (Table 2, Experiment 1) are subjected to a short stability study to investigate whether aggregation will occur during refrigerated storage, or following multiple freeze-thaw (F/T) cycles. Aggregation is assessed by DLS using undiluted samples, and *Rh* values >20nm are deemed to indicate the occurrence of some level of aggregation.

TABLE 3

STABILITY OF AAV2 VECTORS

	Particle radius – Rh (nm)							
Formulation	Pre	4 °C	- 20 °C			- 80 °C		
		5d	1 F/T	5 F/T	10 F/T	1 F/T	5 F/T	10 F/T
CF	14.5	27.0	22.4	56.1	94.5	20.6	57.5	141
TF1	13.8	16.3	TH	TH	TH	TH	TH	TH
TF2	13.8	14.4	14.2	14.0	14.1	13.8	21.3	50.9

Pre: DLS radius measured immediately following 0.2μm filtration.

Vector concentrations (vg/mL): CF: 1.93E13, TF1: 2.38E13, TF2: 2.33E13.

TH: signal intensity is too high to measure because of extensive aggregation.

As shown in Table 3, AAV2-AADC vector prepared in CF shows some aggregation after 5 days of storage at 4 °C, as well as following one or more F/T cycles at -20 or -80°C. For vector prepared in TF1, no aggregation occurs after 5 days at 4°C, but aggregation occurs following a single F/T cycle at -20 or -80 °C as indicated by a DLS signal intensity that is too

high to measure. Visual inspection of these samples reveals slight cloudiness, which is consistent with aggregation. For vector prepared in TF2, no aggregation is observed at 4 °C, or following up to 10 F/T cycles at -20 °C. Some aggregation is observed following 5 and 10 F/T cycles at -80 °C.

AAV activity after storage or F/T cycling in TF2 is assessed as follows. As described above, the high ionic strength, isotonic formulation TF2 effectively prevents vector aggregation during concentration and storage, and therefore represents a promising candidate for further study. An important question is whether preparation and storage of the vector in high ionic strength TF2 would adversely affect its functional activity. To assess this, assays are performed to measure the infectious titer and the transduction efficiency of vectors prepared and stored for an extended period of time in TF2.

For infectivity, a highly sensitive infectivity assay capable of detecting single infectious events is used. Zhen, Z. *et al.* (2004) *Human Gene Ther.* 15: 709-715. AAV2-AADC is prepared in TF2 at a concentration of $6.4x10^{13}$ vg/mL. After being stored for 45 days at 4 °C the preparation has a vector genome to infectious unit ratio (vg/IU) of 13, compared to a value of 16 vg/IU for the reference vector. This difference is not significant given the reported variability of this assay (RSD ~50%).

Transduction efficiency is assessed by measuring the expression of AADC protein by ELISA following transduction of D7/4 cells. FIG. 3 shows no significant difference between vector prepared in TF2 and the reference control for vector input ranging from 10 to 10^5 vg/cell. Together, these data indicate that preparation and storage of AAV2 vectors in high ionic strength TF2 does not have a deleterious effect on vector infectivity or transduction efficiency.

Conclusion

The effect of ionic strength (μ) on virus particle interactions is determined to elucidate the mechanism of vector aggregation. The ionic strength of neutral-buffered isotonic saline (μ = 150mM) is insufficient to prevent aggregation of AAV2 vectors purified by gradient ultracentrifugation or by cation exchange chromatography at concentrations exceeding ~10^{13} particles/mL. Inclusion of sugars (sorbitol, sucrose, mannitol, trehalose, glycerol) at concentrations up to 5% (w/v) or of surfactants Tween80® (1%) or Pluronic® F68 (10%) does not prevent aggregation of vector particles.

In contrast, vector particles remain soluble when elevated ionic strength solutions (μ > 200mM) are used during purification and for final vector formulation. Elevated ionic strength solutions using isotonic excipient concentrations for *in vivo* administration are prepared with salts of multivalent ions, including sodium citrate, sodium phosphate, and magnesium sulfate. An isotonic formulation containing 10mM Tris, 100mM sodium citrate, 0.001% Pluronic® F68, pH 8.0 (μ ~500mM) enables concentration of AAV2-AADC vectors to 6.4x10^{13} vg/mL with no aggregation observed during preparation and following ten freeze-thaw cycles at -20 °C. *See* Table 3, below, and accompanying discussion. AAV2-AADC vectors prepared and stored for an extended period in elevated ionic strength formulation retain high infectivity titer (13 IU/vg) and transduction efficiency.

Nuclease treatment of purified AAV2 vectors reduces the degree of vector aggregation, implicating vector surface nucleic acid impurities in inter-particle interactions. Hence, purification methods to efficiently remove vector surface residual nucleic acids, coupled with the use of elevated ionic strength isotonic formulations, are useful methods to prevent AAV2 vector aggregation.

EXAMPLE 1

AAV PURIFICATION METHODS

AAV2 vectors expressing human coagulation factor IX (FIX) or human amino acid decarboxylase (AADC) are produced by triple transfection of HEK293 cells as previously described (Matsushita, T. *et al.* (1998) *Gene Therapy* **5**: 938-945), with modifications. For the large scale preparations, cells are cultured and transfected in 850 mm^2 roller bottles (Corning). Vectors are purified by one of three methods.

In purification Method 1, modified from Matsushita, transfected HEK293 cells in roller bottles are collected by centrifugation (1000g, 15min), resuspended in 10mM sodium phosphate, 500mM sodium chloride, pH 7.2, and lysed by three freeze / thaw cycles (alternating an ethanol / dry ice bath and a 37°C water bath). The cell lysate is clarified by centrifugation (8,000g, 15 min). The supernatant is then diluted to 200mM NaCl by addition of 10mM sodium phosphate, pH 7.2, and digested with Benzonase® (Merck, Purity Grade 1; 200 U/mL, 1h, 37 °C). The lysate is adjusted to 25mM $CaCl_2$ using a 1M stock solution, and incubated at 4°C for one hour.

The mixture is centrifuged (8,000g, 15 min), and the supernatant containing vector is collected. To precipitate virus from the clarified cell lysate, polyethylene glycol (PEG8000) is added to a final concentration of 8%, the mixture incubated at 4°C for three hours, and then centrifuged (8,000g, 15 min). The pellets containing vector are re-suspended with mixing in 0.15M NaCl, 50mM Hepes, 25mM EDTA, pH 8.0 and incubated at 4°C for 16 hours. The resuspended material is pooled, and solid cesium chloride is added to a final density of 1.40

gm/ml. Vector is then banded by ultracentrifugation (SW28, 27,000rpm, 24h, 20°C) using a Beckman model LE-80 centrifuge. The centrifugation tubes are fractionated, and densities from 1.38 to 1.42 gm/mL containing vector are pooled. This material is banded a second time by ultracentrifugation (NVT65 rotor, 65,000 rpm, 16h, 20°C), and fractions containing purified AAV2 vectors are pooled. To concentrate vector and to perform buffer exchange, vectors in concentrated cesium chloride solution are subjected to ultrafiltration / diafiltration (UF/DF) by tangential flow filtration as described below (Example 2).

In purification Method 2, cell harvests containing AAV are microfluidized and filtered sequentially through 0.65 and 0.22 μm filters (Sartorius). Virus is purified from the clarified cell lysates by cation exchange chromatography using Poros HS50 resin as previously described. U.S. Pat. No. 6,593,123. For the nuclease digestion described in FIG. 2, column-purified vectors are incubated (4h, RT) with 100 U/mL Benzonase and 10 U/mL DNAse I (RNAse free, Roche Diagnostics, Indianapolis, Indiana).

For purification Method 3, AAV2 vectors purified by cation exchange chromatography are subjected to an additional cesium chloride gradient ultracentrifugation step (SW28, 27,000rpm, 20h) to remove empty capsids prior to UF/DF.

Real time quantitative PCR (Q-PCR) is used to quantify AAV preparations as previously described. Sommer, J. M. *et al.* (2003) *Mol. Therapy* **7**: 122-128. Vectors purified by each of the three methods are analyzed by SDS-PAGE / silver staining analysis, and in all cases VP1, VP2 and VP3 are present in the expected ratios, with the capsid proteins representing >95% of total proteins as determined by scanning densitometry. However, unlike gradient-purified AAV2 vectors purified using Methods 1 and 3, vectors purified by Method 2 (column chromatography) contain empty capsids, ranging from 3-10 empty capsids per vector genome.

EXAMPLE 2

ULTRAFILTRATION AND DIAFILTRATION TO DETECT AAV AGGREGATION

Disposable hollow fiber tangential flow filtration devices (Amersham BioSciences 8" Midgee, 100 kDa nominal pore size) are used to concentrate and diafilter AAV2 vectors purified by the methods described above, and for the UF/DF experiments described in Table 2. For all UF/DF procedures a volume of diafiltration buffer corresponding to 10x the product volume is used, and it is added in ~1mL increments to approximate continuous diafiltration. Using this method, the calculated residual CsCl after diafiltration is <0.5mM.

The following three formulations were used for UF/DF: Control Formulation (CF: 140mM sodium chloride, 10mM sodium phosphate, 5% sorbitol, pH 7.3); Test Formulation 1 (TF1: 150mM sodium phosphate, pH7.5); and Test Formulation 2 (TF2: 100 mM sodium citrate, 10mM Tris, pH8.0). For Experiment 1 shown in Table 2, diafiltration is performed at a volume corresponding to a vector concentration of $1x10^{13}$ vg/mL, and following diafiltration the volume is reduced to a value corresponding to $2.5x10^{13}$ vg/mL (assuming no vector loss).

For Experiment 2, diafiltration is performed at a volume corresponding to a $2x10^{13}$ vg/mL, and the volume is then reduced to a value corresponding to $6.7x10^{13}$ vg/mL.

For Experiment 3 (CF ± Bz), AAV2-AADC (approximately $1.2x10^{14}$ vg) is first diafiltered into TF1 (a formulation compatible with nuclease activity) and then passed through a 0.22 μm filter. The titer of this material is determined, and the volume is adjusted to correspond to a concentration of $1x10^{13}$ vg/mL. To 10 mL of this material, $MgCl_2$ is added to a concentration of 2 mM, and then divided into two equal aliquots. One aliquot is incubated with Benzonase (200 U/mL, 4h, RT), and the second is mock-incubated. Each aliquot is then

diafiltered at a volume corresponding to a vector concentration $2x10^{13}$ vg/mL, and then concentrated to a $3.6x10^{13}$ vg/mL target. Following all UF/DF protocols, Pluronic® F-68 (BASF Corp., Mount Olive, NJ) from a 1% stock is added to the vector product to a final concentration of 0.001%, and the solution is passed through a 0.22μm syringe filter (Sartorius). All UF/DF procedures are performed in a laminar flow cabinet.

EXAMPLE 3

MEASUREMENT OF VECTOR AGGREGATION BY DYNAMIC LIGHT SCATTERING

Purified vectors are analyzed for aggregation by dynamic light scattering (DLS) using a Protein Solutions *DynaPro 99* (λ=825.4 nm). Primary data (particle radius – *Rh*, average value measured over 30 cycles, 10 cycles/min) are used for all analyses reported. A "dilution-stress" method is used to assess the effect of varying excipients on vector aggregation. In this method, 80 μL of test diluent is added to 20 μL of vector solution with mixing in the actual cuvette used for DLS measurement, and data collection is initiated within 10 seconds of mixing. Prior to addition of test diluents, the *Rh* value for AAV2 vector preparations is measured and confirmed to be <15 nm to ensure that the starting material is monomeric. Samples that are not 100% monomeric are passed through a 0.22μm syringe disc filter (Sartorius, low protein binding) to remove aggregates.

The osmolarity and ionic strength values given in FIGS. 1 and 2 are calculated using all excipients present in the mixture (i.e. weighted: test diluent (80%) and starting vector formulation (20%)). Osmolarity is calculated according to the equation: Osmolarity = $\sum c_i$, where c_i is the molar concentration of each solute species. The ionic strength (μ) is calculated

according to the equation: $\mu = \frac{1}{2} \sum c_i z_i^2$, where z_i is the charge on each species. In conditions that resulted in vector aggregation (e.g. low μ) a progressive increase in *Rh* is observed over the course of data collection. To validate the use of the average *Rh* measured over the 3 minute interval following dilution as a reliable measure of aggregation, the average rate of increase of *Rh* ($\Delta Rh / \Delta t$) over the same time interval is also determined (not shown). Analysis of $\Delta Rh / \Delta t$ gives results concordant with those obtained using the average *Rh* value reported in FIGS. 1 and 2.

EXAMPLE 4

AAV VIRION INFECTIVITY

Infectivity of AAV2-AADC vectors is determined using a highly sensitive assay as previously described. Zhen, Z. *et al.* (2004) *Human Gene Ther.* 15: 709-715. Briefly, samples are serially diluted (10-fold dilutions, 10 replicates / dilution) and added to D7/4 cells (modified HeLa cells expressing AAV *rep* and *cap*) grown in 96 well tissue culture plates (Falcon, cat. #353227) in DMEM medium containing 10% FBS. Adenovirus (Ad-5, 100 vp/cell) is added to each well to provide helper functions. After 48h, replication of AAV vector in each well is quantified by Q-PCR using transgene-specific primers and probes, and the frequency of infection at limiting dilution is analyzed by the Karber method to calculate the infectivity titer. The test sample is run concurrently with an AAV2-AADC reference previously prepared in CF and stored at -80 °C.

The transduction efficiency of AAV2 vectors is quantified by a whole cell ELISA. D7/4 cells grown in 96 well plates are infected with 10-fold serial dilutions of the test sample and reference vector, corresponding to 10 to 10^5 vg / cell input (5 replicates / dilution). After 48h,

the culture medium is removed, and cells are washed twice with 200 μL PBS (10 mM sodium phosphate, 140mM sodium chloride, pH 7.2). Cells are then permeabilized and fixed by addition of 100μL of PBS containing 0.5% Triton X-100 and 4% paraformaldehyde to each well (15 min). The fixing solution is removed, and the cells are washed twice with PBS containing 0.5% Triton X-100. Non-specific sites are blocked by adding PBS containing 3% bovine serum albumin (BSA) and 0.5% Triton X-100 (60min).

After washing, cells are incubated for one hour with rabbit anti-AADC IgG antibody (Chemicon, AB136), and washed. Cells are then incubated for one hour with alkaline phosphatase-conjugated goat anti-rabbit IgG, and washed. Antibodies are diluted 1:1000 in PBS containing 1% BSA, 0.5% Triton X-100. Substrate (PNPP, Pierce, cat. #34047) is then added (1 mg/mL in 1X diethanolamine substrate buffer, Pierce, cat. #34064), and after incubation for 30min the concentration of cleaved substrate is measured spectrophotometrically (λ=405nm). Human AADC expression as a function of vector input is fitted using a spline curve (SigmaPlot). The AAV2-AADC reference vector is measured concurrently with the test sample.

While preferred illustrative embodiments of the present invention are described, it will be apparent to one skilled in the art that various changes and modifications may be made therein without departing from the invention, and it is intended in the appended claims to cover all such changes and modifications that fall within the true spirit and scope of the invention.

All publications, patents and patent applications referred to herein are hereby incorporated by reference in their entireties.

We claim:

1. A composition for the storage of purified virus particles, comprising:

purified virus particles;

a pH buffer; and

excipients comprising one or more multivalent ions; wherein the ionic strength of the composition is greater than about 200 mM.

2. The composition of claim 1, wherein the purified virus particles are AAV virus particles.

3. The composition of claim 1, wherein one of the one or more multivalent ions is citrate.

4. The composition of claim 1, further comprising Pluronic® F68.

5. The composition of claim 4, wherein the Pluronic® F68 is present at 0.001%.

6. The composition of claim 1, wherein the pH buffer is 10 mM Tris, pH 8.0 and the excipients comprise 100 mM sodium citrate.

7. The composition of claim 1, wherein the average particle radius (*Rh*) of the purified virus particles is less than about 20nm as measured by dynamic light scattering.

8. The composition of claim 1, wherein recovery of the purified virus particles is at least about 90% following filtration of the composition of virions through a 0.22μm filter.

9. A method of preventing aggregation of virions in a preparation of virions, comprising treating said preparation of virions with Benzonase®.

10. The method of claim 9, wherein, after Benzonase® treatment, the average particle radius (*Rh*) of the virions in the preparation of virions is less than about 20nm as measured by dynamic light scattering.

11. The method of claim 9, wherein, after Benzonase® treatment, recovery of the virions is at least about 90% following filtration of the preparation of virions through a 0.22μm filter.

ABSTRACT

Compositions and methods are provided for preparation of concentrated stock solutions of AAV virions without aggregation. Formulations for AAV preparation and storage are high ionic strength solutions (e.g. μ ~500mM) that are nonetheless isotonic with the intended target tissue. This combination of high ionic strength and modest osmolarity is achieved using salts of high valency, such as sodium citrate. AAV stock solutions up to $6.4x10^{13}$ vg/mL are possible using the formulations of the invention, with no aggregation being observed even after ten freeze-thaw cycles. The surfactant Pluronic® F68 may be added at 0.001% to prevent losses of virions to surfaces during handling. Virion preparations can also be treated with nucleases to eliminate small nucleic acid strands on virions surfaces that exacerbate aggregation.

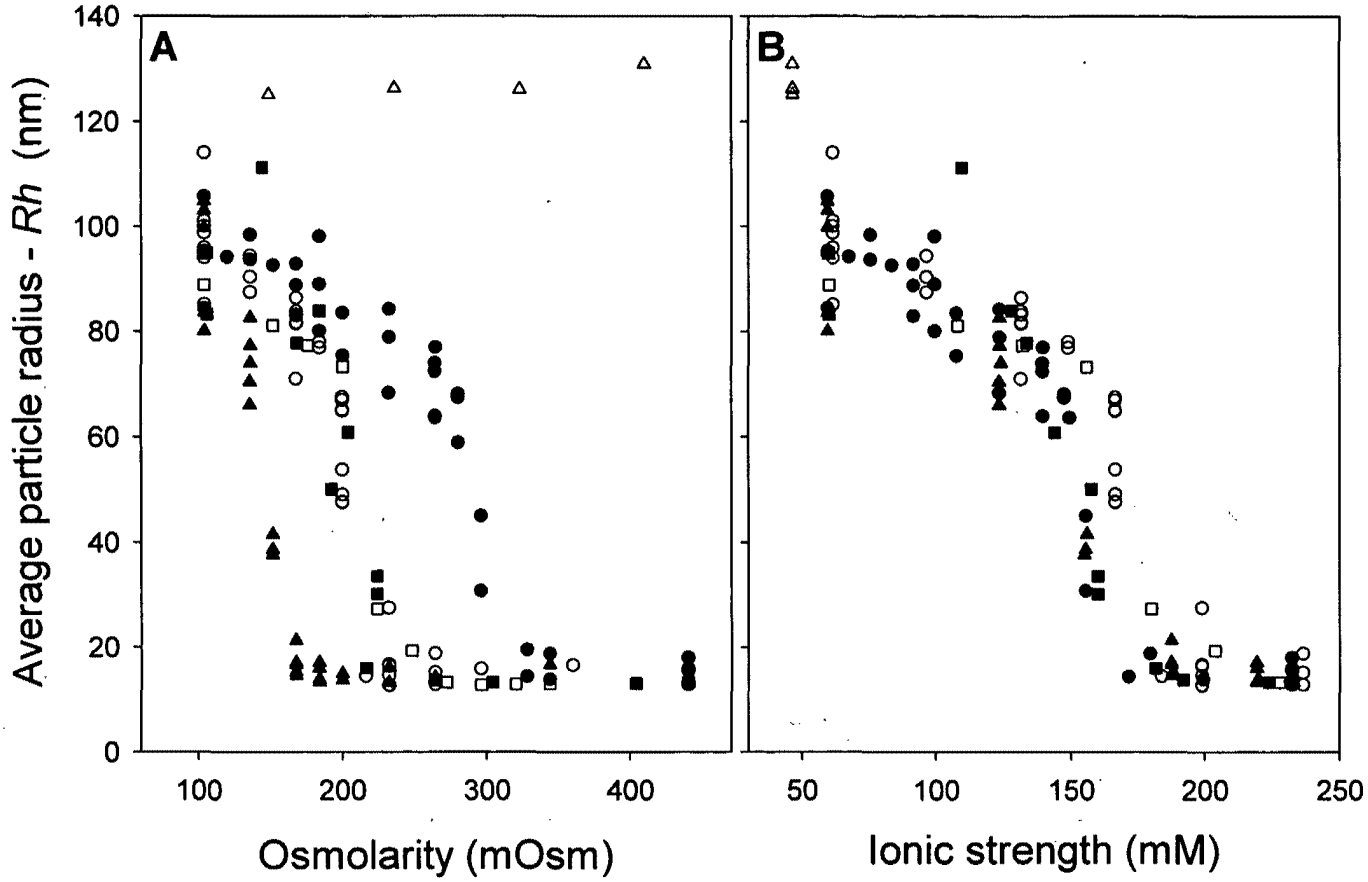
A
B
Average particle radius - Rh (nm)
0
20
40
60
80
100
120
140
100
200
300
400
50
100
150
200
250
Osmolarity (mOsm)
Ionic strength (mM)

FIGURE 1

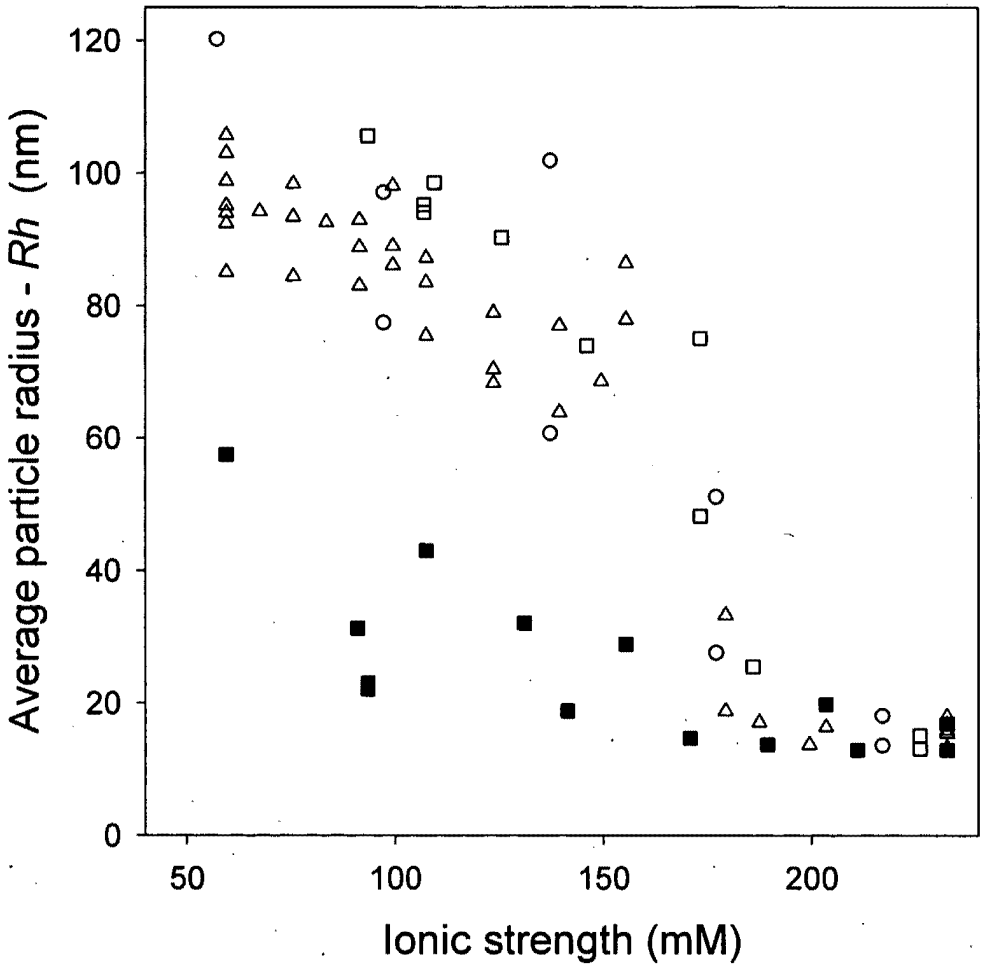
Average particle radius - Rh (nm)
120
100
80
60
40
20
0
50
100
150
200
Ionic strength (mM)

FIGURE 2

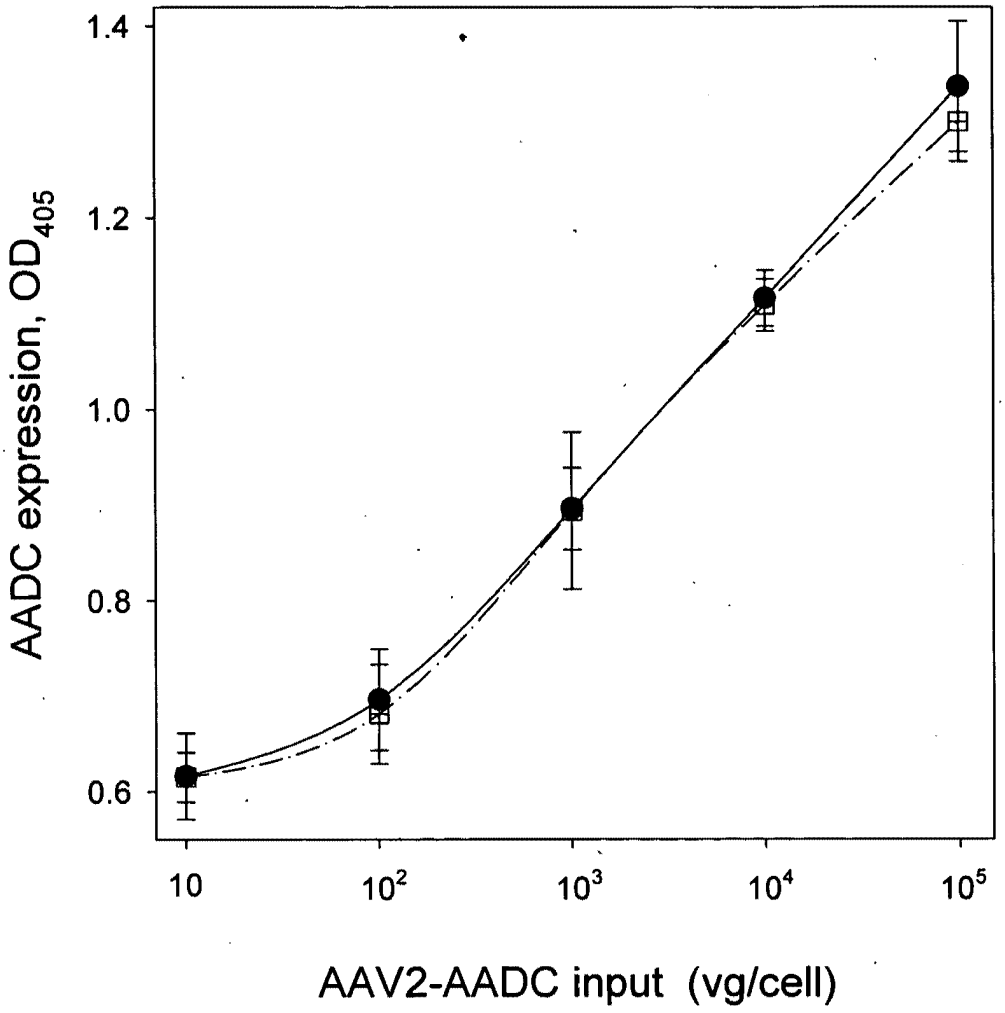
AADC expression, OD405
1.4
1.2
1.0
0.8
0.6
10
10^2
10^3
10^4
10^5
AAV2-AADC input (vg/cell)

FIGURE 3

PATENT APPLICATION SERIAL NO._____

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE
FEE RECORD SHEET

03/23/2010 HDESTA1 00000018 12661553

01 FC:1011	330.00	OP
02 FC:1111	540.00	OP
03 FC:1311	220.00	OP

PTO-1556
(5/87)

*U.S. Government Printing Office: 2002- 489-267/69033

PTO/SB/06 (12-04)

Filing Date: 031910

Approved for use through 7/31/2006. OMB 0651-0032

U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

12/661,553

APPLICATION AS FILED – PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE ($)	SMALL ENTITY FEE ($)	OR	OTHER THAN SMALL ENTITY RATE ($)	OTHER THAN SMALL ENTITY FEE ($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))							330
SEARCH FEE (37 CFR 1.16(k), (i), or (m))							540
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))							220
TOTAL CLAIMS (37 CFR 1.16(i))	11 minus 20 =		X 26 =		OR	X 52 =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	2 minus 3 =		X 110 =			X 220 =	
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $270 ($135 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))			N/A			N/A	
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	1090

APPLICATION AS AMENDED – PART II

AMENDMENT A

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE ($)	SMALL ENTITY ADDI-TIONAL FEE ($)	OR	OTHER THAN SMALL ENTITY RATE ($)	OTHER THAN SMALL ENTITY ADDI-TIONAL FEE ($)
Total (37 CFR 1.16(i))	*	Minus	**	=	X =		OR	X =	
Independent (37 CFR 1.16(h))	*	Minus	***	=	X =		OR	X =	
Application Size Fee (37 CFR 1.16(s))									
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))					N/A		OR	N/A	
					TOTAL ADD'T FEE		OR	TOTAL ADD'T FEE	

AMENDMENT B

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE ($)	SMALL ENTITY ADDI-TIONAL FEE ($)	OR	OTHER THAN SMALL ENTITY RATE ($)	OTHER THAN SMALL ENTITY ADDI-TIONAL FEE ($)
Total (37 CFR 1.16(i))	*	Minus	**	=	X =		OR	X =	
Independent (37 CFR 1.16(h))	*	Minus	***	=	X =		OR	X =	
Application Size Fee (37 CFR 1.16(s))									
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))					N/A		OR	N/A	
					TOTAL ADD'T FEE		OR	TOTAL ADD'T FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".

*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".

The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
12/661,553	03/19/2010	1657	1090	0800-0045.01	11	2

CONFIRMATION NO. 4726

20855
ROBINS & PASTERNAK
1731 EMBARCADERO ROAD
SUITE 230
PALO ALTO, CA 94303

FILING RECEIPT

OC000000040932096

Date Mailed: 04/05/2010

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Applicant(s)

John Fraser Wright, Princeton, NJ;
Guang Qu, Alameda, CA;

Power of Attorney: None

Domestic Priority data as claimed by applicant

This application is a CON of 11/141,996 06/01/2005
which claims benefit of 60/575,997 06/01/2004
and claims benefit of 60/639,222 12/22/2004

Foreign Applications

If Required, Foreign Filing License Granted: 04/01/2010

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 12/661,553**

Projected Publication Date: To Be Determined - pending completion of Corrected Papers

Non-Publication Request: No

Early Publication Request: No

Title

Compositions and methods to prevent AAV vector aggregation

Preliminary Class

435

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

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Title 37, Code of Federal Regulations, 5.11 & 5.15

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
12/661,553	03/19/2010	John Fraser Wright	0800-0045.01

CONFIRMATION NO. 4726

20855
ROBINS & PASTERNAK
1731 EMBARCADERO ROAD
SUITE 230
PALO ALTO, CA 94303

FORMALITIES LETTER

OC000000040932097

Date Mailed: 04/05/2010

NOTICE TO FILE CORRECTED APPLICATION PAPERS

Filing Date Granted

An application number and filing date have been accorded to this application. The application is informal since it does not comply with the regulations for the reason(s) indicated below. Applicant is given TWO MONTHS from the date of this Notice within which to correct the informalities indicated below. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

The required item(s) identified below must be timely submitted to avoid abandonment:

- Replacement drawings in compliance with 37 CFR 1.84 and 37 CFR 1.121(d) are required. The drawings submitted are not acceptable because:
  - The drawings must be reasonably free from erasures and must be free from alterations, overwriting, interlineations, folds, and copy marks. See Figure(s) 1-3.

Applicant is cautioned that correction of the above items may cause the specification and drawings page count to exceed 100 pages. If the specification and drawings exceed 100 pages, applicant will need to submit the required application size fee.

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Office of Data Management, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

Attorney Docket No. 0800-0045.01
PATENT

CERTIFICATE OF MAILING PURSUANT TO 37 CFR § 1.8

I hereby certify pursuant to 37 CFR §1.8 that this correspondence is being transmitted via EFS to the United States Patent and Trademark Office on the date shown below.

Date 4/9/10 Signature Gail Wardwell

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

JOHN FRASER WRIGHT et al.		Examiner:	Not Assigned
Serial No.:	12/661,553	Art Unit:	Not Assigned
Filed:	March 19, 2010	Confirmation No.:	Not Assigned
For:	COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION		

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

INFORMATION DISCLOSURE STATEMENT UNDER 37 C.F.R. §1.97(b)

Sir:

In accordance with the duty of disclosure set forth in 37 C.F.R. §1.56, Applicant(s) hereby submits the following information in conformance with 37 C.F.R. §§1.97 and 1.98.

[] Pursuant to 37 C.F.R. §1.98, a copy of each document cited in the attached Form PTO/SB/08 is enclosed.

[] No copy of the publication _____ listed on the attached Form PTO/SB/08A are being provided because the Office waives the requirement under 37 C.F.R. 1.98 (a) (2) (i) for submitting a copy of each cited U.S. patent and each U. S. patent application publication for all U.S. national patent applications filed after June 30, 2003.

[X] No copies of the publications listed on the attached Form PTO/SB/08A are being provided pursuant to 37 C.F.R. §1.98(d) because the publications were previously cited by or submitted to the Office in prior Application

Serial No. 11/141,996 to which the above-identified application claims priority under 35 U.S.C. §120.

[] Publication(s) _____ listed on the attached Form PTO/SB/08A were cited in a foreign search or examination report corresponding to ____ application serial no. _____ and mailed on _____.

[] Enclosed is a copy of a non-English publication(s) ___. Pursuant to §609 of the M.P.E.P., Applicant submits the attached foreign search or examination report, which cites such non-English language publication(s).

[] Enclosed is a copy of a non-English publication(s) ___. English language publication ___ (copy enclosed) claims priority from this non-English publication.

[] Enclosed is an explanation of non-English publication(s) ___ for which an English translation is not available.

This Information Disclosure Statement is filed within any one of the following time periods:

[] within three months from the filing date of this national application other than a CPA under 37 C.F.R. § 1.53(d);

[] within three months from the date of entry of the national stage as set forth in 37 C.F.R. §1.491 in this international application;

[X] before the mailing date of a first office action on the merits; or

[] before the mailing of a first office action after the filing of a request for continued examination under 37 C.F.R. §1.114.

It is respectfully requested that the Examiner consider the above-noted information and return an initialed copy of the attached Form PTO/SB/08A to the undersigned.

Dated: 4/9/2010

Respectfully submitted,

Robins & Pasternak LLP
1731 Embarcadero Road, Suite 230
Palo Alto, CA 94303
Tel: (650) 493-3400
Fax: (650) 493-3440

By: _____

Roberta L. Robins
Reg. No. 33,208

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PTO/SB/08A (08-00)

Substitute for form 1449A/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** *(use as many sheets as necessary)*				***Complete if Known***	
				Application Number	12/661,553
				Filing Date	March 19, 2010
				First Named Inventor	Wright et al.
				Group Art Unit	Not Assigned
				Examiner Name	Not Assigned
Sheet	1	of	4	Attorney Docket Number	0800-0045.01

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	U.S. Patent Document Number	U.S. Patent Document Kind Code[2] (*if known*)	Name of Patentee or Applicant of Cited Document	Date of Publication of Cited Document MM-DD-YYYY
	A1	6,593,123	435/239	Wright et al.	07-2001
	A2	6,566,118	435/239	Atkinson et al.	05-2003
	A3	6,194,191	435/235.1	Zhang et al.	02-2001
	A4	6,146,874	435/235.1	Zolotukhin et al.	11-2000

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Office[3]	Number[4]	Kind Code[5] (if known)	Name of Patentee or Applicant of Cited Document	Date of Publication of Cited Document MM-DD-YYYY	T[6]

Examiner Signature		Date Considered	

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[2] See attached Kinds of U.S. Patent Documents.
[3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3).
[4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document.
[5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST. 16 if possible.
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				Application Number	12/661,553
				Filing Date	March 19, 2010
				First Named Inventor	Wright et al.
				Group Art Unit	Not Assigned
				Examiner Name	Not Assigned
Sheet	2	of	4	Attorney Docket Number	0800-0045.01

OTHER PRIOR ART – NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[1]
	C1	ADADEYOH et al., "SHORT-TERM FIELD USE AND SHIPPING STABILITY STUDY OF A WILD TYPE AD5 ADENOVIRAL REFERENCE MATERIAL," BioProcessing, 1(3):62-9 (2002)	
	C2	BRAUN et al., "PROTEIN AGGREGATES SEEM TO PLAY A KEY ROLE AMONG THE PARAMETERS INFLUENCING THE ANTIGENICITY OF INTERFERON ALPHA (IFN-ALPHA) IN NORMAL AND TRANSGENIC MICE," Pharm. Res. 14(10):1472-1478 (1997)	
	C3	CHEN et al., "STRATEGIES TO SUPPRESS AGGREGATION OF RECOMBINANT KERATINOCYTE GROWTH FACTOR DURING LIQUID FORMULATION DEVELOPMENT," J. Pharm. Sci. 83(12):1657-1661 (1994)	
	C4	CHENUAUD et al., "AUTOIMMUNE ANEMIA IN MACAQUES FOLLOWING ERYTHROPOIETIN GENE THERAPY," Blood 103(9):3303-3304 (2004)	
	C5	CROYLE et al., "DEVELOPMENT OF FORMULATIONS THAT ENHANCE PHYSICAL STABILITY OF VIRAL VECTORS FOR GENE THERAPY," Gene Therapy 8(17):1281-1290 (2001)	
	C6	DRITTANTI et al., "OPTIMISED HELPER VIRUS-FREE PRODUCTION OF HIGH QUALITY ADENO-ASSOCIATED VIRUS VECTORS", The Journal of Gene Medicine, 3:59-71 (2001)	
	C7	FLOTTE, T. R., "IMMUNE RESPONSES TO RECOMBINANT ADENO-ASSOCIATED VIRUS VECTORS: PUTTING PRECLINICAL FINDINGS INTO PERSPECTIVE," Human Gene Ther. 15(7):716-717 (2004)	
	C8	GAO et al., "ERYTHROPOIETIN GENE THERAPY LEADS TO AUTOIMMUNE ANEMIA IN MACAQUES," Blood 103(9):3300-3302 (2004)	
	C9	HIGH et al., "HUMAN IMMUNE RESPONSES TO AAV-2 CAPSID MAY LIMIT DURATION OF EXPRESSION IN LIVER-DIRECTED GENE TRANSFER IN HUMAN WITH HEMOPHILIS β," Blood, 104(11):121a, Abstract No. 413 (2004)	
	C10	HUANG et al., "ADAAV SUPPORT HIGH-TITER PRODUCTION OF RAAV BUT NOT STABLE," Mol. Therapy 1:S286 (2000)	
	C11	MEIJER et al., "TARGETING OF DRUGS TO THE LIVER," Sem. Liver Dis. 15(3):202-256 (1995)	
	C12	QU et al., "EVIDENCE THAT IONIC INTERACTIONS ARE INVOLVED IN CONCENTRATION-INDUCED AGGREGATION OF RECOMBINANT ADENO-ASSOCIATED VIRUS," Molecular Therapy, 7(5):S348, abstract No. 901 (2003)	

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

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				First Named Inventor	Wright et al.
				Group Art Unit	Not Assigned
				Examiner Name	Not Assigned
Sheet	3	of	4	Attorney Docket Number	0800-0045.01

OTHER PRIOR ART – NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[1]
	C13	QU et al., "SCALING UP PRODUCTION OF RECOMBINANT AAV VECTORS FOR CLINICAL APPLICATIONS," Curr Opin Drug Disc Dev. 3(6):750-755 (2000)	
	C14	SHIRE et al., "CHALLENGES IN THE DEVELOPMENT OF HIGH PROTEIN CONCENTRATION FORMULATIONS," J. Pharm. Sci. 93(6):1390-1402 (2004)	
	C15	SOMMER et al., "QUANTIFICATION OF ADENO-ASSOCIATED VIRUS PARTICLES AND EMPTY CAPSIDS BY OPTICAL DENSITY MEASUREMENT," Mol Ther 7(1):122-128 (2003)	
	C16	STEINBACH et al., "ASSEMBLY OF ADENO-ASSOCIATED VIRUS TYPE 2 CAPSIDS IN VITRO," J Gen. Virol. 78(6):1453-1462 (1997)	
	C17	WANG, W., "INSTABILITY, STABILIZATION, AND FORMULATION OF LIQUID PROTEIN PHARMACEUTICALS," Int. J. Pharm. 185(2):129-188 (1999)	
	C18	WON et al., "STABILIZERS AGAINST HEAT-INDUCED AGGREGATION OF RPR 114849, AN ACIDIC FIBROBLAST GROWTH FACTOR (AFGF)," Int. J. Pharm. 167:25-36 (1998)	
	C19	WRIGHT et al., "RECOMBINANT ADENO-ASSOCIATED VIRUS: FORMULATION CHALLENGES AND STRATEGIES FOR A GENE THERAPY VECTOR," Curr. Opin. Drug Disc. Dev. 6(2):174-178 (2003).	
	C20	WRIGHT et al., "425. FORMULATION DEVELOPMENT FOR AAV2 VECTORS: IDENTIFICATION OF EXCIPIENTS THAT INHIBIT VECTOR AGGREGATION," Mol. Ther. [Online] 9(S1):S163-S163 (2004)	
	C21	WRIGHT et al., "IDENTIFICATION OF FACTORS THAT CONTRIBUTE TO RECOMBINANT AAV2 PARTICLE AGGREGATION AND METHODS TO PREVENT ITS OCCURRENCE DURING VECTOR PURIFICATION AND FORMULATION," Mol. Ther. 12(1):171-178 (2005)	
	C22	XIE et al., "LARGE-SCALE PRODUCTION, PURIFICATION AND CRYSTALLIZATION OF WILD-TYPE ADENO-ASSOCIATED VIRUS-2," J. Virol. Methods 122(1):17-27 (2004)	
	C23	XIE et al., "THE ATOMIC STRUCTURE OF ADENO-ASSOCIATED VIRUS (AAV-2), A VECTOR FOR HUMAN GENE THERAPY," Proc. Natl. Acad. Sci. U.S.A. 99(16):10405-10410 (2002)	
	C24	ZHEN et al., "INFECTIOUS TITER ASSAY FOR ADENO-ASSOCIATED VIRUS VECTORS WITH SENSITIVITY SUFFICIENT TO DETECT SINGLE INFECTIOUS EVENTS," Human Gene Ther. 15:709-715 (2004)	

Examiner Signature		Date Considered	

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				Group Art Unit	Not Assigned
				Examiner Name	Not Assigned
Sheet	4	of	4	Attorney Docket Number	0800-0045.01

OTHER PRIOR ART – NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[1]
	C25	The Term "PURIFY" AND "ABOUT", Merriam-Webster Online Dictionary, at the web- http://www.m-w.com, page 1 and page 2	
	C26	The Definition of the Term "IONIC STRENGTH", Answer.com, at the web- http://www.answers.com, page 1	

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] Unique citation designation number.

[1] Applicant is to place a check mark here if English language Translation attached.

Electronic Acknowledgement Receipt

EFS ID:	7389348
Application Number:	12661553
International Application Number:	
Confirmation Number:	4726
Title of Invention:	Compositions and methods to prevent AAV vector aggregation
First Named Inventor/Applicant Name:	John Fraser Wright
Customer Number:	20855
Filer:	Roberta L. Robins/Gail Wardwell
Filer Authorized By:	Roberta L. Robins
Attorney Docket Number:	0800-0045.01
Receipt Date:	09-APR-2010
Filing Date:	19-MAR-2010
Time Stamp:	17:53:10
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		0800_20100409145803.pdf	501063 4978c1d656bc7459a9e9e7bf78edc418cb075eb8	yes	12

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Transmittal Letter	1	1
	Miscellaneous Incoming Letter	2	3
	Drawings-only black and white line drawings	4	6
	Information Disclosure Statement (IDS) Filed (SB/08)	7	12
Warnings:			
Information:			
	Total Files Size (in bytes):	501063	

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/SB/21 (09-04)

TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Application Number	12/661,553
Filing Date	March 19, 2010
First Named Inventor	John Frasier Wright et al.
Art Unit	Unknown
Examiner Name	Unknown
Attorney Docket Number	0800-0045.01

Total Number of Pages in This Submission: **12**

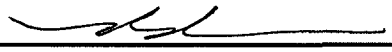
ENCLOSURES *(Check all that apply)*

- ☐ Fee Transmittal Form
  - ☐ Fee Attached
- ☐ Amendment/Reply
  - ☐ After Final
  - ☐ Affidavits/declaration(s)
- ☐ Extension of Time Request
- ☐ Express Abandonment Request
- ☒ Information Disclosure Statement (2 pages) and PTO/SB/08A (4 pages)
- ☐ Certified Copy of Priority Document(s)
- ☐ Reply to Missing Parts/ Incomplete Application
  - ☐ Reply to Missing Parts under 37 CFR 1.52 or 1.53
- ☐ Drawing(s)
- ☐ Licensing-related Papers
- ☐ Petition
- ☐ Petition to Convert to a Provisional Application
- ☐ Power of Attorney, Revocation Change of Correspondence Address
- ☐ Terminal Disclaimer
- ☐ Request for Refund
- ☐ CD, Number of CD(s) _____
  - ☐ Landscape Table on CD
- ☐ After Allowance Communication to TC
- ☐ Appeal Communication to Board of Appeals and Interferences
- ☐ Appeal Communication to TC **(Appeal Notice, Brief, Reply Brief)**
- ☐ Proprietary Information
- ☐ Status Letter
- ☒ Other Enclosure(s) (please identify below):

Copy of Notice to File Corrected Application Papers (2 pages); Replacement Drawings (3 sheets)

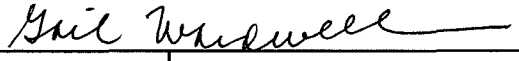
Remarks: **The Commissioner is authorized to charge any additional fees to Deposit Account 18-1648.**

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	Robins & Pasternak LLP		
Signature			
Printed name	Roberta L. Robins		
Date	4/9/2010	Reg. No.	33,208

CERTIFICATE OF TRANSMISSION/MAILING

I hereby certify pursuant to 37 CFR §1.8 that this correspondence is being transmitted via EFS to the United States Patent and Trademark Office on the date shown below.



Signature			
Typed or printed name	Gail Wardwell	Date	4/9/10

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UNITED STATES DEPARTMENT OF COMMERCE
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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
12/661,553	03/19/2010	John Fraser Wright	0800-0045.01

CONFIRMATION NO. 4726

FORMALITIES LETTER

20855
ROBINS & PASTERNAK
1731 EMBARCADERO ROAD
SUITE 230
PALO ALTO, CA 94303

Date Mailed: 04/05/2010

NOTICE TO FILE CORRECTED APPLICATION PAPERS

Filing Date Granted

An application number and filing date have been accorded to this application. The application is informal since it does not comply with the regulations for the reason(s) indicated below. Applicant is given TWO MONTHS from the date of this Notice within which to correct the informalities indicated below. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

The required item(s) identified below must be timely submitted to avoid abandonment:

- Replacement drawings in compliance with 37 CFR 1.84 and 37 CFR 1.121(d) are required. The drawings submitted are not acceptable because:
  - The drawings must be reasonably free from erasures and must be free from alterations, overwriting, interlineations, folds, and copy marks. See Figure(s) 1-3.

Applicant is cautioned that correction of the above items may cause the specification and drawings page count to exceed 100 pages. If the specification and drawings exceed 100 pages, applicant will need to submit the required application size fee.

0800-0045.01
DOCKETED RLR
replacement dwgs 6/5/10

Replies should be mailed to:

Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

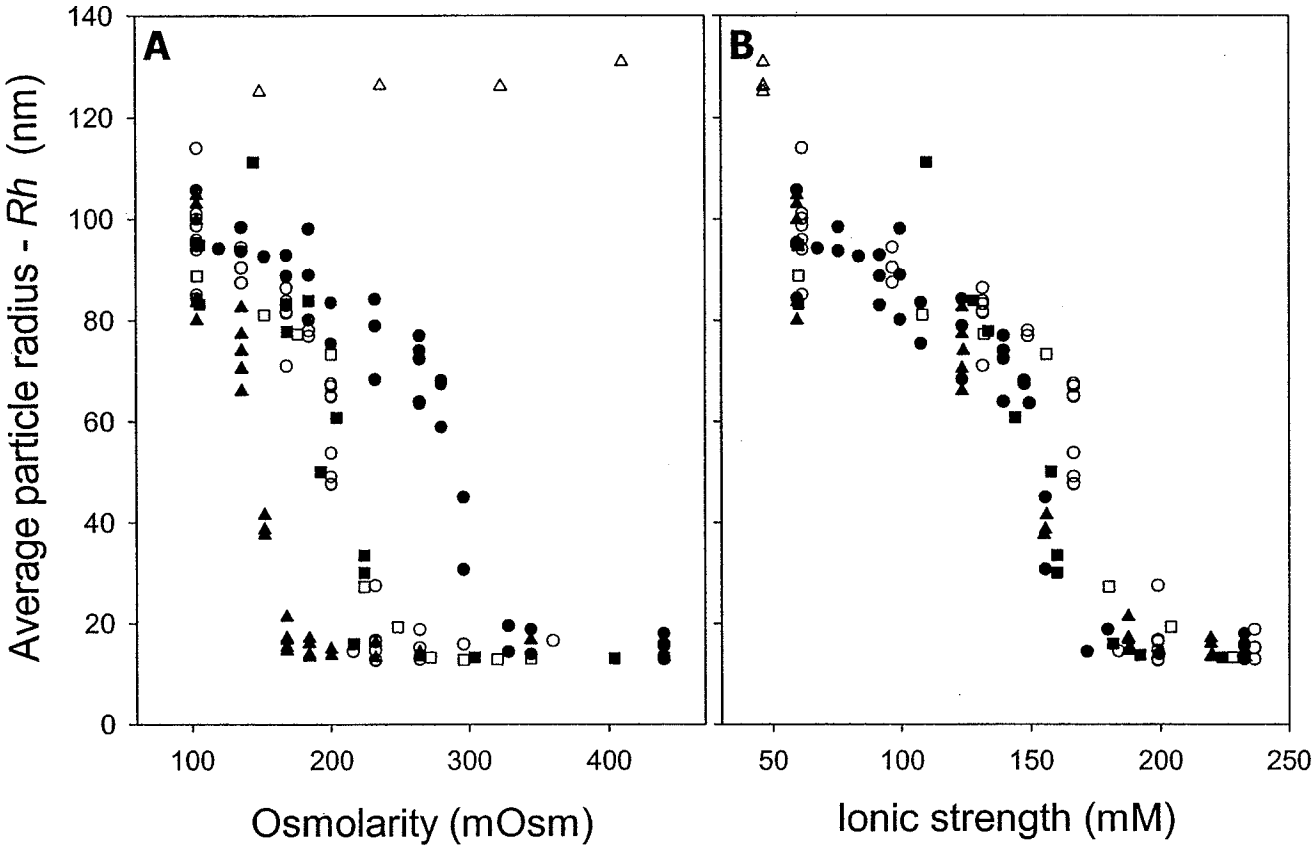
Registered users of EFS-Web may alternatively submit their reply to this notice via EFS-Web.
https://sportal.uspto.gov/authenticate/AuthenticateUserLocalEPF.html

For more information about EFS-Web please call the USPTO Electronic Business Center at **1-866-217-9197** or visit our website at http://www.uspto.gov/ebc.

If you are not using EFS-Web to submit your reply, you must include a copy of this notice.

/nhassani/

Office of Data Management, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101


A
B
Average particle radius - Rh (nm)
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20
40
60
80
100
120
140
100
200
300
400
Osmolarity (mOsm)
50
100
150
200
250
Ionic strength (mM)

FIGURE 1

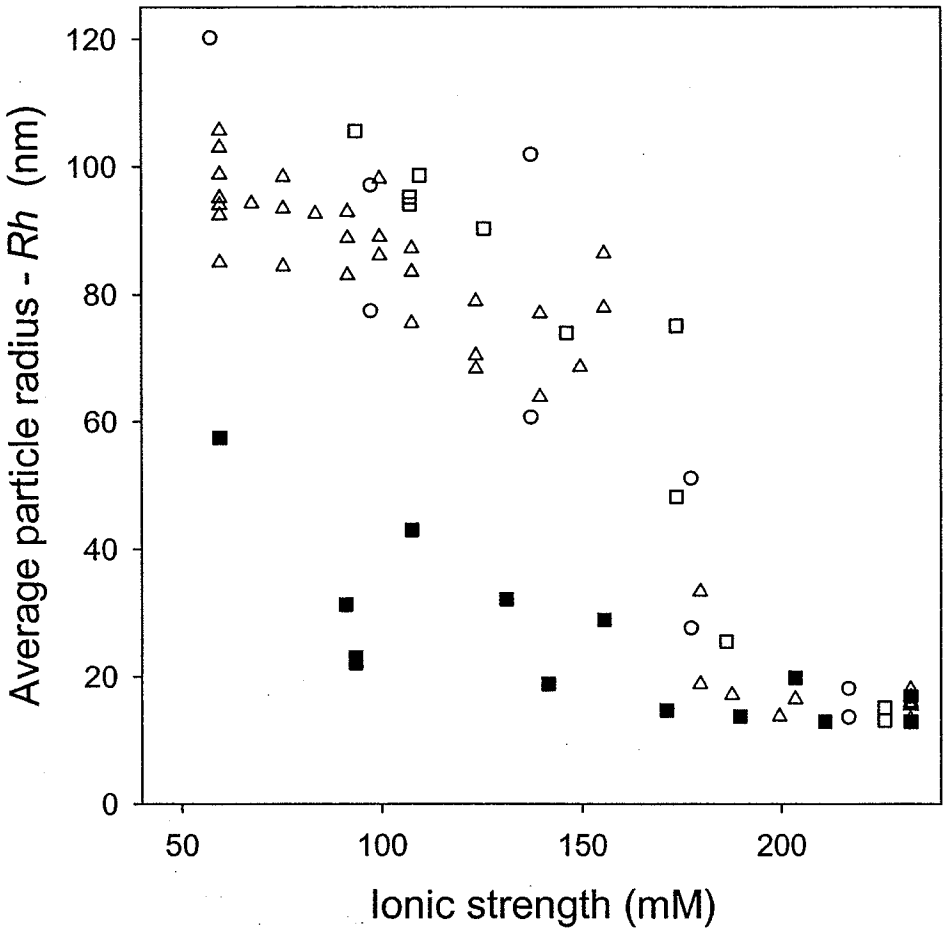
Average particle radius - Rh (nm)
120
100
80
60
40
20
0
50
100
150
200
Ionic strength (mM)

FIGURE 2

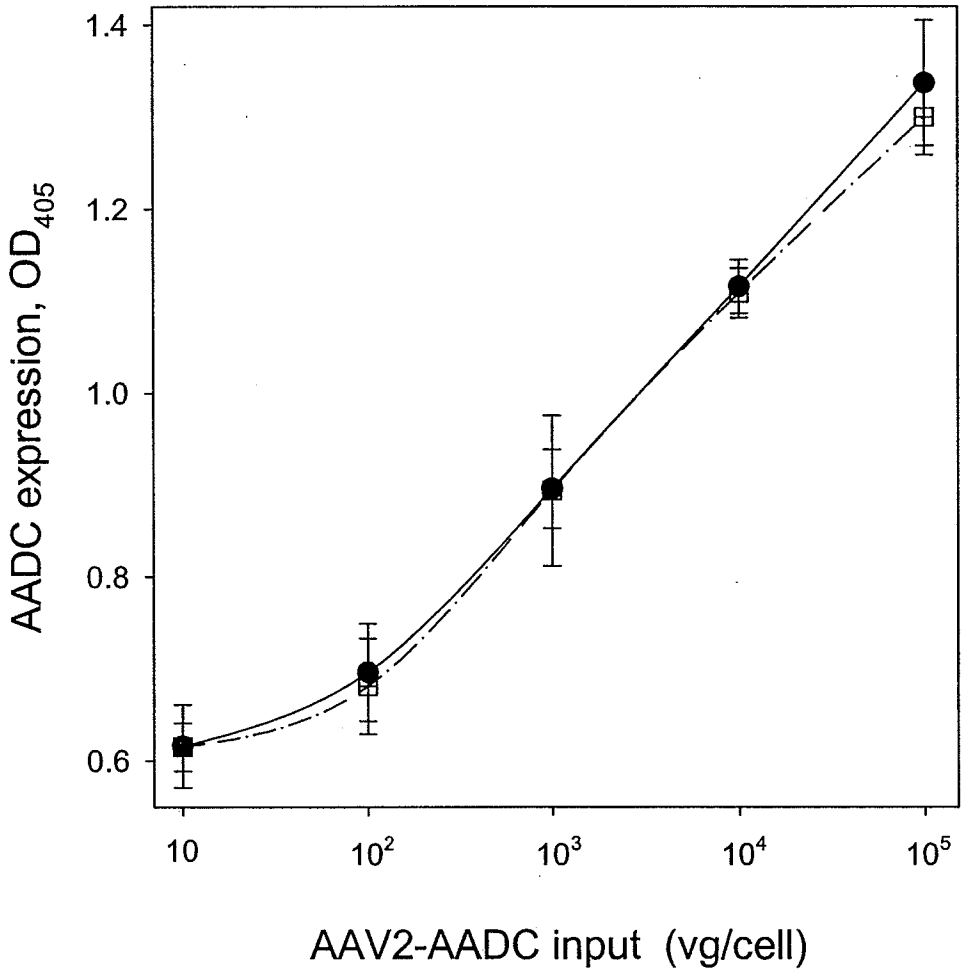
AADC expression, OD405
1.4
1.2
1.0
0.8
0.6
10
10^2
10^3
10^4
10^5
AAV2-AADC input (vg/cell)

FIGURE 3

PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 12/661,553

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	330
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	540
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	220
TOTAL CLAIMS (37 CFR 1.16(i))	11 minus 20 =	*			OR	x 52 =	0.00
INDEPENDENT CLAIMS (37 CFR 1.16(h))	2 minus 3 =	*				x 220 =	0.00
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $270 ($135 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						0.00
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							0.00
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	1090

APPLICATION AS AMENDED - PART II

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT A	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE($)	ADDITIONAL FEE($)		RATE($)	ADDITIONAL FEE($)
AMENDMENT B	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".

*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".

The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.

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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
12/661,553	03/19/2010	1657	1090	0800-0045.01	11	2

CONFIRMATION NO. 4726

UPDATED FILING RECEIPT

20855
ROBINS & PASTERNAK
1731 EMBARCADERO ROAD
SUITE 230
PALO ALTO, CA 94303

OC000000045010039

Date Mailed: 12/17/2010

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Applicant(s)

John Fraser Wright, Princeton, NJ;
Guang Qu, Alameda, CA;

Power of Attorney: None

Domestic Priority data as claimed by applicant

This application is a CON of 11/141,996 06/01/2005 PAT 7,704,721
which claims benefit of 60/575,997 06/01/2004
and claims benefit of 60/639,222 12/22/2004

Foreign Applications

If Required, Foreign Filing License Granted: 04/01/2010

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 12/661,553**

Projected Publication Date: 03/31/2011

Non-Publication Request: No

Early Publication Request: No

Title

Compositions and methods to prevent AAV vector aggregation

Preliminary Class

435

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4158).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

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NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
12/661,553	03/19/2010	John Fraser Wright	0800-0045.01

CONFIRMATION NO. 4726

20855
ROBINS & PASTERNAK
1731 EMBARCADERO ROAD
SUITE 230
PALO ALTO, CA 94303

PUBLICATION NOTICE

OC000000046871581

Title: Compositions and methods to prevent AAV vector aggregation

Publication No.US-2011-0076744-A1
Publication Date:03/31/2011

NOTICE OF PUBLICATION OF APPLICATION

The above-identified application will be electronically published as a patent application publication pursuant to 37 CFR 1.211, et seq. The patent application publication number and publication date are set forth above.

The publication may be accessed through the USPTO's publically available Searchable Databases via the Internet at www.uspto.gov. The direct link to access the publication is currently http://www.uspto.gov/patft/.

The publication process established by the Office does not provide for mailing a copy of the publication to applicant. A copy of the publication may be obtained from the Office upon payment of the appropriate fee set forth in 37 CFR 1.19(a)(1). Orders for copies of patent application publications are handled by the USPTO's Office of Public Records. The Office of Public Records can be reached by telephone at (703) 308-9726 or (800) 972-6382, by facsimile at (703) 305-8759, by mail addressed to the United States Patent and Trademark Office, Office of Public Records, Alexandria, VA 22313-1450 or via the Internet.

In addition, information on the status of the application, including the mailing date of Office actions and the dates of receipt of correspondence filed in the Office, may also be accessed via the Internet through the Patent Electronic Business Center at www.uspto.gov using the public side of the Patent Application Information and Retrieval (PAIR) system. The direct link to access this status information is currently http://pair.uspto.gov/. Prior to publication, such status information is confidential and may only be obtained by applicant using the private side of PAIR.

Further assistance in electronically accessing the publication, or about PAIR, is available by calling the Patent Electronic Business Center at 1-866-217-9197.

Office of Data Managment, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/661,553	03/19/2010	John Fraser Wright	0800-0045.01	4726

20855 7590 09/08/2011

ROBINS & PASTERNAK
1731 EMBARCADERO ROAD
SUITE 230
PALO ALTO, CA 94303

EXAMINER	
SINGH, SATYENDRA K	

ART UNIT	PAPER NUMBER
1653	

MAIL DATE	DELIVERY MODE
09/08/2011	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	12/661,553	WRIGHT ET AL.	
	Examiner	Art Unit	
	SATYENDRA SINGH	1653	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *1* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *19 March 2010*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

5)☒ Claim(s) *1-11* is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☐ Claim(s) _____ is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☒ Claim(s) *1-11* are subject to restriction and/or election requirement.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

12)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Applicant's submission filed on 03/19/2010 is duly acknowledged.

Claims 1-11 are pending in this application, and are subject to election/restriction as follows:

Election/Restrictions

Restriction to one of the following inventions is required under 35 U.S.C. 121:

I. Claims 1-8, drawn to **a composition for the storage of purified virus particles** as recited in claim 1, classified in class 435, subclass 235.1 and various.

II. Claims 9-11, drawn to **a method of preventing aggregation of virions** in a preparation of virions comprising treating said preparation of virions with BENZONASE, classified in class 424, subclass 93.1 and others.

The inventions are distinct, each from the other because of the following reasons:

Inventions of groups I and II are patentably distinct and unrelated. Inventions are unrelated if it can be shown that they are not disclosed as capable of use together and they have different designs, modes of operation, and effects (MPEP § 802.01 and § 806.06). In the instant case, the inventions of groups I and II as claimed have different design, modes of operations and end points/results. The invention of group I as claimed (i.e. the "*composition for the storage of purified virus particles*"; see claim 1, in particular) requires the use of "pH buffer" and "one or more multivalent ions" in a particular "ionic strength", whereas the process invention of group II is specifically directed to a process of "preventing aggregation of virions in a preparation of virions" by "*treating said preparation of virions with BENZONASE*" (an endonuclease), which is completely different than using multivalent ions and pH buffer, as currently recited for the

composition of group I. Thus, the invention of group II does not make the invention of group I, and is unrelated to the product composition of group I, as currently presented.

Restriction for examination purposes as indicated is proper because all these inventions listed in this action are independent or distinct for the reasons given above and there would be a serious search and/or examination burden if restriction were not required because at least the following reason(s) apply:

The search and examination of the above groups of distinct inventions as currently claimed by applicants would be time consuming and burdensome for the examiner as it would require search of different unrelated classes and subclasses (see the different groups above) for the distinct processes as well products as currently claimed. The specific concepts and/or limitations searched for one group of invention would not necessarily provide the pertinent prior art for the others. In addition, the search and examination burden for the examiner lies not only in the search of US Patents, but in the search for literature and foreign patents, and examination of the claim language and specification for compliance with the statutes concerning new matter, distinctness of the inventions, scope of enablement, and various double patenting issues.

Applicant is advised that the reply to this requirement to be complete must include (i) an election of a invention to be examined even though the requirement may be traversed (37 CFR 1.143) **and (ii) identification of the claims encompassing the elected invention**.

The election of an invention may be made with or without traverse. To reserve a right to petition, the election must be made with traverse. If the reply does not distinctly and specifically point out supposed errors in the restriction requirement, the election shall be treated as an election without traverse. Traversal must be presented at the time of election in order to be

considered timely. Failure to timely traverse the requirement will result in the loss of right to petition under 37 CFR 1.144. If claims are added after the election, applicant must indicate which of these claims are readable upon the elected invention.

Should applicant traverse on the ground that the inventions are not patentably distinct, applicant should submit evidence or identify such evidence now of record showing the inventions to be obvious variants or clearly admit on the record that this is the case. In either instance, if the examiner finds one of the inventions unpatentable over the prior art, the evidence or admission may be used in a rejection under 35 U.S.C. 103(a) of the other invention.

Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 CFR 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a request under 37 CFR 1.48(b) and by the fee required under 37 CFR 1.17(i).

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SATYENDRA SINGH whose telephone number is (571)272-8790. The examiner can normally be reached on 9-5MF.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, SUE X. LIU can be reached on 571-272-5539. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Satyendra K. Singh/
Examiner, Art Unit 1653

/Amber D. Steele/
Primary Examiner, Art Unit 1654

CERTIFICATE OF TRANSMISSION PURSUANT TO 37 CFR § 1.8

I hereby certify pursuant to 37 CFR §1.8 that this correspondence is being transmitted via EFS to the United States Patent and Trademark Office on the date shown below.

Date 10/25/11 Signature

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:

JOHN FRASER WRIGHT et al. Confirmation No.: 4726

Application No.: 12/661,553 Art Unit: 1653

Filing Date: March 19, 2010 Examiner: Satyendra K. Singh

Title: COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION

RESPONSE TO RESTRICTION REQUIREMENT

Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

Sir:

This paper is filed in response to the Restriction Requirement mailed September 8, 2011, with an initial response date of October 8, 2011. Accordingly, a one-month extension of time in which to respond is requested and the requisite fee accompanies this response.

The Examiner has required election of one of the following groups of claims:

Group I, claims, 1-8, drawn to a composition for the storage of purified virus particles; and

Group II, claims 9-11, drawn to a method of preventing aggregation of virions in a preparation of virions.

Applicants elect to prosecute the claims of Group 1, claims 1-9 without traverse. Applicants expressly reserve their right under 35 USC §121 to file one or more divisional applications directed to the nonelected subject matter during the pendency of this application.

Respectfully submitted,

Date: 10/25/2011

By: _____
Roberta L. Robins
Registration No. 33,208
Attorney for Applicant

ROBINS & PASTERNAK LLP
1731 Embarcadero Road, Suite 230
Palo Alto, CA 94030
Tel: (650) 493-3400
Fax: (650) 493-3440

Electronic Patent Application Fee Transmittal

Application Number:	12661553
Filing Date:	19-Mar-2010
Title of Invention:	Compositions and methods to prevent AAV vector aggregation
First Named Inventor/Applicant Name:	John Fraser Wright
Filer:	Roberta L. Robins/Denise Vaillancourt
Attorney Docket Number:	0800-0045.01

Filed as Large Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Extension - 1 month with $0 paid	1251	1	150	150

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
			Total in USD ($)	**150**

Electronic Acknowledgement Receipt

EFS ID:	11261307
Application Number:	12661553
International Application Number:	
Confirmation Number:	4726
Title of Invention:	Compositions and methods to prevent AAV vector aggregation
First Named Inventor/Applicant Name:	John Fraser Wright
Customer Number:	20855
Filer:	Roberta L. Robins/Denise Vaillancourt
Filer Authorized By:	Roberta L. Robins
Attorney Docket Number:	0800-0045.01
Receipt Date:	25-OCT-2011
Filing Date:	19-MAR-2010
Time Stamp:	15:40:18
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Credit Card
Payment was successfully received in RAM	$150
RAM confirmation Number	2124
Deposit Account	
Authorized User	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)

1		rrr_20111025124941.pdf	152188 762cb79deb1ede62c3aea9e35fd5b1e8b204a672	yes	4

Multipart Description/PDF files in .zip description		
Document Description	**Start**	**End**
Transmittal Letter	1	1
Extension of Time	2	2
Response to Election / Restriction Filed	3	4

Warnings:

Information:

2	Fee Worksheet (SB06)	fee-info.pdf	30131 89cc78f56a44122046b65c0047e0be9e27886555	no	2

Warnings:

Information:

Total Files Size (in bytes):	182319

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/SB/21 (09-04)

TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Total Number of Pages in This Submission: 4

Application Number	12/661,553
Filing Date	March 19, 2010
First Named Inventor	John Frasier Wright et al.
Art Unit	1653
Examiner Name	Satyendra K. Singh
Attorney Docket Number	0800-0045.01

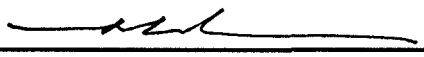
ENCLOSURES *(Check all that apply)*

- ☐ Fee Transmittal Form
  - ☐ Fee Attached
- ☐ Amendment/Reply
  - ☐ After Final
  - ☐ Affidavits/declaration(s)
- ☒ Extension of Time Request (1 pg)
- ☐ Express Abandonment Request
- ☐ Information Disclosure Statement
- ☐ Certified Copy of Priority Document(s)
- ☐ Reply to Missing Parts/ Incomplete Application
  - ☐ Reply to Missing Parts under 37 CFR 1.52 or 1.53
- ☐ Drawing(s)
- ☐ Licensing-related Papers
- ☐ Petition
- ☐ Petition to Convert to a Provisional Application
- ☐ Power of Attorney, Revocation Change of Correspondence Address
- ☐ Terminal Disclaimer
- ☐ Request for Refund
- ☐ CD, Number of CD(s) _____
  - ☐ Landscape Table on CD
- ☐ After Allowance Communication to TC
- ☐ Appeal Communication to Board of Appeals and Interferences
- ☐ Appeal Communication to TC **(Appeal Notice, Brief, Reply Brief)**
- ☐ Proprietary Information
- ☐ Status Letter
- ☒ Other Enclosure(s) (please identify below):

Response to Restriction Requirement (2 pgs)

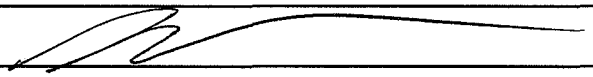
Remarks: **The Commissioner is authorized to charge any additional fees to Deposit Account 18-1648.**

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	Robins & Pasternak LLP		
Signature			
Printed name	Roberta L. Robins		
Date	10/25/2011	Reg. No.	33,208

CERTIFICATE OF TRANSMISSION/MAILING

I hereby certify pursuant to 37 CFR §1.8 that this correspondence is being transmitted via EFS to the United States Patent and Trademark Office on the date shown below.

