UTILITY PATENT APPLICATION TRANSMITTAL

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

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First Inventor	Wright et al.
Title	COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION
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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* 30]
   Both the claims and abstract must start on a new page
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   (for a continuation/divisional with Box 18 completed)
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Name (Print/Type)	Roberta L. Robins	Registration No. (Attorney/Agent)	33,208

0800-0045
PATENT

UTILITY PATENT APPLICATION

COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION

Inventor: John Fraser Wright

Guang Qu

Entity: Small

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

COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims 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, which 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* (ΔRh / Δt) over the same time interval is also determined (not shown). Analysis of ΔRh / Δt 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 method of preventing aggregation of virions in a preparation of virions, comprising adding one or more excipients to the preparation of virions to achieve an ionic strength of at least about 200 mM.

2. The method of claim 1, wherein the virions are AAV virions.

3. The method of claim 1, further comprising treating said preparation of virions with a nuclease.

4. The method of claim 3, wherein the nuclease is Benzonase®.

5. The method of claim 1, wherein one or more of the excipients comprises a multivalent ion.

6. The method of claim 5, wherein the multivalent ion is citrate.

7. The method of claim 1, wherein the osmolarity of the preparations of virions after addition of the one or more excipients is no greater than about 280mOsm.

8. The method of claim 1, wherein, after addition of the one or more excipients, the average particle radius (*Rh*) of the virions in the preparation of virions is less than about 20nm as measured by dynamic light scattering.

9. The method of claim 1, wherein, after addition of the one or more excipients, recovery of the virions is at least about 90% following filtration of the preparations of virions through a 0.22μm filter.

10. 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.

11. The composition of claim 10, wherein the purified virus particles are AAV virus particles.

12. The composition of claim 10, wherein one of the one or more multivalent ions is citrate.

13. The composition of claim 10, further comprising Pluronic® F68.

14. The composition of claim 13, wherein the Pluronic® F68 is present at 0.001%.

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

16. The composition of claim 10, wherein the average particle radius (*Rh*) of the purified virus particles is less than about 20nm as measured by dynamic light scattering.

17. The composition of claim 10, wherein recovery of the purified virus particles is at least about 90% following filtration of the composition of virions through a 0.22μm filter.

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

19. The method of claim 18, 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.

20. The method of claim 18, 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.

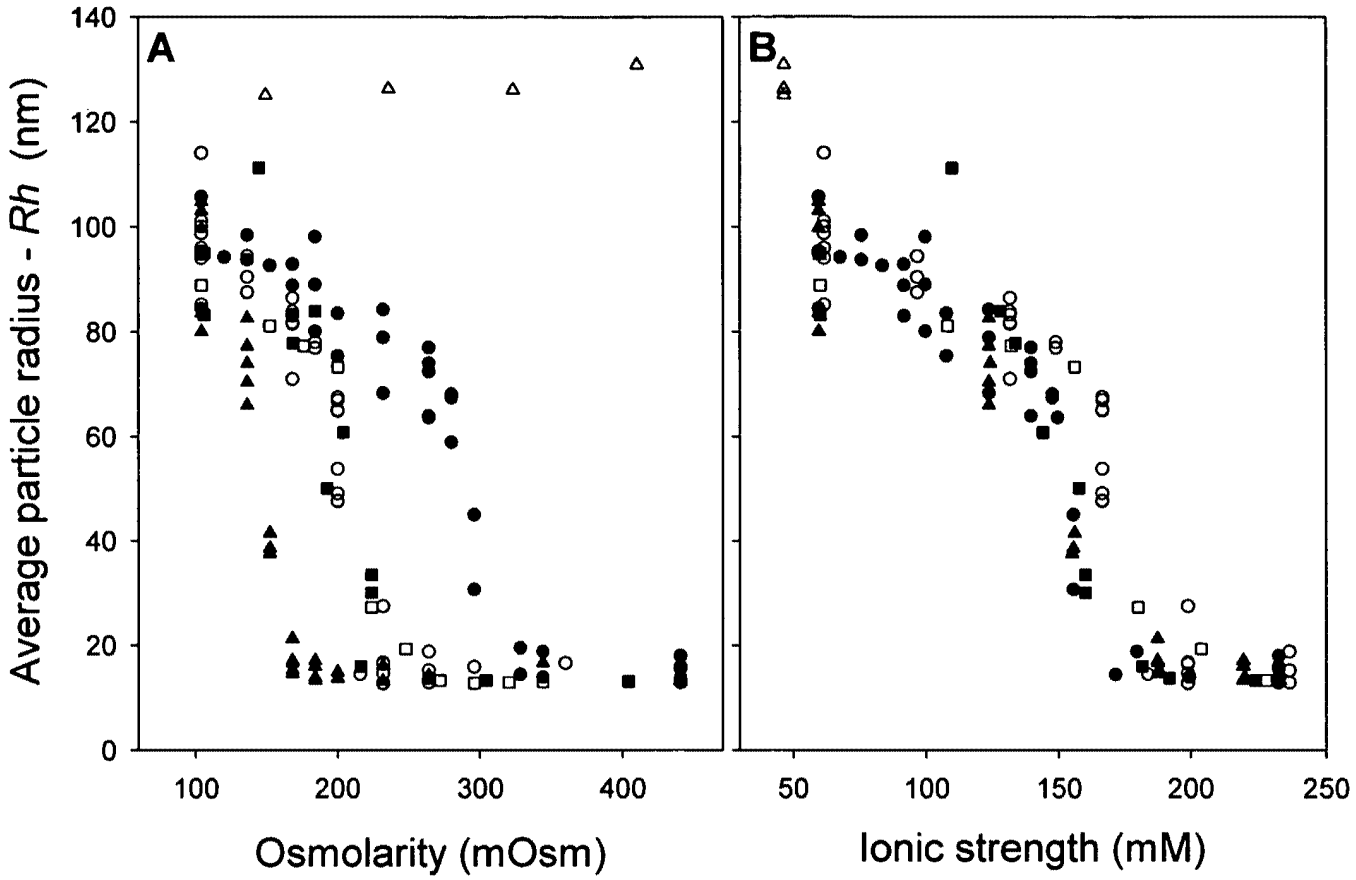
A
B
Average particle radius - Rh (nm)
0
20
40
60
80
100
120
140
100
200
300
400
Osmolarity (mOsm)
50
100
150
200
250
Ionic strength (mM)

FIGURE 1

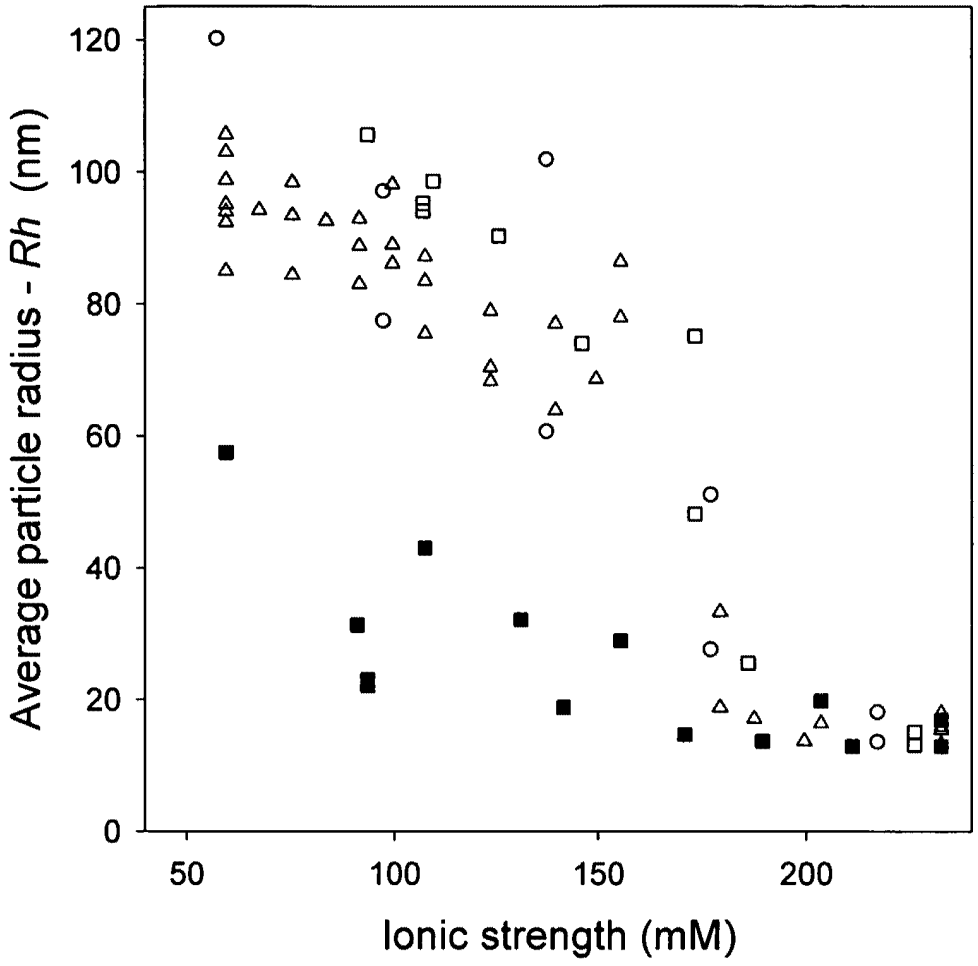
Average particle radius - Rh (nm)
120
100
80
60
40
20
0
50
100
150
200
Ionic strength (mM)

FIGURE 2

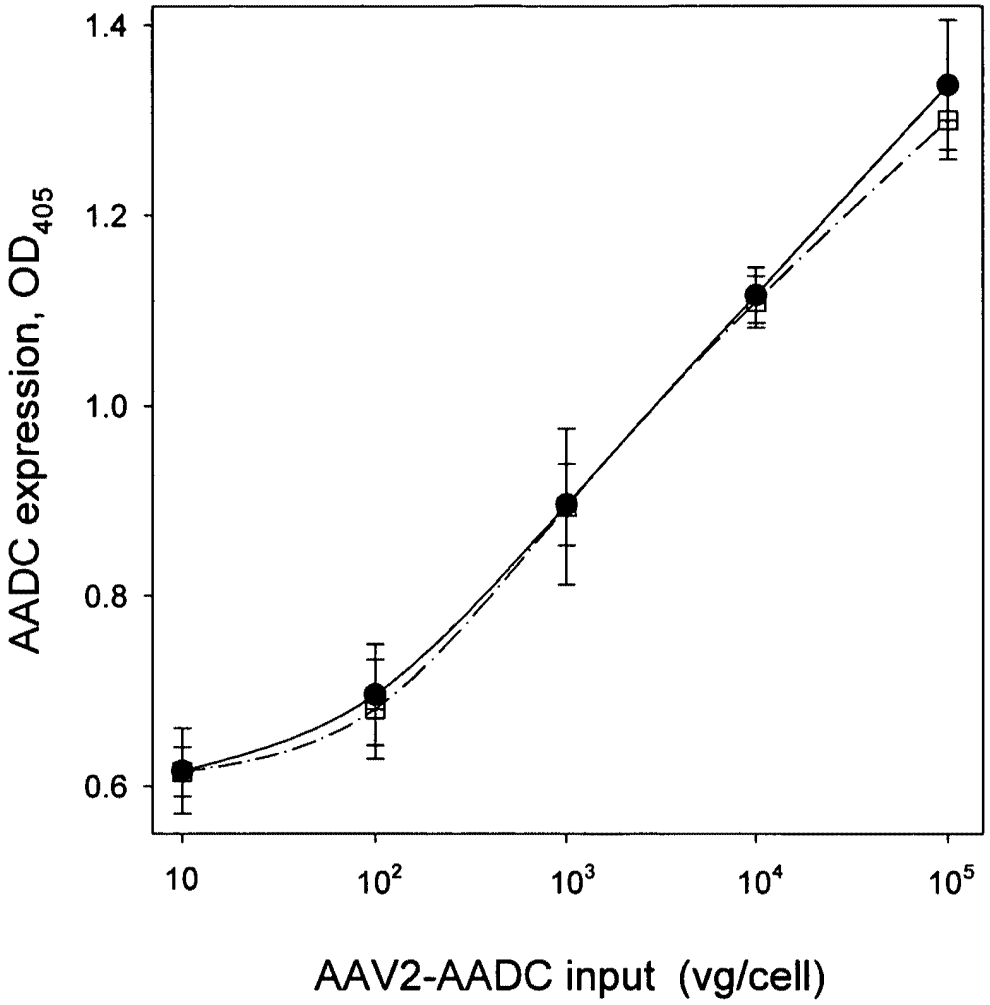
AADC expression, OD405
1.4
1.2
1.0
0.8
0.6
10
10^2
10^3
10^4
10^5
AAV2-AADC input (vg/cell)

FIGURE 3

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
Request for Early Publication?::	No
Request for Non-Publication?::	No
Suggested Drawing Figure::	
Total Drawings Sheets::	3
Small Entity?::	Yes
Latin Name::	
Variety denomination name::	
Petition included?::	No
Petition Type::	
Licensed US Govt. Agency::	
Contract or Grant Numbers::	
Secrecy Order in Parent Appl.?::	No

Applicant Information

Applicant Authority Type::	Inventor
Primary Citizenship Country::	CA
Status::	Full
Given Name::	John
Middle Name::	Fraser
Family Name::	Wright
Name Suffix::	
City of Residence::	Princeton
State or Province of Residence::	New York
Country of Residence::	US
Street of mailing address::	68 River Birch Circle
City of mailing address::	Princeton
State of mailing address::	New York
Country of mailing address::	US
Zip Code of mailing address::	08540
Applicant Authority Type::	Inventor
Primary Citizenship Country::	CN
Status::	Full
Given Name::	Quang
Middle Name::	
Family Name::	Qu
Name Suffix::	
City of Residence::	Alameda
State or Province of Residence::	California
Country of Residence::	US
Street of mailing address::	1103 Regent Street, #D
City of mailing address::	Alameda
State of mailing address::	California
Country of mailing address::	US

Zip Code of mailing address:: 94501

CORRESPONDENCE INFORMATION

Correspondence Customer Number:: 31048

Name:: Roberta L. Robins

Street of mailing address:: 1731 Embarcadero Road

City of mailing address:: Palo Alto

State of mailing address:: CA

Country of mailing address:: U.S.

Zip Code of mailing address:: 94303

Phone Number:: (650) 493-3400

Fax Number:: (650) 493-3440

E-Mail address:: rlr@robinslaw.com

DOMESTIC PRIORITY INFORMATION

Application::	Continuity Type::	Parent Application::	Parent Filing Date::
This Application	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::

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PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 11141996

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))	20 minus 20 =	* —	X =		OR	X =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	3 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))			N/A			N/A	
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	150		TOTAL	

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 ($)	SMALL ENTITY ADDITIONAL FEE ($)	OR	OTHER THAN SMALL ENTITY RATE ($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE ($)
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))					N/A		OR	N/A	
					TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

AMENDMENT B

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)	OR	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))					N/A		OR	N/A	
					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.

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.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

IFW

PTO/SB/21 (09-04)

TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Total Number of Pages in This Submission	5

Application Number	11/141,996
Filing Date	June 1, 2005
First Named Inventor	John Fraser Wright
Art Unit	Unassigned
Examiner Name	Unassigned
Attorney Docket Number	0800-0045

ENCLOSURES *(Check all that apply)*

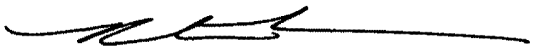
- ☐ Fee Transmittal Form
  - ☐ Fee Attached
- ☐ Amendment/Reply
  - ☐ After Final
  - ☐ Affidavits/declaration(s)
- ☐ Extension of Time Request
- ☐ Express Abandonment Request
- ☐ Information Disclosure Statement
- ☐ Certified Copy of Priority Document(s)
- ☐ Reply to Missing Parts/ Incomplete Application
  - ☐ 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):**

Supplemental ADS (3 pages)
Return Receipt Postcard (1 page)

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

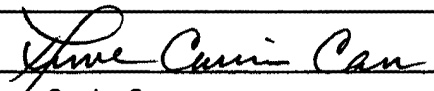
A supplemental ADS is submitted to correct the spelling of the second inventor's name.

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	Robins & Pasternak LLP		
Signature			
Printed name	Roberta L. Robins		
Date	6/16/05	Reg. No.	33,208

CERTIFICATE OF TRANSMISSION/MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on the date shown below.

Signature			
Typed or printed name	Anne Currier Carr	Date	6/16/05

Application Data Sheet

Application Information

Application Number::	11141996
Filing Date::	06/01/05
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
Request for Early Publication?::	No
Request for Non-Publication?::	No
Suggested Drawing Figure::	
Total Drawings Sheets::	3
Small Entity?::	Yes
Latin Name::	
Variety denomination name::	
Petition included?::	No
Petition Type::	
Licensed US Govt. Agency::	
Contract or Grant Numbers::	
Secrecy Order in Parent Appl.?::	No

Applicant Information

Applicant Authority Type::	Inventor
Primary Citizenship Country::	CA
Status::	Full
Given Name::	John
Middle Name::	Fraser
Family Name::	Wright
Name Suffix::	
City of Residence::	Princeton
State or Province of Residence::	New York
Country of Residence::	US
Street of mailing address::	68 River Birch Circle
City of mailing address::	Princeton
State of mailing address::	New York
Country of mailing address::	US
Zip Code of mailing address::	08540
Applicant Authority Type::	Inventor
Primary Citizenship Country::	CN
Status::	Full
Given Name::	Guang
Middle Name::	
Family Name::	Qu
Name Suffix::	
City of Residence::	Alameda
State or Province of Residence::	California
Country of Residence::	US
Street of mailing address::	1103 Regent Street, #D
City of mailing address::	Alameda
State of mailing address::	California
Country of mailing address::	US

Zip Code of mailing address:: 94501

CORRESPONDENCE INFORMATION

Correspondence Customer Number:: 31048

Name:: Roberta L. Robins

Street of mailing address:: 1731 Embarcadero Road

City of mailing address:: Palo Alto

State of mailing address:: CA

Country of mailing address:: U.S.

Zip Code of mailing address:: 94303

Phone Number:: (650) 493-3400

Fax Number:: (650) 493-3440

E-Mail address:: rlr@robinslaw.com

DOMESTIC PRIORITY INFORMATION

Application::	Continuity Type::	Parent Application::	Parent Filing Date::
This Application	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::

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	ATTORNEY DOCKET NUMBER
11/141,996	06/01/2005	John Fraser Wright	0800-0045

CONFIRMATION NO. 5399

31048
ROBINS & PASTERNAK LLP
1731 EMBARCADERO ROAD
SUITE 230
PALO ALTO, CA 94303

FORMALITIES LETTER

OC000000016467878

Date Mailed: 07/07/2005

NOTICE TO FILE MISSING PARTS OF NONPROVISIONAL APPLICATION

FILED UNDER 37 CFR 1.53(b)

Filing Date Granted

Items Required To Avoid Abandonment:

An application number and filing date have been accorded to this application. The item(s) indicated below, however, are missing. Applicant is given **TWO MONTHS** from the date of this Notice within which to file all required items and pay any fees required below to avoid abandonment. 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 statutory basic filing fee is missing.
  Applicant must submit $ **150** *to complete the basic filing fee for a small entity.*
- The oath or declaration is missing. *A properly signed oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date, is required.*
  Note: If a petition under 37 CFR 1.47 is being filed, an oath or declaration in compliance with 37 CFR 1.63 signed by all available joint inventors, or if no inventor is available by a party with sufficient proprietary interest, is required.
- To avoid abandonment, a late filing fee or oath or declaration surcharge as set forth in 37 CFR 1.16(f) of $65 for a small entity in compliance with 37 CFR 1.27, must be submitted with the missing items identified in this letter.

The applicant needs to satisfy supplemental fees problems indicated below.

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

SUMMARY OF FEES DUE:

Total additional fee(s) required for this application is **$565** for a Small Entity

- **$150** Statutory basic filing fee.
- **$65** Late oath or declaration Surcharge.

- The application search fee has not been paid. Applicant must submit **$250** to complete the search fee.
- The application examination fee has not been paid. Applicant must submit **$100** to complete the

examination fee for a small entity in compliance with 37 CFR 1.27

Replies should be mailed to: Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

A copy of this notice MUST be returned with the reply.

Office of Initial Patent Examination (703) 308-1202

PART 3 - OFFICE COPY

OIPE 1659 NOV 0 4 2005 PATENT & TRADEMARK OFFICE

TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

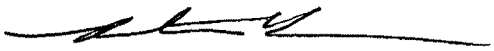
Total Number of Pages in This Submission	20	Application Number	11/141,996
		Filing Date	June 1, 2005
		First Named Inventor	JOHN FRASER WRIGHT et al.
		Art Unit	1646
		Examiner Name	Unassigned
		Attorney Docket Number	0800-0045

ENCLOSURES *(Check all that apply)*

- ☒ **Fee Transmittal Form (duplicate)**
- ☒ **Fee Attached ($790 check)**
- ☐ Amendment/Reply
- ☐ After Final
- ☒ Declaration(s) (2 pages)
- ☒ **Ext of Time Request (duplicate)**
- ☐ 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 (1 page)
- ☐ 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):
  - Supplemental ADS (3 pages)
  - Statement Under 37 CFR 3.73(b) (1 page)
  - COPIES of 2 assigments in chain (4 pages)
  - COPY of Notice of Missing Parts (2 pages)
  - Return Receipt Postcard (1 page)

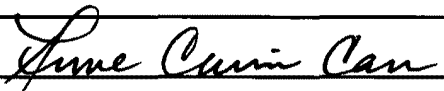
Remarks The Commissioner is authorized to charge any additional fees to Deposit Account 18-1648.

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	Robins & Pasternak LLP		
Signature			
Printed name	Roberta L. Robins		
Date	11/2/05	Reg. No.	33,208

CERTIFICATE OF TRANSMISSION/MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on the date shown below.

Signature			
Typed or printed name	Anne Currier Carr	Date	11/2/2005

PTO/SB/17 (12-04)

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

FEE TRANSMITTAL
For FY 2005

☒ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) **790**

Complete if Known	
Application Number	11/141,996
Filing Date	June 1, 2005
First Named Inventor	JOHN FRASER WRIGHT ET AL.
Examiner Name	Unassigned
Art Unit	1646
Attorney Docket No.	0800-0045

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	300	150	500	250	200	100	**500**
Design	200	100	100	50	130	65	
Plant	200	100	300	150	160	80	
Reissue	300	150	500	250	600	300	
Provisional	200	100	0	0	0	0	

2. EXCESS CLAIM FEES

Fee Description	Fee ($)	Small Entity Fee ($)
Each claim over 20 or, for Reissues, each claim over 20 and more than in the original patent	50	25
Each independent claim over 3 or, for Reissues, each independent claim more than in the original patent	200	100
Multiple dependent claims	360	180

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

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

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

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

Multiple Dependent Claims Fee ($)	Fee Paid ($)
	0

3. APPLICATION SIZE FEE

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).