Signature			
Typed or printed name	Denise M. Vaillancourt	Date	10/25/11

PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a) **FY 2010** *(Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).)*	Docket Number (Optional) 0800-0045.01
Application Number: 12/661,553	Filed: March 19, 2010
For COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION	
Art Unit: 1653	Examiner: Satyendra K. Singh

This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a reply in the above identified application.

The requested extension and fee are as follows (check time period desired and enter the appropriate fee below):

	Fee	Small Entity Fee	
☒ One month (37 CFR 1.17(a)(1))	$150	$75	$ 150
☐ Two months (37 CFR 1.17(a)(2))	$560	$280	$
☐ Three months (37 CFR 1.17(a)(3))	$1270	$635	$
☐ Four months (37 CFR 1.17(a)(4))	$1980	$990	$
☐ Five months (37 CFR 1.17(a)(5))	$2690	$1345	$

☐ Applicant claims small entity status. See 37 CFR 1.27.

☐ A check in the amount of the fee is enclosed.

☒ Payment by credit card.

☐ The Director has already been authorized to charge fees in this application to a Deposit Account.

☒ The Director is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number 18-1648.

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

I am the

☐ applicant/inventor.

☐ assignee of record of the entire interest. See 37 CFR 3.71.
Statement under 37 CFR 3.73(b) is enclosed (Form PTO/SB/96).

☐ attorney or agent of record. Registration Number _____

☒ attorney or agent under 37 CFR 1.34.
Registration number if acting under 37 CFR 1.34 33,208

Signature	Date
	10/25/2011
Roberta L. Robins	(650) 493-3400
Typed or printed name	Telephone Number

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below.

☒ Total of 1 forms are submitted.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/661,553	03/19/2010	John Fraser Wright	0800-0045.01	4726

20855 7590 11/09/2011

ROBINS & PASTERNAK
1731 EMBARCADERO ROAD
SUITE 230
PALO ALTO, CA 94303

EXAMINER	
SINGH, SATYENDRA K	

ART UNIT	PAPER NUMBER
1653	

MAIL DATE	DELIVERY MODE
11/09/2011	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	12/661,553	WRIGHT ET AL.	
	Examiner	Art Unit	
	SATYENDRA SINGH	1653	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *25 October 2011*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

5)☒ Claim(s) *1-11* is/are pending in the application.

5a) Of the above claim(s) *9-11* is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *1-8* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☒ The drawing(s) filed on *09 April 2010* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

12)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *4/9/10*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Election/Restrictions

Applicant's election **without traverse of group I** (**claims 1-8**; directed to the "composition for the storage of purified virus particles") in the reply filed on **10/25/2011** is acknowledged. It is noted that applicants in their response incorrectly state election of "*Group I, claims 1-9*" (see remarks, page 2), which actually encompasses only claims 1-8 as presented in the restriction/election sent previously by the office.

Claims 9-11 have been withdrawn from further considerations.

Claims 1-8 (elected invention of group I) have been examined on their merits in this office action.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

1. Claims 4 and 5 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claims 4 and 5 contain one or more of the trademark/trade names viz: **Pluronic® F68** (a registered trademark). Where a trademark or trade name is used in a claim as a limitation to identify or describe a particular material or product, the claim does not comply with the requirements of 35 U.S.C. 112, second paragraph. See *Ex parte Simpson*, 218 USPQ 1020 (Bd. App. 1982). The claim scope is uncertain since the trademark or trade name cannot be used properly to identify any particular material or product. A trademark or trade name is used to identify a source of goods, and not the goods themselves. Thus, a trademark or

trade name does not identify or describe the goods associated with the trademark or trade name. In the present case, the trademark/trade name is used to identify/describe a commercially available surfactant "Pluronic® F68" and, accordingly, the identification/description is indefinite. Appropriate clarification is required.

2. Claim 5 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claim recites the limitations "wherein the **Pluronic® F68 is present at 0.001%**", which is confusing and ambiguous. It is unclear as to what exactly is encompassed by the claimed recitation of 0.001%, an amount of Pluronic® F68 volume/volume (v/v), or wt/volume (w/v), or any other types of proportion such as weight/weight (w/w), etc. It is also not clear if the amount of Pluronic® F68 in percent represents over the total amount/volume of virion preparation, or any particular part or stage of said composition. Appropriate correction is required.

3. Claim 7 recites the limitation "**the average particle radius**" in line 1. There is insufficient antecedent basis for this limitation in the claim.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

1. Claims 1, 3, 7 and 8 are rejected under 35 U.S.C. 102(b) as being anticipated by Vihinen-Ranta et al (1998; [U]).

Claims are directed to "a composition for the storage of purified virus particles, comprising: purified virus particles; a pH buffer; and excipients comprising one or more multivalent ions; wherein the ionic strength of the composition is greater than about 200 mM (claim 1); wherein one of the one or more multivalent ions is citrate (claim 3); wherein the average particle radius (*Rh*) of the purified virus particles is less than about 20nm as measured by dynamic light scattering (claim 7); and wherein recovery of the purified virus particles is at least about 90% following filtration of the composition of virions through a 0.22μm filter" (claim 8).

Vihinen-Ranta et al (1998) disclose the composition comprising: purified canine parvovirus (CPV) particles (see abstract, in particular); a pH buffer such as citrate buffer, and excipients comprising one or more multivalent ions (such as sodium citrate, and disodium phosphate), wherein the ionic strength of the composition is greater than about 200 mM (such as acid pre-treatment of purified virus particles in 100 mM citric acid and 200 mM Na_2HPO_3, which was neutralized later with 0.5M Na_2HPO_4; see Vihinen-Ranta et al, page 803, right column 1st paragraph, in particular; meets the limitations of claims 1 and 3). The limitations of claims 7 and 8 (average particle radius and percent recovery following filtration) are also met by the prior art as these are taken to be an inherent characteristic features of the purified viral composition as disclosed by Vihinen-Ranta et al. Since, all the components as recited in claims 1 and 7, or claims 1 and 8, are the same as disclosed in the cited prior art, these features will necessarily follow from the composition disclosed in the art.

2. Claims 1, 2, 7 and 8 are rejected under 35 U.S.C. 102(b) as being anticipated by Zolotukhin et al (2000; US 6,146,874; IDS).

Claims are directed to "a composition for the storage of purified virus particles, comprising: purified virus particles; a pH buffer; and excipients comprising one or more multivalent ions; wherein the ionic strength of the composition is greater than about 200 mM (claim 1); wherein the purified virus particles are AAV virus particles (claim 2); wherein the average particle radius (*Rh*) of the purified virus particles is less than about 20nm as measured by dynamic light scattering (claim 7); and wherein recovery of the purified virus particles is at least about 90% following filtration of the composition of virions through a 0.22μm filter" (claim 8).

Zolotukhin et al (2000) disclose the composition comprising: purified recombinant AAV virus particles; a pH buffer such as **phosphate buffered-saline with magnesium chloride and potassium chloride** (PBS-MK); and excipients comprising one or more multivalent ions such as phosphate and magnesium; wherein the ionic strength of the composition is greater than about 200 mM (see Zolotukhin et al, column 11, 4th paragraph, lines 35-40, in particular), wherein they elute the purified rAAV particles in **PBS-MK buffer having 1M NaCl**, i.e. elution buffer. The limitations of claims 7 and 8 (average particle radius and percent recovery following filtration) are also met by the prior art as these limitations are taken to be an inherent characteristic features of the purified viral composition as disclosed by Zolotukhin et al. Since, all the components including purified virus particles, pH buffer and multivalent ions, as recited in claims 1, 2 and 7, or claims 1, 2 and 8, are the same, as disclosed in the cited prior art, these features will necessarily follow from the composition disclosed in the art.

3. Claims 1, 3, 7 and 8 are rejected under 35 U.S.C. 102(b) as being anticipated by Andersson et al (1979; US 4,138,287; [A]).

Andersson et al (1979) disclose the composition comprising: purified hepatitis virus (HB_sAg) particles (see abstract, in particular); a pH buffer such as **Tris-sodium citrate buffer, pH 7.5**, and excipients comprising a multivalent ion such as sodium citrate, wherein the ionic strength of the composition is greater than about 200 mM (see elution of purified virus from column using **0.5 M NaCl in Tris-citrate buffer**; see Andersson et al, example 2 and claim 5, in particular; meets the limitations of claims 1 and 3). The limitations of claims 7 and 8 (average particle radius and percent recovery following filtration) are also met by the prior art as these are taken to be an inherent characteristic features of the purified viral composition as disclosed by Andersson et al. Since, all the components as recited in claims 1 and 7, or claims 1 and 8, are the same as disclosed in the cited prior art, these features will necessarily follow from the composition disclosed in the art.

As per MPEP 2111.01, *during examination, the claims must be interpreted as broadly as their terms reasonably allow. In re American Academy of Science Tech Center, F.3d, 2004 WL 1067528 (Fed. Cir. May 13, 2004)(The USPTO uses a different standard for construing claims than that used by district courts; during examination the USPTO must give claims their broadest reasonable interpretation.). This means that the words of the claim must be given their plain meaning unless applicant has provided a clear definition in the specification. In re Zletz*, 893 F.2d 319, 321, 13 USPQ2d 1320, 1322 (Fed. Cir. 1989).

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* v. *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

1. Claims 1-8 are rejected under 35 U.S.C. 103(a) as being unpatentable over Zolotukhin et al (2000; US 6,146,874; IDS) taken with Andersson et al (1979; US 4,138,287; [A]), Zhang et al (2001; US 6,194,191; IDS) and Chen et al (1994; IDS).

Claims are directed to "a composition (for the storage) of purified virus particles, comprising: purified virus particles; a pH buffer; and excipients comprising one or more multivalent ions; wherein the ionic strength of the composition is greater than about 200 mM (claim 1); wherein the purified virus particles are AAV virus particles (claim 2); wherein one of the one or more multivalent ions is citrate (claim 3); further comprising Pluronic® F68 at 0.001% (claims 4-5); wherein the pH buffer is 10 mM Tris, pH 8.0 and the excipients comprise 100 mM sodium citrate (claim 6); wherein the average particle radius (*Rh*) of the purified virus particles is less than about 20nm as measured by dynamic light scattering (claim 7); and wherein recovery of the purified virus particles is at least about 90% following filtration of the composition of virions through a 0.22μm filter" (claim 8).

The detailed teachings of Zolotukhin et al and Andersson et al have been discussed above, and are further relied upon in the same manner herein.

However, the composition, further comprising **Pluronic® F68** at 0.001% (claims 4-5); and wherein the pH buffer is **10 mM Tris, pH 8.0** and the excipients comprise **100 mM sodium citrate** (claim 6), is not specifically disclosed by the inventions of Zolotukhin et al taken with Andersson et al.

Zhang et al (2001) disclose the use of a surfactant such as **Pluronic® F68** (0.1% in growth medium for adenovirus infection and viral production, etc.; see column 4, 2nd paragraph and columns 53-54, in particular) for production of adenoviral particles in serum-free suspension cultures using spinner flasks, and also use in the cropreservation media (see column 53, last paragraph).

Chen et al (1994) disclose strategies to suppress aggregation of proteins (such as recombinant keratinocyte growth factor) during liquid formulation development (see Chen et al, abstract, table 1-2, in particular) by adding sulfated polysaccharides (such as heparin) and **citrate salts** (such as 0.1 to 0.5 M sodium citrate; see Chen et al, page 1661, left column, in particular) that were found to be effective in preventing protein aggregation. Chen et al also disclose the fact that other negatively charged small ions such as phosphate (a multivalent ion) also have moderate stabilizing effects on preventing aggregation of recombinant keratinocyte growth factor (rhKGF).

Thus, given the disclosure in the cited prior art, a person of ordinary skill in the art would have modified the composition comprising purified AAV virus particles taught by Zolotukhin et al taken with Andersson et al such that said composition additionally comprise a surfactant (to

help prevent aggregation during freezing-thawing cycles, for example) such as Pluronic® F68, as explicitly taught by Zhang et al, and a suitable amount of sodium citrate as a multivalent ion in Tris buffer solution (at a suitable pH; as specifically disclosed by Andersson et al) in order to stabilize the viral particles as suggested by Chen et al (for suppressing/reducing aggregation of fairly unstable proteins such as rhKGF; see discussion above, thus providing a conceptual basis for including multivalent ions in the buffer containing purified AAV particles), in addition to other stabilizing components such as a surfactant. Since, the benefits of including a surfactant and high ionic strength multivalent ions have been disclosed in the cited prior art of Zang et al and Chen et al, an artisan of ordinary skill in the art would have established suitable concentrations required to help stabilize viral preparations with a reasonable expectation of success.

The limitations of claims 7 and 8 (average particle radius and percent recovery following filtration, etc.) are also met by the prior art as these are taken to be intrinsic characteristic features of the purified viral compositions as disclosed by Zolotukhin et al when taken with the teachings of Andersson et al, Zhang et al and Chen et al. Since, all the components as recited in the claims are the same as disclosed and/or suggested in the cited prior art, these features will necessarily follow from the composition disclosed in the art, as they do not structurally change the composition as claimed.

Thus, the invention as a whole would have been *prima facie* obvious to a person of ordinary skill in the art at the time the claimed invention was made.

As per MPEP 2144.06, "*It is prima facie obvious to combine two compositions each of which is taught by the prior art to be useful for the same purpose, in order to form a third composition to be used for the very same purpose.... [T]he idea of combining them flows logically from their*

having been individually taught in the prior art." In re Kerkhoven, 626 F.2d 846, 850, 205 USPQ 1069, 1072 (CCPA 1980).

As per MPEP 2144.06, *In order to rely on equivalence as a rationale supporting an obviousness rejection, the equivalency must be recognized in the prior art, and cannot be based on applicant's disclosure or the mere fact that the components at issue are functional or mechanical equivalents. In re Ruff, 256 F.2d 590, 118 USPQ 340 (CCPA 1958).*

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory obviousness-type double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement.

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

1. **Claims 1-8** are rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over at least claim 1 of U.S. Patent No. **7,701,721 B2** (issued on April 27, 2010 to the same assignee and inventors). Although the conflicting claims are not identical, they are not patentably distinct from each other because issued claim 1 is directed to a method of preventing aggregation of rAAV virion preparations by adding "one or more salts of multivalent ions selected from the group consisting of citrate, phosphate, sulfate and magnesium to said purified virions to produce a preparation of virions with an ionic strength of at least 200 mM", which is suitable for long term storage without significant aggregation problems. Since, the composition disclosed in the issued '721 patent is essentially the same as currently being claimed by applicants in the instant application, an ODP rejection is proper.

Conclusion

NO claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SATYENDRA SINGH whose telephone number is (571)272-8790. The examiner can normally be reached on 9-5MF.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, SUE X. LIU can be reached on 571-272-5539. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Satyendra K. Singh/
Examiner, Art Unit 1653

/SUE LIU/
Supervisory Patent Examiner, Art Unit 1653

Notice of References Cited	Application/Control No. 12/661,553	Applicant(s)/Patent Under Reexamination WRIGHT ET AL.	
	Examiner SATYENDRA SINGH	Art Unit 1653	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
*	A	US-4,138,287	02-1979	Andersson et al.	435/239
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	VIHINEN-RANTA M. et al., Intracellular Route of Canine Parvovirus Entry, JOURNAL OF VIROLOGY, Jan. 1998, vol. 72, No. 1, pages 802-806.
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
S1	987	WRIGHT near3 JOHN	USPAT	OR	OFF	2011/11/05 19:24
S2	1938	WRIGHT near3 JOHN	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2011/11/05 19:24
S3	16	QU near3 GUANG	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2011/11/05 19:24
S4	1944	S2 or S3	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2011/11/05 19:24
S5	18	S4 and ((virion or vira$6 or virus or adenovir$6 or AAV) same (aggreg$6 or precipit$6 or agglutin$6 or stabiliz$6))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2011/11/05 19:27
S6	1	S4 and ((virion or vira$6 or virus or adenovir$6 or AAV) same (aggreg$6 or precipit$6 or agglutin$6 or stabiliz$6) same (multivalent or divalent))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2011/11/05 19:28
S7	6	S4 and ((virion or vira$6 or virus or adenovir$6 or AAV) same (aggreg$6 or precipit$6 or agglutin$6 or stabiliz$6)) and (multivalent or divalent)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2011/11/05 19:29
S8	12	((virion or vira$6 or virus or adenovir$6 or AAV) same (aggreg$6 or precipit$6 or agglutin$6 or stabiliz$6 or sotrag$6) same ((multivalent or divalent) near3 ion))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2011/11/05 19:33
S9	2	((virion or vira$6 or virus or adenovir$6 or AAV) same (aggreg$6 or precipit$6 or agglutin$6 or stabiliz$6 or sotrag$6) same ((multivalent or divalent) near3 ion)).clm.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2011/11/05 19:33
S10	7	((virion or vira$6 or virus or adenovir$6 or AAV) same (stabiliz$6 or sotrag$6) same ((multivalent or divalent) near3 ion))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2011/11/05 19:38
S11	1324	(adenovir$6 or AAV) same (stabiliz$6	US-PGPUB;	OR	OFF	2011/11/05

		or sotrag$6)	USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB			19:39
S12	13	(adenovir$6 or AAV) same (stabiliz$6 or sotrag$6).clm.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2011/11/05 19:39
S13	7	S12 and ((citr$6 or magnes$6 or phosphat$6 or manganese) same buffer)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2011/11/05 19:44
S14	812	((AAV or adenovir$3) near3 (particle or virion)) and ((citr$6 or magnes$6 or phosphat$6 or mangan$6 or sulfate) same buffer)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2011/11/06 11:27
S15	33	((AAV or adenovir$3) near3 (particle or virion)) same ((citr$6 or magnes$6 or phosphat$6 or mangan$6 or sulfate) same buffer)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2011/11/06 11:28
S16	6	((AAV or adenovir$3) near3 (particle or virion)) same ((citr$6 or magnes$6 or phosphat$6 or mangan$6 or sulfate) same buffer same mM)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2011/11/06 11:30
S17	4	(("6146874") or ("6194191") or ("6566118") or ("6593123")).PN.	USPAT; USOCR	OR	OFF	2011/11/06 18:13
S18	4	S17 and (phosphate or magnesium or sulfate or tris or citrate) and buffer	USPAT	OR	OFF	2011/11/06 18:14
S19	367	(AAV or rAAV or adenovirus) same (surfactant or pluronic$2f68 or pluronic$4)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2011/11/06 19:20
S20	126	(AAV or rAAV or adenovirus) same (pluronic$2f68 or pluronic$4)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2011/11/06 19:23
S21	32	(adenovir$3 or AAV or virus) same (tris near3 citrate)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2011/11/06 20:42
S22	1	("4,138,287").PN.	USPAT; USOCR	OR	OFF	2011/11/07 10:34

EAST Search History (Interference)

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Substitute for form 1449A/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** *(use as many sheets as necessary)*				***Complete if Known***	
				Application Number	12/661,553
				Filing Date	March 19, 2010
				First Named Inventor	Wright et al.
				Group Art Unit	Not Assigned
				Examiner Name	Not Assigned
Sheet	1	of	4	Attorney Docket Number	0800-0045.01

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	U.S. Patent Document Number	U.S. Patent Document Kind Code[2] (*if known*)	Name of Patentee or Applicant of Cited Document	Date of Publication of Cited Document MM-DD-YYYY
	A1	6,593,123	435/239	Wright et al.	07-2001
	A2	6,566,118	435/239	Atkinson et al.	05-2003
	A3	6,194,191	435/235.1	Zhang et al.	02-2001
	A4	6,146,874	435/235.1	Zolotukhin et al.	11-2000

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		ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /SS/					

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[4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document.
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Sheet	2	of	4	Attorney Docket Number	0800-0045.01

OTHER PRIOR ART – NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[1]
	C1	ADADEYOH et al., "SHORT-TERM FIELD USE AND SHIPPING STABILITY STUDY OF A WILD TYPE AD5 ADENOVIRAL REFERENCE MATERIAL," BioProcessing, 1(3):62-9 (2002)	
	C2	BRAUN et al., "PROTEIN AGGREGATES SEEM TO PLAY A KEY ROLE AMONG THE PARAMETERS INFLUENCING THE ANTIGENICITY OF INTERFERON ALPHA (IFN-ALPHA) IN NORMAL AND TRANSGENIC MICE," Pharm. Res. 14(10):1472-1478 (1997)	
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	C9	HIGH et al., "HUMAN IMMUNE RESPONSES TO AAV-2 CAPSID MAY LIMIT DURATION OF EXPRESSION IN LIVER-DIRECTED GENE TRANSFER IN HUMAN WITH HEMOPHILIS β," Blood, 104(11):121a, Abstract No. 413 (2004)	
	C10	HUANG et al., "ADAAV SUPPORT HIGH-TITER PRODUCTION OF RAAV BUT NOT STABLE," Mol. Therapy 1:S286 (2000)	
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(use as many sheets as necessary)				Examiner Name	Not Assigned
Sheet	3	of	4	Attorney Docket Number	0800-0045.01

OTHER PRIOR ART – NON PATENT LITERATURE DOCUMENTS			
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	C13	QU et al., "SCALING UP PRODUCTION OF RECOMBINANT AAV VECTORS FOR CLINICAL APPLICATIONS," Curr Opin Drug Disc Dev. 3(6):750-755 (2000)	
	C14	SHIRE et al., "CHALLENGES IN THE DEVELOPMENT OF HIGH PROTEIN CONCENTRATION FORMULATIONS," J. Pharm. Sci. 93(6):1390-1402 (2004)	
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	C21	WRIGHT et al., "IDENTIFICATION OF FACTORS THAT CONTRIBUTE TO RECOMBINANT AAV2 PARTICLE AGGREGATION AND METHODS TO PREVENT ITS OCCURRENCE DURING VECTOR PURIFICATION AND FORMULATION," Mol. Ther. 12(1):171-178 (2005)	
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				Filing Date	March 19, 2010
				First Named Inventor	Wright et al.
				Group Art Unit	Not Assigned
				Examiner Name	Not Assigned
Sheet	4	of	4	Attorney Docket Number	0800-0045.01

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/ss/	C25	The Term "PURIFY" AND "ABOUT", Merriam-Webster Online Dictionary, at the web- http://www.m-w.com, page 1 and page 2 Accessed on 11/30/06 and 12/1/06.	
	C26	The Definition of the Term "IONIC STRENGTH", Answer.com, at the web- http://www.answers.com, page 1 Accessed on 11/30/06.	
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CONFIRMATION NO. 4726

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12/661,553	03/19/2010	435	1653	0800-0045.01

APPLICANTS

John Fraser Wright, Princeton, NJ;
Guang Qu, Alameda, CA;

**** CONTINUING DATA ***************************

This application is a CON of 11/141,996 06/01/2005 PAT 7,704,721
which claims benefit of 60/575,997 06/01/2004
and claims benefit of 60/639,222 12/22/2004

**** FOREIGN APPLICATIONS ***************************

**** IF REQUIRED, FOREIGN FILING LICENSE GRANTED ****

04/01/2010

Foreign Priority claimed ☐ Yes ☑ No 35 USC 119(a-d) conditions met ☐ Yes ☐ No Verified and Acknowledged /SATYENDRA K SINGH/ Examiner's Signature	☐ Met after Allowance Initials	STATE OR COUNTRY	SHEETS DRAWINGS	TOTAL CLAIMS	INDEPENDENT CLAIMS
		NJ	3	11	2

ADDRESS

ROBINS & PASTERNAK
1731 EMBARCADERO ROAD
SUITE 230
PALO ALTO, CA 94303
UNITED STATES

TITLE

Compositions and methods to prevent AAV vector aggregation

FILING FEE RECEIVED		
1090	FEES: Authority has been given in Paper No._____ to charge/credit DEPOSIT ACCOUNT No._____ for following:	☐ All Fees ☐ 1.16 Fees (Filing) ☐ 1.17 Fees (Processing Ext. of time) ☐ 1.18 Fees (Issue) ☐ Other _____ ☐ Credit

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	12661553	WRIGHT ET AL.
	Examiner	Art Unit
	SATYENDRA SINGH	1653

SEARCHED

Class	Subclass	Date	Examiner

SEARCH NOTES

Search Notes	Date	Examiner
EAST: USPAT, USOCR, US-PGPUB, JPO, EPO, DERWENT- ATTACHED	11/6/2011	SKS
INVENTOR SEARCH: PALM, EDAN AND EAST-	11/6/2011	SKS

INTERFERENCE SEARCH

Class	Subclass	Date	Examiner

/SATYENDRA SINGH/ Examiner.Art Unit 1653	

COPY

PTO/SB/81 (04-05)

POWER OF ATTORNEY and CORRESPONDENCE ADDRESS INDICATION FORM

Application Number	11/141,996
Filing Date	June 1, 2005
First Named Inventor	JOHN FRASER WRIGHT
Title	COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION
Art Unit	Unassigned
Examiner Name	Unassigned
Attorney Docket Number	0800-0045

I hereby revoke all previous powers of attorney given in the above-identified application.

I hereby appoint:

☒ Practitioners associated with the Customer Number: **31048**

AND

☒ Practitioner(s) named below:

Name	Registration Number
Roberta L. Robins	33,208
Dahna S. Pasternak	41,411
Susan T. Evans	38,443
Jenny Buchbinder	48,588

as my/our attorney(s) or agent(s) to prosecute the application identified above, and to transact all business in the United States Patent and Trademark Office connected therewith.

Please recognize or change the correspondence address for the above-identified application to:

☐ The address associated with the above-mentioned Customer Number:

OR

☐ The address associated with Customer Number:

OR

☐ Firm or Individual Name			
Address			
City		State	Zip
Country			
Telephone		Email	

I am the:

☐ Applicant/Inventor.

☒ Assignee of record of the entire interest. See 37 CFR 3.71.
Statement under 37 CFR 3.73(b) is enclosed. (Form PTO/SB/96).

SIGNATURE of Applicant or Assignee of Record

Signature	Christina Thomson	Date	10/7/05
Name	M. CHRISTINA THOMSON	Telephone	510-748-7208
Title and Company	VICE PRESIDENT, CORPORATE COUNSEL		

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below*.

☒ *Total of One forms are submitted.

COPY

PTO/SB/96 (09-04)

Attorney Docket No. 0800-0045

STATEMENT UNDER 37 CFR 3.73(b)

Applicant/Patent Owner: John Fraser Wright and Guang Qu

Application No./Patent No.: 11/141,996 Filed/Issue Date: June 1, 2005

Entitled: COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION

Avigen, Inc., a corporation
(Name of Assignee) (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

states that it is:

1. ☒ the assignee of the entire right, title, and interest; or
2. ☐ an assignee of less than the entire right, title and interest.
The extent (by, percentage) of its ownership interest is _____%

in the patent application/patent identified above by virtue of either:

A. ☒ An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel _____, Frame ____, or for which a copy thereof is attached.

OR

B. ☐ A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as shown below:

1. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame _____, or for which a copy thereof is attached.

2. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame _____, or for which a copy thereof is attached.

3. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame _____, or for which a copy thereof is attached.

☐ Additional documents in the chain of title are listed on a supplemental sheet.

☒ Copies of assignments or other documents in the chain of title are attached.
[NOTE: A separate copy (*i.e.*, a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, if the assignment is to be recorded in the records of the USPTO. See MPEP 302.8]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

Christina Thomson — 10/7/05
Signature — Date

M. CHRISTINA THOMSON — 510-748-7208
VICE PRESIDENT, CORPORATE COUNSEL
Printed or Typed Name — Telephone Number

Title

Electronic Acknowledgement Receipt

EFS ID:	12565365
Application Number:	12661553
International Application Number:	
Confirmation Number:	4726
Title of Invention:	Compositions and methods to prevent AAV vector aggregation
First Named Inventor/Applicant Name:	John Fraser Wright
Customer Number:	20855
Filer:	Roberta L. Robins/Denise Vaillancourt
Filer Authorized By:	Roberta L. Robins
Attorney Docket Number:	0800-0045.01
Receipt Date:	17-APR-2012
Filing Date:	19-MAR-2010
Time Stamp:	22:14:10
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		4501_20120417192417.pdf	144901 14e27a75603bfeb1a557a55d031d08c40b30b7cc	yes	3

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Transmittal Letter	1	1
	Power of Attorney	2	2
	Assignee showing of ownership per 37 CFR 3.73(b).	3	3
Warnings:			
Information:			
	Total Files Size (in bytes):	144901	

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
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National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

CERTIFICATE OF TRANSMISSION PURSUANT TO 37 CFR § 1.8

I hereby certify pursuant to 37 CFR §1.8 that this correspondence is being transmitted via EFS to the United States Patent and Trademark Office on the date shown below.

Date 4/17/12 Signature

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:

JOHN FRASER WRIGHT et al. Confirmation No.: 4726

Application No.: 12/661,553 Art Unit: 1653

Filing Date: March 19, 2010 Examiner: Satyendra K. Singh

Title: COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION

TRANSMITTAL

Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

Sir:

The attached Power of Attorney and Statement documents were filed in parent application no. 11/141,996 (now U.S. Patent No. 7,704,721) from which the instant application claims priority. Please enter and use the attached Power of Attorney and Statement for the instant application no. 12/661,553, filed March 19, 2010.

Respectfully submitted,

Date: 4/17/2012 By:

Roberta L. Robins
Registration No. 33,208
Attorney for Applicant

ROBINS & PASTERNAK LLP
1731 Embarcadero Road, Suite 230
Palo Alto, CA 94030
Tel: (650) 493-3400, Fax: (650) 493-3440

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
12/661,553	03/19/2010	John Fraser Wright	0800-0045.01

CONFIRMATION NO. 4726

IMPROPER CPOA LETTER

20855
ROBINS & PASTERNAK
1731 EMBARCADERO ROAD
SUITE 230
PALO ALTO, CA 94303

OC000000053904435

Date Mailed: 04/27/2012

NOTICE REGARDING POWER OF ATTORNEY

This is in response to the Power of Attorney filed 04/17/2012. The Power of Attorney in this application is not accepted for the reason(s) listed below:

- The Power of Attorney is from an assignee and the Certificate required by 37 CFR 3.73(b) has not been received.

/qtran/

Office of Data Management, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

CERTIFICATE OF TRANSMISSION PURSUANT TO 37 CFR § 1.8

I hereby certify pursuant to 37 CFR §1.8 that this correspondence is being transmitted via EFS to the United States Patent and Trademark Office on the date shown below.

Date 5/4/12 Signature

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:

JOHN FRASER WRIGHT et al. Confirmation No.: 4726

Application No.: 12/661,553 Art Unit: 1653

Filing Date: March 19, 2010 Examiner: Satyendra K. Singh

Title: COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION

TRANSMITTAL

Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

Sir:

In response to the Notice Regarding Power of Attorney mailed April 27, 2012 (attached), please find attached a copy of the executed Assignment filed in parent case 11/141,996 and a copy of the documents filed on April 17, 2012 including Transmittal, Power of Attorney and 3.73(b) Statement. The attached documents were filed in parent application no. 11/141,996 (now U.S. Patent No. 7,704,721) from which the instant application claims priority. Please enter and use the attached Power of Attorney and Statement for the instant application no. 12/661,553, filed March 19, 2010.

Respectfully submitted,

Date: 5/4/2012 By:

Roberta L. Robins
Registration No. 33,208
Attorney for Applicant

ROBINS & PASTERNAK LLP
1731 Embarcadero Road, Suite 230
Palo Alto, CA 94030
Tel: (650) 493-3400, Fax: (650) 493-3440

COPY

Atty Dkt No. 0800-0045

ASSIGNMENT

JOINT

THIS ASSIGNMENT, by **John Fraser Wright** and **Guang Qu** (hereinafter referred to as the assignors), residing at Princeton, New Jersey and Alameda, California respectively, witnesseth:

WHEREAS, the said assignors have invented certain new and useful improvements in COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION set forth in an application for Letters Patent of the United States, bearing Application No. 11/141,996 and filed on June 1, 2005; and

WHEREAS, **Avigen, Inc.**, a corporation duly organized under and pursuant to the laws of Delaware, and having its principal place of business at 1301 Harbor Bay Parkway, Alameda, CA 94502 (hereinafter referred to as the assignee) is desirous of acquiring the entire right, title and interest in and to said inventions and said application for Letters Patent of the United States, and in and to any Letters Patent or Patents, United States or foreign, to be obtained therefor and thereon:

NOW THEREFORE, in consideration of One Dollar ($1.00) and other good and sufficient considerations, the receipt of which is hereby acknowledged, the said assignors have sold, assigned, transferred and set over, and by these presents do sell, assign, transfer and set over, unto the assignee, its successors, legal representatives and assigns, the entire right, title and interest in and to the above-mentioned inventions, application for Letters Patent, and any and all Letters Patent or Patents in the United States of America and all foreign countries which may be granted therefor and thereon, and in and to any and all divisions, continuations, and continuations-in-part of said application, or reissues or extensions of said Letters Patent or Patents, and all rights under the International Union for the Protection of Industrial Property, the same to be held and enjoyed by the said assignee, for its own use and behoof and the use and behoof of its successors, legal representatives and assigns, to the full end of the term or terms for which Letters Patent or Patents may be granted, as fully and entirely as the same would have been held and enjoyed by the assignors, had this sale and assignment not been made.

AND for the same consideration, the said assignors hereby covenant and agree to and with the said assignee, its successors, legal representatives and assigns, that, at the time of execution and delivery of these presents, the said assignors are the sole and lawful owners of the entire right, title and interest in and to the said inventions and the application for Letters Patent above-mentioned, and that the same are unencumbered and that the said assignors have good and full right and lawful authority to sell and convey the same in the manner herein set forth.

AND for the same consideration, the said assignors hereby covenant and agree to and with the said assignee, its successors, legal representatives and assigns, that the said assignors will, whenever counsel of the said assignee, or the counsel of its successors, legal representatives and assigns, shall advise that any

proceeding in connection with said inventions, or said application for Letters Patent, or any proceeding in connection with Letters Patent for said inventions in any country, including interference proceedings, is lawful and desirable, or that any division, continuation or continuation-in-part of any application for Letters Patent or any reissue or extension of any Letters Patent, to be obtained thereon, is lawful and desirable, sign all papers and documents, take all lawful oaths, and do all acts necessary or required to be done for the procurement, maintenance, enforcement and defense of Letters Patent for said inventions, without charge to said assignee, its successors, legal representatives and assigns, but at the cost and expense of the said assignee, its successors, legal representatives and assigns.

AND the said assignors hereby request the Commissioner of Patents to issue said Letters Patent of the United States to the said assignee as the assignee of said inventions and the Letters Patent to be issued thereon for the sole use and behoof of the said assignee, its successors, legal representatives and assigns.

Date: 04 08 05

John Fraser Wright

Date: _____

_____
Guang Qu

COPY

Atty Dkt No. 0800-0045

ASSIGNMENT JOINT

THIS ASSIGNMENT, by **John Fraser Wright** and **Guang Qu** (hereinafter referred to as the assignors), residing at Princeton, New Jersey and Alameda, California respectively, witnesseth:

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NOW THEREFORE, in consideration of One Dollar ($1.00) and other good and sufficient considerations, the receipt of which is hereby acknowledged, the said assignors have sold, assigned, transferred and set over, and by these presents do sell, assign, transfer and set over, unto the assignee, its successors, legal representatives and assigns, the entire right, title and interest in and to the above-mentioned inventions, application for Letters Patent, and any and all Letters Patent or Patents in the United States of America and all foreign countries which may be granted therefor and thereon, and in and to any and all divisions, continuations, and continuations-in-part of said application, or reissues or extensions of said Letters Patent or Patents, and all rights under the International Union for the Protection of Industrial Property, the same to be held and enjoyed by the said assignee, for its own use and behoof and the use and behoof of its successors, legal representatives and assigns, to the full end of the term or terms for which Letters Patent or Patents may be granted, as fully and entirely as the same would have been held and enjoyed by the assignors, had this sale and assignment not been made.

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proceeding in connection with said inventions, or said application for Letters Patent, or any proceeding in connection with Letters Patent for said inventions in any country, including interference proceedings, is lawful and desirable, or that any division, continuation or continuation-in-part of any application for Letters Patent or any reissue or extension of any Letters Patent, to be obtained thereon, is lawful and desirable, sign all papers and documents, take all lawful oaths, and do all acts necessary or required to be done for the procurement, maintenance, enforcement and defense of Letters Patent for said inventions, without charge to said assignee, its successors, legal representatives and assigns, but at the cost and expense of the said assignee, its successors, legal representatives and assigns.

AND the said assignors hereby request the Commissioner of Patents to issue said Letters Patent of the United States to the said assignee as the assignee of said inventions and the Letters Patent to be issued thereon for the sole use and behoof of the said assignee, its successors, legal representatives and assigns.

Date: _____

_____
John Fraser Wright

Date: 9/27/2005

_____
Guang Qu

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
12/661,553	03/19/2010	John Fraser Wright	0800-0045.01

CONFIRMATION NO. 4726

IMPROPER CPOA LETTER

20855
ROBINS & PASTERNAK
1731 EMBARCADERO ROAD
SUITE 230
PALO ALTO, CA 94303

Date Mailed: 04/27/2012

NOTICE REGARDING POWER OF ATTORNEY

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/qtran/

Office of Data Management, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

Electronic Acknowledgement Receipt

EFS ID:	12565365
Application Number:	12661553
International Application Number:	
Confirmation Number:	4726
Title of Invention:	Compositions and methods to prevent AAV vector aggregation
First Named Inventor/Applicant Name:	John Fraser Wright
Customer Number:	20855
Filer:	Roberta L. Robins/Denise Vaillancourt
Filer Authorized By:	Roberta L. Robins
Attorney Docket Number:	0800-0045.01
Receipt Date:	17-APR-2012
Filing Date:	19-MAR-2010
Time Stamp:	22:14:10
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		4501_20120417192417.pdf	144901 14e27a75603bfeb1a557a55d031d08c40b30b7cc	yes	3

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Transmittal Letter	1	1
	Power of Attorney	2	2
	Assignee showing of ownership per 37 CFR 3.73(b).	3	3
Warnings:			
Information:			
	Total Files Size (in bytes):	144901	

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
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Date 4/17/12 Signature

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:

JOHN FRASER WRIGHT et al. Confirmation No.: 4726

Application No.: 12/661,553 Art Unit: 1653

Filing Date: March 19, 2010 Examiner: Satyendra K. Singh

Title: COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION

TRANSMITTAL

Commissioner for Patents
P. O. Box 1450
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Sir:

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Respectfully submitted,

Date: 4/17/2012 By:

Roberta L. Robins
Registration No. 33,208
Attorney for Applicant

ROBINS & PASTERNAK LLP
1731 Embarcadero Road, Suite 230
Palo Alto, CA 94030
Tel: (650) 493-3400, Fax: (650) 493-3440

COPY

PTO/SB/81 (04-05)

POWER OF ATTORNEY and CORRESPONDENCE ADDRESS INDICATION FORM

Application Number	11/141,996
Filing Date	June 1, 2005
First Named Inventor	JOHN FRASER WRIGHT
Title	COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION
Art Unit	Unassigned
Examiner Name	Unassigned
Attorney Docket Number	0800-0045

I hereby revoke all previous powers of attorney given in the above-identified application.

I hereby appoint:

☒ Practitioners associated with the Customer Number: **31048**

AND

☒ Practitioner(s) named below:

Name	Registration Number
Roberta L. Robins	33,208
Dahna S. Pasternak	41,411
Susan T. Evans	38,443
Jenny Buchbinder	48,588

as my/our attorney(s) or agent(s) to prosecute the application identified above, and to transact all business in the United States Patent and Trademark Office connected therewith.

Please recognize or change the correspondence address for the above-identified application to:

☐ The address associated with the above-mentioned Customer Number:

OR

☐ The address associated with Customer Number:

OR

☐ Firm or Individual Name			
Address			
City		State	Zip
Country			
Telephone		Email	

I am the:

☐ Applicant/Inventor.

☒ Assignee of record of the entire interest. See 37 CFR 3.71.
Statement under 37 CFR 3.73(b) is enclosed. (Form PTO/SB/96).

SIGNATURE of Applicant or Assignee of Record

Signature	Christina Thomson	Date	10/7/05
Name	M. CHRISTINA THOMSON	Telephone	510-748-7208
Title and Company	VICE PRESIDENT, CORPORATE COUNSEL		

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below*.

☒ *Total of One forms are submitted.

COPY

PTO/SB/96 (09-04)
Attorney Docket No. 0800-0045

STATEMENT UNDER 37 CFR 3.73(b)

Applicant/Patent Owner: John Fraser Wright and Guang Qu

Application No./Patent No.: 11/141,996 Filed/Issue Date: June 1, 2005

Entitled: COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION

Avigen, Inc., a corporation
(Name of Assignee) (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

states that it is:

1. ☒ the assignee of the entire right, title, and interest; or
2. ☐ an assignee of less than the entire right, title and interest.
The extent (by, percentage) of its ownership interest is _____%

in the patent application/patent identified above by virtue of either:

A. ☒ An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel _____, Frame ____, or for which a copy thereof is attached.

OR

B. ☐ A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as shown below:

1. From: _____ To :_____
The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame _____, or for which a copy thereof is attached.

2. From: _____ To :_____
The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame _____, or for which a copy thereof is attached.

3. From: _____ To :_____
The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame _____, or for which a copy thereof is attached.

☐ Additional documents in the chain of title are listed on a supplemental sheet.

☒ Copies of assignments or other documents in the chain of title are attached.

[NOTE: A separate copy (*i.e.*, a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, if the assignment is to be recorded in the records of the USPTO. See MPEP 302.8]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

Christina Thomson
Signature

10/7/05
Date

M. CHRISTINA THOMSON
VICE PRESIDENT,
CORPORATE COUNSEL
Printed or Typed Name

510-748-7208
Telephone Number

Title

Electronic Acknowledgement Receipt

EFS ID:	12709964
Application Number:	12661553
International Application Number:	
Confirmation Number:	4726
Title of Invention:	Compositions and methods to prevent AAV vector aggregation
First Named Inventor/Applicant Name:	John Fraser Wright
Customer Number:	20855
Filer:	Roberta L. Robins/Denise Vaillancourt
Filer Authorized By:	Roberta L. Robins
Attorney Docket Number:	0800-0045.01
Receipt Date:	04-MAY-2012
Filing Date:	19-MAR-2010
Time Stamp:	17:36:53
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Miscellaneous Incoming Letter	poa_20120504141833.pdf	508598 ad8c3f35dfc281f13ea37dfe4a0604131dabfe4d	no	11

Warnings:	
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National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/SB/21 (09-04)

TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Total Number of Pages in This Submission	15

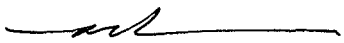
Application Number	12/661,553
Filing Date	March 19, 2010
First Named Inventor	John Frasier Wright et al.
Art Unit	1653
Examiner Name	Satyendra K. Singh
Attorney Docket Number	0800-0045.01

ENCLOSURES *(Check all that apply)*

- ☐ Fee Transmittal Form
  - ☐ Fee Attached
- ☒ Amendment/Reply (13 pgs)
  - ☐ After Final
  - ☐ Affidavits/declaration(s)
- ☒ Extension of Time Request (1 pg)
- ☐ Express Abandonment Request
- ☐ Information Disclosure Statement
- ☐ Certified Copy of Priority Document(s)
- ☐ Reply to Missing Parts/ Incomplete Application
  - ☐ Reply to Missing Parts under 37 CFR 1.52 or 1.53
- ☐ Drawing(s)
- ☐ Licensing-related Papers
- ☐ Petition
- ☐ Petition to Convert to a Provisional Application
- ☐ Power of Attorney, Revocation Change of Correspondence Address
- ☐ Terminal Disclaimer
- ☐ Request for Refund
- ☐ CD, Number of CD(s) _____
  - ☐ Landscape Table on CD
- ☐ After Allowance Communication to TC
- ☐ Appeal Communication to Board of Appeals and Interferences
- ☐ Appeal Communication to TC **(Appeal Notice, Brief, Reply Brief)**
- ☐ Proprietary Information
- ☐ Status Letter
- ☐ Other Enclosure(s) (please identify below):

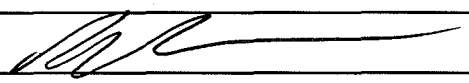
Remarks **The Commissioner is authorized to charge any additional fees to Deposit Account 18-1648.**

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	Robins Law Group		
Signature			
Printed name	Roberta L. Robins		
Date	5/9/2012	Reg. No.	33,208

CERTIFICATE OF TRANSMISSION/MAILING

I hereby certify pursuant to 37 CFR §1.8 that this correspondence is being transmitted via EFS to the United States Patent and Trademark Office on the date shown below.

Signature			
Typed or printed name	Denise M. Vaillancourt	Date	5/9/12

PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a) **FY 2010** *(Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).)*	Docket Number (Optional) 0800-0045.01
Application Number: 12/661,553	Filed: March 19, 2010
For COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION	
Art Unit: 1653	Examiner: Satyendra K. Singh

This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a reply in the above identified application.

The requested extension and fee are as follows (check time period desired and enter the appropriate fee below):

	Fee	Small Entity Fee	
☐ One month (37 CFR 1.17(a)(1))	$150	$75	$
☐ Two months (37 CFR 1.17(a)(2))	$560	$280	$
☒ Three months (37 CFR 1.17(a)(3))	$1270	$635	$ 1270
☐ Four months (37 CFR 1.17(a)(4))	$1980	$990	$
☐ Five months (37 CFR 1.17(a)(5))	$2690	$1345	$

☐ Applicant claims small entity status. See 37 CFR 1.27.

☐ A check in the amount of the fee is enclosed.

☒ Payment by credit card.

☐ The Director has already been authorized to charge fees in this application to a Deposit Account.

☒ The Director is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number 50-5826.

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

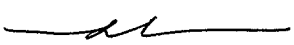
I am the

☐ applicant/inventor.

☐ assignee of record of the entire interest. See 37 CFR 3.71.
Statement under 37 CFR 3.73(b) is enclosed (Form PTO/SB/96).

☐ attorney or agent of record. Registration Number _____

☒ attorney or agent under 37 CFR 1.34.
Registration number if acting under 37 CFR 1.34 33,208



Signature

5/9/2012
Date

Roberta L. Robins
Typed or printed name

(650) 493-3400
Telephone Number

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below.

☒ Total of 1 forms are submitted.

CERTIFICATE OF TRANSMISSION PURSUANT TO 37 CFR § 1.8

I hereby certify pursuant to 37 CFR §1.8 that this correspondence is being transmitted via EFS to the United States Patent and Trademark Office on the date shown below.

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:	Customer No.: 105379
JOHN FRASER WRIGHT et al.	Confirmation No.: 4726
Application No.: 12/661,553	Art Unit: 1653
Filing Date: March 19, 2010	Examiner: Satyendra K. Singh

Title: COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION

AMENDMENT UNDER 37 CFR 1.111

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Sir:

This paper is responsive to the Office Action mailed November 9, 2011, with a shortened statutory period of three months for response. Accordingly, a three-month extension of time in which to respond is requested and a petition and fee therefor accompany this response. Reconsideration of the application is requested in view of the following amendments and remarks.

A **listing of claims** begins on page 2 of this paper.

Remarks begin on page 4 of this paper.

I. AMENDMENT

Amendments to the Claims:

The following listing reflects amendments to the claims and replaces all prior versions and listings of claims in this application.

1. (Currently amended) A composition for the storage of purified adeno-associated virus (AAV) particles, comprising:

purified ~~virus~~ (AAV) particles;

a pH buffer; and

excipients comprising one or more multivalent ions; wherein the ionic strength of the composition is greater than ~~about~~ 200 mM, wherein aggregation of the purified AAV particles in the composition is prevented.

2. (Canceled)

3. (Original) The composition of claim 1, wherein one of the one or more multivalent ions is citrate.

4. (Currently amended) The composition of claim 1, further comprising Pluronic® F68 (ethylene oxide/propylene oxide block copolymer).

5. (Currently amended) The composition of claim 4, wherein the Pluronic® F68 is present at 0.001% (w/v).

6. (Original) The composition of claim 1, wherein the pH buffer is 10 mM Tris, pH 8.0 and the excipients comprise 100 mM sodium citrate.

7. (Currently amended) The composition of claim 1, wherein ~~the average particle radius (*Rh*) of~~ the purified ~~virus~~ AAV particles ~~is~~ have an average particle radius (*Rh*) of less than about 20nm as measured by dynamic light scattering.

8. (Original) The composition of claim 1, wherein recovery of the purified virus particles is at least about 90% following filtration of the composition of virions through a 0.22μm filter.

9-11. (Canceled)

II. REMARKS

Introductory Comments

Claims 1-8 were examined in the Office Action under reply and stand variously rejected under (1) 35 U.S.C. §112, second paragraph (claims 4, 5 and 7); (2) 35 U.S.C. §102 (claims 1-3, 7 and 8); (3) 35 U.S.C. §103(a) (claims 1-8); and (4) the judicially created doctrine of nonstatutory double patenting (claims 1-8). These grounds of rejection are believed to be overcome by this response and are otherwise traversed for reasons discussed in detail below.

Overview of the Above Amendments

Claim 2, and nonelected claims 9-11 have been canceled. Claim 1 has been amended to include the recitations of canceled claim 2. Claim 1 also recites that aggregation of the purified AAV particles in the composition is prevented. Support for this amendment can be found throughout the specification at, e.g., page 5, lines 4-6. Finally, the term "about" has been eliminated from claim 1.

Claim 4 has been amended to include the generic name of Pluronic® F68. See, the accompanying BASF product information sheet. Claim 5 has been amended to recite the units "w/v"). Support for this amendment can be found at, e.g., Table 1 and page 24, lines 2-4. Minor wording changes have been made to claim 7 for antecedent basis purposes.

The foregoing amendments are made without prejudice, without intent to abandon any originally claimed subject matter, and without intent to acquiesce in any rejection of record. Applicants expressly reserve the right to file one or more continuing applications containing the unamended claims.

Rejections Under 35 U.S.C. §112, Second Paragraph

Claims 4 and 5 were rejected under 35 U.S.C. §112, second paragraph, as indefinite based on the use of the trademark Pluronic® F68. As explained above, claims 4 and 5 have been amended to include the generic terminology "ethylene oxide/propylene oxide block copolymer." Thus, this basis for rejection has been overcome and withdrawal thereof is respectfully requested.

Claim 5 was also rejected based on a lack of units for the recitation "0.001%." Applicants have inserted the term "w/v" into the claim. Thus, this basis for rejection has also been overcome.

Claim 7 was rejected for allegedly lacking insufficient antecedent basis. Minor wording changes have been made to overcome this rejection. Based on the foregoing, withdrawal of the rejections under 35 U.S.C. §112, second paragraph is respectfully requested.

Rejections Under 35 U.S.C. §102

Claims 1, 3, 7 and 8 were rejected under 35 U.S.C. §102(b) as anticipated by:

(1) Vihinen-Ranta et al., *J. Virol.* (1998) 72:802-806 ("Vihinen-Ranta"); and

(2) U.S. Patent No. 4,138,287 to Andersson et al. ("Andersson").

Applicants note claim 2 was not subject to this rejection, presumably because neither of Vihinen-Ranta or Andersson pertain to rAAV virions. Claim 1 has been amended to include the recitations from claim 2 regarding rAAV virions. Thus, these bases for rejection have been overcome and withdrawal thereof is respectfully requested.

Claims 1, 2, 7 and 8 were rejected under 35 U.S.C. §102(b) over U.S. Patent No. 6,146,874 to Zolotukhin et al. ("Zolotukhin"). The Office argues Zolotukhin teaches a preparation of rAAV virions with a pH buffer and excipients having an ionic strength of greater than about 200 mM and having multivalent ions. Office Action, page 5. However, applicants respectfully disagree that Zolotukhin anticipates the present claims.

To anticipate a claim, a single source must contain all of the elements of the claim. *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 231 USPQ 81, 90 (Fed. Cir. 1986). *Atlas Powder Co. v. E. I. du Pont De Nemours & Co.*, 224 USPQ 409, 411 (Fed. Cir. 1984). Moreover, the single source must disclose all of the claimed elements "arranged as in the claim." *Richardson v. Suzuki Motor Co.*, 9 USPQ2d 1913, 1920 (Fed. Cir. 1989); *Connell v. Sears Roebuck & Co.*, 220 USPQ 193, 198 (Fed. Cir. 1983). Finally, the law requires identity between the claimed invention and the prior art disclosure. *Kalman v. Kimberly-Clar Corp.* 218 USPQ 781, 789 (Fed. Cir. 1983, cert. denied, 465 U.S. 1026 (1984)). Based on these tenets, Zolotukhin fails to anticipate the present claims.

First, applicants note the Office has applied Zolotukhin under 35 U.S.C. §102(b) against claims 1, 2, 7 and 8, and then combined Zolotukhin with additional art under 35 U.S.C. §103(a) against the same claims. This is improper as a 102 reference **must** disclose each and every element in the claim, without the benefit of additional art. For this reason alone, the rejection must fail.

Moreover, applicants submit Zolotukhin does not teach a composition where the ionic strength of a **purified** preparation of rAAV virions is greater than 200 mM. Rather, in the passage cited by the Examiner to evidence the teaching of excipients greater than 200 mM ionic strength, the rAAV is in the process of being purified. See, for example, Figure 1 of Zolotukhin where all of the steps using such buffers are purification steps. High salt concentrations are not used in the final preparation. Rather, the final AAV product is formulated in Lactated Ringer's buffer. See, column 12, lines 49-54, where virus is concentrated by centrifugation through a BIOMAX 100K filter and desalted into Lactated Ringer's. Only after this step is the virus considered "purified" in the disclosure.

Lactated Ringer's typically includes 130 mM Na+, 4 mM K+, 109 mM Cl-, 28 mM lactate and 1.5 mM Ca++. See, the appended excerpt from Wikipedia that accompanies this response. Using the formula for ionic strength provided in the specification at pages 24-25, bridging paragraph, this translates to an ionic strength of 138.5 mM, not greater than 200 mM as claimed. Thus, Zolotukhin fails to disclose all of the claimed elements arranged as in the claim. Withdrawal of this basis for rejection is therefore respectfully requested.

Rejections Under 35 U.S.C. §103(a)

Claims 1-8 were rejected under 35 U.S.C. §103(a) as being unpatentable over Zolotukhin, taken with Andersson; U.S. Patent No. 6,194,191 to Zhang et al. ("Zhang"); and Chen et al., *J. Pharm. Sci.* (1994) 83:1657-1661 ("Chen").

The Office argues Zolotukhin teaches a preparation of rAAV virions with a pH buffer and excipients having an ionic strength of greater than about 200 mM and having multivalent ions. Office Action, page 5. Andersson is said to disclose a composition of purified HBsAg particles, a pH buffer and excipients comprising a multivalent ion such as sodium citrate wherein the ionic strength of the composition is greater than about 200 mM. With respect to claims 4-6,

the Examiner notes neither of Zolotukhin or Andersson describes a composition further comprising Pluronic (claims 4 and 5) or a composition wherein the pH buffer is 10 mM Tris, pH 8.0 and the excipients comprise 100 mM sodium citrate (claim 6). Office Action, page 8. Zhang is said to disclose the use of Pluronic® F68 for producing adenoviral particles. Chen allegedly discloses strategies to suppress aggregation of keratinocyte growth factor (KGF) in liquid formulations by adding sulfated polysaccharides and citrate salts. Finally, the limitations of claims 7 and 8 are said to be an intrinsic characteristic of the purified viral compositions. However, applicants submit the Office has failed to establish a *prima facie* case of obviousness.

All pending claims pertain to compositions comprising purified rAAV particles, a pH buffer, and excipients comprising one or more multivalent ions, wherein the ionic strength of the composition is greater than 200 mM, such that aggregation of the purified AAV particles in the composition is prevented. None of the cited art, either alone or in combination, teaches or suggests a **purified** rAAV particle preparation with an ionic strength of greater than 200 mM to prevent aggregation.

In particular, Zolotukhin does not recognize that rAAV virions self-aggregate and therefore does not provide suggestions regarding a composition for preventing aggregation of rAAV virions as claimed. This is particularly noteworthy in view of Zolotukhin's recognition (see column 15, lines 22-35) that AAV aggregates with **proteins** in the cell lysate wherein high salt in the **first step** of the iodixanol gradient is used to destabilize these AAV-lysate protein interactions. Moreover, applicants assert that this passage expressly teaches away from the claimed invention because Zolotukhin purposely eliminates high salt concentrations from the remainder of the iodixanol gradient, including the gradient from which the rAAV is collected after centrifugation, because the elimination of high salt is important for subsequent purification steps.

Applicants further note that, even though Zolotukhin recognizes AAV virion-lysate protein aggregation as a problem, it does not teach or suggest that self-aggregation of rAAV virions is a problem at all, let alone a problem in a **purified**, concentrated AAV preparation. In fact, absent applicants' teaching regarding this problem, there is no recognition that rAAV virion self-aggregation is a concern that may be addressed by manipulating ionic strength in any of the cited art.

Furthermore, as explained above, in the Zolotukhin passage cited by the Examiner to evidence the teaching of excipients greater than 200 mM ionic strength, the rAAV is in the process of being purified. See, for example, Figure 1 where all of the steps using such buffers are purification steps. High salt concentrations are not used in the final preparation. Rather, the final AAV product is formulated in Lactated Ringer's buffer. See, column 12, lines 49-54, where virus is concentrated by centrifugation through a BIOMAX 100K filter and desalted into Lactated Ringer's. Only after this step is the virus considered "purified" in the disclosure and, as explained above, Lactated Ringer's has an ionic strength of 138.5 mM. Applicants' purified product, on the other hand, has an ionic strength of greater than 200 mM. There is absolutely no suggestion in Zolotukhin or any of the other cited references that a final preparation with an ionic strength of greater than 200 mM and containing purified virions is desirable or necessary.

The secondary references do not fill the gaps present in Zolotukhin. Applicants reiterate the present claims are directed to compositions of purified **rAAV** particles. None of Andersson, Zhang or Chen even relates to AAV. In this regard, Andersson is directed to methods for isolating Hepatitis B virus (HBV), a virus completely unrelated to AAV. HBV causes a persistent and chronic form of hepatitis and belongs to the *Hepadnaviridae* family. HBV has a circular, partially double-stranded DNA genome. The virus has a virion diameter of 42 nm.

Zhang relates to methods for producing adenoviral vectors. Adenovirus, like HBV, is unrelated to AAV. Adenoviruses are double-stranded DNA viruses, are medium-sized (90–100 nm), and belong to the family *Adenoviridae*. Adenoviruses, like HBV, cause human respiratory diseases, ranging from mild disease to death.

Unlike both HBV and adenovirus, AAV, belongs to the family *Parvoviridae,* and has a 20 nm virion composed of a linear, single-stranded DNA molecule. Moreover, unlike HBV and adenovirus, AAV is not known to cause disease. One of skill in the art of rAAV virion formulations would simply not look to art pertaining to unrelated viruses in order to determine proper conditions to prevent aggregation.

Chen, does not even relate to viruses, but rather pertains to methods for preventing aggregation of keratinocyte growth factor (KGF). There is absolutely no reason to believe that art directed to growth factors is in any way pertinent to virion production.

Applicant respectfully submits the Examiner has failed to establish a *prima facie* case of obviousness because there was no motivation to combine the teachings of Zolotukhin with any of Andersson, Zhang and/or Chen at the time of filing the instant application and, even if such motivation existed, there was no reasonable expectation of a composition containing purified rAAV virions with components that resulted in the prevention of aggregation. Applicants are unaware of, and the Examiner has not cited, any art teaching such a composition. The three secondary references, Andersson, Zhang and Chen, relied on by the Office, have nothing whatsoever to do with AAV. The Office has not provided a motivation to combine formulation conditions from four completely unrelated molecules. Nor has the Office established a reasonable expectation of success in the combination of formulation components to prevent self-aggregation of rAAV particles.

With respect to the Office's assertion regarding claims 7 and 8, namely, that the average particle radius and percent recovery are inherent characteristics, the Examiner is reminded that inherency is not a proper standard on which to base an obviousness rejection. In this regard, it is axiomatic that a retrospective view of inherency is not a substitute for some teaching or suggestion to arrive at the claimed invention. That which may be inherent is not necessarily known, and obviousness **cannot** be predicated on the unknown. See, e.g., *In re Newell*, 13 USPQ2d 1248 (Fed. Cir. 1989).

The combination cited by the Office does not provide evidence that the claimed invention is a "predictable use of prior art elements according to their established functions." *KSR Int'l Co. v. Teleflex, Inc.*, 82 USPQ2d 1385, 1396 (U.S. 2007). Rather, as explained above, the evidence is to the contrary. Applicants submit the Examiner has chosen bits and pieces of the cited references to arrive at the allegation that this combination of references suggests the claimed invention. This is improper. As stated in *KSR*, "a patent composed of several elements is not proved obvious merely by demonstrating that each of its elements was, independently, known in the prior art." *KSR*, page 1396. The Federal Circuit has consistently reversed a finding of obviousness, even when all claimed elements are individually present in the references. See, e.g., *In re Kotzab*, 55 USPQ2d 1313, 1317 (Fed. Cir. 2000). Thus, a rejection cannot be predicated on the mere identification of individual components of claimed limitations. Rather,

particular findings must be made as to the reason the skilled artisan, with no knowledge of the claimed invention, would have selected these components for combination in the manner.

For at least these reasons, withdrawal of the rejections under 35 U.S.C. § 103(a) is respectfully requested.

The Double Patenting Rejection

Claims 1-8 were rejected under the judicially created doctrine of nonstatutory obviousness-type double patenting over claim 1 of U.S. Patent No. 7,704,721[1]. Applicants respectfully traverse. In particular, composition claims corresponding to those pending herein were restricted out in the application that ultimately issued as the '721 patent. Accordingly, it is completely improper to require a Terminal Disclaimer in the present application over the '721 patent. See, MPEP 804.01, citing 35 U.S.C. 121. Accordingly, withdrawal of this basis for rejection is respectfully requested.

[1] Applicants note the Office specified U.S. Patent No. 7,701,721 in the rejection. However, U.S. Patent No. 7,701,721 does not relate to rAAV virion production, is not commonly owned and does not have an inventor in common with the present application. Hence, applicants assume U.S. Patent No. 7,704,721 was actually intended.

III. CONCLUSION

Applicants respectfully submit that the claims are now in condition for allowance and request early notification to that effect. The Examiner is encouraged to contact the undersigned if the Examiner notes any further matters which might be resolved by a telephone interview.

Respectfully submitted,

Date: 5/9/2012

By: _____
Roberta L. Robins
Registration No. 33,208

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2625 Middlefield Road, No. 828
Palo Alto, CA 94306
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Close Window

Pluronic® F 68

Product Info			
Description	**Language**	**Legal Area**	
Product information	EN		View (109k)
Kosher Certificate (pdf)	EN		View (197k)
ISO Certificate	EN		View (329k)
Material Safety Data Sheet	EN	CA	View (36k)
Material Safety Data Sheet	FR	CA	View (38k)
Material Safety Data Sheet	ES	MX	View (41k)
Material Safety Data Sheet	EN	US	View (41k)

Product Attributes	
Description	
Country of origin	USA
Chemical family names	Ethylene Oxide/Propylene Oxide Block Copolymer
Harmonized Tariff Code	3402.13
Business	Consumer & Industrial Specialties
Synonyms	Poloxamer

The Material Safety Data Sheets are based on the legal requirements in the respective country of production. If you make an order, you will also receive the respective Material Safety Data Sheet in the usual country-specific form. All information given is based on our current knowledge and experience and does not represent an assurance of properties or suitability for a specific application. Existing laws and regulations must be observed by the recipient of our products at his own responsibility.

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Lactated Ringer's solution

From Wikipedia, the free encyclopedia
(Redirected from Ringer's lactate)

Lactated Ringer's solution is a solution that is isotonic with blood and intended for intravenous administration. Veterinary administration may also be subcutaneous.

Lactated Ringer's solution is abbreviated as "LR" or "RL". It is also known as **Ringer's lactate solution** (although Ringer's solution technically refers only to the saline component, without lactate). It is very similar, though not identical to, **Hartmann's (Compund Sodium Lactate) Solution**, the ionic concentrations of which differ.

Contents

Ingredients

One liter of Lactated Ringer's Solution contains:

- 130 mEq of sodium ion.
- 109 mEq of chloride ion.
- 28 mEq of lactate.
- 4 mEq of potassium ion.
- 3 mEq of calcium ion.

Generally, the sodium, chloride, potassium and lactate come from NaCl (sodium chloride), $NaC_3H_5O_3$ (sodium lactate), $CaCl_2$ (calcium chloride), and KCl (potassium chloride).

Development of Ringer's Solution

Ringer's saline solution was invented by Sydney Ringer[1] (http://www.whonamedit.com/synd.cfm/2119.html), a British physiologist who was born in 1835 in Norwich, England and died October 14, 1910, in Lastingham, Yorkshire, England. The solution was further modified by Alexis Hartmann, (an american MD of german background with an interest in paediatrics who lived from 1898-1964), for the purpose of treating acidosis in children. Hartmann modified the solution by adding lactate, which while undergoing reactions in Liver, kidney and muscle cells to either produce glucose or be metabolised to water and carbon dioxide, consumes H+ ions thus acting as a base. Thus the solution became known as 'lactated Ringer's Solution' and later, 'Hartmann's solution' [2] (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=9165959&dopt=Abstract)

Therapy

Lactated Ringer's Solution is often used for fluid resuscitation after a blood loss due to trauma, surgery, or a burn injury. It is also used to induce urination in patients with renal failure.

Electronic Patent Application Fee Transmittal

Application Number:	12661553
Filing Date:	19-Mar-2010
Title of Invention:	Compositions and methods to prevent AAV vector aggregation
First Named Inventor/Applicant Name:	John Fraser Wright
Filer:	Roberta L. Robins/Denise Vaillancourt
Attorney Docket Number:	0800-0045.01

Filed as Large Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Extension - 3 months with $0 paid	1253	1	1270	1270

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
			Total in USD ($)	**1270**

Electronic Acknowledgement Receipt

EFS ID:	12737418
Application Number:	12661553
International Application Number:	
Confirmation Number:	4726
Title of Invention:	Compositions and methods to prevent AAV vector aggregation
First Named Inventor/Applicant Name:	John Fraser Wright
Customer Number:	20855
Filer:	Roberta L. Robins/Denise Vaillancourt
Filer Authorized By:	Roberta L. Robins
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Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)

1		PTO_20120509104740.pdf	691698 e8f1a58afbe02a63079f2b21ffdbdea00a7fd73f	yes	15

Multipart Description/PDF files in .zip description		
Document Description	**Start**	**End**
Transmittal Letter	1	1
Extension of Time	2	2
Applicant Arguments/Remarks Made in an Amendment	3	15

Warnings:

Information:

2	Fee Worksheet (SB06)	fee-info.pdf	30262 761d78ed0d49a6fb32b174162f20d37ff95014fb	no	2

Warnings:

Information:

Total Files Size (in bytes):	721960

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New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/SB/06 (07-06)
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PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number	Filing Date	☐ To be Mailed
12/661,553	03/19/2010	

APPLICATION AS FILED – PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY ☐ RATE ($)	SMALL ENTITY ☐ FEE ($)	OR	OTHER THAN SMALL ENTITY RATE ($)	OTHER THAN SMALL ENTITY FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =		OR	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =			X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	

APPLICATION AS AMENDED – PART II

AMENDMENT	05/09/2012	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE ($)	SMALL ENTITY ADDITIONAL FEE ($)	OR	OTHER THAN SMALL ENTITY RATE ($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 7	Minus	** 20	= 0	X $ =		OR	X $60=	0
	Independent (37 CFR 1.16(h))	* 1	Minus	***3	= 0	X $ =		OR	X $250=	0
	☐ Application Size Fee (37 CFR 1.16(s))									
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	0

AMENDMENT		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =		OR	X $ =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =		OR	X $ =	
	☐ Application Size Fee (37 CFR 1.16(s))									
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".

*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".

The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

Legal Instrument Examiner:
/KELLY HARRIS/

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/661,553	03/19/2010	John Fraser Wright	0800-0045.01	4726

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EXAMINER	
SINGH, SATYENDRA K	
ART UNIT	PAPER NUMBER
1657	

MAIL DATE	DELIVERY MODE
07/17/2012	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	12/661,553	WRIGHT ET AL.	
	Examiner	Art Unit	
	SATYENDRA SINGH	1657	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *09 May 2012*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

5)☒ Claim(s) *1 and 3-8* is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *1 and 3-8* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

10)☒ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

12)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Applicant's submission filed on 05/09/2012 is duly acknowledged.

Claims 2, and 9-11 (invention of group II) have been canceled by applicants.

Claims 1 and 3-8 (elected invention of group I), as currently amended, have been examined on their merits in this office action.

Objection to Specification

The disclosure is objected to for the following informalities:

Page 1, first paragraph of the instant disclosure should be amended to acknowledge the issued patent US 7,704,721 from application 11/141,996. Appropriate correction is required.

Claim Rejections - 35 USC § 112- withdrawn

In view of the current amendments presented in claims 4, 5 and claim 7, the 112-second rejections, as set forth in the previous office action, have been withdrawn by the examiner.

Claim Rejections - 35 USC § 102- withdrawn

In view of current amendments to claim 1, the rejection of claims 1, 3, 7 and 8 under 35 U.S.C. 102(b) over cited references of Vihinen-Ranta et al (1998) and Andersson et al (1979), as previously made by the examiner, have been withdrawn.

Claim Rejections - 35 USC § 102-maintained

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

1. **Claims 1, 7 and 8** (as currently amended) are/remain rejected under 35 U.S.C. 102(b) as being anticipated by **Zolotukhin et al** (2000; US 6,146,874; IDS).

Claims are directed to "a composition for the storage of purified adeno-associated virus (AAV) particles, comprising: purified AAV particles; a pH buffer; and excipients comprising one or more multivalent ions; wherein the ionic strength of the composition is greater than 200 mM, wherein aggregation of the purified AAV particles in the composition is prevented (claim 1); wherein the purified AAV particles have an average particle radius (*Rh*) of less than about 20 nm as measured by dynamic light scattering (instant claim 7 as amended); and wherein recovery of the purified virus particles is at least about 90% following filtration of the composition of virions through a 0.22 μm filter" (claim 8).

Zolotukhin et al (2000) disclose the composition comprising: purified recombinant AAV virus particles; a pH buffer such as **phosphate buffered-saline with magnesium chloride and potassium chloride** (PBS-MK); and excipients comprising one or more multivalent ions such as phosphate and magnesium; wherein the ionic strength of the composition is greater than 200 mM (see Zolotukhin et al, column 11, 4th paragraph, lines 35-40, in particular), wherein they elute the purified rAAV particles in **PBS-MK buffer having 1M NaCl**, i.e. elution buffer. The limitations of claims 7 and 8 (average particle radius and percent recovery following filtration) are also met by the prior art as these limitations are taken to be an inherent characteristic features of the purified viral composition as disclosed by Zolotukhin et al. Since, all the components including purified virus particles, pH buffer and multivalent ions, as recited in claims 1 and 7, or claims 1 and 8, are the same, as disclosed in the cited prior art, these features will necessarily follow from the composition disclosed in the art.

As per MPEP 2111.01, *during examination, the claims must be interpreted as broadly as their terms reasonably allow. In re American Academy of Science Tech Center, F.3d, 2004 WL 1067528 (Fed. Cir. May 13, 2004)(The USPTO uses a different standard for construing claims than that used by district courts; during examination the USPTO must give claims their broadest reasonable interpretation.). This means that the words of the claim must be given their plain meaning unless applicant has provided a clear definition in the specification. In re Zletz*, 893 F.2d 319, 321, 13 USPQ2d 1320, 1322 (Fed. Cir. 1989).

Response to 102(b) Arguments over Zolotukhin et al

Applicant's arguments filed on 5/9/2012 (as they pertain to the 102(b) rejection of claims 1, 2, 7 and 8) have been fully considered but they are not persuasive for the following reasons:

Applicants argument that "… *Zolotukhin does not teach a composition where the ionic strength of a purified preparation of rAAV virions is greater than 200 mM. Rather, in the passage cited by the Examiner to evidence the teaching of excipients greater than 200 mM ionic strength, the rAAV is* ***in the process of being purified****. See, for example, Figure 1.... considered "purified" in the disclosure*" (see remarks, page 6, 2nd and 3rd paragraphs), is duly noted and considered. However, it is noted that the term "purified" is not specifically defined in the instant disclosure, as being tied to any particular step and/or degree of purification of the AAV particles, or for that matter number of virions associated with such composition as claimed. The cited reference of Zolotukhin et al disclose, *albeit* during the process of purification the composition comprising purified rAAV virus particles, a pH buffer such as phosphate buffered-saline with magnesium chloride and potassium chloride (PBS-MK used as elution buffer containing 1M NaCl), and excipients comprising multivalent ions such phosphate and magnesium, wherein the ionic strength of said composition is greater than 200mM, and therefore anticipate the claimed invention as currently presented. The arguments regarding Lactate Ringer's composition and ionic strength as made by the applicants is not found to be persuasive because Zolotukhin et al

disclose all the elements of the claimed composition. In addition, since the components including purified AAV particles, pH buffer and multivalent ions, as recited in instant claim 1 are the same, as disclosed in the cited prior art, the features recited in claims 7 and 8 would necessarily follow from the composition disclosed in the art.

Claim Rejections - 35 USC § 103- maintained

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* v. *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

1. Claims **1 and 3-8** (as amended) are/remain rejected under 35 U.S.C. 103(a) as being unpatentable over Zolotukhin et al (2000; US 6,146,874; IDS) taken with Andersson et al (1979; US 4,138,287; [A]), Zhang et al (2001; US 6,194,191; IDS) and Chen et al (1994; IDS).

Claims are directed to "a composition (for the storage) of purified adeno-associated virus (AAV) particles, comprising: purified AAV particles; a pH buffer; and excipients comprising one or more multivalent ions; wherein the ionic strength of the composition is greater than 200

mM, wherein aggregation of the purified AAV particles in the composition is prevented (instant claim 1); wherein one of the one or more multivalent ions is citrate (claim 3); further comprising Pluronic® F68 (ethylene oxide/propylene oxide block copolymer) at 0.001% (w/v) (claims 4-5); wherein the pH buffer is 10 mM Tris, pH 8.0 and the excipients comprise 100 mM sodium citrate (claim 6); wherein the purified AAV particles have an average particle radius (*Rh*) of less than about 20 nm as measured by dynamic light scattering (instant claim 7 as amended); and wherein recovery of the purified virus particles is at least about 90% following filtration of the composition of virions through a 0.22 μm filter" (claim 8).

The detailed teachings of Zolotukhin et al have been discussed above, and are further relied upon in the same manner herein.

Andersson et al (1979) disclose the composition comprising purified hepatitis virus (HB_sAg) particles (see abstract, in particular), a pH buffer such as Tris-sodium citrate buffer, pH 7.5, and excipients comprising a multivalent ion such as **sodium citrate**, wherein the ionic strength of the composition is greater than about 200 mM (see elution of purified virus from column using 0.5 M NaCI in Tris-citrate buffer; see Andersson et al, example 2 and claim 5, in particular; meets the limitations of claims 1 and 3).

However, the composition, further comprising **Pluronic® F68** at 0.001% w/v (claims 4-5); and wherein the pH buffer is **10 mM Tris, pH 8.0** and the excipients comprise **100 mM sodium citrate** (claim 6), is not specifically disclosed by the inventions of Zolotukhin et al taken with Andersson et al.

Zhang et al (2001) disclose the use of a surfactant such as **Pluronic® F68** (0.1% in growth medium for adenovirus infection and viral production, etc.; see column 4, 2nd paragraph and columns 53-54, in particular) for production of adenoviral particles in serum-free suspension cultures using spinner flasks, and also use in the cryopreservation media (see column 53, last paragraph).

Chen et al (1994) disclose strategies to suppress aggregation of proteins (such as recombinant keratinocyte growth factor) during liquid formulation development (see Chen et al, abstract, table 1-2, in particular) by adding sulfated polysaccharides (such as heparin) and **citrate salts** (such as 0.1 to 0.5 M sodium citrate; see Chen et al, page 1661, left column, in particular) that were found to be effective in preventing protein aggregation. Chen et al also disclose the fact that other negatively charged small ions such as phosphate (a multivalent ion) also have moderate stabilizing effects on preventing aggregation of recombinant keratinocyte growth factor (rhKGF).

Thus, given the disclosure in the cited prior art, a person of ordinary skill in the art would have modified the composition comprising purified AAV virus particles taught by Zolotukhin et al taken with Andersson et al such that said composition additionally comprise a surfactant (to help prevent aggregation during freezing-thawing cycles, for example) such as Pluronic® F68, as explicitly taught by Zhang et al, and a suitable amount of sodium citrate as a multivalent ion in Tris buffer solution (at a suitable pH; as specifically disclosed by Andersson et al) in order to stabilize the viral particles as suggested by Chen et al (for suppressing/reducing aggregation of fairly unstable proteins such as rhKGF; see discussion above, thus providing a conceptual basis for including multivalent ions in the buffer containing purified AAV particles), in addition to

other stabilizing components such as a surfactant. Since, the benefits of including a surfactant and high ionic strength multivalent ions have been disclosed in the cited prior art of Zhang et al and Chen et al, an artisan of ordinary skill in the art would have established suitable concentrations required to help stabilize viral preparations (i.e. by preventing aggregation, etc.) with a reasonable expectation of success.

The limitations of claims 7 and 8 (average particle radius and percent recovery following filtration, etc.) are also met by the prior art as these are taken to be intrinsic characteristic features of the purified viral compositions as disclosed by Zolotukhin et al when taken with the teachings of Andersson et al, Zhang et al and Chen et al. Since, all the components of the product, as recited in the claims are the same as disclosed and/or suggested in the cited prior art, these features will necessarily follow from the composition disclosed in the art, as they do not structurally change the composition as claimed.

Thus, the invention as a whole would have been *prima facie* obvious to a person of ordinary skill in the art at the time the claimed invention was made.

As per MPEP 2144.06, "*It is prima facie obvious to combine two compositions each of which is taught by the prior art to be useful for the same purpose, in order to form a third composition to be used for the very same purpose.... [T]he idea of combining them flows logically from their having been individually taught in the prior art." In re Kerkhoven, 626 F.2d 846, 850, 205 USPQ 1069, 1072 (CCPA 1980).*

As per MPEP 2144.06, *In order to rely on equivalence as a rationale supporting an obviousness rejection, the equivalency must be recognized in the prior art, and cannot be based on applicant's disclosure or the mere fact that the components at issue are functional or mechanical equivalents. In re Ruff, 256 F.2d 590, 118 USPQ 340 (CCPA 1958).*

Response to Arguments over 103(a) Rejection

Applicant's arguments filed 05/09/2012 (as they pertain to the obviousness rejection of record) have been fully considered but they are not persuasive for the following reasons of record:

Applicants argument that "*(N)one of the cited art, either alone or in combination, teaches or suggests a purified rAAV particle preparation with an ionic strength of greater than 200 mM to prevent aggregation*" (see remarks, page 7), is duly noted and considered. However it is not found to be persuasive because Zolotukhin et al disclose such a product composition as recited in instant claim 1 (see 102b rejection of record, above). The argument that Zolotukhin et al "*expressly teaches away from the claimed invention because Zolotukhin purposely eliminates high salt concentrations from the remainder of the iodixanol gradient...*" (see remarks, page 7, 3rd paragraph), is not found to be persuasive because instant claims are directed to product composition that has been fully disclosed in the cited art of record, *albeit* as an intermediate composition during the process of preparation of said rAAV particles. Thus, applicants argument that "*...even though Zolotukhin recognizes AAV virion-lysate protein aggregation as a problem, it does not teach or suggest that self-aggregation of rAAV virions is a problem at all, let alone a problem in a purified, concentrated AAV preparation*", is noted and fully considered but is not found to be persuasive for the same reasons of record. The arguments regarding Lactate Ringer's composition and its ionic strength as made by the applicants (see remarks page 8) is also not found be persuasive because Zolotukhin et al disclose all the elements of the claimed composition. Moreover, it is noted that the term "**purified**" is not specifically defined in the instant disclosure, as being tied to any particular step and/or degree of purification of the

AAV particles in said composition, as claimed. The cited reference of Zolotukhin et al disclose (*albeit* during the process of purification as an intermediate) the composition comprising purified rAAV virus particles, a pH buffer such as phosphate buffered-saline with magnesium chloride and potassium chloride (PBS-MK used as elution buffer containing 1M NaCl), and excipients comprising multivalent ions such phosphate and magnesium, wherein the ionic strength of said composition is greater than 200mM, and therefore meet the limitations of the claimed invention as currently presented in claim 1. Regarding the cited references of Andersson et al, Zhang et al and Chen et al, applicants seem to argue that they are non-analogous art and do not relate to AAV composition at hand (see remarks, page 8), and that "*.. .there was no motivation to combine the teachings of Zolotukhin with any of Andersson, Zhang and/or Chen at the time of filing the instant application and, even if such motivation existed, there was no reasonable expectation of a composition containing purified rAAV virions with components that resulted in the prevention of aggregation*", which is fully considered, but is not found to be persuasive because (as also discussed in the rejection of record above) the problems of viral particle aggregation has been known and/or recognized in the art (see disclosure of Zolotukhin et al, above), specifically as suggested by the disclosure of Chen et al (when taken in combination with Andersson et al and Zhang et al) that salts of multivalent ions such as citrate were found to be effective in preventing protein aggregation. They also disclose the fact that other negatively charged small ions such as phosphate (also a multivalent ion) also have moderate stabilizing effect on preventing aggregation of recombinant keratinocyte growth factor. Since, AAV virions are encapsulated in capsid proteins, an artisan of ordinary skill in the art would have been motivated to use multivalent ions such as citrate, phosphate, etc. (with or without suitable surfactants such as

Pluronic® F68) in order to stabilize the purified preparation of AAV virions, with a reasonable expectation of success. In the absence of evidence/data to the contrary, the limitations presented in instant claims 7 and 8 are taken to be intrinsic to the composition disclosed in the cited art of record (see arguments on page 9, 2nd paragraph), as all the components recited in instant claim 1 have been disclosed and/or fully suggested by the combined disclosure of the cited art of record. Applicants are advised to amend claim 1 in order to reflect the novelty of the invention commensurate in the scope of the claims and showing in the disclosure of record.

Double Patenting- Maintained

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory obviousness-type double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting

ground provided the conflicting application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement.

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

Applicants are apprised that The United States Patent and Trademark Office now provides for an **eTerminal Disclaimer in EFS-Web**. For more information please see http://www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp. The new eTerminal Disclaimer provides applicants with many advantages and promotes greater efficiency in the patent examination process. This web-based eTerminal Disclaimer can be filled out completely online through web-screens and no EFS-Web fillable forms are required. eTerminal Disclaimers are auto-processed and approved immediately upon submission if the request meets all of the requirements.

Fees must be paid immediately which will then provide users more financial flexibility. A paper filed Terminal Disclaimer requires a fee but does not guarantee a Terminal Disclaimer approval. Each eTerminal Disclaimer filed requires a single terminal disclaimer fee, but can include up to 50 "reference applications" and 50 "prior patents".

1. **Claims 1 and 3-8** (as presented) are/remain rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over at least **claim 1** of U.S. Patent No. **7,704,721 B2** (issued on April 27, 2010 to the same assignee and inventors). Although the conflicting claims are not identical, they are not patentably distinct from each other because issued claim 1 is directed to a method of preventing aggregation of rAAV virion preparations by adding "one or more salts of multivalent ions selected from the group consisting of citrate, phosphate, sulfate and magnesium to said purified virions to produce a preparation of virions with an ionic strength of at least 200 mM", which is suitable for long term storage without significant aggregation problems. Since, the composition disclosed in the issued '721

patent is essentially similar in scope to the composition as currently being claimed by applicants in the instant application, an ODP rejection is deemed proper.

Response to ODP Arguments

Applicant's arguments filed 5/9/2012 (regarding the ODP rejection of record) have been fully considered but they are not persuasive for the following reasons of record:

First, it is noted that applicant correction that it was patent **7,704,721** over which the ODP rejection was made, is acknowledged and appreciated. Regarding the ODP arguments that "*…composition claims corresponding to those pending herein were restricted out in the application that ultimately issued as the '721 patent. Accordingly, it is completely improper to require a Terminal Disclaimer in the present application over the '721 patent. See, MPEP 804.01, citing 35 U.S.C. 121*", it is noted that instant application has been filed as a CON of the 11/141,996 (allowed and issued as US 7,704,721; see page 1 of the instant disclosure filed on 3/19/2010). Moreover, the issued method claims deal with a composition prepared using a nuclease, whereas the composition as currently claimed only requires AAV particles in a buffer containing one or more multivalent ions having ionic strength as recited in claim 1. Thus, instant application has not been treated as a true DIV of earlier application 11/141,996, even though a restriction was made between methods and the product. Since, the composition as claimed is fully contemplated in the issued patent '721, and is co-extensive in scope, the ODP rejection is deemed proper.

Conclusion

NO claims are allowed.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SATYENDRA SINGH whose telephone number is (571)272-8790. The examiner can normally be reached on 9-5MF.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, JON P. WEBER can be reached on 571-272-0925. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Satyendra K. Singh/
Examiner, Art Unit 1657

/JON P WEBER/
Supervisory Patent Examiner, Art Unit 1657

EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
S22	1	("4,138,287").PN.	USPAT; USOCR	OR	OFF	2011/11/07 10:34
S23	1	("7704721").PN.	USPAT; USOCR	OR	OFF	2012/07/10 17:27
S24	1004	WRIGHT near3 JOHN	USPAT	OR	OFF	2012/07/10 17:30
S25	1970	WRIGHT near3 JOHN	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2012/07/10 17:30
S26	19	QU near3 GUANG	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2012/07/10 17:30
S27	1977	S25 or S26	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2012/07/10 17:30
S28	18	S27 and ((virion or vira$6 or virus or adenovir$6 or AAV) same (aggreg$6 or precipit$6 or agglutin$6 or stabiliz$6))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2012/07/10 17:30
S29	1	S27 and ((virion or vira$6 or virus or adenovir$6 or AAV) same (aggreg$6 or precipit$6 or agglutin$6 or stabiliz$6) same (multivalent or divalent))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2012/07/10 17:30
S30	6	S27 and ((virion or vira$6 or virus or adenovir$6 or AAV) same (aggreg$6 or precipit$6 or agglutin$6 or stabiliz$6)) and (multivalent or divalent)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2012/07/10 17:30
S31	13	((virion or vira$6 or virus or adenovir$6 or AAV) same (aggreg$6 or precipit$6 or agglutin$6 or stabiliz$6 or sotrag$6) same ((multivalent or divalent) near3 ion))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2012/07/10 17:30
S32	2	((virion or vira$6 or virus or adenovir$6 or AAV) same (aggreg$6 or precipit$6 or agglutin$6 or stabiliz$6 or sotrag$6) same ((multivalent or divalent) near3 ion)).clm.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2012/07/10 17:30
S33	7	((virion or vira$6 or virus or adenovir$6 or AAV) same (stabiliz$6	US-PGPUB; USPAT; USOCR;	OR	OFF	2012/07/10 17:30

		or sotrag$6) same ((multivalent or divalent) near3 ion))	FPRS; EPO; JPO; DERWENT; IBM_TDB			
S34	1399	(adenovir$6 or AAV) same (stabiliz$6 or sotrag$6)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2012/07/10 17:30
S35	15	(adenovir$6 or AAV) same (stabiliz$6 or sotrag$6).clm.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2012/07/10 17:30
S36	9	S35 and ((citr$6 or magnes$6 or phosphat$6 or manganese) same buffer)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2012/07/10 17:30
S37	853	((AAV or adenovir$3) near3 (particle or virion)) and ((citr$6 or magnes$6 or phosphat$6 or mangan$6 or sulfate) same buffer)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2012/07/10 17:30
S38	35	((AAV or adenovir$3) near3 (particle or virion)) same ((citr$6 or magnes$6 or phosphat$6 or mangan$6 or sulfate) same buffer)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2012/07/10 17:30
S39	8	((AAV or adenovir$3) near3 (particle or virion)) same ((citr$6 or magnes$6 or phosphat$6 or mangan$6 or sulfate) same buffer same mM)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2012/07/10 17:30
S40	4	(("6146874") or ("6194191") or ("6566118") or ("6593123")).PN.	USPAT; USOCR	OR	OFF	2012/07/10 17:30
S41	4	S40 and (phosphate or magnesium or sulfate or tris or citrate) and buffer	USPAT	OR	OFF	2012/07/10 17:30
S42	396	(AAV or rAAV or adenovirus) same (surfactant or pluronic$2f68 or pluronic$4)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2012/07/10 17:30
S43	141	(AAV or rAAV or adenovirus) same (pluronic$2f68 or pluronic$4)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2012/07/10 17:30
S44	34	(adenovir$3 or AAV or virus) same (tris near3 citrate)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2012/07/10 17:30
S45	1	("4,138,287").PN.	USPAT; USOCR	OR	OFF	2012/07/10 17:30
S46	4	(("6146874") or ("6194191") or ("6566118") or ("6593123")).PN.	USPAT; USOCR	OR	OFF	2012/07/11 15:05
S47	3	S46 and aggregat$6	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO;	OR	OFF	2012/07/11 15:05

			DERWENT; IBM_TDB			

EAST Search History (Interference)

< This search history is empty>

7/ 11/ 2012 4:18:45 PM
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Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	12661553	WRIGHT ET AL.
	Examiner	Art Unit
	SATYENDRA SINGH	1657

SEARCHED

Class	Subclass	Date	Examiner

SEARCH NOTES

Search Notes	Date	Examiner
EAST: USPAT, USOCR, US-PGPUB, JPO, EPO, DERWENT- ATTACHED	11/6/2011	SKS
INVENTOR SEARCH: PALM, EDAN AND EAST-	11/6/2011	SKS
EAST: USPAT, USOCR, US-PGPUB, JPO, EPO, DERWENT- UPDATED & ATTACHED	7/11/2012	SKS
INVENTOR SEARCH: PAL, EDAN AND EAST- UPDATED	7/11/2012	SKS

INTERFERENCE SEARCH

Class	Subclass	Date	Examiner

/SATYENDRA SINGH/ Examiner.Art Unit 1657	

Request for Continued Examination (RCE) Transmittal

Address to:
Mail Stop RCE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Application Number	12/661,553
Filing Date	March 19, 2010
First Named Inventor	John Fraser Wright, et al.
Art Unit	1653
Examiner Name	Satyendra K. Singh
Attorney Docket Number	0800-0045.01

This is a Request for Continued Examination (RCE) under 37 CFR 1.114 of the above-identified application.
Request for Continued Examination (RCE) practice under 37 CFR 1.114 does not apply to any utility or plant application filed prior to June 8, 1995, or to any design application. See Instruction Sheet for RCEs (not to be submitted to the USPTO) on page 2.

1. Submission required under 37 CFR 1.114 Note: If the RCE is proper, any previously filed unentered amendments and amendments enclosed with the RCE will be entered in the order in which they were filed unless applicant instructs otherwise. If applicant does not wish to have any previously filed unentered amendment(s) entered, applicant must request non-entry of such amendment(s).

   a. ☐ Previously submitted. If a final Office action is outstanding, any amendments filed after the final Office action may be considered as a submission even if this box is not checked.
      i. ☐ Consider the arguments in the Appeal Brief or Reply Brief previously filed on _____
      ii. ☐ Other _____

   b. ☒ Enclosed
      i. ☒ Amendment/Reply
      ii. ☐ Affidavit(s)/ Declaration(s)
      iii ☐ Information Disclosure Statement (IDS)
      iv. ☐ Other _____

2. Miscellaneous

   a. ☐ Suspension of action on the above-identified application is requested under 37 CFR 1.103(c) for a period of ___months. (Period of suspension shall not exceed 3 months; Fee under 37 CFR 1.17(i) required)

   b. ☐ Other _____

3. Fees The RCE fee under 37 CFR 1.17(e) is required by 37 CFR 1.114 when the RCE is filed.

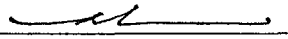
   a. ☒ The Director is hereby authorized to charge the following fees, or credit any overpayments, to Deposit Account No. 50-5826. **I have enclosed a duplicate copy of this sheet.**
      i. ☐ RCE fee required under 37 CFR 1.17(e)
      ii. ☐ Extension of time fee (37 CFR 1.136 and 1.17)
      iii. ☒ Other **any fees not already included**

   b. ☐ Check in the amount of $ _____ enclosed

   c. ☒ Payment by credit card

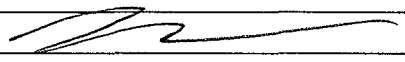
WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization of PTO-2038.

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT REQUIRED

Signature		Date	1/17/2013
Name (Print /Type)	Roberta L. Robins	Registration No.	33,208

CERTIFICATE OF MAILING OR TRANSMISSION

I hereby certify pursuant to 37 CFR §1.8 that this correspondence is being transmitted via EFS to the United States Patent and Trademark Office on the date shown below.

Signature			
Name (Print /Type)	Denise M. Vaillancourt	Date	1/17/13

PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a) **FY 2012** *(Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).)*	Docket Number (Optional) 0800-0045.01
Application Number: 12/661,553	Filed: March 19, 2010
For COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION	
Art Unit: 1653	Examiner: Satyendra K. Singh

This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a reply in the above identified application.

The requested extension and fee are as follows (check time period desired and enter the appropriate fee below):

	Fee	Small Entity Fee	
☐ One month (37 CFR 1.17(a)(1))	$150	$75	$
☐ Two months (37 CFR 1.17(a)(2))	$570	$285	$
☒ Three months (37 CFR 1.17(a)(3))	$1290	$645	$ 1290
☐ Four months (37 CFR 1.17(a)(4))	$2010	$1005	$
☐ Five months (37 CFR 1.17(a)(5))	$2730	$1365	$

☐ Applicant claims small entity status. See 37 CFR 1.27.

☐ A check in the amount of the fee is enclosed.

☒ Payment by credit card.

☐ The Director has already been authorized to charge fees in this application to a Deposit Account.

☒ The Director is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number 50-5826.

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

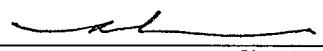
I am the

☐ applicant/inventor.

☐ assignee of record of the entire interest. See 37 CFR 3.71.
Statement under 37 CFR 3.73(b) is enclosed (Form PTO/SB/96).

☐ attorney or agent of record. Registration Number _____

☒ attorney or agent under 37 CFR 1.34.
Registration number if acting under 37 CFR 1.34 33,208



Signature

1/17/2013
Date

Roberta L. Robins
Typed or printed name

(650) 493-3400
Telephone Number

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below.

☒ Total of 1 forms are submitted.

CERTIFICATE OF TRANSMISSION PURSUANT TO 37 CFR § 1.8

I hereby certify pursuant to 37 CFR §1.8 that this correspondence is being transmitted via EFS to the United States Patent and Trademark Office on the date shown below.

Date 1/17/13 Signature

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:	Customer No.: 105379
JOHN FRASER WRIGHT et al.	Confirmation No.: 4726
Application No.: 12/661,553	Art Unit: 1653
Filing Date: March 19, 2010	Examiner: Satyendra K. Singh

Title: COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION

SUBMISSION UNDER 37 CFR 1.114

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Sir:

This Submission under 37 C.F.R. §1.114 is being filed in response to the Final Office Action mailed July 17, 2012. A request for an extension of time, as well as the requisite fee, accompany this Submission. Applicants request reconsideration of the above-referenced patent application in view of the following amendments and remarks.

Amendments to the **specification** begin on page 2 of this paper.

A **listing of claims** begins on page 3 of this paper.

Remarks begin on page 5 of this paper.

I. AMENDMENT

Amendments to the Specification

Please amend the first paragraph of the Specification at page 1 to read as follows:

This application is a continuation of U.S. application serial no. 11/141,996, now U.S. Patent No. 7,704,721, from which application priority is claimed pursuant to 35 U.S.C. §120; which application claims the benefit under 35 U.S.C. § 119(e) of provisional applications 60/575,997 filed June 1, 2004 and 60/639,222 filed December 22, 2004. The foregoing applications are hereby incorporated by reference in their entireties.

Amendments to the Claims:

The following listing reflects amendments to the claims and replaces all prior versions and listings of claims in this application.

1. (Currently amended) A composition for the storage of purified adeno-associated virus (AAV) particles, comprising:

purified (AAV) particles at a concentration exceeding $1x10^{13}$ vg/ml up to $6.4x10^{13}$ vg/ml,;

a pH buffer; and

excipients comprising one or more multivalent ions; wherein the ionic strength of the composition is greater than 200 mM, wherein aggregation of the purified AAV particles in the composition is prevented.

2. (Canceled)

3. (Original) The composition of claim 1, wherein one of the one or more multivalent ions is citrate.

4. (Previously presented) The composition of claim 1, further comprising Pluronic® F68 (ethylene oxide/propylene oxide block copolymer).

5. (Previously presented) The composition of claim 4, wherein the Pluronic® F68 is present at 0.001% (w/v).

6. (Original) The composition of claim 1, wherein the pH buffer is 10 mM Tris, pH 8.0 and the excipients comprise 100 mM sodium citrate.

7. (Previously presented) The composition of claim 1, wherein the purified AAV particles have an average particle radius (*Rh*) of less than about 20nm as measured by dynamic light scattering.

8. (Original) The composition of claim 1, wherein recovery of the purified virus particles is at least about 90% following filtration of the composition of virions through a 0.22μm filter.

9-11. (Canceled)

II. REMARKS

Introductory Comments

Claims 1 and 3-8 were examined in the Office Action under reply and stand variously rejected under (1) 35 U.S.C. §102 (claims 1, 7 and 8); (2) 35 U.S.C. §103(a) (claims 1 and 3-8); and (3) the judicially created doctrine of nonstatutory double patenting (claims 1 and 3-8). These grounds of rejection are believed to be overcome by this response and are otherwise traversed for reasons discussed in detail below.

Applicants note with appreciation the withdrawal of the previous rejections under 35 U.S.C. §112, second paragraph.

Overview of the Above Amendments

Claim 1 has been amended to recite that the concentration of purified rAAV virions in the composition exceeds $1x10^{13}$ vg/ml up to $6.4x10^{13}$ vg/ml. Support for this recitation can be found throughout the specification at, for example, page 6, lines 5-6 and in the examples.

The foregoing amendment is made without prejudice, without intent to abandon any originally claimed subject matter, and without intent to acquiesce in any rejection of record. Applicants expressly reserve the right to file one or more continuing applications containing the unamended claims.

Rejections Under 35 U.S.C. §102

Claims 1, 7 and 8 were rejected under 35 U.S.C. §102(b) over U.S. Patent No. 6,146,874 to Zolotukhin et al. ("Zolotukhin"). The Office argues Zolotukhin teaches a preparation of purified rAAV virions with a pH buffer and excipients comprising one or more multivalent ions, wherein the ionic strength of the composition is greater than 200 mM. Office Action, page 3. However, Applicants respectfully disagree that Zolotukhin anticipates the present claims.

Applicants reiterate the Office has applied Zolotukhin under 35 U.S.C. §102(b) against claims 1, 7 and 8, and then combined Zolotukhin with additional art under 35 U.S.C. §103(a) against the same claims. Implicitly, then, the Examiner appears to be acknowledging that

Zolotukhin does not disclose each and every element in claims 1, 7 and 8 without the benefit of additional art. For this reason alone, the rejection must fail.

Moreover, Applicants submit Zolotukhin does not teach a composition as currently claimed, including purified AAV particles exceeding more than $1x10^{13}$ up to $6.4x10^{13}$ vg/ml, wherein the ionic strength of the composition including such purified particles is greater than 200 mM. Rather, in the passage at column 11, lines 35-40, cited by the Examiner to evidence purified rAAV particles in excipients greater than 200 mM ionic strength, the rAAV is in the process of being purified. The Examiner appears to agree with this assessment in the rejection under 35 U.S.C. §103(a), acknowledging that the disclosure relied on in Zolotukhin relates to "an intermediate composition during the process of preparation of rAAV particles." Office Action, page 9. High salt concentrations as claimed are not used in the Zolotukhin's preparation. On the contrary, as previously explained, Zolotukhin's final, purified AAV product is formulated in Lactated Ringer's buffer (see, column 12, lines 49-54) which according to the formula for ionic strength in the present specification, provides an ionic strength of 138.5 mM, not greater than 200 mM as claimed.

Thus, Zolotukhin fails to disclose all of the claimed elements arranged as in the claim. Withdrawal of this basis for rejection is therefore respectfully requested.

Rejections Under 35 U.S.C. §103(a)

Claims 1 and 3-8 remain rejected under 35 U.S.C. §103(a) as being unpatentable over Zolotukhin, taken with U.S. Patent No. 4,138,287 to Andersson et al. ("Andersson"); U.S. Patent No. 6,194,191 to Zhang et al. ("Zhang"); and Chen et al., *J. Pharm. Sci.* (1994) 83:1657-1661 ("Chen").

The Office applies Zolotukhin as above. Andersson is said to disclose a composition of purified HBsAg particles, a pH buffer and excipients comprising a multivalent ion such as sodium citrate wherein the ionic strength of the composition is greater than about 200 mM. The Examiner correctly notes neither of Zolotukhin or Andersson describes a composition further comprising Pluronic (claims 4 and 5) or a composition wherein the pH buffer is 10 mM Tris, pH 8.0 and wherein the excipients comprise 100 mM sodium citrate (claim 6). Office Action, page 6. Zhang is said to disclose the use of Pluronic® F68 for producing adenoviral particles. Office

Action, page 7. Chen allegedly discloses strategies to suppress aggregation of keratinocyte growth factor (KGF) in liquid formulations by adding sulfated polysaccharides and citrate salts. Office Action, page 7. Finally, the limitations of claims 7 and 8 are said to be an intrinsic characteristic of the purified viral compositions. However, Applicants submit the Office has failed to establish a *prima facie* case of obviousness.

The Office disputes Applicants' previous arguments alleging "the instant claims are directed to product composition that has been fully disclosed in the cited art of record, ***albeit* as an intermediate composition during the process of preparation of rAAV particles**." Office Action, page 9, emphasis added. The Examiner argues the term "purified" is not specifically defined by Applicants as being tied to any particular step and/or degree of purification of the AAV particles in the composition. Office Action, pages 9-10, bridging paragraph. The Office concludes the combined teachings in the cited prior art references of record disclose and/or fully suggest all the claimed elements, including the recognition of the problem of self aggregation of rAAV virions associated with purified preparations of the virions. However, Applicants submit that all elements of the claimed invention are not taught or suggested by the cited combination.

To reiterate, all pending claims pertain to compositions for storing purified rAAV particles such that aggregation of rAAV particles in the composition is prevented. The concentration of purified rAAV particles in the preparation exceeds $1x10^{13}$ up to $6.4x10^{13}$ vg/ml. One or more excipients is also present in the composition such that an ionic strength of at least 200 mM is achieved. None of the cited art, either alone or in combination, teaches or suggests such a composition.

First, all claims now define the purity level of the rAAV particles in the composition. Thus, it is clear that highly purified rAAV particles are present in the claimed composition which has an ionic strength exceeding 200 mM. As the Examiner has expressly acknowledged, the passages in Zolotukhin relied upon to evidence the teaching of excipients greater than 200 mM ionic strength, relate to intermediate steps where the rAAV is in the process of being purified. See, for example, Figure 1 where all of the steps using such buffers are purification steps. High salt concentrations are not used in Zolotukhin's final preparation. Rather, the final AAV product is formulated in Lactated Ringer's buffer. See, column 12, lines 49-54, where virus is concentrated by centrifugation through a BIOMAX 100K filter and desalted into Lactated

Ringer's. Only after this step is the virus considered "purified" in Zolotukhin's disclosure and, as explained above, Lactated Ringer's has an ionic strength of 138.5 mM. Applicants' purified product, on the other hand, has an ionic strength of at least 200 mM. There is absolutely no suggestion in Zolotukhin or any of the other cited references that a final preparation with an ionic strength of at least 200 mM and more than $1x10^{13}$ up to $6.4x10^{13}$ vg/ml is desirable or necessary.

Moreover, contrary to the Office's assertions, Zolotukhin does not recognize that rAAV virions **self-aggregate** and therefore does not provide any suggestions regarding a composition as claimed. Rather, Zolotukhin recognizes AAV aggregates with **proteins** in the cell lysate and uses high salt in the **first step** of the iodixanol gradient to destabilize AAV-lysate protein interactions. High salt concentrations are purposefully eliminated from the remainder of the iodixanol gradient, including the gradient from which the rAAV is collected after centrifugation, because the elimination of high salt is important for subsequent purification steps. See, column 15, lines 22-35 of Zolotukhin. Accordingly, this passage expressly teaches away from Applicants' invention. Absent Applicants' teaching regarding this problem, there is no recognition that rAAV virion self-aggregation is a concern that may be addressed by manipulating ionic strength in any of the cited art.

The secondary references do not fill the gaps present in Zolotukhin. Applicants reiterate the present claims are directed to compositions of purified **rAAV** particles with an ionic strength of at least 200 mM and more than $1x10^{13}$ up to $6.4x10^{13}$ vg/ml. None of Andersson, Zhang or Chen even relates to AAV and hence would not suggest a composition with the particular purity and ionic strength as claimed. Andersson is directed to methods for isolating Hepatitis B virus (HBV), a virus completely unrelated to AAV. Zhang relates to methods for producing adenoviral vectors. Adenovirus, like HBV, is unrelated to AAV. A skilled artisan in the AAV field would not look to art pertaining to unrelated viruses in order to determine proper conditions to prevent aggregation at the recited purity levels.

As for Chen, this reference does not relate to viruses, but rather pertains to methods for preventing aggregation of keratinocyte growth factor (KGF). There is absolutely no reason to believe that art directed to growth factors is in any way pertinent to virion production. Moreover, Chen used sulfated polysaccharides in combination with citrate to prevent aggregation. There is no suggestion in Chen to use citrate alone, or to provide a composition as

claimed in order to prevent aggregation of rAAV virions, wherein the composition includes more than $1x10^{13}$ up to $6.4x10^{13}$ vg/ml of rAAV virions.

With respect to the Office's assertion regarding claims 7 and 8, namely, that the average particle radius and percent recovery are inherent characteristics, Applicants reiterate inherency is not a proper standard on which to base an obviousness rejection. It is axiomatic that a retrospective view of inherency is not a substitute for some teaching or suggestion to arrive at the claimed invention. That which may be inherent is not necessarily known, and obviousness **cannot** be predicated on the unknown. See, e.g., *In re Newell*, 13 USPQ2d 1248 (Fed. Cir. 1989).

Applicants' composition provides a commercially viable solution for providing high amounts of rAAV particles in stored compositions. As explained at page 20, second paragraph of the specification and in Table 3, rAAV particles prepared and stored in elevated ionic strength solutions remain soluble and can be stored for an extended period.

Applicants continue to submit the Examiner has chosen bits and pieces of the cited references to arrive at the allegation that this combination of references suggests the claimed invention. It is axiomatic that statements in the prior art must be considered in the context of the teaching of the entire reference, and that rejection of claims **cannot** be predicated on mere identification in a reference of individual components of claimed limitations. In this regard, the Federal Circuit has consistently reversed a finding of obviousness, even when all claimed elements are individually present in the references. *See, e.g., In re Kotzab*, 55 USPQ2d 1313, 1317 (CAFC 2000, emphasis added):

> While the test for establishing an implicit teaching, motivation or suggestion is what the combination of these two statements [in the reference] would have suggested to those of ordinary skill in the art, the two statements cannot be viewed in the abstract. Rather, they must be considered in the context of the teaching of the entire reference. Further, a rejection **cannot** be predicated on the mere identification [in the reference] of individual components of claimed limitations. Rather, particular findings must be made as to the reason the skilled artisan, with no knowledge of the claimed invention, would have selected these components for combination in the manner claimed.

Virtually all inventions are combinations of elements that can be individually identified in multiple references. See, e.g., *In re Rouffet*, 47 USPQ2d 1453 (Fed. Cir. 1998), noting that the

Office cannot rely on a high level of skill in the art to overcome the differences between the selected elements in the references, it cannot rely on a high level of skill in the art to provide the necessary motivation.

As explained in Section 2143.01 of the MPEP, the mere fact that references can be combined or modified does not render the resultant combination obvious, unless the prior art also suggests the desirability of the combination. Since the suggestion or motivation to combine the references to arrive at the claimed invention is not in the references, the Examiner is required to cite to some knowledge generally available to one of ordinary skill in the art for the motivation to combine the references. It is respectfully submitted that the Examiner has not provided such knowledge. Instead, the Examiner has merely asserted that it would have been obvious to combine the various methods of the cited art to arrive at Applicants' specifically claimed composition that prevents aggregation of rAAV virions in a purified preparation.

For at least these reasons, withdrawal of the rejections under 35 U.S.C. § 103(a) is respectfully requested.

The Double Patenting Rejection

Claims 1 and 3-8 remain rejected under the judicially created doctrine of nonstatutory obviousness-type double patenting over claim 1 of U.S. Patent No. 7,704,721. Applicants respectfully traverse.

Obviousness-type double patenting requires that the claimed subject matter is **not patentably distinct** from the subject matter in the cited patent. As previously explained, the Examiner considered the method and composition claims patentably distinct in a Restriction Requirement in USSN 11/941,996 which ultimately matured into the '721 patent. In particular, the Office required Applicants to elect, *inter alia*, between methods of preventing aggregation of virions (Group I) and compositions for the storage of purified virus particles (Group II). The composition claims included in Group II were identical to claims 1-8 as filed in the present application. Additionally, the Patent Office required restriction in the present application between the currently elected composition claims and methods of preventing aggregation of virions.

The restriction requirements quoted above clearly set forth the subject matter and the claims that the PTO considered patentably distinct. Accordingly, the present double patenting rejection is in direct contradiction to the Examiner's previous position regarding the claims.

Moreover, Applicants dispute the Examiner's argument that the composition as claimed is co-extensive in scope with the methods issued in the '721 patent. The '721 patent claims are directed to methods of preventing aggregation of rAAV virions which include specific steps in addition to the use of multivalent ions, such as (1) providing a lysate comprising rAAV virions; and (2) purifying rAAV virions from the lysate using ultracentrifugation and/or chromatography. The present composition claims require no such steps.

Based on the foregoing, Applicants submit the claims in the '721 patent and those in the present application are patentably distinct. Thus, the double patenting rejection is believed to be in error and withdrawal thereof is respectfully requested.

III. CONCLUSION

Applicants respectfully submit that the claims are now in condition for allowance and request early notification to that effect. The Examiner is encouraged to contact the undersigned if the Examiner notes any further matters which might be resolved by a telephone interview.

Respectfully submitted,

Date: 1/17/2013

By: _____
Roberta L. Robins
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Electronic Patent Application Fee Transmittal

Application Number:	12661553
Filing Date:	19-Mar-2010
Title of Invention:	Compositions and methods to prevent AAV vector aggregation
First Named Inventor/Applicant Name:	John Fraser Wright
Filer:	Roberta L. Robins/Denise Vaillancourt
Attorney Docket Number:	0800-0045.01

Filed as Large Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Extension - 3 months with $0 paid	1253	1	1290	1290

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
			Total in USD ($)	**1290**

Electronic Acknowledgement Receipt

EFS ID:	14731105
Application Number:	12661553
International Application Number:	
Confirmation Number:	4726
Title of Invention:	Compositions and methods to prevent AAV vector aggregation
First Named Inventor/Applicant Name:	John Fraser Wright
Customer Number:	20855
Filer:	Roberta L. Robins/Denise Vaillancourt
Filer Authorized By:	Roberta L. Robins
Attorney Docket Number:	0800-0045.01
Receipt Date:	17-JAN-2013
Filing Date:	19-MAR-2010
Time Stamp:	17:58:47
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Credit Card
Payment was successfully received in RAM	$1290
RAM confirmation Number	5467
Deposit Account	
Authorized User	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)

1		roa.pdf	574079 78619bc8578a81703bb767fa6e0c810d5a418d4b	yes	13

Multipart Description/PDF files in .zip description		
Document Description	**Start**	**End**
Request for Continued Examination (RCE)	1	1
Extension of Time	2	2
Applicant Arguments/Remarks Made in an Amendment	3	13

Warnings:

Information:

2	Fee Worksheet (SB06)	fee-info.pdf	30322 c9c20b4eba97eea004ed23c6f09c2684c11a96e9	no	2

Warnings:

Information:

Total Files Size (in bytes):	604401

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/SB/06 (07-06)
Approved for use through 1/31/2007. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number	Filing Date	☐ To be Mailed
12/661,553	03/19/2010	

APPLICATION AS FILED – PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY ☐ RATE ($)	SMALL ENTITY ☐ FEE ($)	OR	OTHER THAN SMALL ENTITY RATE ($)	OTHER THAN SMALL ENTITY FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =		OR	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =			X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	

APPLICATION AS AMENDED – PART II

AMENDMENT 01/17/2013	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE ($)	SMALL ENTITY ADDITIONAL FEE ($)	OR	OTHER THAN SMALL ENTITY RATE ($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	* 7	Minus	** 20	= 0	X $ =		OR	X $62=	0
Independent (37 CFR 1.16(h))	* 1	Minus	***3	= 0	X $ =		OR	X $250=	0
☐ Application Size Fee (37 CFR 1.16(s))									
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
					TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	0

AMENDMENT	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)		RATE ($)	ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =		OR	X $ =	
Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =		OR	X $ =	
☐ Application Size Fee (37 CFR 1.16(s))									
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
					TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".

*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".

The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

Legal Instrument Examiner:
/FLORENCE PATTERSON/

Document code: WFEE

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/661,553	03/19/2010	John Fraser Wright	0800-0045.01	4726

20855 7590 08/15/2013

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EXAMINER	
SINGH, SATYENDRA K	

ART UNIT	PAPER NUMBER
1657	

MAIL DATE	DELIVERY MODE
08/15/2013	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No. 12/661,553	Applicant(s) WRIGHT ET AL.	
	Examiner SATYENDRA SINGH	Art Unit 1657	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *17 January 2013*.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

5)☒ Claim(s) *1 and 3-8* is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *1 and 3-8* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some * c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)

2) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

4) ☐ Other: _____.

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on **01/17/2013** has been entered.

Claims 2, and 9-11 (invention of group II) were previously canceled by applicants.

Claims 1 and 3-8 (elected invention of group I), as currently amended, have been examined on their merits in this office action.

Objection to Specification-Withdrawn

In view of current amendments to page 1 first paragraph of the specification, the objection as previously made by the examiner has been withdrawn.

Claim Rejections - 35 USC § 102-Withdrawn

In view of current amendments to claim 1, the rejection of **Claims 1, 7 and 8** under 35 U.S.C. 102(b) as being anticipated by **Zolotukhin et al** (2000; US 6,146,874), as previously made by the examiner has been withdrawn.

Claim Rejections - 35 USC § 103

The following is a quotation of pre-AIA 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under pre-AIA 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.

2. Ascertaining the differences between the prior art and the claims at issue.

3. Resolving the level of ordinary skill in the pertinent art.

4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

This application currently names **joint inventors**. In considering patentability of the claims under pre-AIA 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of pre-AIA 35 U.S.C. 103(c) and potential pre-AIA 35 U.S.C. 102(e), (f) or (g) prior art under pre-AIA 35 U.S.C. 103(a).

1. **Claims 1 and 3-8** (as currently amended) **are/remain** rejected under 35 U.S.C. 103(a) as being unpatentable over Zolotukhin et al (2000; US 6,146,874) taken with Andersson et al (1979; US 4,138,287), Zhang et al (2001; US 6,194,191) and Chen et al (1994).

Claims (as amended) are directed to "a composition (for the storage) of purified adeno-associated virus (AAV) particles, comprising: purified (AAV) particles at a concentration exceeding 1 x 10^{13} vg/ml up to 6.4x 10^{13} vg/ml; a pH buffer; and excipients comprising one or

more multivalent ions; wherein the ionic strength of the composition is greater than 200 mM, wherein aggregation of the purified AAV particles in the composition is prevented (instant claim 1); wherein one of the one or more multivalent ions is citrate (claim 3); further comprising Pluronic® F68 (ethylene oxide/propylene oxide block copolymer) at 0.001% (w/v) (claims 4-5); wherein the pH buffer is 10 mM Tris, pH 8.0 and the excipients comprise 100 mM sodium citrate (claim 6); wherein the purified AAV particles have an average particle radius (*Rh*) of less than about 20 nm as measured by dynamic light scattering (instant claim 7); and wherein recovery of the purified virus particles is at least about 90% following filtration of the composition of virions through a 0.22 μm filter." (claim 8).

Zolotukhin et al (2000) disclose the composition comprising: purified recombinant AAV virus particles; a pH buffer such as **phosphate buffered-saline with magnesium chloride and potassium chloride** (PBS-MK); and excipients comprising one or more multivalent ions such as phosphate and magnesium; wherein the ionic strength of the composition is greater than 200 mM (see Zolotukhin et al, column 11, 4th paragraph, lines 35-40, in particular), wherein they elute the purified rAAV particles in **PBS-MK buffer having 1M NaCl**, i.e. elution buffer; and wherein they disclose the fact that highly purified stocks having titers up to **about 10^{13} particles/ml** are obtained using their purification steps within 24 hours or less (see abstract, and column 16, 1st paragraph, in particular), and in fact has the potential to produce **10^{14} virus particles**. In addition Zolotukhin et al also disclose the problems facing the purification of high titer AAV particles specifically related to the **problem of aggregation**, which was alleviated and/or reduced significantly by the use of high concentrations of salts (i.e. high ionic strength buffer

such as 1M NaCl in PBS-MK; see column 3, last paragraph; column 15, 3rd paragraph, for examples) during purification, wherein the excess salt can be later removed, if required for downstream applications.

However, the composition having AAV particles at a concentration exceeding 1 x 10^{13} vg/ml up to 6.4x 10^{13} vg/ml (see instant claim 1), further comprising **Pluronic® F68** at 0.001% w/v (claims 4-5); and wherein the pH buffer is **10 mM Tris, pH 8.0** and the excipients comprise **100 mM sodium citrate** (claim 6), is not explicitly exemplified (although the number of virus particles potentially purified have been disclosed to be in the vicinity of **about 10^{13} and about 10^{14}**; see Zolotukhin et al above) and/or disclosed by the inventions of Zolotukhin et al.

Andersson et al (1979) disclose the composition comprising purified hepatitis virus (HB_sAg) particles (see abstract, in particular), a pH buffer such as Tris-sodium citrate buffer, pH 7.5, and excipients comprising a multivalent ion such as **sodium citrate**, wherein the ionic strength of the composition is greater than about 200 mM (see elution of purified virus from column using 0.5 M NaCl in Tris-citrate buffer; see Andersson et al, example 2 and claim 5, in particular).

Zhang et al (2001) disclose the use of a surfactant such as **Pluronic® F68** (0.1% in growth medium for adenovirus infection and viral production, etc.; see column 4, 2nd paragraph and columns 53-54, in particular) for production of adenoviral particles in serum-free suspension cultures using spinner flasks, and also use in the cryopreservation media (see column 53, last paragraph).

Chen et al (1994) disclose strategies to suppress aggregation of proteins (such as recombinant keratinocyte growth factor) during liquid formulation development (see Chen et al,

abstract, table 1-2, in particular) by adding sulfated polysaccharides (such as heparin) and **citrate salts** (such as **0.1 to 0.5 M sodium citrate**; see Chen et al, page 1661, left column, in particular) that were found to be effective in preventing protein aggregation. Chen et al also disclose the fact that other negatively charged small ions such as phosphate (a multivalent ion) also have moderate stabilizing effects **on preventing aggregation** of recombinant keratinocyte growth factor (rhKGF).

Thus, given the disclosure in the cited prior art, a person of ordinary skill in the art would have modified the composition comprising purified AAV virus particles taught by Zolotukhin et al taken such that said composition additionally comprise a surfactant (to help prevent aggregation during freezing-thawing cycles, for example) such as Pluronic® F68, as explicitly taught by Zhang et al, and a suitable amount of sodium citrate as a multivalent ion in Tris buffer solution (at a suitable pH; as specifically disclosed by Andersson et al) in order to stabilize the viral particles as suggested by Chen et al (for suppressing/reducing aggregation of fairly unstable proteins such as rhKGF; see discussion above, thus providing a conceptual basis for including multivalent ions in the buffer containing purified AAV particles), in addition to other stabilizing components such as a surfactant. Since, the benefits of including a surfactant and high ionic strength multivalent ions have been disclosed in the cited prior art of Zhang et al and Chen et al, an artisan of ordinary skill in the art would have established suitable concentrations required to help stabilize viral preparations (i.e. by preventing aggregation, etc.) with a reasonable expectation of success, especially given the disclosure from Zolotukhin et al, for example, for the use of high salt concentrations for reducing the potential aggregation of virus particles (see discussion of Zolotukhin et al, above). Such modification in the use of suitable concentrations of

the multivalent ions (such as sodium citrate) for reducing aggregation in place of high salt concentrations (i.e. 1 M NaCl) used by Zolotukhin et al would have been therefore obvious and fully contemplate by an artisan of ordinary skill in the art, given the combined disclosure provided by the cited references of Andersson et al, Zhang et al and Chen et al, as discussed above.

The limitations of claims 7 and 8 (average particle radius and percent recovery following filtration, etc.) are also met by the prior art as these are taken to be intrinsic characteristic features of the purified viral compositions as disclosed by Zolotukhin et al when taken with the teachings of Andersson et al, Zhang et al and Chen et al. Since, all the components of the product, as recited in the claims are the same as disclosed and/or suggested in the cited prior art, these features will necessarily follow from the composition disclosed in the art, as they do not structurally change the composition as claimed.

Thus, the invention as a whole would have been *prima facie* obvious to a person of ordinary skill in the art at the time the claimed invention was made.

As per MPEP 2144.06, "*It is prima facie obvious to combine two compositions each of which is taught by the prior art to be useful for the same purpose, in order to form a third composition to be used for the very same purpose.... [T]he idea of combining them flows logically from their having been individually taught in the prior art." In re Kerkhoven, 626 F.2d 846, 850, 205 USPQ 1069, 1072 (CCPA 1980).*

As per MPEP 2144.06, *In order to rely on equivalence as a rationale supporting an obviousness rejection, the equivalency must be recognized in the prior art, and cannot be based on applicant's disclosure or the mere fact that the components at issue are functional or mechanical equivalents. In re Ruff, 256 F.2d 590, 118 USPQ 340 (CCPA 1958).*

Response to Applicant's Arguments

Applicant's arguments filed **01/17/2013** (as they pertain to the prior art rejection of record) have been fully considered but they are not persuasive for the following reasons of record:

Regarding the 103a rejection of record, applicants argue that "*…all pending claims pertain to compositions for storing purified rAAV particles such that aggregation of rAAV particles in the composition is prevented. The concentration of purified rAAV particles in the preparation exceeds 1 x 10^{13} up to 6.4x 10^{13} vg/ml. One or more excipients is also present in the composition such that an ionic strength of at least 200 mM is achieved. None of the cited art, either alone or in combination, teaches or suggests such a composition*" (see remarks, page 7), which is noted and fully considered. However, as discussed in the obviousness rejection above, the cited prior art of Zolotukhin et al disclose the range of viral particles that can be purified up to or greater than 10^{14} vp/ml (see column 6, 1st paragraph,; column 16, 1st paragraph, in particular), and therefore, when taken with the disclosure from Andersson et al, Zhang et al and Chen et al for the benefits of using suitable concentrations of a multivalent ions such as sodium citrate, a person of ordinary skill in the art would have fully contemplated the modification in the buffer composition disclosed by Zolotukhin et al such that it uses multivalent ions as stabilizing buffer excipient in order to prevent viral aggregation, as already intended by the disclosure of Zolotukhin et al and supported by Zhang et al and Chen et al, as discussed above.

The argument that "*…Zhang relates to methods for producing adenoviral vectors. Adenovirus, like HBV, is unrelated to AAV. A skilled artisan in the AAV field would not look to art pertaining to unrelated viruses in order to determine proper conditions to prevent*

aggregation at the recited purity levels", is noted and considered, but is not found to be persuasive because the problems of viral particle aggregation has been well known and/or recognized in the art (see disclosure of Zolotukhin et al, as discussed above), specifically as suggested by the disclosure of Chen et al (when taken in combination with Andersson et al and Zhang et al) that salts of multivalent ions such as citrate were found to be effective in preventing protein aggregation. They also disclose the fact that other negatively charged small ions such as phosphate (also a multivalent ion) also have moderate stabilizing effect on preventing aggregation of recombinant keratinocyte growth factor. Since, AAV virions are also encapsulated in capsid proteins, an artisan of ordinary skill in the art, at the time this invention was made, would have been motivated to use multivalent ions such as citrate, phosphate, etc. (in place of very high salt concentrations as used by Zolotukhin et al that are generally removed before clinical applications; with or without suitable surfactants such as Pluronic® F68) in order to stabilize the purified preparation of AAV virions, with a reasonable expectation of success. In addition, applicants have not provided evidence/data on the record that demonstrates that such modification would not have been feasible or applicable to the viral formulation and/or buffer system used by Zolotukhin et al when taken with the disclosure of Andersson et al, Zhang et al and Chen et al, as discussed in the 103a rejection, above. Therefore, the argument that "*.. As for Chen, this reference does not relate to viruses, but rather pertains to methods for preventing aggregation of keratinocyte growth factor (KGF). There is absolutely no reason to believe that art directed to growth factors is in any way pertinent to virion production…*" (see remarks, page 8), is fully considered, but is not found to be persuasive for the above discussed reasons of record. The argument regarding the intrinsic features as recited in claims 7 and 8 (i.e. the

average particle radius and recovery following filtration) are also not found to be persuasive because such features are taken to be intrinsic in the composition disclosed by the cited art of record, as the use of suitable concentration of multivalent ions in the buffer reduce and/or prevent viral aggregation in order to provide said features as currently recited in the claims of record.

Thus, the 103(a) rejection of record is properly made and maintained.

Double Patenting- Withdrawn

In view of applicant's remarks (see remarks, dated 01/17/2013, pages 10-11) regarding the ODP rejection over US 7,704,721 B2 (issued to Wright et al on April 27, 2010) as previously made by the examiner, the rejection of record has been fully withdrawn.

Conclusion

NO claims are currently allowed.

Pertinent art:

1. **Evans et al.** (published on 08/26/2004; **US 2004/0166122 A1**; cited as ref. [A] on PTO 892 form) - Adenovirus formulations (disclose stable viral formulations for gene therapy and other clinical applications generally comprising up to about $1x10^{13}$ viral particles/ml in a suitable buffer, a sugar, a salt, a divalent cation, a non-ionic detergent as well as sodium citrate, wherein the typical formulations comprise 5 mM Tris buffer, pH 7.5, 250 mM NaCl, sucrose to provide suitable osmolarity in the range of 200-800 mOsm/L, $MgCl_2$ in the range of 0.1mM to about 10 mM, 0.001% to about 2% of polysorbate-80 as surfactant, and sodium citrate at about 10 mM; see page 8, example 1, in particular; paragraphs [0051], [0056], [0060], [0079], entire disclosure at pages 5-6, in particular and claims).

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SATYENDRA SINGH whose telephone number is (571)272-8790. The examiner can normally be reached on 9-5MF.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, JON P. WEBER can be reached on 571-272-0925. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Satyendra K. Singh/
Examiner, Art Unit 1657

Notice of References Cited	Application/Control No. 12/661,553	Applicant(s)/Patent Under Reexamination WRIGHT ET AL.	
	Examiner SATYENDRA SINGH	Art Unit 1657	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
*	A	US-2004/0166122	08-2004	Evans et al.	424/204.1
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
S49	6	benzonase.clm.	USPAT	OR	OFF	2012/07/11 16:27
S50	2051	WRIGHT near3 JOHN	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2013/08/10 11:28
S51	23	QU near3 GUANG	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2013/08/10 11:28
S52	2059	S50 or S51	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2013/08/10 11:28
S53	8	S52 and ((virion or vira$6 or virus or adenovir$6 or AAV) same (aggreg$6 or precipit$6 or agglutin$6 or stabiliz$6)) and (multivalent or divalent)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2013/08/10 11:28
S54	37	((AAV or adenovir$3) near3 (particle or virion)) same ((citr$6 or magnes$6 or phosphat$6 or mangan$6 or sulfate) same buffer)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2013/08/10 11:31
S55	6	S54 and (number near3 particl$3)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2013/08/10 11:32
S56	822	(adenovir$6 or AAV) same (number near3 particl$3)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2013/08/10 11:35
S57	67	(adenovir$6 or AAV) same (number near3 particl$3) same (buffer or citric or citrate)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2013/08/10 11:35
S58	0	(adenovir$6 or AAV) same (number near3 particl$3) same (buffer or citric or citrate).clm.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2013/08/10 11:36
S59	55	S57 and (storage or stabiliz$6)	US-PGPUB; USPAT; USOCR;	OR	OFF	2013/08/10 11:37

			FPRS; EPO; JPO; DERWENT; IBM_TDB			
S60	1	S57 and (storage or stabiliz$6) and (ionic near3 (concentrat$6 or strength))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2013/08/10 11:38
S61	152	(stock near3 solution) same (adenovir$6 or AAV)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2013/08/10 11:44
S62	3	(stock near3 solution) same (adenovir$6 or AAV) same (particle or virion)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2013/08/10 11:44
S63	1	("6,146,874").PN.	USPAT; USOCR	OR	OFF	2013/08/11 13:59
S64	983	(adenovir$6 or AAV or adeno-associated) same (purif$3 or stock or storage) same (titre or number)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2013/08/11 17:21
S65	6	(adenovir$6 or AAV or adeno-associated) same (purif$3 or stock or storage) same (titre or number).clm.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2013/08/11 17:21
S66	2	(adenovir$6 or AAV or adeno-associated) same (purif$3 or stock or storage) same (titre or number) same (citric or citrate)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2013/08/11 17:23
S67	2	("20040166122").PN.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2013/08/11 17:44

EAST Search History (Interference)

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8/ 12/ 2013 11:13:32 AM
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Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	12661553	WRIGHT ET AL.
	Examiner	Art Unit
	SATYENDRA SINGH	1657

CPC- SEARCHED		
Symbol	Date	Examiner

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES		
Search Notes	Date	Examiner
EAST: USPAT, USOCR, US-PGPUB, JPO, EPO, DERWENT- ATTACHED	11/6/2011	SKS
INVENTOR SEARCH: PALM, EDAN AND EAST-	11/6/2011	SKS
EAST: USPAT, USOCR, US-PGPUB, JPO, EPO, DERWENT- UPDATED & ATTACHED	7/11/2012	SKS
INVENTOR SEARCH: PAL, EDAN AND EAST- UPDATED	7/11/2012	SKS
EAST: USPAT, USOCR, US-PGPUB, JPO, EPO, DERWENT- UPDATED & ATTACHED	8/12/2013	SKS
INVENTOR SEARCH: PALM, EDAN AND EAST- UPDATED	8/12/2013	SKS

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner

/SATYENDRA SINGH/ Examiner.Art Unit 1657	

TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Total Number of Pages in This Submission	10

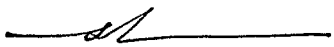
Application Number	12/661,553
Filing Date	March 19, 2010
First Named Inventor	John Frasier Wright et al.
Art Unit	1653
Examiner Name	Satyendra K. Singh
Attorney Docket Number	0800-0045.01

ENCLOSURES *(Check all that apply)*

- ☐ Fee Transmittal Form
  - ☐ Fee Attached
- ☒ Amendment/Reply (8 pgs)
  - ☐ After Final
  - ☐ Affidavits/declaration(s)
- ☒ Extension of Time Request (1 pg)
- ☐ Express Abandonment Request
- ☐ Information Disclosure Statement
- ☐ Certified Copy of Priority Document(s)
- ☐ Reply to Missing Parts/ Incomplete Application
  - ☐ Reply to Missing Parts under 37 CFR 1.52 or 1.53
- ☐ Drawing(s)
- ☐ Licensing-related Papers
- ☐ Petition
- ☐ Petition to Convert to a Provisional Application
- ☐ Power of Attorney, Revocation Change of Correspondence Address
- ☐ Terminal Disclaimer
- ☐ Request for Refund
- ☐ CD, Number of CD(s) _____
  - ☐ Landscape Table on CD
- ☐ After Allowance Communication to TC
- ☐ Appeal Communication to Board of Appeals and Interferences
- ☐ Appeal Communication to TC **(Appeal Notice, Brief, Reply Brief)**
- ☐ Proprietary Information
- ☐ Status Letter
- ☐ Other Enclosure(s) (please identify below):

Remarks **The Commissioner is authorized to charge any additional fees to Deposit Account** 50-5826.

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	Robins Law Group		
Signature			
Printed name	Roberta L. Robins		
Date	Feb. 18, 2014	Reg. No.	33,208

CERTIFICATE OF TRANSMISSION/MAILING

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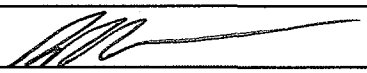
Signature			
Typed or printed name	Denise M. Vaillancourt	Date	2/18/14

PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a) **FY 2014** *(Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).)*	Docket Number (Optional) 0800-0045.01
Application Number: 12/661,553	Filed: March 19, 2010
For COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION	
Art Unit: 1653	Examiner: Satyendra K. Singh

This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a reply in the above identified application.

The requested extension and fee are as follows (check time period desired and enter the appropriate fee below):

	Fee	Small Entity Fee	
☐ One month (37 CFR 1.17(a)(1))	$200	$100	$
☐ Two months (37 CFR 1.17(a)(2))	$600	$300	$
☒ Three months (37 CFR 1.17(a)(3))	$1400	$700	$ 1400
☐ Four months (37 CFR 1.17(a)(4))	$2200	$1100	$
☐ Five months (37 CFR 1.17(a)(5))	$3000	$1500	$

☐ Applicant claims small entity status. See 37 CFR 1.27.

☐ A check in the amount of the fee is enclosed.

☒ Payment by credit card.

☐ The Director has already been authorized to charge fees in this application to a Deposit Account.

☒ The Director is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number 50-5826.

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

I am the

☐ applicant/inventor.

☐ assignee of record of the entire interest. See 37 CFR 3.71.
Statement under 37 CFR 3.73(b) is enclosed (Form PTO/SB/96).

☐ attorney or agent of record. Registration Number _____

☒ attorney or agent under 37 CFR 1.34.
Registration number if acting under 37 CFR 1.34 33,208

Signature	FEB. 18, 2014 Date
Roberta L. Robins Typed or printed name	(650) 493-3400 Telephone Number

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below.

☒ Total of 1 forms are submitted.

CERTIFICATE OF TRANSMISSION PURSUANT TO 37 CFR § 1.8

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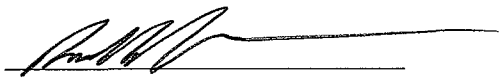
Date 2/18/14 Signature 

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:	Customer No.: 105379
JOHN FRASER WRIGHT et al.	Confirmation No.: 4726
Application No.: 12/661,553	Art Unit: 1653
Filing Date: March 19, 2010	Examiner: Satyendra K. Singh

Title: COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION

AMENDMENT UNDER 37 CFR 1.111

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Sir:

This paper is responsive to the Office Action mailed August 15, 2013, with a shortened statutory period of three months for response. Accordingly, a request for an extension of time, as well as the requisite fee, accompany this response. Applicants request reconsideration of the above-referenced patent application in view of the following amendments and remarks.

A **listing of claims** begins on page 2 of this paper.

Remarks begin on page 4 of this paper.

I. AMENDMENT

Amendments to the Claims:

The following listing reflects amendments to the claims and replaces all prior versions and listings of claims in this application.