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: Surcharge for Late Oath or Declaration	**65**
Other: Petition to Extend Two Months (Small Entity)	**225**

SUBMITTED BY

Signature	[signature]	Registration No. (Attorney/Agent) 33,208	Telephone (650) 493-3400
Name (Print/Type)	Roberta L. Robins		Date 11/2/05

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	ATTORNEY DOCKET NUMBER
11/141,996	06/01/2005	John Fraser Wright	0800-0045

CONFIRMATION NO. 5399

FORMALITIES LETTER

OC000000016467878

31048
ROBINS & PASTERNAK LLP
1731 EMBARCADERO ROAD
SUITE 230
PALO ALTO, CA 94303

Date Mailed: 07/07/2005

NOTICE TO FILE MISSING PARTS OF NONPROVISIONAL APPLICATION

FILED UNDER 37 CFR 1.53(b)

Filing Date Granted

Items Required To Avoid Abandonment:

An application number and filing date have been accorded to this application. The item(s) indicated below, however, are missing. Applicant is given **TWO MONTHS** from the date of this Notice within which to file all required items and pay any fees required below to avoid abandonment. 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 statutory basic filing fee is missing.
  Applicant must submit $ **150** *to complete the basic filing fee for a small entity.*
- The oath or declaration is missing. *A properly signed oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date, is required.*
  Note: If a petition under 37 CFR 1.47 is being filed, an oath or declaration in compliance with 37 CFR 1.63 signed by all available joint inventors, or if no inventor is available by a party with sufficient proprietary interest, is required.
- To avoid abandonment, a late filing fee or oath or declaration surcharge as set forth in 37 CFR 1.16(f) of $65 for a small entity in compliance with 37 CFR 1.27, must be submitted with the missing items identified in this letter.

The applicant needs to satisfy supplemental fees problems indicated below.

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

SUMMARY OF FEES DUE:

11/07/2005 MAHMED1 00000028 11141996

01 FC:2011	150.00 OP
02 FC:2111	250.00 OP
03 FC:2311	100.00 OP
04 FC:2051	65.00 OP

Total additional fee(s) required for this application is **$565** for a Small Entity

- **$150** Statutory basic filing fee.
- **$65** Late oath or declaration Surcharge.

- The application search fee has not been paid. Applicant must submit **$250** to complete the search fee.
- The application examination fee has not been paid. Applicant must submit **$100** to complete the

examination fee for a small entity in compliance with 37 CFR 1.27

Replies should be mailed to: Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

A copy of this notice MUST be returned with the reply.

Office of Initial Patent Examination (703) 308-1202
PART 2 - COPY TO BE RETURNED WITH RESPONSE

Application Data Sheet

Application Information

Application Number::	11141996
Filing Date::	06/01/05
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
Request for Early Publication?::	No
Request for Non-Publication?::	No
Suggested Drawing Figure::	
Total Drawings Sheets::	3
Small Entity?::	Yes
Latin Name::	
Variety denomination name::	
Petition included?::	No
Petition Type::	
Licensed US Govt. Agency::	
Contract or Grant Numbers::	
Secrecy Order in Parent Appl.?::	No

Applicant Information

Applicant Authority Type::	Inventor
Primary Citizenship Country::	Canada
Status::	Full Capacity
Given Name::	John
Middle Name::	Fraser
Family Name::	Wright
Name Suffix::	
City of Residence::	Princeton
State or Province of Residence::	New Jersey
Country of Residence::	US
Street of mailing address::	68 River Birch Circle
City of mailing address::	Princeton
State of mailing address::	New Jersey
Country of mailing address::	US
Zip Code of mailing address::	08540

Applicant Authority Type::	Inventor
Primary Citizenship Country::	US
Status::	Full Capacity
Given Name::	Guang
Middle Name::	
Family Name::	Qu
Name Suffix::	
City of Residence::	Alameda
State or Province of Residence::	California
Country of Residence::	US
Street of mailing address::	1103 Regent Street, #D
City of mailing address::	Alameda
State of mailing address::	California

Country of mailing address:: US

Zip Code of mailing address:: 94501

CORRESPONDENCE INFORMATION

Correspondence Customer Number:: 31048

Name:: Roberta L. Robins

Street of mailing address:: 1731 Embarcadero Road

City of mailing address:: Palo Alto

State of mailing address:: CA

Country of mailing address:: U.S.

Zip Code of mailing address:: 94303

Phone Number:: (650) 493-3400

Fax Number:: (650) 493-3440

E-Mail address:: rlr@robinslaw.com

DOMESTIC PRIORITY INFORMATION

Application::	Continuity Type::	Parent Application::	Parent Filing Date::
This Application	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:: Avigen, Inc.

Street of mailing address:: 1301 Harbor Bay Parkway

City of mailing address:: Alameda

State of mailing address:: CA

Country of mailing address:: US

Zip Code of mailing address:: 94502

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

Signature: _____ Citizen of: Canada

Inventor two: Guang Qu Date:

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.

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.

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.

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

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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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: _____

_____
John Fraser Wright

Date: 9/27/2005

_____
Guang Qu

PTO/SB/22 (12-04)

PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a) FY 2005 *(Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).)*	Docket Number (Optional) 0800-0045
Application Number 11/141,996	Filed June 1, 2005
For COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION	
Art Unit 1648	Examiner Unassigned

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))	$120	$60	$
☒ Two months (37 CFR 1.17(a)(2))	$450	$225	$ 225
☐ Three months (37 CFR 1.17(a)(3))	$1020	$510	$
☐ Four months (37 CFR 1.17(a)(4))	$1590	$795	$
☐ Five months (37 CFR 1.17(a)(5))	$2160	$1080	$

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

☒ A check **including** the amount of the fee is enclosed.

☐ Payment by credit card. Form PTO-2038 is attached.

☐ 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. I have enclosed a duplicate copy of this sheet.

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	Date
	11/2/05
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 One forms are submitted **in duplicate**.

11/07/2005 MAHMED1 00000028 11141996

05 FC:2252 225.00 OP

PTO/SB/22 (12-04)

PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a) FY 2005 *(Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).)*	Docket Number (Optional) 0800-0045
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☐ Four months (37 CFR 1.17(a)(4))	$1590	$795	$
☐ Five months (37 CFR 1.17(a)(5))	$2160	$1080	$

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

☒ A check **including** the amount of the fee is enclosed.

☐ Payment by credit card. Form PTO-2038 is attached.

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

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Signature	Date
	11/2/05
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 One forms are submitted **in duplicate**.

11/07/2005 MAHMED1 00000028 11141996

05 FC:2252 225.00 OP

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.
11/141,996	06/01/2005	John Fraser Wright	0800-0045	5399

31048 7590 01/23/2006

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

EXAMINER	
SINGH, SATYENDRA K	
ART UNIT	**PAPER NUMBER**
1651	

DATE MAILED: 01/23/2006

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

Office Action Summary	Application No.	Applicant(s)	
	11/141,996	WRIGHT ET AL.	
	Examiner	Art Unit	
	Satyendra K. Singh	1651	

-- 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 *01 June 2005*.

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

3)☐ 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

4)☒ Claim(s) *1-20* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☐ Claim(s) _____ is/are rejected.

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

8)☒ Claim(s) *1-20* are subject to restriction and/or election requirement.

Application Papers

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

10)☐ 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).

11)☐ 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

12)☐ 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-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Election/Restrictions

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

I. Claims 1-9, drawn to **a method of preventing aggregation of virions,** comprising adding one or more excipient to the preparation of virions to achieve an ionic strength of at least about 200 mM, classified in class 424, subclass 233.1 and others.

II. Claims 10-17, drawn 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, classified in class 435, subclass 235.1 and others.

III. Claims 18-20, 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.

2. Inventions of groups I and III are patentably distinct from each other because they recite different and distinct method steps, which lead to different and distinct end products/results. Invention of group III is directed to a process of preventing aggregation of virions in a preparation of virions by treating the said preparation with Benzonase, whereas the invention of group I does not require such a treatment step in the process as claimed. On the other hand, the invention of group I requires the limitation of adding one or more excipient to the preparation of virions to achieve an ionic strength of at least about 200 mM, whereas, the process of group III does not.

3. Inventions of group II and groups (I and III) are distinct from one another because the inventions of group I and III are directed towards distinct processes, whereas the

invention of group II is directed to a composition for the storage of purified virus particles, and requires the limitation of having a pH buffer unlike the inventions of group I and III which are distinct processes of preventing aggregation of virions (as discussed supra).

4. The inventions listed above are independent and distinct from one another as they have acquired a separate status in the art and require independent searches, particularly with regard to the literature searches. Clearly, a reference that would anticipate one of the above groups would not necessarily anticipate or even make obvious any of the others.

An undue burden would ensue from the examination of multiple methods, which have distinct steps and end points. Burden 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, scope of enablement, and double patenting issues.

Because these inventions are distinct for the reasons given above and the literature search required for one Group is not required for the other group, restriction for examination purposes as indicated is proper.

Applicant is advised that the reply to this requirement to be complete must include an election of the invention to be examined even though the requirement be traversed (37 CFR 1.143).

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).

5. The examiner has required restriction between **product and process claims**. Where applicant elects claims directed to the product, and a product claim is subsequently found allowable, withdrawn process claims that depend from or otherwise include all the limitations of the allowable product claim will be rejoined in accordance with the provisions of M.P.E.P. § 821.04. **Process claims that depend from or otherwise include all the limitations of the patentable product** will be entered as a matter of right if the amendment is presented prior to final rejection or allowance, whichever is earlier. Amendments submitted after final rejection are governed by 37 C.F.R. 1.116; amendments submitted after allowance are governed by 37 C.F.R. 1.312.

In the event of rejoinder, the requirement for restriction between the product claims and the rejoined process claims will be withdrawn, and the rejoined process claims will be fully examined for patentability in accordance with 37 C.F.R. 1.104. Thus, to be allowable, the rejoined claims must meet all criteria for patentability including the requirements of 35U.S.C. §§101, 102, 103, and 112. Until an elected product claim is found allowable, an otherwise proper restriction requirement between product claims and process claims may be maintained. Withdrawn process claims that are not commensurate in scope with an allowed product claim will not be rejoined. See "Guidance on Treatment of Product and Process Claims in light of *In re Ochiai*, *In re Brouwer* and 35 U.S.C. § 103(b)," 1184 O.G. 86 (March 26, 1996). Additionally, in order

to maintain the right to rejoinder in accordance with the above policy, Applicant is advised that the process claims should be amended during prosecution either to maintain dependency on the product claims or to otherwise include the limitations of the product claims. **Failure to do so may result in a loss of the right to rejoinder**.

Further, note that the protection against double patenting rejections of 35 U.S.C. 121 does not apply where the restriction requirement is withdrawn by the examiner before the patent issues. See M.P.E.P. § 804.01.

6. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Satyendra K. 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, Michael G. Wityshyn can be reached on 571-272-0926. 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).

Satyendra K. Singh
Patent Examiner
Art Unit 1651
Phone: 571-272-8790

SANDRA E. SAUCIER
PRIMARY EXAMINER

Index of Claims

Application/Control No.	Applicant(s)/Patent under Reexamination
11/141,996	WRIGHT ET AL.
Examiner	**Art Unit**
Satyendra K. Singh	1651

√	Rejected	–	(Through numeral) Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

Claim		Date								
Final	Original	1/17/06								
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PTO/SB/21 (09-04)

OIPE FEB 03 2006 PATENT & TRADEMARK OFFICE

TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Total Number of Pages in This Submission: 6

Application Number	11/141,996
Filing Date	June 1, 2005
First Named Inventor	JOHN FRASER WRIGHT et al.
Art Unit	1651
Examiner Name	Satyendra K. Singh
Attorney Docket Number	0800-0045

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
  - Statement (2 pages)
  - PTO/SB/08B (2 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):
  - Copies of all Cited References (pages not counted)
  - Return Receipt Postcard (1 page)

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	1/31/06	Reg. No.	33,208

CERTIFICATE OF TRANSMISSION/MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on the date shown below.

Signature			
Typed or printed name	Anne Currier Carr	Date	Jan. 31, 2006

Attorney Docket No. 0800-0045
PATENT

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to the Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on January 31, 2006

By: Anne Currier Carr
Anne Currier Carr

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

JOHN FRASER WRIGHT et al.		Examiner:	Satyendra K. Singh
Serial No.:	11/141,996	Art Unit:	1651
Filed:	June 1, 2005	Confirmation No.:	5399
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.

[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.

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: 1/31/06

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

PTO/SB/08B (08-03)
Approved for use through 06/30/2006. OMB 0651-0031
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

Substitute for form 1449B/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(use as many sheets as necessary)

Sheet 1 of 2

Complete if Known	
Application Number	11/141,996
Filing Date	June 1, 2005
First Named Inventor	JOHN FRASER WRIGHT et al.
Art Unit	1651
Examiner Name	Satyendra K. Singh
Attorney Docket Number	0800-0045

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[2]
	CA	Braun, A. 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," (1997) Pharm. Res. 14(10):1472-8.	
	CB	Chen, B. et al., "Strategies to suppress aggregation of recombinant keratinocyte growth factor during liquid formulation development," (1994) J. Pharm. Sci. 83(12):1657-1661.	
	CC	Chenuaud, P. et al., "Autoimmune anemia in macaques following erythropoietin gene therapy," (2004) Blood 103(9):3303-4.	
	CD	Croyle, M. A. et al., "Development of formulations that enhance physical stability of viral vectors for gene therapy," (2001) Gene Therapy 8(17):1281-90.	
	CE	Flotte, T. R., "Immune responses to recombinant adeno-associated virus vectors: putting preclinical findings into perspective," (2004) Human Gene Ther. 15(7):716-7.	
	CF	Gao, G. et al., "Erythropoietin gene therapy leads to autoimmune anemia in macaques," (2004) Blood 103(9):3300-2.	
	CG	Huang, J. et al. "AdAAV support High-titer Production of rAAV but not Stable," (2000) Mol. Therapy 1:S286.	
	CH	Meijer, et al., "Targeting of drugs to the liver," (1995) Sem. Liver Dis. 15(3):202-56.	
	CI	Shire, S. J. et al., "Challenges in the development of high protein concentration formulations," (2004) J. Pharm. Sci. 93(6):1390-402.	
	CJ	Wang, W., "Instability, stabilization, and formulation of liquid protein pharmaceuticals," (1999) Int. J. Pharm. 185(2):129-188.	
	CK	Won, C. M. et al., "Stabilizers against heat-induced aggregation of RPR 114849, an acidic fibroblast growth factor (aFGF)," (1998) Int. J. Pharm. 167:25-36.	

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] Applicant's unique citation designation number (optional). [2] Applicant is to place a check mark here if English language Translation is attached.

PTO/SB/08B (08-03)
Approved for use through 06/30/2006. 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 contains a valid OMB control number

Substitute for form 1449B/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(use as many sheets as necessary)

Sheet 2 of 2

Complete if Known	
Application Number	11/141,996
Filing Date	June 1, 2005
First Named Inventor	JOHN FRASER WRIGHT et al.
Art Unit	1651
Examiner Name	Satyendra K. Singh
Attorney Docket Number	0800-0045

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[2]
	CL	Wright, J. F. et al., "Recombinant adeno-associated virus: formulation challenges and strategies for a gene therapy vector," (2003) Curr. Opin. Drug Disc. Dev. 6(2):174-8.	
	CM	Xie, Q. et al., "Large-scale production, purification and crystallization of wild-type adeno-associated virus-2," (2004) J. Virol. Methods 122(1):17-27.	
	CN	Xie, Q. et al., "The atomic structure of adeno-associated virus (AAV-2), a vector for human gene therapy," (2002) Proc. Natl. Acad. Sci. U.S.A. 99(16):10405-10.	
	CO	Zhen, Z. et al., "Infectious Titer Assay for Adeno-Associated Virus Vectors with Sensitivity Sufficient to Detect Single Infectious Events," (2004) Human Gene Ther. 15:709-15.	

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] Applicant's unique citation designation number (optional). [2] Applicant is to place a check mark here if English language Translation is attached.

Atty Dkt No: 0800-0045
PATENT

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, P. O. Box 1450, Alexandria, VA 22313-1450 on:

2/17/06
Date

Denise M. Vaillancourt

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:

JOHN FRASER WRIGHT et al.

Confirmation No.: 5399

Application No.: 11/141,996

Art Unit: 1651

Filing Date: June 1, 2005

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 January 23, 2006, with an initial response date of February 23, 2006. Accordingly, this response is timely filed.

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

Group I, claims, 1-9, drawn to a method of preventing aggregation of virions comprising adding one or more excipient to the preparation of virions to achieve an ionic strength of at least about 200 mM;

Group II, claims, 10-17, drawn to a composition for the storage of purified virus particles, comprising purified virus particles; a pH buffer; and exicipients comprising one or more multivalent ions; wherein the ionic strength of the composition is greater thann about 200 mM; and

Group III, claims 18-20, drawn to a method of preventing aggregation of virions in a preparation of virions, comprising treating said preparation of virions with Benzonase.

In response to the restriction requirement, 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: 2/17/06

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

PLUS Search Results for S/N 11141996, Searched April 04, 2006

The Patent Linguistics Utility System (PLUS) is a USPTO automated search system for U.S. Patents from 1971 to the present. PLUS is a query-by-example search system which produces a list of patents that are most closely related linguistically to the application searched. This search was prepared by the staff of the Scientific and Technical Information Center, SIRA.

6475753
6265636
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     (FILE 'HOME' ENTERED AT 11:20:14 ON 11 MAY 2006)

     FILE 'CAPLUS' ENTERED AT 11:20:22 ON 11 MAY 2006
                E WRIGHT JOHN FRASER/AU 25
L1             3 S (E3)
                E QU GUANG/AU 25
L2            15 S (E3)

     FILE 'BIOSIS' ENTERED AT 11:26:37 ON 11 MAY 2006
L3             0 S L1 AND L2

     FILE 'CAPLUS, BIOSIS, MEDLINE' ENTERED AT 11:27:31 ON 11 MAY 2006
L4             4 S L1
L5            25 S L2
L6             3 S L4 AND L5
L7           418 S VIRION? (L) AAV
L8        834371 S AAV OR ADENO?
L9           418 S L7 AND L8
L10       468404 S AGGREGAT? OR (LOSS (L) TITER)
L11            2 S L9 AND L10
L12           35 S AAV AND AGGREGAT?
L13           23 DUP REM L12 (12 DUPLICATES REMOVED)
L14            4 S AAV AND (CITRATE OR MULTIVALEN? (L) ION?)
L15            3 DUP REM L14 (1 DUPLICATE REMOVED)

     FILE 'STNGUIDE' ENTERED AT 11:42:22 ON 11 MAY 2006

     FILE 'BIOSIS, MEDLINE, CAPLUS' ENTERED AT 11:43:40 ON 11 MAY 2006
L16         2864 S (AAV OR ADENO?) AND (BENZONASE OR NUCLEASE)
L17           13 S L16 AND CITRATE
L18           13 DUP REM L17 (0 DUPLICATES REMOVED)
L19            1 S AAV AND BENZONASE AND (CITRATE OR CITRIC?)
L20            3 S AAV AND (LIGHT (L) SCATTER?)
L21            2 DUP REM L20 (1 DUPLICATE REMOVED)
L22           27 S VIRION? AND RH
L23            0 S L22 AND AAV
L24           16 DUP REM L22 (11 DUPLICATES REMOVED)
L25          762 S VIRION? AND (PARTICLE (L) RADI? OR HYDRODYNAM? (L) RADI?)
L26            9 S L25 AND AAV
L27            4 DUP REM L26 (5 DUPLICATES REMOVED)

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COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                   135.27     309.23

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)          SINCE FILE      TOTAL
                                                       ENTRY    SESSION
CA SUBSCRIBER PRICE                                   -11.25     -39.75

 SESSION WILL BE HELD FOR 60 MINUTES
STN INTERNATIONAL SESSION SUSPENDED AT 12:01:10 ON 11 MAY 2006
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     (FILE 'HOME' ENTERED AT 09:40:15 ON 13 MAY 2006)

     FILE 'CA' ENTERED AT 09:41:13 ON 13 MAY 2006
L1        178119 S AAV OR ADENO-ASSOCIATED? OR ADENO?
L2        149600 S (MULTIVALEN? (3A) ION?) OR (CITRAT? OR CITRIC?)
L3         24911 S BENZONASE OR NUCLEASE
L4            14 S L1 AND L2 AND L3

     FILE 'BIOSIS, MEDLINE, CAPLUS' ENTERED AT 09:45:37 ON 13 MAY 2006
L5            16 S L4
L6            16 DUP REM L5 (0 DUPLICATES REMOVED)
L7           484 S (VIRUS? OR VIRION? OR VIRAL) (L) PARTICL? (P) (AGGREGAT? OR P
L8             7 S L7 AND (CITRATE OR CITRIC?)
L9             2 S (VIRUS? OR VIRAL OR VIRION?) AND  AAV AND (CITRATE OR CITRIC?
L10        29046 S CAPSID (P) (VIRUS? OR VIRAL OR VIRION?)
L11           26 S L2 AND L10
L12            2 S L11 AND (AGGREGAT? OR PRECIPITAT?)
L13           19 DUP REM L11 (7 DUPLICATES REMOVED)

     FILE 'STNGUIDE' ENTERED AT 10:08:21 ON 13 MAY 2006

=> log y
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                     0.90     224.91

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)          SINCE FILE      TOTAL
                                                       ENTRY    SESSION
CA SUBSCRIBER PRICE                                     0.00     -21.75

STN INTERNATIONAL LOGOFF AT 10:17:20 ON 13 MAY 2006
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EAST Search History

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
S1	1	("6593123").PN.	USPAT; USOCR	OR	OFF	2006/05/15 09:02
S2	1	("6566118").PN.	USPAT; USOCR	OR	OFF	2006/05/10 14:51
S3	886	aav and aggregat$5 and virion$2	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/05/10 14:52
S4	863	aav and aggregat$5 and virion$2 and prevent$5	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/05/10 14:53
S5	885	aav and aggregat$5 and virion$2 and (avoid$5 or prevent$5 or reduc$5)	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/05/10 14:53
S6	207	aav and aggregat$5 and virion$2 and (avoid$5 or prevent$5 or reduc$5).clm.	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/05/11 12:26
S7	207	aav and aggregat$5 and virion$2 and (avoid$5 or prevent$5 or reduc$5).clm.	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/05/11 12:26
S8	17	S7 and benzonase	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/05/11 13:53
S9	97	S7 and (citrate or citric)	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/05/11 12:27
S10	9	S7 and benzonase and (citrate or citric)	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/05/11 14:38
S11	14	(prevent$5 or reduc$5) near5 aggregat$5 near5 virion	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/05/11 14:40
S12	2	S11 and (citrate or citric)	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/05/11 14:42

EAST Search History

S13	61	aggregat$5 same virion same (prevent$5 or reduc$5)	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/05/11 14:44
S14	3	S13 same aav	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/05/11 14:45
S15	1	S13 same (benzonase or nuclease)	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/05/11 14:46
S16	10	aav near5 aggregat$5	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/05/12 17:56
S17	342	aav and (prevent$5 near5 aggregat$5)	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/05/12 17:56
S18	197	aav and (prevent$5 near5 aggregat$5) and (nuclease or benzonase)	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/05/12 17:57
S19	163	aav and (prevent$5 near5 aggregat$5) and (nuclease or benzonase) and (multivalen$5 or citrate or citric)	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/05/12 17:57
S20	168	aav and (prevent$5 near5 aggregat$5) and (nuclease or benzonase) and (multivalen$5 or citrate or citric or oxalate or oxalic)	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/05/12 17:58
S21	32	aav and (prevent$5 near5 aggregat$5) and (nuclease or benzonase) and (multivalen$5 or citrate or citric or oxalate or oxalic) and virion	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/05/12 17:59
S22	28	aav and (prevent$5 near5 aggregat$5) and (nuclease or benzonase) and (multivalen$5 or citrate or citric or oxalate or oxalic) and virion and filter	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/05/12 17:59
S23	2378	(citrate or citric) and (aav or virion)	USPAT	OR	ON	2006/05/13 12:03
S24	1	(citrate or citric) near5 (aav or virion)	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/05/13 11:43

EAST Search History

S25	124	(citrate or citric) same (aav or virion)	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/05/13 11:49
S26	1	(citrate or citric) same (aav or virion) same nuclease	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/05/13 11:44
S27	0	(citrate or citric) same (aav or virion) same benzonase	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/05/13 11:44
S28	26	S25 and (aav near5 purif$8)	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/05/13 11:49
S29	10	(citrate or citric) and (aav or virion) and benzonase	USPAT	OR	ON	2006/05/13 12:04
S30	1	("6194191").PN.	USPAT; USOCR	OR	OFF	2006/05/13 15:58
S31	1	S30 and aav and benzonase	USPAT	OR	ON	2006/05/13 15:59
S32	1	S30 and aav and benzonase and aggregat$5	USPAT	OR	ON	2006/05/13 16:00
S33	1	S30 and aav and benzonase and aggregat$5 and filter and recovery	USPAT	OR	ON	2006/05/13 16:00
S34	1	S30 and aav and benzonase and aggregat$5 and filter and recovery and nuclease	USPAT	OR	ON	2006/05/13 16:00
S35	1	S30 and aav and benzonase and aggregat$5 and filter and recovery and nuclease and (citrate or citric)	USPAT	OR	ON	2006/05/13 16:13
S36	1	aav same nm same (particle adj radius)	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/05/15 09:03
S37	295	aav same nm	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/05/15 09:03
S38	3	S37 same radius	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/05/15 09:03

UNITED STATES PATENT AND TRADEMARK OFFICE

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/141,996	06/01/2005	John Fraser Wright	0800-0045	5399

31048 7590 05/18/2006

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

EXAMINER	
SINGH, SATYENDRA K	

ART UNIT	PAPER NUMBER
1651	

DATE MAILED: 05/18/2006

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

Office Action Summary	Application No.	Applicant(s)	
	11/141,996	WRIGHT ET AL.	
	Examiner	Art Unit	
	Satyendra K. Singh	1651	

-- 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 *23 February 2006*.

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

3)☐ 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

4)☒ Claim(s) *1-9* is/are pending in the application.

4a) Of the above claim(s) *10-20* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-9* is/are rejected.

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

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

Application Papers

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

10)☒ The drawing(s) filed on *01 June 2005* 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).

11)☐ 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

12)☐ 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-1449 or PTO/SB/08) Paper No(s)/Mail Date *2/3/2006*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Applicant's response filed with the office on Feb 23rd 2006 is duly acknowledged.

Claims 10-20 (the inventions of groups II and III) are withdrawn from further consideration.

Claims 1-9 (the invention of group I) are examined on their merits in this office action.

Election/Restrictions

Applicant's election without traverse of group I (claims 1-9) in the reply filed on Feb 23rd 2006 is acknowledged.

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.

Claim 4 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 4 contains one or more of the trademark/trade name(s) viz: **Benzonase** (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.

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 1-5 are rejected under 35 U.S.C. 102(b) as being anticipated by Zolotukhin et al (US 6,146,874; [A]).

Claims are directed to a method of preventing aggregation of virions in a **preparation of virions**, comprising adding one or more **excipients** to the preparation of virions to achieve an **ionic strength** of at least about 200 mM (claim 1); wherein the virions are **AAV** (adeno-associated virus) virions (claim 2); wherein the method further comprises treating said preparation of virions with a **nuclease** (claim 3); wherein the nuclease is **Benzonase** (a registered trademark; claim 4); and wherein the one or more of the excipients comprises a **multivalent ion** (claim 5).

Zolotukhin et al [A] teach a preparation of virions (AAV virions; see abstract, summary of the invention, column 9-10, in particular) comprising excipients having ionic strength of at least about 200mM and having multivalent ions (such as salt and buffers; see Zolotukhin et al, column 10, 1 M NaCl-PBS-MgCl2 and KCl buffer containing multivalent ions such as magnesium and phosphate; see also applicant's exemplification of multivalent ions at page 20, 2nd paragraph, in particular). Zolotukhin et al teach a preparation of virions wherein a nuclease (such as Benzonase; see Zolotukhin et al, column 10, 1st paragraph, in particular) has been added to the cell lysate and incubated for nuclease treatment for 30 min at 37 ^{0}C. Since the invention as

claimed (i.e. the method of preventing aggregation of virions in a preparation of virions) requires only the step of "adding" one or more of the exicipients to the virion preparation in order to prevent the aggregation of virions of the composition (which is taught by Zolotukhin et al), the referenced invention anticipates the claimed process.

2. Claims 1, 3-5 are rejected under 35 U.S.C. 102(b) as being anticipated by Zhang et al (US 6,689,600 B1; [B]).

Claims are directed to a method of preventing aggregation of virions in a **preparation of virions**, comprising adding one or more **excipients** to the preparation of virions to achieve an **ionic strength** of at least about 200 mM (claim 1); wherein the method further comprises treating said preparation of virions with a **nuclease** (claim 3); wherein the nuclease is **Benzonase** (a registered trademark; claim 4); and wherein the one or more of the excipients comprises a **multivalent ion** (claim 5).

Zhang et al [B] teach a method of preventing aggregation of virions (such as adenovirus AdCMVp53; see Zhang et al, abstract, summary of the invention, columns 32, 41-42, in particular) comprising adding one or more excipients to the preparation of virions to achieve an ionic strength of at least 200 mM (such as salts/multivalent ions including NaCl and $MgCl_2$ in a buffer or Dullbecco's phosphate buffered saline; see Zhang et al, column 41, section on *Clarification and filtration* and *Concentration and diafiltration*, in particular), wherein the concentrated/diafiltered virion preparation is treated with a nuclease (such as Benzonase; see Zhang et al, column 42, 2nd paragraph, in particular) to reduce agglomeration (i.e. aggregation) induced by the presence of nucleic acids (see Zhang et al, column 32, section on *Removing nucleic acid contaminants*, in particular).

3. Claims 1-5 and 7 are rejected under 35 U.S.C. 102(b) as being anticipated by Atkinson et al (US 6,566,118 B1; [C]).

Claims are directed to a method of preventing aggregation of virions in a **preparation of virions**, comprising adding one or more **excipients** to the preparation of virions to achieve an **ionic strength** of at least about 200 mM (claim 1); wherein the virions are **AAV** (adeno-associated virus) virions (claim 2); wherein the method further comprises treating said preparation of virions with a **nuclease** (claim 3); wherein the nuclease is **Benzonase** (a registered trademark; claim 4); wherein the one or more of the excipients comprises a **multivalent ion** (claim 5); and wherein the **osmolarity** of the preparations of virions after addition of the one or more excipients is no greater than about 280 mOsm (claim 7).

Atkinson et al [C] teach a method for generating high titer helper-free preparations of released recombinant AAV vectors (see Atkinson et al, abstract, summary of the invention, Figures 13, 19, 32, 36 and37, columns 41-42, examples 7 and 8, in particular) wherein the preparation of AAV virions is added with one or more excipients to achieve an ionic strength of at least about 200 mM (see column 55, last paragraph, in particular) containing multivalent ions (such as NaCl, $MgCl_2$, EDTA), wherein the virion preparation has been treated with a nuclease (such as Benzonase; see Atkinson et al, example 7 and 8, in particular), and wherein the osmolarity of the preparations of virions after additions of the one or more excipients is no greater than about 280 mOsm (see Atkinson et al, example 19, claims 6, 12, 14 and 20, in particular). Since the invention as claimed (i.e. the method of preventing aggregation of virions in a preparation of virions) requires only the step of "adding" one or more of the exicipients to the virion preparations in order to prevent the aggregation of virions of the composition (which is taught by Atkinson et al), the referenced invention anticipates the claimed process.

4. Claims 1-5 are rejected under 35 U.S.C. 102(b) as being anticipated by Drittanti et al [U].

Claims are directed to a method of preventing aggregation of virions in a **preparation of virions**, comprising adding one or more **excipients** to the preparation of virions to achieve an **ionic strength** of at least about 200 mM (claim 1); wherein the virions are **AAV** (adeno-associated virus) virions (claim 2); wherein the method further comprises treating said preparation of virions with a **nuclease** (claim 3); wherein the nuclease is **Benzonase** (a registered trademark; claim 4); and wherein the one or more of the excipients comprises a **multivalent ion** (claim 5).

Drittanti et al [U] teach preparation of high quality adeno-associated virus vectors (AAV virions; see abstract, *Materials & Methods*, in particular) comprising excipients having ionic strength of at least about 200mM and having multivalent ions (such as salt and buffers; see Drittanti et al, page 61, left column, uses lysis buffer containing a multivalent ion such as magnesium; see also applicant's exemplification of multivalent ions at page 20, 2nd paragraph, in particular). Drittanti et al teach a preparation of virions wherein a nuclease (such as Benzonase; see Drittanti et al, page 61, left column, 4th paragraph, in particular) has been added to the cell lysate containing virions and incubated for nuclease treatment for 30 min at 37 ^{0}C. Since the invention as claimed (i.e. the method of preventing aggregation of virions in a preparation of virions) requires only the step of "adding" one or more of the exicipients to the virion preparation in order to prevent the aggregation of virions of the composition (which is taught by Drittanti et al, see above), the referenced invention anticipates the claimed process.

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.

Claims 1-9 are rejected under 35 U.S.C. 103(a) as being unpatentable over Zolotukhin et al (US 6,146,874; [A]) or Atkinson et al (US 6,566,118 B1; [C]), or Drittanti et al [U] in view of Qu et al [V] and Chen et al (IDS).

Claims are directed to a method of preventing aggregation of virions in a **preparation of virions**, comprising adding one or more **excipients** to the preparation of virions to achieve an **ionic strength** of at least about 200 mM (claim 1); wherein the virions are **AAV** (adeno-associated virus) virions (claim 2); wherein the method further comprises treating said preparation of virions with a **nuclease** (claim 3); wherein the nuclease is **Benzonase** (a registered trademark; claim 4); wherein the one or more of the excipients comprises a **multivalent ion** (claim 5); wherein the multivalent ion is **citrate** (claim 6); wherein the **osmolarity** of the preparations of virions after addition of the one or more excipients is no greater than about 280 mOsm (claim 7); wherein, after addition of the one or more excipients, the **averge particle radius** (*Rh*) of the virions in

the preparation of virions is lees than about 20 nm as measured by dynamic light scattering (claim 8); and wherein, after addition of the one or more excipients, **recovery** of the virions is at least about 90% following filtration of the preparation of virions through a **0.22μm filter** (claim 9).

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

However, a method of preventing aggregation of virions comprising adding one or more excipients to the preparation of virions comprising a multivalent ion, **citrate**, is not explicitly disclosed by the inventions of Zolotukhin et al, Atkinson et al, or Drittanti et al.

Qu et al [V] disclose the problems associated with the concentration-induced aggregation of recombinant AAV virions, and highlight the role of ionic interactions (along with other types of interactions, presumably hydrophobic and other inter-particle interactions) in the preparation of concentrated stocks of vectors used for human gene therapy (see Qu et al, abstract, entire document). Qu et al demonstrate (using dynamic light scattering, size-exclusion chromatography, and by quantification of loss of titer following 0.2 μm filtration of the virions) that the aggregation was concentration dependent, and typically occurred when the concentrations of virions exceeded the range 0.5×10^{14} cp/ml for column purified preparations (see Qu et al, abstract). More importantly, Qu et al also demonstrate that changes in buffer pH values resulted in reversal of this aggregation phenomenon, and thus suggested that ionic bridges between charged amino acids (Glu, Asp, Lys, presumably of the viral capsid protein) on the surface of vector particles contribute to the inter-particle interactions, and work in concert with hydrophobic and other types of inter-particle interactions (as the virions

were found to be stable in a solution of 3M CsCl at neutral pH) to result in such concentration-dependent aggregation of the viral particles.

Chen et al (IDS) 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).

Therefore, it would have been obvious to a person of ordinary skill in the art at the time this invention was made to modify the process of preparation of virions as taught by Drittanti et al or Atkinson et al or Zolotukhin et al, such that the preparation of virions is added with one or more excipients such as multivalent ion (citrate salt) as explicitly suggested by the combined disclosures of Qu et al and Chen et al, in order to prevent the aggregation of virions by providing higher ionic strength preparations.

One of ordinary skill in the art would have been motivated to modify the virion preparations of Zolotukhin et al, or Atkinson et al, or Drittanti et al by adding sodium citrate to the preparation of virions because (1) Qu et al explicitly identify the problem of particle aggregation in standard preparations of AAV virions, and suggests the role of ionic interactions (among other type of interactions involved) in the concentration-induced aggregation of virions, and (2) Chen et al demonstrate that using multivalent

ions such as citrate salts can effectively reduce/suppress aggregation of fairly unstable proteins (such as rhKGF; see discussion above), thus providing a conceptual as well as practical basis for such modification of the preparations obtained by the invention of Zolotukhin et al, or Atkinson et al, or Drittanti et al.

The person of ordinary skill in the art would have had a reasonable expectation of success when adding citrate as a multivalent ion in order to prevent the aggregation of virions as obtained by the process taught by Zolotukhin et al, or Atkinson et al, or Drittanti et al, because Chen et al explicitly demonstrates the use of salts of citrate in the stabilization of highly unstable protein formulations, such as rhKGF. Since, the viral capsid (outer coat) proteins have been shown to undergo similar concentration-induced aggregation phenomenon (as disclosed/suggested by Qu et al; see discussion above), and since Qu et al suggest the role of ionic inter-particle interactions in such aggregation, one of ordinary skill in the art would have been motivated to adapt the strategies taught by Chen et al (i.e. incorporation of multivalent ion such as citrate), and would have had a reasonable expectation of success in avoiding such aggregation in the preparations of virions.

The limitations of claim 9 (wherein the recovery of the virions is at least about 90% after filtration through a 0.22 μm filter) would have been a matter of routine optimization for an artisan of ordinary skill in the art as evident by the fact that Drittanti et al (see, page 61 right column), Atkinson, et al (see column 54, 2nd paragraph, in particular), or Zolotukhin et al (see column 5-6, in particular) disclose the step of

filtration and recovery of the virions through 0.22 μm filter, and provide the basis for optimization of such method steps.

Similarly, the limitations of claim 8 (wherein the average particle radius of the virions is less than about 20 nm as measured by dynamic light scattering) would have been a matter of routine optimization for an artisan of ordinary skill in the art (as evident by the fact that Qu et al disclose the process of using dynamic light scattering as one of the techniques for measuring/assessing the size of AAV virion aggregates and thus quantifying the loss of virions following 0.2 μm filtration step; see Qu et al, abstract), and the skilled artisan recognizing the fact that such optimization are routine part of the process for development of such stable clinical formulations for gene therapy (as disclosed by the inventions of Zolotukhin et al, or Atkinson et al, or Drittanti et al).

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

Pertinent art not relied upon in the rejections

1. Wu et al. (US 6,689,600 B1), Formulation of adenovirus for gene therapy, abstract, columns 6, 12-14, 19-20, in particular).

2. Croyle et al. (US 6,399,385 B1), Method for rapid PEG-modification of viral vectors, compositions for enhanced gene transduction, compositions with enhanced physical stability, and uses therefor, abstract, summary of the invention, table 3, in particular).

3. Orlov et al. Macroscopic aggregation of Tobacco Mosaic Virus coat protein, Biochemistry (Moscow), 2001, 66(2): 154-162, especially abstract, Materials & Methods, page 157, in particular).

Conclusion

No claims are allowed.

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

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Michael Wityshyn can be reached on 571-272-0926. 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).

Satyendra K. Singh
Patent Examiner
Art Unit 1651
Ph: 571-272-8790

JEAN C. WITZ
PRIMARY EXAMINER

PTO/SB/08B (08-03)
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(use as many sheets as necessary)

Sheet 1 of 2

Complete If Known	
Application Number	11/141,996
Filing Date	June 1, 2005
First Named Inventor	JOHN FRASER WRIGHT et al.
Art Unit	1651
Examiner Name	Satyendra K. Singh
Attorney Docket Number	0800-0045

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 [2]
SS	CA	Braun, A. 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," (1997) Pharm. Res. 14(10):1472-8.	
	CB	Chen, B. et al., "Strategies to suppress aggregation of recombinant keratinocyte growth factor during liquid formulation development," (1994) J. Pharm. Sci. 83(12):1657-1661.	
	CC	Chenuaud, P. et al., "Autoimmune anemia in macaques following erythropoietin gene therapy," (2004) Blood 103(9):3303-4.	
	CD	Croyle, M. A. et al., "Development of formulations that enhance physical stability of viral vectors for gene therapy," (2001) Gene Therapy 8(17):1281-90.	
	CE	Flotte, T. R., "Immune responses to recombinant adeno-associated virus vectors: putting preclinical findings into perspective," (2004) Human Gene Ther. 15(7):716-7.	
	CF	Gao, G. et al., "Erythropoietin gene therapy leads to autoimmune anemia in macaques," (2004) Blood 103(9):3300-2.	
	CG	Huang, J. et al. "AdAAV support High-titer Production of rAAV but not Stable," (2000) Mol. Therapy 1:S286.	
	CH	Meijer, et al., "Targeting of drugs to the liver," (1995) Sem. Liver Dis. 15(3):202-56.	
	CI	Shire, S. J. et al., "Challenges in the development of high protein concentration formulations," (2004) J. Pharm. Sci. 93(6):1390-402.	
	CJ	Wang, W., "Instability, stabilization, and formulation of liquid protein pharmaceuticals," (1999) Int. J. Pharm. 185(2):129-188.	
SS	CK	Won, C. M. et al., "Stabilizers against heat-induced aggregation of RPR 114849, an acidic fibroblast growth factor (aFGF)," (1998) Int. J. Pharm. 167:25-36.	

Examiner Signature	/Satyendra Singh/	Date Considered	05/13/2006

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

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(use as many sheets as necessary)

Sheet 2 of 2

Complete if Known	
Application Number	11/141,996
Filing Date	June 1, 2005
First Named Inventor	JOHN FRASER WRIGHT et al.
Art Unit	1651
Examiner Name	Satyendra K. Singh
Attorney Docket Number	0800-0045

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[2]
SS	CL	Wright, J. F. et al., "Recombinant adeno-associated virus: formulation challenges and strategies for a gene therapy vector," (2003) Curr. Opin. Drug Disc. Dev. 6(2):174-8.	
	CM	Xie, Q. et al., "Large-scale production, purification and crystallization of wild-type adeno-associated virus-2," (2004) J. Virol. Methods 122(1):17-27.	
	CN	Xie, Q. et al., "The atomic structure of adeno-associated virus (AAV-2), a vector for human gene therapy," (2002) Proc. Natl. Acad. Sci. U.S.A. 99(16):10405-10.	
SS	CO	Zhen, Z. et al., "Infectious Titer Assay for Adeno-Associated Virus Vectors with Sensitivity Sufficient to Detect Single Infectious Events," (2004) Human Gene Ther. 15:709-15.	

Examiner Signature	/Satyendra Singh/	Date Considered	05/13/2006

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

Notice of References Cited	Application/Control No. 11/141,996	Applicant(s)/Patent Under Reexamination WRIGHT ET AL.	
	Examiner Satyendra K. Singh	Art Unit 1651	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
*	A	US-6,146,874	11-2000	Zolotukhin et al.	435/235.1
*	B	US-6,194,191	02-2001	Zhang et al.	435/235.1
*	C	US-6,566,118	05-2003	Atkinson et al.	435/239
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	E	US-			
	F	US-			
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	I	US-			
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FOREIGN PATENT DOCUMENTS

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	N					
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NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Drittanti L. et al. Optimised helper virus-free production of high quality adeno-associated virus vectors, The Journal of Gene Medicine, 2001, 3: 59-71. entire document.
	V	Qu G. et al. Evidence that ionic interactions are involved in concentration-induced aggregation of recombinant adeno-associated virus, Molecular Therapy, 2003, 7(5): S348, abstract No. 901.
	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.

Index of Claims

Application/Control No.	Applicant(s)/Patent under Reexamination
11/141,996	WRIGHT ET AL.
Examiner	**Art Unit**
Satyendra K. Singh	1651

√	Rejected	–	(Through numeral) Cancelled	N	Non-Elected	A	Appeal
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Claim		Date							
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Search Notes	Application/Control No.	Applicant(s)/Patent under Reexamination
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	Examiner	Art Unit
	Satyendra K. Singh	1651

SEARCHED			
Class	Subclass	Date	Examiner

INTERFERENCE SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES (INCLUDING SEARCH STRATEGY)		
	DATE	EXMR
EAST: USPAT, PG-PUB, USOCR, JPO, EPO, DERWENT ATTCHED SEPARATELY.	5/13/2006	SKS
STN: CA, CAPLUS, BIOSIS, MEDLINE ATTACHED SEPARATELY.	5/13/2006	SKS
INVENTOR SEARCH: PALM &STN	5/13/2006	SKS

Attorney Docket No. 0800-0045
PATENT

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

JOHN FRASER WRIGHT et al.		Examiner:	Satyendra K. Singh
Serial No.:	11/141,996	Art Unit:	1651
Filed:	June 1, 2005	Confirmation No.:	5399
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.