1. (Currently amended) A composition for the storage of purified adeno-associated virus (AAV) particles, comprising:

purified (AAV) particles at a concentration exceeding $1x10^{13}$ vg/ml up to $6.4x10^{13}$ vg/ml[[,]];

a pH buffer, wherein the pH of the composition is between 7.5 and 8.0; and

excipients comprising one or more multivalent ions; wherein the ionic strength of the composition is greater than 200 mM, wherein aggregation of the purified AAV particles in the composition is prevented.

2. (Canceled)

3. (Original) The composition of claim 1, wherein one of the one or more multivalent ions is citrate.

4. (Previously presented) The composition of claim 1, further comprising Pluronic® F68 (ethylene oxide/propylene oxide block copolymer).

5. (Previously presented) The composition of claim 4, wherein the Pluronic® F68 is present at 0.001% (w/v).

6. (Original) The composition of claim 1, wherein the pH buffer is 10 mM Tris, pH 8.0 and the excipients comprise 100 mM sodium citrate.

7. (Previously presented) The composition of claim 1, wherein the purified AAV particles have an average particle radius (*Rh*) of less than about 20nm as measured by dynamic light scattering.

8. (Original) The composition of claim 1, wherein recovery of the purified virus particles is at least about 90% following filtration of the composition of virions through a 0.22μm filter.

9-11. (Canceled)

II. REMARKS

Introductory Comments

Claims 1 and 3-8 were examined in the Office Action under reply and stand rejected solely under 35 U.S.C. §103(a). This ground of rejection is believed to be overcome by this response and is otherwise traversed for reasons discussed in detail below.

Applicants note with appreciation the withdrawal of the previous rejections under 35 U.S.C. §102 and under the judicially created doctrine of nonstatutory double patenting.

Overview of the Above Amendments

Claim 1 has been amended to eliminate a typographical error and to recite that the pH of the composition is between 7.5 and 8.0. Support for this recitation can be found throughout the specification at, e.g., in the examples.

The foregoing amendment is made without prejudice, without intent to abandon any originally claimed subject matter, and without intent to acquiesce in any rejection of record. Applicants expressly reserve the right to file one or more continuing applications containing the unamended claims.

Rejection Under 35 U.S.C. §103(a)

Claims 1 and 3-8 remain rejected under 35 U.S.C. §103(a) as being unpatentable over U.S. Patent No. 6,146,874 to Zolotukhin et al. ("Zolotukhin"), taken with U.S. Patent No. 4,138,287 to Andersson et al. ("Andersson"); U.S. Patent No. 6,194,191 to Zhang et al. ("Zhang"); and Chen et al., *J. Pharm. Sci.* (1994) 83:1657-1661 ("Chen").

The Office reiterates the previous rejection and disputes Applicants' arguments, asserting the primary reference, Zolotukhin, discloses that viral particles can be purified up to or greater than 10^{14} vp/ml and Andersson et al., Zhang et al. and Chen et al. disclose the benefits of using suitable concentrations of multivalent ions as stabilizing buffer excipients in order to prevent viral aggregation. Office Action, page 6. The Examiner also dismisses Applicants' arguments regarding the additional references as directed to non-relevant art, alleging:

> Since AAV virions are also encapsulated in capsid proteins, an artisan of ordinary skill in the art, at the time this invention was made, would have been motivated to use multivalent ions such as citrate, phosphate, etc. (in place of very high salt concentrations as used by Zolotukhin et al that are generally removed before clinical applications; with or without suitable surfactants such as Pluronic® F68) in order to stabilize the purified preparation of AAV virions, with a reasonable expectation of success.

Office Action, page 9. However, Applicants continue to submit the Office has failed to establish a *prima facie* case of obviousness.

First, Applicants do not agree that self-aggregation of AAV virions would necessarily be prevented using the same mechanisms as used for other non-related viruses and proteins. Applicants direct the Examiner's attention to Qu et al., *Molec. Ther.* (2003) 5:S348, Abstract 901 ("Qu") of record herein and which was successfully overcome in the parent application, USSN 11/141,996. Qu proposed that ion bridges between charged amino acids on the surface of vector particles contribute to inter-particle interactions. However, as explained at page 9, lines 18-20 of the instant specification, contrary to the above, it has been found that amino acids with charged side chains are not effective in preventing AAV2 vector aggregation beyond their contribution to ionic strength. Additionally, Qu specifically states that other types of interactions may play a role in aggregation and concludes: "In conjunction with further elucidation of the mechanism(s) of AAV vector aggregation, these observations will facilitate formulation development for optimal large-scale vector purification and clinical use." Thus, Qu explicitly acknowledges that the causes of self-aggregation of rAAV virions had not yet been determined and further work was needed in order to provide a viable purification procedure. Thus, the Examiner's implication that rAAV virion self-aggregation is based on a simple, universal mechanism, is misplaced.

Additionally, Zolotukhin does not recognize that rAAV virions self-aggregate and therefore does not provide any suggestions regarding a composition as claimed. Rather, Zolotukhin recognizes AAV aggregates with cellular debris, especially proteins present during an **intermediate stage** of purification. Further, Zolotukhin actually teaches away from the use of high ionic strength because it describes the use of I M NaCl as "unnecessary or unwarranted" in the other steps of the purification process, and it teaches that it is desirable to "remove or reduce the concentration of salt ... prior to use of, or further purification of, the rAAV." See, column 3,

line 63 to column 4, line 5 of Zolotukhin. Thus, Zolotukhin uses high salt in the **first step** of the iodixanol gradient to destabilize AAV-lysate protein interactions. High salt concentrations are purposefully eliminated from the remainder of the iodixanol gradient, including the gradient from which the rAAV is collected after centrifugation, because the elimination of high salt is important for subsequent purification steps. See, column 15, lines 22-35 of Zolotukhin. Absent Applicants' teaching regarding the problem of rAAV self-aggregation, there is no recognition that this is a concern that may be addressed by manipulating ionic strength in any of the cited art.

The secondary references do not cure the defects present in Zolotukhin. The present claims are directed to compositions of purified **rAAV** particles with an ionic strength of at least 200 mM and more than $1x10^{13}$ up to $6.4x10^{13}$ vg/ml. Additionally, the pH of the composition is between 7.5 and 8.0. None of Andersson, Zhang or Chen even relates to AAV and hence would not suggest a composition with the particular purity, ionic strength and pH as claimed. As explained above, the mechanisms of rAAV virion self-aggregation was not understood at the time of the invention. Hence, relying on the teachings of the secondary references to provide the motivation to make a composition as claimed, in order to prevent self-aggregation of rAAV virions, is misplaced.

With respect to the Office's continued assertion regarding claims 7 and 8, namely, that the average particle radius and percent recovery are intrinsic characteristics, Applicants again assert that inherency is not a proper standard on which to base an obviousness rejection. It is axiomatic that a retrospective view of inherency is not a substitute for some teaching or suggestion to arrive at the claimed invention. That which may be inherent is not necessarily known, and obviousness **cannot** be predicated on the unknown. See, e.g., *In re Newell*, 13 USPQ2d 1248 (Fed. Cir. 1989).

Applicants' composition provides a commercially viable solution for providing high amounts of rAAV particles in stored compositions. As explained at page 20, second paragraph of the specification and in Table 3, rAAV particles prepared and stored in elevated ionic strength solutions remain soluble and can be stored for an extended period.

Finally, Applicants' claims now also recite that the pH of the composition is between 7.5 and 8.0. As acknowledged by the Examiner in the parent application, such a composition was considered nonobvious over the art.

For at least the above reasons, withdrawal of the rejections under 35 U.S.C. § 103(a) is respectfully requested.

III. CONCLUSION

Applicants respectfully submit that the claims are now in condition for allowance and request early notification to that effect. The Examiner is encouraged to contact the undersigned if the Examiner notes any further matters which might be resolved by a telephone interview.

Respectfully submitted,

Date: FEB. 18, 2014

By: _____
Roberta L. Robins
Registration No. 33,208

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response against AAV-2, yet retain the high gene delivery efficiency inherent to AAV-2, could surmount the problem of pre-existing anti-AAV neutralizing antibodies in a significant fraction of the human population, and may present opportunities for readministration. Finally, not only do these libraries provide useful mutants directly applicable to gene therapy applications, but they also offer a way to continue to dissect AAV biology.

900. Packaging of Host Cell and Plasmid DNA into Recombinant Adeno-Associated Virus Particles Produced by Triple Transfection

Peter H. Smith,[1] J. Fraser Wright,[1] Guang Qu,[1] Susannah Patarroyo-White,[2] Amy Parker,[2] Jurg M. Sommer.[1]
[1]Development, Avigen, Inc., Alameda, CA; [2]Applications Research, Avigen, Inc., Alameda, CA.

In this study we characterized residual host cell and plasmid DNA impurities in preparations of highly purified AAV vectors. AAV-hFIX16, a recombinant AAV2 vector containing the coding sequence for human coagulation factor IX under the control of the human alpha-1 antitrypsin (hAAT) promotor, was produced by triple plasmid transfection of HEK293 cells and purified by sequential cation and anion exchange chromatography. Reverse packaging of the vector plasmid was eliminated by using an oversized plasmid backbone of approximately 7000 base pairs. As determined by real-time quantitative PCR (Q-PCR), residual HEK293 and plasmid DNA ranged from 1-5% of total vector DNA in preparations purified by chromatography. The levels of DNA impurities in the final product were not reduced by in-process or final treatment with Benzonase or DNase I. Column chromatography-purified vector was fractionated by CsCl density gradient centrifugation to further characterize the DNA impurities. Increasing amounts of HEK293 and plasmid DNA were observed in fractions ranging in density from 1.32 gm/mL (density of empty AAV capsids) to 1.38 gm/mL (density of vector particles). Southern blot analysis of gradient fractions demonstrated that the average size of residual DNA in each fraction ranged from 600 nucleotides near the empty capsid band to a maximal size of about 4,500 nucleotides in fractions containing intact vector particles. Negligible amounts of residual DNA were associated with fractions of a density higher than that of intact vector. These results suggest that AAV packages single-stranded HEK293 or plasmid DNA fragments of various sizes up to the packaging limit of AAV. Preferential (2 to 5-fold) packaging of vector plasmid sequences over Adenovirus helper plasmid or rep/cap-encoding plasmid sequences was observed. The data indicate that the amount of non-vector DNA in AAV vectors purified by column chromatography can be reduced three to five-fold by an additional gradient fractionation step, but that the remaining host cell and plasmid DNA fragments are approximately the same size as the vector genomes. Additional studies are required to more fully characterize packaged non-vector DNA, and to further optimize production methods to reduce residual DNA impurities in AAV vectors.

901. Evidence That Ionic Interactions Are Involved in Concentration-Induced Aggregation of Recombinant Adeno-Associated Virus

Guang Qu,[1] Christopher Connolly,[1] Alicia Koblansky,[1] Jurg M. Sommer,[1] Alan McClelland, J. Fraser Wright.[1]

Recombinant adeno-associated viruses (rAAV) are promising vectors for human gene therapy, and have demonstrated excellent safety and promising efficacy in pre-clinical and clinical studies. A key requirement for successful development of AAV vectors is to establish a reliable and cost-effective process to generate material of high purity and titer. Aggregation of vector particles may occur during purification, and result in reduced yield and deleterious effects following *in vivo* administration (eg reduced efficacy, increased immunogenicity). Huang *et al.* (*Mol Therapy* (2000) 1:S286) previously reported that AAV vector particles undergo concentration-dependent aggregation. In this study vector aggregation has been further characterized. We examined aggregation during tangential flow filtration, a process step used to concentrate and diafilter purified vectors at large-scale. Aggregation was assessed by dynamic light scattering, size-exclusion chromatography, and by quantification of loss following 0.2μm filtration. We observed that aggregation was capsid particle (cp) concentration-dependent, and typically occurred when concentrations exceed the range $0.5\text{-}1.0 \times 10^{14}$ cp/mL for column purified vectors. Considerable variability in the concentration at which aggregation occurred was observed, which may be attributable to variability in the levels of empty capsids, and in levels of DNA and/or protein impurities in the vector preparations. To investigate the mechanism(s) of AAV vector aggregation, we assessed the effect of surfactants and buffer pH on this phenomenon. Neither Polysorbate 80 (0.1%) nor Pluronic F68 (0.1%) added to vector in phosphate buffered saline, pH 7.2, affected the concentration at which aggregation was observed relative to control vector lacking surfactant. Adjusting the pH to values ≤4.5 or ≥10 resulted in reversal of concentration-induced vector aggregation, suggesting that ion bridges between charged amino acids (Glu, Asp, Lys) on the surface of vector particles contribute to inter-particle interactions. However, vector aggregates were also observed to be stable in approximately 3M CsCl (neutral pH), suggesting that other types of interactions play a role. In conjunction with further elucidation of the mechanism(s) of AAV vector aggregation, these observations will facilitate formulation development for optimal large-scale vector purification and clinical use.

902. Construction and Analysis of Truncated Muscle-Specific Promoters (Muscle Creatine Kinase Promoter)

Bing Wang,[1] Liqiao Zhou,[1] Juan Li,[1] Xiao Xiao.[1,2]
[1]Dept. of Molecular Genetics and Biochemistry & Gene Therapy Center, University of Pittsburgh, Pittsburgh, PA, United States; [2]Dept. of Orthopaedic Surgery, University of Pittsburgh, Pittsburgh, PA, United States.

Muscle is readily accessibly for direct injection of gene therapy vectors for the treatment of both muscle- and non-muscle diseases. The CMV promoter is not the ideal promoter for muscular dystrophy gene therapy, because it renders gene expression in non-muscle cells (such as APCs), which may potentially elicit an immune response against the transgene product. While tissue-specific promoters are highly desirable in gene therapy practice, those promoters are generally large in size and less active than viral promoters such as the CMV promoter. Large promotes are not well suited for AAV vectors. Our aim is to develop some highly compact, highly active yet highly tissue-specific promoters. The commonly used muscle-specific promoter (MCK) and its derivatives developed by Dr. Hauschka's lab are well known for the high tissue-specificity and moderate activities. The major regulatory regions include a muscle-specific enhancer and a 358-bp proximal promoter. Here we have constructed chimeric promoters containing one, two or three modified MCK enhancer with the minimal MCK promoter. We have compared the promoter activity and tissue-specificity of these promoters in differentiated and undifferentiated muscle cells in vitro and muscle tissues in vivo. Our results showed that the levels of of Luciferase activity achieved by the chimeric promoters, especially the modified construct dMCK (including two modified enhancers), tMCK (including three modified enhancers), were significantly higher (> 10 fold) than the original MCK promoter. We have also shown

Electronic Patent Application Fee Transmittal

Application Number:	12661553
Filing Date:	19-Mar-2010
Title of Invention:	Compositions and methods to prevent AAV vector aggregation
First Named Inventor/Applicant Name:	John Fraser Wright
Filer:	Roberta L. Robins/Denise Vaillancourt
Attorney Docket Number:	0800-0045.01

Filed as Large Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Extension - 3 months with $0 paid	1253	1	1400	1400

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
			Total in USD ($)	**1400**

Electronic Acknowledgement Receipt

EFS ID:	18230294
Application Number:	12661553
International Application Number:	
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First Named Inventor/Applicant Name:	John Fraser Wright
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1		roa.pdf	498447 cd6f2984881b91832d6a0f142b9289ad5e6dec1b	yes	10

Multipart Description/PDF files in .zip description		
Document Description	**Start**	**End**
Transmittal Letter	1	1
Extension of Time	2	2
Applicant Arguments/Remarks Made in an Amendment	3	10

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2	Fee Worksheet (SB06)	fee-info.pdf	30197 0a7672778ecb1f5435ec30c016cb335e18331201	no	2

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If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

<u>National Stage of an International Application under 35 U.S.C. 371</u>
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

<u>New International Application Filed with the USPTO as a Receiving Office</u>
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/SB/06 (09-11)
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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 12/661,553	Filing Date 03/19/2010	☐ To be Mailed

ENTITY: ☒ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	

APPLICATION AS AMENDED – PART II

AMENDMENT **02/18/2014**

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	* 7	Minus	** 20	= 0	X $80 =	0
Independent (37 CFR 1.16(h))	* 1	Minus	***3	= 0	X $420 =	0
☐ Application Size Fee (37 CFR 1.16(s))						
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
					TOTAL ADD'L FEE	0

AMENDMENT

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =	
Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =	
☐ Application Size Fee (37 CFR 1.16(s))						
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
					TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

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12/661,553	03/19/2010	John Fraser Wright	0800-0045.01	4726

20855 7590 05/21/2014

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EXAMINER	
SINGH, SATYENDRA K	
ART UNIT	PAPER NUMBER
1657	

MAIL DATE	DELIVERY MODE
05/21/2014	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No. 12/661,553	Applicant(s) WRIGHT ET AL.	
	Examiner SATYENDRA SINGH	Art Unit 1657	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *2/8/2014*.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5)☒ Claim(s) *1 and 3-8* is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *1 and 3-8* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☐ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

4) ☐ Other: _____.

The present application is being examined under the pre-AIA first to invent provisions.

DETAILED ACTION

Applicant's submission filed on **02/18/2014** is duly acknowledged.

Claims 2, and 9-11 (invention of group II) were previously canceled by applicants.

Claims 1 and 3-8 (elected invention of group I), as currently amended, have been examined on their merits in this office action.

The following is a **NON-FINAL action** on the pending claims as currently amended by applicants:

Claim Rejections - 35 USC § 101- NEW

35 U.S.C. 101 reads as follows:

> Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

The claimed invention is **not directed to patent eligible subject matter**. Based upon an analysis with respect to the claim as a whole, claim(s) 1 and 3-8 do not recite something significantly different than a judicial exception. The rationale for this determination is explained below:

Claim 1 is directed to the following product composition:

"**A composition** for the storage of purified adeno-associated virus (AAV) particles, comprising:

purified (AAV) particles at a concentration exceeding 1 x 10^{13} vg/ml up to 6.4x 10^{13} vg/ml;

a pH buffer, wherein the pH of the composition is between 7.5 and 8.0; and

excipients comprising one or more multivalent ions; wherein the ionic strength of the composition is greater than 200 mM, wherein aggregation of the purified AAV particles in the composition is prevented."

It is noted that instant claims are directed to a product composition comprising purified AAV particles (i.e. virions or viral particles) in combination with excipients such as buffer and salt(s) (see also instant claim 3 and 6, in particular) that appears to be a composition comprising a natural AAV product that does not seem to be "**markedly different in structure**" from the naturally occurring AAV particles. The elements/components (i.e. excipients such as pH buffer, salts, detergent, etc.; see claims 3-6, in particular) recited in the claims do not seem to effectuate significant structural change in the purified viral particles *per se*, as claimed (see also claims 7-8 directed to intrinsic features of the purified AAV preparation), and are known in the art for use in buffering and/or stabilization of viral preparations in the prior art (see the art rejection based on the cited prior art references, as discussed below), therefore instant invention reads on a natural product, i.e. a judicial exception.

Thus, based upon an analysis with respect to the claim as a whole, claims 1 and 3-8 (as presented) do not recite something significantly different than a judicial exception, and are therefore deemed to be patent ineligible (see MPEP 2106).

Appropriate correction is required.

Claim Rejections - 35 USC § 103- Made/Maintained

The following is a quotation of pre-AIA 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the

> invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under pre-AIA 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.

2. Ascertaining the differences between the prior art and the claims at issue.

3. Resolving the level of ordinary skill in the pertinent art.

4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

This application currently names **joint inventors**. In considering patentability of the claims under pre-AIA 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of pre-AIA 35 U.S.C. 103(c) and potential pre-AIA 35 U.S.C. 102(e), (f) or (g) prior art under pre-AIA 35 U.S.C. 103(a).

1. **Claims 1 and 3-8** (as currently amended) **are/remain** rejected under 35 U.S.C. 103(a) as being unpatentable over Zolotukhin et al (2000; US 6,146,874) taken with Andersson et al (1979; US 4,138,287), Zhang et al (2001; US 6,194,191) and Chen et al (1994).

Claims (as currently amended) are directed to "a composition (for the storage) of purified adeno-associated virus (AAV) particles, comprising: purified (AAV) particles at a

concentration exceeding 1 x 10^{13} vg/ml up to 6.4x 10^{13} vg/ml; a pH buffer, wherein the pH of the composition is between 7.5 and 8.0; and excipients comprising one or more multivalent ions; wherein the ionic strength of the composition is greater than 200 mM, wherein aggregation of the purified AAV particles in the composition is prevented (instant claim 1); wherein one of the one or more multivalent ions is citrate (claim 3); further comprising Pluronic® F68 (ethylene oxide/propylene oxide block copolymer) at 0.001% (w/v) (claims 4-5); wherein the pH buffer is 10 mM Tris, pH 8.0 and the excipients comprise 100 mM sodium citrate (claim 6); wherein the purified AAV particles have an *average particle radius* (*Rh*) of less than about 20 nm as measured by dynamic light scattering (instant claim 7); and wherein *recovery of the purified virus particles* is at least about 90% following filtration of the composition of virions through a 0.22 μm filter." (claim 8).

Zolotukhin et al (2000) disclose the composition comprising: purified **recombinant** AAV virus particles; a pH buffer such as **phosphate buffered-saline with magnesium chloride and potassium chloride** (PBS-MK); and excipients comprising one or more multivalent ions such as phosphate and magnesium; wherein the ionic strength of the composition is greater than 200 mM (see Zolotukhin et al, column 11, 4th paragraph, lines 35-40, in particular), wherein they elute the purified rAAV particles in **PBS-MK buffer having 1M NaCl**, i.e. elution buffer; and wherein they disclose the fact that highly purified stocks having titers up to **about 10^{13} particles/ml** are obtained using their purification steps within 24 hours or less (see abstract, and column 16, 1st paragraph, in particular), and in fact has the potential to produce **10^{14} virus particles**. In addition Zolotukhin et al also disclose the problems facing the purification of high titer AAV particles specifically related to the **problem of aggregation**, which was alleviated

and/or reduced significantly by the use of high concentrations of salts (i.e. high ionic strength buffer such as 1M NaCl in PBS-MK; see column 3, last paragraph; column 15, 3rd paragraph, for examples) during purification, wherein the excess salt can be later removed, if required for downstream applications.

However, the composition having AAV particles at a concentration exceeding 1 x 10^{13} vg/ml up to 6.4x 10^{13} vg/ml (see instant claim 1), further comprising **Pluronic® F68** at 0.001% w/v (claims 4-5); and wherein the pH buffer is **10 mM Tris, pH 8.0** and the excipients comprise **100 mM sodium citrate** (claims 1 and 6), is not explicitly exemplified and/or disclosed by the inventions of Zolotukhin et al (although the number of virus particles potentially purified have been disclosed to be in the vicinity of **about 10^{13} and about 10^{14}**; see Zolotukhin et al above).

Andersson et al (1979) disclose the composition comprising purified hepatitis virus (HB_sAg) particles (see abstract, in particular), a pH buffer such as Tris-sodium citrate buffer, pH 7.5, and excipients comprising a multivalent ion such as **sodium citrate**, wherein the ionic strength of the composition is greater than about 200 mM (see elution of purified virus from column using 0.5 M NaCl in Tris-citrate buffer; see Andersson et al, example 2 and claim 5, in particular).

Zhang et al (2001) disclose the use of a surfactant such as **Pluronic® F68** (0.1% in growth medium for adenovirus infection and viral production, etc.; see column 4, 2nd paragraph and columns 53-54, in particular) for production of adenoviral particles in serum-free suspension cultures using spinner flasks, and also use in the cryopreservation media (see column 53, last paragraph).

Chen et al (1994) disclose strategies to suppress aggregation of proteins (such as recombinant keratinocyte growth factor) during liquid formulation development (see Chen et al, abstract, table 1-2, in particular) by adding sulfated polysaccharides (such as heparin) and **citrate salts** (such as **0.1 to 0.5 M sodium citrate**; see Chen et al, page 1661, left column, in particular) that were found to be effective in preventing protein aggregation. Chen et al also disclose the fact that other negatively charged small ions such as phosphate (a multivalent ion) also have moderate stabilizing effects **on preventing aggregation** of recombinant keratinocyte growth factor (rhKGF).

Thus, given the disclosure in the cited prior art, a person of ordinary skill in the art would have modified the composition comprising purified recombinant AAV virus particles taught by Zolotukhin et al such that said composition additionally comprise a surfactant (to help prevent aggregation during freezing-thawing cycles, for example) such as Pluronic® F68, as explicitly taught by Zhang et al, and a suitable amount of sodium citrate as a multivalent ion in Tris buffer solution (at a suitable pH; as specifically disclosed by Andersson et al) in order to stabilize the viral particles as suggested by Chen et al (*albeit* for suppressing/reducing aggregation of fairly unstable proteins such as rhKGF; see discussion above, thus providing a conceptual basis for including multivalent ions in the buffer containing purified recombinant AAV particles having capsid proteins), in addition to other stabilizing components such as a surfactant. Since, the benefits of including a surfactant and high ionic strength multivalent ions have been disclosed in the cited prior art of Zhang et al and Chen et al, an artisan of ordinary skill in the art would have established suitable concentrations required to help stabilize purified recombinant AAV viral preparations (i.e. for preventing aggregation, etc.) with a reasonable expectation of success,

especially given the disclosure from Zolotukhin et al, for example, for the use of high salt concentrations for reducing the potential aggregation of virus particles (see discussion of Zolotukhin et al, above). Such modification in the use of suitable concentrations of the multivalent ions (such as sodium citrate) for reducing aggregation in place of high salt concentrations (i.e. 1 M NaCl) used by Zolotukhin et al would have been therefore obvious and fully contemplate by an artisan of ordinary skill in the art, given the combined disclosure provided by the cited references of Andersson et al, Zhang et al and Chen et al, as discussed above.

The limitations of claims 7 and 8 (average particle radius and percent recovery following filtration, etc.) are also met by the prior art as these are taken to be intrinsic features of the purified AAV viral compositions as disclosed by Zolotukhin et al when taken with the teachings of Andersson et al, Zhang et al and Chen et al. Since, all the components of the product, as recited in the claims are the same as disclosed and/or suggested in the cited prior art, these features will necessarily follow from the composition disclosed in the art, as they do not structurally change the composition as claimed.

Thus, the invention as a whole would have been *prima facie* obvious to a person of ordinary skill in the art at the time the claimed invention was made.

As per MPEP 2144.06, "*It is prima facie obvious to combine two compositions each of which is taught by the prior art to be useful for the same purpose, in order to form a third composition to be used for the very same purpose.... [T]he idea of combining them flows logically from their having been individually taught in the prior art." In re Kerkhoven, 626 F.2d 846, 850, 205 USPQ 1069, 1072 (CCPA 1980).*

As per MPEP 2144.06, *In order to rely on equivalence as a rationale supporting an obviousness rejection, the equivalency must be recognized in the prior art, and cannot be based on applicant's disclosure or the mere fact that the components at issue are functional or mechanical equivalents. In re Ruff, 256 F.2d 590, 118 USPQ 340 (CCPA 1958).*

Response to Applicant's 103(a) Rejection Arguments

Applicant's arguments filed on **02/18/2014** (as they pertain to the prior art rejection over pending claims) have been fully considered but they are not persuasive for the following reasons of record:

First, it is noted that instant claims are drawn to a judicial exception (i.e. preparation comprising a naturally occurring AAV particles) as discussed above in the 101 rejection above.

Regarding the 103a rejection of record, applicant's argument that "*…Qu explicitly acknowledges that the causes of self-aggregation of rAAV virions had not yet been determined and further work was needed in order to provide a viable purification procedure. Thus, the Examiner's implication that rAAV virion self-aggregation is based on a simple, universal mechanism, is misplaced*" (see remarks on page 5 regarding the abstract by Au et al, 2003; not relied upon in the instant rejection of record), is duly noted and considered. However first, it is noted that instant claims are directed to any AAV particles (not necessarily limited to recombinant AAV discussed by Qu et al. Secondly, applicant's allegation that " *Examiner's implication that rAAV virion self-aggregation is based on a simple, universal mechanism"* does not represent required evidence and/or data providing sufficient reasons in order to obviate the rejection of record. As discussed in the 103a rejection of record, the cited prior art of Zolotukhin et al disclose the range of viral particles that can be purified up to or greater than 10^{14} vp/ml (see column 6, 1st paragraph,; column 16, 1st paragraph, in particular), and therefore, when taken with the disclosure from Andersson et al, Zhang et al and Chen et al for the benefits of using suitable concentrations of a multivalent ions such as sodium citrate under suitable pH conditions, a person of ordinary skill in the art would have fully contemplated the modification in the buffer

composition disclosed by Zolotukhin et al such that it uses multivalent ions as stabilizing buffer excipient in order to reduce/prevent viral aggregation, as already intended by the disclosure of Zolotukhin et al and supported by Zhang et al and Chen et al, as discussed above.

Applicant's argument that "*…Zolotukhin does not recognize that rAAV virions self-aggregate and therefore does not provide any suggestions regarding a composition as claimed. Rather, Zolotukhin recognizes AAV aggregates with cellular debris, especially proteins present during an intermediate stage of purification*" (see remarks, page 5, last paragraph) is also not found to be persuasive because instant claim is drawn to a composition, not a process of "*preventing self-aggregation of AAV particles*", and since the components required to provide such desired effects (i.e. in terms of stabilization of the viral preparation) have already been disclosed and/or suggested by the combined teachings in the cited prior art for the same purposes of reducing aggregation (irrespective of the mode of viral aggregation), a person of ordinary skill in the viral vector purification art would have fully contemplated such adjustments in the concentrations of excipients/surfactants and/or pH as already suggested in the art of record. Moreover, viral capsid proteins would be reasonably assumed to be interacting with each other, as well as other proteins and/or cellular debris contained in the preparation of Zolotukin et al, and therefore, a person of ordinary skill in the art, at the time this invention was made, would have reasonably contemplated the teachings related to protein-based aggregation of particles (see teachings of Chen et al, above), and would have employed multivalent ions in suitable concentration under appropriate pH condition, as disclosed by the combined teachings and/or suggestions of the cited art of record.

Applicant's arguments regarding the limitations of claims 7 and 8, that "*…Applicants again assert that inherency is not a proper standard on which to base an obviousness rejection. It is axiomatic that a retrospective view of inherency is not a substitute for some teaching or suggestion to arrive at the claimed invention…*" (see remarks on page 6), is duly noted and considered. However, applicants have not provided any data or evidence on the record to demonstrate that under the conditions as claimed, such average particle radius would not be an intrinsic feature of the composition comprising purified AAV particles (i.e. would be structurally different), to which the percent filtration recovery would be considered an added feature, as already contemplated in the cited art of record.

Applicants seem to argue benefit of the composition as disclosed in the instant specification of record on page 20 and Table 3 (see remarks, page 6), which is duly noted and considered. However, it is noted that the scope of the showing must be commensurate with the scope of claims to consider evidence probative of unexpected results, for example. *In re Dill, 202 USPQ 805 (CCPA, 1979), In re Lindner 173 USPQ 356 (CCPA 1972), In re Hyson, 172 USPQ 399 (CCPA 1972), In re Boesch, 205 USPQ 215, (CCPA 1980), In re Grasselli, 218 USPQ 769 (Fed. Cir. 1983), In re Clemens, 206 USPQ 289 (CCPA 1980).* It should be clear that the probative value of the data is not commensurate in scope with the degree of protection sought by the instant claims. Thus, the argument that "*….Applicants' claims now also recite that the pH of the composition is between 7.5 and 8.0. As acknowledged by the Examiner in the parent application, such a composition was considered nonobvious over the art*", is also not found to be persuasive because the claims in the parent case were directed to the "*method of preventing*

aggregation of recombinant AAV virions", the scope of which is very different from the product claims under consideration.

Thus, the 103(a) rejection of record is properly made and/or maintained.

Applicants are advised to amend claims appropriately in order to limit the scope of the claims commensurate with the unexpected results in order to further the prosecution of this case.

Conclusion

NO claims are allowed.

Pertinent art:

1. **Evans et al.** (published on 08/26/2004; **US 2004/0166122 A1**; previously cited by the examiner) - Adenovirus formulations (disclose stable **viral vector formulations** for gene therapy and other clinical applications generally comprising up to about $1x10^{13}$ viral particles/ml in a suitable buffer, a sugar, a salt, a divalent cation, a non-ionic detergent as well as sodium citrate, wherein the typical formulations comprise 5 mM Tris buffer, pH 7.5, 250 mM NaCl, sucrose to provide suitable osmolarity in the range of 200-800 mOsm/L, $MgCl_2$ in the range of 0.1mM to about 10 mM, 0.001% to about 2% of polysorbate-80 as surfactant, and sodium citrate at about 10 mM; see page 8, example 1, in particular; paragraphs [0051], [0056], [0060], [0079], entire disclosure at pages 5-6, in particular and claims).

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SATYENDRA SINGH whose telephone number is (571)272-8790. The examiner can normally be reached on 9-5MF.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, JON P. WEBER can be reached on 571-272-0925. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/SATYENDRA SINGH/
Primary Examiner,
Art Unit 1657

EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
S67	2	("20040166122").PN.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2013/08/11 17:44
S68	4476	WRIGHT near3 JOHN	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2014/03/28 19:52
S69	69	QU near3 GUANG	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2014/03/28 19:52
S70	4502	S68 or S69	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2014/03/28 19:52
S71	29	S70 and ((virion or vira$6 or virus or adenovir$6 or AAV) same (aggreg$6 or precipit$6 or agglutin$6 or stabiliz$6))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2014/03/28 19:52
S72	9	S70 and ((virion or vira$6 or virus or adenovir$6 or AAV) same (aggreg$6 or precipit$6 or agglutin$6 or stabiliz$6) same (ionic near3 strength))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2014/03/28 19:53
S73	262	((virion or vira$6 or virus or adenovir$6 or AAV) same (aggreg$6 or precipit$6 or agglutin$6 or stabiliz$6) same (ionic near3 strength))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2014/03/28 19:54
S74	9	((virion or vira$6 or virus or	US-PGPUB;	OR	OFF	2014/03/28

		adenovir$6 or AAV) same (aggreg$6 or precipit$6 or agglutin$6 or stabiliz$6) same (ionic near3 strength)).clm.	USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB			19:54
S75	1223	((virion or vira$6 or virus or adenovir$6 or AAV) same (aggreg$6 or precipit$6 or agglutin$6 or stabiliz$6) same (citric or citrate or multivalen$3 or divalen$3 or trivalen$3))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2014/03/28 20:00
S76	17	((virion or vira$6 or virus or adenovir$6 or AAV) same (aggreg$6 or precipit$6 or agglutin$6 or stabiliz$6) same (citric or citrate or multivalen$3 or divalen$3 or trivalen$3)).clm.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2014/03/28 20:00
S77	335	((virion or vira$6 or virus or adenovir$6 or AAV) same (citric or citrate or multivalen$3 or divalen$3 or trivalen$3)).clm.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2014/03/28 20:04
S78	62	((adenovir$6 or AAV) same (citric or citrate or multivalen$3 or divalen$3 or trivalen$3)).clm.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2014/03/28 20:04
S79	110	((adenovir$6 or AAV) same (manganese or phosphate or citric or citrate or multivalen$3 or divalen$3 or trivalen$3)).clm.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2014/03/28 20:07
S80	11	((adenovir$6 or AAV) same (manganese or phosphate or citric or citrate or multivalen$3 or divalen$3 or trivalen$3) same (millimolar or molar or mm)).clm.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2014/03/28 20:10
S85	70	(adenovir$6 or AAV or rAAV or adeno-associat$6 or adenoassoc$6) same (phosphate or phosphoric or citrate or citric or sulfate or magnesium) same (molar or millimolar or (ionic near3 strength)) same buffer	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/03/28 21:42
S86	7	(adenovir$6 or AAV or rAAV or adeno-associat$6 or adenoassoc$6) same (phosphate or phosphoric or citrate or citric or sulfate or magnesium) same (molar or millimolar or (ionic near3 strength))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT;	OR	ON	2014/03/28 21:43

		same buffer same (storag$6 or stabil$6 or stable)	IBM_TDB			
S87	4	(("6593123") or ("7261544") or ("7704721") or ("8137948")).PN.	US-PGPUB; USPAT; USOCR	OR	OFF	2014/03/29 18:03
S88	4476	WRIGHT near3 JOHN	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2014/03/29 18:05
S89	69	QU near3 GUANG	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2014/03/29 18:05
S90	4502	S88 or S89	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2014/03/29 18:05
S91	58	S90 and (aav or adenoassociat$6 or virion or (aav near3 particle))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/03/29 18:07
S92	25	S90 and (aav or adenoassociat$6 or virion or (aav near3 particle)) and (citrate or phosphate or magnesium or sulfate)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/03/29 18:07
S93	5	S90 and (aav or adenoassociat$6 or virion or (aav near3 particle)) and (citrate or phosphate or magnesium or sulfate).clm.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/03/29 18:07
S94	18	S90 and ((aav or adenoassociat$6 or virion or (aav near3 particle)) same (citrate or phosphate or magnesium or sulfate))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/03/29 18:09
S95	434451	(rAAV particle)	USPAT	OR	OFF	2014/03/31 10:26
S96	682	purified near3 (rAAV particle)	USPAT	OR	OFF	2014/03/31 10:26
S97	44	purified near3 (rAAV particle).clm.	USPAT	OR	OFF	2014/03/31 10:27

S98	2	(composition or formulation) same (purified near3 (rAAV particle)).clm.	USPAT	OR	OFF	2014/03/31 10:27
S99	1	(composition or formulation) same (purified near3 AAV).clm.	USPAT	OR	OFF	2014/03/31 10:28
S100	6	(purified same (recombinant near3 AAV)).clm.	USPAT	OR	OFF	2014/03/31 10:32
S101	1	("6015686").PN.	USPAT; USOCR	OR	OFF	2014/03/31 11:36
S102	77	(adenoassociated or AAV) SAME (citrate or citric)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2014/03/31 11:49
S103	0	(adenoassociated or AAV) SAME (citrate or citric).clm.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2014/03/31 11:49
S104	25	(adenoassociated or AAV) SAME (citrate or citric) same buffer	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2014/03/31 11:50
S105	4566	WRIGHT near3 JOHN	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2014/05/19 10:28
S106	69	QU near3 GUANG	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2014/05/19 10:28
S107	4592	S105 or S106	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2014/05/19 10:28
S108	19	S107 and ((adenovir$6 or AAV) same (aggreg$6 or precipit$6 or agglutin$6 or stabiliz$6))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2014/05/19 10:29

EAST Search History (Interference)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
S81	99	(virion or virus or adenovir$6 or AAV or rAAV or adeno-associat$6 or (vir$6 near3 particle)) same ((phosphate or phosphoric or citrate or citric) near3 buffer) same (molar or millimolar or (ionic near3 strength))	US-PGPUB; USPAT; UPAD	OR	OFF	2014/03/28 21:09
S82	2	(virion or virus or adenovir$6 or AAV or rAAV or adeno-associat$6 or (vir$6 near3 particle)) same ((phosphate or phosphoric or citrate or citric) near3 buffer) same (molar or millimolar or (ionic near3 strength)).clm.	US-PGPUB; USPAT; UPAD	OR	OFF	2014/03/28 21:09
S83	33	(adenovir$6 or AAV or rAAV or adeno-associat$6 or (vir$6 near3 particle)) same ((phosphate or phosphoric or citrate or citric) near3 buffer) same (molar or millimolar or (ionic near3 strength))	US-PGPUB; USPAT; UPAD	OR	OFF	2014/03/28 21:14
S84	10	(adenovir$6 or AAV or rAAV or adeno-associat$6 or (vir$6 near3 particle)) same ((phosphate or phosphoric or citrate or citric) near3 buffer) same (molar or millimolar or (ionic near3 strength) same (stor$6 or stable or stabiliz$3))	US-PGPUB; USPAT; UPAD	OR	OFF	2014/03/28 21:17

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Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	12661553	WRIGHT ET AL.
	Examiner	Art Unit
	SATYENDRA SINGH	1657

CPC- SEARCHED		
Symbol	Date	Examiner

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES		
Search Notes	Date	Examiner
EAST: USPAT, USOCR, US-PGPUB, JPO, EPO, DERWENT- ATTACHED	11/6/2011	SKS
INVENTOR SEARCH: PALM, EDAN AND EAST-	11/6/2011	SKS
EAST: USPAT, USOCR, US-PGPUB, JPO, EPO, DERWENT- UPDATED & ATTACHED	7/11/2012	SKS
INVENTOR SEARCH: PAL, EDAN AND EAST- UPDATED	7/11/2012	SKS
EAST: USPAT, USOCR, US-PGPUB, JPO, EPO, DERWENT- UPDATED & ATTACHED	8/12/2013	SKS
INVENTOR SEARCH: PALM, EDAN AND EAST- UPDATED	8/12/2013	SKS
EAST: USPAT, USOCR, US-PGPUB, JPO, EPO, DERWENT- UPDATED & ATTACHED	5/19/2014	SKS
INVENTOR SEARCH: PALM, EDAN AND EAST- UPDATED	5/19/2014	SKS

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner

	/SATYENDRA SINGH/ Primary Examiner.Art Unit 1657

Attorney Docket No. 0800-0045.01 **PATENT**

I hereby certify pursuant to 37 CFR §1.8 that this correspondence is being transmitted via EFS to the United States Patent and Trademark Office on the date shown below.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of John Fraser Wright et al.

Serial No.: 12/661,553 Examiner: Satyendra K. Singh

Confirmation No.: 4726 Art Unit: 1653

Filed: March 19, 2010

For: COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

INFORMATION DISCLOSURE STATEMENT UNDER 37 C.F.R. §1.97(c)

In accordance with the duty of disclosure set forth in 37 C.F.R. §1.56, Applicant(s) hereby submits the following information in conformance with 37 C.F.R. §§1.97 and 1.98.

[X] Pursuant to 37 C.F.R. §1.98, a copy of each document cited in the attached Form PTO/SB/08 is enclosed.

[] No copy of the publication _____ listed on the attached Form PTO/SB/08A are being provided because the Office waives the requirement under 37 C.F.R. 1.98 (a) (2) (i) for submitting a copy of each cited U.S. patent and each U. S. patent application publication for all U.S. national patent applications filed after June 30, 2003.

[] No copies of the publications listed on the attached Form PTO/SB/08A are being provided pursuant to 37 C.F.R. §1.98(d) because the publications were previously cited by or submitted to the Office in prior Application Serial No. ___ to which the above-identified application claims priority under 35 U.S.C. §120.

[] Publication(s) _____ listed on the attached Form PTO/SB/08A were cited in a foreign search or examination report corresponding to ___ application serial no. _____ and mailed on _____.

[] Enclosed is a copy of a non-English publication(s) ___. Pursuant to §609 of the M.P.E.P., Applicant submits the attached foreign search or examination report, which cites such non-English language publication(s).

[] Enclosed is a copy of a non-English publication(s) ___. English language publication ___ (copy enclosed) claims priority from this non-English publication.

[] Enclosed is an explanation of non-English publication(s) ___ for which an English translation is not available.

[] Enclosed is an English translation of non-English publication(s) ___cited in the attached Form PTO/SB/08A.

[] Enclosed is a copy of pending patent Application Serial No. ___.

This Information Disclosure Statement is filed after the period specified in 37 C.F.R. § 1.97(b), but before the mailing of:

[X] a final action under 37 C.F.R. § 1.113;

[] a notice of allowance under 37 C.F.R. § 1.113; or

[] an action that otherwise closes prosecution in this application.

In accordance with 37 C.F.R. § 1.97(c) also enclosed is:

[X] Fee under 37 C.F.R. § 1.17(p) in the amount of $180.00; or

[] Statement as specified in 37 C.F.R. § 1.97(e):

[] Each item of information contained in the Information Disclosure Statement cited herein was first cited in a communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing date of the Information Disclosure Statement; or

[] No item of information contained in the Information Disclosure Statement submitted herewith was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the undersigned, having made a reasonable inquiry, no item of information contained in the Information Disclosure Statement was known to any individual designated in 37

C.F.R. § 1.56(c) more than three months prior to the filing date of the Information Disclosure Statement.

It is respectfully requested that the Examiner consider the above-noted information and return an initialed copy of the attached Form PTO/SB/08A to the undersigned.

Dated: Nov. 14, 2014

Respectfully submitted,

Robins Law Group
2625 Middlefield Road. No. 828
Palo Alto, CA 94306
Tel: (650) 493-3400
Fax: (650) 493-3440

By: _____

Roberta L. Robins
Reg. No. 33,208

PTO/SB/08a (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 1

Complete if Known	
Application Number	12/661,553
Filing Date	March 19, 2010
First Named Inventor	Wright et al.
Art Unit	1657
Examiner Name	Satyendra Singh
Attorney Docket Number	0800-0045.01

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		US-			
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FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]-Number[4]-Kind Code[5] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
	C27	WO99/61643	12-02-1999	University of Florida		

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:	Customer No.: 105379
JOHN FRASER WRIGHT et al.	Confirmation No.: 4726
Application No.: 12/661,553	Art Unit: 1653
Filing Date: March 19, 2010	Examiner: Satyendra K. Singh

Title: COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION

AMENDMENT UNDER 37 CFR 1.111

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Sir:

This paper is responsive to the Office Action mailed May 21, 2014, with a shortened statutory period of three months for response. Accordingly, an extension of time in which to respond is requested and the requisite fee accompanies this response. Applicants request reconsideration of the above-referenced patent application in view of the following amendments and remarks.

A **listing of claims** begins on page 2 of this paper.

Remarks begin on page 4 of this paper.

I. AMENDMENT

Amendments to the Claims:

The following listing reflects amendments to the claims and replaces all prior versions and listings of claims in this application.

1. (Currently amended) A composition for the storage of purified, recombinant adeno-associated virus (AAV) particles, comprising:

purified, recombinant ~~(AAV)~~ AAV particles at a concentration exceeding $1x10^{13}$ vg/ml up to $6.4x10^{13}$ vg/ml;

a pH buffer, wherein the pH of the composition is between 7.5 and 8.0; and

excipients comprising one or more multivalent ions; wherein the ionic strength of the composition is greater than 200 mM, wherein aggregation of the purified AAV particles in the composition is prevented.

2. (Canceled)

3. (Original) The composition of claim 1, wherein one of the one or more multivalent ions is citrate.

4. (Previously presented) The composition of claim 1, further comprising Pluronic® F68 (ethylene oxide/propylene oxide block copolymer).

5. (Previously presented) The composition of claim 4, wherein the Pluronic® F68 is present at 0.001% (w/v).

6. (Original) The composition of claim 1, wherein the pH buffer is 10 mM Tris, pH 8.0 and the excipients comprise 100 mM sodium citrate.

7. (Currently amended) The composition of claim 1, wherein the purified, recombinant AAV particles have an average particle radius (*Rh*) of less than about 20nm as measured by dynamic light scattering.

8. (Currently amended) The composition of claim 1, wherein recovery of the purified, recombinant virus particles is at least about 90% following filtration of the composition of virions through a 0.22μm filter.

9-11. (Canceled)

II. REMARKS

Introductory Comments

Claims 1 and 3-8 were examined in the Office Action under reply and stand rejected under (1) 35 U.S.C. §101; and (2) 35 U.S.C. §103(a). These rejections are believed to be overcome by this response and are otherwise traversed for reasons discussed in detail below.

Overview of the Above Amendments

Claims 1, 7 and 8 have been amended to clarify that the purified AAV particles are "recombinant" AAV particles. Support for this recitation can be found throughout the specification at, e.g., page 8, lines 6-7. Claim 1 has also been amended to correct a minor typographical error.

The foregoing amendments are made without prejudice, without intent to abandon any originally claimed subject matter, and without intent to acquiesce in any rejection of record. Applicants expressly reserve the right to file one or more continuing applications containing the unamended claims.

Rejection Under 35 U.S.C. §101

Claims 1 and 3-8 were rejected under 35 U.S.C. §101 as being directed to nonpatentable subject matter. The Office alleges the claims "do not recite something significantly different than a judicial exception" because they appear to cover "a composition comprising a natural AAV product that does not seem to be '**markedly different in structure**' from the naturally occurring AAV particles." Office Action, page 3, emphasis in original. Applicants submit the present claims indeed recite patentable subject matter.

As explained above, the claims have been amended to recite that the AAV particles are "recombinant" particles. It is well known that such particles represent an artificial AAV which does not contain any AAV rep and cap genes that encode viral replication and structural proteins, respectively. Rather, these genes are replaced with a gene or construct of interest which is flanked by the ITRs which include cis-acting elements necessary for replication and packaging.

Accordingly, a "recombinant AAV particle" is not an AAV product as found in nature. Based on the foregoing, withdrawal of the rejection under 35 U.S.C. §101 is respectfully requested.

Rejection Under 35 U.S.C. §103(a)

Claims 1 and 3-8 remain rejected under 35 U.S.C. §103(a) as being unpatentable over U.S. Patent No. 6,146,874 to Zolotukhin et al. ("Zolotukhin"), taken with U.S. Patent No. 4,138,287 to Andersson et al. ("Andersson"); U.S. Patent No. 6,194,191 to Zhang et al. ("Zhang"); and Chen et al., *J. Pharm. Sci.* (1994) 83:1657-1661 ("Chen").

The Office reiterates the previous rejection and disputes Applicants' arguments, asserting the primary reference, Zolotukhin, discloses that viral particles can be purified up to or greater than 10^{14} vp/ml and Andersson et al., Zhang et al. and Chen et al. allegedly disclose the benefits of using suitable concentrations of multivalent ions under suitable pH conditions. Office Action, page 9. With respect to these secondary references, the Examiner dismisses Applicants' citation of Qu et al., *Molec. Ther.* (2003) 5:S348, Abstract 901 ("Qu") which Applicants pointed out evidenced that the causes of self-aggregation of AAV virions had not yet been determined. The Examiner alleges:

> [F]irst, it is noted that instant claims are directed to any AAV particles not necessarily limited to recombinant AAV discussed by Qu et al. Secondly, applicant's allegation that 'Examiner's implication that rAAV virion self-aggregation is based on a simple, universal mechanism' does not represent required evidence and or data providing sufficient reasons in order to obviate the rejection of record.

Office Action, page 9. However, Applicants continue to submit the Office has failed to establish a *prima facie* case of obviousness.

As explained above, Applicants' claims are directed to compositions comprising "recombinant" AAV particles. Qu's abstract is also directed to recombinant AAV particles. Contrary to the Office's assertion above, this article, when read in combination with the present application, does evidence that it would **not** be obvious to provide a composition as claimed. To reiterate, Qu proposed that ion bridges between charged amino acids on the surface of recombinant AAV particles contribute to inter-particle interactions. However, page 9, lines 18-

20 of the instant application explain that, contrary to the above, it has been found that amino acids with charged side chains are not effective in preventing AAV vector aggregation beyond their contribution to ionic strength. Additionally, Qu specifically states other types of interactions may play a role in aggregation and concludes: "In conjunction with further elucidation of the mechanism(s) of AAV vector aggregation, these observations will facilitate formulation development for optimal large-scale vector purification and clinical use." Thus, Qu **explicitly** acknowledges that the causes of self-aggregation of **rAAV virions** had not yet been determined and further work was needed in order to provide a viable composition as claimed "wherein aggregation of the purified AAV particles in the composition is prevented." Accordingly, contrary to the Examiner's allegation, Applicants have indeed provided evidence in order to obviate the assertion that the references provide a motivation to formulate recombinant AAV particles in a composition as claimed.

To reiterate, the secondary references which are **not** even related to AAV would not suggest a composition with the particular purity, ionic strength and pH as alleged by the Office as causes of aggregation of recombinant AAV particles **were not known** prior to Applicants' invention. Hence, relying on the teachings of the secondary references which do not pertain to recombinant AAV particles, to provide the motivation to make a composition as claimed, in order to prevent self-aggregation of rAAV virions, is misplaced.

The Examiner also disputes Applicants' argument that Zolotukhin does not recognize that rAAV virions self-aggregate and therefore does not provide any suggestions regarding a composition as claimed, asserting this argument is unpersuasive because the claims are drawn to compositions and not to a process of preventing self-aggregation. The Examiner further asserts:

> [T]he components required to provide such desired effects i.e. in terms of stabilization of the viral preparation have already been disclosed and/or suggested by the combined teachings in the cited prior art for the same purposes of reducing aggregation (irrespective of the mode of viral aggregation), a person of ordinary skill in the viral vector purification art would have fully contemplated such adjustments in the concentrations of excipients/surfactants and/or pH as already suggested in the art of record.

Office Action, page 10. Applicants disagree with this assertion.

First, although the claims relate to compositions, not methods, the claims explicitly require that "aggregation of the purified AAV particles in the composition is prevented."

Accordingly, Applicants' unique mixture of components in the purified composition as claimed must be factored into the assessment of obviousness. To reiterate, Zolotukhin teaches away from the use of high ionic strength because it teaches that it is desirable to "remove or reduce the concentration of salt ... prior to use of, or further purification of, the rAAV." See, column 3, line 63 to column 4, line 5 of Zolotukhin. Thus, high salt concentrations are purposefully eliminated from later purification steps, and hence from the final product. Moreover, Applicants have provided evidence that the causes of aggregation of recombinant AAV particles **were not known** prior to Applicants' invention and hence a composition as claimed would not be obvious in view of Zolotokhin in combination with the secondary references which are not in any way directed to recombinant AAV preparations. Absent Applicants' teaching regarding the problem of rAAV self-aggregation, there is no recognition that this is a concern that may be addressed by manipulating ionic strength in any of the cited art.

With respect to the Office's continued assertion regarding claims 7 and 8, namely, that the average particle radius and percent recovery are intrinsic characteristics, the Office argues "applicants have not provided any data or evidence on the record to demonstrate that under conditions as claimed, such average particle radius would not be an intrinsic feature of the composition comprising purified particles." Office Action, page 11. However, this argument assumes Zolotukhin's final composition is the same as Applicants' composition since none of the secondary references pertain to recombinant AAV particles. In fact, the Examiner recognizes Zolotukhin's final composition, as well as the compositions of the additional cited art, are not the same as Applicants' since the previous rejections under 35 U.S.C. §102 over Zolotukhin and Andersson were withdrawn.

Even if appropriate, the fact that a certain result or characteristic may occur or be present in the prior art is not sufficient to establish the inherency of that result or characteristic. *In re Rijckaert,* 28 USPQ2d 1955, 1957 (Fed. Cir. 1993) (reversed rejection because inherency was based on what would result due to optimization of conditions, not what was necessarily present in the prior art); *In re Oelrich,* 212 USPQ 323, 326 (CCPA 1981). "To establish inherency, the extrinsic evidence 'must make clear that the missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill. Inherency, however, may not be established by probabilities or possibilities. The mere fact

that a certain thing may result from a given set of circumstances is not sufficient.' " *In re Robertson,* 49 USPQ2d 1949, 1950-51 (Fed. Cir. 1999) (citations omitted).

The Examiner further disputes Applicants' showing that the claimed composition provides a commercially viable solution for providing high amounts of recombinant AAV particles in stored compositions. The Examiner states "the scope of the showing must be commensurate with the scope of claims to consider evidence probative of unexpected results." This is indeed the case. As explained at page 20, second paragraph of the specification and in Table 3, recombinant AAV particles prepared and stored in elevated ionic strength solutions (greater than 200 mM as claimed) remain soluble with no aggregation and can be stored for an extended period.

Finally, the Examiner dismisses the fact that Applicants' current claims also recite that the pH of the composition is between 7.5 and 8.0 as in the issued parent case. Indeed, the recitation "wherein the pH of the purified preparation of rAAV virions is between 7.5 and 8.0" was added to the parent claims in an Examiner's amendment. Applicants are aware the present claims relate to compositions and not methods, however, as explained above, the composition is one where "aggregation of the purified AAV particles in the composition is prevented" and the causes of aggregation, and hence the proper formulation for preventing aggregation, were unknown prior to Applicants' discovery.

Accordingly, the combination cited by the Office does not provide evidence that the claimed invention is a "predictable use of prior art elements according to their established functions." *KSR Int'l Co. v. Teleflex, Inc.*, 82 USPQ2d 1385, 1396 (U.S. 2007). Rather, as explained above, the evidence is to the contrary. Applicants submit the Examiner has chosen bits and pieces of the cited references to arrive at the allegation that this combination of references suggests the claimed invention. This is improper. As stated in *KSR*, "a patent composed of several elements is not proved obvious merely by demonstrating that each of its elements was, independently, known in the prior art." *KSR*, page 1396; see also, *In re Kotzab*, 55 USPQ2d 1313, 1317 (Fed. Cir. 2000). A rejection cannot be predicated on the mere identification of individual components of claimed limitations. Rather, particular findings must be made as to the reason the skilled artisan, with no knowledge of the claimed invention, would have selected these components for combination in the manner.

Additionally, as set forth in MPEP 2142, impermissible hindsight must be avoided and the conclusion of obviousness must be reached on the basis of the facts gleaned from the prior art. Should the Examiner continue to maintain the rejection over the cited combination, the only conclusion that can be drawn is that the rejection is premised on an impermissible hindsight reconstruction of the invention based on Applicants' disclosure. As stated by the Court of Appeals for the Federal Circuit, "[i]t is impermissible to use the claimed invention as an instruction manual or 'template' to piece together the teachings of the prior art so that the claimed invention is rendered obvious." *In re Fritch*, 23 USPQ2d 1780, 1784 (Fed. Cir. 1992). See, also, *In re Fine*, 5 USPQ2d 1596, 1600 (Fed. Cir. 1988): "One cannot use hindsight reconstruction to pick and choose among isolated disclosures in the prior art to deprecate the claimed invention." Thus, it is insufficient merely to show that some or all of the elements of the invention are present in the prior art and possess characteristics of the elements of the instant invention.

For at least the above reasons, withdrawal of the rejections under 35 U.S.C. § 103(a) is respectfully requested.

III. CONCLUSION

Applicants respectfully submit that the claims are now in condition for allowance and request early notification to that effect. The Examiner is encouraged to contact the undersigned if the Examiner notes any further matters which might be resolved by a telephone interview.

Respectfully submitted,

Date: Nov. 18, 2014

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(54) Title: METHOD OF PREPARING RECOMBINANT ADENO–ASSOCIATED VIRUS COMPOSITIONS BY USING AN IODIXANANOL GRADIENT

(57) Abstract

Disclosed are methods for the isolation and purification of high–titer recombinant adeno–associated virus (rAAV) compositions. Also disclosed are methods for reducing or eliminating the concentration of helper adenovirus in rAAV samples. Methods are disclosed that provide highly–purified rAAV stocks having titers up to about 10^{13} particles/ml at particle–to–infectivity ratios of less than 100 in processes that are accomplished about 24 hours or less.

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DESCRIPTION

METHOD OF PREPARING RECOMBINANT ADENO-ASSOCIATED VIRUS COMPOSITIONS BY USING AN IODIXANANOL GRADIENT

1.0 BACKGROUND OF THE INVENTION

The present application claims the priority of United States Provisional Patent Application Serial No. 60/086,898 filed May 27, 1998, the entire disclosure of which is incorporated herein by reference without disclaimer. The government may have certain rights in the present invention pursuant to grant numbers PO1 HL59412 and PO1 NS36302 from the National Institutes of Health.

1.1 FIELD OF THE INVENTION

The present invention relates generally to the field of virology, and in particular, to methods for preparing highly-purified, high-titer recombinant adeno-associated virus compositions. In certain embodiments, the invention concerns the use of equilibrium density centrifugation techniques, affinity chromatographic media, and in certain embodiments anion- and cation-exchange resins, to remove rAAV particles from solution and to prepare highly purified viral stocks for use in a variety of investigative, diagnostic and therapeutic regimens. Methods are also provided for purifying rAAVs from solution and for reducing the concentration of adenovirus in rAAV stocks.

1.2 DESCRIPTION OF RELATED ART

1.2.1 ADENO-ASSOCIATED VIRUS

Adeno-associated virus-2 (AAV) is a human parvovirus which can be propagated both as a lytic virus and as a provirus (Cukor *et al.*, 1984; Hoggan *et al.*, 1972). The viral genome consists of linear single-stranded DNA (Rose *et al.*, 1969), 4679 bases long (Srivastava *et al.*, 1983), flanked by inverted terminal repeats of 145 bases (Lusby *et al.*, 1982). For lytic growth AAV requires co-infection with a helper virus. Either adenovirus (Ad; Atchinson *et al.*, 1965; Hoggan, 1965; Parks *et al.*, 1967) or herpes simplex virus (HSV; Buller *et al.*, 1981) can supply helper function. Without helper, there is no evidence of AAV-specific replication or gene expression (Rose *et al.*,

1972; Carter *et al.*, 1983; Carter *et al.*, 1983). When no helper is available, AAV can persist as an integrated provirus (Hoggan, 1965; Berns *et al.*, 1975; Handa *et al.*, 1977; Cheung *et al.*, 1980; Berns *et al.*, 1982).

Integration apparently involves recombination between AAV termini and host sequences and most of the AAV sequences remain intact in the provirus. The ability of AAV to integrate into host DNA is apparently an inherent strategy for insuring the survival of AAV sequences in the absence of the helper virus. When cells carrying an AAV provirus are subsequently superinfected with a helper, the integrated AAV genome is rescued and a productive lytic cycle occurs (Hoggan, 1965).

AAV sequences cloned into prokaryotic plasmids are infectious (Samulski *et al.*, 1982). For example, when the wild type AAV/pBR322 plasmid, pSM620, is transfected into human cells in the presence of adenovirus, the AAV sequences are rescued from the plasmid and a normal AAV lytic cycle ensues (Samulski *et al.*, 1982). This renders it possible to modify the AAV sequences in the recombinant plasmid and, then, to grow a viral stock of the mutant by transfecting the plasmid into human cells (Samulski *et al.*, 1983; Hermonat *et al.*, 1984). AAV contains at least three phenotypically distinct regions (Hermonat *et al.*, 1984). The *rep* region codes for one or more proteins that are required for DNA replication and for rescue from the recombinant plasmid, while the *cap* and *lip* regions appear to code for AAV capsid proteins and mutants within these regions are capable of DNA replication (Hermonat *et al.*, 1984). It has been shown that the AAV termini are required for DNA replication (Samulski *et al.*, 1983).

The construction of two *E. coli* hybrid plasmids, each of which contains the entire DNA genome of AAV, and the transfection of the recombinant DNAs into human cell lines in the presence of helper adenovirus to successfully rescue and replicate the AAV genome has been described (Laughlin *et al.*, 1983; Tratschin *et al.*, 1984a; 1984b).

1.2.2 Conventional Methods for Preparing Recombinant AAV

Recombinant adeno-associated virus (rAAV) has been demonstrated to be a useful vector for efficient and long-term gene transfer in a variety of tissues, including lung (Flotte, 1993), muscle (Kessler, 1996; Xiao and Samulski, 1996; Clark *et al.*, 1997; Fisher *et al.*, 1997), brain (Kaplitt, 1994; Klein, 1998) retina (Flannery, 1997; Lewin *et*

al., 1998), and liver (Snyder, 1997). It has also been demonstrated to evade the immune response of the host by failing to transduce dendritic cells (Jooss *et al.*, 1998). Phase I clinical trails are underway for cystic fibrosis rAAV-mediated gene therapy (Flotte *et al.*, 1996; Wagner *et al.*, 1998). Yet in spite of these promising developments one of the problems that remains to be solved is that vector production remains very laborious.

Currently rAAV is most often produced by co-transfection of rAAV vector plasmid and wt AAV helper plasmid into Ad-infected 293 cells (Hermonat and Muzyczka, 1984). Recent improvements in AAV helper design (Li *et al.*, 1997) as well as construction of non-infectious mini-Ad plasmid helper (Grimm *et al.*, 1998; Xiao *et al.*, 1998; Salvetti, 1998) have eliminated the need for Ad infection, and made it possible to increase the yield of rAAV up to 10^5 particles per transfected cell in a crude lysate. Scalable methods of rAAV production that do not rely on DNA transfection have also been developed (Chiorini *et al.*, 1995; Conway *et al.*, 1997; Inoue and Russell, 1998; Clark *et al.*, 1995). These methods, which generally involve the construction of producer cell lines and helper virus infection, are suitable for high-volume production.

However, little progress has been made on the downstream purification of rAAV. The conventional protocol involves the stepwise precipitation of rAAV using ammonium sulfate, followed by two or preferably, three rounds of CsCl density gradient centrifugation. Each round of CsCl centrifugation involves fractionation of the gradient and probing fractions for rAAV by dot-blot hybridization or by PCR™ analysis. No only does it require two weeks to complete, but the current protocol often results in poor recovery of the vector and poor virus quality. The growing demand for different rAAV stocks often strains the limited capacities of vector production facilities. There is, therefore, a clear need for a protocol that will reduce the preparation time substantially without sacrificing the quality and/or purity of the final product.

2.0 SUMMARY OF THE INVENTION

In a first embodiment, the invention concerns a method of purifying a recombinant adeno-associated virus. In general, the method comprises centrifuging a sample containing or suspected of containing recombinant adeno-associated virus through at least a first iodixanol gradient, and collecting the purified virus or at least a

first fraction comprising the recombinant adeno-associated virus, from the gradient. Preferably the gradient is a discontinuous gradient, although the inventors contemplate the formulation of continuous iodixanol gradients that also provide purification of rAAV compositions. In certain aspects of the invention, multiple iodixanol gradients, for example at least a second, at least a third and/or at least a fourth iodixanol gradient, are used to purify the recombinant adeno-associated virus.

In an exemplary discontinuous iodixanol gradient, the gradient comprises an about 15% iodixanol step, an about 25% iodixanol step, an about 40% iodixanol step, and an about 60% iodixanol step. Optionally, the gradient may contain steps having lower concentrations of iodixanol, and likewise, the gradient may contain steps that have higher concentrations of iodixanol. Naturally, the concentrations of each step do not need to be exact, but can vary slightly depending upon the particular formulation and preparation of each step. The inventors have shown that most rAAV particles will band in an iodixanol gradient at a level corresponding to a percentage of iodixanol approximately equal to 52%, although depending upon the number of viral particles loaded on the gradient and the volume and capacity of the gradient, the range of concentrations at which purified rAAV particles may be found may range on the order of from about 50% to about 53%, or from about 50% to about 54%, 55%, 56%, 57%, 58%, 59% and even up to and including about 60% iodixanol. Likewise, the range of concentrations at which the rAAV particles may be isolated following centrifugation may be on the order of from about 55% down to and including about 49%, about 48%, about 47%, about 46%, about 45%, about 44%, about 43%, about 42%, about 41% or about 40% or so iodixanol. Naturally, all concentrations in the range of from about 40% to about 60% are contemplated to be useful in recovering purified rAAV particles from the centrifuged gradient. As such, all intermediate concentrations including about 41%, about 42%, about 43%, about 44%, about 45%, about 46%, about 47%, about 48%, about 49%, about 50%, about 51%, about 52%, about 53%, about 54%, about 55%, about 56%, about 57%, about 58%, and about 59% or so are contemplated to be useful in the practice of the present invention for recovering purified rAAV particles from the centrifuged gradient.

When step gradients are utilized, it is convenient to include in the gradient steps that encompass or "bracket" the range of optimal recovery of virus. For example, in a 25%/40%/60% step gradient, the 40% band comprises the virus, and this fraction is then removed for recovery of the virus composition. The design of both continuous and discontinuous gradients is well-known to those of skill in the art, and those having benefit of the present specification may readily prepare iodixanol gradients of sufficient capacity and range to isolate a band of purified rAAV particles from the gradient following centrifugation.

In certain embodiments, to improve the yield and/or recovery of virus particles from such a gradient, one may add to one or more steps of the gradient one or more salts to reduce or prevent aggregation of the virus and any cellular debris or proteins, polypeptides, *etc.* which may be present in the crude sample. In an exemplary embodiment, the inventors have shown that the addition of salt to the 15% iodixanol step in a discontinuous gradient improves the recovery of virus particles from an iodixanol gradient. As an example, the addition of NaCl to a final concentration of about 1 M in the 15% step was found by the inventors to be particularly advantageous in recovery of purified rAAV particles from the 40% step of such a gradient. While addition of one or more salts to one or more of the other steps in the gradient may be performed as required, in most instances, the inventors have shown that the presence of salt in other steps were either unnecessary or unwarranted. In situations where one or more salts are added to a layer which comprises the rAAV particles, following centrifugation it may be desirable to remove or reduce the concentration of salt in such a fraction prior to use of, or further purification of, the rAAV. Such removal may readily be achieved by dialysis, microconcentration, ultrafiltration, and the like.

In alternative embodiments, the inventors contemplate that the gradient may optionally comprise one or more additional compositions to permit further, or enhanced purification of rAAV particles. Such compositions may include derivatives of iodixanol, iodixanol analogs, iodixanol-derived compounds, and/or compounds having centrifugation properties similar to, equal to, or superior to, iodixanol-alone compositions. Depending upon the particular composition added to the gradient, the relative position of the purified particles in the gradient may vary from that in which

iodixanol alone is used (*i.e.* approximately 52% iodixanol), but such variance is readily overcome in the design of the gradient, and does not preclude the isolation of the rAAV from the particular density in the gradient where such virus particles are banded following centrifugation. Likewise, when one or more compositions are added to the iodixanol gradient, the centrifugation time, centrifugal force, and/or banding position within the gradient of the viral particles may be varied depending upon the particular application. Any such variations, improvements, or alterations in the composition of the iodixanol gradient are also contemplated to fall within the scope of this invention, and such modifications to the gradient will be apparent to those of skill in the art given the benefit of the teachings of the instant specification.