[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.

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: 5/15/06

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

PTO/SB/08B (08-03)
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(use as many sheets as necessary)

Sheet 1 of 1

Complete if Known	
Application Number	11/141,996
Filing Date	June 1, 2005
First Named Inventor	JOHN FRASER WRIGHT et al.
Art Unit	1651
Examiner Name	Satyendra K. Singh
Attorney Docket Number	0800-0045

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[2]
	CA	Adadevoh et al., "SHORT-TERM FIELD USE AND SHIPPING STABILITY STUDY OF A WILD TYPE AD5 ADENOVIRAL REFERENCE MATERIAL," BioProcessing, 1(3):62-9 (2002)	
	CB	High, K. A. 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)	
	CC	Qu, G. et al., "EVIDENCE THAT IONIC INTERACTIONS ARE INVOLVED IN CONCENTRATION-INDUCED AGGREGATION OF RECOMBINANT ADENO-ASSOCIATED VIRUS," Mol. 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.
[1] Applicant's unique citation designation number (optional). [2] Applicant is to place a check mark here if English language Translation is attached.

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, P. O. Box 1450, Alexandria, VA 22313-1450 on:

9/18/06
Date

Denise M. Vaillancourt

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:

JOHN FRASER WRIGHT et al.

Confirmation No.: 5399

Application No.: 11/141,996

Art Unit: 1651

Filing Date: June 1, 2005

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 18, 2006, with a shortened statutory period of three months for response. Accordingly, a one-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 method of preventing aggregation of recombinant adeno-associated virus (rAAV) virions in a purified preparation of rAAV virions, comprising:

providing a lysate comprising rAAV virions;

purifying rAAV virions from the lysate using ultracentrifugation and/or chromatography;

adding one or more excipients to the purified preparation of virions to achieve an ionic strength of at least about 200 mM.

2. (Canceled)

3. (Currently amended) The method of claim 1, further comprising treating said purified preparation of virions with a nuclease.

4. (Currently amended) The method of claim 3, wherein the nuclease is an endonuclease from *Serratia marcescens* (Benzonase®).

5. (Original) The method of claim 1, wherein one or more of the excipients comprises a multivalent ion.

6. (Original) The method of claim 5, wherein the multivalent ion is citrate.

7. (Original) The method of claim 1, wherein the osmolarity of the preparations of virions after addition of the one or more excipients is no greater than about 280mOsm.

8. (Original) The method of claim 1, wherein, after addition of the one or more excipients, the average particle radius (Rh) of the virions in the preparation of virions is less than about 20nm as measured by dynamic light scattering.

9. (Original) The method of claim 1, wherein, after addition of the one or more excipients, recovery of the virions is at least about 90% following filtration of the preparations of virions through a 0.22μm filter.

10-20. (Canceled)

21. (New) The method of claim 3, wherein rAAV virions are purified from the lysate using cesium chloride gradient ultracentrifugation.

22. (New) The method of claim 3, wherein rAAV virions are purified from the lysate using cation exchange chromatography.

23. (New) The method of claim 3, wherein rAAV virions are purified from the lysate using cation exchange chromatography and cesium chloride gradient ultracentrifugation.

24. (New) The method of claim 3, further comprising diafiltering the purified rAAV virions to achieve an ionic strength of at least about 200 mM.

II. REMARKS

Introductory Comments

Claims 1-9 were examined in the Office Action under reply and stand variously rejected under (1) 35 U.S.C. §112, second paragraph (claim 4); (2) 35 U.S.C. §102(b) (claims 1-5 and 7); and (3) 35 U.S.C. §103(a) (claims 1-9). 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 withdrawn claims 10-20 have been canceled. Claim 1 has been amended to incorporate the substance of canceled claim 2 and further recites that the method comprises preventing aggregation of rAAV virions in a purified preparation of rAAV virions. Method steps directed to providing a lysate comprising rAAV virions and purifying rAAV virions from the lysate using ultracentrifugation and/or chromatography have also been added to claim 1.

Claim 3 has been amended to track the language of amended claim 1 and claim 4 has been amended to add the generic synonym for Benzonase®. As shown in the appended excerpt from the Sigma-Aldrich catalogue, Benzonase® is an endonuclease from *Serratia marcescens*.

New claims 21-24 have been added and recite various purification steps.

Support for the foregoing amendments and new claims can be found throughout the specification at, e.g., page 7, second full paragraph; pages 15-16; and Examples 1 and 2.

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. §112, Second Paragraph

Claim 4 was rejected as indefinite based on the use of the trademark Benzonase®. As explained above, claim 4 has been amended to insert the synonym for Benzonase®. Thus, this basis for rejection has been overcome and withdrawal thereof is respectfully requested.

Rejections Under 35 U.S.C. §102

Claims 1-5 were rejected under 35 U.S.C. §102(b) as anticipated by U.S. Patent No. 6,146,874 to Zolotukhin et al. ("Zolotukhin"). The Office argues Zolotukhin teaches a preparation of rAAV virions with excipients having an ionic strength of at least about 200 mM and having multivalent ions. The Office further alleges Zolotukhin teaches that a nuclease is added to the cell lysate. 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.

In particular, Zolotukhin does not teach a method where the ionic strength of a **purified** preparation of rAAV virions is raised to at least about 200 mM. Rather, the passage at column 10, lines 45-48 referred to by the Examiner, describes adding excipients to a crude preparation of virions in order to purify the virions using iodixanol density gradient centrifugation. This crude preparation had not yet been subjected to purification steps as presently claimed. See, column 10, lines 1-6. Additionally, the Examiner is confusing molarity with ionic strength in the rejections. Even though the two concepts are expressed in the same units (e.g. mM), they are not the same thing. In particular, molarity is a measure of the number of gram-molecular weights of a compound present (dissolved) in one liter of solution. Ionic strength, on the other hand, is the measure of the average electrostatic interactions among ions in an electrolyte and is equal to one-half the sum of the terms obtained by multiplying the molar concentration of each ion by its valence squared. See, pages 24-25, bridging paragraph of applicants' specification. Moreover, as explained at columns 9-10, bridging paragraph of Zolotukhin, benzonase was added directly

to the cell lysate and not to a purified preparation of virions obtained from the lysate as presently claimed in claims 2, 3 and claims dependent thereon. 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.

Claims 1 and 3-5 were rejected under 35 U.S.C. §102(b) as anticipated by U.S. Patent No. 6,194,191 to Zhang et al. ("Zhang")[1]. Applicants note claim 2, reciting that the virions are rAAV virions, was not subject to this rejection. The claims have been amended to incorporate the subject matter of claim 2. Thus, this basis for rejection no longer applies and withdrawal thereof is respectfully requested.

Claims 1-5 and 7 were rejected under 35 U.S.C. §102(b) as anticipated by U.S. Patent No. 6,566,118 to Atkinson et al. ("Atkinson"). The Office alleges Atkinson teaches a preparation of AAV virions with an excipient to achieve an ionic strength of at least about 200 mM, where the preparation has been treated with a nuclease and wherein the osmolarity of the preparation is not greater than about 280 mOsm. However, Atkinson is not believed to anticipate the present claims.

As explained above, applicants' claims pertain to methods of preventing aggregation of rAAV virions that comprise adding one or more excipients to a **purified** preparation of rAAV virions in order to achieve an ionic strength of at least about 200 mM. As noted by the Office, Atkinson teaches a preparation of AAV virions at column 55, final paragraph wherein the AAV is concentrated and diafiltered into "TMEG + 100 mM NaCl." The composition of this buffer is taught at column 33, lines 50-52. However, the Office again appears to be confusing the concepts of molarity and ionic strength as Atkinson does not teach or suggest that the buffer has an ionic strength of at least 200 mM. Additionally, the osmolarity of Atkinson's preparation is adapted in a bioreactor, well before purification, in order to promote release of the virus into the cell culture. See, columns 41-42 of Atkinson. Further, with respect to claims 2, 3 and claims dependent thereon, Atkinson adds benzonase directly to an AAV lysate, not to a purified preparation. See, examples 7 and 8. Thus, Atkinson also fails to teach the subject invention. Withdrawal of this basis for rejection is respectfully requested.

[1] The Office Action indicates the rejection is over U.S. Patent No. 6,689,600. However, the PTO-892 Form cites U.S. Patent No. 6,194,191. Applicants assume the '191 patent was intended as the '600 patent is in the name of Wu et al. and the '191 patent is in the name of Zhang et al.

Finally, claims 1-5 were rejected under 35 U.S.C. §102(b) as anticipated by Drittanti et al, *J. Gene Med.* (2001) 3:59-71 ("Drittanti'). Drittanti is said to teach a preparation of AAV virions with excipients having an ionic strength of at least about 200 mM; the use of a lysis buffer containing a multivalent ion; and a cell lysate containing virions and incubated with a nuclease. However, as with the art cited above, Drittanti's procedure fails to include a step of raising the ionic strength of a **purified** preparation of rAAV virions. As explained at page 61, second column, second full paragraph of Drittanti, the purified fractions were dialyzed against a Ringer-lactate buffer. 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 at least 200 mM as claimed. Additionally, with respect to claims 2, 3 and claims dependent thereon, Drittanti adds benzonase directly to the lysate and not to a purified rAAV virion preparation. Thus, Drittanti also fails to anticipate the claimed invention and withdrawal of this basis for rejection is respectfully requested.

Rejections Under 35 U.S.C. §103(a)

Claims 1-9 were rejected under 35 U.S.C. §103(a) as unpatentable over Zolotukhin, Atkinson or Drittanti, in view of Qu et al., *Molec. Ther.* (2003) 5:S348, Abstract 901 ("Qu") and Chen et al., *J. Pharm. Sci.* (1994) 83:1657-1661 ("Chen"). Zolotukhin, Atkinson and Drittanti are applied as above. Qu is said to disclose the problems associated with rAAV virion aggregation and allegedly demonstrates that changes in buffer pH values result in reversal of aggregation. Qu is further characterized as suggesting that ionic bridges between charged amino acids on the surface of vector particles contribute to inter-particle interactions and work in concert with other types of inter-particle interactions to result in concentration-dependent aggregation of viral particles. Office Action, pages 8-9, bridging paragraph. 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 9. The Office concludes:

> [I]t would have been obvious to a person of ordinary skill in the art at the time this invention was made to modify the process of preparation of virions as taught

> by Drittanti et al or Atkinson et al or Zolotukhin et al, such that the preparation of virions is added with one or more excipients such as multivalent ion (citrate salt) as explicitly suggested by the combined disclosures of Qu et al and Chen et al, in order to prevent the aggregation of virions by providing higher ionic strength preparations.

Office Action, page 9. However, applicants do not agree with these assertions and respectfully traverse the Office's rejection and supporting remarks.

In order to render claims obvious, the burden is on the Office to establish a *prima facie* case of obviousness for which three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the references. Second, there must be a reasonable expectation of success. Finally, the prior art references must teach or suggest all the claim limitations. *In re Vaeck* , 20 USPQ2d 1438 (Fed. Cir. 1991). Applicants submit that the Office has failed to satisfy these criteria and therefore has not presented a *prima facie* case of obviousness.

In particular, all pending claims pertain to methods of preventing aggregation of rAAV virions in a **purified** preparation. The rAAV virions are purified from a lysate using ultracentrifugation and/or chromatography. One or more excipients are then added to achieve an ionic strength of at least about 200 mM. In certain embodiments, the purified preparation is treated with a nuclease. None of the cited art, either alone or in combination, teaches or suggests the method as claimed.

As explained above, in making the various rejections, the Examiner has mistakenly equated molarity with ionic strength. However, the two physical properties are very different. Molarity is a measure of the number of gram-molecular weights of a compound present (dissolved) in one liter of solution. Ionic strength is the measure of the average electrostatic interactions among ions in an electrolyte and is equal to one-half the sum of the terms obtained by multiplying the molar concentration of each ion by its valence squared. Keeping this in mind, none of Zolotukhin, Atkinson or Drittanti adds one or more excipients to a **purified** rAAV virion preparation to achieve an ionic strength as claimed. Additionally, none of the cited art adds a nuclease to an already purified preparation.

For example, the buffers used by Zolotukhin and referred to by the Examiner in the Office Action are provided as the first step in a discontinuous step gradient purification procedure. See, column 10, line 25 to column 11, line 4. There is absolutely no suggestion to provide such buffers in order to prevent aggregation. Thus, the Examiner appears to rely on Zolotukhin for an inherent showing that use of such a buffer during a purification procedure would inherently prevent aggregation. However, the unsubstantiated assumption that Zolotukhin's method may have the "inherent property" of preventing aggregation is of no import. 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). Moreover, Zolotukhin adds benzonase to a cell lysate (see, column 9, line 65 to column 10, line 3) not to a purified rAAV preparation.

Similarly, Atkinson fails to teach or suggest applicants' claimed method. There is no disclosure in Atkinson regarding adding excipients to a **purified** preparation of rAAV virions to achieve an ionic strength of at least 200 mM in order to prevent aggregation. Rather, as explained above, the passage referred to by the Examiner at column 55, final paragraph discloses diafiltering a preparation of AAV virions into a TMEG buffer + 100 mM NaCl, the components of which are described at column 33, lines 50-52. However, the Office again appears to be confusing the concepts of molarity and ionic strength as Atkinson does not teach or suggest that the buffer has an ionic strength of at least 200 mM. Further, the osmolarity of Atkinson's preparation, referred to by the Examiner, is adapted in the bioreactor, well before purification, in order to promote release of the virus into the cell culture. See, columns 41-42 of Atkinson. Again, assuming *arguendo* that this salt adjustment indeed results in reduced aggregation, this is not a proper basis for rejection as obviousness cannot be properly based on a purported inherent property. Finally, as with Zolotukhin, Atkinson adds benzonase directly to an AAV lysate, not to a purified preparation. See, examples 7 and 8.

Drittanti also adds benzonase directly to a lysate and not to a purified preparation of rAAV virions. Moreover, the multivalent ions referred to in the Office Action are present in a lysis buffer and are therefore clearly not applied to purified rAAV virions. There is absolutely no indication that the addition of multivalent ions in a lysis buffer results in reduced aggregation,

as claimed. Although Drittanti dialyses purified fractions against a lactated Ringer's buffer, as explained above, such a buffer does not have an ionic strength of at least 200 mM.

Thus, none of the primary references teaches or suggests the claimed invention. Qu and Chen fail to cure the defects of the primary references. In particular, Qu merely proposes a mechanism for AAV vector aggregation. The Examiner asserts Qu suggests that ion bridges between charged amino acids on the surface of vector particles contribute to inter-particle interactions. If anything, this might lead one of skill in the art to postulate that high concentrations of free amino acids could block vector particle interactions. However, as explained at page 9, lines 18-20 of the specification, 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 states that further work is needed in order to provide a viable purification procedure. At best, then, Qu could be considered an invitation to experiment and such is not a proper basis for an obviousness rejection.

Chen also does not make up for the failures of the primary references and Qu. 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. Moreover, Chen used sulfated polysaccharides in combination with citrate to prevent aggregation. There is no suggestion in Chen to use citrate alone, or to carry out a purification protocol as claimed in order to prevent aggregation of rAAV virions.

Applicants' process, on the other hand, provides a commercially viable method for producing high amounts of rAAV virions. As explained at pages 16-17 of the specification and in Table 2, vector recovery using applicants' claimed methods results in yields of more than 90%.

The Office Action has failed to identify the requisite teaching or motivation from the prior art to arrive at applicants' invention. Without the benefit of applicants' disclosure, there is

no motivation or suggestion to one of ordinary skill in the art to combine the cited references to render the methods claimed. Thus, the instant grounds of rejection are improper. Reconsideration and withdrawal of the §103 rejections 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: 9/18/06

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

ROBINS & PASTERNAK LLP
1731 Embarcadero Road, Suite 230
Palo Alto, CA 94303
Telephone: (650) 493-3400
Facsimile: (650) 493-3440

PTO/SB/22 (12-04)

PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a) FY 2005 *(Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).)*	Docket Number (Optional) 0800-0045
Application Number 11/141,996	Filed June 1, 2005
For COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION	
Art Unit 1651	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))	$120	$60	$ 120
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☐ Applicant claims small entity status. See 37 CFR 1.27.

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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	9/18/06 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 One forms are submitted.

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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	11/141,996
Filing Date	June 1, 2005
First Named Inventor	JOHN FRASER WRIGHT et al.
Art Unit	1651
Examiner Name	Satyendra K. Singh
Attorney Docket Number	0800-0045

ENCLOSURES *(Check all that apply)*

- ☐ **Fee Transmittal Form**
- ☒ **Fee Attached**
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  - ☐ After Final
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SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	Robins & Pasternak LLP		
Signature			
Printed name	Roberta L. Robins		
Date	9/18/06	Reg. No.	33,208

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PTO/SB/06 (12-04)
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PATENT APPLICATION FEE DETERMINATION RECORD
Substitute for Form PTO-875

Application or Docket Number: 11141996

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), (l), 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))	20 minus 20 =	* —	x =		OR	x =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	3 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))			N/A			N/A	
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	150		TOTAL	

APPLICATION AS AMENDED – PART II

9/26/06

AMENDMENT A	(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 ($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE ($)
9/26/06									
Total (37 CFR 1.16(i))	* 12	Minus	** 20	0	x 25 =		OR	x =	
Independent (37 CFR 1.16(h))	* 1	Minus	*** 3	0	x 100 =		OR	x =	
Application Size Fee (37 CFR 1.16(s))							OR		
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))					N/A		OR	N/A	
					TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

AMENDMENT B	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)	OR	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))					N/A		OR	N/A	
					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.

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.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

EAST Search History

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
S38	3	S37 same radius	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/05/15 09:03
S39	210	rAAV and (citrate or citric or citr$5) and (purif$5 or isolat$5 or preparat$6)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/11/29 17:38
S40	210	rAAV and (citrate or citric or citr$5) and (purif$5 or isolat$5 or preparat$6)".clm"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/11/29 17:38
S41	92	rAAV and (citrate or citric or citr$5) and (purif$5 or isolat$5 or preparat$6).clm.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/11/29 17:38
S42	20	rAAV and (citrate or citric or citr$5) and purif$5.clm.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/11/29 17:41
S43	0	rAAV same (citrate or citric or citr$5).clm.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/11/29 17:42
S44	0	(adenoassoc$8 or AAV or rAAV) same (citrate or citric or citr$5).clm.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/11/29 17:42
S45	40	(virion or virus or adenoassoc$8 or AAV or rAAV) same (citrate or citric or citr$5).clm.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/11/29 17:43
S46	3	S45 and (prevent$6 or reduc$6 or decreas$6 or eliminat$6) and (clump$6 or aggregat$6)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/11/29 17:44

EAST Search History

S47	19355	(process$6 or method or step) near10 (prevent$6 or reduc$6 or decreas$6 or eliminat$6 or avoid$6) near10 (aggregat$6 or clump$6 or precipitat$6 or agglutinat$6)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/11/29 17:49
S48	2	(process$6 or method or step) near10 (prevent$6 or reduc$6 or decreas$6 or eliminat$6 or avoid$6) near10 (aggregat$6 or clump$6 or precipitat$6 or agglutinat$6) near10 (aav or raav or adenoassoc$6)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/11/29 17:50

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/141,996	06/01/2005	John Fraser Wright	0800-0045	5399

31048 7590 12/07/2006

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

EXAMINER	
SINGH, SATYENDRA K	

ART UNIT	PAPER NUMBER
1657	

DATE MAILED: 12/07/2006

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

TH

Office Action Summary	Application No.	Applicant(s)	
	11/141,996	WRIGHT ET AL.	
	Examiner	Art Unit	
	Satyendra K. 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 *21 September 2006*.

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

3)☐ 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

4)☒ Claim(s) *1,3-9 and 21-24* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,3-9 and 21-24* is/are rejected.

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

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

Application Papers

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

10)☒ The drawing(s) filed on *01 June 2005* 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).

11)☐ 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

12)☐ 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 *5/18/06*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Applicant's response and amendments to the claims filed with the office on September 21st 2006 is duly acknowledged.

Claims 2 and 10-20 are canceled by applicant's amendments to the claims.

Claims 1-9 (group I) and newly added claims 21-24 are examined on their merits in this office action.

This is a **new ground of rejection** necessitated by applicant's amendments to the claims.

Claim Rejections - 35 USC § 112

The following is a quotation of the first 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.

Claims 1 and 3 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 recite the limitation **"purified"** preparation of rAAV virions, which is confusing and ambiguous. The broader claim 1 recites the method steps of "purifying" rAAV virions from the lysate using ultracentrifugation **and/or** chromatography, but does not provide as to what "degree of purification" is required by the limitation as claimed in instant invention. The instant disclosure uses various purification steps (see instant specification, page 21, example 1, in particular) to achieve various degree of purified vectors that are either further treated by nuclease or added with one or more excipients, but do not disclose an explicit definition of the term "purified" applicable for the

preparation of rAAV virions. Thus, to an artisan of ordinary skill, it would be unclear as to what degree or level of purification is required, and as to what elements or components of the crude viral lysate are being removed by the process step of purification (the extent/degree of purification, and the types of undesirable elements removed by such techniques will vary depending on the process being used; i.e. using ultracentrifugation **and/or** chromatography) and by the limitation as claimed (see interpretations of the term "purify" in prior art, "to make pure" or "to clear from material defilement or imperfection", or "to free from undesirable elements"; Merriam-Webster Online Dictionary, [U2]). Thus, the use of the term "purified" in context with the preparation of rAAV virions, as presented in the claims, renders the invention ambiguous to one of ordinary skill in the art, and is therefore deemed indefinite. Appropriate explanation/correction is required.

Since, claims 4-9 and 21-24 directly or indirectly depend from the broader claim 1 and 3, they are also 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.

For examination purposes, the instant claims are being interpreted by the examiner (for the term "purified"), as encompassing a rAAV virion preparation that has been obtained through the separation of the virions from the lysate using an ultracentrifugation and/or chromatographic step (irrespective of the degree/quality of the purification achieved).

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 and 5 (as currently amended) are rejected under 35 U.S.C. 102(b) as being anticipated by Zolotukhin et al (US 6,146,874; [A]) as evidenced by Merriam-Webster Online Dictionary [U2] and prior art [V2].