In a second embodiment, the invention relates to a method for purifying rAAV particles that comprises contacting a sample containing the virus with at least a first matrix that comprises heparin, under conditions effective and for a period of time sufficient to permit binding of the virus to the matrix, removing any unbound proteins or contaminants from the matrix, and then subsequently collecting or eluting the virus from the matrix. In exemplary embodiments, the matrix comprises heparin agarose type I or heparin agarose type II-S, although the inventors contemplate the use of any heparin composition or combinations thereof demonstrated to be effective in binding the rAAV, and thus removing it from a solution that is contacted with such a matrix. Preferably, the matrix is an affinity chromotographic medium, that may be comprised within a column, a syringe, a microfilter, or microaffinity column, or alternatively may be comprised within an HPLC affinity column. The matrix may be formed of any material suitable for the preparation of a heparin affinity matrix, and may, for example, be formulated as a resin, bead, agarose, acrylamide, glass, fiberglass, plastic, polyester, methacrylate, cellulose, sepharose, sephacryl, and/or the like. In fact, the inventors contemplate that the matrix may be fashioned out of any suitable material that forms a solid or semi solid support, and that permits the adsorption, ionic bonding, covalent linking, crosslinking, derivatization, or other attachment of a heparin moiety to the support matrix. Indeed, the art of affinity chromatographic medium preparation is sufficiently advanced so that a skilled artisan could readily prepare a suitable heparin affinity medium for use in purifying the rAAV particles

using the methods disclosed herein. For example, the inventors have shown that an HPLC affinity column containing a crosslinked polyhydroxylated polymer derivatized with one or more heparin functional groups was useful in the purification of rAAV from a solution contacted with such a column.

Elution of the bound virus to the affinity column may be achieved in any manner convenient to the skilled practitioner, and may include, for example, the use of one or more elution buffers such as a salt buffer, to collect the virus from the column. In an exemplary embodiment, the inventors utilized a 1 M NaCl solution to elute the virus from the column. Prior to elution, the column comprising the bound virus may be washed with one or more washing or equilibrating buffers prior to elution of the virus from the column.

The use of an affinity column to purify rAAV particles may be used alone, or may be combined with the iodixanol gradient as described above to further increase the purification of the rAAV composition. One or more affinity columns may be utilized prior to the density gradient centrifugation purification method, and/or one or more affinity columns may be utilized after the purification through iodixanol gradients. In an exemplary embodiment, a cellular lysate containing rAAV particles is subjected to iodixanol centrifugation, and the fraction of the gradient containing the partially-purified rAAV is then contacted with at least one heparin affinity column to increase the total purity of the rAAV preparation.

Likewise, following either or both of the aforementioned purification methods, the rAAV composition obtained may be subjected to further purification, dialysis, concentration, and/or the like. In an exemplary embodiment, the partially-purified rAAV preparation may be further purified by contacting a fraction or sample containing or comprising recombinant adeno-associated virus with a hydrophobic matrix, under conditions effective to permit interaction of hydrophobic species (proteins or other contaminants) with the hydrophobic matrix, and collecting the non-interacting virus from the hydrophobic matrix. Preferred are hydrophobic matrices that comprise phenyl groups, for example phenyl sepharose, phenyl sepharose 6 fast flow (low sub) or phenyl sepharose 6 (high sub). In certain

embodiments, rAAV that has been partially purified by heparin affinity chromatography is further purified by hydrophobic interaction chromatography.

In other embodiments, the partially-purified rAAV preparation may be further purified by subjecting the viral sample to one or more cesium chloride equilibrium density gradients, and collecting from the gradient(s) the fraction(s) comprising the purified virus. The virus may then optionally be further purified by dialysis, microfiltration, microconcentration, and/or precipitation. Additionally, the virus may be further purified by contacting the virus with one or more ion exchange chromatography media, and eluting the virus from the media using one or more suitable elution buffers. Such an ion exchange chromatography medium may comprise a cation or an anion exchange medium. An exemplary cation exchange medium comprises at least one negatively-charged sulfonic group.

Contaminants that may be present in the sample containing the recombinant adeno-associated virus include, but are not limited to, viruses, such as adenovirus or herpes simplex virus, proteins, polypeptides, peptides, nucleic acids, cell extracts, growth medium, or combinations thereof. The methods of the present invention serve to reduce or eliminate one or more, or in certain embodiments all of the contaminants in a given recombinant adeno-associated virus sample. In preferred embodiments, the rAAV is about 70%, about 80%, about 90%, about 95%, about 98%, about 99%, about 99.5% or more pure as judged by any of a variety of assays and analytical techniques that are known to those of skill in the art, including, but not limited to gel electrophoresis and staining and/or spectroscopy.

In certain embodiments, the invention provides methods for the preparation of highly-purified rAAV compositions comprising greater than about 10^{10} rAAV particles/ml. In exemplary embodiments, such methods have been demonstrated useful in the preparation of viral compositions comprising greater than about 10^{11}, 10^{12}, and even greater than about 10^{13} or 10^{14} particles/ml. In other embodiments, the invention provides methods for the preparation of rAAV compositions having a particle-to-infectivity ratio of less than about 100, and in certain aspects less than about 90, about 80, about 70, about 60, about 50 about 40, about 30, about 20 about

10, about 5, or in certain exemplary embodiments rAAV compositions having a particle-to-infectivity ratio of about 1.

The process for preparing highly-purified and/or highly-infectious viral preparations generally comprise the steps of centrifuging a sample containing recombinant adeno-associated virus through an iodixanol gradient, collecting from the iodixanol gradient at least a first fraction comprising the recombinant adeno-associated virus, contacting the at least a first fraction comprising the recombinant adeno-associated virus with a matrix comprising heparin, under conditions effective to permit binding of the virus to the matrix, removing non-bound species from the matrix, and eluting the virus from the matrix. Other methods for isolating rAAV provided by the present invention comprise the steps of centrifuging a sample containing or suspected of containing recombinant adeno-associated virus through an iodixanol gradient, collecting the purified virus from the gradient, contacting the virus collected from the gradient with a matrix comprising heparin, under conditions effective to permit binding of the virus to the matrix, collecting the virus from the matrix, subjecting the virus collected from the matrix to at least a first cesium chloride equilibrium density gradient, and collecting from the gradient a fraction comprising the highly-purified rAAV composition.

Additional methods of isolating a recombinant adeno-associated virus are also provided in the present invention. These methods generally comprises the steps of centrifuging a sample containing recombinant adeno-associated virus through an iodixanol gradient, collecting from the iodixanol gradient at least a first fraction comprising the recombinant adeno-associated virus, contacting the at least a first fraction comprising the recombinant adeno-associated virus with a matrix comprising heparin, under conditions effective to permit binding of the virus to the matrix, removing at least a first non-bound species from the matrix, eluting the virus from the matrix, contacting the eluted virus with a hydrophobic matrix, under conditions effective to permit interaction of hydrophobic species with the hydrophobic matrix, and collecting the non-interacting virus from the hydrophobic matrix.

Further methods generally comprise the steps of centrifuging a sample suspected of containing recombinant adeno-associated virus through an iodixanol

gradient, collecting the purified virus from the gradient, contacting the virus collected from the gradient with a first matrix comprising heparin, under conditions effective to permit binding of the virus to the matrix, collecting the virus from the first matrix, contacting the virus collected from the first matrix with a second matrix comprising an anion exchange medium, and collecting from the second matrix a fraction comprising the purified virus.

In another embodiment, the invention provides a method of preparing recombinant adeno-associated virus. The method generally involves subjecting a sample suspected of containing recombinant adeno-associated virus to centrifugation through an iodixanol gradient, and collecting the virus from a fraction of the gradient corresponding to a concentration of iodixanol of about 40%. Such a gradient may be formed as described above, and may be prepared either as a continuous or a discontinuous gradient. In the case of discontinuous gradients, the gradient will preferably include at least an about 15% iodixanol step, an about 25% iodixanol step, an about 40% iodixanol step, and an about 60% iodixanol step, with the virus being isolatable from the 40% iodixanol step following centrifugation. Following recovery of the banded rAAV particles, the virus may be further purified using the heparin affinity chromatographic methods disclosed herein, and/or be optionally further purified via CsCl gradient centrifugation, anion exchange chromatography, cation exchange chromatography, affinity chromatography, or precipitation.

The invention also provides methods for reducing or eliminating adenovirus from a recombinant adeno-associated virus composition contaminated with adenovirus. The method generally comprises centrifuging a sample containing or suspected of containing both recombinant adeno-associated virus and adenovirus through one or more iodixanol gradients as described herein, and collecting the recombinant adeno-associated virus from the gradient. The concentration of adenovirus may be further reduced in such a sample by a number of methods, including, but not limited to, further purification on a heparin affinity column and/or a hydrophobic interaction column, by heating the sample, or alternatively, by anion exchange chromatography as described herein.

A method for reducing the concentration of adenovirus in a recombinant adeno-associated virus composition is also provided that generally involves centrifuging a sample containing recombinant adeno-associated virus through an iodixanol gradient, collecting from the iodixanol gradient at least a first fraction comprising the recombinant adeno-associated virus, contacting the at least a first fraction comprising the recombinant adeno-associated virus with a matrix comprising heparin, under conditions effective to permit binding of the virus to the matrix, removing any non-bound species from the matrix, and eluting the virus from the matrix.

A further aspect of the invention is the preparation of a high-titer rAAV composition. The method generally comprises the steps of: centrifuging a sample or rAAV through an iodixanol gradient, collecting the purified recombinant adeno-associated virus from the gradient; contacting the partially-purified recombinant adeno-associated virus collected from the gradient with a matrix comprising heparin, under conditions effective to permit binding of the recombinant adeno-associated virus to the matrix, and collecting the recombinant adeno-associated virus from the matrix. The purified rAAV composition eluted from the matrix may also be optionally further purified, such as in the case of the preparation of high-titer viral stocks, by contacting the sample with a matrix comprising an anion exchange medium, under conditions effective to permit binding of the recombinant adeno-associated virus to the matrix, and collecting the purified recombinant adeno-associated virus from the matrix, preferably by elution.

The present invention thus also provides recombinant adeno-associated virus compositions, prepared by any one or more of the methods described herein. Generally, the invention provides at least a first recombinant adeno-associated virus composition, prepared by applying a sample containing recombinant adeno-associated virus to an iodixanol gradient, and collecting from the gradient at least a first fraction comprising the recombinant adeno-associated virus.

Also provided by the present invention are kits comprising combinations of the recombinant adeno-associated virus isolation media described herein. Generally, the kits comprise, in a suitable container, iodixanol and a matrix comprising heparin.

In certain preferred aspects, the iodixanol is formulated as an iodixanol gradient. In other kits of the present invention, the matrix comprises heparin agarose type I or heparin agarose type II-S. Additional kits of the invention further comprise a hydrophobic matrix, such as a matrix comprising phenyl groups, exemplified by phenyl sepharose.

3.0 BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

FIG. 1. rAAV purification flow chart.

FIG. 2. Iodixanol step gradient for the purification of rAAV. Shown is a plot of the refractive index (vertical axis) of one ml-fractions (fraction number, horizontal axis) collected from the bottom of a tube after a 1 hour spin.

FIG. 3A and FIG. 3B. HPLC purification of the iodixanol fraction of rAAV-UF5, monitored at 231 nm. The absorbance at 231 nm (A_{231}) is shown on the left vertical axis, time (min) is shown on the horizontal axis, and the ratio of diluent B (%B) is shown on the right vertical axis. **FIG. 3A.** POROS® HE/M chromatography. **FIG. 3B.** UNO™ S1 cation exchange chromatography. The dotted line indicates the shape of the gradient. Elution time is shown in min above the respective peaks.

4.0 DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

Recently, it has been shown that the transduction of cells by wt AAV was mediated through the heparan sulfate proteoglycan receptor (Summerford and Samulski, 1998). In order to develop an efficient and simple protocol for purification of rAAV, the inventors developed heparin affinity column chromatography, which significantly simplifies and expedites the production of rAAV. To efficiently bind the virus to the affinity media the inventors have also introduced a new pre-purification technique - centrifugation of the crude viral lysate through a pre-formed gradient of the non-ionic gradient media iodixanol. The present invention provides for the first time protocols

which permit the completion of rAAV purification in one day and produces viral stocks sufficiently pure for pre-clinical and/or clinical studies. The inventors have shown that use of these new purification techniques permit an increase in the yield of purified virus by at least 10-fold over conventional methods, resulting in highly-purified, high-titer stocks (10^{12}-10^{13} particles/ml), equivalent to at least about 10^4-10^5 particles per cell, as well as improved viral infectivity and more rapid purification.

5.0 EXAMPLES

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

5.1 EXAMPLE 1 -- METHODS FOR PRODUCTION OF RAAV COMPOSITIONS

5.1.1 MATERIALS AND METHODS

5.1.1.1 CELLS

Low passage number (P29-35) 293 cells were propagated in DMEM/10% FBS. The C12 cell line (Clark *et al.*, 1995) was maintained in the presence of 0.5 mg/ml G418, while the Cre8 cell line (Hardy *et al.*, 1997) was propagated in DMEM supplemented with 200 μg/ml G418.

5.1.1.2 CONSTRUCTION OF RECOMBINANT PLASMIDS

The construction of pTR-UF5 was described earlier (Klein, 1998). To produce the vector containing the enhanced blue fluorescent mutant of green fluorescent protein (gfp; Heim and Tsien, 1996), the inventors have introduced the Tyr-145-Phe mutation into pTR-UFB background (Zolotukhin *et al.*, 1996) using Quick Change site-Directed Mutagenesis kit (Stratagene, La Jolla, CA). The resulting plasmid was termed pTR-

UF6. To construct the rAd-UF7 vector, the inventors substituted the rAAV cassette from pTR-UF5 for the CMV promoter fragment in pAdlox (Hardy *et al.*, 1997). The infectious rAd-UF7 was rescued essentially as described by Hardy *et al.* (1997). QC-PCR™ standard template pdl-*neo* was constructed as described earlier (Conway *et al.*, 1997). The primers used to detect rAAV were:

5′-TATGGGATCGGCCATTGAAC-3′ (SEQ ID NO:1) and

5′-CCTGATGCTCTTCGTCCAGA-3′ (SEQ ID NO:2).

5.1.1.3 PRODUCTION OF RAAV

To produce rAAV, a triple co-transfection procedure was used to introduce a rAAV vector plasmid (pTR-UF5 or pTR-UF6) together with pACG2 AAV helper (Li *et al.*, 1997) and pXX6 Ad helper (Xiao *et al.*, 1998) at a 1:1:1 molar ratio. Alternatively, rAAV vector plasmid was co-transfected with the helper plasmid pDG carrying the AAV *rep* and *cap* genes, as well as Ad helper genes, required for rAAV replication/packaging(Grimm *et al.*, 1998). Plasmid DNA used in the transfection was purified by conventional alkaline lysis/CsCl gradient protocol.

The transfection was carried out as follows: 293 cells (P33) were split 1:2 the day prior to the experiment, so that, when transfected, the cell confluence was about 75-80%. Ten 15-cm plates were transfected as one batch. To make $CaPO_4$-precipitate 180 μg of pACG2 were mixed with 180 μg of pTR-UF5 and 540 μg of pXX6 in a total volume of 12.5 ml of 0.25 M $CaCl_2$. The old media was removed from the cells and the formation of the $CaPO_4$-precipitate was initiated by adding 12.5 ml of 2 × HBS pH 7.05 (pre-warmed at 37°C) to the DNA/$CaCl_2$ solution. The DNA was incubated for 1 min, at which time the formation of the precipitate was stopped by transferring the mixture into pre-warmed 200 ml of DMEM-10% FBS. Twenty-two ml of the media was immediately dispensed into each plate and cells were incubated at 37°C for 48 h. The $CaPO_4$-precipitate was allowed to stay on the cells during the whole incubation period without compromising cell visibility. Forty-eight hours post-transfection cells were harvested by centrifugation at 1,140 × *g* for 10 min; the media was discarded unless specified otherwise. Cells were then lysed in 15 ml of 0.15 M NaCl - 50 mM Tris HCl pH 8.5 by 3 freeze/thaw cycles in dry ice-ethanol and 37°C baths. Benzonase

(Nycomed Pharma A/S, pure grade) was added to the mixture (50 U/ml final concentration) and the lysate was incubated for 30 min at 37°C. The crude lysate was clarified by centrifugation at 3,700 × *g* for 20 min and the virus-containing supernatant was further purified by iodixanol density gradient centrifugation.

5.1.1.4 CONVENTIONAL PURIFICATION PROTOCOL

rAAV was purified essentially as described earlier (Snyder *et al.*, 1996) with the following modifications. The virus pellet after the second ammonium sulfate cut was resuspended in total of 39 ml of 1.37 g/ml CsCl/PBS and subjected to an 18 h spin in 60 Ti rotor (Beckman Instruments, Somerset, NJ) at 255,600 × *g* at 15°C. The gradient was fractionated from the bottom of the tube and aliquots of the middle ten fractions were screened for rAAV by PCR™. Positive fractions were pooled, diluted to 13 ml with the CsCl solution of the same density and centrifuged in an 80 Ti rotor (Beckman Instruments, Somerset, NJ) at 391,600 × *g* for 3.5 h at 15°C. After fractionation of the gradient, the positive fractions were identified by PCR™ and pooled. The virus then was concentrated/dialyzed using the ULTRAFREE-15 centrifugal filter device BIOMAX-100K (Millipore, Bedford, MA).

5.1.1.5 PREPARATION OF IODIXANOL DENSITY GRADIENT

A typical discontinuous step gradient was formed by underlayering and displacing the less dense cell lysate with Iodixanol 5,5'[(2-hydroxy-1-3-propanediyl)-bis(acetylamino]bis[N,N'-bis[2,3dihydroxypropyl-2,4,6-triiodo-1,3-benzenecarboxamide], prepared using the 60% (w/v) sterile solution of OptiPrep (Nycomed). Specifically, 15 ml of the clarified lysate were transferred into a Quick-Seal Ultra-Clear 25 × 89 mm centrifuge tube (Beckman Instruments, Somerset, NJ) using a syringe equipped with a 1.27 × 89 mm spinal needle. Care was taken to avoid bubbles, which would interfere with subsequent filling and sealing of the tube. A two-channel variable speed peristaltic pump, Model EP-1 (Bio-Rad Laboratories, Hercules, CA), was equipped with PharMed 1.6 mm ID tubing with two additional 15 cm pieces of silicon 1.6 mm ID tubing attached at both sides of the pump head frame assembly. Each tubing line was equipped at both sides with a 100 µl microcapillary borosilicate glass pipet (Fisher, Pittsburgh,

PA). Two pipets at one end of both channels were simultaneously placed into 50 ml screw cap conical tubes (Sarstedt).

Eighteen ml of the solution (9 ml per one centrifuge tube) containing 15% iodixanol-1 M NaCl-PBS-MK (1 × PBS-1 mM $MgCl_2$, 2.5 mM KCl) were transferred into the tube and the pump was started at 4 ml/min. Both channels were primed with the iodixanol solution down to the tip of the glass pipet at the other end of the line, at which time the pump was stopped and the two pipets were inserted into two centrifuge tubes containing cell lysate. The tips of the pipets were placed at the bottom of the tubes and the pump was started to dispense the first density step. Care was taken to introduce no air bubbles into the tubing, which could disturb the density layers. With about a drop of the first density step solution left in the tube the pump was stopped and 12 ml of the second density step (6 ml per one centrifuge tube) containing 25% iodixanol-PBS-MK-Phenol Red (2.5 µl of 0.5% stock solution per ml of the iodixanol solution) were added to the same 50 ml tube. The dispensing of the second step was resumed as described above, followed by the third step, consisting of 10 ml (5 ml per one centrifuge tube) of 40% iodixanol-PBS-MK, and, finally, by 10 ml (5 ml per one centrifuge tube) of 60% iodixanol containing Phenol Red (at the same concentration as the 25% step, 0.01 µg/ml). The two microcapillary pipets then were carefully withdrawn and the tubes were filled with PBS-MK buffer. Therefore, each gradient consisted of (from the bottom up): 5 ml 60%, 5 ml 40%, 6 ml 25%, 9 ml of 15% iodixanol, the last density step containing 1 M NaCl.

Tubes were sealed and centrifuged in a Type 70 Ti rotor (Beckman Instruments, Somerset, NJ) at 350,000 × *g* for 1 h at 18°C. The Phenol Red serves to distinguish the alternating density steps. About 4 ml of the clear 40% step was aspirated after puncturing the tube on the side with a syringe equipped with an 18 gauge needle with the bevel uppermost. A similar amount was removed as 0.75 to 1 ml fractions upon harvest. The virus was further purified as described below and shown in FIG. 1.

5.1.1.6 PURIFICATION OF rAAV USING CsCl GRADIENT CENTRIFUGATION

The rAAV-containing iodixanol fraction was further purified using a conventional CsCl gradient. To form the gradient 4.5 ml of virus in iodixanol were

mixed with 35 ml of CsCl (1.37 g/ml in PBS), transferred into a Quick-Seal 25 × 89 mm centrifuge tubes (Beckman Instruments, Somerset, NJ) and centrifuged in a Type 60 rotor (Beckman Instruments, Somerset, NJ) at 214,800 × *g* overnight at 18°C. The gradient was processed as described above.

5.1.1.7 PURIFICATION OF RAAV USING HEPARIN AFFINITY CHROMATOGRAPHY

The binding, washing and elution conditions were identical for all Heparin-ligand affinity media used. Typically, a pre-packed 2.5 ml Heparin agarose Type I column (Sigma Chemical, St. Louis, MO) was equilibrated with 20 ml of PBS-MK under gravity. Alternatively, the columns were placed inside 15 ml screw cap conical tubes (Sarstedt) and spun in a low speed centrifuge Type J6-HC (Beckman Instruments, Somerset, NJ) at 200 rpm for 5 min. After each spin the flowthrough was discarded and fresh buffer was added to repeat the washing three more times. The iodixanol fraction containing virus was applied to the pre-equilibrated column under gravity and the column was washed with 10 ml of the PBS-MK buffer either under gravity or in the spin column mode. The rAAV was eluted with the same buffer containing 1 M NaCl under gravity. After applying the elution buffer, the first 2 ml of the eluant were discarded, and the virus was collected in the subsequent 3.5 ml of the elution buffer. Conventional Heparin columns that were not prepacked were loaded and eluted in a similar manner.

Alternatively, the Heparin agarose columns were placed into screw-type valves of the Visiprep Solid Phase Extraction (SPE) Vacuum Manifold (Supelco). The manifold valves were equipped with disposable Teflon valve liner guides, designed to eliminate the possibility of cross-contamination from one sample to the next in the same manifold port. Each guide was placed into 15 ml screw cap conical tube (Sarstedt) used as the collection vessel. This arrangement ensures that all surfaces that come in contact with the sample can be replaced following each chromatography. Chromatography was performed with house vacuum attached to the manifold's vacuum gauge, using less than 1 cm H_2O (-1" Hg) vacuum. Precise flow control through each column was provided by rotating the independent, screw-type valves built into the cover. Up to 12 samples could be purified simultaneously using the 12-Port Model manifold.

For the ACTI-Disk 50 filter disk chromatography, the binding of the virus in 40% iodixanol was performed in the upward fashion, *i.e.*, the flow of the solution was directed against gravity from the bottom part of the filter assembly towards the top using a peristaltic pump. Once applied, the filter assembly was turned up side down and chromatography was resumed in a regular downward fashion with gravity.

5.1.1.8 PURIFICATION OF rAAV USING HPLC CHROMATOGRAPHY

System Gold (Beckman Instruments, Somerset, NJ) hardware installed inside a biosafety cabinet was used to further purify the iodixanol fraction of virus. Only biocompatible polyetheretherketone(PEEK) tubing and fittings were used to process the samples. The chromatography was monitored at 231 nm. The virus in 4 to 5 ml of iodixanol was directly loaded onto a column using 5 ml injection loop. When the volume of the sample exceeded 5 ml, multiple successive injections were performed, each followed by washing with 5 ml (injection loop dwell volume) of mobile phase. Two different columns were successfully used to purify the virus.

5.1.1.9 UNO™ S1 CATION-EXCHANGE CHROMATOGRAPHY

UNO™ S1 column (Bio-Rad Laboratories, Hercules, CA) contained "Continuous Bed" support (bed volume 1.3 ml) derivatized with strongly acidic negatively charged-SO_3 sulfonic groups. The column was pre-equilibrated with solvent A (PBS-MK buffer). The virus sample was loaded at 0.5 ml/min and the column was washed with solvent A until the iodixanol-induced absorption was reduced to near background levels. A 0-1 M gradient of NaCl in PBS-MK was applied over 36 min (15 column volumes) and the virus was eluted as a double UV absorption peak, which was collected manually.

5.1.1.10 POROS® HE HEPARIN AFFINITY CHROMATOGRAPHY

POROS® HE/M heparin column (Boehringer Mannheim Biochemicals, Indianapolis, IN) contained particles coated with a crosslinked polyhydroxylated polymer (bed volume 1.7 ml) derivatized with heparin functional groups. The chromatography conditions were essentially the same as described for the UNO™ S1

column, except that a 0-0.5 M Na_2SO_4 in PBS-MK gradient was applied (15 column volumes) at a flow rate of 1 ml/min. A single UV absorption peak of a virus was collected manually.

5.1.1.11 PHENYL SEPHAROSE HYDROPHOBIC INTERACTION CHROMATOGRAPHY

Phenyl Sepharose (Pharmacia Biotech) is a highly cross-linked agarose (6%, spherical) that is substituted with approximately 20 μmol (low sub) or 40 μmol (high sub) of phenyl per ml of gel. The column is equilibrated with a high ionic strength buffer (salt concentration just below that employed for salting out proteins, for example 1.7 M $(NH_4)_2SO_4$) at a flow rate of about 400 cm/h. The rAAV does not interact with the Phenyl Sepharose, and is eluted in the void volume of the column, while certain contaminating proteins interact with the column and are thus retained.

5.1.1.12 CONCENTRATION OF RAAV

The virus was concentrated and desalted by centrifugation through a BIOMAX 100 K filter (Millipore, Bedford, MA) according to the manufacturer's instructions. The high salt buffer was changed by repeatedly diluting concentrated virus with Lactated Ringer's solution and repeating the centrifugation.

5.1.1.13 QUANTITATIVE COMPETITIVE PCR™ (QC-PCR™) ASSAY FOR DETERMINING RAAV PHYSICAL PARTICLES

The purified viral stock was first treated with DNase I to digest any contaminating unpackaged DNA. Ten μl of a purified virus stock was incubated with 10 U of DNase I (Boehringer Mannheim Biochemicals, Indianapolis, IN) in a 100 μl reaction mixture, containing 50 mM Tris HCl, pH 7.5, 10 mM $MgCl_2$ for 1 h at 37°C. At the end of the reaction, 10 μl of 10X Proteinase K buffer (10 mM Tris HCl, pH 8.0, 10 mM EDTA, 1% SDS final concentration) was added, followed by the addition of 1 μl of Proteinase K (18.6 mg/ml, Boehringer Mannheim Biochemicals, Indianapolis, IN). The mixture was incubated at 37°C for one h. Viral DNA was purified by phenol/chloroform extraction (twice), followed by chloroform extraction and ethanol

precipitation using 10 μg of glycogen as a carrier. The DNA pellet was resuspended in 100 μl of H_2O and dilutions were made to use in the QC-PCR™ assay.

The PCR™ reaction mixtures each contained 1 μl of the diluted viral DNA and two-fold serial dilutions of the internal standard plasmid DNA pdl-*neo*. The most reliable range of the dilution standard DNA was found to be between 1 and 100 pg. An aliquot of each reaction was then analyzed by 2% agarose gel electrophoresis, until two PCR™ products were resolved. The analog image of the ethidium bromide (EtBr)-stained gel was digitized using an ImageStore 7500 system (UVP). The densities of the target and competitor bands in each lane were measured using ZERO-Dscan Image Analysis System, version 1.0 (Scanalytics) and the respective ratios were plotted as a function of the standard DNA concentration. A ratio of 1, at which the number of viral DNA molecules equals the number of standard competitor DNA was used to derive the respective DNA concentration of the virus stock, which was the value of the line at the X intercept.

5.1.1.14 INFECTIOUS CENTER ASSAY TO DETERMINE RAAV VIRUS TITER

A modification of the previously published protocol (McLaughlin *et al.*, 1988) was used to measure the ability of the virus to infect C12 cells (Clark *et al.*, 1995), unpackage, and replicate. Briefly, C12 cells were plated in a 96-well dish at about 75% confluence and infected with Ad5 at the multiplicity of infection (M.O.I.) of 20. One μl of serially diluted rAAV to be titered was added to each well, whereupon cells were incubated for 42 h. Cells infected with rAAV-UF5 were visually scored using the fluorescence microscope. To calculate the titer by hybridization, cells were harvested and processed essentially as described earlier (McLaughlin *et al.*, 1988).

5.1.1.15 PROTEIN CONCENTRATION

The protein concentration in rAAV samples was determined using the NanoOrange™ Protein Quantitation Kit (Molecular Probes). The fluorescence in the sample was measured using the Laboratory Fluorometer Model TD-700 (Turner Designs). To estimate the purity of various virus fractions, virus was electrophoresed on

12% SDS acrylamide gels for 5 hours at 200 volts under standard buffer conditions and visualized by silver staining.

5.1.2 RESULTS

The history of the rAAV as a gene delivery vector is not without controversy. While some investigators in the field report efficient rAAV-mediated transduction, others have found strong dependence of the transduction upon Ad helper virus contaminants (Ferrari *et al.*, 1996), wt AAV contaminants (McLaughlin *et al.*, 1988; Samulski *et al.*, 1989) or mitotic or growth state of the cells being transduced (Russel *et al.*, 1994). A pseudotransduction artifact has been also reported when using crude rAAV viral preparations (Alexander *et al.*, 1997).

Some of the variability in rAAV transduction *in vivo* is undoubtedly due to the intrinsic properties of the target cells. Some targets for example, do not have the high affinity heparin proteoglycan receptor (Summerford and Samulski, 1998) and others may be incapable of efficiently synthesizing the transcriptionally active form of the rAAV genome (Ferrari *et al.*, 1996; Fisher *et al.*, 1996). However, much of the variation is also due to the methods used for purifying rAAV and the contaminants that are present in the final preparation. In general, there has been a correlation between the success of AAV vectors and the ability to generate high-titer virus free of contaminants. Under optimal conditions, as few as 10-40 infectious particles of rAAV have been found to be sufficient to transduce one cell *in vivo* (Klein *et al.*, 1998; Peel *et al.*, 1997; Lewin *et al.*, 1998).

Recent advances in design of wt AAV and mini Ad helper plasmids have made it possible to produce high-titer rAAV free of Ad contamination. Although the current transient transfection protocol for producing rAAV yields up to about 10^4-10^5 rAAV particles per cell in crude lysates, relatively little attention has been paid to downstream purification. Most laboratories continue to use sequential CsCl centrifugation. Not only does it take several weeks to complete, it often results in loss of up to 90% of virus. Furthermore, the final stock is often contaminated with cell or serum proteins, which may compromise subsequent interpretation of the data by triggering an *in vivo* immune response. While the quality of such vector preparations may be useful in some

laboratory studies, and perhaps even some additional pre-clinical applications, they are unsuitable for clinical studies using rAAV that require highly-purified vector stocks containing few if any contaminating substances.

5.1.2.1 PRODUCTION OF RAAV

To produce rAAV, the inventors used the transient Ca-phosphate-mediated co-transfection protocol, delivering three plasmids (rAAV vector pTR-UF5 (Zolotukhin *et al.*, 1996), wt AAV helper pACG2 (Li *et al.*, 1997) and Ad helper pXX6 (Xiao *et al.*, 1998)). Alternatively the helper plasmid pDG was used to provide all genes required to propagate rAAV (Grimm *et al.*, 1998). To streamline the protocol the $CaPO_4$/DNA precipitate was left in the media for the whole incubation period of 48 h. This did not compromise cell viability, but did increase the transfection efficiency at least two-fold. The transfection efficiency routinely reached 60% as judged by GFP fluorescence. After harvesting the cells, virus was extracted by freezing and thawing the cells and clarified by low speed centrifugation. The use of sonication, microfluidizing, and detergent extraction (for example, deoxycholate) did not appear to significantly increase the viral yield.

5.1.2.2 IODIXANOL DENSITY STEP GRADIENT

Tamayose and co-authors have recently described a Cellulofine sulfate chromatography protocol as a method of purification and concentration of the rAAV from the crude lysate (Tamayose *et al.*, 1996). However, using this method the inventors repeatedly failed to quantitatively bind rAAV in the crude lysate. It appeared that rAAV and cell proteins could form aggregates in lysate. These complexes fail to display uniform biochemical properties, which makes it difficult to develop a purification strategy. It also leads to poor recovery of the virus at all purification stages. Finally, this nonspecific interaction results in contamination with Ad proteins even after several rounds of CsCl gradient centrifugation.

The bulk purification of the crude is, therefore, a very important stage in rAAV purification. In the conventional protocol it is usually done by stepwise NH_4SO_4 precipitation (Snyder *et al.*, 1996). Although this simple procedure could be used to

concentrate the virus, the NH_4SO_4 precipitation makes a poor purification step. The residual ammonium sulfate salt in the protein pellet also interferes with subsequent ion-exchange chromatography procedure. The dialysis at this purification stage leads to the aggregation and precipitation of proteins, resulted in poor recovery of rAAV. The combination of NH_4SO_4 precipitation and hydrophobic interaction Phenyl-sepharose chromatography was also employed, although this approach also failed to produce a purified virus without sizeable loss of the infectivity. To solve the problem, the inventors introduced a new step into rAAV production protocol - iodixanol density gradient, which efficiently pre-purifies the virus from the crude cell extract.

Iodixanol is an iodinated density gradient media originally produced as an X-ray contrast compound for injection into humans and, as such, it has been subjected to rigorous screening and clinical testing. It is non-toxic to cells; indeed; cells can be grown in the presence of 30% iodixanol for 3 days with no subsequent effect on the viability of cells. Unlike CsCl and sucrose gradients commonly used for fractionating macromolecules, iodixanol solutions can be made iso-osmotic at all densities. This property makes iodixanol an ideal media for analysis and downstream purification steps. Because of its non-ionic and inert nature, electrophoretic analysis and virus infectivity assays can be carried out on gradient fractions directly in the presence of iodixanol. Since the viscosity of iodixanol solutions is also lower than those of sucrose of the same density, it is also possible to use the iodixanol fractions directly in subsequent chromatography purification steps without dialysis or dilution.

As mentioned earlier, rAAV aggregates with proteins in cell lysate, which changes its buoyant density and makes it distribute along the whole length of the gradient. This confounded initial attempts to purify rAAV using discontinuous iodixanol gradients. The inventors, however, devised a preformed multiple density step gradient that included 1 M NaCl in the first 15% step. The inventors reasoned that high concentrations of salt would destabilize ionic interactions between macromolecules, and reduce aggregation of rAAV particles with cell lysate material. High salt concentrations were excluded, however, from the rest of the iodixanol gradient in order to permit the virus to band under iso-osmotic conditions, which was important for subsequent purification steps.

The banding density of the purified rAAV-UF5 was approximately 1.415 g/ml, which corresponded to an about 52% concentration of iodixanol. The inventors therefore incorporated a 40% iodixanol step (1.21 g/ml) as a cut-off target step to accommodate rAAV/protein complexes trailing at slightly lower densities, followed by a 60% step that acts as a cushion for any rAAV containing a full length genome. To locate the 40% density step after the centrifugation, the inventors stained the upper 25% and lower 60% density steps with Phenol Red dye.

A plot of the refractive index at the end of a 1 hour run is shown in FIG. 2. rAAV was distributed through the 40% density step and could be recovered by inserting a syringe needle at about 2 mm below the 60%-40% density junction. The bulk of the rAAV bands within the 40% density step (fractions 5-8, FIG. 2). The heavy band at the 40%-to-25% density interface consisted mostly of cellular proteins and contained less than 5% of input rAAV, as judged by FCA. A small amount of the rAAV also bands at the 40%-60% density junction (fraction 5, FIG. 2). Approximately 75-80% of the rAAV in the crude lysate is recovered in the iodixanol fraction (Table I).

The nucleic acid/protein ratio in the rAAV-UF5 is different from wt AAV because of the size of the DNA packaged: 3400 bases in rAAV-UF5 vs. 4680 in wt AAV, or approximately 73% of the wt AAV size. Using the same protocol with no modifications, the inventors purified about 15 different rAAV vectors with the size of the packaged genome ranging from 3 to 5 kb. Regardless of the size, there was no substantial difference in the banding pattern of rAAV. Therefore, no modification of the protocol, accounting for the size of rAAV genome, is required.

To determine the resolving capacity of the iodixanol gradient, the inventors loaded into separate tubes virus-containing lysates obtained from 1.56×10^8 cells, 3.12×10^8 cells, or 4.68×10^8 cells, corresponding to 5, 10 or 15 large 15-cm culture plates, respectively. rAAV was aspirated as described, and aliquots of each sample that were equivalent to 1.73×10^6 cells were subjected to SDS-gel electrophoresis. The three viral capsid proteins VP1, VP2, and VP3 constituted the major protein species at all concentrations, even in the tube with the most concentrated lysate. In further studies, however, the inventors routinely loaded the lysate from 10 plates per gradient. In the scale-up protocol the viral lysate from 3.1×10^9 cell (one hundred 15-cm plates) could

be pre-purified in one Ti 70 rotor during single one-hour run. Such run could potentially produce 10^{14} virus particles, or about 10^{12} infectious particles.

It is also possible to concentrate and purify rAAV from the media supernatant using the iodixanol gradient (FIG. 1). To do this, the inventors precipitated the bulk of proteins and virus from the media using conventional precipitation with 50% ammonium sulfate. The pellet was further resuspended in PBS-MK buffer and subjected to regular iodixanol gradient purification. This procedure, however, is optional, since at the time of harvesting cells 48 h post-transfection the majority of the virus (about 90%) (Grimm *et al.*, 1998; Xiao *et al.*, 1998) is associated with cell pellet.

Iodixanol proved to be an excellent bulk purification method that accomplished at least three things. Crude lysate was purified by at least 100 fold and when Ad helper was present, Ad contamination was reduced by a factor of 100. The virus was concentrated in a non-ionic and relatively non-viscous medium that could be loaded on virtually any kind of chromatographic matrix. Finally, iodixanol prevented rAAV aggregation and the associated loss of virus that accompanies most other bulk purification and column chromatography methods. Typically, 70-80% of the starting infectious units are recovered following iodixanol gradient fractionation (Table I), and unlike other purification methods, this step was more reproducible.

5.1.2.3 METHODS FOR SEPARATING ADENOVIRUS FROM RAAV

The production of rAAV by transient co-transfection with a mini Ad plasmid is an efficient but laborious protocol. Although it eliminates the problem of removing Ad virus from the rAAV crude lysate, it requires up to 1 mg of plasmid DNA (combined), for transfection of 10 plates. Furthermore, it is not readily amenable to the industrial large-scale production using suspension cell culture. An ideal production system would consist of rAAV proviral cell line, induced to rescue and replicate by infection with a helper virus carrying the rep/cap functions, such as an HSV amplicon (Conway *et al.*, 1997), or rAd. For downstream purification the HSV helper could be separated from rAAV by simple filtration due to the considerable size difference (Conway *et al.*, 1997) or by exposure to high salt. In case of Ad, rAAV is usually separated by a combination of CsCl gradient centrifugation and heat treatment, both approaches suffering from

drawbacks. The inventors were interested in whether the newly introduced iodixanol gradient could be combined with ion exchange chromatography columns (FIG. 1) to separate rAAV and Ad without heat inactivation of the latter.

To address this issue, the inventors prepared pTR-UF6. This construct is identical to pTR-UF5 except that the *gfp* cDNA contains a Tyr-145-Phe mutation in the pTR-UFB background described previously (Zolotukhin *et al.*, 1996) and fluoresces blue. At the time of co-transfection of 293 cells with pTR-UF6 and pDG, they were also infected with rAd-UF7 at an M.O.I. of 10. rAd-UF7 is a recombinant E1-E3 deleted Ad vector that contains the *gfp/neo* cassette from pTR-UF5 and fluoresces green. The use of these two constructs together permitted the monitoring of infections with rAAV (pTR-UF6) and rAd (rAD-UF7) in the same GFP fluorescence assay by scoring for blue or green cells. Cells infected with rAAV fluoresce blue, while cells infected with rAd (or both viruses) fluoresce green.

Cells transfected with pTR-UF6 and infected with rAD-UF7 were processed exactly as described for the purification of rAAV using iodixanol gradient. The gradient was fractionated after puncturing the bottom of the tube and 25 μl aliquots from each fraction were subjected to the SDS acrylamide gel electrophoresis and Western analysis with polyclonal anti-Ad antibodies. More than 99% of the Ad, as judged by the fluorescence assay, banded in the gradient with densities lower than 1.4 g/ml. rAAV, on the other hand, banded in fractions 5-8 (FIG. 2; densities of 1.4 to 1.415 g/ml) and were clearly separated from the Ad. The crude lysate contained 4.5×10^{10} pfu of rAd-UF7 (as determined by the fluorescence cell assay). After the iodixanol gradient the titer of the rAd-UF7 dropped to 4.2×10^{8} pfu. Although iodixanol gradient efficiently separated rAAV/rAd mixture and reduced the titer of rAd by two logs, further purification steps were studied to further separate rAd.

To reduce Ad contamination further, column chromatography was used as a second step in purification following the iodixanol gradient. To compare the effectiveness of the various column chromatography steps, rAAV-UF5 was prepared from 1×10^{9} cells as described above, using pDG helper plasmid. The crude lysate was purified using the iodixanol step gradient and virus-containing fractions were pooled. The pooled fractions were then split into equal portions and virus was purified using

four different methods illustrated in FIG. 1: (1) CsCl density gradient centrifugation, (2) heparin affinity chromatography, (3) HPLC heparin affinity chromatography, and (4) HPLC cation exchange chromatography. The purification steps were monitored by measuring rAAV titers, both physical and infectious, as well as protein concentration in virus samples generated by each purification step (Table 1). For purposes of comparison, a second batch of virus was purified by the commonly used method of ammonium sulfate precipitation followed by two consecutive CsCl gradients (Table 1).

5.1.2.4 HEPARIN AFFINITY CHROMATOGRAPHY

Heparinized supports have been successfully used for the purification of many heparin-binding macromolecules, including viruses such as CMV (Neyts *et al.*, 1992). Heparin is the glucosaminoglycan moiety covalently bound to the protein core of proteoglycans (PG). It is closely related to heparan sulfate (HS), which constitutes the glycosaminoglycan (GAG) chain of the HS proteoglycan (HSPG). The latter has been shown to be a cell surface receptor mediating AAV infection (Summerford and Samulski, 1998). Covalent binding of heparin molecules to the matrix through its reducing end mimics the orientation of the naturally occurring GAGs (Nadcarni *et al.*, 1994). To take advantage of the structural similarities between heparin and HS, heparin affinity chromatography was utilized to further purify rAAV.

Heparin is a heterogeneous carbohydrate molecule composed of long unbranched polysaccharides modified by sulfations and acetylations. The degree of sulfation strongly correlates with the virus-binding capacity of HS (Herold *et al.*, 1995). It, therefore, was anticipated that heparinized matrices from different vendors would display different affinity towards rAAV. Thus, to develop the method the inventors tested several heparin ligand-containing media, including ACTI-Disk 50 (Arbor Technologies, Inc.), Affi-Gel Heparin Gel (Bio-Rad Laboratories, Hercules, CA), Heparin-Agarose Type I, Heparin-Agarose Type II-S and, finally, Heparin Agarose Type III-S, the last three manufactured by Sigma Chemical, St. Louis, MO. Although ACTI-Disk 50 was found to bind rAAV quantitatively, it was not used in the actual production protocol, since the manufacturer discontinued this product. Affi-Gel Heparin gel and Heparin Agarose Type III-S columns failed to bind at least 50% of the virus and,

therefore, were excluded from further consideration. Heparin-Agarose Type I and Heparin-Agarose Type II-S pre-packed 2.5 ml columns were efficient in retaining and subsequently releasing rAAV. The Type II-S column, however, was found to be less selective, binding many cell proteins along with the virus. The Heparin-Agarose Type I was the best among those tested in terms of binding specificity and virus recovery, and was used in further studies as described below.

rAAV-UF5 purity at different stages of purification was analyzed by silver stained SDS acrylamide gel electrophoresis. The iodixanol-purified fraction prepared from cells transfected with pTR-UF5/pDG was directly applied to a Heparin-agarose Type I column and eluted with 1 M NaCl as described above. The 1 M NaCl fraction contained 35% of the input rAAV (Table 1), which was more than 95% pure, as judged by the silver stained SDS gel analysis. The Heparin-agarose affinity fraction of rAAV was consistently more pure than virus purified by the conventional protocol using ammonium sulfate, followed by two rounds of CsCl gradient centrifugation.

TABLE 1

rAAV-UF5 TITERS AND PROTEIN CONCENTRATION AT DIFFERENT STEPS OF THE PURIFICATION PROTOCOL[a]

	Purification step	Particles by dot blot, 10^{11}	Particles by QC PCR™, 10^{11}	Infectious particles by ICA, 10^9	Infectious particles by FCA, 10^9	Particle-to-infect. ratio[b]	Infect. Units per Cell[c]	Particle recovery, %[d]	Infectious particles yield, %[c]
1	3× Frz./thaw lys.	57	103	69	62.7	90.8	209	100	100
2	Iodixanol	44	82	32.3	51	86	170	76	81
3	Iodixanol/CsCl	5.7	2.5	4	3.6	158	12	8.4	6
4	Iodixanol/ Heparin agarose	20	63	32	35	56	117	35	56
5	Iodixanol/HPLC POROS® HE/M	15	16	12	20	73	67	26	32
6	Iodixanol/HPLC UNO™ S1	19	13	20	20	95	67	33	32
7	2 × CsCl	7	6	4.8	2.9	241	1		

[a]The yield of rAAV and protein concentrations in each row are normalized to 3×10^8 cells (ten 15 cm plates).

[b]The particle-to-infectivity ratio was calculated using numbers obtained by dot blot assay and FCA.

[c]Calculated using FCA

[d]Calculated using dot blot assay

5.1.2.5 PURIFICATION OF rAAV USING HPLC CHROMATOGRAPHY

Two different HPLC columns, UNO™ S1 and POROS® HE/M heparin, were tested to further purify the iodixanol fraction of rAAV (FIG. 3A and FIG. 3B). Both columns were successful in removing most of the protein contaminants that remained in the iodixanol fraction. The UNO™ S1 purification yielded rAAV-UF5 that was more than 99% pure as judged by SDS acrylamide electrophoresis. Curiously two rAAV peaks were obtained during UNO™ S1 fractionation (FIG. 3B). Both peaks were found to contain rAAV that was indistinguishable both by SDS-gel electrophoresis analysis and by GFP fluorescence assay.

Both HPLC columns used in the study produced rAAV, comparable both in terms of purity and yield. POROS® HE/M column produced a slightly more infectious virus, which is not surprising, since the purification process involves binding to heparin, structurally similar to native AAV receptor. From the practical point of view, HPLC Heparin column is easier to use, it allows for a higher back pressure and, therefore, higher flow rates. It also cleared off iodixanol in the flowthrough much faster (30 min vs. 45 min, FIG. 3A and FIG. 3B). Finally, it performed consistently, producing essentially identical chromatograms for as many as 10 different virus runs (the maximum tried). This kind of performance is very important for GMP validation of a production protocol.

Having established that both the UNO™-S1 and POROS® HE/M columns could be used successfully to purify rAAV, the inventors determined whether they also would separate adenovirus from AAV in preparations grown in the presence of Ad virus. To this end, the rAAV-UF6/rAd-UF7 mixture (described above) was purified by iodixanol gradient centrifugation and then subjected to HPLC POROS® HE/M affinity chromatography under the conditions described above. The majority of the contaminating rAD-UF7 was found in the flowthrough. The peak of rAAV-UF6 contained 8×10^5 pfu of rAd, as compared to 3×10^{10} infectious units (IU) of rAAV-UF6 particles. Thus, the rAd titer in the mixed stock was decreased from 4.5×10^{10} in the crude lysate, to 4.2×10^8 in the iodixanol fraction, to the 8×10^5 after the HPLC affinity step. The same degree of separation was achieved with conventional chromatography using Heparin-agarose Type I. In contrast, UNO™ S1 cation exchange

chromatography failed to separate rAd and rAAV. Additional data indicates that the mixture could be further separated using UNO™ Q1 anion exchange HPLC column.

5.1.2.6 IODIXANOL PLUS CSCL DENSITY GRADIENT

The use of an iodixanol step gradient followed by a CsCl gradient was compared with the conventional use of two consecutive CsCl gradients (Table 1). The iodixanol plus CsCl protocol produced rAAV with purity that was comparable to iodixanol followed by column chromatography. Both methods produced rAAV that was significantly purer than virus that had undergone only two consecutive CsCl gradients. However the rAAV produced by conventional CsCl purification generally had higher particle-to-infectivity ratios (200-1000) than the methods described herein (Table 1). Furthermore, rAAV that had undergone even one CsCl centrifugation (Table 1, row 3) had a higher particle-to-infectivity ratio than virus that had not been exposed to CsCl (Table 1, rows 4-6). These observations suggest that treatment with CsCl leads to reduced viral infectivity.

Taken together, the data show that a combination of iodixanol plus heparin affinity chromatography (either heparin agarose or heparin HPLC) has unique advantages as a method for purifying rAAV. To compare this method directly with the current method for rAAV purification, a crude rAAV virus stock was prepared and the two methods of purification were compared side by side with the same starting material, *i.e.*, ammonium sulfate fractionation followed by two CsCl gradients vs. iodixanol fractionation followed by heparin agarose chromatography (Table 2). A significant increase in recovery of vector was seen with the iodixanol/heparin protocol, resulting from an approximately 5 fold higher recovery of vector particles and over a 100 fold increase in infectivity. Expressed as the ratio of infectious particles to total particles, the virus prepared by CsCl centrifugation had a significantly higher ratio than virus prepared by the iodixanol protocol, approximately 1700 vs 67 (Table 1). Furthermore, as expected, the virus prepared by the conventional CsCl method was significantly less pure than that prepared by iodixanol/heparin.

TABLE 2

COMPARISON OF IODIXANOL/HEPARIN AGAROSE AND NH_4SO_4/CSCL PURIFICATION

Purification	Particles by QC PCR™ 10^{11}	Infectious Units by FCA 10^9	Particle-to-Infectivity Ratio
NH_4SO_4/2 × CsCl	0.2	0.012	1667
Iodixanol/Heparin Agarose	1.0	1.5	67

Following iodixanol gradient fractionation, rAAV was sufficiently free of cellular protein such that it displayed reproducible chromatographic behavior during subsequent purification. Two types of columns have been identified that are capable of purifying rAAV approximately 10-100 fold, heparin sulfate and sulfate cation exchange resins. Both types of material could be used successfully in the HPLC format and displayed recoveries of 40-70 % (Table 1). By contrast, CsCl purification of the iodixanol fraction resulted in the recovery of as little as 7 % of the starting infectious units. Therefore, methods have been identified that increase the yield of infectious rAAV by at least ten-fold in this step.

Importantly, neither iodixanol fractionation nor column chromatography on heparin or cation exchange resins had a significant effect on the particle-to-infectivity ratio of rAAV. In contrast, the use of CsCl gradients generally had the detrimental effect of increasing the particle-to-infectivity ratio. If CsCl were the only method used for purification, the increase could be dramatic. The particle-to-infectivity ratios of rAAV that had been purified by iodixanol and heparin affinity ranged from as low as 26 to 73 (Table 1). The particle-to-infectivity ratio of rAAV that had been purified by iodixanol and CsCl was approximately 158 (Table 1). Finally, virus that had been purified only by ammonium sulfate fractionation and sequential CsCl centrifugation had particle-to-infectivity ratios of 241 to 1600 (Tables 1 and 2).

Thus, the inventors have identified methods for producing pure, high titer rAAV that are significantly better in yield and quality of material produced than the conventional methods currently in use. One of these methods, an iodixanol step gradient followed by a conventional heparin agarose column has consistently resulted in

overall recoveries of greater than 50% of the starting material, and produces virus that is better than 99% pure, with particle-to-infectivity ratios less than 100:1. Furthermore, the method allows the purification of rAAV in one day.

5.1.2.7 IODIXANOL PLUS HEPARIN AFFINITY AND PHENYL SEPHAROSE CHROMATOGRAPHY

The use of hydrophobic interaction chromatography (HIC) in the further purification of rAAV was investigated using Phenyl Sepharose gel (Pharmacia Biotech). rAAV that was initially purified on an iodixanol gradient and Heparin-Sepharose chromatography, as described above, was loaded onto a Phenyl Sepharose column. The rAAV does not interact with the Phenyl Sepharose, and is present in the supernatant (bulk purification) or elutes in the void volume (column purification). Several proteins present in the rAAV sample from the iodixanol/heparin purification, in particular several proteins between 45 and 60 kDa and large proteins or aggregates of greater than about 116 kDa, interacted with the Phenyl Sepharose, and were retained in the gel.

5.1.2.8 CHARACTERIZATION OF THE PURIFIED RAAV

5.1.2.8.1 RAAV TITERING

An important index of virus quality is the ratio of the physical particles to the infectious particles in a given preparation. To characterize the purification steps and the quality of the virus obtained using different methods, the inventors used two independent assays to titer both physical and infectious rAAV particles. For physical particle titers, the inventors used a conventional dot-blot assay and a QC PCR™ assay. For the infectivity titer, the inventors used fluorescence cell assay (FCA), which scored for the expression of GFP, and infectious center assay (ICA). In order to avoid adventitious contamination of rAAV stocks with wt AAV, the use of wt AAV was eliminated from all protocols, including the ICA. For the ICA and FCA, the inventors used the C12 cell line (Clark *et al.*, 1995), which contains integrated wt AAV *rep* and *cap* genes. Ad5, which was used to co-infect C12 along with rAAV, was titered using the same C12 cell line in a serial dilution cytopathic effect (CPE) assay. The amount of

Ad producing well-developed CPE in 48 h on C12 cells was used to provide helper function in both the ICA and FCA assays.

Both physical particle titers and infectious particle titers, each obtained by two independent titering methods, were generally in agreement, differing in most cases by a factor of 2 or less (Table 1). The particle-to-infectivity ratios ranged from 56 to 240. rAAV purified by iodixanol/Heparin affinity chromatography had the lowest (Table 1, Rows 4 and 5). rAAV purified exclusively by using CsCl centrifugation had the lowest infectivity, which is probably due to the deleterious effect of hyper-osmotic conditions of a gradient (Table 1, compare crude lysate in Row 1 and CsCl-purified virus, Rows 3 and 7). In extreme cases some CsCl-grade rAAV preparations had the respective ratios of 1000 or higher, while HPLC/heparin affinity purified stocks had ratios as low as 26.

5.1.2.8.2 rAAV Recovery

To compare the effectiveness of the column chromatography steps in a single study, rAAV-UF5 has been prepared from fifty 15 cm plates as described, using the pDG helper plasmid. The crude lysate was pre-purified using 5 tubes of iodixanol gradient and virus-containing fractions were pooled. The pooled fractions were then split and virus was purified using 5 different methods (FIG. 1). The inventors monitored the purification steps by measuring rAAV titers, both physical and infectious, as well as protein concentration in virus samples (Table 1). The total amount of the virus in the crude lysate was assumed to represent a 100% of virus, available for purification. The iodixanol gradient centrifugation step reduces the amount of protein in the sample 1,577 fold. Therefore, the degree of purification achieved at the first purification step is 1,214 times, if one takes into account the yield of viral particles.

5.1.2.8.3 Comparison of Helper Plasmids

Recently three independent groups described the construction of a new generation of helper plasmids, pXX6, (Xiao *et al.*, 1998), pACG2 (Li *et al.*, 1997), pDG (Grimm *et al.*, 1998) and pAdΔ (Salvetti, 1998), which modulate the synthesis of Rep78/68 and supply Ad helper functions from non-infectious, non-packagable mini-Ad plasmids. The inventors had the opportunity to evaluate side-by-side two systems,

namely pACG2/pXX6 vs. pDG. In the studies both systems performed well, the pACG2/pXX6 yielding about 10^{15} particles of rAAV per ml of the purified stock per starting size run of ten 15 cm plates, with the "wild-type" replication-competent AAV contamination at about 3 to 4 logs lower than recombinant virus titer. pDG, on the other hand, produced somewhat lower titers, 3-4 $\times$ 10^{12} particles/ml, with no detectable "wt" AAV contamination, as judged by the ICA, done on 293 cells with Ad5 helper.

In conclusion, the developed protocol is very efficient, routinely yielding 30-40% of the total virus in the original crude lysate. The recovery of the virus in conventional CsCl protocol in the studies never exceeded 10%. The infectivity of iodixanol/heparin-purified virus is exceptional with the particle-to infectivity ratios consistently lower than 1:100. On the other hand, the respective ratio for the CsCl-purified virus stays within 1:200-1000 range. The inventors, therefore developed the method which increases the overall yield of the infectious rAAV by at least ten-fold.

In short, the inventors have developed protocols for the purification of rAAV that are versatile and efficient. rAAV, purified by any of these approaches, is highly infectious and practically free of contaminants. It is affordable for an average research lab (iodixanol/Heparin-agaroseprotocol), or it could be adopted for a GMP production facility (iodixanol/HPLC chromatography protocol). The use of such techniques make broader gene therapy applications of rAAV feasible.

6.0 REFERENCES

The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

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All of the compositions and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of

preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

WHAT IS CLAIMED IS:

1. A method of isolating a recombinant adeno-associated virus, comprising applying a sample containing recombinant adeno-associated virus to an iodixanol gradient, and collecting said recombinant adeno-associated virus from said gradient.

2. The method of claim 1, wherein said iodixanol gradient is a discontinuous gradient.

3. The method of claim 2, wherein said iodixanol gradient comprises an about 15% iodixanol step, an about 25% iodixanol step, an about 40% iodixanol step, and an about 60% iodixanol step.

4. The method of claim 3, wherein said recombinant adeno-associated virus is collected from said 40% iodixanol step.

5. The method of claim 3, wherein said 15% iodixanol step further comprises about 1 M NaCl.

6. The method of claim 1, wherein said iodixanol gradient is subjected to centrifugation after applying said sample.

7. The method of claim 1, further comprising contacting said recombinant adeno-associated virus with a matrix comprising heparin, under conditions

effective to permit binding of said virus to said matrix, removing non-bound species from said matrix, and eluting said virus from said matrix.

8. The method of claim 7, wherein said matrix comprises heparin agarose type I or heparin agarose type II-S.

9. The method of claim 7, wherein said matrix is comprised within an HPLC column.

10. The method of claim 7, wherein said virus is eluted from said matrix with a solution comprising about 1 M NaCl.

11. The method of claim 1, further comprising contacting said recombinant adeno-associated virus with a hydrophobic matrix, under conditions effective to permit interaction of hydrophobic species with said hydrophobic matrix, and collecting the non-interacting virus from said hydrophobic matrix.

12. The method of claim 11, wherein said hydrophobic matrix comprises phenyl groups.

13. The method of claim 12, wherein said hydrophobic matrix is phenyl-sepharose.

14. The method of claim 1, further comprising applying said recombinant adeno-associated virus to a cesium chloride equilibrium density gradient, and collecting said recombinant adeno-associated virus from said gradient.

15. The method of claim 1, further comprising contacting said recombinant adeno-associated virus with at least a first ion exchange chromatography medium, under conditions effective to permit interaction of said virus with said medium, removing non-interacting species from said medium, and eluting said virus from said medium.

16. The method of claim 1, wherein said sample further comprises a virus.

17. The method of claim 16, wherein said sample further comprises an adenovirus.

18. The method of claim 1, wherein said sample further comprises at least a first polypeptide or protein.

19. The method of claim 1, wherein said sample further comprises a cell extract or a growth medium.

20. A method of isolating a recombinant adeno-associated virus, comprising the steps of:

a) centrifuging a sample containing recombinant adeno-associated virus through an iodixanol gradient;

b) collecting from said iodixanol gradient at least a first fraction comprising said recombinant adeno-associated virus;

c) contacting said at least a first fraction comprising said recombinant adeno-associated virus with a matrix comprising heparin, under conditions effective to permit binding of said virus to said matrix;

d) removing non-bound species from said matrix; and

e) eluting said virus from said matrix.

21. A method of isolating a recombinant adeno-associated virus, comprising the steps of:

a) centrifuging a sample containing recombinant adeno-associated virus through an iodixanol gradient;

b) collecting from said iodixanol gradient at least a first fraction comprising said recombinant adeno-associated virus;

c) contacting said at least a first fraction comprising said recombinant adeno-associated virus with a matrix comprising heparin, under conditions effective to permit binding of said virus to said matrix;

d) removing non-bound species from said matrix;

e) eluting said virus from said matrix;

f) contacting the eluted virus with a hydrophobic matrix, under conditions effective to permit interaction of hydrophobic species with said hydrophobic matrix; and

g) collecting the non-interacting virus from said hydrophobic matrix.

22. A method for reducing or eliminating adenovirus from a recombinant adeno-associated virus composition contaminated with adenovirus, comprising applying a sample containing recombinant adeno-associated virus and adenovirus to an iodixanol gradient, and collecting from said gradient at least a first fraction comprising said recombinant adeno-associated virus.

23. A method of producing a recombinant adeno-associated virus having a particle-to-infectivity ratio of less than about 100 to 1, comprising the steps of:

a) centrifuging a sample containing recombinant adeno-associated virus through an iodixanol gradient;.

b) collecting from said iodixanol gradient at least a first fraction comprising said recombinant adeno-associated virus;

c) contacting said at least a first fraction comprising said recombinant adeno-associated virus with a matrix comprising heparin, under conditions effective to permit binding of said virus to said matrix;

d) removing non-bound species from said matrix; and

e) eluting said virus from said matrix.

24. Recombinant adeno-associated virus, prepared by applying a sample containing recombinant adeno-associated virus to an iodixanol gradient, and collecting said recombinant adeno-associated virus from said gradient.

25. A kit comprising, in a suitable container, iodixanol, a matrix comprising heparin and instructions for isolating recombinant adeno-associated virus.

26. The kit of claim 25, wherein said iodixanol is formulated as an iodixanol gradient.

27. The kit of claim 25, wherein said matrix comprises heparin agarose type I or heparin agarose type II-S.

28. The kit of claim 25, further comprising a hydrophobic matrix.

29. The kit of claim 28, wherein said hydrophobic matrix comprises phenyl groups.

30. The kit of claim 29, wherein said hydrophobic matrix is phenyl-sepharose.

rAAV Purification Flow Chart

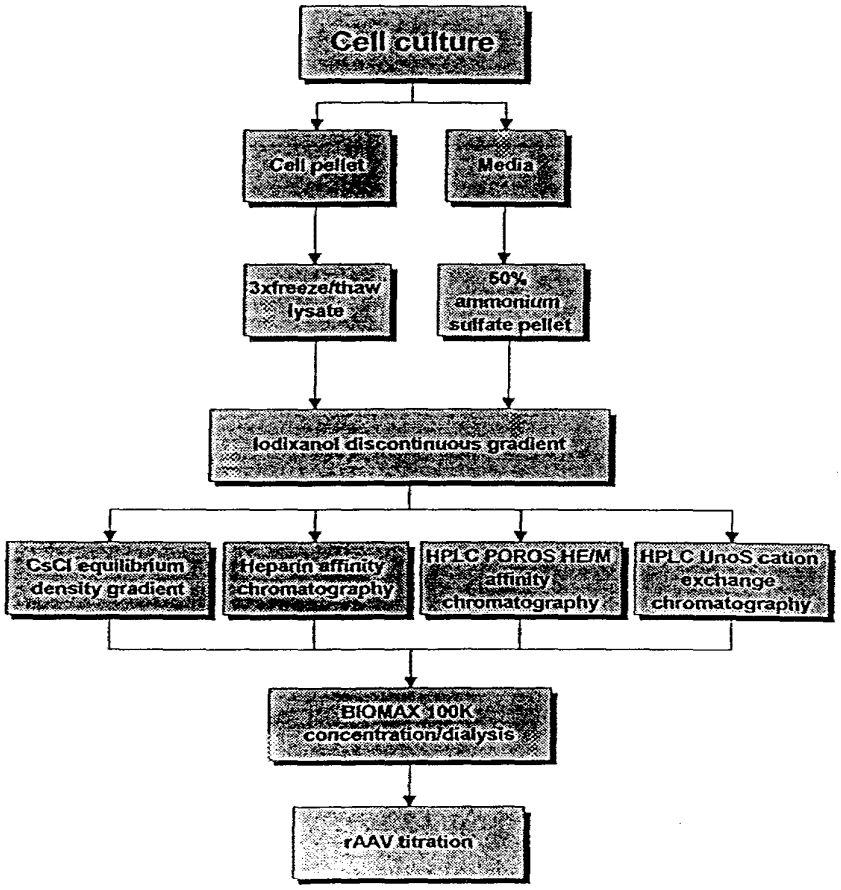

FIG. 1

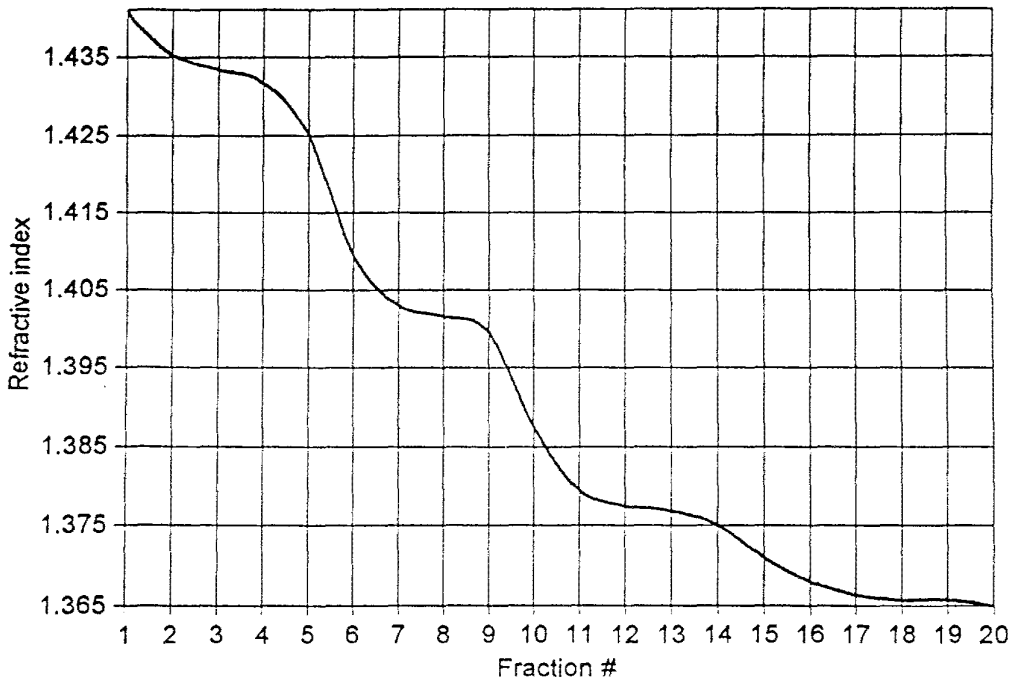
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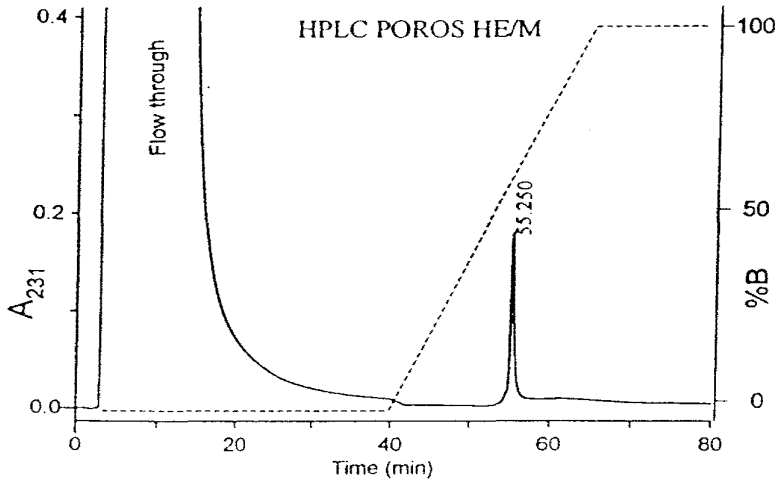
FIG. 2

FIG. 3A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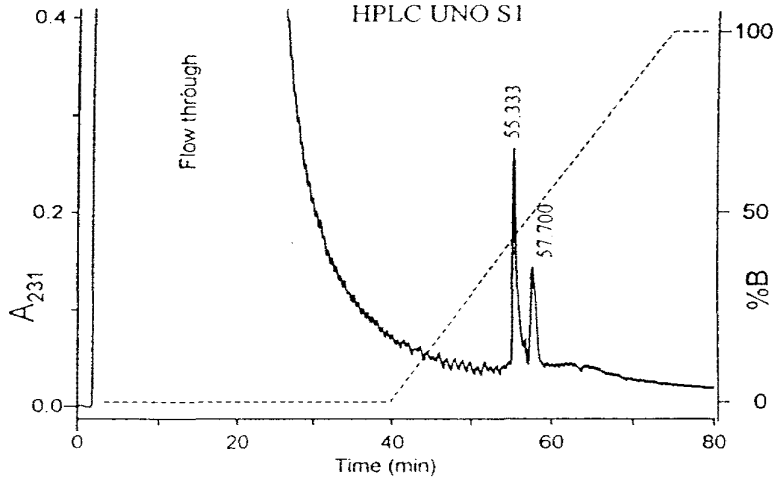
Time (min)

FIG. 3B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Time (min)

SEQUENCE LISTING

<110> ZOLOTUKHIN, SERGEI
BYRNE, BARRY J.
MUZYCZKA, NICHOLAS

<120> METHOD OF PREPARING RECOMBINANT ADENO-ASSOCIATED VIRUS COMPOSITIONS

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INTERNATIONAL SEARCH REPORT

Interr ›nal Application No
PCT/US 99/11945

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/86 C12N7/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	ZOLOTUKHIN, S. ET AL: "Recombinant adeno -associated virus purification using novel methods improves infectious titer and yield." GENE THERAPY, (JUNE, 1999) VOL. 6, NO. 6, PP. 973-985. , XP002116593 ---	
P,X	HERMENS, W. T. J. M. C. ET AL: "Purification of high titer adeno - associated virus vectors for gene delivery in the brain." SOCIETY FOR NEUROSCIENCE ABSTRACTS, (1998) VOL. 24, NO. 1-2, PP. 1308. MEETING INFO.: 28TH ANNUAL MEETING OF THE SOCIETY FOR NEUROSCIENCE, PART 2 LOS ANGELES, CALIFORNIA, USA NOVEMBER 7-12, 1998 , XP002116594 abstract --- -/--	1,6,24

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 September 1999	12/10/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Espen, J

2

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INTERNATIONAL SEARCH REPORT

Interr ›nal Application No
PCT/US 99/11945

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 00524 A (GUILLAUME JEAN MARC ;BLANCHE FRANCIS (FR); RHONE POULENC RORER SA) 8 January 1998 (1998-01-08) page 12 page 17; claim 31 -----	1,6,15, 18,19

2

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

ormation on patent family members

Intern nal Application No
PCT/US 99/11945

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9800524 A	08-01-1998	FR 2750433 A	02-01-1998
		AU 3447097 A	21-01-1998
		CA 2258158 A	08-01-1998
		CZ 9804383 A	17-03-1999
		NO 986202 A	15-02-1999

Form PCT/ISA/210 (patent family annex) (July 1992)

Electronic Patent Application Fee Transmittal

Application Number:	12661553
Filing Date:	19-Mar-2010
Title of Invention:	Compositions and methods to prevent AAV vector aggregation
First Named Inventor/Applicant Name:	John Fraser Wright
Filer:	Roberta L. Robins/Denise Vaillancourt
Attorney Docket Number:	0800-0045.01

Filed as Large Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Extension - 3 months with $0 paid	1253	1	1400	1400

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
Submission- Information Disclosure Stmt	1806	1	180	180
		Total in USD ($)		**1580**

Electronic Acknowledgement Receipt

EFS ID:	20729135
Application Number:	12661553
International Application Number:	
Confirmation Number:	4726
Title of Invention:	Compositions and methods to prevent AAV vector aggregation
First Named Inventor/Applicant Name:	John Fraser Wright
Customer Number:	20855
Filer:	Roberta L. Robins/Denise Vaillancourt
Filer Authorized By:	Roberta L. Robins
Attorney Docket Number:	0800-0045.01
Receipt Date:	18-NOV-2014
Filing Date:	19-MAR-2010
Time Stamp:	14:42:46
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Credit Card
Payment was successfully received in RAM	$1580
RAM confirmation Number	952
Deposit Account	505826
Authorized User	ROBINS, ROBERTA L.