Claims are generally directed to a method of preventing aggregation of rAAV virions in a **purified** preparation of virions, comprising providing a lysate comprising rAAV virions; purifying rAAV virions from the lysate using ultracentrifugation **and/or** chromatography; adding one or more excipients to the purified preparation of virions to achieve an ionic strength of at least **about** 200 mM (amended claim 1); and wherein the one or more of the excipients comprises a multivalent ion (claim 5).

Zolotukhin et al [A] teach a preparation of virions (AAV virions; see abstract, summary of the invention, column 9-10, in particular) comprising excipients having ionic strength of at least **about** 200mM and having multivalent ions (such as salt and buffers; see Zolotukhin et al, column 10, 1 M NaCl-PBS-MgCl2 and KCl buffer containing multivalent ions such as magnesium and phosphate; see also applicant's exemplification of multivalent ions at page 20, 2nd paragraph, in particular). The limitations of purifying rAAV virions from the lysate using ultracentrifugation and/or chromatography is met as Zolotukhin et al teach the step of purifying the crude lysate through Iodixanol density gradient which uses ultracentrifugation step, and further purifying the rAAV virions obtained from the above Iodixanol gradient by using a chromatographic step, wherein the "purified" preparation of virions (for example, bound

to column such as Heparin-ligand affinity media; see Zolotukhin et al, the entire columns 10 and 11, in particular) was added with one or more excipients (such as PBS-MK with 1M NaCl for elution from the column; see Zolotukhin et al, column 10, lines 36-37, in particular).

The limitation of adding one or more excipients to the "purified" virion preparation of rAAV to achieve an ionic strength of at least about 200 mM is also met by the method of Zolotukhin et al because they teach such adjustment in the ionic strength of the purified vector preparation by using a final concentration and desalting step (using a Biomax 100K filter and Lactated Ringer's solution that has calculated ionic strength of 183.5 mM; see Zolotukhin et al, column 11, lines 49-54, in particular and prior art [V2], page 1, in particular) in order to achieve the **ionic strength** of at least **about** 200 mM (see the interpretation of the term "about" which to an artisan of ordinary skill in the art means "in the vicinity of"; see prior art Merriam-Webster Dictionary [U2], page 2, in particular).

2. Claims 1 and 5 are rejected under 35 U.S.C. 102(b) as being anticipated by Atkinson et al (US 6,566,118 B1; [C]) as evidenced by Merriam-Webster Online Dictionary [U2] and prior art [V2].

Claims are generally directed to a method of preventing aggregation of rAAV virions in a **purified** preparation of virions, comprising providing a lysate comprising rAAV virions; purifying rAAV virions from the lysate using ultracentrifugation **and/or** chromatography; adding one or more excipients to the purified preparation of virions to achieve an ionic strength of at least **about** 200 mM (amended claim 1); and wherein the one or more of the excipients comprises a multivalent ion (claim 5).

Atkinson et al [C] teach a method for generating high titer helper-free preparations of released recombinant AAV vectors (see Atkinson et al, abstract,

summary of the invention, Figures 13, 19, 32, 36 and 37, columns 41-42, examples 7, in particular) wherein the preparation of AAV virions is added with one or more excipients to achieve an ionic strength of at least **about** 200 mM (see column 55, last paragraph, in particular; also, see the interpretation of the term "about" which to an artisan of ordinary skill in the art means "in the vicinity of"; see prior art Merriam-Webster Dictionary [U2], page 2, in particular) containing multivalent ions (such as NaCl, **Mg**Cl_2, EDTA).

The limitations of purifying rAAV virions from the lysate using ultracentrifugation and/or chromatography is met by the method of Atkinson et al because they teach the method steps (see Atkinson et al, example 7, columns 53, 54 and 55, in particular), wherein the crude lysate is subjected to nuclease treatment and CsCl gradient runs, or subjected to anion and/or cation exchange column chromatography before being pooled, and finally concentrated and diafiltered using Modified Ringer's Balanced salt solution having 5% glycerol, which is further concentrated 10-fold (i.e. the step of adding one or more excipients) on a 300K molecular weight cut off membrane (see Atkinson et al, column 54, lines 38-49, in particular), and thus, meets the claimed limitation (see prior art [V2] for the definition of ionic strength, in particular).

3. Claims 1 and 5 are rejected under 35 U.S.C. 102(b) as being anticipated by Drittanti et al [U] as evidenced by Merriam-Webster Online Dictionary [U2] and prior art [V2].

Claims are generally directed to a method of preventing aggregation of rAAV virions in a **purified** preparation of virions, comprising providing a lysate comprising rAAV virions; purifying rAAV virions from the lysate using ultracentrifugation **and/or** chromatography; adding one or more excipients to the purified preparation of virions to achieve an ionic strength of at least

about 200 mM (amended claim 1); and wherein the one or more of the excipients comprises a multivalent ion (claim 5).

Drittanti et al [U] teach preparation of high quality adeno-associated virus vectors (AAV virions; see abstract, *Materials & Methods*, in particular) comprising providing a lysate containing rAAV virions; purifying the virions from the lysate using anion and cation exchange column chromatography in tendem (see Drittanti et al, section on Materials & Methods, "Downstream processing", page 61; and figure 4, in particular), wherein the rAAV virions were recovered/eluted by adding 300 mM NaCl through a 0-1M NaCl linear gradient (molar concentration of the salt which is equivalent to a calculated ionic strength of 300 mM; see prior art [V2] for the definition of "ionic strength"). Furthermore, the limitations of adding one or more excipients to the "purified" rAAV virions is also met by the method of Drittanti et al because they teach the fact that purified and pooled fractions of the virions were dialysed against Ringer-lactate buffer which has an ionic strength of at least **about** 200 mM (Lactated Ringer's solution, calculated ionic strength of 183.5mM, see the prior art [V2]; also see the definition of the term "about" which means "in the vicinity of"; prior art [U2], page 2, in particular); and wherein the excipients comprise a multivalent ion (such as calcium and lactate).

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.

Claims 1, 3-9 and 21-24 are/remain rejected under 35 U.S.C. 103(a) as being unpatentable over Zolotukhin et al (US 6,146,874; [A]) or Atkinson et al (US 6,566,118 B1; [C]), or Drittanti et al [U] in view of Qu et al [V] and Chen et al (IDS).

Claims are generally directed to a method of preventing aggregation of rAAV virions in a purified preparation of virions, comprising providing a lysate comprising rAAV virions; purifying rAAV virions from the lysate using ultracentrifugation and/or chromatography; and adding one or more excipients to the preparation of virions to achieve an ionic strength of at least about 200 mM (see specific recitations of the instant claims 1, 3-9, and 21-24).

The teachings of Zolotukhin et al, Atkinson et al, and Drittanti et al (as evidenced by Merriam-Webster Online Dictionary [U2] and prior art [V2]) have been discussed above and are further relied upon in the same manner, herein. Each of the prior art references cited supra teach a method step for the treatment of rAAV virion with a nuclease (such as Benzonase; see Zolotukhin et al, column 10, 1st paragraph; Atkinson

et al, example 7 and 8, in particular; and Drittanti et al, page 61, left column, 4th paragraph, in particular), albeit as a starting step in the purification of the virions. In addition, the process steps such as ultracentrifugation (CsCl gradient), chromatography (anion and cation exchange), and diafilteration (as recited in claims 1 and 21-24) are used and explicitly disclosed by the prior art references cited (see Zolotukhin et al, figure 1, columns 10-12, in particular; see Atkinson et al, example 7, column 53, in particular; and Drittanti et al, page 61, and figure 4, page 66, in particular) for the process of purification of rAAV virions, and for stabilizing the purified virion preparations obtained.

However, a method of preventing aggregation of "purified" preparation of rAAV virions comprising adding one or more excipients to said preparation of virions comprising a multivalent ion, **citrate**, is not explicitly disclosed by the inventions of Zolotukhin et al, Atkinson et al, or Drittanti et al.

Qu et al [V] disclose the problems associated with the concentration-induced aggregation of recombinant AAV virions, and highlight the role of ionic interactions (along with other types of interactions, presumably hydrophobic and other inter-particle interactions) in the preparation of concentrated stocks of vectors used for human gene therapy (see Qu et al, abstract, entire document). Qu et al demonstrate (using dynamic light scattering, size-exclusion chromatography, and by quantification of loss of titer following 0.2 μm filtration of the virions) that the aggregation was concentration dependent, and typically occurred when the concentrations of virions exceeded the range 0.5×10^{14} cp/ml for column purified preparations (see Qu et al, abstract). More

importantly, Qu et al also demonstrate that changes in buffer pH values resulted in reversal of this aggregation phenomenon, and thus suggested that ionic bridges between charged amino acids (Glu, Asp, Lys, presumably of the viral capsid protein) on the surface of vector particles contribute to the inter-particle interactions, and work in concert with hydrophobic and other types of inter-particle interactions (as the virions were found to be stable in a solution of 3M CsCl at neutral pH) to result in such concentration-dependent aggregation of the viral particles.

Chen et al (IDS) 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).

Therefore, it would have been obvious to a person of ordinary skill in the art at the time this invention was made to modify the process of preparation of virions as taught by Drittanti et al or Atkinson et al or Zolotukhin et al, such that the preparation of virions is added with one or more excipients such as multivalent ion (citrate salt) as explicitly suggested by the combined disclosures of Qu et al and Chen et al, in order to prevent the aggregation of virions by providing higher ionic strength preparations.

One of ordinary skill in the art would have been motivated to modify the virion preparations of Zolotukhin et al, or Atkinson et al, or Drittanti et al by adding sodium citrate to the preparation of virions because (1) Qu et al explicitly identify the problem of particle aggregation in standard preparations of AAV virions, and suggests the role of ionic interactions (among other type of interactions involved) in the concentration-induced aggregation of virions, and (2) Chen et al demonstrate that using multivalent ions such as citrate salts can effectively reduce/suppress aggregation of fairly unstable proteins (such as rhKGF; see discussion above), thus providing a conceptual as well as practical basis for such modification of the preparations obtained by the invention of Zolotukhin et al, or Atkinson et al, or Drittanti et al.

The person of ordinary skill in the art would have had a reasonable expectation of success when adding citrate as a multivalent ion in order to prevent the aggregation of virions as obtained by the process taught by Zolotukhin et al, or Atkinson et al, or Drittanti et al, because Chen et al explicitly demonstrates the use of salts of citrate in the stabilization of highly unstable protein formulations, such as rhKGF. Since, the viral capsid (outer coat) proteins have been shown to undergo similar concentration-induced aggregation phenomenon (as disclosed/suggested by Qu et al; see discussion above), and since Qu et al suggest the role of ionic inter-particle interactions in such aggregation, one of ordinary skill in the art would have been motivated to adapt the strategies taught by Chen et al (i.e. incorporation of multivalent ion such as citrate), and would have had a reasonable expectation of success in avoiding such aggregation in the preparations of virions.

The limitation of claim 7 (wherein the osmolarity of the preparation of virions after addition of the one or more excipients is no greater than about 280 mOsm) would have been a matter of routine optimization to an artisan of ordinary skill in the art as evidenced by the fact that each of the prior art references have used Lactated or modified Ringer's balanced salt solution to concentrate the final purified rAAV product (i.e. using the step of concentration and diafilteration against Ringer's solution; see Zolotukhin et al, example 1, column 12, lines 49-54, in particular; Atkinson et al, column 54, lines42-44, in particular; and Drittanti et al, page 61, right column, 4th paragraph, in particular) in order to match the physiological osmolarity (for example, of plasma about 300 mOsm, for use in *in vivo* applications in animal models).

The limitations of claim 9 (wherein the recovery of the virions is at least about 90% after filtration through a 0.22 μm filter) would have been a matter of routine optimization for an artisan of ordinary skill in the art as evident by the fact that Drittanti et al (see, page 61 right column), Atkinson, et al (see column 54, 2nd paragraph, in particular), or Zolotukhin et al (see column 5-6, in particular) disclose the step of filtration and recovery of the virions through 0.22 μm filter, and provide the basis for optimization of such method steps.

The limitations of claim 8 (wherein the average particle radius of the virions is less than about 20 nm as measured by dynamic light scattering) would have been a matter of routine optimization for an artisan of ordinary skill in the art (as evident by the fact that Qu et al disclose the process of using dynamic light scattering as one of the techniques for measuring/assessing the size of AAV virion aggregates and thus

quantifying the loss of virions following 0.2 μm filtration step; see Qu et al, abstract), and the skilled artisan recognizing the fact that such optimization are routine part of the process for development of such stable clinical formulations for gene therapy (as disclosed by the inventions of Zolotukhin et al, or Atkinson et al, or Drittanti et al).

The limitations of claims 3, and 21-24 would have been a matter arrangement of method steps, which have been explicitly disclosed and employed by the prior art references relied upon in the rejection (see teachings of the primary references, supra), and therefore, in the absence of evidence to the contrary, one of ordinary skill in the art can further incorporate a nuclease treatment step followed by an ultracentrifugation and/or chromatographic step(s) used for purification of rAAV virions from crude cell lysates containing rAAV virions, under conditions that are already known to an artisan of ordinary skill in the art by the disclosures provided in the prior art, as discussed supra.

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

As per MPEP 2144.04 (Arrangement of process steps), Ex parte Rubin, 128 USPQ 440 (Bd. App. 1959) (Prior art reference disclosing a process of making a laminated sheet wherein a base sheet is first coated with a metallic film and thereafter impregnated with a thermosetting material was held to render prima facie obvious claims directed to a process of making a laminated sheet by reversing the order of the prior art process steps.). See also In re Burhans, 154 F.2d 690, 69 USPQ 330 (CCPA 1946) (selection of any order of performing process steps is prima facie obvious in the absence of new or unexpected results); In re Gibson, 39 F.2d 975, 5 USPQ 230 (CCPA 1930) (Selection of any order of mixing ingredients is prima facie obvious.).

As per MPEP 2144.05 [R3], II. OPTIMIZATION OF RANGES - A. Optimization Within Prior Art Conditions or Through Routine Experimentation: Generally, differences in concentration or temperature will not support the patentability of subject matter encompassed by the prior art unless there is evidence indicating such concentration or temperature is critical. "[W]here 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, 105 USPQ 233, 235 (CCPA 1955).

As per MPEP 2144, [T]the rationale to modify or combine the prior art does not have to be expressly stated in the prior art; the rationale may be expressly or impliedly contained in the prior art or it may be reasoned from knowledge generally available to one of ordinary skill in the art, established scientific principles, or legal precedent established by prior case law. 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).

Response to Arguments

Applicant's arguments filed with the office on September 21st 2006 (as they pertain to the rejections of record) have been fully considered but they are not persuasive for the following reasons of record.

Claims are generally directed to a method of preventing aggregation of rAAV virions in a purified preparation of virions comprising providing a lysate comprising rAAV virions; purifying rAAV virions from the lysate using ultracentrifugation and/or chromatography; adding one or more excipients to the purified preparation of virions to achieve an ionic strength of at least about 200 mM.

Applicant's arguments regarding the anticipation rejections made with disclosures of prior art references (Zolotukhin et al, Atkinson et al, and Drittanti et al; see discussion supra) over rejected claims are fully considered but are moot in view of the new grounds of rejection made in this office action. The amended claims 1 and 5 are still deemed to be anticipated by the prior art references (Zolotukhin et al, Atkinson et al, and Drittanti et al) because they teach the method steps of providing a lysate containing rAAV virions, purifying the virions using ultracentrifugation and/or chromatography, and adding one or more excipients to the "purified" virion preparation in order to achieve an ionic strength of at least **about** 200 mM. Applicant's argument (see applicant's remarks, page 7, 1st paragraph, in particular) that Lactated Ringer's solution (used by the prior art references relied upon; it is to be noted that the calculated ionic strength of this solution is **183.5 mM**, and dies not translate to 138.5 mM, as

pointed out by the applicants in their remarks and evidentiary supports filed with the office) does not meet the limitation of **at least 200 mM** is found to be accurate. However, the instant claims recite the limitation of "at least **about**" 200 mM in the claimed invention (i.e. meaning in the vicinity of 200 mM), and therefore, the claimed process is anticipated by the prior art. Moreover, the prior art discloses the fact that after concentration of the final purified virions, the "purified" product can be further concentrated 10 fold using diafilteration and ultrafiltration process steps and Modified Ringer's solution (see Atkinson et al, column 54, in particular), and thus, meets the claimed limitation. Since the active method steps (providing lysate containing rAAV virions, purifying the virions using ultracentrifugation and/or chromatographic techniques, and adding one or more excipients to the purified virion preparation) of the claimed invention are met by the prior art references, as discussed supra, the prior art references are deemed to be anticipatory to the process of preventing aggregation of purified rAAV virions, as claimed.

Applicant's arguments regarding the obviousness rejection of record over pending claims were fully considered but were not found to be persuasive. Applicants argue that there is no motivation to combine the references (see applicant's remarks, pages 8-10, in general). Please note that motivation to combine is found not only in the direct teachings of the prior art but may be found also in the nature of the problem to be solved and the knowledge of persons of ordinary skill in the art, see Ruiz v. A.B. Chance Co., 357 F.3d 1270, 69 USPQ2d 1686 (2004) and *In re* Rouffet, 149 F.3d 1350, 47 USPQ2d (1998). Thus, the motivation in the instant case flows from the nature of

the problem to be solved which is the same in the cited references, namely preventing the aggregation of purified rAAV virions with high titers, which are known to an artisan of ordinary skill in the art to be prone to aggregation (see Zolotukhin et al, column 3, last paragraph and column 15, 3rd paragraph, in particular; the disclosure of Qu et al, title and the abstract, in particular).

As per MPEP 2143, obviousness can only 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 explicitly or implicitly in the references themselves or in the knowledge generally available to one of ordinary skill in the art. *"The test for an implicit showing is what the combined teachings, knowledge of one of ordinary skill in the art, and the nature of the problem to be solved as a whole would have suggested to those of ordinary skill in the art." In re Kotzab, 217 F.3d 1365, 1370, 55 USPQ2d 1313,1317 (Fed. Cir. 2000). See also In re Lee, 277 F.3d 1338, 1342-44, 61 USPQ2d 1430, 1433-34 (Fed. Cir. 2002)* (discussing the importance of relying on objective evidence and making specific factual findings with respect to the motivation to combine references); 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).

Since, the prior art disclosures (Zolotukhin et al, Atkinson et al, Drittanti et al in view of Qu et al, in particular, and Chen et al in general) clearly reveal the problem associated with the aggregation of rAAV virions at various levels and steps of production and purification starting from cellular lysate containing the rAAV virions (see Zolotukhin et al, and Qu et al; see discussion supra), and in fact, suggest/incorporate

two distinct solutions (viz: nuclease treatment to remove extraneous/undesired nucleic acid fragments usually employing *Serratia marcescens* endonuclease, Benzonase® to treat the crude cellular lysate containing viral particles; and increasing salt concentrations and/or ionic strength of the solution containing rAAV viral particles using NaCl or other ionic salt-containing buffer compositions; see Zolotukhin et al, column 10 and column 15, in particular), the invention as claimed would have been obvious to a person of ordinary skill in the art at the time this invention was made. The disclosures of Qu et al regarding the role of ionic interparticle interactions of side chains/charges of amino acids in the viral capsid protein, and the suggestions of using a multivalent ion (such as citrate) to suppress protein aggregation during liquid formulation development lend support for the argument (in the absence of an evidence of criticality) that an artisan of ordinary skill would have had a strong motivation (and a reasonable expectation of success) for modifying the process of producing purified rAAV virions by employing the method steps (such as optimizing ionic strength of the purified viral solution, and incorporating a nuclease step after purifying the virions using method steps of ultracentrifugation and/or chromatography), and thus preventing the aggregation of purified rAAV preparations, as claimed in the instant invention.

Therefore, the pending claims are properly rejected under 35 USC 103(a).

Pertinent Prior art not relied upon in the rejections

1. Wu et al. (US 6,689,600 B1), Formulation of adenovirus for gene therapy (see abstract, columns 6, 12-14, 19-20, in particular).
2. Croyle et al. (US 6,399,385 B1), Method for rapid PEG-modification of viral vectors, compositions for enhanced gene transduction, compositions with

enhanced physical stability, and uses therefor (see abstract, summary of the invention, table 3, in particular).

3. Orlov et al. Macroscopic aggregation of Tobacco Mosaic Virus coat protein, *Biochemistry* (Moscow), 2001, 66(2): 154-162 (especially, abstract, Materials & Methods, page 157, in particular).

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 K. 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
Patent Examiner
Art Unit 1657

SANDRA E. SAUCIER
PRIMARY EXAMINER

PTO/SB/08B (08-03)
Approved for use through 06/30/2006. OMB 0651-0031
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(use as many sheets as necessary)

Sheet 1 of 1

Complete if Known	
Application Number	11/141,996
Filing Date	June 1, 2005
First Named Inventor	JOHN FRASER WRIGHT et al.
Art Unit	1651
Examiner Name	Satyendra K. Singh
Attorney Docket Number	0800-0045

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[2]
SS	CA	Adadevoh et al., "SHORT-TERM FIELD USE AND SHIPPING STABILITY STUDY OF A WILD TYPE AD5 ADENOVIRAL REFERENCE MATERIAL," BioProcessing, 1(3):62-9 (2002)	
SS	CB	High, K. A. 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)	
SS	CC	Qu, G. et al., "EVIDENCE THAT IONIC INTERACTIONS ARE INVOLVED IN CONCENTRATION-INDUCED AGGREGATION OF RECOMBINANT ADENO-ASSOCIATED VIRUS," Mol. Therapy 7(5):S348, Abstract No. 901 (2003)	

Examiner Signature	/Satyendra Singh/	Date Considered	11/29/2006

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

Notice of References Cited	Application/Control No. 11/141,996	Applicant(s)/Patent Under Reexamination WRIGHT ET AL.	
	Examiner Satyendra K. 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-			
	B	US-			
	C	US-			
	D	US-			
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	F	US-			
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FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
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NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U2	The term "PURIFY" AND "ABOUT" Merriam-Webster Online Dictionary, at the web- http://www.m-w.com, page 1 and page 2.
	V2	The definition of the term "Ionic strength", Answer.com, at the web- http://www.answers.com, page 1.
	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.