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

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Multipart Description/PDF files in .zip description

Document Description	Start	End
Transmittal Letter	1	1
Extension of Time	2	2
Information Disclosure Statement (IDS) Form (SB08)	3	6
Applicant Arguments/Remarks Made in an Amendment	7	16

Warnings:					
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		Total Files Size (in bytes):	3220836		

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New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

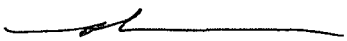
Application Number	12/661,553
Filing Date	March 19, 2010
First Named Inventor	John Frasier Wright et al.
Art Unit	1653
Examiner Name	Satyendra K. Singh
Total Number of Pages in This Submission: 16	Attorney Docket Number: 0800-0045.01

ENCLOSURES *(Check all that apply)*

- ☐ Fee Transmittal Form
  - ☐ Fee Attached
- ☒ Amendment/Reply (10 pgs)
  - ☐ After Final
  - ☐ Affidavits/declaration(s)
- ☒ Extension of Time Request (1 pg)
- ☐ Express Abandonment Request
- ☒ Information Disclosure Statement (4 pgs)
- ☐ Certified Copy of Priority Document(s)
- ☐ Reply to Missing Parts/ Incomplete Application
  - ☐ Reply to Missing Parts under 37 CFR 1.52 or 1.53
- ☐ Drawing(s)
- ☐ Licensing-related Papers
- ☐ Petition
- ☐ Petition to Convert to a Provisional Application
- ☐ Power of Attorney, Revocation Change of Correspondence Address
- ☐ Terminal Disclaimer
- ☐ Request for Refund
- ☐ CD, Number of CD(s) _____
  - ☐ Landscape Table on CD
- ☐ After Allowance Communication to TC
- ☐ Appeal Communication to Board of Appeals and Interferences
- ☐ Appeal Communication to TC **(Appeal Notice, Brief, Reply Brief)**
- ☐ Proprietary Information
- ☐ Status Letter
- ☐ Other Enclosure(s) (please identify below):

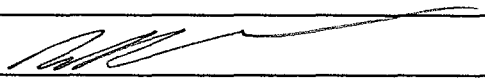
Remarks: **The Commissioner is authorized to charge any additional fees to Deposit Account 50-5826.**

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	Robins Law Group		
Signature			
Printed name	Roberta L. Robins		
Date	Nov. 18, 2014	Reg. No.	33,208

CERTIFICATE OF TRANSMISSION/MAILING

I hereby certify pursuant to 37 CFR §1.8 that this correspondence is being transmitted via EFS to the United States Patent and Trademark Office on the date shown below.

Signature			
Typed or printed name	Denise M. Vaillancourt	Date	11/18/14

PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a) **FY 2014** *(Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).)*	Docket Number (Optional) 0800-0045.01
Application Number: 12/661,553	Filed: March 19, 2010
For COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION	
Art Unit: 1653	Examiner: Satyendra K. Singh

This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a reply in the above identified application.

The requested extension and fee are as follows (check time period desired and enter the appropriate fee below):

	Fee	Small Entity Fee	
☐ One month (37 CFR 1.17(a)(1))	$200	$100	$
☐ Two months (37 CFR 1.17(a)(2))	$600	$300	$
☒ Three months (37 CFR 1.17(a)(3))	$1400	$700	$ 1400
☐ Four months (37 CFR 1.17(a)(4))	$2200	$1100	$
☐ Five months (37 CFR 1.17(a)(5))	$3000	$1500	$

☐ Applicant claims small entity status. See 37 CFR 1.27.

☐ A check in the amount of the fee is enclosed.

☒ Payment by credit card.

☐ The Director has already been authorized to charge fees in this application to a Deposit Account.

☒ The Director is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number 50-5826.

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

I am the

☐ applicant/inventor.

☐ assignee of record of the entire interest. See 37 CFR 3.71.
Statement under 37 CFR 3.73(b) is enclosed (Form PTO/SB/96).

☒ attorney or agent of record. Registration Number 33,208

☐ attorney or agent under 37 CFR 1.34.
Registration number if acting under 37 CFR 1.34 _____

Signature	Nov. 18, 2014 Date
Roberta L. Robins Typed or printed name	(650) 493-3400 Telephone Number

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below.

☒ Total of 1 forms are submitted.

PTO/SB/06 (09-11)
Approved for use through 1/31/2014. OMB 0651-0032
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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 12/661,553	Filing Date 03/19/2010	☐ To be Mailed

ENTITY: ☒ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	

APPLICATION AS AMENDED – PART II

AMENDMENT **11/18/2014**

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	* 7	Minus	** 20	= 0	X $80 =	0
Independent (37 CFR 1.16(h))	* 1	Minus	***3	= 0	X $420 =	0
☐ Application Size Fee (37 CFR 1.16(s))						
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
					TOTAL ADD'L FEE	0

AMENDMENT

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =	
Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =	
☐ Application Size Fee (37 CFR 1.16(s))						
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
					TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".

*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".

The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

LIE
/KIM DOWNING/

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/661,553	03/19/2010	John Fraser Wright	0800-0045.01	4726

20855 7590 01/27/2015

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EXAMINER	
SINGH, SATYENDRA K	

ART UNIT	PAPER NUMBER
1657	

MAIL DATE	DELIVERY MODE
01/27/2015	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Examiner-Initiated Interview Summary	Application No.	Applicant(s)	
	12/661,553	WRIGHT ET AL.	
	Examiner	Art Unit	
	SATYENDRA SINGH	1657	

All participants (applicant, applicant's representative, PTO personnel):

(1) *SATYENDRA SINGH*. (3)_____.

(2) *ROBERTA ROBINS (ATTORNEY)*. (4)_____.

Date of Interview: *22 January 2015*.

Type: ☒ Telephonic ☐ Video Conference
☐ Personal [copy given to: ☐ applicant ☐ applicant's representative]

Exhibit shown or demonstration conducted: ☒ Yes ☐ No.
If Yes, brief description: *see a copy of the proposed EXAM AMEND faxed to attorney Robins on 12/23/14*.

Issues Discussed ☒101 ☐112 ☐102 ☒103 ☒Others
(For each of the checked box(es) above, please describe below the issue and detailed description of the discussion)

Claim(s) discussed: *1, in particular*.

Identification of prior art discussed: *of the record*.

Substance of Interview
(For each issue discussed, provide a detailed description and indicate if agreement was reached. Some topics may include: identification or clarification of a reference or a portion thereof, claim interpretation, proposed amendments, arguments of any applied references etc...)

after cosiderations of applicant's arguments (and discussion with SPE, Jon Weber regarding 101 rejection of record; dated 12/18/14), examiner called applicant's attorney of record Miss Roberta Robins (ph=650-493-3400 x303) and provided with (via fascimile) a proposed Examiner's Amendment to pending claims (the scope of which was found to be tentatively allowable; see attached copy of the EXAM AMEND faxed to attorney Robins on 12/23/2014) for applicant's quick considerations. Attorney Robins informed the examiner that applicants are on leave and once they respond, she will contact the examiner. Upon multiple calling by the examiner (from 1/7/15 to 1/21/15), attorney Robins told the examiner that for some reasons, she was not able to contact the applicants, and that they are not responding to her emails.
On 01/22/15, in the absence of any response from the applicants, examiner called the attorney Robins again, and was informed that applicants have not yet responded, and she suggested the examiner to go ahead and issue an appropriate office action, as she is not able to get in touch with the applicants. Examiner told attorney Robins that in this situation, a FINAL rejection will be mailed by the office soon.

Applicant recordation instructions: It is not necessary for applicant to provide a separate record of the substance of interview.

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

☒ Attachment

/SATYENDRA SINGH/ Primary Examiner, Art Unit 1657	

Office Action Summary	Application No. 12/661,553	Applicant(s) WRIGHT ET AL.	
	Examiner SATYENDRA SINGH	Art Unit 1657	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *11/18/14*.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5)☒ Claim(s) *1 and 3-8* is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *1 and 3-8* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☒ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date *11/18/14*.

3) ☒ Interview Summary (PTO-413) Paper No(s)/Mail Date. *1/22/15*.

4) ☐ Other: _____.

The present application is being examined under the pre-AIA first to invent provisions.

DETAILED ACTION

Applicant's submission filed on **11/18/2014** has been entered.

Claims 2, and 9-11 (invention of group II) were previously canceled by applicants.

Claims 1 and 3-8 (elected invention of group I), as currently amended, have been examined on their merits in this office action.

Examiner-Initiated Interview

In the spirit of compact prosecution, an attempt by the examiner was made to offer applicants the allowable scope of the claimed invention (see attached interview summary, and the attached copy of the proposed examiner's amendment faxed to the Attorney of record **Miss Roberta Robins** on 12/23/2014). However, no response from the applicants was received by attorney Robins, and therefore, finally no agreement could be reached.

Claim Rejections - 35 USC § 101- Withdrawn

In view of applicant's arguments (see remarks, page 4) and amendment to claim 1, the 101 rejection of record, as previously made by the examiner, has been withdrawn. The "recombinant AAV particles" have been taken as "recombinant AAV **vector** particles" for the purposes of this office action hereinafter.

Claim Rejections - 35 USC § 103- Maintained

The following is a quotation of pre-AIA 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under pre-AIA 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.

2. Ascertaining the differences between the prior art and the claims at issue.

3. Resolving the level of ordinary skill in the pertinent art.

4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

This application currently names **joint inventors**. In considering patentability of the claims under pre-AIA 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of pre-AIA 35 U.S.C. 103(c) and potential pre-AIA 35 U.S.C. 102(e), (f) or (g) prior art under pre-AIA 35 U.S.C. 103(a).

1. **Claims 1 and 3-8** (as currently amended) **remain** rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Zolotukhin et al (2000; US 6,146,874) taken with Andersson et al (1979; US 4,138,287), Zhang et al (2001; US 6,194,191) and Chen et al (1994).

Claims (as currently amended) are directed to "a composition (for the storage) of purified, recombinant adeno-associated virus (AAV) particles, comprising: purified , recombinant (AAV) particles at a concentration exceeding 1 x 10^{13} vg/ml up to 6.4x 10^{13} vg/ml;

a pH buffer, wherein the pH of the composition is between 7.5 and 8.0; and excipients comprising one or more *multivalent ions*; wherein the ionic strength of the composition is greater than 200 mM, wherein aggregation of the purified AAV particles in the composition is prevented (instant claim 1); wherein one of the one or more multivalent ions is citrate (claim 3); further comprising Pluronic® F68 (ethylene oxide/propylene oxide block copolymer) at 0.001% (w/v) (claims 4-5); wherein the pH buffer is 10 mM Tris, pH 8.0 and the excipients comprise 100 mM sodium citrate (claim 6); wherein the purified AAV particles have an *average particle radius* (*Rh*) of less than about 20 nm as measured by dynamic light scattering (instant claim 7); and wherein *recovery of the purified, recombinant virus particles* is at least about 90% following filtration of the composition of virions through a 0.22 μm filter." (claim 8).

Zolotukhin et al (2000) disclose the composition comprising: purified **recombinant** AAV (rAAV) virus particles; a pH buffer such as **phosphate buffered-saline with magnesium chloride and potassium chloride** (PBS-MK); and excipients comprising one or more multivalent ions such as phosphate and magnesium; wherein the ionic strength of the composition is greater than 200 mM (see Zolotukhin et al, column 11, 4th paragraph, lines 35-40, in particular), wherein they elute the purified rAAV particles in **PBS-MK buffer having 1M NaCl**, i.e. elution buffer; and wherein they disclose the fact that highly purified stocks having titers up to **about 10^{13} particles/ml** are obtained using their purification steps within 24 hours or less (see abstract, and column 16, 1st paragraph, in particular), and in fact has the potential to produce **10^{14} virus particles**. In addition Zolotukhin et al also disclose the problems facing the purification of high titer AAV particles specifically related to the **problem of aggregation**, which was alleviated and/or reduced significantly by the use of high concentrations of salts (i.e.

high ionic strength buffer such as 1M NaCl in PBS-MK; see column 3, last paragraph; column 15, 3rd paragraph, for examples) during purification, wherein the excess salt can be later removed, if required for downstream applications.

However, the composition having AAV particles at a concentration exceeding 1 x 10^{13} vg/ml up to 6.4x 10^{13} vg/ml (see instant claim 1), further comprising **Pluronic® F68** at 0.001% w/v (claims 4-5); and wherein the pH buffer is **10 mM Tris, pH 8.0** and the excipients comprise **100 mM sodium citrate** (claims 1 and 6), is not explicitly exemplified and/or disclosed by the inventions of Zolotukhin et al (although the number of virus particles potentially purified have been disclosed to be in the vicinity of **about 10^{13} and about 10^{14}**; see Zolotukhin et al above).

Andersson et al (1979) disclose the composition comprising purified hepatitis virus (HB_sAg) particles (see abstract, in particular), a pH buffer such as Tris-sodium citrate buffer, pH 7.5, and excipients comprising a multivalent ion such as **sodium citrate**, wherein the ionic strength of the composition is greater than about 200 mM (see elution of purified virus from column using 0.5 M NaCl in Tris-citrate buffer; see Andersson et al, example 2 and claim 5, in particular).

Zhang et al (2001) disclose the use of a surfactant such as **Pluronic® F68** (0.1% in growth medium for adenovirus infection and viral production, etc.; see column 4, 2nd paragraph and columns 53-54, in particular) for production of adenoviral particles in serum-free suspension cultures using spinner flasks, and also use in the cryopreservation media (see column 53, last paragraph).

Chen et al (1994) disclose strategies to suppress aggregation of proteins (such as recombinant keratinocyte growth factor) during liquid formulation development (see Chen et al,

abstract, table 1-2, in particular) by adding sulfated polysaccharides (such as heparin) and **citrate salts** (such as **0.1 to 0.5 M sodium citrate**; see Chen et al, page 1661, left column, in particular) that were found to be effective in preventing protein aggregation. Chen et al also disclose the fact that other negatively charged small ions such as phosphate (a multivalent ion) also have moderate stabilizing effects **on preventing aggregation** of recombinant keratinocyte growth factor (rhKGF).

Thus, given the disclosure in the cited prior art, a person of ordinary skill in the art would have modified the composition comprising purified recombinant AAV virus particles taught by Zolotukhin et al such that said composition additionally comprise a surfactant (to help prevent aggregation during freezing-thawing cycles, for example) such as Pluronic® F68, as explicitly taught by Zhang et al, and a suitable amount of sodium citrate as a multivalent ion in Tris buffer solution (at a suitable pH; as specifically disclosed by Andersson et al) in order to stabilize the viral particles as suggested by Chen et al (*albeit* for suppressing/reducing aggregation of fairly unstable proteins such as rhKGF; see discussion above, thus providing a conceptual basis for including multivalent ions in the buffer containing purified recombinant AAV particles having capsid proteins), in addition to other stabilizing components such as a surfactant. Since, the benefits of including a surfactant and high ionic strength multivalent ions have been disclosed in the cited prior art of Zhang et al and Chen et al, an artisan of ordinary skill in the art would have established suitable concentrations required to help stabilize purified recombinant AAV viral preparations (i.e. for preventing aggregation, etc.) with a reasonable expectation of success, especially given the disclosure from Zolotukhin et al, for example, for the use of high salt concentrations for reducing the potential aggregation of virus particles (see discussion of

Zolotukhin et al, above). Such modification in the use of suitable concentrations of the multivalent ions (such as sodium citrate) for reducing aggregation in place of high salt concentrations (i.e. 1 M NaCl) used by Zolotukhin et al would have been therefore obvious and fully contemplated by an artisan of ordinary skill in the art, given the combined disclosure provided by the cited references of Andersson et al, Zhang et al and Chen et al, as discussed above.

The limitations of claims 7 and 8 (average particle radius and percent recovery following filtration, etc.) are also met by the prior art as these are taken to be intrinsic features of the purified AAV viral compositions as disclosed by Zolotukhin et al when taken with the teachings of Andersson et al, Zhang et al and Chen et al. Since, all the components of the product, as recited in the claims are the same as disclosed and/or suggested in the cited prior art, these features will necessarily follow from the composition disclosed in the art, as they do not structurally change the composition as claimed.

Thus, the invention as a whole would have been *prima facie* obvious to a person of ordinary skill in the art at the time the claimed invention was made.

As per MPEP 2144.06, "*It is prima facie obvious to combine two compositions each of which is taught by the prior art to be useful for the same purpose, in order to form a third composition to be used for the very same purpose.... [T]he idea of combining them flows logically from their having been individually taught in the prior art." In re Kerkhoven, 626 F.2d 846, 850, 205 USPQ 1069, 1072 (CCPA 1980).*

As per MPEP 2144.06, *In order to rely on equivalence as a rationale supporting an obviousness rejection, the equivalency must be recognized in the prior art, and cannot be based on applicant's disclosure or the mere fact that the components at issue are functional or mechanical equivalents. In re Ruff, 256 F.2d 590, 118 USPQ 340 (CCPA 1958).*

Response to Applicant's Arguments

Applicant's arguments filed on **11/18/2014** (as they pertain to the prior art rejection of record) have been fully considered but they are not persuasive for the following reasons of record:

Regarding the 103a rejection of record, applicants essentially argue that there is no motivation and/or suggestion to combine the cited prior art of record, and earlier cited abstract of Qu et al shows that there is no clear understanding for the causes of self-aggregation of purified rAAV particles (see remarks, pages 5-7), which is duly note d and considered. However, it is noted that Qu et al 2003 abstract has not been currently relied upon in the instant rejection of record. In response to applicant's argument that there is no teaching, suggestion, or motivation to combine the references, the examiner recognizes that obviousness may be established by combining or modifying the teachings of the prior art to produce the claimed invention where there is some teaching, suggestion, or motivation to do so found either in the references themselves or in the knowledge generally available to one of ordinary skill in the art. See *In re Fine*, 837 F.2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988), *In re Jones*, 958 F.2d 347, 21 USPQ2d 1941 (Fed. Cir. 1992), and *KSR International Co. v. Teleflex, Inc.*, 550 U.S. 398, 82 USPQ2d 1385 (2007). In this case, as discussed in the 103a rejection of record, the cited prior art of Zolotukhin et al disclose the range of viral particles that can be purified up to or greater than 10^{14} vp/ml (see column 6, 1st paragraph; column 16, 1st paragraph, in particular), and therefore, when taken with the disclosure from Andersson et al, Zhang et al and Chen et al for the benefits of using suitable concentrations of a multivalent ions such as sodium citrate under suitable pH conditions, a person of ordinary skill in the art would have fully contemplated the modification

in the buffer composition disclosed by Zolotukhin et al such that it uses multivalent ions as stabilizing buffer excipient in order to reduce/prevent viral aggregation, as already recognized and/or intended by the disclosure of Zolotukhin et al (see column 3, last paragraph,; column 14,,last paragraphs, for instances) and supported by the teachings of Zhang et al and Chen et al, as discussed above.

Regarding the limitations of claims 7 and 8, applicants appear to argue that the average particle radius and the percent recovery are not intrinsic properties of the composition (see remarks, page 7), which is duly considered. However, if the components of the composition have been fully disclosed and/or suggested in the art, one would reasonably presume that the resulting characteristics of the composition comprising rAAV particles (such as average particle radius, and recovery, etc.) stabilized with suitable concentrations of multivalent ions such as citrate under similar conditions of number of particles, buffer, pH and temperature, would provide the same characteristics, as currently being claimed by the applicants. Moreover, instant claim 1 is not limited to such characteristics, as currently argued by applicants.

Applicant's arguments regarding the beneficial aspects and scope of the claimed composition and unexpected results (see remarks, page 8), is duly noted and fully considered. However, the scope of the showing must be commensurate with the scope of claims to consider evidence probative of unexpected results, for example. *In re Dill, 202 USPQ 805 (CCPA, 1979), In re Lindner 173 USPQ 356 (CCPA 1972), In re Hyson, 172 USPQ 399 (CCPA 1972), In re Boesch, 205 USPQ 215, (CCPA 1980), In re Grasselli, 218 USPQ 769 (Fed. Cir. 1983), In re Clemens, 206 USPQ 289 (CCPA 1980).* It should be clear that the probative value of the data is not commensurate in scope with the degree of protection sought by the instant claims.

Applicants are advised to amend scope of the claims (as already suggested by the examiner during the examiner-initiated interview, attached herewith) in order to commensurate with the showings, for favorable considerations in future prosecution.

Conclusion

NO claims are allowed.

Pertinent art:

1. **Evans et al.** (published on 08/26/2004; **US 2004/0166122 A1**; previously cited by the examiner) - Adenovirus formulations (disclose stable **viral vector formulations** for gene therapy and other clinical applications generally comprising up to about $1x10^{13}$ viral particles/ml in a suitable buffer, a sugar, a salt, a divalent cation, a non-ionic detergent as well as sodium citrate, wherein the typical formulations comprise 5 mM Tris buffer, pH 7.5, 250 mM NaCl, sucrose to provide suitable osmolarity in the range of 200-800 mOsm/L, $MgCl_2$ in the range of 0.1mM to about 10 mM, 0.001% to about 2% of polysorbate-80 as surfactant, and sodium citrate at about 10 mM; see page 8, example 1, in particular; paragraphs [0051], [0056], [0060], [0079], entire disclosure at pages 5-6, in particular and claims).

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period

will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SATYENDRA SINGH whose telephone number is (571)272-8790. The examiner can normally be reached on 9-5MF.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, JON P. WEBER can be reached on 571-272-0925. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/SATYENDRA SINGH/
Primary Examiner,
Art Unit 1657

Examiner-Initiated Interview Summary	Application No.	Applicant(s)	
	12/661,553	WRIGHT ET AL.	
	Examiner	Art Unit	
	SATYENDRA SINGH	1657	

All participants (applicant, applicant's representative, PTO personnel):

(1) *SATYENDRA SINGH*. (3)_____.

(2) *ROBERTA ROBINS (ATTORNEY)*. (4)_____.

Date of Interview: *22 January 2015*.

Type: ☒ Telephonic ☐ Video Conference
☐ Personal [copy given to: ☐ applicant ☐ applicant's representative]

Exhibit shown or demonstration conducted: ☒ Yes ☐ No.
If Yes, brief description: *see a copy of the proposed EXAM AMEND faxed to attorney Robins on 12/23/14*.

Issues Discussed ☒101 ☐112 ☐102 ☒103 ☒Others
(For each of the checked box(es) above, please describe below the issue and detailed description of the discussion)

Claim(s) discussed: *1, in particular*.

Identification of prior art discussed: *of the record*.

Substance of Interview
(For each issue discussed, provide a detailed description and indicate if agreement was reached. Some topics may include: identification or clarification of a reference or a portion thereof, claim interpretation, proposed amendments, arguments of any applied references etc...)

after cosiderations of applicant's arguments (and discussion with SPE, Jon Weber regarding 101 rejection of record; dated 12/18/14), examiner called applicant's attorney of record Miss Roberta Robins (ph=650-493-3400 x303) and provided with (via fascimile) a proposed Examiner's Amendment to pending claims (the scope of which was found to be tentatively allowable; see attached copy of the EXAM AMEND faxed to attorney Robins on 12/23/2014) for applicant's quick considerations. Attorney Robins informed the examiner that applicants are on leave and once they respond, she will contact the examiner. Upon multiple calling by the examiner (from 1/7/15 to 1/21/15), attorney Robins told the examiner that for some reasons, she was not able to contact the applicants, and that they are not responding to her emails.
On 01/22/15, in the absence of any response from the applicants, examiner called the attorney Robins again, and was informed that applicants have not yet responded, and she suggested the examiner to go ahead and issue an appropriate office action, as she is not able to get in touch with the applicants. Examiner told attorney Robins that in this situation, a FINAL rejection will be mailed by the office soon.

Applicant recordation instructions: It is not necessary for applicant to provide a separate record of the substance of interview.

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

☒ Attachment

/SATYENDRA SINGH/ Primary Examiner, Art Unit 1657	

EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
S108	19	S107 and ((adenovir$6 or AAV) same (aggreg$6 or precipit$6 or agglutin$6 or stabiliz$6))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2014/05/19 10:29
S109	4648	WRIGHT near3 JOHN	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2015/01/23 10:47
S110	79	QU near3 GUANG	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2015/01/23 10:47
S111	4675	S109 or S110	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2015/01/23 10:47
S112	19	S111 and ((adenovir$6 or AAV) same (aggreg$6 or precipit$6 or agglutin$6 or stabiliz$6))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2015/01/23 10:47
S113	9	S112 and (citric or citrate or multivalent or multival$6)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2015/01/23 10:49
S114	2305	((AAV or adenovir$3) near3 (particle or virion)) and (citr$6 or magnes$6 or phosphat$6 or mangan$6 or sulfate)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2015/01/23 14:04
S115	170	((AAV or adenovir$3) near3 (particle or virion)) same (citr$6 or magnes$6 or phosphat$6 or mangan$6 or sulfate)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2015/01/23 14:05
S116	7	((AAV or adenovir$3) near3 (particle or virion)) same (citr$6 or magnes$6 or phosphat$6 or mangan$6 or sulfate).clm.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2015/01/23 14:05

EAST Search History (Interference)

<This search history is empty>

1/23/2015 2:07:04 PM
C:\Users\ssingh3\Documents\EAST\Workspaces\12661553-all-dbs.wsp

UNITED STATES PATENT AND TRADEMARK OFFICE

Facsimile Transmission

To:	**Name:**	ROBERTA L. ROBINS
	Company:	
	Fax Number:	6504933440
	Voice Phone:	
From:	**Name:**	
	Voice Phone:	

37 C.F.R. 1.6 sets forth the types of correspondence that can be communicated to the Patent and Trademark Office via facsimile transmissions. Applicants are advised to use the certificate of facsimile transmission procedures when submitting a reply to a non-final or final Office action by facsimile (37 CFR 1.8(a)).

Fax Notes:

Hi Attorney Robins,
after considerations of applicant's claim amendments and arguments prsented on 11/18/14, I am sending you by fax, a proposed Examiner's Amendments to pending claims (the scope of which has been tentatively found to be allowable) for applicant's quick considerations. Pl. let me know at your earlieast, if there are any issues with the claims currently being amended and considered for allowance (pref. by COB 12/26/2014).
Sincerely,
Satyendra Singh
Examiner, AU 1657

Date and time of transmission: Tuesday, December 23, 2014 3:39:24 PM
Number of pages including this cover sheet: 04

The present application is being examined under the pre-AIA first to invent provisions.

DRAFT

DETAILED ACTION

Applicant's submission filed on **11/18/2014** has been entered.

Claims 2, and 9-11 (invention of group II) were previously canceled by applicants.

Claims 1 and 3-8 (elected invention of group I), as currently amended, have been examined on their merits in this office action.

PROPOSED EXAMINER'S AMENDMENT

An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in a telephone interview with **Roberta L. Robins** (attorney of record) on December xxxx 2014.

The application has been amended as follows:

In The Claims

Claim 3 has been **canceled** by this Examiner's Amendment.

Claims 1 and 4-8 have been allowed by this Examiner's amendment as follows:

Claims 1, 4, 5, 7 and 8 have been amended, and the entire set of allowed claims have been recited below:

1. (Currently amended) A composition for the storage of purified, recombinant adeno-associated virus (AAV) vector particles, comprising:

purified, recombinant AAV vector particles at a concentration exceeding $1x10^{13}$ vg/ml up to $6.4x10^{13}$ vg/ml;

a pH buffer, wherein the pH of the composition is between 7.5 and 8.0; and

excipients comprising one or more multivalent ions selected from the group consisting of citrate, sulfate, magnesium, and phosphate; wherein the ionic strength of the composition is greater than 200 mM, and wherein ~~aggregation of~~ the purified AAV vector particles are stored in the composition ~~is prevented~~ without significant aggregation.

4. (Currently amended) The composition of claim 1, further comprising ethylene oxide/propylene oxide block copolymer Pluronic® F68 ~~(ethylene oxide/propylene oxide block copolymer)~~.

5. (Currently amended) The composition of claim 4, wherein the Pluronic® F68 is present at a concentration of 0.001% (w/v).

6. (Original) The composition of claim 1, wherein the pH buffer is 10 mM Tris, pH 8.0 and the excipients comprise 100 mM sodium citrate.

7. (Currently amended) The composition of claim 1, wherein the purified, recombinant AAV vector particles have an average particle radius (Rh) of less than about 20nm as measured by dynamic light scattering.

8. (Currently amended) The composition of claim 1, wherein recovery of the purified, recombinant virus particles is at least about 90% following filtration of the composition of ~~virions~~ said AAV vector particles through a 0.22μm filter.

Conclusion

Claims 1 and 4-8 are being allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SATYENDRA SINGH whose telephone number is (571)272-8790. The examiner can normally be reached on 9-5MF.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, JON P. WEBER can be reached on 571-272-0925. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

SATYENDRA SINGH
Primary Examiner,
Art Unit 1657

PTO/SB/08a (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 1

Complete if Known	
Application Number	12/661,553
Filing Date	March 19, 2010
First Named Inventor	Wright et al.
Art Unit	1657
Examiner Name	Satyendra Singh
Attorney Docket Number	0800-0045.01

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		US-			
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FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]-Number[4]-Kind Code[5] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
	C27	WO99/61643	12-02-1999	University of Florida		
		ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /SS/				

Examiner Signature	/Satyendra Singh/	Date Considered	12/30/2014

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. [1] Applicant's unique citation designation number (optional). [2] See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached.

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	12661553	WRIGHT ET AL.
	Examiner	Art Unit
	SATYENDRA SINGH	1657

CPC- SEARCHED		
Symbol	Date	Examiner

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES		
Search Notes	Date	Examiner
EAST: USPAT, USOCR, US-PGPUB, JPO, EPO, DERWENT- ATTACHED	11/6/2011	SKS
INVENTOR SEARCH: PALM, EDAN AND EAST-	11/6/2011	SKS
EAST: USPAT, USOCR, US-PGPUB, JPO, EPO, DERWENT- UPDATED & ATTACHED	7/11/2012	SKS
INVENTOR SEARCH: PAL, EDAN AND EAST- UPDATED	7/11/2012	SKS
EAST: USPAT, USOCR, US-PGPUB, JPO, EPO, DERWENT- UPDATED & ATTACHED	8/12/2013	SKS
INVENTOR SEARCH: PALM, EDAN AND EAST- UPDATED	8/12/2013	SKS
EAST: USPAT, USOCR, US-PGPUB, JPO, EPO, DERWENT- UPDATED & ATTACHED	5/19/2014	SKS
INVENTOR SEARCH: PALM, EDAN AND EAST- UPDATED	5/19/2014	SKS
DISCUSSION: SPE Jon Weber- regarding 101 rejection	12/18/14	SKS
EAST: USPAT, USOCR, US-PGPUB, JPO, EPO, DERWENT- UPDATED & ATTACHED	1/23/15	SKS
INVENTOR SEARCH: PALM, EDAN AND EAST- UPDATED	1/23/15	SKS

	/SATYENDRA SINGH/ Primary Examiner.Art Unit 1657

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner

	/SATYENDRA SINGH/ Primary Examiner.Art Unit 1657

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NOTICE OF ALLOWANCE AND FEE(S) DUE

20855 7590 02/03/2015

PASTERNAK PATENT LAW
1900 EMBARCADERO ROAD
SUITE 211
PALO ALTO, CA 94303

EXAMINER
SINGH, SATYENDRA K

ART UNIT	PAPER NUMBER
1657	

DATE MAILED: 02/03/2015

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/661,553	03/19/2010	John Fraser Wright	0800-0045.01	4726

TITLE OF INVENTION: Compositions and methods to prevent AAV vector aggregation

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$960	$0	$0	$960	05/04/2015

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 1/2 the amount of undiscounted fees, and micro entity fees are 1/2 the amount of small entity fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail **Mail Stop ISSUE FEE**
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

20855 7590 02/03/2015
PASTERNAK PATENT LAW
1900 EMBARCADERO ROAD
SUITE 211
PALO ALTO, CA 94303

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission
I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/661,553	03/19/2010	John Fraser Wright	0800-0045.01	4726

TITLE OF INVENTION: Compositions and methods to prevent AAV vector aggregation

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$960	$0	$0	$960	05/04/2015

EXAMINER	ART UNIT	CLASS-SUBCLASS
SINGH, SATYENDRA K	1657	435-239000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).
- ❑ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
- ❑ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.
1 _____
2 _____
3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE (B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ❑ Individual ❑ Corporation or other private group entity ❑ Government

4a. The following fee(s) are submitted:
- ❑ Issue Fee
- ❑ Publication Fee (No small entity discount permitted)
- ❑ Advance Order - # of Copies _____

4b. Payment of Fee(s): **(Please first reapply any previously paid issue fee shown above)**
- ❑ A check is enclosed.
- ❑ Payment by credit card. Form PTO-2038 is attached.
- ❑ The director is hereby authorized to charge the required fee(s), any deficiency, or credits any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)
- ❑ Applicant certifying micro entity status. See 37 CFR 1.29 — NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.
- ❑ Applicant asserting small entity status. See 37 CFR 1.27 — NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.
- ❑ Applicant changing to regular undiscounted fee status. — NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/661,553	03/19/2010	John Fraser Wright	0800-0045.01	4726

20855 7590 02/03/2015

PASTERNAK PATENT LAW
1900 EMBARCADERO ROAD
SUITE 211
PALO ALTO, CA 94303

EXAMINER	
SINGH, SATYENDRA K	

ART UNIT	PAPER NUMBER
1657	

DATE MAILED: 02/03/2015

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Applicant-Initiated Interview Summary	Application No.	Applicant(s)	
	12/661,553	WRIGHT ET AL.	
	Examiner	Art Unit	
	SATYENDRA SINGH	1657	

All participants (applicant, applicant's representative, PTO personnel):

(1) *SATYENDRA SINGH*. (3)_____.

(2) *ROBERTA ROBINS (ATTORNEY)*. (4)_____.

Date of Interview: *28 January 2015*.

Type: ☒ Telephonic ☐ Video Conference
☐ Personal [copy given to: ☐ applicant ☐ applicant's representative]

Exhibit shown or demonstration conducted: ☒ Yes ☐ No.
If Yes, brief description: *previously discussed EXAM AMEND that was faxed on 12/23/14 to attorney Robins (see IMIS dated 1/27/15, already on record)*.

Issues Discussed ☐101 ☐112 ☐102 ☒103 ☒Others
(For each of the checked box(es) above, please describe below the issue and detailed description of the discussion)

Claim(s) discussed: *of the record*.

Identification of prior art discussed: *of the record*.

Substance of Interview
(For each issue discussed, provide a detailed description and indicate if agreement was reached. Some topics may include: identification or clarification of a reference or a portion thereof, claim interpretation, proposed amendments, arguments of any applied references etc...)

After the FINAL rejection was mailed by the office on 1/27/2015, applicant's attorney Miss Roberta Robins called the examiner on 01/28/2015, and informed that she has finally received the response from applicants, and they have agreed to the proposed EXAM. AMEND. as previously offered by the examiner on 12/23/14 (see paper IMIS dated 1/27/15 already on the record), and even if the FINAL rejection has been mailed, Examiner is authorized amend the claims per proposed examiner's amendments that were discussed before.

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☐ Attachment

/SATYENDRA SINGH/ Primary Examiner, Art Unit 1657	

Summary of Record of Interview Requirements

Manual of Patent Examining Procedure (MPEP), Section 713.04, Substance of Interview Must be Made of Record
A complete written statement as to the substance of any face-to-face, video conference, or telephone interview with regard to an application must be made of record in the application whether or not an agreement with the examiner was reached at the interview.

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Paragraph (b)

In every instance where reconsideration is requested in view of an interview with an examiner, a complete written statement of the reasons presented at the interview as warranting favorable action must be filed by the applicant. An interview does not remove the necessity for reply to Office action as specified in §§ 1.111, 1.135. (35 U.S.C. 132)

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All business with the Patent or Trademark Office should be transacted in writing. The personal attendance of applicants or their attorneys or agents at the Patent and Trademark Office is unnecessary. The action of the Patent and Trademark Office will be based exclusively on the written record in the Office. No attention will be paid to any alleged oral promise, stipulation, or understanding in relation to which there is disagreement or doubt.

The action of the Patent and Trademark Office cannot be based exclusively on the written record in the Office if that record is itself incomplete through the failure to record the substance of interviews.

It is the responsibility of the applicant or the attorney or agent to make the substance of an interview of record in the application file, unless the examiner indicates he or she will do so. It is the examiner's responsibility to see that such a record is made and to correct material inaccuracies which bear directly on the question of patentability.

Examiners must complete an Interview Summary Form for each interview held where a matter of substance has been discussed during the interview by checking the appropriate boxes and filling in the blanks. Discussions regarding only procedural matters, directed solely to restriction requirements for which interview recordation is otherwise provided for in Section 812.01 of the Manual of Patent Examining Procedure, or pointing out typographical errors or unreadable script in Office actions or the like, are excluded from the interview recordation procedures below. Where the substance of an interview is completely recorded in an Examiners Amendment, no separate Interview Summary Record is required.

The Interview Summary Form shall be given an appropriate Paper No., placed in the right hand portion of the file, and listed on the "Contents" section of the file wrapper. In a personal interview, a duplicate of the Form is given to the applicant (or attorney or agent) at the conclusion of the interview. In the case of a telephone or video-conference interview, the copy is mailed to the applicant's correspondence address either with or prior to the next official communication. If additional correspondence from the examiner is not likely before an allowance or if other circumstances dictate, the Form should be mailed promptly after the interview rather than with the next official communication.

The Form provides for recordation of the following information:
- Application Number (Series Code and Serial Number)
- Name of applicant
- Name of examiner
- Date of interview
- Type of interview (telephonic, video-conference, or personal)
- Name of participant(s) (applicant, attorney or agent, examiner, other PTO personnel, etc.)
- An indication whether or not an exhibit was shown or a demonstration conducted
- An identification of the specific prior art discussed
- An indication whether an agreement was reached and if so, a description of the general nature of the agreement (may be by attachment of a copy of amendments or claims agreed as being allowable). Note: Agreement as to allowability is tentative and does not restrict further action by the examiner to the contrary.
- The signature of the examiner who conducted the interview (if Form is not an attachment to a signed Office action)

It is desirable that the examiner orally remind the applicant of his or her obligation to record the substance of the interview of each case. It should be noted, however, that the Interview Summary Form will not normally be considered a complete and proper recordation of the interview unless it includes, or is supplemented by the applicant or the examiner to include, all of the applicable items required below concerning the substance of the interview.

A complete and proper recordation of the substance of any interview should include at least the following applicable items:
1) A brief description of the nature of any exhibit shown or any demonstration conducted,
2) an identification of the claims discussed,
3) an identification of the specific prior art discussed,
4) an identification of the principal proposed amendments of a substantive nature discussed, unless these are already described on the Interview Summary Form completed by the Examiner,
5) a brief identification of the general thrust of the principal arguments presented to the examiner,
   (The identification of arguments need not be lengthy or elaborate. A verbatim or highly detailed description of the arguments is not required. The identification of the arguments is sufficient if the general nature or thrust of the principal arguments made to the examiner can be understood in the context of the application file. Of course, the applicant may desire to emphasize and fully describe those arguments which he or she feels were or might be persuasive to the examiner.)
6) a general indication of any other pertinent matters discussed, and
7) if appropriate, the general results or outcome of the interview unless already described in the Interview Summary Form completed by the examiner.

Examiners are expected to carefully review the applicant's record of the substance of an interview. If the record is not complete and accurate, the examiner will give the applicant an extendable one month time period to correct the record.

Examiner to Check for Accuracy

If the claims are allowable for other reasons of record, the examiner should send a letter setting forth the examiner's version of the statement attributed to him or her. If the record is complete and accurate, the examiner should place the indication, "Interview Record OK" on the paper recording the substance of the interview along with the date and the examiner's initials.

Notice of Allowability	Application No. 12/661,553	Applicant(s) WRIGHT ET AL.	
	Examiner SATYENDRA SINGH	Art Unit 1657	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to *11/18/14; and interview on 1/28/15*.
   ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on_____.
2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.
3. ☒ The allowed claim(s) is/are *1 and 4-8*. As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov .
4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

   Certified copies:

   a) ☐ All b) ☐ Some *c) ☐ None of the:
   1. ☐ Certified copies of the priority documents have been received.
   2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
   3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).
6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☐ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
4. ☒ Interview Summary (PTO-413), Paper No./Mail Date *1/28/15* .
5. ☒ Examiner's Amendment/Comment
6. ☐ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____.

/SATYENDRA SINGH/
Primary Examiner, Art Unit 1657

The present application is being examined under the pre-AIA first to invent provisions.

DETAILED ACTION

Applicant's submission filed on **11/18/2014** is duly acknowledged.

Claims 2, and 9-11 (invention of group II) were previously canceled by applicants.

Claims 1 and 3-8 (elected invention of group I), as currently amended, have been examined on their merits in this office action.

WITHDRAWAL OF FINALITY

In view of applicant's acceptance of the previously proposed Examiner's Amendment (see IMIS and Examiner-initiated interview summary, dated 01/27/15 of record) to the pending claims, the FINALITY of the previous rejection is withdrawn.

EXAMINER'S AMENDMENT

An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in a telephone interview with **Miss Roberta L. Robins** (attorney of record) on **January 28th 2015** (see also attached interview summary regarding the previously proposed Examiner's Amendment to claims that was finally agreed upon on 01/28/15 by the applicants, after the FINAL rejection was sent by the office on 01/27/15).

The application has been amended as follows:

In The Claims

Claim 3 has been **canceled** by this Examiner's Amendment.

Claims 1 and 4-8 have been allowed by this Examiner's amendment as follows:

Claims 1, 4, 5, 7 and 8 have been amended, and the allowed claims have been recited below:

1. (Currently amended) A composition for the storage of purified, recombinant adeno-associated virus (AAV) vector particles, comprising:

purified, recombinant AAV vector particles at a concentration exceeding $1x10^{13}$ vg/ml up to $6.4x10^{13}$ vg/ml;

a pH buffer, wherein the pH of the composition is between 7.5 and 8.0; and

excipients comprising one or more multivalent ions selected from the group consisting of citrate, sulfate, magnesium, and phosphate; wherein the ionic strength of the composition is greater than 200 mM, and wherein ~~aggregation of~~ the purified AAV vector particles are stored in the composition ~~is prevented~~ without significant aggregation.

4. (Currently amended) The composition of claim 1, further comprising ethylene oxide/propylene oxide block copolymer Pluronic® F68 ~~(ethylene oxide/propylene oxide block copolymer)~~.

5. (Currently amended) The composition of claim 4, wherein the Pluronic® F68 is present at a concentration of 0.001% (w/v).

6. (Original) The composition of claim 1, wherein the pH buffer is 10 mM Tris, pH 8.0 and the excipients comprise 100 mM sodium citrate.

7. (Currently amended) The composition of claim 1, wherein the purified, recombinant AAV vector particles have an average particle radius (Rh) of less than about 20nm as measured by dynamic light scattering.

8. (Currently amended) The composition of claim 1, wherein recovery of the purified, recombinant virus particles is at least about 90% following filtration of the composition of ~~virions~~ said AAV vector particles through a 0.22μm filter.

Conclusion

Claims 1 and 4-8 have been allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SATYENDRA SINGH whose telephone number is (571)272-8790. The examiner can normally be reached on 9-5MF.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, JON P. WEBER can be reached on 571-272-0925. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/SATYENDRA SINGH/
Primary Examiner,
Art Unit 1657

Applicant-Initiated Interview Summary	Application No.	Applicant(s)	
	12/661,553	WRIGHT ET AL.	
	Examiner	Art Unit	
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All participants (applicant, applicant's representative, PTO personnel):

(1) *SATYENDRA SINGH*. (3)_____.

(2) *ROBERTA ROBINS (ATTORNEY)*. (4)_____.

Date of Interview: *28 January 2015*.

Type: ☒ Telephonic ☐ Video Conference
☐ Personal [copy given to: ☐ applicant ☐ applicant's representative]

Exhibit shown or demonstration conducted: ☒ Yes ☐ No.
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6) a general indication of any other pertinent matters discussed, and
7) if appropriate, the general results or outcome of the interview unless already described in the Interview Summary Form completed by the examiner.

Examiners are expected to carefully review the applicant's record of the substance of an interview. If the record is not complete and accurate, the examiner will give the applicant an extendable one month time period to correct the record.

Examiner to Check for Accuracy

If the claims are allowable for other reasons of record, the examiner should send a letter setting forth the examiner's version of the statement attributed to him or her. If the record is complete and accurate, the examiner should place the indication, "Interview Record OK" on the paper recording the substance of the interview along with the date and the examiner's initials.

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	12661553	WRIGHT ET AL.
	Examiner	**Art Unit**
	SATYENDRA SINGH	1657

CPC						
Symbol					**Type**	**Version**
C12N		7	/	00	F	2013-01-01
C12N		2750	/	14151	A	2013-01-01
			/			
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			/			
			/			

CPC Combination Sets							
Symbol					**Type**	**Set**	**Ranking**	**Version**
			/					
			/					

NONE		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	6	
/SATYENDRA SINGH/ Primary Examiner.Art Unit 1657	01/30/15	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	NONE

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	12661553	WRIGHT ET AL.
	Examiner	**Art Unit**
	SATYENDRA SINGH	1657

US ORIGINAL CLASSIFICATION		INTERNATIONAL CLASSIFICATION	
CLASS	SUBCLASS	CLAIMED	NON-CLAIMED

CROSS REFERENCE(S)					
CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)				

NONE		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	6	
/SATYENDRA SINGH/ Primary Examiner.Art Unit 1657	01/30/15	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	NONE

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	12661553	WRIGHT ET AL.
	Examiner	**Art Unit**
	SATYENDRA SINGH	1657

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original
1	1														
2	4														
3	5														
4	6														
5	7														
6	8														

NONE

(Assistant Examiner) (Date)

Total Claims Allowed:

6

/SATYENDRA SINGH/
Primary Examiner.Art Unit 1657 01/30/15

(Primary Examiner) (Date)

O.G. Print Claim(s)	O.G. Print Figure
1	NONE

EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
S116	7	((AAV or adenovir$3) near3 (particle or virion)) same (citr$6 or magnes$6 or phosphat$6 or mangan$6 or sulfate).clm.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2015/01/23 14:05
S117	4649	WRIGHT near3 JOHN	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2015/01/30 09:58
S118	79	QU near3 GUANG	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2015/01/30 09:58
S119	4676	S117 or S118	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2015/01/30 09:58
S120	19	S119 and ((adenovir$6 or AAV) same (aggreg$6 or precipit$6 or agglutin$6 or stabiliz$6))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2015/01/30 09:58

EAST Search History (Interference)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
S121	1631	(buffer same (multivalent or citrate or citric)) and ((adenovir$6 or AAV) same (aggreg$6 or precipit$6 or agglutin$6 or stabiliz$6))	US-PGPUB; USPAT; UPAD	OR	OFF	2015/01/30 09:59
S122	20	(buffer same (multivalent or citrate or citric)) same ((adenovir$6 or AAV) same (aggreg$6 or precipit$6 or agglutin$6 or stabiliz$6))	US-PGPUB; USPAT; UPAD	OR	OFF	2015/01/30 09:59
S123	0	(buffer same (multivalent or citrate or citric)) same ((adenovir$6 or AAV) same (aggreg$6 or precipit$6 or agglutin$6 or stabiliz$6)).clm.	US-PGPUB; USPAT; UPAD	OR	OFF	2015/01/30 09:59
S124	9846	((ionic near3 strength) same (multivalent or citrate or citric or phosphate or sulfate or magnesium)) and ((adenovir$6 or AAV) and (aggreg$6 or precipit$6 or agglutin$6 or stabiliz$6))	US-PGPUB; USPAT; UPAD	OR	OFF	2015/01/30 10:08
S125	4	((ionic near3 strength) same (multivalent or citrate or citric or phosphate or sulfate or magnesium)) same ((adenovir$6 or AAV) same (aggreg$6 or precipit$6 or agglutin$6 or stabiliz$6))	US-PGPUB; USPAT; UPAD	OR	OFF	2015/01/30 10:08

1/30/2015 10:10:26 AM

C:\ Users\ ssingh3\ Documents\ EAST\ Workspaces\ 12661553-all-dbs.wsp

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	12661553	WRIGHT ET AL.
	Examiner	Art Unit
	SATYENDRA SINGH	1657

CPC- SEARCHED		
Symbol	Date	Examiner

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES		
Search Notes	Date	Examiner
EAST: USPAT, USOCR, US-PGPUB, JPO, EPO, DERWENT- ATTACHED	11/6/2011	SKS
INVENTOR SEARCH: PALM, EDAN AND EAST-	11/6/2011	SKS
EAST: USPAT, USOCR, US-PGPUB, JPO, EPO, DERWENT- UPDATED & ATTACHED	7/11/2012	SKS
INVENTOR SEARCH: PAL, EDAN AND EAST- UPDATED	7/11/2012	SKS
EAST: USPAT, USOCR, US-PGPUB, JPO, EPO, DERWENT- UPDATED & ATTACHED	8/12/2013	SKS
INVENTOR SEARCH: PALM, EDAN AND EAST- UPDATED	8/12/2013	SKS
EAST: USPAT, USOCR, US-PGPUB, JPO, EPO, DERWENT- UPDATED & ATTACHED	5/19/2014	SKS
INVENTOR SEARCH: PALM, EDAN AND EAST- UPDATED	5/19/2014	SKS
DISCUSSION: SPE Jon Weber- regarding 101 rejection	12/18/14	SKS
EAST: USPAT, USOCR, US-PGPUB, JPO, EPO, DERWENT- UPDATED & ATTACHED	1/23/15	SKS
INVENTOR SEARCH: PALM, EDAN AND EAST- UPDATED	1/23/15	SKS
EAST: USPAT, USOCR, US-PGPUB, JPO, EPO, DERWENT- UPDATED & ATTACHED	1/30/15	SKS
INVENTOR SEARCH: PALM, EDAN AND EAST- UPDATED	1/30/15	SKS

	/SATYENDRA SINGH/ Primary Examiner.Art Unit 1657

INTERFERENCE SEARCH			
US Class/ CPC Symbol	**US Subclass / CPC Group**	**Date**	**Examiner**
	TEXT-LIMITED INTERFERENCE SEARCH IN EAST ONLY	1/30/15	SKS

	/SATYENDRA SINGH/ Primary Examiner.Art Unit 1657

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail **Mail Stop ISSUE FEE**
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

20855 7590 02/03/2015
PASTERNAK PATENT LAW
1900 EMBARCADERO ROAD
SUITE 211
PALO ALTO, CA 94303

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission
I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

Denise Vaillancourt	(Depositor's name)
	(Signature)
May 1, 2015	(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/661,553	03/19/2010	John Fraser Wright	0800-0045.01	4726

TITLE OF INVENTION: Compositions and methods to prevent AAV vector aggregation

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$960	$0	$0	$960	05/04/2015

EXAMINER	ART UNIT	CLASS-SUBCLASS
SINGH, SATYENDRA K	1657	435-239000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).
☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 Roberta L. Robins
2 Robins Law Group
3

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE: Genzyme Corporation

(B) RESIDENCE: (CITY and STATE OR COUNTRY): Framingham, MA

Please check the appropriate assignee category or categories (will not be printed on the patent) : ☐ Individual ☒ Corporation or other private group entity ☐ Government

4a. The following fee(s) are submitted:
☒ Issue Fee
☐ Publication Fee (No small entity discount permitted)
☐ Advance Order - # of Copies _____

4b. Payment of Fee(s): **(Please first reapply any previously paid issue fee shown above)**
☐ A check is enclosed.
☒ Payment by credit card. Form PTO-2038 is attached.
☒ The director is hereby authorized to charge the required fee(s), any deficiency, or credits any overpayment, to Deposit Account Number 50-5826 (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)
☐ Applicant certifying micro entity status. See 37 CFR 1.29
NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.
☐ Applicant asserting small entity status. See 37 CFR 1.27
NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.
☐ Applicant changing to regular undiscounted fee status.
NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature /Roberta L. Robins/ Date May 1, 2015

Typed or printed name Roberta L. Robins Registration No. 33,208

Electronic Patent Application Fee Transmittal

Application Number:	12661553
Filing Date:	19-Mar-2010
Title of Invention:	Compositions and methods to prevent AAV vector aggregation
First Named Inventor/Applicant Name:	John Fraser Wright
Filer:	Roberta L. Robins/Denise Vaillancourt
Attorney Docket Number:	0800-0045.01

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Utility Appl Issue Fee	1501	1	960	960

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Miscellaneous:				
			Total in USD ($)	**960**

Electronic Acknowledgement Receipt

EFS ID:	22231876
Application Number:	12661553
International Application Number:	
Confirmation Number:	4726
Title of Invention:	Compositions and methods to prevent AAV vector aggregation
First Named Inventor/Applicant Name:	John Fraser Wright
Customer Number:	20855
Filer:	Roberta L. Robins/Denise Vaillancourt
Filer Authorized By:	Roberta L. Robins
Attorney Docket Number:	0800-0045.01
Receipt Date:	01-MAY-2015
Filing Date:	19-MAR-2010
Time Stamp:	14:57:24
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Credit Card
Payment was successfully received in RAM	$960
RAM confirmation Number	1272
Deposit Account	505826
Authorized User	ROBINS, ROBERTA L.

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Issue Fee Payment (PTO-85B)	if.pdf	85862 22f03d28700ea9ea5df653f245ef3a230be0fcb5	no	1
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	30386 ec61404e2767c29f883d4a74eeb9c7bcb630ef17	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	116248		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Please type a plus sign (+) inside this box → [+]

PTO/SB/08A (08-00)

Substitute for form 1449A/PTO				*Complete if Known*	
INFORMATION DISCLOSURE STATEMENT BY APPLICANT				**Application Number**	12/661,553
				Filing Date	March 19, 2010
				First Named Inventor	Wright et al.
				Group Art Unit	Not Assigned
(use as many sheets as necessary)				Examiner Name	Not Assigned
Sheet	1	of	4	Attorney Docket Number	0800-0045.01

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	U.S. Patent Document Number	U.S. Patent Document Kind Code[2] (*if known*)	Name of Patentee or Applicant of Cited Document	Date of Publication of Cited Document MM-DD-YYYY
Change(s) applied to document, /R.S./ 3/3/2015	A1	6,593,123	435/239	Wright et al. July 15, 2003	~~07-2001~~
	A2	6,566,118	435/239	Atkinson et al.	05-2003
	A3	6,194,191	435/235.1	Zhang et al.	02-2001
	A4	6,146,874	435/235.1	Zolotukhin et al.	11-2000

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Office[3]	Number[4]	Kind Code[5] (if known)	Name of Patentee or Applicant of Cited Document	Date of Publication of Cited Document MM-DD-YYYY	T[6]
		~~ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /SS/~~					

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] Unique citation designation number.
[2] See attached Kinds of U.S. Patent Documents.
[3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3).
[4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document.
[5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST. 16 if possible.
[6] Applicant is to place a check mark here if English language Translation is attached.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/661,553	06/09/2015	9051542	0800-0045.01	4726

20855 7590 05/20/2015

PASTERNAK PATENT LAW
1900 EMBARCADERO ROAD
SUITE 211
PALO ALTO, CA 94303

ISSUE NOTIFICATION

The projected patent number and issue date are specified above.

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(application filed on or after May 29, 2000)

The Patent Term Adjustment is 0 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (http://pair.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Application Assistance Unit (AAU) of the Office of Data Management (ODM) at (571)-272-4200.

APPLICANT(s) (Please see PAIR WEB site http://pair.uspto.gov for additional applicants):

John Fraser Wright, Princeton, NJ;
Guang Qu, Alameda, CA;

COMBINED POWER OF ATTORNEY BY ASSIGNEE
AND STATEMENTS UNDER 37 CFR §§ 3.73 (b) AND 3.71

Genzyme Corporation (hereinafter "Assignee") having a place of business at **450 Water Street, Cambridge, Massachusetts, 02141**, states that it is the assignee of the entire right, title and interest in the patent listed below by virtue of either an assignment from the inventor(s), or by chain of title from the inventor(s), to the Assignee, recorded at the specified reel and frame numbers listed below, or attached hereto:

Patent No.	Issue Date	Title	Assignment Recordation
9,051,542	June 9, 2015	Compositions and methods to prevent AAV vector aggregation	Reel 039960 Frame 0383 Reel 039960 Frame 0444

As required by 37 CFR § 3.73(b)(1)(i), the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR § 3.11.

Genzyme Corporation hereby appoints **Amanda K. Antons**, Registration No. **65,236**, and the **Dechert LLP** practitioners associated with **Customer Number 37509** as its attorneys and agents with full power of substitution and revocation, to prosecute the above-captioned patent, and to transact all business in the USPTO connected therewith, said appointment being to the exclusion of the inventor(s) and his/her attorney(s) in accordance with the provisions of 37 CFR § 3.71; provided that if any one of said attorneys or agents ceases to be affiliated with the law firm of Dechert LLP as partner, employee or of counsel, such attorney or agent's appointment as attorney and all powers derived therefrom shall terminate on the date such attorney or agent ceases being so affiliated.

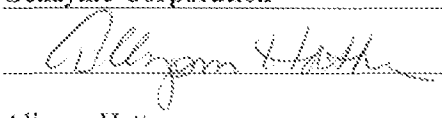
Please direct all correspondence address for the above-identified application to:

☒ Customer Number **37509**
Dechert LLP, 1095 Avenue of the Americas, New York, NY 10036-6797
Telephone: 212.698.3500

The undersigned, whose title is supplied below, is authorized to act on behalf of the Assignee.

Assignee: **Genzyme Corporation**

Date: June 14, 2023

Signed: 

Print Name: Allyson Hatton

Print Title: Principal Counsel, Attorney-in-Fact on behalf of Genzyme Corporation

Electronic Patent Application Fee Transmittal

Application Number:	12661553
Filing Date:	19-Mar-2010
Title of Invention:	Compositions and methods to prevent AAV vector aggregation
First Named Inventor/Applicant Name:	John Fraser Wright
Filer:	Blaine Motove Hackman/Sean Hynes
Attorney Docket Number:	0800-0045.01

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
STATUTORY OR TERMINAL DISCLAIMER	1814	1	170	170
		Total in USD ($)		**170**

Electronic Acknowledgement Receipt

EFS ID:	48159861
Application Number:	12661553
International Application Number:	
Confirmation Number:	4726
Title of Invention:	Compositions and methods to prevent AAV vector aggregation
First Named Inventor/Applicant Name:	John Fraser Wright
Customer Number:	20855
Filer:	Blaine Motove Hackman/Sean Hynes
Filer Authorized By:	Blaine Motove Hackman
Attorney Docket Number:	0800-0045.01
Receipt Date:	15-JUN-2023
Filing Date:	19-MAR-2010
Time Stamp:	16:44:37
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	$170
RAM confirmation Number	E20236EG45209909
Deposit Account	
Authorized User	

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal Letter	Transmittal_Form.pdf	151032 2b160bad89147fabc7b44e26534b3e2ad932a8a2	no	1
Warnings:					
Information:					
2	Statutory disclaimers per Manual of Patent Examining Procedure(MPEP) 1490.	Disclaimer_Form.pdf	95543 2cf3cced215283ed11822f8379b397f79e5e08c6	no	1
Warnings:					
Information:					
3	Power of Attorney	POA.pdf	3008783 0ffb1675302589e4a530563cd9a11fba326ca5b2	no	1
Warnings:					
Information:					
4	Fee Worksheet (SB06)	fee-info.pdf	38141 34e6b18af1c2996ee2feb892c0841e7280b72ced	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	3293499		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Doc Code: TRAN.LET
Document Description: Transmittal Letter

PTO/SB/21 (07-09)
Approved for use through 05/31/2024. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Total Number of Pages in This Submission	

Application Number	12/661,553
Filing Date	March 19, 2010
First Named Inventor	John Fraser Wright
Art Unit	1657
Examiner Name	SATYENDRA K SINGH
Attorney Docket Number	

ENCLOSURES (*Check all that apply*)

- ☐ Fee Transmittal Form
  - ☐ Fee Attached
- ☐ Amendment/Reply
  - ☐ After Final
  - ☐ Affidavits/declaration(s)
- ☐ Extension of Time Request
- ☐ Express Abandonment Request
- ☐ Information Disclosure Statement
- ☐ Certified Copy of Priority Document(s)
- ☐ Reply to Missing Parts/ Incomplete Application
  - ☐ Reply to Missing Parts under 37 CFR 1.52 or 1.53
- ☐ Drawing(s)
- ☐ Licensing-related Papers
- ☐ Petition
- ☐ Petition to Convert to a Provisional Application
- ☐ Power of Attorney, Revocation Change of Correspondence Address
- ☐ Terminal Disclaimer
- ☐ Request for Refund
- ☐ CD, Number of CD(s) _____
  - ☐ Landscape Table on CD
- ☐ After Allowance Communication to TC
- ☐ Appeal Communication to Board of Appeals and Interferences
- ☐ Appeal Communication to TC **(Appeal Notice, Brief, Reply Brief)**
- ☐ Proprietary Information
- ☐ Status Letter
- ☒ Other Enclosure(s) (please Identify below):

Statutory Disclaimer; and Combined Power of Attorney by Assignee and Statements under 37 CFR §§ 3.73(c) and 3.71

Remarks

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	DECHERT LLP		
Signature	/Amanda K. Antons/		
Printed name	Amanda K. Antons		
Date	June 15, 2023	Reg. No.	65,236

PTO/SB/43 (07-09)
Approved for use through 11/30/2020. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

DISCLAIMER IN PATENT UNDER 37 CFR 1.321(a)

Name of Patentee	Docket Number (Optional)
GENZYME CORPORATION	
Patent Number	**Date Patent Issued**
9,051,542	June 9, 2015

Title of Invention: Compositions and methods to prevent AAV vector aggregation

I hereby disclaim the following complete claims in the above identified patent:

1 and 2

The extent of my interest in said patent is (if assignee of record, state liber and page, or reel and frame, where assignment is recorded): Reel: 39960 Frame: 0444

The fee for this disclaimer is set forth in 37 CFR 1.20(d).

- [ ] Patentee claims small entity status. See 37 CFR 1.27.
- [ ] Small entity status has already been established in this case, and is still proper.
- [ ] A check in the amount of the fee is enclosed.
- [ ] Payment by credit card. Form PTO-2038 is attached.
- [x] The Director is hereby authorized to charge any fees which may be required or credit any overpayment to Deposit Account No. 50-2778 .

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

Signed at Chicago , State of IL ,
this 15th day of June 2023 .

/Amanda K. Antons/
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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
12/661,553	03/19/2010	John Fraser Wright	0800-0045.01

CONFIRMATION NO. 4726

IMPROPER CPOA LETTER

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Date Mailed: 06/22/2023

NOTICE REGARDING POWER OF ATTORNEY

This is in response to the power of attorney filed 06/15/2023. The power of attorney in this application is not accepted for the reason(s) listed below:

The power of attorney filed 06/15/2023 has not been accepted because the power of attorney must be signed by the applicant for patent. See 37 CFR 1.32(b)(4).

- The person or entity attempting the change to power of attorney is not the applicant of record in the application. Any request to change the applicant once the applicant has been specified must include (1) an application data sheet (ADS) specifying the new applicant in the Applicant Information section, and (2) a statement under 37 CFR 3.73(c) (USPTO Form PTO/AIA/96 or an equivalent) to show chain of title to the new applicant. The ADS must contain markings to show the information that is being changed, with underlining for insertions and strike-through or brackets for text removed. See 37 CFR 1.76(c)(2).

Because the request to change or update the applicant cannot be accepted, the power of attorney is not properly signed by the applicant and cannot be accepted. The change to applicant must be acceptable before the new applicant can appoint power of attorney.

/sltorres/

Office of Data Management, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

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UNITED STATES

Disclaimer

9,051,542 B2 - John Fraser Wright, Princeton, NJ (US); Guang Qu, Alameda, CA (US). COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION. Patent dated June 9, 2015. Disclaimer filed June 16, 2023, by the assignee, Genzyme Corporation.

I hereby disclaim the following complete claims 1 and 2, of said patent.

(Official Gazette, August 22, 2023)

Trials@uspto.gov
571-272-7822

Paper 20
Entered: August 30, 2023

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

NOVARTIS GENE THERAPIES, INC. & NOVARTIS PHARMACEUTICALS CORPORATION,
Petitioners,
v.

GENZYME CORPORATION,
Patent Owner.