Index of Claims

Application/Control No.	Applicant(s)/Patent under Reexamination
11/141,996	WRIGHT ET AL.
Examiner	**Art Unit**
Satyendra K. Singh	1651

√	Rejected	–	(Through numeral) Cancelled	N	Non-Elected	A	Appeal
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Claim		Date							
Final	Original	1/17/06	5/13/06	11/30/06					
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	5	+	√	√					
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Search Notes	Application/Control No.	Applicant(s)/Patent under Reexamination	
	11/141,996	WRIGHT ET AL.	
	Examiner	Art Unit	
	Satyendra K. Singh	1651	

SEARCHED			
Class	Subclass	Date	Examiner

INTERFERENCE SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES (INCLUDING SEARCH STRATEGY)		
	DATE	EXMR
EAST: USPAT, PG-PUB, USOCR, JPO, EPO, DERWENT ATTCHED SEPARATELY.	5/13/2006	SKS
STN: CA, CAPLUS, BIOSIS, MEDLINE ATTACHED SEPARATELY.	5/13/2006	SKS
INVENTOR SEARCH: PALM &STN UPDATED.	12/1/2006	SKS
EAST: USPAT, USOCR, US-PGPUB, JPO, EPO, DERWENT- UPDATED & ATTACHED SEPARATELY..	12/1/2006	SKS

PTO/SB/31 (09-04)

NOTICE OF APPEAL FROM THE EXAMINER TO THE BOARD OF PATENT APPEALS AND INTERFERENCES	Docket Number (Optional) 0800-0045

VIA EFS

In re Application of Wright et al.	
Application Number 11/141,996	Filed June 1, 2005
For COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION	
Art Unit 1657	Examiner S.K. SINGH

Applicant hereby **appeals** to the Board of Patent Appeals and Interferences from the last decision of the examiner.

The fee for this Notice of Appeal is (37 CFR 41.20(b)(1)) $ 500.00

☐ Applicant claims small entity status. See 37 CFR 1.27. Therefore, the fee shown above is reduced by half, and the resulting fee is: $ _____

☐ 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. I have enclosed a duplicate copy of this sheet.

☒ The Director is hereby authorized to charge any **additional** fees which may be required, or credit any overpayment to Deposit Account No. 18-1648. I have enclosed a duplicate copy of this sheet.

☒ A petition for an extension of time under 37 CFR 1.136(a) (PTO/SB/22) is enclosed.

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.

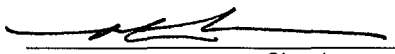
I am the

☐ 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 acting under 37 CFR 1.34. Registration number if acting under 37 CFR 1.34. _____



Signature

Roberta L. Robins

Typed or printed name

650 493-3400

Telephone number

5/22/07

Date

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.

PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a) **FY 2005** *(Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).)*	Docket Number (Optional) 0800-0045
Application Number: 11/141,996	Filed: June 1, 2005
For COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION	
Art Unit: 1657	Examiner: S.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))	$120	$60	$
☐ Two months (37 CFR 1.17(a)(2))	$450	$225	$
☒ Three months (37 CFR 1.17(a)(3))	$1020	$510	$ 1020
☐ Four months (37 CFR 1.17(a)(4))	$1590	$795	$
☐ Five months (37 CFR 1.17(a)(5))	$2160	$1080	$

☐ 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 additional fees which may be required, or credit any overpayment, to Deposit Account Number 18-1648. I have enclosed a duplicate copy of this sheet.

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☐ 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

5/22/07
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.

Electronic Patent Application Fee Transmittal

Application Number:	11141996
Filing Date:	01-Jun-2005
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

Filed as Large Entity

Utility Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Notice of appeal	1401	1	500	500
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension - 3 months with $0 paid	1253	1	1020	1020
Miscellaneous:				
			Total in USD ($)	**1520**

Electronic Acknowledgement Receipt

EFS ID:	1801385
Application Number:	11141996
International Application Number:	
Confirmation Number:	5399
Title of Invention:	Compositions and methods to prevent AAV vector aggregation
First Named Inventor/Applicant Name:	John Fraser Wright
Customer Number:	31048
Filer:	Roberta L. Robins/Denise Vaillancourt
Filer Authorized By:	Roberta L. Robins
Attorney Docket Number:	0800-0045
Receipt Date:	22-MAY-2007
Filing Date:	01-JUN-2005
Time Stamp:	18:25:33
Application Type:	Utility

Payment information:

Submitted with Payment	yes
Payment was successfully received in RAM	$1520
RAM confirmation Number	18248
Deposit Account	

File Listing:

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

1		noa.pdf	89666	yes	2

Multipart Description/PDF files in .zip description		
Document Description	**Start**	**End**
Notice of Appeal Filed	1	1
Extension of Time	2	2

Warnings:

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2	Fee Worksheet (PTO-06)	fee-info.pdf	8315	no	2

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Information:

Total Files Size (in bytes):	97981

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/22 (11-07)
Approved for use through 11/30/2007. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a) FY 2006 *(Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).)*	Docket Number (Optional) 1039.US
Application Number 11/141,996	Filed June 1, 2005
For COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION	
Art Unit 1657	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))	$120	$60	$____
☐ Two months (37 CFR 1.17(a)(2))	$460	$230	$____
☐ Three months (37 CFR 1.17(a)(3))	$1050	$525	$____
☐ Four months (37 CFR 1.17(a)(4))	$1640	$820	$____
☒ Five months (37 CFR 1.17(a)(5))	$2230	$1115	$2230

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

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

☐ Payment by credit card. Form PTO-2038 is attached.

☐ 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 07-1074 . I have enclosed a duplicate copy of this sheet.

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.

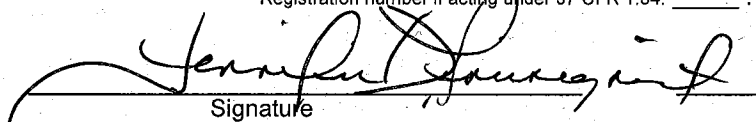
I am the

☐ 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 54,498

☐ attorney or agent under 37 CFR 1.34.
Registration number if acting under 37 CFR 1.34. _____ .



Signature — December 26, 2007 (Date)

Jennifer D. Tousignant — Typed or printed name

508.270.2499 — 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 _____ forms are submitted.

PTO/SB/22 (11-07)
Approved for use through 11/30/2007. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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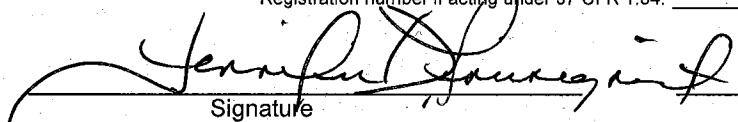
☒ attorney or agent of record. Registration Number 54,498

☐ attorney or agent under 37 CFR 1.34.
Registration number if acting under 37 CFR 1.34. _____ .



Signature — December 26, 2007 (Date)

Jennifer D. Tousignant — Typed or printed name

508.270.2499 — 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 _____ forms are submitted.

PTO/SB/30 (11-07)
Approved for use through 11/30/2007. OMB 0651-0031
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

Request For Continued Examination (RCE) Transmittal

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

Application Number	11/141,996
Filing Date	June 1, 2005
First Named Inventor	Wright, John Fraser
Art Unit	1657
Examiner Name	Satyendra K. Singh
Attorney Docket Number	1039.US

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 C.F.R. 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 C.F.R. 1.103(c) for a period of _____months. (Period of suspension shall not exceed 3 months; Fee under 37 C.F.R. 1.17(i) required)

b. ☐ Other _____

3. Fees The RCE fee under 37 C.F.R. 1.17(e) is required by 37 C.F.R. 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. 07-1074 . I have enclosed a duplicate copy of this sheet.

i. ☒ RCE fee required under 37 C.F.R. 1.17(e)
ii. ☒ Extension of time fee (37 C.F.R. 1.136 and 1.17)
iii. ☐ Other _____

b. ☐ Check in the amount of $ _____ enclosed

c. ☐ Payment by credit card (Form PTO-2038 enclosed)

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.

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT REQUIRED

Signature	Jennifer D. Tousignant	Date	December 26, 2007
Name (Print/Type)	Jennifer D. Tousignant	Registration No.	54,498

CERTIFICATE OF MAILING OR TRANSMISSION

I hereby certify that this correspondence is being deposited with the United States Postal Service ~~with sufficient postage as first class mail in an envelope addressed to: Mail Stop RCE~~, Commissioner for Patents, P. O. Box 1450, Alexandria, VA 22313-1450 or facsimile transmitted to the U.S. Patent and Trademark Office on the date shown below. EFS Web 12/26/07

Signature	Jennifer D. Tousignant		
Name (Print /Type)	Jennifer D. Tousignant	Date	December 26, 2007

This collection of information is required by 37 CFR 1.114. 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 1.11 and 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: Mail Stop RCE, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Patent
Our Docket: 1039US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: WRIGHT et al.	)	Art Unit: 1657
Serial No.: 11/141,996	)	Examiner: Satyendra K. Singh
Filed: June 1, 2005	)	
For: Compositions and Methods to Prevent AAV Vector Aggregation	)	

Mailstop RCE
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

I HEREBY CERTIFY UNDER 37 CFR § 1.8 THAT THIS CORRESPONDENCE IS BEING DEPOSITED ELECTRONICALLY THE UNITED STATES PATENT AND TRADEMARK OFFICE AT:
http://www.uspto.gov/ebc/index.html .

December 26, 2007 /Jennifer D. Tousignant/

Date **Signature of person depositing correspondence**

REQUEST FOR CONTINUED EXAMINATION UNDER 37 C.F.R. § 1.114

Sir:

This Request for Continued Examination under 37 C.F.R. § 1.114 is being filed in response to the Final Office Action mailed on December 7, 2007 in connection with the above-identified application. A response to the Final Office Action was originally due on June 7, 2007. A Notice of Appeal was filed in the above-referenced case on May 22, 2007. An Appeal Brief was due on July 22, 2007. Applicants are hereby filing a Five Month Petition for Extension of Time thereby extending the due date to December 22, 2007, which falls on a Saturday thereby extending the date to the following Wednesday, December 26, 2007 since Monday, December 24, 2007 and Tuesday, December 25, 2007 were federal holidays.

In lieu of an Appeal Brief, Applicants are filing herein a Request for Continued Examination under 37 C.F.R. § 1.114. In accordance with 37 C.F.R. § 1.114, Applicants are filing with this request a submission comprising a response to the outstanding Final Office Action with amended claims and the required fee. Accordingly, this request and submission are timely filed. Applicants respectfully request withdrawal of the finality of the Office Action and entry and consideration of this submission.

I. Remarks

Claims 1, 3-9, and 21-24 are currently pending.

Claims 1 and 3 have been amended with this response. Support for these amendments is found throughout the instant specification and particularly at page 9, 10, 14, 15, 17, and 20. Accordingly, Applicants do not believe that these amendments add new matter. Entry of the amendments is respectfully requested.

II. Claim rejections under 35 U.S.C. § 112

Claims 1, 3, 4-9, and 21-24 stand rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for allegedly failing to point out and distinctly claim the subject matter which Applicants regard as the invention. In particular, the Office has concluded that the term "purified" is confusing and ambiguous because it would be unclear to an artisan of ordinary skill to what degree or level of purification is required. Applicants respectfully traverse.

In order to further prosecution, Applicants have amended the claims to delete the term "purified" and to define with more particularity the instantly claimed subject matter. Accordingly, withdrawal of the rejection is respectfully requested.

III. Claim rejections under 35 U.S.C. § 102(b)

A) Claims 1 and 5 stand rejected under 35 U.S.C. § 102(b) as being allegedly anticipated by Zolotukhin et al. (U.S. Patent 6,146,874) as evidenced by Merriam-Webster Online Dictionary and prior art (V2). Applicants respectfully traverse. Zolotukhin et al. cannot anticipate the instant invention because it does not teach the addition of one or more excipients to achieve an ionic strength of at least 200 mM to the rAAV virions following purification as now required by the instant claims.

Zolotukhin et al. teaches the use of excipients in rAAV purification and down-stream processing in two ways. First, the reference uses 1M NaCl/PBS/ 1 mM MgCl2/ 2.5 mM KCl in one of the iodixanol gradients that are utilized to purify rAAV from a cell lysate. Second, the reference teaches the concentration and desalting of the rAAV into Lactated Ringer's Buffer, which has an ionic strength of 138.5 mM. The instant claims differ from these teachings provided by Zolotukhin et al.

The instant claims require the addition of one or more excipients to achieve an ionic strength of at least 200 mM to the rAAV virions after purification of the virion from the cell lysate, wherein purification can be by centrifugation or chromatography. As the 1M NaCl/PBS/ 1 mM MgCl2/ 2.5 mM KCl is utilized in one of the iodixanol gradients used to purify the rAAV, this teaching cannot anticipate the instant claims.

With respect to the teaching relating to the concentration and desalting of the rAAV into Lactated Ringer's Buffer, this cannot anticipate the instant claims because they require an ionic strength of at least 200 mM. Lactated Ringer's Buffer has an ionic strength of 138.5 mM. Accordingly, this teaching cannot anticipate the instant claims.

Accordingly, Applicants respectfully request withdrawal of the instant rejection.

B) Claims 1 and 5 stand rejected under 35 U.S.C. § 102(b) as being allegedly anticipated by Atkinson et al. (U.S. Patent 6,566,118) as evidenced by Merriam-Webster Online Dictionary and prior art (V2). Atkinson et al. cannot anticipate the instant invention because it does not teach the addition of one or more excipients to achieve an ionic strength of at least 200 mM to the rAAV virions as now required by the instant claims.

Atkinson et al. teaches the concentration rAAV into Ringer's Balanced Salt Solution, which Applicants believe are the same as Lactated Ringer's Solution without the lactate. Based on Applicants' calculations, Lactated Ringer's Buffer has an ionic strength of 138.5 mM. Without lactate, Ringer's Balanced Salt Solution would have an ionic strength of less than 138.5 mM. The instant claims differ from these teachings because they require the addition of one or more excipients to achieve an ionic strength of at least 200 mM. Accordingly, this teaching cannot anticipate the instant claims. Applicants respectfully request withdrawal of the instant rejection.

C) Claims 1 and 5 stand rejected under 35 U.S.C. § 102(b) as being allegedly anticipated by Drittani et al., J. Gene Med. 2001; 3: 59-71 as evidenced by Merriam-Webster Online Dictionary and prior art (V2). Drittani et al. cannot anticipate the instant invention because it does not teach the addition of one or more excipients to achieve an ionic strength of at least 200 mM to the rAAV virions following purification as now required by the instant claims.

The instant claims require the addition of one or more excipients to achieve an ionic strength of at least 200 mM to the rAAV virions after purification of the virion from the cell lysate, wherein purification

can be by centrifugation or chromatography. In Drittani et al., a NaCl gradient is utilized to elute rAAV during chromatography. As the NaC is utilized during purification, this teaching cannot anticipate the instant claims.

In addition, Drittani et al. utilize Lactated Ringer's Buffer to dialyze the rAAV. Based on Applicants' calculations, Lactated Ringer's Buffer has an ionic strength of 138.5 mM. The instant claims differ from these teachings because they require the addition of one or more excipients to achieve an ionic strength of at least 200 mM. Accordingly, this teaching cannot anticipate the instant claims. Applicants respectfully request withdrawal of the instant rejection.

IV. Claim rejections under 35 U.S.C. § 103(a)

Claims 1, 3-9 and 21-24 stand rejected under 35 U.S.C. § 103(a) as being allegedly unpatentable over Zolotukhin et al. or Atkinson et al. or Drittani et al. in view of Qu et al. and Chen et al. Applicants respectfully traverse because a prima facie case of obviousness requires that the prior art references teach or suggest all claim limitations. The cited prior art does not teach or suggest the use of one or more excipients to achieve an ionic strength of at least 200 mM following purification as now required by the instant claims. Absent this element in the prior art, the Office has failed to establish a prima facie case of obviousness for claims 1, 3-9, and 21-24. Applicants respectfully request withdrawal of the instant rejection.

Further, Applicants respectfully assert that the Office has also failed to establish a prima facie case of obviousness with respect to claims 3 and 4. The instant claims have been amended to clarify that purified rAAV are those virions that have been separated from a lysate comprising said virions. Accordingly, claims 3 and 4 are drawn to the instant method that treats purified virions with nuclease to prevent virion aggregation.

The Office has failed to provide a reference that teaches or suggests the use of nuclease treatment with purified rAAV. The references cited by the Office treat the cell lysate with nuclease, e.g. use nuclease treatment prior to purification. As noted by the instant specification at page 9, last paragraph, such early treatment with nuclease does not reduce aggregation.

Absent this element in the prior art, the Office has failed to establish a prima facie case of obviousness for claims 3 and 4 as such a case requires that the prior art references teach or suggest all claim limitations. Applicants respectfully request withdrawal of the instant rejection.

V. Claim Amendments under 37 C.F.R. § 1.121

1. (Currently amended) A method of preventing aggregation of recombinant adeno-associated virus (rAAV) virions in a ~~purified~~ preparation of rAAV virions, comprising:

1) providing a lysate comprising rAAV virions;

2) purifying rAAV virions from the lysate using ultracentrifugation and/or chromatography, wherein said virions are purified; and

3) adding one or more excipients to ~~the~~ said purified ~~preparation of~~ virions to achieve an ionic strength of at least ~~about~~ 200 mM.

2. (Canceled)

3. (Currently amended) The method of claim 1, further comprising treating said purified ~~preparation of~~ virions with a nuclease.

4. (Previously presented) The method of claim 3, wherein the nuclease is an endonuclease from *Serratia marcescens* (Benzonase®)

5. (Original) The method of claim 1, wherein one or more of the excipients comprises a multivalent ion.

6. (Original) The method of claim 1, wherein the multivalent ion is citrate.

7. (Original) The method of claim 1, wherein the osmolarity of the preparations of virions after addition of the one or more excipients is no greater than about 280mOsm.

8. (Original) The method of claim 1, wherein, after addition of the one or more excipients, the average particle radius (Rh) of the virions in the preparation of virions is less than about 20nm as measured by dynamic light scattering.

9. (Original) The method of claim 1, wherein, after addition of the one or more excipients, recovery of the virions is at least about 90% following filtration of the preparation of virions through 0.22µm filter.

10-20. (canceled)

21. (Previously presented) The method of claim 3, wherein rAAV virions are purified from the lysate using cesium chloride gradient ultracentrifugation.

22. (Previously presented) The method of claim 3, wherein rAAV virions are purified from the lysate using cation exchange chromatography.

23. (Previously presented) The method of claim 3, wherein rAAV virions are purified from the lysate using cation exchange chromatography and cesium chloride gradient ultracentrifugation.

24. (Previously presented) The method of claim 3, further comprising diafiltering the purified rAAV virions to achieve an ionic strength of at least about 200 mM.

VI. Conclusion

No fee is deemed necessary in connection with the filing of this communication. However, if any fee is required, authorization is hereby given to charge the amount of any such fee to Deposit Account No. 07-1074.

Respectfully submitted,

December 26, 2007
Date

/Jennifer D. Tousignant/
Jennifer D. Tousignant
Attorney for Applicants
Registration No. 54,498
Telephone: (508) 270-2499
Facsimile: (508) 872-5415

GENZYME CORPORATION
15 Pleasant Street Connector
P.O. Box 9322
Framingham, Massachusetts 01701-9322

Electronic Patent Application Fee Transmittal

Application Number:	11141996
Filing Date:	01-Jun-2005
Title of Invention:	Compositions and methods to prevent AAV vector aggregation
First Named Inventor/Applicant Name:	John Fraser Wright
Filer:	Jennifer D. Tousignant.
Attorney Docket Number:	0800-0045

Filed as Large Entity

Utility 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 - 5 months with $0 paid	1255	1	2230	2230

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
Request for continued examination	1801	1	810	810
		Total in USD ($)		**3040**

Electronic Acknowledgement Receipt

EFS ID:	2642390
Application Number:	11141996
International Application Number:	
Confirmation Number:	5399
Title of Invention:	Compositions and methods to prevent AAV vector aggregation
First Named Inventor/Applicant Name:	John Fraser Wright
Customer Number:	31048
Filer:	Jennifer D. Tousignant.
Filer Authorized By:	
Attorney Docket Number:	0800-0045
Receipt Date:	26-DEC-2007
Filing Date:	01-JUN-2005
Time Stamp:	23:59:01
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$3040
RAM confirmation Number	3337
Deposit Account	071074
Authorized User	

File Listing:

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

1		1039forms122607.pdf	139876 085869a4f0d456a1b420ae697942114e72ca54de	yes	2

Multipart Description/PDF files in .zip description		
Document Description	**Start**	**End**
Request for Continued Examination (RCE)	1	1
Extension of Time	2	2

Warnings:

Information:

2	Amendment Submitted/Entered with Filing of CPA/RCE	1039RCE122607.pdf	88320 99a9701d3d52f2e2b6a34e69e6dce0d0d6ace2ca	no	7

Warnings:

Information:

3	Fee Worksheet (PTO-06)	fee-info.pdf	8310 1cf2b1645c83c6d83450f2d36119b6aca07948e9	no	2

Warnings:

Information:

Total Files Size (in bytes):	236506

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.

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New International Application Filed with the USPTO as a Receiving Office
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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
11/141,996	06/01/2005	

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 12/26/2007	(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))	* 12	Minus	** 20	=	X $ =		OR	X $ =	
Independent (37 CFR 1.16(h))	* 1	Minus	***3	=	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	

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:
Katrina S. Turner

UNITED STATES PATENT AND TRADEMARK OFFICE

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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ROBINS & PASTERNAK LLP
1731 EMBARCADERO ROAD
SUITE 230
PALO ALTO, CA 94303

EXAMINER	
SINGH, SATYENDRA K	

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1657	

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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)	
	11/141,996	WRIGHT ET AL.	
	Examiner	Art Unit	
	SATYENDRA K. 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 *26 December 2007*.

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

3)☐ 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

4)☒ Claim(s) *1,3-9 and 21-24* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,3-9 and 21-24* is/are rejected.

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

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

Application Papers

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

10)☒ The drawing(s) filed on *01 June 2005* 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).

11)☐ 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

12)☐ 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

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 December 26th 2007 has been entered.

Claims 2 and 10-20 have been canceled by applicant's previous amendments to the claims.

Claims 1, 3-9 and 21-24 (group I, as currently amended) are examined on their merits in this office action.

Improper claim amendment

The amendment to the claims filed on December 26th 2007, does not comply with the requirements of 37 CFR 1.121(c) because the claim listing does not contain a listing of all the claims ever presented for examination in this case, as well as **their status**. Amendments to the claims filed on or after July 30, 2003 must comply with 37 CFR 1.121(c) which states (emphasis added):

> (c) *Claims*. Amendments to a claim must be made by rewriting the entire claim with all changes (e.g., additions and deletions) as indicated in this subsection, except when the claim is being canceled. Each amendment document that includes a change to an existing claim, cancellation of an existing claim or addition of a new claim, must include a complete listing of all claims ever presented, including the text of all pending and withdrawn claims, in the application. The claim listing, including the text of the claims, in the amendment document will serve to replace all prior versions of the claims, in the application. In the claim listing, the status of every claim must be indicated after its claim number by using one of the

following identifiers in a parenthetical expression: (Original), (Currently amended), (Canceled), (Withdrawn), (Previously presented), (New), and (Not entered).