IPR2023-00608
Patent 9,051,542 B2

Before JEFFREY N. FREDMAN, SHERIDAN K. SNEDDEN, and JAMES A. TARTAL, *Administrative Patent Judges.*

SNEDDEN, *Administrative Patent Judge.*

DECISION
Denying Institution of *Inter Partes* Review
35 U.S.C. § 314

I. INTRODUCTION

A. Background and Summary

Novartis Gene Therapies, Inc. and Novartis Pharmaceuticals Corporation (collectively, "Petitioner") filed a Petition requesting an *inter partes* review of claims 1, 2, 5, and 6 of U.S. Patent No. 9,051,542 B2 ("the '542 patent," Ex. 1001). Paper 2 ("Pet."). Genzyme Corporation ("Patent Owner") filed a Preliminary Response to the Petition. Paper 14 ("Prelim. Resp."). In its Preliminary Response, Patent Owner indicates that claims 1 and 2 are disclaimed, so only claims 5 and 6 remain challenged. *Id.* at 3.

With our authorization, Petitioner filed a Reply to Patent Owner's Preliminary Response (Paper 17) and Patent Owner filed a Sur-reply (Paper 18).

To institute an *inter partes* review, we must determine that the information presented in the Petition shows "a reasonable likelihood that the petitioner would prevail with respect to at least 1 of the claims challenged in the petition." 35 U.S.C. § 314(a) (2018). The Supreme Court has held that a decision to institute under 35 U.S.C. § 314 may not institute on less than all claims challenged in the petition. *SAS Inst., Inc. v. Iancu*, 138 S. Ct. 1348, 1359–60 (2018). After considering the evidence and arguments presented in the Petition, we determine that Petitioner has not demonstrated a reasonable likelihood of success in proving that either claim 5 or claim 6 of the '542 patent is unpatentable.

B. Real Parties in Interest

Petitioner asserts that Novartis Gene Therapies, Inc. and Novartis Pharmaceuticals Corporation are the real parties in interest. Pet. 67. Patent Owner asserts that "Sanofi, the ultimate parent company of Genzyme

Corporation, Genzyme Corporation, and Aventis, Inc. are the real parties-in-interest." Paper 6, 2.

C. Related Matters

The parties indicate that the '542 patent is asserted against Petitioner in *Genzyme Corporation et al. v. Novartis Gene Therapies, Inc. et al.*, Case No. 1:21-cv-01736 (D. Del.), filed December 10, 2021. Pet. 67–68; Paper 6, 2. Petitioner also filed a petition for *inter partes* review in IPR2023-00609 seeking to challenge claims 5 and 6 of the '542 patent on other grounds. Pet. 68.

D. The '542 patent (Ex. 1001)

The '542 patent is titled "Compositions and Methods to Prevent AAV Vector Aggregation," and issued on June 9, 2015, from U.S. Patent Application No. 12/661,553, filed March 19, 2010. Ex. 1001, codes (21), (22), (45), (54). The '542 patent "relates to compositions and methods of preparing and storing AAV [(adeno-associated virus)] virions that prevent aggregation." *Id.* at 1:17–19. According to the '542 patent, "[t]he solubility of purified AAV2 virus particles is limited, and aggregation of AAV2 particles has been described as a problem." *Id.* at 1:41–46 (citing, e.g., Wright et al., "Recombinant Adeno-Associated Virus: Formulation Challenges and Strategies for a Gene Therapy Vector," *Curr. Opin. Drug Disc. Dev.* 6(2):174–178 (2003) (Ex. 1007, "Wright"); Croyle, et al., "Development of Formulations That Enhance Physical Stability of Viral Vectors for Gene Therapy," *Gene Ther.*, 8:1281–1290 (2001) (Ex. 1013, "Croyle")).

In particular, the '542 patent discloses high ionic strength solutions that are isotonic with the intended target tissue. *Id.* at code (57). The

"combination of high ionic strength and modest osmolarity is achieved using salts of high valency, such as sodium citrate." *Id.*

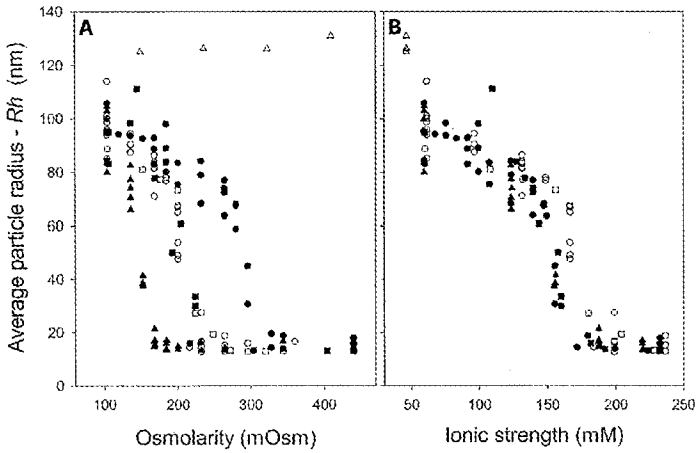
The '542 patent further explains as follows:

> The present invention is based in part on the observation that solution ionic strength is an important parameter in AAV vector aggregation, implicating the involvement of ionic interactions between virus particles in the aggregation process. The observation that elevated ionic strength increases AAV2 [AAV serotype 2] vector solubility regardless of the identity of the charged excipient supports the hypothesis that ionic strength of solution per se, rather than interactions involving a specific ionic species, is the relevant physico-chemical parameter. A threshold ionic strength of at least 200 mM is required to prevent aggregation at vector particle concentrations examined herein.

Id. at 4:53–64. The '542 patent additionally states as follows:

> In embodiments of the present invention the exponential relationship of ionic strength with charge valency is used to develop isotonic formulations with high ionic strengths. Salt species with multiple charge valencies (e.g. salts of sulfate, citrate, and phosphate) that are commonly used as excipients in human parenteral formulations can provide the level of ionic strength needed to prevent AAV2 vector aggregation when used at isotonic concentrations. While isotonic (150 mM) sodium chloride has an ionic strength of 150 mM, a value insufficient to maintain AAV2 solubility at high vector concentrations, isotonic sodium citrate, with an ionic strength of ~500 mM, can support AAV2 vector concentrations of at least 6.4×10^{13} vg/mL without aggregation.

Id. at 5:7–20. Figures 1A and 1B of the '542 patent are reproduced below.



Figures 1A and 1B present the results of a vector aggregation study that tracked aggregation as a function of two parameters, osmolarity (Figure 1A) and ionic strength (Figure 1B) for buffer compositions of sodium chloride (●), sodium citrate (○), sodium phosphate (■), sodium sulfate (□), magnesium sulfate (▲), and glycerol (Δ), and. *Id.* at 6:63–65, 12:33–67 (Example 3), FIGS. 1A, 1B. "Average particle radius is measured by dynamic light scattering (DLS) following vector dilution in varying concentrations of excipients buffered with 10 mM sodium phosphate at pH 7.5." *Id.* at 4:18–28. "Rh values >20 nm are deemed to indicate the occurrence of some level of aggregation." *Id.* at 9:25–27.

The results of Figure 1A, which plots vector aggregation as a function of the osmolarity of selected excipients, are explained as follows:

> For charged species a concentration-dependent inhibition of AAV2 vector aggregation is observed. Salts with multivalent ions achieve a similar degree of inhibition of aggregation at lower concentrations than monovalent sodium chloride. For example, magnesium sulfate prevents aggregation at >200 mOsm whereas sodium chloride requires ≧350 mOsm to achieve a similar effect. Sodium citrate, sodium sulfate, and sodium phosphate are intermediate in their potency to prevent vector aggregation.

Id. at 6:65–7:8.

Figure 1B shows data from the same experiment "plotted as a function of the calculated ionic strength, rather than osmolarity, for each excipient." *Id.* at 7:18–20. Figure 1B's plot of particle radius versus ionic strength shows that "vector aggregation is prevented when ionic strength is ~200 mM or greater regardless of which salt is used." *Id.* at 7:21–22. "These data suggested that the ionic strength (μ) of a solution . . . is the primary factor affecting aggregation." *Id.* at 7:22–25.

The '542 patent discloses the results of a study assessing "the effects of elevated ionic strength and nuclease treatment on AAV2 vector aggregation at a larger scale, using methods to induce and quantify vector aggregation that are relevant to preparative scale vector purification" in Table 2. *Id.* at 8:1–5.

Table 2 of the '542 patent is reproduced, in part, below.

TABLE 2

AAV VECTOR RECOVERY AT PROCESS SCALE

Experiment	Formulation	μ (mM)	Target (vg/mL)	Actual (vg/mL)	Yield % (RSD)
1	CF	160	2.5E13	1.93E13	77 (6.6)
1	TF1	310	2.5E13	2.38E13	95 (7.4)
1	TF2	510	2.5E13	2.33E13	93 (7.4)
2	CF	160	6.7E13	3.98E13	59 (6.0)
2	TF2	510	6.7E13	6.42E13	96 (4.4)

Table 2 shows the results for three solutions of AAV2-AADC vectors filtered through a 0.22 μm filter. *Id.* at 8:1–10, 11:53–12:29. The three solutions are as follows:

> Control Formulation (CF: 140 mM sodium chloride, 10 mM sodium phosphate, 5% sorbitol, pH 7.3); Test Formulation 1 (TF1: 150 mM sodium phosphate, pH 7.5); and Test Formulation 2 (TF2: 100 mM sodium citrate, 10 mM Tris, pH 8.0).

Id. at 11:66–12:3. In Experiment 1, the samples contained 2.5×10^{13} vg/ml vector, and, in Experiment 2, the samples contained 6.7×10^{13} vg/ml vector. *Id.* at 12:4–12. Table 2 shows recoveries exceeded 90% following filtration in formulations TF1 and TF2 having ionic strengths greater than 200 mM, whereas recovery from CF formulations, having ionic strength of 160 mM, was only 77% and 59% for experiments 1 and 2, respectively. *Id.* at 8:19–56.

The '542 patent also discloses the results of a study assessing "stability after storage or freeze-thaw (F/T) cycling is assessed in buffers of the present invention." *Id.* at 9:19–27. Particle radius was measured by dynamic light scattering (DLS) to determine the presence of aggregates. *Id.*

Table 3, reproduced below, summarizes the results of the study.

TABLE 3

STABILITY OF AAV2 VECTORS

	Particle radius - Rh (nm)							
Formu-		4° C.	−20° C.			−80° C.		
lation	Pre	5 d	1 F/T	5 F/T	10 F/T	1 F/T	5 F/T	10 F/T
CF	14.5	27.0	22.4	56.1	94.5	20.6	57.5	141
TF1	13.8	16.3	TH	TH	TH	TH	TH	TH
TF2	13.8	14.4	14.2	14.0	14.1	13.8	21.3	50.9

Pre: DLS radius measured immediately following 0.2 μm filtration.
Vector concentrations (vg/mL): CF: 1.93E13, TF1: 2.38E13, TF2: 2.33E13.
TH: signal intensity is too high to measure because of extensive aggregation.

According to the '542 patent, Table 3 provides data showing as follows:

> AAV2-AADC vector prepared in CF shows some aggregation after 5 days of storage at 4° C., as well as following one or more F/T cycles at −20 or −80° C. For vector prepared in TF1, no aggregation occurs after 5 days at 4° C., but aggregation occurs following a single F/T cycle at −20 or −80° C. as indicated by a DLS signal intensity that is too high to measure. Visual

> inspection of these samples reveals slight cloudiness, which is consistent with aggregation. For vector prepared in TF2, no aggregation is observed at 4° C., or following up to 10 F/T cycles at −20° C. Some aggregation is observed following 5 and 10 F/T cycles at −80° C.

Id. at 9:29–55. According to Patent Owner, the results of the studies disclosed in the '542 patent "confirmed the importance of increased ionic strength in preventing aggregation." Prelim. Resp. 13 (citing Ex. 1001, 10:29–43 (stating "[t]he effect of ionic strength [] on virus particle interactions is determined to elucidate the mechanism of vector aggregation")).

E. The Challenged Claims

Challenged Claims 5 and 6 are reproduced below, along with claim 1 from which they depend.

> 1. A composition for the storage of purified, recombinant adeno-associated virus (AAV) vector particles, comprising:
>
> > purified, recombinant AAV vector particles at a *concentration exceeding 1×10^{13} vg/ml up to 6.4×10^{13} vg/ml*;[1]

[1] The units of measurements used in the art to measure the titer of AAV compositions are explained in the Petition as follows:

> The titer of AAV compositions can be measured in vector genomes (vg)/ml, genome copies (gc)/ml, capsid particles (cp)/ml, or virus particles (vp)/ml. Ex. 1025, ¶¶35. The first two are used interchangeably, since both represent the number of functional vectors containing the therapeutic gene. *Id.*, ¶¶36-37. By contrast, the latter two measurements include particles that are incomplete, damaged, or lacking genetic material. Ex. 1009, [00281]; Ex. 1025, ¶36.

Pet. 12.

a pH buffer, wherein the pH of the composition is between 7.5 and 8.0; and

excipients comprising one or more multivalent ions selected from the group consisting of citrate, sulfate, magnesium, and phosphate; wherein *the ionic strength of the composition is greater than 200 mM*, and wherein the purified AAV vector particles are stored in the composition without significant aggregation.

5. The composition of claim 1, *wherein the purified, recombinant AAV vector particles have an average particle radius (Rh) of less than about 20 nm as measured by dynamic light scattering.*

6. The composition of claim 1, *wherein recovery of the purified, recombinant virus particles is at least about 90% following filtration of the composition of said AAV vector particles through a 0.22 μm filter.*

Ex. 1001, 14:15–28, 34–41 (emphasis added to highlight disputed elements).

F. Evidence

Petitioner relies upon information that includes the following.

Ex. 1003, Evans, WO 01/66137 A1, published Sept. 13, 2000 ("Evans").

Ex. 1004, Frei et al., WO 99/41416, published Aug. 19, 1999 ("Frei").

Ex. 1005, Huang J., Gao, et al., "Aggregation of AAV vectors, its Impact on Liver directed Gene Transfer and Development of Vector Formulations to Prevent and Dissolve Aggregation and Enhance Gene Transfer Efficiency," MOL THER. 1:S286 (2000) ("Huang").

Ex. 1006, Mingozzi, et al., "Improved Hepatic Gene Transfer by Using an Adeno-Associated Virus Serotype 5 Vector," J VIROL. Vol. 76, No. 20, pp. 10497–502 (2002) ("Mingozzi").

Ex. 1007, Wright et al., "Recombinant Adeno-Associated Virus: Formulation Challenges and Strategies for a Gene Therapy

Vector," CURR. OPIN. DRUG DISC. DEV. 6(2):174–178 (2003) ("Wright").

Petitioner also relies upon the Declaration of Mansoor M. Amiji, R.Ph., Ph.D. (Ex. 1025) to support its contentions.

Patent Owner relies upon the Declaration of Martyn C. Davies, D.Sc., Ph.D. (Ex. 2004) to support its contentions.

G. Asserted Grounds of Unpatentability

In the Petition, Petitioner challenges claims 1, 2, 5, and 6 on the following grounds:

Ground	Claim(s) Challenged	35 U.S.C. §[2]	Reference(s)/Basis
1	1, 5, 6	103	Evans, Huang, Mingozzi
2	2	103	Evans, Wright, Huang, Mingozzi
3	1, 2, 5, 6	103	Frei, Huang, Mingozzi

Pet. 4. After the Petition was filed, Patent Owner subsequently explained that "[c]laims 1 and 2 were disclaimed to streamline issues for the Board, because only claims 5 and 6 are asserted for infringement in the co-pending litigation." Prelim. Resp. 3 n.3. Accordingly, for the purposes of this Decision, we consider only Petitioner's Grounds 1 and 3 as directed to challenged claims 5 and 6.

H. Claim Construction

We interpret a claim "using the same claim construction standard that would be used to construe the claim in a civil action under 35 U.S.C.

[2] The Leahy-Smith America Invents Act ("AIA") included revisions to 35 U.S.C. §103 that became effective on March 16, 2013. We apply the pre-AIA version of §103 here, because the application identified in the '542 patent was filed before the effective date of the AIA. *See* Ex. 1001, code (22).

282(b)." 37 C.F.R. § 42.100(b) (2019). Under this standard, we construe the claim "in accordance with the ordinary and customary meaning of such claim as understood by one of ordinary skill in the art and the prosecution history pertaining to the patent." *Id.*

Petitioner asserts that the claim terms require no express construction. Pet. 17. Patent Owner does not challenge Petitioner's position. Prelim. Resp. 14.

Having considered the parties' positions and evidence of record, we determine that no express construction of any claim term is necessary to determine whether to institute *inter partes* review. *Nidec Motor Corp. v. Zhongshan Broad Ocean Motor Co.*, 868 F.3d 1013, 1017 (Fed. Cir. 2017) ("[W]e need only construe terms 'that are in controversy, and only to the extent necessary to resolve the controversy.'" (quoting *Vivid Techs., Inc. v. Am. Sci. & Eng'g, Inc.*, 200 F.3d 795, 803 (Fed. Cir. 1999))). To the extent further discussion of the meaning of any claim term is necessary to our decision, we provide that discussion below in our analysis of the asserted grounds of unpatentability.

I. Level of Ordinary Skill in the Art

The level of ordinary skill in the art usually is evidenced by the prior art references themselves. *See Okajima v. Bourdeau*, 261 F.3d 1350, 1355 (Fed. Cir. 2001); *In re GPAC Inc.*, 57 F.3d 1573, 1579 (Fed. Cir. 1995).

Petitioner proposes that a person of ordinary skill in the art ("POSA") at the time of the invention

> would have possessed at least a BS in biology, chemistry, chemical engineering, biochemistry, pharmaceutical science, or a related discipline, with ≥4 years of industry, laboratory, and/or clinical experience in formulating or developing dispersions for therapeutic biologics, such as proteins or vectors for gene

> delivery. Such person may be familiar with, or consult with someone familiar with, the development and/or administration of viral vectors for gene therapy. Ex. 1025, ¶82.

Pet. 16–17. Patent Owner does not dispute Petitioner's proposal about the POSA's qualifications. Prelim. Resp. 2 n.2.

For this Decision, we adopt and apply Petitioner's proposal for the person of ordinary skill in the art level, which appears to be consistent with the level of skill reflected in the asserted prior art and the '542 patent.

II. ANALYSIS

"In an [*inter partes* review], the petitioner has the burden from the onset to show with particularity why the patent it challenges is unpatentable." *Harmonic Inc. v. Avid Tech., Inc.*, 815 F.3d 1356, 1363 (Fed. Cir. 2016) (citing 35 U.S.C. § 312(a)(3) (requiring inter partes review petitions to identify "with particularity . . . the evidence that supports the grounds for the challenge to each claim")). This burden of persuasion never shifts to the patent owner. *See Dynamic Drinkware, LLC v. Nat'l Graphics, Inc.*, 800 F.3d 1375, 1378 (Fed. Cir. 2015). Moreover, a petitioner should not "place the burden on [the Board] to sift through information presented by the Petitioners, determine where each element [of the challenged claims] is found in [the cited references], and identify any differences between the claimed subject matter and the teachings of [the cited references.]" *Google Inc. and Twitter, Inc. v. EveryMD.com LLC*, IPR2014-00347, Paper 9 at 25 (PTAB May 22, 2014).

The question of obviousness is resolved on the basis of underlying factual determinations including: (1) the scope and content of the prior art; (2) any differences between the claimed subject matter and the prior art; (3) the level of ordinary skill in the art; and (4) objective evidence of

nonobviousness.[3] *Graham v. John Deere Co.*, 383 U.S. 1, 17–18 (1966).

The obviousness inquiry also typically requires an analysis of "whether there was an apparent reason to combine the known elements in the fashion claimed by the patent at issue." *KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398, 418 (2007) (citing *In re Kahn*, 441 F.3d 977, 988 (Fed. Cir. 2006) (requiring "articulated reasoning with some rational underpinning to support the legal conclusion of obviousness")). A petitioner cannot prove obviousness with "mere conclusory statements." *In re Magnum Oil Tools Int'l, Ltd.*, 829 F.3d 1364, 1380 (Fed. Cir. 2016). Rather, a petitioner must articulate a sufficient reason why a person of ordinary skill in the art would have combined the prior art references. *In re NuVasive, Inc.*, 842 F.3d 1376, 1382 (Fed. Cir. 2016).

We analyze the asserted grounds of unpatentability in accordance with these principles to determine whether Petitioner has met its burden to establish a reasonable likelihood of success at trial.

A. Summary of Cited Prior Art

1. Evans

Evans discloses viral compositions for use in gene therapy. Ex. 1003, Abstract, 1:15–19. Evans teaches buffer conditions to maintain its compositions for potential human parenteral administration. Ex.1003, 1:15–19. Evans explains that "[a]n ongoing challenge in the field of gene therapy and vaccine research is to generate liquid virus formulations which are stable for longer periods of time within a useful temperature range." *Id.* at 1:16–19, 28–30.

[3] Patent Owner does not present any objective evidence of nonobviousness (i.e., secondary considerations) for the challenged claims.

Evans discloses that its compositions comprise a buffer, a salt, a divalent cation, and a non-ionic detergent. Ex. 1003, 1:19–21. Evans further discloses the identity of and concentration ranges for those components. *See* Ex. 1003, 8:22–11:4. Evans also discloses that the compositions support virus concentrations of about 1 x 10^7 to 1 x 10^{13} vp/ml. Ex. 1003, 8:5–11.

Evans claims a virus composition comprising a purified virus with a concentration of about 1 x 10^7 to 1 x 10^{13} vp/ml, a buffer acceptable for human parenteral use at a pH of about 7.5–8.5, sodium chloride at about 25mM–250mM, a divalent cation selected from $MgCl_2$ and $CaCl_2$ at about 0.1mM–5mM, and a non-ionic detergent. Ex. 1003, 36 (claim 5). Evans teaches that its compositions may be used with AAV. Ex. 1003, 3:12–14; 7:16–18.

2. Huang

Huang, an abstract titled "Aggregation of AAV Vectors, its impact on Liver directed Gene Transfer and Development of Vector Formulations to Prevent and Dissolve Aggregation and Enhance Gene Transfer Efficiency," states that "to achieve high level of gene transfer and ensure the safety of vector administration it is desirable to deliver high doses of vector in small volumes." Ex. 1005, S286. According to Huang, "at high concentrations, AAV virions form aggregates of different sizes in a range of different buffer systems and storage conditions." *Id.* Huang states that "when the vector titer reached 5-10 x 10^{13} GCs/ml, gene transfer efficiency was 10-100 folds lower at the same dose as compared to the vector whose titer was 1-5 x 10^{12} GCs/ml. *Id.* Huang states that "a series of formulation studies were performed to prevent and dissolve AAV aggregation," and reported "a 30–

50% reduction in the size of aggregates size at high vector concentrations" for some of the compositions. *Id.*

3. Mingozzi

Mingozzi, titled Improved Hepatic Gene Transfer by Using Adeno-Associated Virus Serotype 5 Vector, states that "AAV vectors do not contain viral coding sequences and have been shown to efficiently transfer genes to nondividing target cells," and that "[a]n excellent safety profile combined with reduced potential for activation of inflammatory or cellular immune responses has made this vector system attractive for clinical application and treatment of genetic disorders." Ex. 1006, 10497. According to Mingozzi, purification of AAV-2 and AAV-5 vectors "by repeated CsCl gradient centrifugation" yielded concentrations of $>10^{13}$ vg/ml. *Id.*

4. Wright

Wright teaches that AAV "is a promising vector for human gene transfer" and has "received considerable attention in the field of gene therapy, because of [its] ability to mediate long-term gene transfer in the absence of significant toxicity." Ex. 1007, 174. Wright teaches that "because AAV and adenovirus are both non-enveloped viruses developed as gene transfer vectors, studies on the latter can provide guidance for AAV vector formulation development." *Id.*

Wright notes that "[t]he mechanism of vector aggregation is not well understood, and purification conditions that may affect aggregation include buffer ionic strength and pH, shear and vector concentration." *Id.* at 175. Wright discloses that

> Our and other research teams have observed that freeze-thaw cycling exacerbates vector aggregation, and can lead to aggregation at vector concentrations significantly lower than

> 10^{14} cp/ml. For example, using dynamic light scattering, we observed that highly purified vector preparations at concentrations of 5×10^{13} cp/ml that are stable in a non-aggregated, monomeric state when stored at 2 to 8°C, can be induced to undergo some aggregation following a single freeze-thaw cycle to -20°C.

Id. Wright notes that "[r]educed yield is one of the deleterious consequences of aggregation during the vector purification process" and notes that "loss of rAAV following a 0.2-μm filtration step correlates with the extent of vector aggregation." *Id.*

Wright teaches that "empty capsids, whose size and surface characteristics are similar to that of genome-containing vector particles, contribute to particle aggregation, and their presence may result in aggregation at lower vector genome (vg) concentrations than would be observed in their absence." *Id.* (citation omitted).

Wright further discloses that "[a]ssuming that full vector particles and empty capsids aggregate by a similar mechanism (an assumption that requires testing), a preparation of AAV vectors containing a 10-fold excess of empty capsids should have a similar risk of aggregation at concentrations of $\geq 10^{13}$ vg/ml (corresponding to $\geq 10^{14}$ cp/ml)." *Id.* at 175–176.

5. Frei

Frei discloses viral formulations comprising polyhydroxy hydrocarbon for use in gene therapy. Ex. 1004, Abstract, 1:15–20. Frei identifies "a critical need to develop formulations that stabilize relatively high concentrations of virus," and discloses a buffered formulation that stabilizes high concentrations of recombinant virus for use in gene therapy and maintains viability after storage. *Id.* at 4:26–36, 7:7–11, 8:27–29, 8:34–36. Frei discloses that its compositions comprise a buffer system that

maintains a pH of about 7.0–8.5 despite storage between -80°C and 27°C. *Id.* at 6:21–24. Frei's compositions include pharmaceutically acceptable divalent metal salt stabilizers, and Frei teaches that magnesium salts are particularly preferred in an amount of about 0.1 mg/ml to 1 mg/ml. *Id.* at 5:31–36. Pharmaceutically acceptable monovalent salt stabilizers are also included, and Frei discloses that sodium chloride in an amount of 0.6 mg/ml to 10.0 mg/ml is preferred. *Id.* at 5:37–6:6. Frei further teaches that "the formulation of the present invention can maintain stability of the virus at concentrations ranging up to 1 x 10^{13} particles/mL." *Id.* at 7:9–11. Frei's example of a virus composition ("Example D-1") comprises purified adenovirus at a concentration of 1.6 x 10^{13} vp/ml, in 20 mM NaPi buffer, 100 mM NaCl, 2 mM $MgCl_2$, 2% sucrose, and 10% glycerol, having pH 8 at 2–10°C. *Id.* at 22:17–31.

B. Ground 1: Obviousness of Claims 5 and 6 over the Combination of Evans, Huang, and Mingozzi

Petitioner asserts that claims 5 and 6 are unpatentable as obvious over Evans, Huang, and Mingozzi. Pet. 23–46. Patent Owner disputes Petitioner's contentions. Prelim. Resp. 14–44.

For the reasons set forth below, we determine that Petitioner has not shown a reasonable likelihood of establishing that at least one of the challenged claims is unpatentable as obvious over Evans, Huang, and Mingozzi.

1. Petitioner's Contentions

With regard to Challenged Claim 5, Petitioner contends that the "average particle radius" limitation is an "inherent characteristic feature of the purified viral composition." Pet. 41–42.

Petitioner next directs our attention to Evans's claim 5, which is directed to a virus composition containing a "divalent cation [] selected from the group consisting of $MgCl_2$ and $CaCl_2$ in an amount from about 0.1 mM to about 5 mM." Ex. 1003, 36 (claim 5); Pet. 42. Petitioner further contends that

> The '542 patent admits that AAV2 particles have a diameter of ~26nm (Ex.1001, 1:29-38). Because Evans's claim 5 composition prevented aggregation, a POSA would have reasonably expected AAV particles stored therein would have an Rh of <~20 nm measured by DLS. Indeed, the '542 patent does not identify anything critical about the recited radius range other than it being exemplary of no aggregation. *Id.*, 9:25-27 ("Rh values >20 nm are deemed to indicate the occurrence of some level of aggregation.").

Pet. 42. Petitioner contends that a person of ordinary skill in the art would have been motivated to minimize any potential aggregation in Evans's claim 5 because it was known that virus aggregation reduces gene transfer efficiency and other potentially deleterious consequences. *Id.* at 42–43 (citing Ex. 1005, S286; Ex. 1007, 176). Petitioner further contends that

> A POSA would have reasonably expected success in minimizing particle size in view of Huang's teaching that its optimized compositions "could lead to a 30-50% reduction in the size of aggregates at high vector concentrations." Ex.1005, S286. Indeed, "no signs of settling or precipitation" were observed for prior art adenovirus compositions stored in a high ionic strength buffer over a 7-day period (Ex.1009, [00369]), and a POSA would have understood that AAV "is significantly more stable than the adenovirus" used in Liu (Ex.1013, 1283); Ex.1025, ¶¶197-198. Thus, only routine optimization would be required to obtain an average AAV Rh of <20nm in Evans's claim 5 composition. Ex.1025, ¶¶ 199-201.

Pet. 43 (citing *Senju Pharm. Co. v. Lupin Ltd.*, 780 F.3d 1337, 1353 (Fed. Cir. 2015) (invalidating a claim directed to "a product of routine optimization that would have been obvious to one of skill in the art.")).

With regard to claim 6, Petitioner contends

> a POSA would have been motivated to minimize any potential aggregation in Evans's claim 5 formulation, since both Wright and Huang linked aggregation to reduced functional activity of AAV vectors. Ex.1007, 176; Ex.1005, S286. Thus, a POSA would have been motivated to maximize virus recovery from a 0.22μm filter through routine optimization of the known stabilization factors in Evans's claim 5 composition. Ex.1025, ¶ 205.

Pet. 45. Petitioner further contends that

> A POSA also would have reasonably expected success in maximizing particle recovery after filtration because POSA knew that Huang taught its optimized compositions "could lead to a 30-50% reduction in the size of aggregates at high vector concentrations" (Ex.1005, S286), Liu observed "no signs of settling or precipitation" for adenovirus particles stored in a high ionic strength buffer over a 7-day period (Ex.1009, [00369]), and Croyle taught that AAV "is significantly more stable than the adenovirus" (Ex.1013, 1283). Thus, only routine optimization would be required to improve AAV recovery following filtration of Evans's claim 5 formulation through a 0.22μm filter. Ex.1025, ¶¶ 206-209.

Pet. 45–46.

2. Patent Owner's Contentions

Patent Owner contends that Petitioner "does not submit any evidence that the particle radius and product recovery elements of claims 5 and 6, respectively, would inherently result from the claimed combination." Prelim. Resp. 16.

Patent Owner contends that Petitioner's reliance on Evans's disclosure of "a virus concentration in the range from about $1x10^7$ vp/mL to about

$1x10^{13}$ vp/mL" to argue that the uppermost endpoint of this range ($1x10^{13}$ vp/mL ± 5%) overlaps with the scope of the claims assumes that 100% of the particles contain vector genomes and that Petitioner "provides no basis for why the POSA would make such an assumption." *Id.* at 17 (citing Pet. 30 ("Assuming that 100% of the particles contain vector genomes, Evans's claim 5 composition therefore comprises viral particles at a concentration exceeding $1x10^{13}$ vg/ml.")). Rather, according to Patent Owner,

> Dr. Amiji's declaration indicates that the POSA would not have assumed that Evans's viral particle compositions were free of empty capsids, and instead would have assumed the opposite—that as many as 90% of capsids in a given composition are empty. [Ex. 2004] ¶¶72-73. Dr. Amiji states that "Wright [Ex. 1007] teaches that $\geq 10^{14}$ capsid particles (cp)/ml corresponds to $\geq 10^{13}$ vg/ml)," indicating as much as 10-fold excess in empty capsids. [Ex. 1025] ¶119 (citing Ex. 1007, 176).

Id. (emphasis omitted).

Patent Owner contends that

> [Petitioner] fails to establish that the POSA would have been motivated to develop a composition comprising an rAAV "concentration exceeding $1x10^{13}$ vg/ml," "one or more multivalent ions selected from … citrate, sulfate, magnesium, and phosphate," with an ionic strength "greater than 200mM." [Ex. 2004] ¶¶ 75–79.

Id. at 18. Patent Owner notes that Petitioner relies on "Mingozzi to argue that the POSA would been motivated to administer 'doses of $3.2x10^{13}$ vg for a 60kg human' at a 'concentration exceeding $1x10^{13}$ vg/ml.'" *Id.* (citing Pet. 31). Patent Owner contends, however, that Mingozzi "says nothing about any formulations for AAV vectors let alone anything about ionic strength or multivalent ions." *Id.* at 18–19 (citing Ex. 2004 ¶ 78).

Patent Owner further argues that Petitioner's arguments that the claimed ionic strength range—greater than 200 mM—would have been

achieved by through routine optimization misapplies obviousness case law. Prelim. Resp. 20–21 (citing Pet. 35–36). In particular, Patent Owner contends that "[f]or a range to be obvious, a parameter must first be recognized as a 'result-effective variable,' before the determination of the optimum or workable ranges of that variable might be characterized as routine experimentation. *Id.* at 21 (citing *In re Antonie*, 559 F.2d 618, 620 (CCPA 1977)). Petitioner, however, "fails to identify any disclosure in Evans, Huang, and/or Mingozzi suggesting that ionic strength would impact rAAV aggregation" and failed to establish ionic strength as a "result-effective variable." *Id.* Moreover, Patent Owner contends that Petitioner

> mischaracterizes Wright (Ex. 1007) to support its contention that "ionic strength . . . likely affects vector aggregation." Wright stated that the "mechanism of vector aggregation is not well understood, and purification conditions that may affect aggregation include buffer ionic strength and pH, shear and vector concentration." Ex. 1007, 175. Novartis never explains how Wright's statement that factors causing vector aggregation were "not well understood"—followed by a non-exclusive list of conditions that may impact aggregation—was an indication that "ionic strength . . . likely affects vector aggregation." *Id.*; Davies, ¶¶ 80-81.

Id. at 21–22 (emphasis omitted).[4] According to Patent Owner, "Wright also fails to teach or suggest ionic strength as a results-effective variable for rAAV aggregation." *Id.* at 22 (citing Ex. 2004 ¶¶ 84–85).

[4] We note that while Petitioner does not rely on Wright for its obviousness challenge in Ground 1, Petitioner relies on Wright for its argument that "ionic strength was a known condition that likely affects vector aggregation." Pet. 36 (citing Ex. 1007, 175; Ex. 1025 ¶¶ 175–177).

3. Discussion

Claims 5 and 6 require, respectively, that the composition does not exhibit significant aggregation as determined by particle radius (claim 5) and by percent product recovery following filtration (claim 6). Petitioner's arguments that those elements of claims 5 and 6 are inherent properties to AAV2 particles misses what is required by those claims, because each of those elements of the claims are used as a measure of aggregation achieved by the claimed compositions. Pet. 41–42, 44, 60; Ex. 2004 ¶¶ 126, 130–134; Ex. 1001, 4:61–5:25, 8:19–44, 9:25–27. For example, the '542 patent explains that the effect of ionic strength on aggregation was assessed by measuring vector recovery after filtration through a 0.22 μm filter. Ex. 1001, 8:1–10, 11:53–12:29 (Example 2); Ex. 2004 ¶ 133. Thus, Petitioner's arguments that particle radius (claim 5) and percent product recovery following filtration (claim 6) are inherent properties is insufficient to prove obviousness.

To prove inherency in the context of obviousness "[a] party must . . . meet a high standard . . . the limitation at issue necessarily must be present, or the natural result of the combination of elements explicitly disclosed by the prior art." *PAR Pharm., Inc. v. TWI Pharms., Inc.*, 773 F.3d 1186, 1195–96 (Fed. Cir. 2014). To that point, Petitioner fails to provide sufficient evidence such as prior art or testing evidence to show that the combination of Evans, Huang, and Mingozzi would result in a composition having the recited aggregation outcomes. For example, the '542 patent explains that compositions having ionic strength greater than 200 mM surprisingly resulted in recoveries exceeding 90%, whereas compositions having ionic strengths below 200 mM resulted in recoveries below 80%. Ex. 1001, 8:1–

10, 11:53–12:29 (Example 2); Ex. 2004 ¶ 133. Petitioner fails to submit any evidence that the particle radius and product recovery elements of claims 5 and 6, respectively, would necessarily be present, or the natural result of the combination of teachings explicitly disclosed by Evans, Huang, and Mingozzi.

Petitioner also argues that adjusting the ionic strength of the composition would have been a matter of routine optimization. Pet. 36 (citing Ex. 1003, 11:13–19; Ex. 1025 ¶¶ 61–71, 178–182), 43 (citing Ex. 1025 ¶¶ 199–201), 45 (Ex. 1025 ¶¶ 205–209). We are not persuaded by Petitioner's routine optimization argument as applied to the claimed ionic strength range. We acknowledge that "where the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." *In re Aller*, 220 F.2d 454, 456 (CCPA 1955). We also acknowledge, however, an "exception" to this *Aller* rule where "the parameter optimized was not recognized to be a result-effective variable." *In re Antonie*, 559 F.2d 618, 620 (CCPA 1977). Here, Petitioner fails to establish a known relationship between ionic strength and viral particle aggregation. Rather, the evidence of record teaches that "[t]he mechanism of vector aggregation is not well understood, and purification conditions that may affect aggregation include buffer ionic strength and pH, shear and vector concentration." Ex. 1007, 175; Prelim. Resp. 21; Ex. 2004 ¶¶ 84–85. Additionally, as explained in detail by Patent Owner, data presented in Evans does not establish any clear relationship between ionic strength and maintained infectivity after storage. Prelim. Resp 22–27. Accordingly, on this record, we determine that

Petitioner fails to establish ionic strength as a result-effective variable for rAAV aggregation.

For at least the reasons discussed above, we are not persuaded that Petitioner has shown a reasonable likelihood of establishing that either claim 5 or claim 6 is unpatentable as obvious over Evans, Huang, and Mingozzi. Accordingly, Petitioner has not demonstrated a reasonable likelihood of prevailing on Ground 1.

C. Ground 3: Obviousness of Claims 5 and 6 over the Combination of Frei, Huang, and Mingozzi

Petitioner asserts that claims 5 and 6 are unpatentable as obvious over Frei, Huang, and Mingozzi. Pet. 47–61. Patent Owner disputes Petitioner's contentions. Prelim. Resp. 50–60.

As in Ground 1, Petitioner contends that the particle radius and product recovery elements of claims 5 and 6 would inherently result from the claimed combination and additionally that a "selection of an appropriate ionic strength for a therapeutic composition is a matter of routine optimization." Pet. 54–55, 59–61. We are unpersuaded by Petitioner's contentions for the same reasons discussed above in Ground 1 because those contentions are similarly unsupported by the evidence of record. *See, e.g.*, Pet. 55 (relying on Wright for the premise that ionic strength is a parameter that may affect vector aggregation).

Accordingly, we determine that Petitioner has not shown a reasonable likelihood of establishing that at least one of the challenged claims is unpatentable as obvious over Frei, Huang, and Mingozzi.

III. CONCLUSION

After considering the evidence and arguments of record, we determine that Petitioner has not demonstrated a reasonable likelihood of prevailing

with respect to any claim challenged in the Petition.[5] Accordingly, we do not institute an *inter partes* review.

IV. ORDER

In consideration of the foregoing, it is hereby:

ORDERED that the Petition is *denied* as to all challenged claims, and no trial is instituted.

[5] Because we deny the Petition on the merits, we do not reach Patent Owner's argument for discretionary denial under 35 U.S.C. § 314(a) or § 325(d). Prelim. Resp. 44–50, 61–67.

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Paper No. 22
Date: August 30, 2023

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

NOVARTIS GENE THERAPIES, INC. &
NOVARTIS PHARMACEUTICALS CORPORATION,
Petitioners,

v.

GENZYME CORPORATION,
Patent Owner.

IPR2023-00609
Patent 9,051,542 B2

Before JEFFREY N. FREDMAN, SHERIDAN K. SNEDDEN, and JAMES A. TARTAL, *Administrative Patent Judges*.

TARTAL, *Administrative Patent Judge*.

DECISION
Denying Institution of *Inter Partes* Review
35 U.S.C. § 314

I. INTRODUCTION

Novartis Gene Therapies, Inc. and Novartis Pharmaceuticals Corporation (collectively, "Petitioner")[1] filed a Petition pursuant to 35 U.S.C. §§ 311–319 requesting an *inter partes* review of claims 1, 2, 5, and 6 of U.S. Patent No. 9,051,542 B2 (Ex. 1001, "the '542 patent"). Paper 2 ("Pet."). Genzyme Corporation ("Patent Owner")[2] filed a Preliminary Response. Paper 16. In its Preliminary Response, Patent Owner states that it has disclaimed claims 1 and 2 of the '542 patent. *Id.* at 3 (citing Ex. 2015 (the "Disclaimer"); 37 C.F.R. § 42.107(e)). In light of the Disclaimer, only claims 5 and 6 of the '542 patent remain at issue in this proceeding. *See* 37 C.F.R. § 42.107(e) (stating that "[n]o *inter partes* review will be instituted based on disclaimed claims"). With our prior authorization, Petitioner filed a Reply to Patent Owner's Preliminary Response (Paper 19) and Patent Owner filed a Sur-reply (Paper 20).

We have authority to determine whether to institute an *inter partes* review. 35 U.S.C. § 314(b) (2018); 37 C.F.R. § 42.4(a) (2021). An *inter partes* review may not be instituted "unless . . . the information presented in the petition . . . shows that there is a reasonable likelihood that the petitioner would prevail with respect to at least 1 of the claims challenged in the petition." 35 U.S.C. § 314(a); *see also PGS Geophysical AS v. Iancu*, 891 F.3d 1354, 1360 (Fed. Cir. 2018) (stating that the decision whether to institute *inter partes* review requires "a simple yes-or-no institution choice respecting a petition, embracing all challenges included in the petition").

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[1] Petitioner identifies no additional real parties in interest related to Petitioner. Pet. 63.

[2] Patent Owner states that Sanofi and Aventis, Inc. are additional real parties in interest. Paper 6, 2.

Upon consideration of the evidence and arguments presented, we conclude that the information does not demonstrate a reasonable likelihood that Petitioner would prevail in showing the unpatentability of either claim 5 or claim 6 of the '542 patent. Accordingly, we do not institute an *inter partes* review.

II. BACKGROUND

A. The '542 Patent

The '542 patent is titled "Compositions and Methods to Prevent AAV Vector Aggregation," and issued on June 9, 2015, from U.S. Patent Application No. 12/661,553, filed March 19, 2010. Ex. 1001, codes (21), (22), (45), (54). The '542 patent "relates to compositions and methods of preparing and storing AAV [(adeno-associated virus)] virions that prevent aggregation." *Id.* at 1:17–19. According to the '542 patent, "[t]he solubility of purified AAV2 virus particles is limited, and aggregation of AAV2 particles has been described as a problem." *Id.* at 1:41–46 (citing, e.g., Wright et al., "Recombinant Adeno-Associated Virus: Formulation Challenges and Strategies for a Gene Therapy Vector," *Curr. Opin. Drug Disc. Dev.* 6(2):174–178 (2003) (Ex. 1007, "Wright"); Croyle, et al., "Development of Formulations That Enhance Physical Stability of Viral Vectors for Gene Therapy," *Gene Ther.*, 8:1281–1290 (2001) (Ex. 1013, "Croyle")).

In particular, the '542 patent discloses high ionic strength solutions that are isotonic with the intended target tissue. *Id.* at code (57). The "combination of high ionic strength and modest osmolarity is achieved using salts of high valency, such as sodium citrate." *Id.*

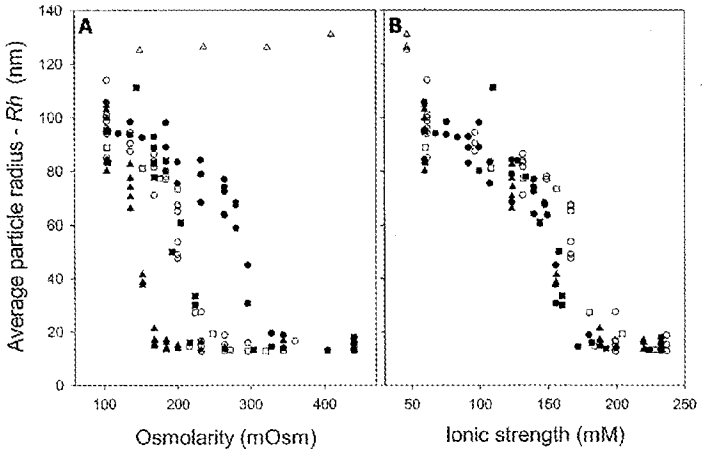
The '542 patent further explains as follows:

> The present invention is based in part on the observation that solution ionic strength is an important parameter in AAV vector aggregation, implicating the involvement of ionic interactions between virus particles in the aggregation process. The observation that elevated ionic strength increases AAV2 [AAV serotype 2] vector solubility regardless of the identity of the charged excipient supports the hypothesis that ionic strength of solution per se, rather than interactions involving a specific ionic species, is the relevant physico-chemical parameter. A threshold ionic strength of at least 200 mM is required to prevent aggregation at vector particle concentrations examined herein.

Id. at 4:53–64. The '542 patent additionally states as follows:

> In embodiments of the present invention the exponential relationship of ionic strength with charge valency is used to develop isotonic formulations with high ionic strengths. Salt species with multiple charge valencies (e.g. salts of sulfate, citrate, and phosphate) that are commonly used as excipients in human parenteral formulations can provide the level of ionic strength needed to prevent AAV2 vector aggregation when used at isotonic concentrations. While isotonic (150 mM) sodium chloride has an ionic strength of 150 mM, a value insufficient to maintain AAV2 solubility at high vector concentrations, isotonic sodium citrate, with an ionic strength of ~500 mM, can support AAV2 vector concentrations of at least 6.4×10^{13} vg/mL without aggregation.

Id. at 5:7–20. Figures 1A and 1B of the '542 patent are reproduced below.



Figures 1A and 1B present the results of a vector aggregation study that tracked aggregation as a function of two parameters, osmolarity (Figure 1A) and ionic strength (Figure 1B) for buffer compositions of sodium chloride (●), sodium citrate (○), sodium phosphate (■), sodium sulfate (□), magnesium sulfate (▲), and glycerol (Δ), and. *Id.* at 6:63–65, 12:33–67 (Example 3), FIGS. 1A, 1B. "Average particle radius is measured by dynamic light scattering (DLS) following vector dilution in varying concentrations of excipients buffered with 10 mM sodium phosphate at pH 7.5." *Id.* at 4:18–28. "Rh values >20 nm are deemed to indicate the occurrence of some level of aggregation." *Id.* at 9:25–27.

The results of Figure 1A, which plots vector aggregation as a function of the osmolarity of selected excipients, are explained as follows:

> For charged species a concentration-dependent inhibition of AAV2 vector aggregation is observed. Salts with multivalent ions achieve a similar degree of inhibition of aggregation at lower concentrations than monovalent sodium chloride. For example, magnesium sulfate prevents aggregation at >200 mOsm whereas sodium chloride requires ≧350 mOsm to achieve a similar effect. Sodium citrate, sodium sulfate, and sodium phosphate are intermediate in their potency to prevent vector aggregation.

Id. at 6:65–7:8.

Figure 1B shows data from the same experiment "plotted as a function of the calculated ionic strength, rather than osmolarity, for each excipient." *Id.* at 7:18–20. Figure 1B's plot of particle radius versus ionic strength shows that "vector aggregation is prevented when ionic strength is ~200 mM or greater regardless of which salt is used." *Id.* at 7:21–22. "These data suggested that the ionic strength (μ) of a solution . . . is the primary factor affecting aggregation." *Id.* at 7:22–25.

The '542 patent discloses the results of a study assessing "the effects of elevated ionic strength and nuclease treatment on AAV2 vector aggregation at a larger scale, using methods to induce and quantify vector aggregation that are relevant to preparative scale vector purification" in Table 2. *Id.* at 8:1–5.

Table 2 of the '542 patent is reproduced, in part, below.

TABLE 2

AAV VECTOR RECOVERY AT PROCESS SCALE

Experiment	Formulation	μ (mM)	Target (vg/mL)	Actual (vg/mL)	Yield % (RSD)
1	CF	160	2.5E13	1.93E13	77 (6.6)
1	TF1	310	2.5E13	2.38E13	95 (7.4)
1	TF2	510	2.5E13	2.33E13	93 (7.4)
2	CF	160	6.7E13	3.98E13	59 (6.0)
2	TF2	510	6.7E13	6.42E13	96 (4.4)

Table 2 shows the results for three solutions of AAV2-AADC vectors filtered through a 0.22 μm filter. *Id.* at 8:1–10, 11:53–12:29. The three solutions are as follows:

> Control Formulation (CF: 140 mM sodium chloride, 10 mM sodium phosphate, 5% sorbitol, pH 7.3); Test Formulation 1 (TF1: 150 mM sodium phosphate, pH 7.5); and Test Formulation 2 (TF2: 100 mM sodium citrate, 10 mM Tris, pH 8.0).

Id. at 11:66–12:3. In Experiment 1, the samples contained 2.5×10^{13} vg/ml vector, and, in Experiment 2, the samples contained 6.7×10^{13} vg/ml vector. *Id.* at 12:4–12. Table 2 shows recoveries exceeded 90% following filtration in formulations TF1 and TF2 having ionic strengths greater than 200 mM, whereas recovery from CF formulations, having ionic strength of 160 mM, was only 77% and 59% for experiments 1 and 2, respectively. *Id.* at 8:19–56.

The '542 patent also discloses the results of a study assessing "stability after storage or freeze-thaw (F/T) cycling is assessed in buffers of

the present invention." *Id.* at 9:19–27. Particle radius was measured by dynamic light scattering (DLS) to determine the presence of aggregates. *Id.*

Table 3, reproduced below, summarizes the results of the study.

TABLE 3

STABILITY OF AAV2 VECTORS

	Particle radius - Rh (nm)							
Formu-		4° C.	−20° C.			−80° C.		
lation	Pre	5 d	1 F/T	5 F/T	10 F/T	1 F/T	5 F/T	10 F/T
CF	14.5	27.0	22.4	56.1	94.5	20.6	57.5	141
TF1	13.8	16.3	TH	TH	TH	TH	TH	TH
TF2	13.8	14.4	14.2	14.0	14.1	13.8	21.3	50.9

Pre: DLS radius measured immediately following 0.2 μm filtration.
Vector concentrations (vg/mL): CF: 1.93E13, TF1: 2.38E13, TF2: 2.33E13.
TH: signal intensity is too high to measure because of extensive aggregation.

According to the '542 patent, Table 3 provides data showing as follows:

> AAV2-AADC vector prepared in CF shows some aggregation after 5 days of storage at 4° C., as well as following one or more F/T cycles at −20 or −80° C. For vector prepared in TF1, no aggregation occurs after 5 days at 4° C., but aggregation occurs following a single F/T cycle at −20 or −80° C. as indicated by a DLS signal intensity that is too high to measure. Visual inspection of these samples reveals slight cloudiness, which is consistent with aggregation. For vector prepared in TF2, no aggregation is observed at 4° C., or following up to 10 F/T cycles at −20° C. Some aggregation is observed following 5 and 10 F/T cycles at −80° C.

Id. at 9:29–55. According to Patent Owner, the results of the studies disclosed in the '542 patent "confirmed the importance of increased ionic strength in preventing aggregation." Prelim. Resp. 12 (citing Ex. 1001, 10:29–43 (stating "[t]he effect of ionic strength [] on virus particle interactions is determined to elucidate the mechanism of vector aggregation")).

B. Claims at Issue

Claims 5 and 6 of the '542 patent are at issue in this proceeding. Claims 5 and 6 each depend directly from disclaimed claim 1. Ex. 1001, 14:34–41. Claims 5 and 6 are reproduced below, along with disclaimed independent claim 1 from which they each depend.

> 1. A composition for the storage of purified, recombinant adeno-associated virus (AAV) vector particles, comprising:
>
> purified, recombinant AAV vector particles at a concentration exceeding 1×10^{13} vg/ml up to 6.4×10^{13} vg/ml;[3]
>
> a pH buffer, wherein the pH of the composition is between 7.5 and 8.0; and
>
> excipients comprising one or more multivalent ions selected from the group consisting of citrate, sulfate, magnesium, and phosphate; wherein the ionic strength of the composition is greater than 200 mM, and wherein the purified AAV vector particles are stored in the composition without significant aggregation.
>
> 5. The composition of claim 1, wherein the purified, recombinant AAV vector particles have an average particle radius (Rh) of less than about 20 nm as measured by dynamic light scattering.
>
> 6. The composition of claim 1, wherein recovery of the purified, recombinant virus particles is at least about 90% following

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[3] The units of measurements used in the art to measure the titer of AAV compositions are explained in the Petition as follows:

> The titer of AAV compositions can be measured in vector genomes (vg)/ml, genome copies (gc)/ml, capsid particles (cp)/ml, or virus particles (vp)/ml. Ex. 1025, ¶35. The first two are used interchangeably, since both represent the number of functional vectors containing the therapeutic gene. *Id.*, ¶¶36–37. By contrast, the latter two measurements include particles that are incomplete, damaged, or lacking genetic material. Ex. 1009, [00281]; Ex. 1025, ¶36.

Pet. 12.

filtration of the composition of said AAV vector particles through a 0.22 μm filter.

Ex. 1001, 14:15–28, 34–41.

C. *Asserted Grounds of Unpatentability*

In the Petition, Petitioner asserts that claims 1, 2, 5, and 6 of the '542 patent are unpatentable. Pet. 4. As noted above, after the Petition was filed, Patent Owner explained that it disclaimed claims 1 and 2 of the '542 patent. Prelim. Resp. 3 (citing Ex. 2015). Accordingly, our determination of whether to institute *inter partes* review is based on Petitioner's allegations directed to claims 5 and 6. *See* 37 C.F.R. § 42.107(e). Petitioner challenges claims 5 and 6 on the following grounds:

Claim(s) Challenged	35 U.S.C. §[4]	Reference(s)/Basis
5, 6	103	Liu,[5] Huang,[6] Mingozzi[7]
5, 6	103	Lochrie,[8] Huang, Mingozzi, Johnson,[9] Liu

[4] The Leahy-Smith America Invents Act ("AIA") included revisions to 35 U.S.C. §103 that became effective on March 16, 2013. We apply the pre-AIA version of §103 here, because the application identified in the '542 patent was filed before the effective date of the AIA. *See* Ex. 1001, code (22).

[5] WO 03/039459 A2, published May 15, 2003 (Ex. 1009, "Liu").

[6] Huang J, Gao, et al., "Aggregation of AAV Vectors, its Impact on Liver-directed Gene Transfer and Development of Vector Formulations to Prevent and Dissolve Aggregation and Enhance Gene Transfer Efficiency," MOL THER. 1:S286 (2000) (Ex. 1005, "Huang").

[7] Mingozzi, et al., "Improved Hepatic Gene Transfer by Using an Adeno-Associated Virus Serotype 5 Vector," J VIROL. 76:10497–502 (2002) (Ex. 1006, "Mingozzi").

[8] WO 03/046142 A2, published May 6, 2003 ("Ex. 1010, "Lochrie").

[9] F. B. Johnson and A. S. Bodily, *Effects of Environmental pH on Adenovirus-Associated Virus (39085)*, Procs. of the Soc. for Experimental Biology and Med., pp. 585-90 (1975) (Ex. 1019, "Johnson").

Pet. 4. Petitioner relies on the supporting Declaration of Mansoor M. Amiji, R.Ph., Ph.D., dated February 22, 2023. Ex. 1025. Patent Owner relies on the Declaration of Martyn C. Davies, dated June 15, 2023. Ex. 2004.

D. Related Proceedings

The Parties indicate that the '542 patent is asserted against Petitioner in *Genzyme Corporation et al. v. Novartis Gene Therapies, Inc. et al.*, Case No. 1:21-cv-01736 (D. Del.), filed December 10, 2021. Pet. 63; Paper 6, 2. Petitioner also filed a petition for *inter partes* review in IPR2023-00608 seeking to challenge claims of the '542 patent on other grounds. *Id.*

III. ANALYSIS

A. Legal Standards for Obviousness

A patent claim is unpatentable for obviousness if the differences between the claimed subject matter and the prior art are such that the subject matter, as a whole, would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. *KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398, 406 (2007). In *Graham v. John Deere Co.*, 383 U.S. 1 (1966), the Supreme Court set out a framework for assessing obviousness that requires consideration of four factors: (1) the "level of ordinary skill in the pertinent art," (2) the "scope and content of the prior art," (3) the "differences between the prior art and the claims at issue," and (4) "secondary considerations" of nonobviousness such as "commercial success, long felt but unsolved needs, failure of others, etc." *Id.* at 17–18; *KSR*, 550 U.S. at 407.

"Whether an ordinarily skilled artisan would have been motivated to modify the teachings of a reference is a question of fact." *WBIP, LLC v. Kohler Co.*, 829 F.3d 1317, 1327 (Fed. Cir. 2016) (citations omitted). The

Supreme Court made clear that we apply "an expansive and flexible approach" to the question of obviousness. *KSR*, 550 U.S. at 415. Whether a patent claiming the combination of prior art elements would have been obvious is determined by whether the improvement is more than the predictable use of prior art elements according to their established functions. *Id.* at 417. To support this conclusion, however, it is not enough to show merely that the prior art includes separate references covering each separate limitation in a challenged claim. *Unigene Labs., Inc. v. Apotex, Inc.*, 655 F.3d 1352, 1360 (Fed. Cir. 2011). Rather, obviousness additionally requires that a person of ordinary skill at the time of the invention "would have selected and combined those prior art elements in the normal course of research and development to yield the claimed invention." *Id.*

In determining whether there would have been a motivation to combine prior art references to arrive at the claimed invention, it is insufficient to simply conclude the combination would have been obvious without identifying any reason why a person of skill in the art would have made the combination. *Metalcraft of Mayville, Inc. v. Toro Co.*, 848 F.3d 1358, 1366 (Fed. Cir. 2017). Moreover, in determining the differences between the prior art and the claims, the question under 35 U.S.C. § 103 is not whether the differences themselves would have been obvious, but whether the claimed invention as a whole would have been obvious. *Litton Indus. Prods., Inc. v. Solid State Sys. Corp.*, 755 F.2d 158, 164 (Fed. Cir. 1985) ("It is elementary that the claimed invention must be considered as a *whole* in deciding the question of obviousness."); *see also Stratoflex, Inc. v. Aeroquip Corp.*, 713 F.2d 1530, 1537 (Fed. Cir. 1983) ("[T]he question under 35 U.S.C. § 103 is not whether the differences themselves would have been obvious. Consideration of differences, like each of the findings set

forth in Graham, is but an aid in reaching the ultimate determination of whether the claimed invention as a whole would have been obvious."). "[W]here a party argues a skilled artisan would have been motivated to combine references, it must show the artisan 'would have had a reasonable expectation of success from doing so.'" *Arctic Cat Inc. v. Bombardier Recreational Prods. Inc.*, 876 F.3d 1350, 1360–61 (Fed. Cir. 2017) (quoting *In re Cyclobenzaprine Hydrochloride Extended-Release Capsule Patent Litig.*, 676 F.3d 1063, 1068–69 (Fed. Cir. 2012)).

B. Level of Ordinary Skill in the Art

In determining the level of ordinary skill in the art, various factors may be considered, including the "type of problems encountered in the art; prior art solutions to those problems; rapidity with which innovations are made; sophistication of the technology; and educational level of active workers in the field." *In re GPAC Inc.*, 57 F.3d 1573, 1579 (Fed. Cir. 1995) (citation omitted). Petitioner proposes that a person of ordinary skill in the art at the time of the invention would have possessed the following level of skill:

> at least a BS in biology, chemistry, chemical engineering, biochemistry, pharmaceutical science, or a related discipline, with ≥4 years of industry, laboratory, and/or clinical experience in formulating or developing dispersions for therapeutic biologics, such as proteins or vectors for gene delivery. Such person may be familiar with, or consult with someone familiar with, the development and/or administration of viral vectors for gene therapy. Ex. 1025, ¶82.

Pet. 16–17. Patent Owner does not dispute Petitioner's proposed level of ordinary skill in the art. Prelim. Resp. 2 n.2.

We find that the '542 patent and the cited prior art references reflect a level of skill at the time of the claimed invention that is consistent with the

level of skill proposed by Petitioner. *See Okajima v. Bourdeau*, 261 F.3d 1350, 1355 (Fed. Cir. 2001). Accordingly, for purposes of this Decision, we adopt Petitioner's proposed definition for a person of ordinary skill in the art.

C. Claim Construction

We apply the same claim construction standard that would be used to construe the claim in a civil action under 35 U.S.C. § 282(b). 37 C.F.R. § 42.100(b). Under that standard, claim terms "are generally given their ordinary and customary meaning" as would have been understood by a person of ordinary skill in the art at the time of the invention. *Phillips v. AWH Corp.*, 415 F.3d 1303, 1312–13 (Fed. Cir. 2005) (en banc). "In determining the meaning of the disputed claim limitation, we look principally to the intrinsic evidence of record, examining the claim language itself, the written description, and the prosecution history, if in evidence." *DePuy Spine, Inc. v. Medtronic Sofamor Danek, Inc.*, 469 F.3d 1005, 1014 (Fed. Cir. 2006) (citing *Phillips*, 415 F.3d at 1312–17). Extrinsic evidence is "less significant than the intrinsic record in determining 'the legally operative meaning of claim language.'" *Phillips*, 415 F.3d at 1317.

Petitioner asserts that "[f]or this proceeding, no terms require express construction," states that the Petition "analyzes the claim terms under their 'plain and ordinary meaning.'" Pet. 17. Patent Owner does not challenge Petitioner's position. Prelim. Resp. 13. We agree that it is not otherwise necessary to address the express interpretation of any claim term for purposes of this Decision. *See Wellman, Inc. v. Eastman Chem. Co.*, 642 F.3d 1355, 1361 (Fed. Cir. 2011) ("[C]laim terms need only be construed 'to the extent necessary to resolve the controversy'" (quoting *Vivid Techs., Inc. v. Am. Sci. & Eng'g, Inc.*, 200 F.3d 795, 803 (Fed. Cir. 1999))). To the

extent further discussion of the meaning of any claim term is necessary to this Decision, we provide that discussion below in our analysis of the asserted grounds of unpatentability.

D. Alleged Obviousness Over Liu, Huang, and Mingozzi

Petitioner contends the subject matter of claims 5 and 6 would have been obvious over Liu, Huang, and Mingozzi. Pet. 22–43. Patent Owner disputes Petitioner's contentions. Prelim. Resp. 14–36. For the reasons set forth below, we determine that Petitioner has not shown a reasonable likelihood of establishing that either claim 5 or claim 6 is unpatentable as obvious over Liu, Huang, and Mingozzi.

1. Summary of Liu

Liu, titled "Viral Vector Production Methods and Compositions," describes methods of preparing viral vector particles and compositions, including "storage compositions" that "effectively maintain a stable population of adenoviral vector particles during the viral vector particle production and/or purification process." Ex. 1009 ¶ 365. Under the heading "Example 17," Liu teaches "an adenoviral vector particle composition comprising a population of adenoviral vector particles in a temporary storage buffer (25mM Tris, 300mM NaCl, 5mM MgCl2, 0.0025% polysorbate 80, 5% trehalose, pH 7.5)." *Id.* ¶ 366. According to Liu, the composition was maintained at about 4°C for 7 days in the temporary storage buffer and "showed no signs of settling or precipitation" and "no significant decrease in particle number over the 7 day test period." *Id.* ¶ 368.

2. Summary of Huang

Huang, an abstract titled "Aggregation of AAV Vectors, its impact on Liver-directed Gene Transfer and Development of Vector Formulations to

Prevent and Dissolve Aggregation and Enhance Gene Transfer Efficiency," states that "[t]o achieve high level of gene transfer and ensure the safety of vector administration it is desirable to deliver high doses of vector in small volumes." Ex. 1005, S286. According to Huang, "at high concentrations, AAV virions form aggregates of different sizes in a range of different buffer systems and storage conditions." *Id.* Huang states that "when the vector titer reached 5-10 x 10^{13} GCs/ml, gene transfer efficiency was 10-100 folds lower at the same dose as compared to the vector whose titer was 1-5 x 10^{12} GCs/ml." *Id.* Huang states that "a series of formulation studies were performed to prevent and dissolve AAV aggregation," and reported "a 30–50% reduction in the size of aggregates size at high vector concentrations" for some of the compositions. *Id.*

3. Summary of Mingozzi

Mingozzi, titled "Improved Hepatic Gene Transfer by Using Adeno-Associated Virus Serotype 5 Vector," states that "AAV vectors do not contain viral coding sequences and have been shown to efficiently transfer genes to nondividing target cells," and that "[a]n excellent safety profile combined with reduced potential for activation of inflammatory or cellular immune responses has made this vector system attractive for clinical application and treatment of genetic disorders." Ex. 1006, 10497. According to Mingozzi, purification of AAV-2 and AAV-5 vectors "by repeated CsCl gradient centrifugation" yielded concentrations of >10^{13} vg/ml. *Id.*

4. Claims 5 and 6

Claim 5 recites a composition of claim 1, "wherein the purified, recombinant AAV vector particles have an average particle size radius (Rh) of less than about 20 nm as measured by dynamic light scattering [DLS]"

(the "average particle radius limitation"). Ex. 1001, 14:34–37. Claim 6 recites a composition of claim 1, "wherein recovery of the purified, recombinant virus particles is at least about 90% following filtration of the composition of said AAV vector particles through a 0.22μm filter" (the "percent filtration limitation"). *Id.* at 14:38–41. Together, the average particle radius limitation and the percent filtration limitation are referred to as the "aggregate limitations."

Petitioner's contentions are based in part on the composition described in Liu's Example 17, described above, which Petitioner concedes does not teach the "recombinant AAV vector particles at a concentration exceeding 1×10^{13} vg/ml," as required by claim 1. Pet. 29 ("Assuming that 100% of the particles contained vector genomes, Liu's composition had an initial concentration of $3.24x10^{8}$ vg/mL") (citing Ex. 1025 ¶ 285). Petitioner reasons that a person of ordinary skill in the art would have had reason to increase "Liu's vector production and concentration" in light of the teachings of Mingozzi and Huang, with a reasonable expectation of success in attaining the concentration required by claim 1. *Id.* at 30–33. Petitioner relies on "Liu's Example 17 composition, as modified by Huang and Mingozzi" in its contentions directed to the aggregate limitations of claims 5 and 6. Pet. 40–43.

In particular, Petitioner asserts that Patent Owner stated in a filing in district court that the aggregate limitations "provide different methods of ensuring that there is no substantial aggregation." *Id.* at 39, 41 (quoting Ex. 1023, 72). From this, Petitioner asserts that "[i]f true," then "because Liu's compositions prevent aggregation," the limitations of claims 5 and 6 provide "no patentable weight." *Id.* at 39, 41. Further, according to Petitioner, during prosecution the Examiner concluded that the aggregate

limitations were inherent characteristics of the purified viral composition. *Id.* at 39, 41–42 (citing Ex. 1002, 86–88, 91, 146, 151, 154, 188, 191, 220, 318). Petitioner maintains that Patent Owner's failure to dispute the Examiner's conclusions during prosecution "constitutes a binding admission." *Id.* (citing *TorPharm, Inc. v. Ranbaxy Pharms., Inc.*, 336 F.3d 1322, 1330 (Fed. Cir. 2003)).

Petitioner further argues with regard to claim 5 as follows:

> The '542 patent admits that AAV2 particles have a diameter of ~26nm (Ex.1001, 1:29-38); thus, a [person of ordinary skill in the art] would have reasonably expected that, because Liu's compositions prevented aggregation, AAV particles stored therein and in obvious variants of Liu's Example 17 composition also have an Rh of less than about 20nm measured by DLS. Indeed, the '542 patent does not identify anything critical about the recited radius range other than it being exemplary of no aggregation. Ex.1001, 9:25-27 ("Rh values >20 nm are deemed to indicate the occurrence of some level of aggregation.").

Pet. 39–40. Petitioner contends that a person of ordinary skill in the art would have been motivated to minimize any potential aggregation in Liu's composition "through routine optimization of known stabilization factors," because it was known that virus aggregation reduced gene transfer efficiency and caused potentially deleterious consequences. *Id.* at 40 (citing Ex. 1005, S286; Ex. 1007, 176). Petitioner also argues as follows:

> A [person of ordinary skill in the art] would have reasonably expected success in minimizing particle size based on Huang's teaching that formulation optimization "could lead to a 30-50% reduction in the size of aggregates at high vector concentrations." Ex.1005, S286. Indeed, Liu taught that its experiments "demonstrate that viral vector compositions can be stably stored in the temporary storage buffers of the invention for extended periods of time" and that reduced aggregation can be achieved by "addition of surfactants." Ex.1009, [00371],

> [00263]. And a [person of ordinary skill in the art] would have understood that AAV "is significantly more stable than the adenovirus." Ex.1013, 1283. Thus, at most, only routine optimization would be required to obtain an average AAV Rh <20nm using the obvious variants of Liu's Example 17 composition discussed above. Ex.1025, ¶¶320-322.