(1) *Claim listing*. All of the claims presented in a claim listing shall be presented in ascending numerical order. Consecutive claims having the same status of "canceled" or "not entered" may be aggregated into one statement (*e.g.*, Claims 1–5 (canceled)). The claim listing shall commence on a separate sheet of the amendment document and the sheet(s) that contain the text of any part of the claims shall not contain any other part of the amendment.

(2) *When claim text with markings is required*. All claims being currently amended in an amendment paper shall be presented in the claim listing, **indicate a status of "currently amended**," and **be submitted with markings to indicate the changes** that have been made relative to the immediate prior version of the claims. The text of any added subject matter must be shown **by underlining the added text**. The text of any deleted matter must be shown by strike-through except that double brackets placed before and after the deleted characters may be used to show deletion of five or fewer consecutive characters. The text of any deleted subject matter must be shown by being placed within double brackets if strike-through cannot be easily perceived. Only claims having the status of "currently amended," or "withdrawn" if also being amended, shall include markings. If a withdrawn claim is currently amended, its status in the claim listing may be identified as "withdrawn—currently amended."

(3) *When claim text in clean version is required*. The text of all pending claims not being currently amended shall be presented in the claim listing in clean version, *i.e.*, without any markings in the presentation of text. The presentation of a clean version of any claim having the status of "original," "withdrawn" or "previously presented" will constitute an assertion that it has not been changed relative to the immediate prior version, except to omit markings that may have been present in the immediate prior version of the claims of the status of "withdrawn" or "previously presented." Any claim added by amendment must be indicated with the status of "new" and presented in clean version, *i.e.*, without any underlining.

(4) *When claim text shall not be presented; canceling a claim*.

(i) No claim text shall be presented for any claim in the claim listing with the status of "canceled" or "not entered."

(ii) Cancellation of a claim shall be effected by an instruction to cancel a particular claim number. Identifying the status of a claim in the claim listing as "canceled" will constitute an instruction to cancel the claim.

(5) *Reinstatement of previously canceled claim*. A claim which was previously canceled may be reinstated only by adding the claim as a "new" claim with a new claim number.

As noted above, the amendment under consideration herein fails to comply with 37 CFR 1.121 because the claim listing does not contain a listing of all the claims ever presented for examination in this case, as well as their status (see instant claim 6, that is indicated as being "original" even though the claim has been amended by applicants to depend from the broader claim 1, instead of from claim 5, as originally filed and prosecuted). Thus, the amendment could be considered non-responsive. However, in the interest of compact prosecution the amendment at issue will not be considered non-

compliant. However, any future responses failing to comply with 37 CFR 1.121 will be held non-compliant, and will not be considered.

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. Claim 24 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 24 recites "ionic strength of at least **about** 200 mM", which is confusing. It is unclear as to what exactly is encompassed by the method claim as presented by applicants. Claim 1 (from which claim 24 ultimately depends from) requires "adding one or more excipients to said purified virions to achieve an ionic strength of at least 200 mM" (i.e. 200 mM or more), however, it is unclear as to how the method step of nuclease treatment and further comprising "diafiltering" changes the ionic strength to "at least **about** 200 mM". Since, the term "about" encompasses an ionic strength "in the vicinity of" 200 mM (for example, less than 200 mM such as180 mM, 190 mM, etc.), in the absence of any such guidance in the instant specification, the claimed invention is deemed indefinite. Appropriate explanation/correction is required.

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, 3-9 and 21-24 (as currently amended) are rejected under 35 U.S.C. 103(a) as being unpatentable over Zolotukhin et al (US 6,146,874; [A]) taken with Atkinson et al (US 6,566,118 B1; [C]) and Drittanti et al [U] in view of Qu et al [V] and Chen et al (IDS), and further in view of Wright et al (US 6,593,123 B1; [A2]).

> Claims are generally directed to **a method of preventing aggregation of rAAV virions** in a preparation of rAAV virions, comprising 1) providing a lysate comprising rAAV virions; 2) purifying rAAV virions from the lysate using ultracentrifugation and/or chromatography, wherein said virions are purified; and 3) adding one or more excipients to said purified virions to achieve an ionic strength of at least 200 mM (see also specific recitations of the instant claims 3-9, and 21-24).

Zolotukhin et al [A] teach a preparation of virions (rAAV virions; see abstract, summary of the invention, column 9-10, in particular) comprising excipients having ionic strength of at least 200mM and having multivalent ions (such as salt and buffers; see Zolotukhin et al, column 10, 1 M NaCl-PBS-MgCl2 and KCl buffer containing multivalent ions such as magnesium and phosphate; see also applicant's exemplification of multivalent ions at page 20, 2nd paragraph, in particular). The limitations of purifying rAAV virions from the lysate using ultracentrifugation and/or chromatography is met as Zolotukhin et al teach the step of purifying the crude lysate through Iodixanol density gradient which uses ultracentrifugation step, and further purifying the rAAV virions obtained from the above Iodixanol gradient by using a chromatographic step, wherein the "purified" preparation of virions (for example, bound to column such as Heparin-ligand affinity media; see Zolotukhin et al, the entire columns 10 and 11, in particular) was added with one or more excipients (such as PBS-MK with 1M NaCl for elution from the column; see Zolotukhin et al, column 11, lines 36-37, in particular).

Atkinson et al [C] teach a method for generating high titer helper-free preparations of released recombinant AAV vectors (rAAV; see Atkinson et al, abstract, summary of the invention, Figures 13, 19, 32, 36 and 37, columns 41-42, examples 7, in particular) wherein the preparation of AAV virions is added with one or more excipients to achieve an ionic strength of at least 200 mM (see column 55, last paragraph, in particular) containing multivalent ions (such as NaCl, **Mg**Cl_2, EDTA). The limitations of purifying rAAV virions from the lysate using ultracentrifugation and/or chromatography is met by the method of Atkinson et al because they teach the method steps (see Atkinson et al, example 7, columns 53, 54 and 55, in particular), wherein the crude lysate is subjected to nuclease treatment and CsCl gradient runs, or subjected to anion and/or cation exchange column chromatography before being pooled, and finally concentrated and diafiltered using Modified Ringer's Balanced salt solution having 5% glycerol, which is further concentrated 10-fold (i.e. the step of adding one or more excipients) on a 300K molecular weight cut off membrane (see Atkinson et al, column 54, lines 38-49, in particular), and thus, meets the claimed limitation.

Drittanti et al [U] teach preparation of high quality adeno-associated virus vectors (rAAV virions; see abstract, *Materials & Methods*, in particular) comprising providing a lysate containing rAAV virions; purifying the virions from the nuclease (Benzonase) treated cell lysate using anion and cation exchange column chromatography in tendem (see Drittanti et al, section on Materials & Methods, "Downstream processing", page 61; and figure 4, in particular), wherein the rAAV virions were recovered/eluted by adding 300 mM NaCl through a 0-1M NaCl linear gradient in 100 mM HEPES buffer.

Each of the prior art references cited supra teach a method step for the treatment of rAAV virion with a nuclease (such as Benzonase; see Zolotukhin et al, column 10, 1st paragraph; Atkinson et al, example 7 and 8, in particular; and Drittanti et al, page 61, left column, 4th paragraph, in particular), albeit as a starting step in the purification of the virions. In addition, the process steps such as ultracentrifugation (CsCl gradient), chromatography (anion and cation exchange), and diafilteration (as recited in claims 1 and 21-24) are used and explicitly disclosed by the prior art references cited (see Zolotukhin et al, figure 1, columns 10-12, in particular; see Atkinson et al, example 7, column 53, in particular; and Drittanti et al, page 61, and figure 4, page 66, in particular)

for the process of purification of rAAV virions, and for stabilizing the purified virion preparations obtained.

However, a method of preventing aggregation of preparation of rAAV virions comprising adding one or more excipients to said preparation of virions comprising a multivalent ion, **citrate**, wherein the method further comprises treating said purified virions (i.e. after step 2) as recited in instant claim 1 as currently amended) with a nuclease, such as Benzonase (see instant claim 4), is not explicitly disclosed by the inventions of Zolotukhin et al when taken with Atkinson et al and Drittanti et al.

Qu et al [V] disclose the problems associated with the concentration-induced aggregation of recombinant AAV virions, and highlight the role of ionic interactions (along with other types of interactions, presumably hydrophobic and other inter-particle interactions) in the preparation of concentrated stocks of vectors used for human gene therapy (see Qu et al, abstract, entire document). Qu et al demonstrate (using dynamic light scattering, size-exclusion chromatography, and by quantification of loss of titer following 0.2 micron filtration of the virions) that the aggregation was concentration dependent, and typically occurred when the concentrations of virions exceeded the range 0.5 x 10^{14} cp/ml for column purified preparations (see Qu et al, abstract). More importantly, Qu et al also demonstrate that changes in buffer pH values resulted in reversal of this aggregation phenomenon, and thus suggested that ionic bridges between charged amino acids (Glu, Asp, Lys, presumably of the viral capsid protein) on the surface of vector particles contribute to the inter-particle interactions, and work in concert with hydrophobic and other types of inter-particle interactions (as the virions were found to be stable in a solution of 3M CsCl at neutral pH) to result in such concentration-dependent aggregation of the viral particles.

Chen et al (IDS) 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).

Therefore, it would have been obvious to a person of ordinary skill in the art at the time this invention was made to modify the process of preparation of virions as taught by Drittanti et al or Atkinson et al or Zolotukhin et al, such that the preparation of virions is added with one or more excipients such as multivalent ion (citrate salt) as

explicitly suggested by the combined disclosures of Qu et al and Chen et al, in order to prevent the aggregation of virions which are similarly protein containing, by providing higher ionic strength preparations.

One of ordinary skill in the art would have been motivated to modify the virion preparations of Zolotukhin et al, or Atkinson et al, or Drittanti et al by adding sodium citrate to the preparation of virions because (1) Qu et al explicitly identify the problem of particle aggregation in standard preparations of AAV virions, and suggests the role of ionic interactions (among other type of interactions involved) in the concentration-induced aggregation of virions, and (2) Chen et al demonstrate that using multivalent ions such as citrate salts can effectively reduce/suppress aggregation of fairly unstable proteins (such as rhKGF; see discussion above), thus providing a conceptual as well as practical basis for such modification of the preparations obtained by the invention of Zolotukhin et al, or Atkinson et al, or Drittanti et al.

The person of ordinary skill in the art would have had a reasonable expectation of success when adding citrate as a multivalent ion in order to prevent the aggregation of virions as obtained by the process taught by Zolotukhin et al, or Atkinson et al, or Drittanti et al, because Chen et al explicitly demonstrates the use of salts of **citrate** in the stabilization of highly unstable protein formulations, such as rhKGF. Since, the viral capsid (outer coat) proteins have been shown to undergo similar concentration-induced aggregation phenomenon (as disclosed/suggested by Qu et al; see discussion above), and since Qu et al suggest the role of ionic inter-particle interactions in such aggregation, one of ordinary skill in the art would have been motivated to adapt the

strategies taught by Chen et al (i.e. incorporation of multivalent ion such as citrate), and would have had a reasonable expectation of success in avoiding such aggregation in the preparations of virions.

The limitation of claim 7 (wherein the osmolarity of the preparation of virions after addition of the one or more excipients is no greater than about 280 mOsm) would have been a matter of routine optimization to an artisan of ordinary skill in the art as evidenced by the fact that each of the prior art references have used Lactated or modified Ringer's balanced salt solution to concentrate the final purified rAAV product (i.e. using the step of concentration and diafilteration against Ringer's solution; see Zolotukhin et al, example 1, column 12, lines 49-54, in particular; Atkinson et al, column 54, lines 42-44, in particular; and Drittanti et al, page 61, right column, 4th paragraph, in particular) in order to match the physiological osmolarity (for example, of plasma about 300 mOsm, for use in *in vivo* applications in animal models).

The limitations of claim 9 (wherein the recovery of the virions is at least about 90% after filtration through a 0.22 micron filter) would have been a matter of routine optimization for an artisan of ordinary skill in the art as evident by the fact that Drittanti et al (see, page 61 right column), Atkinson, et al (see column 54, 2nd paragraph, in particular), or Zolotukhin et al (see column 5-6, in particular) disclose the step of filtration and recovery of the virions through 0.22 micron filter, and provide the basis for optimization of such method steps.

The limitations of claim 8 (wherein the average particle radius of the virions is less than about 20 nm as measured by dynamic light scattering) would have been a

matter of routine optimization for an artisan of ordinary skill in the art (as evident by the fact that Qu et al disclose the process of using dynamic light scattering as one of the techniques for measuring/assessing the size of AAV virion aggregates and thus quantifying the loss of virions following 0.2 micron filtration step; see Qu et al, abstract), and the skilled artisan recognizing the fact that such optimization are routine part of the process for development of such stable clinical formulations for gene therapy (as disclosed by the inventions of Zolotukhin et al taken with Atkinson et al and Drittanti et al).

However, a method of preventing aggregation of a preparation of rAAV virions, further comprising treating said purified virions (i.e. after step 2) as recited in instant claim 1 as currently amended) with a nuclease, such as Benzonase (see instant claims 3 and 4), is not explicitly disclosed by the inventions of Zolotukhin et al when taken with Atkinson et al and Drittanti et al (in view of Qu et al and Chen et al).

Wright et al [A2] teach a method for large scale rAAV production and purification (see abstract, summary of the invention, columns 3-4, and 11-15), and also disclose the fact that "typically DNA is digested with nuclease, such as DNase I or Benzonase before the purification process", however, they also suggest an alternative embodiment wherein, “the digestion may occur **before, after, or during** the first chromatographic purification step” (see column 15, section “*Nucleic Acid Digestion*”, in particular).

Thus, given the detailed disclosure of the method steps in the cited prior art references (as discussed above), and the fact that prior art (i.e. Wright et al) discloses the fact that "nuclease treatment" can be used to prevent aggregation induced by contaminating DNA fragments at any stage of the purification of rAAV virions including after purification, it would have been clearly obvious to a person of ordinary skill in the art to further modify the method as disclosed by Zolotukhin et al when taken with Atkinson et al and Drittanti et al to include a step of nuclease digestion with Benzonase

after the ultracentrifugation and/or chromatographic step as explicitly suggested by the disclosure of Wright et al with a reasonable expectation of success, as evidenced by the fact that such method steps have routinely been used in the cited prior art (see teachings of Zolotukhin et al when taken with Atkinson et al and Drittanti et al) in the removal of contaminating DNA fragments from the viral preparations, albeit during cell lysis stage. In the absence of any evidence to the contrary, an artisan of ordinary skill in the clinical art would have been motivated to modify the method of Zolotukhin et al when taken with Atkinson et al and Drittanti et al by further incorporating such a nuclease treatment step after the virions have been for example, semi-purified using a chromatographic and/or ultracentrifugation steps, with a reasonable expectation of success.

Similarly, the limitations of claims 21-24 (arrangement of method steps well known in the prior art for the purification of rAAV virions; see teachings of cited references as discussed above) would have been a matter arrangement of method steps, which have been explicitly disclosed and employed by the prior art references relied upon in the rejection (see teachings of the primary references, supra), and therefore, in the absence of evidence to the contrary, one of ordinary skill in the art can further incorporate a nuclease treatment step followed by an ultracentrifugation and/or chromatographic step(s) used for purification of rAAV virions from crude cell lysates containing rAAV virions, under conditions that are already known to an artisan of ordinary skill in the art by the disclosures provided in the prior art, as discussed supra.

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

As per MPEP 2144.04 (Arrangement of process steps), Ex parte Rubin, 128 USPQ 440 (Bd. App. 1959) (Prior art reference disclosing a process of making a laminated sheet wherein a base sheet is first coated with a metallic film and thereafter impregnated with a thermosetting material was held to render prima facie obvious claims directed to a process of making a laminated sheet by reversing the order of the prior art process steps.). See also In re Burhans, 154 F.2d 690, 69 USPQ 330 (CCPA 1946) (selection of any order of performing process steps is prima facie obvious in the absence of new or unexpected results); In re Gibson, 39 F.2d 975, 5 USPQ 230 (CCPA 1930) (Selection of any order of mixing ingredients is prima facie obvious.).

As per MPEP 2144.05 [R3], II. OPTIMIZATION OF RANGES - A. Optimization Within Prior Art Conditions or Through Routine Experimentation: Generally, differences in concentration or temperature will not support the patentability of subject matter encompassed by the prior art unless there is evidence indicating such concentration or temperature is critical. "[W]here 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, 105 USPQ 233, 235 (CCPA 1955).

As per MPEP 2144, [T]the rationale to modify or combine the prior art does not have to be expressly stated in the prior art; the rationale may be expressly or impliedly contained in the prior art or it may be reasoned from knowledge generally available to one of ordinary skill in the art, established scientific principles, or legal precedent established by prior case law. 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).

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 Arguments

Applicant's arguments with respect to claims 1, 3-9, and 21-24 (as they pertain to the cited prior art rejections of record) have been considered but are moot in view of the new ground(s) of rejection.

However, applicants argue the following against the obviousness rejection of record (see remarks, page 4):

> "Further, Applicants respectfully assert that the Office has also failed to establish a prima facie case of obviousness with respect to claims 3 and 4. The instant claims have been amended to clarify that purified rAAV are those virions that have been separated from a lysate comprising said virions. Accordingly, claims 3 and 4 are drawn to the instant method that treats purified virions with nuclease to prevent virion aggregation.
>
> The Office has failed to provide a reference that teaches or suggests the use of nuclease treatment with purified rAAV. The references cited by the Office treat the cell lysate with nuclease, e.g. use nuclease treatment prior to purification. As noted by the instant specification at page 9, last paragraph, such early treatment with nuclease does not reduce aggregation. Absent this element in the prior art, the Office has failed to establish a prima facie case of obviousness for claims 3 and 4 as such a case requires that the prior art references teach or suggest all claim limitations. Applicants respectfully request withdrawal of the instant rejection."

In response, it is noted that such modification of the prior art method for preventing aggregation of rAAV virions as to incorporate a nuclease treatment step (such as DNase I or Benzonase) after semi-purification of virions would have been a method step clearly obvious to an artisan of ordinary skill in the art, as evidenced by the disclosure of, for example, of Zolotukhin et al (see column 12, last paragraph, in particular) wherein a purified viral stock is first treated with DNase I (a nuclease treatment step to remove any contaminating DNA fragments) before further downstream applications and use. However, as discussed above in the obviousness rejection in record, such modifications are explicitly suggested and disclosed by the prior art reference of Wright et al (US 6,593,123 B1; column 15, lines 22-33, in particular), which has been cited as a matter of record, now.

Therefore, the pending claims (as currently amended) are properly rejected under 35 USC 103(a) over the cited prior art.

Pertinent Prior art not relied upon in the rejections

1. Wu et al. (US 6,689,600 B1), Formulation of adenovirus for gene therapy (see abstract, columns 6, 12-14, 19-20, in particular).

2. Croyle et al. (US 6,399,385 B1), Method for rapid PEG-modification of viral vectors, compositions for enhanced gene transduction, compositions with enhanced physical stability, and uses therefor (see abstract, summary of the invention, table 3, in particular).

3. Orlov et al. Macroscopic aggregation of Tobacco Mosaic Virus coat protein, *Biochemistry* (Moscow), 2001, 66(2): 154-162 (especially, abstract, Materials & Methods, page 157, in particular).

Conclusion

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Satyendra K. 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

/Irene Marx/

Primary Examiner, Art Unit 1651

Notice of References Cited	Application/Control No. 11/141,996	Applicant(s)/Patent Under Reexamination WRIGHT ET AL.	
	Examiner SATYENDRA K. 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-6,593,123	07-2003	Wright 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	
	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.

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	11141996	WRIGHT ET AL.
	Examiner	Art Unit
	SATYENDRA K SINGH	1657

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIM		DATE								
Final	Original	02/12/2008								
	1	✓								
	2	-								
	3	✓								
	4	✓								
	5	✓								
	6	✓								
	7	✓								
	8	✓								
	9	✓								
	10	-								
	11	-								
	12	-								
	13	-								
	14	-								
	15	-								
	16	-								
	17	-								
	18	-								
	19	-								
	20	-								
	21	✓								
	22	✓								
	23	✓								
	24	✓								

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	11141996	WRIGHT ET AL.
	Examiner	**Art Unit**
	SATYENDRA K SINGH	1657

SEARCHED			
Class	**Subclass**	**Date**	**Examiner**

SEARCH NOTES		
Search Notes	**Date**	**Examiner**
EAST: USPAT, USOCR, US-PGPUB, JPO, EPO, DERWENT- UPDATED & ATTACHED.	2/12/08	SKS
INVENTOR SEARCH: PALM & EAST- UPDATED	2/13/08	SKS

INTERFERENCE SEARCH			
Class	**Subclass**	**Date**	**Examiner**

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CONFIRMATION NO. 5399

SERIAL NUMBER	FILING or 371(c) DATE RULE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
11/141,996	06/01/2005	435	1657	0800-0045

APPLICANTS

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

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

This appln 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 **** ** SMALL ENTITY **
07/06/2005

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	20	3

ADDRESS

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

TITLE

Compositions and methods to prevent AAV vector aggregation

FILING FEE RECEIVED		
565	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

EAST Search History

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
S48	2	(process$6 or method or step) near10 (prevent$6 or reduc$6 or decreas$6 or eliminat$6 or avoid$6) near10 (aggregat$6 or clump$6 or precipitat$6 or agglutinat$6) near10 (aav or raav or adenoassoc$6)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/11/29 17:50
S49	574	(AAV near3 virion$3)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2008/02/12 14:35
S50	11	aggreagat$6 same (prevent$6 or stabiliz$6)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2008/02/12 14:36
S51	21713	purif$6 and centrifug$6 and (column or chromatograph$6) and (ionic near3 strength)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2008/02/12 14:39
S52	2361	purif$6 and ultracentrifug$6 and (column or chromatograph$6) and (ionic near3 strength)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2008/02/12 14:39
S53	18	S49 and S52	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2008/02/12 14:40
S54	18	(rAAV or AAV) and (ionic near3 strength).clm.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2008/02/12 14:42
S55	72	(rAAV or AAV) and (ionic near3 strength) and (ultracentrifug$6 or chromatograph$6).clm.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2008/02/12 14:43

S56	9	(wright near3 john near3 fraser)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2008/02/13 14:43
S57	11	guang near3 qu	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2008/02/13 14:45

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Date 8/19/08 Signature

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:

JOHN FRASER WRIGHT et al. Confirmation No.: 5399

Application No.: 11/141,996 Art Unit: 1657

Filing Date: June 1, 2005 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 February 19, 2008, 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. (Previously presented) A method of preventing aggregation of recombinant adeno-associated virus (rAAV) virions in a preparation of rAAV virions, comprising:

1) providing a lysate comprising rAAV virions;

2) purifying rAAV virions from the lysate using ultracentrifugation and/or chromatography, wherein said virions are purified; and

3) adding one or more excipients to said purified virions to produce a preparation of virions with ~~achieve~~ an ionic strength of at least 200 mM, wherein the concentration of purified rAAV virions in said preparation exceeds $1x10^{13}$ vg/ml.