Pet. 41 (citing *Senju Pharm. Co. v. Lupin Ltd.*, 780 F.3d 1337, 1353 (Fed. Cir. 2015) (invalidating a claim directed to "a product of routine optimization that would have been obvious to one of skill in the art")).

With regard to claim 6, Petitioner argues as follows:

> Since the inventors acknowledged in Wright that "loss of rAAV following a 0.2-μm filtration step correlates with the extent of vector aggregation" (Ex.1007, 175), a [person of ordinary skill in the art] would have reasonably expected that at least 90% of the AAV particles stored without observable aggregation in Liu's Example 17 composition, as modified by Huang and Mingozzi, will be recovered following filtration through a 0.22μm filter. Ex.1025, ¶324.

Pet. 41–42. Petitioner contends that the '542 patent "does not identify anything critical about the recited recovery rate," and that a person of ordinary skill in the art "would have been motivated to minimize any potential aggregation in Liu's modified Example 17 composition," because "Wright and Huang linked aggregation to reduced functional activity of AAV vectors." *Id.* at 42–43 (citing Ex. 1005, S286; Ex. 1007, 176). Petitioner reasons that a person of ordinary skill in the art "would have been motivated to maximize virus recovery from a 0.22μm filter through routine optimization of known stabilization factors," and would reasonably have expected success through "only routine optimization" for the following reasons:

> Huang taught optimized formulations "could lead to a 30-50% reduction in the size of aggregates at high vector concentrations" (Ex.1005, S286), Liu observed "no signs of settling or

> precipitation" for adenovirus particles stored in a high ionic strength buffer over a 7 day period (Ex. 1009, [00369]), and Croyle taught that AAV "is significantly more stable than the adenovirus" (Ex. 1013, 1283).

Id. at 43.

In opposition, Patent Owner argues, in part, that Petitioner fails to show the asserted combination of references teaches: "(1) the rAAV 'concentration exceeding $1x10^{13}$vg/ml up to $6.4x10^{13}$vg/ml'; (2) 'the ionic strength of the composition is greater than 200 mM'; and (3) the respective aggregate limitations of claims 5 and 6." Prelim. Resp. 13. According to Patent Owner, Liu's compositions have a viral particle concentration several orders of magnitude below the claimed vector genome concentration and a person of ordinary skill in the art would have understood that "Liu's visual assessment would be unable to detect significant aggregation to the levels recited in claims 5 and 6." *Id.* at 14 (citing Pet. 24, 29; Ex. 1009 ¶¶ 126, 186, 369, 371; Ex. 1025 ¶ 285; Ex. 2004 ¶¶ 64–65). With regard to Huang, Patent Owner argues that the reported 30–50% reduction in the size of aggregates "does not explain what this size reduction means in terms of particle radius or any other metric, does not disclose any information about its formulations, and never suggests that its stored rAAV compositions could remain free of significant aggregation." *Id.* at 14–15 (citing Ex. 2004 ¶ 67). Patent Owner further argues that Mingozzi does not addresses aggregation, rAAV formulations, or storage. *Id.* at 15 (citing Ex. 2004 ¶ 68).

First, we agree with Patent Owner that Petitioner fails to sufficiently show a reasonable likelihood of prevailing in its assertion that the aggregate limitations carry no patentable weight. *See* Prelim. Resp. 31–32; Pet. 39, 41. As Patent Owner explains, the aggregate limitations "'provide[] the criteria by which the' composition for the storage of purified rAAV vector particles

'is analyzed.'" *Id.* (quoting *In re Jasinski*, 508 F. App'x 950, 952 (Fed. Cir. 2013) (citing *Vizio, Inc. v. Int'l Trade Comm'n*, 605 F.3d 1330, 1340 (Fed. Cir. 2010) (holding that claims language going to "the essence or a fundamental characteristic of the claimed invention" was "properly construed as a limitation")). The "wherein" clauses in claims 5 and 6 are reasonably assigned patentable weight because they impose structural limitations that requires the AAV vectors to have particular aggregation properties. *See Griffin v. Bertina*, 285 F.3d 1029, 1033 (Fed. Cir. 2002) (stating that "the Board did not err in giving limiting effect to the 'wherein' clauses" because they give "meaning and purpose to the manipulative steps"). As Patent Owner explains, the aggregate limitations "relate to the quantification of rAAV aggregation (or the absence of aggregates) more accurately than other methods of detection, including visual inspection and analytical methods such as A320/A260 absorbance." Prelim. Resp. 32 (citing Ex. 2005 ¶¶ 45–50; Ex. 2009).

Second, we agree with Patent Owner that Petitioner fails to sufficiently show a reasonable likelihood of prevailing in its assertion that Patent Owner's decision not to dispute the Examiner's conclusion that the aggregate limitations were inherent characteristics of the purified viral composition during prosecution constitutes a binding admission. Pet. 39, 41–42; Prelim. Resp 32–33. Patent Owner explains that it disputed the Examiner's rejection, was not obligated to advance redundant arguments for patentability before the Examiner, and is not limited in subsequent proceedings to advancing only arguments in support of patentability previously made during prosecution. Prelim. Resp. 33 (citing *Woods v. DeAngelo Marine Exhaust, Inc.*, 692 F.3d 1272, 1287 (Fed. Cir. 2012); *Tor Pharm*, 336 F.3d at 1330).

Third, we agree with Patent Owner that Petitioner fails to sufficiently show a reasonable likelihood of prevailing in its assertion that the aggregate limitations were inherently taught by the asserted art. Pet. 38–43; Prelim. Resp. 34–36. To prove inherency in the context of obviousness, a party must "meet a high standard" and establish that "the limitation at issue necessarily must be present, or the natural result of the combination of elements explicitly disclosed by the prior art." *PAR Pharm., Inc. v. TWI Pharms., Inc.*, 773 F.3d 1186, 1195–96 (Fed. Cir. 2014).

In regard to claim 5, we agree with Patent Owner that Petitioner "*fails* to produce any evidence that for the claimed composition 'the purified, recombinant AAV vector particles' would necessarily and inevitably have had 'an average particle radius (Rh) of less than about 20 nm as measured by [DLS].'" Prelim. Resp. 34–35. Petitioner offers no testing or other persuasive evidence in support of its contentions, arguing instead that a person of ordinary skill in the art "would have reasonably expected" Liu's compositions to meet the average particle radius limitation of claim 5. *See* Pet. 39–40.

In regard to claim 6, we also agree with Patent Owner that Petitioner offers no sufficient evidence to show "Liu's Example 17 composition, as modified by Huang and Mingozzi," would necessarily and inevitably result in "at least about 90%" recovery of the "purified, recombinant virus particles following filtration of the composition of said AAV vector particles through a 0.22μm filter." Prelim. Resp. 35–36. Patent Owner argues that Petitioner "cannot possibly meet the high standard for inherency relying on Liu's Example 17, which applies only visual methods that the [person of ordinary skill in the art] would have understood could not determine the presence (or absence) of significant aggregation to the degree of claim 6's product

recovery following filtration requirement." Prelim. Resp. 36 (citing Ex. 2004 ¶ 42). In support, Patent Owner explains that the '542 patent discloses "compositions having ionic strength greater than 200 mM surprisingly resulted in recoveries exceeding 90%, whereas compositions having ionic strengths below 200 mM resulted in recoveries below 80%" and that "[w]ithin the variability of the assays used, vector was recovered fully at both target concentrations using TF2, indicating that aggregation was prevented." Prelim. Resp. 35–36 (quoting Ex. 1001, 8:19–46) (emphasis omitted). Petitioner offers no testing or other persuasive evidence in support of its contentions, arguing instead that a person of ordinary skill in the art "would have reasonably expected that, because Liu's compositions prevented aggregation, AAV particles stored therein and in obvious variants of Liu's Example 17 composition also have an Rh of less than about 20nm measured by DLS." Pet. 39–40.

Petitioner fails to submit sufficient persuasive evidence that either the average particle radius limitation of claim 5 or the percent filtration limitation of claim 6 would necessarily be present in, or the natural result of, the asserted combination of teachings in Liu, Huang, and Mingozzi. Merely asserting a person of ordinary skill in the art "would have reasonably expected" Liu's composition, as modified by Huang and Mingozzi, to meet the aggregation limitations of either claim 5 or claim 6 does not sufficiently support a finding of inherency as to those limitations and the evidence relied upon by Petitioner is not sufficient to show the alleged "expectation" would have been reasonable.

Fourth, we find insufficient and not persuasive Petitioner's conclusory assertions that "only routine optimization" with a reasonable expectation of success in light of the asserted art would have been required to obtain a

composition meeting the aggregation limitations. *See* Pet. 41 ("only routine optimization would be required to obtain an average AAV Rh <20nm using the obvious variants of Liu's Example 17 composition") (citing Ex. 1025 ¶¶ 320–322), 43 ("only routine optimization would be required to improve AAV recovery following filtration of Liu's modified Example 17 composition through a 0.22μm filter") (citing Ex. 1025 ¶¶ 326–329). Petitioner offers scant evidence or explanation of what "optimization" was necessary, or why it would have been obvious to do so at the time of the invention. *See* Pet. 40 ("To the extent modifications to Lin's composition" would have been "required to achieve the features of claim 5," a person of ordinary skill in the art "would have been motivated to make *such changes*") (emphasis added). Petitioner then suggests "such changes" are merely "optimization of known stabilization factors." *Id.* Neither the Petition nor Dr. Amiji on behalf of Petitioner adequately explains what such optimization would have entailed or whether it would have been within the ability of a person of ordinary skill in the art at the time of the invention. *See* Ex. 1025 ¶ 319 (referring to "known aggregation reduction tools" without citation to any supporting prior art and asserting Liu taught "reducing potential aggregation by addition of surfactants"). In regard to the alleged expectation of success, we also find persuasive the testimony of Dr. Davies who notes that a person of ordinary skill in the art would have understood that "rAAV undergoes concentration-dependent aggregation," and that the "nature of the interparticle interactions that result in aggregation has not been well characterized." Ex. 2004 ¶¶ 97, 100 (quoting Ex. 1007, 17, 176).

Petitioner's contentions and supporting evidence fail to show a reasonable likelihood that the aggregate limitations of claims 5 and 6 would have been attainable with a reasonable expectation of success merely based

on a desire to optimize Liu's composition, as allegedly already modified by the teachings of Huang and Mingozzi.

5. Showing of a Reasonable Likelihood of Success

For at least the reasons discussed above, we are not persuaded that Petitioner has shown a reasonable likelihood of establishing that either claim 5 or claim 6 is unpatentable as obvious over Liu, Huang, and Mingozzi

E. Alleged Obviousness Over Lochrie, Huang, Mingozzi, Johnson, and Liu

Petitioner contends the subject matter of claims 5 and 6 would have been obvious over Lochrie, Huang, Mingozzi, Johnson, and Liu. Pet. 43–57. Patent Owner disputes Petitioner's contentions. Prelim. Resp. 36–50. For the reasons set forth below, we determine that Petitioner has not shown a reasonable likelihood of establishing that either claim 5 or claim 6 is unpatentable as obvious over Lochrie, Huang, Mingozzi, Johnson, and Liu.

1. Summary of Lochrie

Lochrie, titled "Methods for Producing Stocks of Recombinant AAV Virons," is directed to "efficient and commercially viable methods for producing stocks of rAAV [recombinant adeno-associated virus] virions with reduced amounts of empty capsids." Ex. 1010, 5:2–4. Lochrie explains that "[a]fter culturing the host cells with the necessary components for rAAV production, the host cell is harvested and a crude extract is produced." *Id.* at 4:5–6. According to Lochrie, "[t]he resulting preparation will contain, among other components, AAV capsids with genomes containing the heterologous gene (i.e., 'packaged capsids') and AAV capsids lacking genomes (i.e., 'empty capsids')." *Id.* at 4:6–9. Lochrie states that in some embodiments "the method produces a stock of rAAV virions

substantially free of empty AAV capsids, such as a stock wherein at least 75 % to about 99 % or more of the AAV virions present are packaged AAV capsids." *Id.* at 7:1–4. In one example of a specific embodiment, Lochrie states that "[h]uman embryonic kidney-293 cells . . . were used as host cells for the production of rAAV virions" and "72 hr post-transfection, 293 cells were disrupted by microfluidization," the crude lysate was collected and subjected to a two-step filtration, and then "the clarified lysate" was purified using column chromate chromatography. *Id.* at 28:28–29, 29:1–6; *see also* Pet. 21. According to Lochrie, "[t]he rAAV virions were eluted with buffer containing 20 mM NaH2PO4 and 350 mM NaCl," and "[t]he eluant was formulated in 20 mM NaH2PO4, 150 mM NaCl, 5% sorbitol, and 0.1 % Tween-80, at pH 7.4 at a concentration of 4 x 10^{12} vector genomes/milliliter (vg/mL)." Ex. 1010, 29:6–9.

2. Summary of Johnson

Johnson, titled "Effects of Environmental pH on Adenovirus-Associated Virus (39085)," describes a study that found that "AAV infectivity titrations, virus production, and induction of FA stainable antigen regions are all influenced by environmental pH." Ex. 1019, 589. Johnson states that "t]he greatest effect of pH appeared to be its influence on the aggregation of the viral particles." *Id.* Johnson states that the effect of pH on the aggregation of AAV particles was examined by exposing purified AAV particles to preparations of PTA [phosphotungstic acid]." *Id.* Johnson reports that at pH 7.5 "the virus particles occurred singly and were evenly distributed," and that at pH 7.2 and all lower pH's tested "the particles were aggregated and were not evenly distributed in the field but were found in clumps, between which were large empty spaces." *Id.* at 589. According to

Johnson, AAV particles "associate into increasingly large aggregates as the environmental pH is lowered." *Id.* at 585.

3. Claims 5 and 6

With regard to claim 1, from which claims 5 and 6 depend, according to Petitioner, "Lochrie's Example 2 provides an rAAV composition meeting all but three of the limitations of challenged claim 1, each of which differ only slightly in value than the recited elements." Pet. 44 (citing Ex. 1025 ¶ 331). Petitioner further argues that "Huang and Mingozzi describe AAV compositions having the recited concentration ("a concentration exceeding $1x10^{13}$vg/ml"), Johnson teaches the recited pH (pH "between 7.5 and 8.0"), and Liu teaches the recited ionic strength (ionic strength "greater than 200mM"). *Id.* More particularly in regard to claims 5 and 6, Petitioner concedes that the aggregation limitations are not expressly taught by Lochrie or any other of the asserted references. Pet. 55–57. Instead, as with the first ground based on Liu discussed above, Petitioner maintains without persuasive supporting evidence that the limitations were inherent and that a person of ordinary skill in the art "would have reasonably expected" the aggregate limitations to have been satisfied and would have arrived at a composition satisfying the limitation through "routine optimization." Pet. 55–57 (substantially relying on Petitioner's arguments set forth in the Petition under the first ground based on Liu). Patent Owner argues, in part, that Petitioner's contentions in the second ground based on Lochrie fail for

the same reasons as Petitioner's contentions in the first ground based on Liu, which we addressed above.[10] Prelim. Resp. 49–50.

4. Showing of a Reasonable Likelihood of Success

We agree with Patent Owner and find, for substantially the same reasons discussed above in regard to Petitioner's contentions based on Liu in the first ground, that Petitioner's contentions and supporting evidence fail to show a reasonable likelihood of establishing that either claim 5 or claim 6 is unpatentable as obvious over Lochrie, Huang, Mingozzi, Johnson, and Liu. *See supra* III.D.

IV. CONCLUSION

For the foregoing reasons, we determine that Petitioner does not demonstrate a reasonable likelihood of prevailing with respect to either

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[10] Patent Owner also argues that Petitioner fails to demonstrate the ionic strength was a recognized result effective variable. Prelim. Resp. 40–42 (citing Pet. 52 (Petitioner arguing that a person of ordinary skill in the art would have been motivated to increase the ionic strength of Lochrie's composition to prevent aggregation)). We recognize that "where the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." *In re Aller*, 220 F.2d 454, 456 (CCPA 1955). We also recognize, however, an "exception" to this *Aller* rule where "the parameter optimized was not recognized to be a result-effective variable." *In re Antonie*, 559 F.2d 618, 620 (CCPA 1977). Here, Petitioner fails to establish a known relationship between ionic strength and viral particle aggregation. Rather, the evidence of record teaches that "[t]he mechanism of vector aggregation is not well understood," and while buffer ionic strength is identified as a condition that may affect aggregation, the evidence of record does to show a known relationship between vector aggregation and buffer ionic strength. *See* Prelim. Resp. 41; Ex. 1007, 175; Ex. 2001 ¶ 120. Accordingly, on this record, we agree with Patent Owner that Petitioner fails to sufficiently show for purposes of institution that ionic strength was recognized as a result-effective variable for rAAV aggregation.

claim 5 or claim 6 of the '542 patent.[11] Accordingly, we do not institute *inter partes* review of the '542 patent.

IV. ORDER

Upon consideration of the record before us, it is:

ORDERED that the Petition is *denied* and no trial is instituted.

[11] Because we deny the Petition on the merits, we do not reach Patent Owner's argument for discretionary denial under 35 U.S.C. § 314(a). *See* Prelim. Resp. 51–57.

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Paper 20
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UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

NOVARTIS GENE THERAPIES, INC. & NOVARTIS PHARMACEUTICALS CORPORATION,
Petitioners,
v.

GENZYME CORPORATION,
Patent Owner.

IPR2023-00608
Patent 9,051,542 B2

Before JEFFREY N. FREDMAN, SHERIDAN K. SNEDDEN, and JAMES A. TARTAL, *Administrative Patent Judges.*

SNEDDEN, *Administrative Patent Judge.*

DECISION
Denying Institution of *Inter Partes* Review
35 U.S.C. § 314

I. INTRODUCTION

A. Background and Summary

Novartis Gene Therapies, Inc. and Novartis Pharmaceuticals Corporation (collectively, "Petitioner") filed a Petition requesting an *inter partes* review of claims 1, 2, 5, and 6 of U.S. Patent No. 9,051,542 B2 ("the '542 patent," Ex. 1001). Paper 2 ("Pet."). Genzyme Corporation ("Patent Owner") filed a Preliminary Response to the Petition. Paper 14 ("Prelim. Resp."). In its Preliminary Response, Patent Owner indicates that claims 1 and 2 are disclaimed, so only claims 5 and 6 remain challenged. *Id.* at 3.

With our authorization, Petitioner filed a Reply to Patent Owner's Preliminary Response (Paper 17) and Patent Owner filed a Sur-reply (Paper 18).

To institute an *inter partes* review, we must determine that the information presented in the Petition shows "a reasonable likelihood that the petitioner would prevail with respect to at least 1 of the claims challenged in the petition." 35 U.S.C. § 314(a) (2018). The Supreme Court has held that a decision to institute under 35 U.S.C. § 314 may not institute on less than all claims challenged in the petition. *SAS Inst., Inc. v. Iancu*, 138 S. Ct. 1348, 1359–60 (2018). After considering the evidence and arguments presented in the Petition, we determine that Petitioner has not demonstrated a reasonable likelihood of success in proving that either claim 5 or claim 6 of the '542 patent is unpatentable.

B. Real Parties in Interest

Petitioner asserts that Novartis Gene Therapies, Inc. and Novartis Pharmaceuticals Corporation are the real parties in interest. Pet. 67. Patent Owner asserts that "Sanofi, the ultimate parent company of Genzyme

Corporation, Genzyme Corporation, and Aventis, Inc. are the real parties-in-interest." Paper 6, 2.

C. Related Matters

The parties indicate that the '542 patent is asserted against Petitioner in *Genzyme Corporation et al. v. Novartis Gene Therapies, Inc. et al.*, Case No. 1:21-cv-01736 (D. Del.), filed December 10, 2021. Pet. 67–68; Paper 6, 2. Petitioner also filed a petition for *inter partes* review in IPR2023-00609 seeking to challenge claims 5 and 6 of the '542 patent on other grounds. Pet. 68.

D. The '542 patent (Ex. 1001)

The '542 patent is titled "Compositions and Methods to Prevent AAV Vector Aggregation," and issued on June 9, 2015, from U.S. Patent Application No. 12/661,553, filed March 19, 2010. Ex. 1001, codes (21), (22), (45), (54). The '542 patent "relates to compositions and methods of preparing and storing AAV [(adeno-associated virus)] virions that prevent aggregation." *Id.* at 1:17–19. According to the '542 patent, "[t]he solubility of purified AAV2 virus particles is limited, and aggregation of AAV2 particles has been described as a problem." *Id.* at 1:41–46 (citing, e.g., Wright et al., "Recombinant Adeno-Associated Virus: Formulation Challenges and Strategies for a Gene Therapy Vector," *Curr. Opin. Drug Disc. Dev.* 6(2):174–178 (2003) (Ex. 1007, "Wright"); Croyle, et al., "Development of Formulations That Enhance Physical Stability of Viral Vectors for Gene Therapy," *Gene Ther.*, 8:1281–1290 (2001) (Ex. 1013, "Croyle")).

In particular, the '542 patent discloses high ionic strength solutions that are isotonic with the intended target tissue. *Id.* at code (57). The

"combination of high ionic strength and modest osmolarity is achieved using salts of high valency, such as sodium citrate." *Id.*

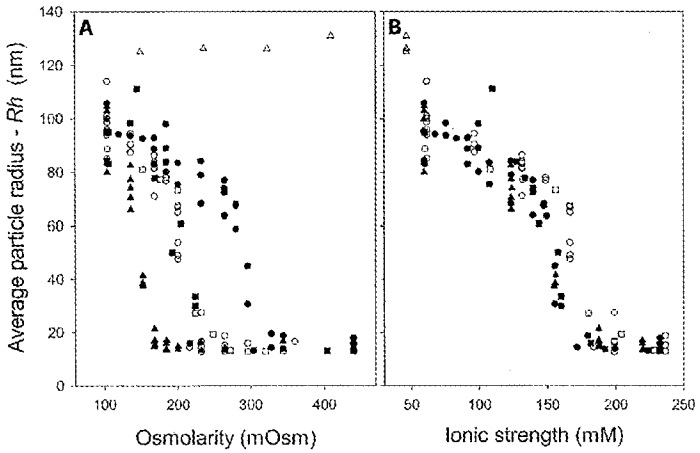
The '542 patent further explains as follows:

> The present invention is based in part on the observation that solution ionic strength is an important parameter in AAV vector aggregation, implicating the involvement of ionic interactions between virus particles in the aggregation process. The observation that elevated ionic strength increases AAV2 [AAV serotype 2] vector solubility regardless of the identity of the charged excipient supports the hypothesis that ionic strength of solution per se, rather than interactions involving a specific ionic species, is the relevant physico-chemical parameter. A threshold ionic strength of at least 200 mM is required to prevent aggregation at vector particle concentrations examined herein.

Id. at 4:53–64. The '542 patent additionally states as follows:

> In embodiments of the present invention the exponential relationship of ionic strength with charge valency is used to develop isotonic formulations with high ionic strengths. Salt species with multiple charge valencies (e.g. salts of sulfate, citrate, and phosphate) that are commonly used as excipients in human parenteral formulations can provide the level of ionic strength needed to prevent AAV2 vector aggregation when used at isotonic concentrations. While isotonic (150 mM) sodium chloride has an ionic strength of 150 mM, a value insufficient to maintain AAV2 solubility at high vector concentrations, isotonic sodium citrate, with an ionic strength of ~500 mM, can support AAV2 vector concentrations of at least 6.4×10^{13} vg/mL without aggregation.

Id. at 5:7–20. Figures 1A and 1B of the '542 patent are reproduced below.



Figures 1A and 1B present the results of a vector aggregation study that tracked aggregation as a function of two parameters, osmolarity (Figure 1A) and ionic strength (Figure 1B) for buffer compositions of sodium chloride (●), sodium citrate (○), sodium phosphate (■), sodium sulfate (□), magnesium sulfate (▲), and glycerol (Δ), and. *Id.* at 6:63–65, 12:33–67 (Example 3), FIGS. 1A, 1B. "Average particle radius is measured by dynamic light scattering (DLS) following vector dilution in varying concentrations of excipients buffered with 10 mM sodium phosphate at pH 7.5." *Id.* at 4:18–28. "Rh values >20 nm are deemed to indicate the occurrence of some level of aggregation." *Id.* at 9:25–27.

The results of Figure 1A, which plots vector aggregation as a function of the osmolarity of selected excipients, are explained as follows:

> For charged species a concentration-dependent inhibition of AAV2 vector aggregation is observed. Salts with multivalent ions achieve a similar degree of inhibition of aggregation at lower concentrations than monovalent sodium chloride. For example, magnesium sulfate prevents aggregation at >200 mOsm whereas sodium chloride requires ≧350 mOsm to achieve a similar effect. Sodium citrate, sodium sulfate, and sodium phosphate are intermediate in their potency to prevent vector aggregation.

Id. at 6:65–7:8.

Figure 1B shows data from the same experiment "plotted as a function of the calculated ionic strength, rather than osmolarity, for each excipient." *Id.* at 7:18–20. Figure 1B's plot of particle radius versus ionic strength shows that "vector aggregation is prevented when ionic strength is ~200 mM or greater regardless of which salt is used." *Id.* at 7:21–22. "These data suggested that the ionic strength (μ) of a solution . . . is the primary factor affecting aggregation." *Id.* at 7:22–25.

The '542 patent discloses the results of a study assessing "the effects of elevated ionic strength and nuclease treatment on AAV2 vector aggregation at a larger scale, using methods to induce and quantify vector aggregation that are relevant to preparative scale vector purification" in Table 2. *Id.* at 8:1–5.

Table 2 of the '542 patent is reproduced, in part, below.

TABLE 2

AAV VECTOR RECOVERY AT PROCESS SCALE

Experiment	Formulation	μ (mM)	Target (vg/mL)	Actual (vg/mL)	Yield % (RSD)
1	CF	160	2.5E13	1.93E13	77 (6.6)
1	TF1	310	2.5E13	2.38E13	95 (7.4)
1	TF2	510	2.5E13	2.33E13	93 (7.4)
2	CF	160	6.7E13	3.98E13	59 (6.0)
2	TF2	510	6.7E13	6.42E13	96 (4.4)

Table 2 shows the results for three solutions of AAV2-AADC vectors filtered through a 0.22 μm filter. *Id.* at 8:1–10, 11:53–12:29. The three solutions are as follows:

> Control Formulation (CF: 140 mM sodium chloride, 10 mM sodium phosphate, 5% sorbitol, pH 7.3); Test Formulation 1 (TF1: 150 mM sodium phosphate, pH 7.5); and Test Formulation 2 (TF2: 100 mM sodium citrate, 10 mM Tris, pH 8.0).

Id. at 11:66–12:3. In Experiment 1, the samples contained 2.5×10^{13} vg/ml vector, and, in Experiment 2, the samples contained 6.7×10^{13} vg/ml vector. *Id.* at 12:4–12. Table 2 shows recoveries exceeded 90% following filtration in formulations TF1 and TF2 having ionic strengths greater than 200 mM, whereas recovery from CF formulations, having ionic strength of 160 mM, was only 77% and 59% for experiments 1 and 2, respectively. *Id.* at 8:19–56.

The '542 patent also discloses the results of a study assessing "stability after storage or freeze-thaw (F/T) cycling is assessed in buffers of the present invention." *Id.* at 9:19–27. Particle radius was measured by dynamic light scattering (DLS) to determine the presence of aggregates. *Id.*

Table 3, reproduced below, summarizes the results of the study.

TABLE 3

STABILITY OF AAV2 VECTORS

	Particle radius - Rh (nm)							
Formu-		4° C.	−20° C.			−80° C.		
lation	Pre	5 d	1 F/T	5 F/T	10 F/T	1 F/T	5 F/T	10 F/T
CF	14.5	27.0	22.4	56.1	94.5	20.6	57.5	141
TF1	13.8	16.3	TH	TH	TH	TH	TH	TH
TF2	13.8	14.4	14.2	14.0	14.1	13.8	21.3	50.9

Pre: DLS radius measured immediately following 0.2 μm filtration.
Vector concentrations (vg/mL): CF: 1.93E13, TF1: 2.38E13, TF2: 2.33E13.
TH: signal intensity is too high to measure because of extensive aggregation.

According to the '542 patent, Table 3 provides data showing as follows:

> AAV2-AADC vector prepared in CF shows some aggregation after 5 days of storage at 4° C., as well as following one or more F/T cycles at −20 or −80° C. For vector prepared in TF1, no aggregation occurs after 5 days at 4° C., but aggregation occurs following a single F/T cycle at −20 or −80° C. as indicated by a DLS signal intensity that is too high to measure. Visual

> inspection of these samples reveals slight cloudiness, which is consistent with aggregation. For vector prepared in TF2, no aggregation is observed at 4° C., or following up to 10 F/T cycles at −20° C. Some aggregation is observed following 5 and 10 F/T cycles at −80° C.

Id. at 9:29–55. According to Patent Owner, the results of the studies disclosed in the '542 patent "confirmed the importance of increased ionic strength in preventing aggregation." Prelim. Resp. 13 (citing Ex. 1001, 10:29–43 (stating "[t]he effect of ionic strength [] on virus particle interactions is determined to elucidate the mechanism of vector aggregation")).

E. The Challenged Claims

Challenged Claims 5 and 6 are reproduced below, along with claim 1 from which they depend.

> 1. A composition for the storage of purified, recombinant adeno-associated virus (AAV) vector particles, comprising:
>
> > purified, recombinant AAV vector particles at a *concentration exceeding 1×10^{13} vg/ml up to 6.4×10^{13} vg/ml*;[1]

[1] The units of measurements used in the art to measure the titer of AAV compositions are explained in the Petition as follows:

> The titer of AAV compositions can be measured in vector genomes (vg)/ml, genome copies (gc)/ml, capsid particles (cp)/ml, or virus particles (vp)/ml. Ex. 1025, ¶¶35. The first two are used interchangeably, since both represent the number of functional vectors containing the therapeutic gene. *Id.*, ¶¶36-37. By contrast, the latter two measurements include particles that are incomplete, damaged, or lacking genetic material. Ex. 1009, [00281]; Ex. 1025, ¶36.

Pet. 12.

> a pH buffer, wherein the pH of the composition is between 7.5 and 8.0; and
>
> excipients comprising one or more multivalent ions selected from the group consisting of citrate, sulfate, magnesium, and phosphate; wherein *the ionic strength of the composition is greater than 200 mM*, and wherein the purified AAV vector particles are stored in the composition without significant aggregation.
>
> 5. The composition of claim 1, *wherein the purified, recombinant AAV vector particles have an average particle radius (Rh) of less than about 20 nm as measured by dynamic light scattering.*
>
> 6. The composition of claim 1, *wherein recovery of the purified, recombinant virus particles is at least about 90% following filtration of the composition of said AAV vector particles through a 0.22 μm filter.*

Ex. 1001, 14:15–28, 34–41 (emphasis added to highlight disputed elements).

F. Evidence

Petitioner relies upon information that includes the following.

Ex. 1003, Evans, WO 01/66137 A1, published Sept. 13, 2000 ("Evans").

Ex. 1004, Frei et al., WO 99/41416, published Aug. 19, 1999 ("Frei").

Ex. 1005, Huang J., Gao, et al., "Aggregation of AAV vectors, its Impact on Liver directed Gene Transfer and Development of Vector Formulations to Prevent and Dissolve Aggregation and Enhance Gene Transfer Efficiency," MOL THER. 1:S286 (2000) ("Huang").

Ex. 1006, Mingozzi, et al., "Improved Hepatic Gene Transfer by Using an Adeno-Associated Virus Serotype 5 Vector," J VIROL. Vol. 76, No. 20, pp. 10497–502 (2002) ("Mingozzi").

Ex. 1007, Wright et al., "Recombinant Adeno-Associated Virus: Formulation Challenges and Strategies for a Gene Therapy

Vector," CURR. OPIN. DRUG DISC. DEV. 6(2):174–178 (2003) ("Wright").

Petitioner also relies upon the Declaration of Mansoor M. Amiji, R.Ph., Ph.D. (Ex. 1025) to support its contentions.

Patent Owner relies upon the Declaration of Martyn C. Davies, D.Sc., Ph.D. (Ex. 2004) to support its contentions.

G. Asserted Grounds of Unpatentability

In the Petition, Petitioner challenges claims 1, 2, 5, and 6 on the following grounds:

Ground	Claim(s) Challenged	35 U.S.C. §[2]	Reference(s)/Basis
1	1, 5, 6	103	Evans, Huang, Mingozzi
2	2	103	Evans, Wright, Huang, Mingozzi
3	1, 2, 5, 6	103	Frei, Huang, Mingozzi

Pet. 4. After the Petition was filed, Patent Owner subsequently explained that "[c]laims 1 and 2 were disclaimed to streamline issues for the Board, because only claims 5 and 6 are asserted for infringement in the co-pending litigation." Prelim. Resp. 3 n.3. Accordingly, for the purposes of this Decision, we consider only Petitioner's Grounds 1 and 3 as directed to challenged claims 5 and 6.

H. Claim Construction

We interpret a claim "using the same claim construction standard that would be used to construe the claim in a civil action under 35 U.S.C.

[2] The Leahy-Smith America Invents Act ("AIA") included revisions to 35 U.S.C. §103 that became effective on March 16, 2013. We apply the pre-AIA version of §103 here, because the application identified in the '542 patent was filed before the effective date of the AIA. *See* Ex. 1001, code (22).

282(b)." 37 C.F.R. § 42.100(b) (2019). Under this standard, we construe the claim "in accordance with the ordinary and customary meaning of such claim as understood by one of ordinary skill in the art and the prosecution history pertaining to the patent." *Id.*

Petitioner asserts that the claim terms require no express construction. Pet. 17. Patent Owner does not challenge Petitioner's position. Prelim. Resp. 14.

Having considered the parties' positions and evidence of record, we determine that no express construction of any claim term is necessary to determine whether to institute *inter partes* review. *Nidec Motor Corp. v. Zhongshan Broad Ocean Motor Co.*, 868 F.3d 1013, 1017 (Fed. Cir. 2017) ("[W]e need only construe terms 'that are in controversy, and only to the extent necessary to resolve the controversy.'" (quoting *Vivid Techs., Inc. v. Am. Sci. & Eng'g, Inc.*, 200 F.3d 795, 803 (Fed. Cir. 1999))). To the extent further discussion of the meaning of any claim term is necessary to our decision, we provide that discussion below in our analysis of the asserted grounds of unpatentability.

I. Level of Ordinary Skill in the Art

The level of ordinary skill in the art usually is evidenced by the prior art references themselves. *See Okajima v. Bourdeau*, 261 F.3d 1350, 1355 (Fed. Cir. 2001); *In re GPAC Inc.*, 57 F.3d 1573, 1579 (Fed. Cir. 1995).

Petitioner proposes that a person of ordinary skill in the art ("POSA") at the time of the invention

> would have possessed at least a BS in biology, chemistry, chemical engineering, biochemistry, pharmaceutical science, or a related discipline, with ≥4 years of industry, laboratory, and/or clinical experience in formulating or developing dispersions for therapeutic biologics, such as proteins or vectors for gene

> delivery. Such person may be familiar with, or consult with someone familiar with, the development and/or administration of viral vectors for gene therapy. Ex. 1025, ¶82.

Pet. 16–17. Patent Owner does not dispute Petitioner's proposal about the POSA's qualifications. Prelim. Resp. 2 n.2.

For this Decision, we adopt and apply Petitioner's proposal for the person of ordinary skill in the art level, which appears to be consistent with the level of skill reflected in the asserted prior art and the '542 patent.

II. ANALYSIS

"In an [*inter partes* review], the petitioner has the burden from the onset to show with particularity why the patent it challenges is unpatentable." *Harmonic Inc. v. Avid Tech., Inc.*, 815 F.3d 1356, 1363 (Fed. Cir. 2016) (citing 35 U.S.C. § 312(a)(3) (requiring inter partes review petitions to identify "with particularity . . . the evidence that supports the grounds for the challenge to each claim")). This burden of persuasion never shifts to the patent owner. *See Dynamic Drinkware, LLC v. Nat'l Graphics, Inc.*, 800 F.3d 1375, 1378 (Fed. Cir. 2015). Moreover, a petitioner should not "place the burden on [the Board] to sift through information presented by the Petitioners, determine where each element [of the challenged claims] is found in [the cited references], and identify any differences between the claimed subject matter and the teachings of [the cited references.]" *Google Inc. and Twitter, Inc. v. EveryMD.com LLC*, IPR2014-00347, Paper 9 at 25 (PTAB May 22, 2014).

The question of obviousness is resolved on the basis of underlying factual determinations including: (1) the scope and content of the prior art; (2) any differences between the claimed subject matter and the prior art; (3) the level of ordinary skill in the art; and (4) objective evidence of

nonobviousness.[3] *Graham v. John Deere Co.*, 383 U.S. 1, 17–18 (1966).

The obviousness inquiry also typically requires an analysis of "whether there was an apparent reason to combine the known elements in the fashion claimed by the patent at issue." *KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398, 418 (2007) (citing *In re Kahn*, 441 F.3d 977, 988 (Fed. Cir. 2006) (requiring "articulated reasoning with some rational underpinning to support the legal conclusion of obviousness")). A petitioner cannot prove obviousness with "mere conclusory statements." *In re Magnum Oil Tools Int'l, Ltd.*, 829 F.3d 1364, 1380 (Fed. Cir. 2016). Rather, a petitioner must articulate a sufficient reason why a person of ordinary skill in the art would have combined the prior art references. *In re NuVasive, Inc.*, 842 F.3d 1376, 1382 (Fed. Cir. 2016).

We analyze the asserted grounds of unpatentability in accordance with these principles to determine whether Petitioner has met its burden to establish a reasonable likelihood of success at trial.

A. Summary of Cited Prior Art

1. Evans

Evans discloses viral compositions for use in gene therapy. Ex. 1003, Abstract, 1:15–19. Evans teaches buffer conditions to maintain its compositions for potential human parenteral administration. Ex.1003, 1:15–19. Evans explains that "[a]n ongoing challenge in the field of gene therapy and vaccine research is to generate liquid virus formulations which are stable for longer periods of time within a useful temperature range." *Id.* at 1:16–19, 28–30.

[3] Patent Owner does not present any objective evidence of nonobviousness (i.e., secondary considerations) for the challenged claims.

Evans discloses that its compositions comprise a buffer, a salt, a divalent cation, and a non-ionic detergent. Ex. 1003, 1:19–21. Evans further discloses the identity of and concentration ranges for those components. *See* Ex. 1003, 8:22–11:4. Evans also discloses that the compositions support virus concentrations of about 1 x 10^7 to 1 x 10^{13} vp/ml. Ex. 1003, 8:5–11.

Evans claims a virus composition comprising a purified virus with a concentration of about 1 x 10^7 to 1 x 10^{13} vp/ml, a buffer acceptable for human parenteral use at a pH of about 7.5–8.5, sodium chloride at about 25mM–250mM, a divalent cation selected from $MgCl_2$ and $CaCl_2$ at about 0.1mM–5mM, and a non-ionic detergent. Ex. 1003, 36 (claim 5). Evans teaches that its compositions may be used with AAV. Ex. 1003, 3:12–14; 7:16–18.

2. Huang

Huang, an abstract titled "Aggregation of AAV Vectors, its impact on Liver directed Gene Transfer and Development of Vector Formulations to Prevent and Dissolve Aggregation and Enhance Gene Transfer Efficiency," states that "to achieve high level of gene transfer and ensure the safety of vector administration it is desirable to deliver high doses of vector in small volumes." Ex. 1005, S286. According to Huang, "at high concentrations, AAV virions form aggregates of different sizes in a range of different buffer systems and storage conditions." *Id.* Huang states that "when the vector titer reached 5-10 x 10^{13} GCs/ml, gene transfer efficiency was 10-100 folds lower at the same dose as compared to the vector whose titer was 1-5 x 10^{12} GCs/ml. *Id.* Huang states that "a series of formulation studies were performed to prevent and dissolve AAV aggregation," and reported "a 30–

50% reduction in the size of aggregates size at high vector concentrations" for some of the compositions. *Id.*

3. Mingozzi

Mingozzi, titled Improved Hepatic Gene Transfer by Using Adeno-Associated Virus Serotype 5 Vector, states that "AAV vectors do not contain viral coding sequences and have been shown to efficiently transfer genes to nondividing target cells," and that "[a]n excellent safety profile combined with reduced potential for activation of inflammatory or cellular immune responses has made this vector system attractive for clinical application and treatment of genetic disorders." Ex. 1006, 10497. According to Mingozzi, purification of AAV-2 and AAV-5 vectors "by repeated CsCl gradient centrifugation" yielded concentrations of $>10^{13}$ vg/ml. *Id.*

4. Wright

Wright teaches that AAV "is a promising vector for human gene transfer" and has "received considerable attention in the field of gene therapy, because of [its] ability to mediate long-term gene transfer in the absence of significant toxicity." Ex. 1007, 174. Wright teaches that "because AAV and adenovirus are both non-enveloped viruses developed as gene transfer vectors, studies on the latter can provide guidance for AAV vector formulation development." *Id.*

Wright notes that "[t]he mechanism of vector aggregation is not well understood, and purification conditions that may affect aggregation include buffer ionic strength and pH, shear and vector concentration." *Id.* at 175. Wright discloses that

> Our and other research teams have observed that freeze-thaw cycling exacerbates vector aggregation, and can lead to aggregation at vector concentrations significantly lower than

> 10^{14} cp/ml. For example, using dynamic light scattering, we observed that highly purified vector preparations at concentrations of 5×10^{13} cp/ml that are stable in a non-aggregated, monomeric state when stored at 2 to 8°C, can be induced to undergo some aggregation following a single freeze-thaw cycle to -20°C.

Id. Wright notes that "[r]educed yield is one of the deleterious consequences of aggregation during the vector purification process" and notes that "loss of rAAV following a 0.2-μm filtration step correlates with the extent of vector aggregation." *Id.*

Wright teaches that "empty capsids, whose size and surface characteristics are similar to that of genome-containing vector particles, contribute to particle aggregation, and their presence may result in aggregation at lower vector genome (vg) concentrations than would be observed in their absence." *Id.* (citation omitted).

Wright further discloses that "[a]ssuming that full vector particles and empty capsids aggregate by a similar mechanism (an assumption that requires testing), a preparation of AAV vectors containing a 10-fold excess of empty capsids should have a similar risk of aggregation at concentrations of $\geq 10^{13}$ vg/ml (corresponding to $\geq 10^{14}$ cp/ml)." *Id.* at 175–176.

5. Frei

Frei discloses viral formulations comprising polyhydroxy hydrocarbon for use in gene therapy. Ex. 1004, Abstract, 1:15–20. Frei identifies "a critical need to develop formulations that stabilize relatively high concentrations of virus," and discloses a buffered formulation that stabilizes high concentrations of recombinant virus for use in gene therapy and maintains viability after storage. *Id.* at 4:26–36, 7:7–11, 8:27–29, 8:34–36. Frei discloses that its compositions comprise a buffer system that

maintains a pH of about 7.0–8.5 despite storage between -80°C and 27°C. *Id.* at 6:21–24. Frei's compositions include pharmaceutically acceptable divalent metal salt stabilizers, and Frei teaches that magnesium salts are particularly preferred in an amount of about 0.1 mg/ml to 1 mg/ml. *Id.* at 5:31–36. Pharmaceutically acceptable monovalent salt stabilizers are also included, and Frei discloses that sodium chloride in an amount of 0.6 mg/ml to 10.0 mg/ml is preferred. *Id.* at 5:37–6:6. Frei further teaches that "the formulation of the present invention can maintain stability of the virus at concentrations ranging up to 1 x 10^{13} particles/mL." *Id.* at 7:9–11. Frei's example of a virus composition ("Example D-1") comprises purified adenovirus at a concentration of 1.6 x 10^{13} vp/ml, in 20 mM NaPi buffer, 100 mM NaCl, 2 mM $MgCl_2$, 2% sucrose, and 10% glycerol, having pH 8 at 2–10°C. *Id.* at 22:17–31.

B. Ground 1: Obviousness of Claims 5 and 6 over the Combination of Evans, Huang, and Mingozzi

Petitioner asserts that claims 5 and 6 are unpatentable as obvious over Evans, Huang, and Mingozzi. Pet. 23–46. Patent Owner disputes Petitioner's contentions. Prelim. Resp. 14–44.

For the reasons set forth below, we determine that Petitioner has not shown a reasonable likelihood of establishing that at least one of the challenged claims is unpatentable as obvious over Evans, Huang, and Mingozzi.

1. Petitioner's Contentions

With regard to Challenged Claim 5, Petitioner contends that the "average particle radius" limitation is an "inherent characteristic feature of the purified viral composition." Pet. 41–42.

Petitioner next directs our attention to Evans's claim 5, which is directed to a virus composition containing a "divalent cation [] selected from the group consisting of $MgCl_2$ and $CaCl_2$ in an amount from about 0.1 mM to about 5 mM." Ex. 1003, 36 (claim 5); Pet. 42. Petitioner further contends that

> The '542 patent admits that AAV2 particles have a diameter of ~26nm (Ex.1001, 1:29-38). Because Evans's claim 5 composition prevented aggregation, a POSA would have reasonably expected AAV particles stored therein would have an Rh of <~20 nm measured by DLS. Indeed, the '542 patent does not identify anything critical about the recited radius range other than it being exemplary of no aggregation. *Id.*, 9:25-27 ("Rh values >20 nm are deemed to indicate the occurrence of some level of aggregation.").

Pet. 42. Petitioner contends that a person of ordinary skill in the art would have been motivated to minimize any potential aggregation in Evans's claim 5 because it was known that virus aggregation reduces gene transfer efficiency and other potentially deleterious consequences. *Id.* at 42–43 (citing Ex. 1005, S286; Ex. 1007, 176). Petitioner further contends that

> A POSA would have reasonably expected success in minimizing particle size in view of Huang's teaching that its optimized compositions "could lead to a 30-50% reduction in the size of aggregates at high vector concentrations." Ex.1005, S286. Indeed, "no signs of settling or precipitation" were observed for prior art adenovirus compositions stored in a high ionic strength buffer over a 7-day period (Ex.1009, [00369]), and a POSA would have understood that AAV "is significantly more stable than the adenovirus" used in Liu (Ex.1013, 1283); Ex.1025, ¶¶197-198. Thus, only routine optimization would be required to obtain an average AAV Rh of <20nm in Evans's claim 5 composition. Ex.1025, ¶¶ 199-201.

Pet. 43 (citing *Senju Pharm. Co. v. Lupin Ltd.*, 780 F.3d 1337, 1353 (Fed. Cir. 2015) (invalidating a claim directed to "a product of routine optimization that would have been obvious to one of skill in the art.")).

With regard to claim 6, Petitioner contends

> a POSA would have been motivated to minimize any potential aggregation in Evans's claim 5 formulation, since both Wright and Huang linked aggregation to reduced functional activity of AAV vectors. Ex.1007, 176; Ex.1005, S286. Thus, a POSA would have been motivated to maximize virus recovery from a 0.22μm filter through routine optimization of the known stabilization factors in Evans's claim 5 composition. Ex.1025, ¶ 205.

Pet. 45. Petitioner further contends that

> A POSA also would have reasonably expected success in maximizing particle recovery after filtration because POSA knew that Huang taught its optimized compositions "could lead to a 30-50% reduction in the size of aggregates at high vector concentrations" (Ex.1005, S286), Liu observed "no signs of settling or precipitation" for adenovirus particles stored in a high ionic strength buffer over a 7-day period (Ex.1009, [00369]), and Croyle taught that AAV "is significantly more stable than the adenovirus" (Ex.1013, 1283). Thus, only routine optimization would be required to improve AAV recovery following filtration of Evans's claim 5 formulation through a 0.22μm filter. Ex.1025, ¶¶ 206-209.

Pet. 45–46.

2. Patent Owner's Contentions

Patent Owner contends that Petitioner "does not submit any evidence that the particle radius and product recovery elements of claims 5 and 6, respectively, would inherently result from the claimed combination." Prelim. Resp. 16.

Patent Owner contends that Petitioner's reliance on Evans's disclosure of "a virus concentration in the range from about $1x10^7$ vp/mL to about

$1x10^{13}$ vp/mL" to argue that the uppermost endpoint of this range ($1x10^{13}$ vp/mL ± 5%) overlaps with the scope of the claims assumes that 100% of the particles contain vector genomes and that Petitioner "provides no basis for why the POSA would make such an assumption." *Id.* at 17 (citing Pet. 30 ("Assuming that 100% of the particles contain vector genomes, Evans's claim 5 composition therefore comprises viral particles at a concentration exceeding $1x10^{13}$ vg/ml.")). Rather, according to Patent Owner,

> Dr. Amiji's declaration indicates that the POSA would not have assumed that Evans's viral particle compositions were free of empty capsids, and instead would have assumed the opposite—that as many as 90% of capsids in a given composition are empty. [Ex. 2004] ¶¶72-73. Dr. Amiji states that "Wright [Ex. 1007] teaches that ≥ 10^{14} capsid particles (cp)/ml corresponds to ≥ 10^{13} vg/ml)," indicating as much as 10-fold excess in empty capsids. [Ex. 1025] ¶119 (citing Ex. 1007, 176).

Id. (emphasis omitted).

Patent Owner contends that

> [Petitioner] fails to establish that the POSA would have been motivated to develop a composition comprising an rAAV "concentration exceeding $1x10^{13}$ vg/ml," "one or more multivalent ions selected from … citrate, sulfate, magnesium, and phosphate," with an ionic strength "greater than 200mM." [Ex. 2004] ¶¶ 75–79.

Id. at 18. Patent Owner notes that Petitioner relies on "Mingozzi to argue that the POSA would been motivated to administer 'doses of $3.2x10^{13}$ vg for a 60kg human' at a 'concentration exceeding $1x10^{13}$ vg/ml.'" *Id.* (citing Pet. 31). Patent Owner contends, however, that Mingozzi "says nothing about any formulations for AAV vectors let alone anything about ionic strength or multivalent ions." *Id.* at 18–19 (citing Ex. 2004 ¶ 78).

Patent Owner further argues that Petitioner's arguments that the claimed ionic strength range—greater than 200 mM—would have been

achieved by through routine optimization misapplies obviousness case law. Prelim. Resp. 20–21 (citing Pet. 35–36). In particular, Patent Owner contends that "[f]or a range to be obvious, a parameter must first be recognized as a 'result-effective variable,' before the determination of the optimum or workable ranges of that variable might be characterized as routine experimentation. *Id.* at 21 (citing *In re Antonie*, 559 F.2d 618, 620 (CCPA 1977)). Petitioner, however, "fails to identify any disclosure in Evans, Huang, and/or Mingozzi suggesting that ionic strength would impact rAAV aggregation" and failed to establish ionic strength as a "result-effective variable." *Id.* Moreover, Patent Owner contends that Petitioner

> mischaracterizes Wright (Ex. 1007) to support its contention that "ionic strength . . . likely affects vector aggregation." Wright stated that the "mechanism of vector aggregation is not well understood, and purification conditions that may affect aggregation include buffer ionic strength and pH, shear and vector concentration." Ex. 1007, 175. Novartis never explains how Wright's statement that factors causing vector aggregation were "not well understood"—followed by a non-exclusive list of conditions that may impact aggregation—was an indication that "ionic strength . . . likely affects vector aggregation." *Id.*; Davies, ¶¶ 80-81.

Id. at 21–22 (emphasis omitted).[4] According to Patent Owner, "Wright also fails to teach or suggest ionic strength as a results-effective variable for rAAV aggregation." *Id.* at 22 (citing Ex. 2004 ¶¶ 84–85).

[4] We note that while Petitioner does not rely on Wright for its obviousness challenge in Ground 1, Petitioner relies on Wright for its argument that "ionic strength was a known condition that likely affects vector aggregation." Pet. 36 (citing Ex. 1007, 175; Ex. 1025 ¶¶ 175–177).

3. Discussion

Claims 5 and 6 require, respectively, that the composition does not exhibit significant aggregation as determined by particle radius (claim 5) and by percent product recovery following filtration (claim 6). Petitioner's arguments that those elements of claims 5 and 6 are inherent properties to AAV2 particles misses what is required by those claims, because each of those elements of the claims are used as a measure of aggregation achieved by the claimed compositions. Pet. 41–42, 44, 60; Ex. 2004 ¶¶ 126, 130–134; Ex. 1001, 4:61–5:25, 8:19–44, 9:25–27. For example, the '542 patent explains that the effect of ionic strength on aggregation was assessed by measuring vector recovery after filtration through a 0.22 μm filter. Ex. 1001, 8:1–10, 11:53–12:29 (Example 2); Ex. 2004 ¶ 133. Thus, Petitioner's arguments that particle radius (claim 5) and percent product recovery following filtration (claim 6) are inherent properties is insufficient to prove obviousness.

To prove inherency in the context of obviousness "[a] party must . . . meet a high standard . . . the limitation at issue necessarily must be present, or the natural result of the combination of elements explicitly disclosed by the prior art." *PAR Pharm., Inc. v. TWI Pharms., Inc.*, 773 F.3d 1186, 1195–96 (Fed. Cir. 2014). To that point, Petitioner fails to provide sufficient evidence such as prior art or testing evidence to show that the combination of Evans, Huang, and Mingozzi would result in a composition having the recited aggregation outcomes. For example, the '542 patent explains that compositions having ionic strength greater than 200 mM surprisingly resulted in recoveries exceeding 90%, whereas compositions having ionic strengths below 200 mM resulted in recoveries below 80%. Ex. 1001, 8:1–

10, 11:53–12:29 (Example 2); Ex. 2004 ¶ 133. Petitioner fails to submit any evidence that the particle radius and product recovery elements of claims 5 and 6, respectively, would necessarily be present, or the natural result of the combination of teachings explicitly disclosed by Evans, Huang, and Mingozzi.

Petitioner also argues that adjusting the ionic strength of the composition would have been a matter of routine optimization. Pet. 36 (citing Ex. 1003, 11:13–19; Ex. 1025 ¶¶ 61–71, 178–182), 43 (citing Ex. 1025 ¶¶ 199–201), 45 (Ex. 1025 ¶¶ 205–209). We are not persuaded by Petitioner's routine optimization argument as applied to the claimed ionic strength range. We acknowledge that "where the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." *In re Aller*, 220 F.2d 454, 456 (CCPA 1955). We also acknowledge, however, an "exception" to this *Aller* rule where "the parameter optimized was not recognized to be a result-effective variable." *In re Antonie*, 559 F.2d 618, 620 (CCPA 1977). Here, Petitioner fails to establish a known relationship between ionic strength and viral particle aggregation. Rather, the evidence of record teaches that "[t]he mechanism of vector aggregation is not well understood, and purification conditions that may affect aggregation include buffer ionic strength and pH, shear and vector concentration." Ex. 1007, 175; Prelim. Resp. 21; Ex. 2004 ¶¶ 84–85. Additionally, as explained in detail by Patent Owner, data presented in Evans does not establish any clear relationship between ionic strength and maintained infectivity after storage. Prelim. Resp 22–27. Accordingly, on this record, we determine that

Petitioner fails to establish ionic strength as a result-effective variable for rAAV aggregation.

For at least the reasons discussed above, we are not persuaded that Petitioner has shown a reasonable likelihood of establishing that either claim 5 or claim 6 is unpatentable as obvious over Evans, Huang, and Mingozzi. Accordingly, Petitioner has not demonstrated a reasonable likelihood of prevailing on Ground 1.

C. Ground 3: Obviousness of Claims 5 and 6 over the Combination of Frei, Huang, and Mingozzi

Petitioner asserts that claims 5 and 6 are unpatentable as obvious over Frei, Huang, and Mingozzi. Pet. 47–61. Patent Owner disputes Petitioner's contentions. Prelim. Resp. 50–60.

As in Ground 1, Petitioner contends that the particle radius and product recovery elements of claims 5 and 6 would inherently result from the claimed combination and additionally that a "selection of an appropriate ionic strength for a therapeutic composition is a matter of routine optimization." Pet. 54–55, 59–61. We are unpersuaded by Petitioner's contentions for the same reasons discussed above in Ground 1 because those contentions are similarly unsupported by the evidence of record. *See, e.g.*, Pet. 55 (relying on Wright for the premise that ionic strength is a parameter that may affect vector aggregation).

Accordingly, we determine that Petitioner has not shown a reasonable likelihood of establishing that at least one of the challenged claims is unpatentable as obvious over Frei, Huang, and Mingozzi.

III. CONCLUSION

After considering the evidence and arguments of record, we determine that Petitioner has not demonstrated a reasonable likelihood of prevailing

with respect to any claim challenged in the Petition.[5] Accordingly, we do not institute an *inter partes* review.

IV. ORDER

In consideration of the foregoing, it is hereby:

ORDERED that the Petition is *denied* as to all challenged claims, and no trial is instituted.

[5] Because we deny the Petition on the merits, we do not reach Patent Owner's argument for discretionary denial under 35 U.S.C. § 314(a) or § 325(d). Prelim. Resp. 44–50, 61–67.

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PTO/SB/26a (02-14)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

TERMINAL DISCLAIMER IN A PATENT OR PROCEEDING IN VIEW OF ANOTHER PATENT

Docket Number (Optional)

Application/Control Number: 12/661,553
Filing Date: 03/19/2010
First Named Inventor: John Fraser Wright
Title: COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION
Patent No.: 9,051,542

The patentee, Genzyme Corportation, owner of 100 percent interest in the instant patent hereby disclaims, except as provided below, the terminal part of the statutory term of the instant patent which would extend beyond the expiration date of the full statutory term of patent No. 7,704,721 (the "**reference** patent"), as the term of said **reference** patent is presently shortened by any terminal disclaimer. The patentee hereby agrees that the instant patent shall be enforceable only for and during such period that the instant patent and the **reference** patent are commonly owned. This agreement runs with the instant patent and is binding upon the grantee, its successors or assigns.

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1. ☐ The current ownership was established by the filing of a statement under 37 CFR 3.73 during prosecution of the application that issued as the instant patent.

2. ☐ The instant patent was issued from an application filed on or after September 16, 2012, and the current patent owner was the applicant under 37 CFR 1.46.

3. ☑ A statement under 37 CFR 3.73 is attached herewith. Form PTO/SB/96 or PTO/AIA/96, as appropriate, may be used.

II. Authorization for Terminal Disclaimer - Check either box 1 or 2 below, if appropriate:

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2. ☑ The undersigned is an attorney or agent of record. Reg. No. 78607

/David M. Lee/	September 11, 2023
Signature	Date
David M. Lee	617-728-7100
Typed or printed name	Telephone number

☑ The terminal disclaimer fee under 37 CFR 1.20(d) is included.

NOTE: Submit multiple forms if more than one signature is required, see below.*

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COMBINED POWER OF ATTORNEY BY ASSIGNEE
AND STATEMENTS UNDER 37 CFR §§ 3.73 (b) AND 3.71

Genzyme Corporation (hereinafter "Assignee") having a place of business at **450 Water Street, Cambridge, Massachusetts, 02141**, states that it is the assignee of the entire right, title and interest in the patent listed below by virtue of either an assignment from the inventor(s), or by chain of title from the inventor(s), to the Assignee, recorded at the specified reel and frame numbers listed below, or attached hereto:

Patent No.	Issue Date	Title	Assignment Recordation
9,051,542	June 9, 2015	Compositions and methods to prevent AAV vector aggregation	Reel 039960 Frame 0383 Reel 039960 Frame 0444

As required by 37 CFR § 3.73(b)(1)(i), the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR § 3.11.

Genzyme Corporation hereby appoints **Amanda K. Antons**, Registration No. **65,236**, and the **Dechert LLP** practitioners associated with **Customer Number 37509** as its attorneys and agents with full power of substitution and revocation, to prosecute the above-captioned patent, and to transact all business in the USPTO connected therewith, said appointment being to the exclusion of the inventor(s) and his/her attorney(s) in accordance with the provisions of 37 CFR § 3.71; provided that if any one of said attorneys or agents ceases to be affiliated with the law firm of Dechert LLP as partner, employee or of counsel, such attorney or agent's appointment as attorney and all powers derived therefrom shall terminate on the date such attorney or agent ceases being so affiliated.

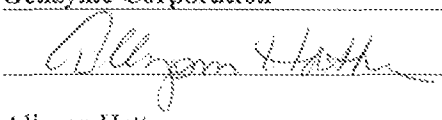
Please direct all correspondence address for the above-identified application to:

☒ Customer Number **37509**
Dechert LLP, 1095 Avenue of the Americas, New York, NY 10036-6797
Telephone: 212.698.3500

The undersigned, whose title is supplied below, is authorized to act on behalf of the Assignee.

Assignee: **Genzyme Corporation**

Date: June 14, 2023

Signed: 

Print Name: Allyson Hatton

Print Title: Principal Counsel, Attorney-in-Fact on behalf of Genzyme Corporation

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ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
12/661,553	**09/11/2023 03:32:28 PM ET**	**0800-0045.01**

Title of Invention

Compositions and methods to prevent AAV vector aggregation

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	9051542
CONFIRMATION #	4726	FILED BY	Kerri Leary
PATENT CENTER #	62771702	FILING DATE	03/19/2010
CUSTOMER #	37509	FIRST NAMED INVENTOR	John Fraser Wright
CORRESPONDENCE ADDRESS	-	AUTHORIZED BY	David Lee

Documents

TOTAL DOCUMENTS: 3

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
199648_Terminal_Disclaimer_sb0026a_9051542_signed.pdf	2	Terminal Disclaimer Filed	102 KB
199648_3_73b_Genzyme_Corporation_9051542_signed.pdf	2	Assignee showing of ownership per 37 CFR 3.73	99 KB
199648_3_73b_Genzyme_Corporation_Power_of_Attorney_9051542_signed.PDF	1	Power of Attorney	64 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
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199648_3_73b_Genzyme_Corporation_Power_of_Attorney_9051542_signed.PDF	7FF77A6DE7119AA1EF93085A4FE0B762C40370480E43753A4FABFC812FC7337EF08212CAB14E6BFB9CCA254A6F1CCCB0D882CFD0272BC28E1D8DEE950FCD7B06

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If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application

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APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
12/661,553	**09/11/2023 03:32:28 PM ET**	**0800-0045.01**

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Compositions and methods to prevent AAV vector aggregation

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PATENT CENTER #	62771702	AUTHORIZED BY	David Lee
CUSTOMER #	37509	FILING DATE	03/19/2010
CORRESPONDENCE ADDRESS	-	FIRST NAMED INVENTOR	John Fraser Wright

Payment Information

PAYMENT METHOD	PAYMENT TRANSACTION ID	PAYMENT AUTHORIZED BY
DA / 502778	E20239AF33157037	Kerri Leary

FEE CODE	DESCRIPTION	ITEM PRICE($)	QUANTITY	ITEM TOTAL($)
1814	STATUTORY DISCLAIMER, INCLUDING TERMINAL DISCLAIMER	170.00	1	170.00
			TOTAL AMOUNT:	**$170.00**

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PTO/SB/96 (11-18)
Approved for use through XX/XX/XXXX. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

STATEMENT UNDER 37 CFR 3.73(b)

Applicant/Patent Owner: Genzyme Corporation

Application No./Patent No.: 9,051,542 Filed/Issue Date: 06/09/2015

Titled: COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION

Genzyme Corporation, a corporation

(Name of Assignee) (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.

states that it is:

1. ☒ the assignee of the entire right, title, and interest in;

2. ☐ an assignee of less than the entire right, title, and interest in (The extent (by percentage) of its ownership interest is _____ %); or

3. ☐ the assignee of an undivided interest in the entirety of (a complete assignment from one of the joint inventors was made)

the patent application/patent identified above, by virtue of either:

A. ☐ An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel_____, Frame_____, or a copy* is attached.

OR

B. ☒ A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as follows:

1. From: John Fraser Wright and Guang Qu To: Avigen Inc

The document was recorded in the United States Patent and Trademark Office at Reel 039960, Frame 0383, or a copy* is attached.

2. From: Avigen Inc To: Genzyme Corporation

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3. From: _____ To: _____

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☐ Additional documents in the chain of title are listed on a supplemental sheet(s).

☒ *As required by 37 CFR 3.73(b)(1)(i), if a copy/copies is/are attached, the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11.

[NOTE: A separate copy (*i.e.*, a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP 302.]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

/David M. Lee/ September 11, 2023

Signature Date

David M. Lee 78,607

Printed or Typed Name Title or Registration Number

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
12/661,553	03/19/2010	John Fraser Wright	0800-0045.01

CONFIRMATION NO. 4726

POA ACCEPTANCE LETTER

37509
DECHERT LLP
Three Byrant Park
1095 Avenue of the Americas
New York, NY 10036-6797

OC0000000061744123

Date Mailed: 09/12/2023

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 06/15/2023.

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/sltorres/

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CONFIRMATION NO. 4726

37509
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New York, NY 10036-6797

POA ACCEPTANCE LETTER

OC000000062308734

Date Mailed: 09/26/2023

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/jfgerrety/

Application Number	Application/Control No.	Applicant(s)/Patent under Reexamination	
* 12/661,553 *	12/661,553	Wright et al.	
	Examiner	**Art Unit**	
	SINGH, SATYENDRA K	1657	

Document Code - DISQ	**Internal Document - DO NOT MAIL**

TERMINAL DISCLAIMER	☐ APPROVED	☑ DISAPPROVED
Date Filed: **11 September 2023**	**This patent is subject to a Terminal Disclaimer**	

Approved/Disapproved by:

/PAMELA A YOUNG/

Technology Center: OPLC

Telephone: (571)272-3622

• The applicant spelling is incorrect:
The applicant cited on the TD should be cited exactly as cited on the ADS form, in its entirety.

Note: Please correct and submit TD. No fee required.

Application Number	Application/Control No.	Applicant(s)/Patent under Reexamination	
* 12/661,553 *	12/661,553	Wright et al.	
	Examiner	**Art Unit**	
	SINGH, SATYENDRA K	1657	

Document Code - DISQ	**Internal Document - DO NOT MAIL**

TERMINAL DISCLAIMER	☑ APPROVED	☐ DISAPPROVED
Date Filed: **12 October 2023**	**This patent is subject to a Terminal Disclaimer**	

Approved/Disapproved by:

/PAMELA A YOUNG/

Technology Center: OPLC

Telephone: (571)272-3622

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ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
12/661,553	**10/12/2023 02:26:41 PM ET**	**0800-0045.01**

Title of Invention

Compositions and methods to prevent AAV vector aggregation

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	9051542
CONFIRMATION #	4726	FILED BY	Kerri Leary
PATENT CENTER #	62983119	FILING DATE	03/19/2010
CUSTOMER #	37509	FIRST NAMED INVENTOR	John Fraser Wright
CORRESPONDENCE ADDRESS	-	AUTHORIZED BY	David Lee

Documents

TOTAL DOCUMENTS: 1

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
199648_Terminal_Disclaimer_sb0026a_9051542_signed_10_12_2023.pdf	2	Terminal Disclaimer Filed	102 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
199648_Terminal_Disclaimer_sb0026a_9051542_signed_10_12_2023.pdf	730EBF1A68C3757C87B4B05AA1388F0A9BBF865D728F87B0F440C9ADFBDD744EF6C0AE3284908BB57BD3E3B11389E5E247463954715E0805CC7EA34EC0B1DD7F

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National Stage of an International Application under 35 U.S.C. 371

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PTO/SB/26a (02-14)
Approved for use through 05/31/2024. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

TERMINAL DISCLAIMER IN A PATENT OR PROCEEDING IN VIEW OF ANOTHER PATENT

Docket Number (Optional)

Application/Control Number: 12/661,553
Filing Date: 03/19/2010
First Named Inventor: John Fraser Wright
Title: COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION
Patent No.: 9,051,542

The patentee, Genzyme Corporation, owner of 100 percent interest in the instant patent hereby disclaims, except as provided below, the terminal part of the statutory term of the instant patent which would extend beyond the expiration date of the full statutory term of patent No. 7,704,721 (the "**reference** patent"), as the term of said **reference** patent is presently shortened by any terminal disclaimer. The patentee hereby agrees that the instant patent shall be enforceable only for and during such period that the instant patent and the **reference** patent are commonly owned. This agreement runs with the instant patent and is binding upon the grantee, its successors or assigns.

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1. ☑ The current ownership was established by the filing of a statement under 37 CFR 3.73 during prosecution of the application that issued as the instant patent.

2. ☐ The instant patent was issued from an application filed on or after September 16, 2012, and the current patent owner was the applicant under 37 CFR 1.46.

3. ☐ A statement under 37 CFR 3.73 is attached herewith. Form PTO/SB/96 or PTO/AIA/96, as appropriate, may be used.

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2. ☑ The undersigned is an attorney or agent of record. Reg. No. 78607

/David M. Lee/	October 12, 2023
Signature	Date
David M. Lee	617-728-7100
Typed or printed name	Telephone number

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