2. (Canceled)

3. (Previously presented) The method of claim 1, further comprising treating said purified virions with a nuclease.

4. (Previously presented) The method of claim 3, wherein the nuclease is an endonuclease from *Serratia marcescens* (Benzonase®).

5. (Original) The method of claim 1, wherein one or more of the excipients comprises a multivalent ion.

6. (Currently amended) The method of claim ~~4~~ 5, wherein the multivalent ion is citrate.

7. (Currently amended) The method of claim 1, wherein the osmolarity of the preparations of virions after addition of the one or more excipients is no greater than about 280mOsm.

8. (Original) The method of claim 1, wherein, after addition of the one or more excipients, the average particle radius (Rh) of the virions in the preparation of virions is less than about 20nm as measured by dynamic light scattering.

9. (Currently amended) The method of claim 1, wherein, after addition of the one or more excipients, recovery of the virions is at least about 90% following filtration of the preparations of virions through a 0.22μm filter.

10-20. (Canceled)

21. (Previously presented) The method of claim 3, wherein rAAV virions are purified from the lysate using cesium chloride gradient ultracentrifugation.

22. (Previously presented) The method of claim 3, wherein rAAV virions are purified from the lysate using cation exchange chromatography.

23. (Previously presented) The method of claim 3, wherein rAAV virions are purified from the lysate using cation exchange chromatography and cesium chloride gradient ultracentrifugation.

24. (Currently amended) The method of claim 3, further comprising diafiltering the purified rAAV virions to achieve an ionic strength of at least ~~about~~ 200 mM.

II. REMARKS

Introductory Comments

Claims 1, 3-9 and 21-24 were examined in the Office Action under reply and stand variously rejected under (1) 35 U.S.C. §112, second paragraph (claim 24); and (2) 35 U.S.C. §103(a) (claims 1, 3-9 and 21-24). 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 and 35 U.S.C. §102(b).

Overview of the Above Amendments

Claim 1 has been amended to provide proper antecedent basis for dependent claims 7-9 and recites that a "preparation of virions" is produced in step 3. Additionally, the preparation is characterized by having a concentration of purified rAAV virions that exceeds $1x10^{13}$ vg/ml. Support for this recitation can be found throughout the specification at, for example, page 6, line 5 and in the examples.

Claim 6 has been amended to depend from claim 5 rather than claim 1 and claim 24 has been amended to delete the term "about."

Claims 7 and 9 have been amended to correct a 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.

Claim Objections

The Office objected to the previous claim amendments, noting that claim 6 had been amended to depend from claim 1 but was indicated as being an original claim. Claim 6 has been amended to correct this typographical error which was introduced in the previous response. Applicants apologize for any confusion this inadvertent error caused.

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

Claim 24 was rejected as indefinite based on the use of the term "about." This term has been deleted from claim 24. Thus, this basis for rejection has been overcome and withdrawal thereof is respectfully requested.

Rejections Under 35 U.S.C. §103(a)

Claims 1, 3-9 and 21-24 were 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. 6,566,118 to Atkinson et al. ("Atkinson") and Drittanti et al, *J. Gene Med.* (2001) 3:59-71 ("Drittanti'); in view of Qu et al., *Molec. Ther.* (2003) 5:S348, Abstract 901 ("Qu") and Chen et al., *J. Pharm. Sci.* (1994) 83:1657-1661 ("Chen"), and further in view of U.S. Patent No. 6,593,123 to Wright et al. ("Wright").

The Office argues each of the references of Zolotukhin, Atkinson and Drittanti teach a method step for the treatment of rAAV virions with a nuclease and that process steps such as ultracentrifugation, chromatography and diafiltration are used and disclosed by each of these references. Office Action, page 6. Qu is cited for disclosing problems associated with concentration-induced aggregation of rAAV virions and Chen is cited for disclosing strategies to suppress aggregation of proteins. Office Action, page 7. Finally, Wright is cited for teaching a method for large scale production of rAAV virions and the use of a nuclease. However, applicants submit the Office has failed to establish a prima facie case of obviousness.

All pending claims pertain to methods of preventing aggregation of rAAV virions in a **purified** preparation. The rAAV virions are purified from a lysate using ultracentrifugation and/or chromatography. One or more excipients are then added to achieve an ionic strength of at least 200 mM and the concentration of purified rAAV virions in the preparation exceeds $1x10^{13}$ vg/ml. In certain embodiments, the purified preparation is treated with a nuclease. None of the cited art, either alone or in combination, teaches or suggests adding one or more excipients to a **purified** rAAV virion preparation to achieve an ionic strength of at least 200 mM wherein the preparation includes more than $1x10^{13}$ vg/ml, as claimed. For this reason alone, the cited combination fails to render the claims obvious.

In particular, Zolotukhin (1) does not recognize that rAAV virions self-aggregate and (2) therefore does not provide suggestions regarding a method 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 applicants' 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 **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, in each of the Zolotukhin passages 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 in the previous responses, 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 $1x10^{13}$ vg/ml is desirable or necessary.

Similarly, Atkinson fails to provide the requisite teaching or suggestion to arrive at the instant invention. The Office references Figures 13, 19, 32, 36 and 37 to evidence that excipients

are added to a preparation of AAV virions to achieve an ionic strength of at least 200 mM. However, as in Zolotukhin, there is no disclosure in Atkinson regarding adding excipients to a **purified** preparation of rAAV virions to achieve an ionic strength of at least 200 mM where the purified preparation with more than $1x10^{13}$ vg/ml. Indeed, as with Zolotukhin, **all** of the steps cited by the Office, wherein the buffers utilized may have an ionic strength of greater than 200mM, are steps mid-purification. Furthermore, as with all of the references cited by the Office, the purified AAV preparations disclosed in Atkinson are formulated in a final buffer consisting of Modified Ringer's Solution with 5% glycerol (for example, see Atkinson at column 54, lines 33-44). As noted before, Modified Ringer's Solution does not have an ionic strength of at least 200 mM.

Finally, the Office cites Figures 13, 32, 36 and 37 of Atkinson as relevant to the instant invention. These Figures pertain to the adjustment of ionic strength in cell culture media, well before purification, in order to promote release of the virus into the cell culture without cell lysis. See, columns 41-42 of Atkinson. Thus, Atkinson, as with Zolotukhin, fails to teach or suggest a method where excipients are added to a **purified** preparation of rAAV virions to achieve an ionic strength of at least 200 mM in order to prevent aggregation and to result in a purified preparation with more than $1x10^{13}$ vg/ml.

Drittanti also fails to teach or suggest these elements of the claimed invention. The Office argues rAAV virions are recovered/eluted by adding 300 mM NaCl through a 0-1M NaCl linear gradient in 100 mM HEPES buffer. However, as with Zolotukhin and Atkinson, this step is not a final purification step. Rather, as explained at page 61, second column, third full paragraph, the final preparation is in Ringer-lactate buffer, and as explained above, this buffer does not have an ionic strength of at least 200 mM. Drittanti, as with Zolotukhin and Atkinson, does not teach or suggest a method where excipients are added to a **purified** preparation of rAAV virions to achieve an ionic strength of at least 200 mM in order to prevent aggregation and to result in a purified preparation with more than $1x10^{13}$ vg/ml. Thus, none of the primary references teaches or suggests the claimed invention.

The secondary references of Qu and Chen fail to cure the defects of the primary references. Qu merely proposes a mechanism for AAV vector aggregation. The Examiner asserts Qu suggests that ion bridges between charged amino acids on the surface of vector

particles contribute to inter-particle interactions. At best, this teaching might lead one of skill in the art to adjust the pH of the final formulation, which Qu expressly teaches results "in reversal of concentration-induced vector aggregation". Alternatively, the skilled artisan might postulate that high concentrations of free amino acids could block vector particle interactions. However, as explained at page 9, lines 18-20 of the specification, 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 states that further work is needed in order to provide a viable purification procedure.

Chen also does not make up for the failures of the primary references and Qu. 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. Moreover, Chen used sulfated polysaccharides in combination with citrate to prevent aggregation. There is no suggestion in Chen to use citrate alone, or to carry out a purification protocol as claimed in order to prevent aggregation of rAAV virions and to result in a purified preparation with more than $1x10^{13}$ vg/ml of rAAV virions.

Finally, Wright does not provide the elements missing from Zolotukhin, Atkinson, Drittanti, Qu and Chen. Wright nowhere mentions that self-aggregation of rAAV virions occurs and hence does not teach or suggest methods for preventing aggregation of rAAA virions as claimed. Wright uses high salt concentrations initially to promote release of rAAV virions from cells and in some mid-purification steps. The only discussion in Wright regarding adding excipients to the final purified product is in Example 9. However, unlike applicants' purification method, the excipient added is phosphate-buffered saline, pH 7.1, containing 5% sorbitol. This buffer does not have an ionic strength of at least 200 mM.

Thus, Wright, as with all of the references discussed above, does not teach or suggest a method for preventing aggregation of rAAV virions where excipients are added to a purified

preparation of rAAV virions to achieve an ionic strength of at least 200 mM and to result in a purified preparation with more than $1x10^{13}$ vg/ml.

Applicants' process, on the other hand, provides a commercially viable method for producing high amounts of rAAV virions. As explained at pages 16-17 of the specification and in Table 2, vector recovery using applicants' claimed methods results in yields of more than 90%.

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. The very fact that the Examiner relies on six references in making the present obviousness rejection is evidence of the above. 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.

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: 8/19/08

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

ROBINS & PASTERNAK LLP
1731 Embarcadero Road, Suite 230
Palo Alto, CA 94303
Telephone: (650) 493-3400
Facsimile: (650) 493-3440

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In Re Application of:	Examiner: SINGH, Satyendra K.
JOHN FRASER WRIGHT, et al.	Group Art Unit: 1657
Serial No.: 11/141,996	Confirmation No.: 5399
Filing Date: June 1, 2005	Customer No.: 20855
Title: COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION	

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

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

Dear Sirs:

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 copies of publications B1 and C1-C5 cited in the attached Form PTO/SB/08 are enclosed.

[] No copy of the U.S. publications listed on the attached Form PTO/SB/08A is 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.

[X] Publications B1 and C1-C5 listed on the attached Form PTO/SB/08A were cited in a foreign search or examination report corresponding to EP application serial no. 05755269.7 and mailed on April 1, 2008.

[] 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

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Respectfully submitted,

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

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

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U.S. PATENT DOCUMENTS

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OTHER PRIOR ART – NON PATENT LITERATURE DOCUMENTS

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	C1	QU, *et al.*, "Scaling Up Production of Recombinant AAV Vectors for Clinical Applications," *Curr Opin Drug Disc Dev* 3(6):750-755 (2000)	
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(71) Applicant *(for all designated States except US)*: UNIVERSITY OF FLORIDA [US/US]; 1938 W. University Avenue, Gainesville, FL 32603 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: ZOLOTUKHIN, Sergei [US/US]; 1122 S.W. 96th Street, Gainesville, FL 32607 (US). BYRNE, Barry, J. [US/US]; 7902 S.W. 45th Lane, Gainesville, FL 32608 (US). MUZYCZKA, Nicholas [US/US]; 9837 S.W. 67th Drive, Gainesville, FL 32608 (US).

(74) Agent: HIBLER, David, W.; Williams, Morgan & Amerson, P.C., Suite 250, 7676 Hillmont, Houston, TX 77040 (US).

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(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.

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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 x 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.

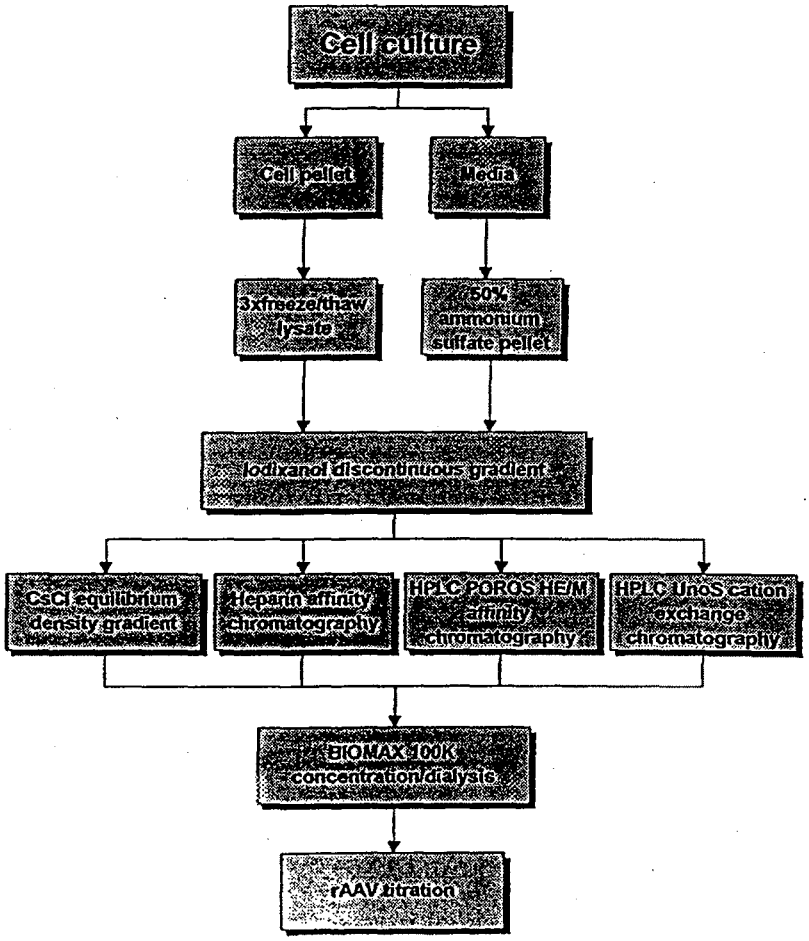
27. The kit of claim 25, wherein said matrix comprises heparin agarose type I or heparin agarose type II-S.

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



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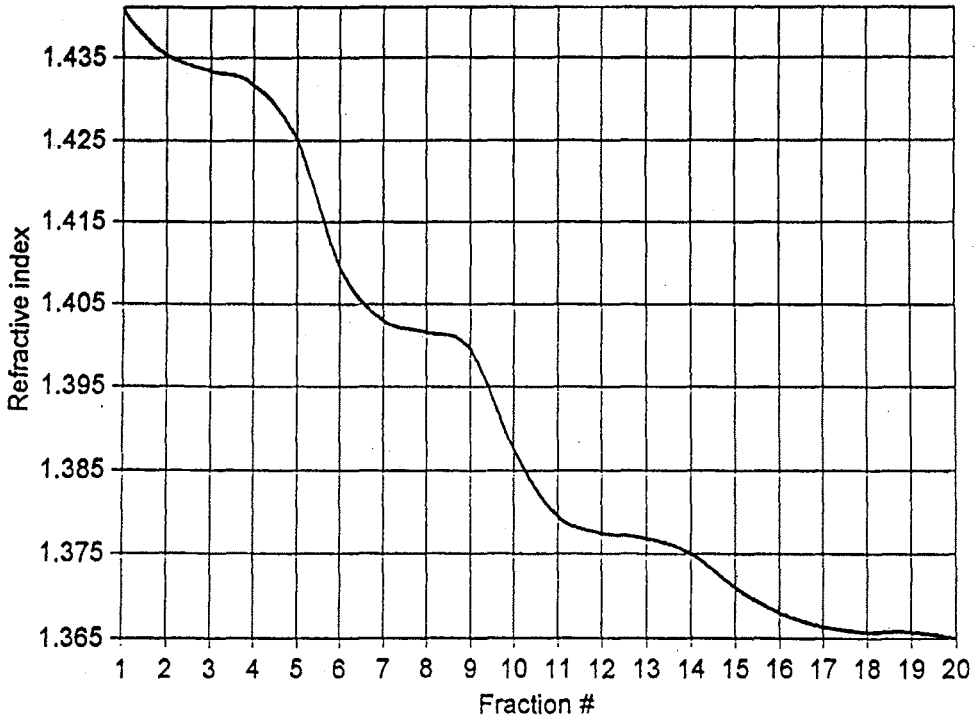
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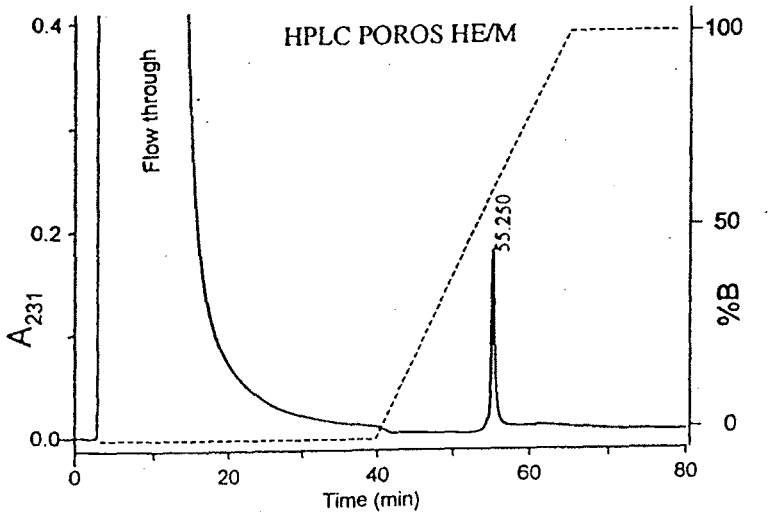
FIG. 2

FIG. 3A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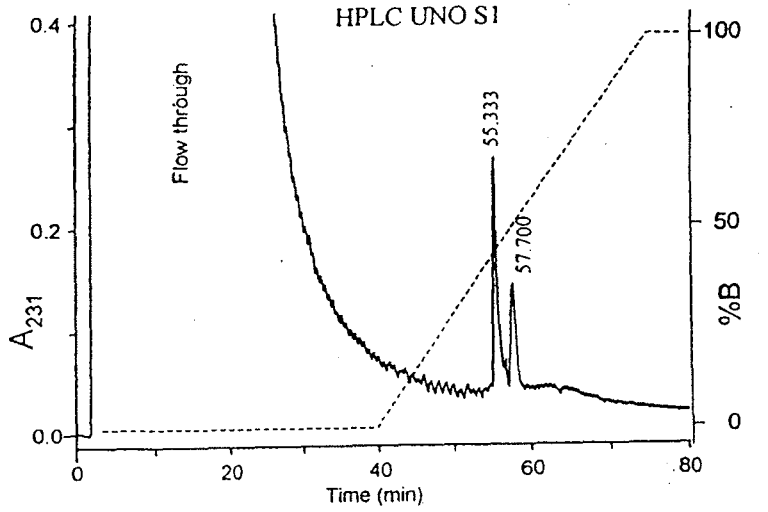
60
80

FIG. 3B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SEQUENCE LISTING

<110> ZOLOTUKHIN, SERGEI
BYRNE, BARRY J.
MUZYCZKA, NICHOLAS

<120> METHOD OF PREPARING RECOMBINANT ADENO-ASSOCIATED VIRUS COMPOSITIONS

<130> 4300.007800/4300.007810

<140> UNKNOWN
<141> 1999-05-27

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<151> 1998-05-27

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<170> PatentIn Ver. 2.0

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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

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

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

Electronic Patent Application Fee Transmittal

Application Number:	11141996
Filing Date:	01-Jun-2005
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

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	1050	1050

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
Submission- Information Disclosure Stmt	1806	1	180	180
		Total in USD ($)		**1230**

Electronic Acknowledgement Receipt

EFS ID:	3805970
Application Number:	11141996
International Application Number:	
Confirmation Number:	5399
Title of Invention:	Compositions and methods to prevent AAV vector aggregation
First Named Inventor/Applicant Name:	John Fraser Wright
Customer Number:	31048
Filer:	Roberta L. Robins/Denise Vaillancourt
Filer Authorized By:	Roberta L. Robins
Attorney Docket Number:	0800-0045
Receipt Date:	19-AUG-2008
Filing Date:	01-JUN-2005
Time Stamp:	17:23:14
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Credit Card
Payment was successfully received in RAM	$1230
RAM confirmation Number	4215
Deposit Account	
Authorized User	

File Listing:

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

1		roaids_20080819141322.pdf	6984624 9425833896bf9e418418a90e1dbd21369f92345b	yes	104

Multipart Description/PDF files in .zip description

Document Description	Start	End
Miscellaneous Incoming Letter	1	1
Extension of Time	2	2
Amendment - After Non-Final Rejection	3	3
Claims	4	5
Applicant Arguments/Remarks Made in an Amendment	6	12
Information Disclosure Statement Letter	13	15
Information Disclosure Statement (IDS) Filed (SB/08)	16	16
Foreign Reference	17	72
NPL Documents	73	78
NPL Documents	79	88
NPL Documents	89	95
NPL Documents	96	96
NPL Documents	97	104

Warnings:

Information:

2	Fee Worksheet (PTO-06)	fee-info.pdf	31869 8545fe6e70914153d2a39960ce72b4fd56248adf	no	2

Warnings:

Information:

Total Files Size (in bytes):	7016493

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	104

Application Number	11/141,996
Filing Date	June 1, 2005
First Named Inventor	John Fraser Wright
Art Unit	Unassigned
Examiner Name	Unassigned
Attorney Docket Number	0800-0045

ENCLOSURES *(Check all that apply)*

- ☐ Fee Transmittal Form
  - ☐ Fee Attached
- ☒ Amendment/Reply (10 pages)
  - ☐ After Final
  - ☐ Affidavits/declaration(s)
- ☒ Extension of Time Request (1 page)
- ☐ 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):

Information Disclosure Statement (3 pages)
PTO/SB/08A (1 page)
Copies of 6 references cited (total 88 pages)

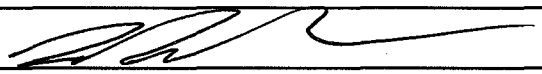
Remarks The Commissioner is authorized to charge any additional fees to Deposit Account 18-1648.

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	Robins & Pasternak LLP		
Signature			
Printed name	Roberta L. Robins		
Date	8/19/08	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	8/19/08

PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a) FY 2008 *(Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).)*	Docket Number (Optional) 0800-0045
Application Number 11/141,996	Filed June 1, 2005
For COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION	
Art Unit 1651	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))	$120	$60	$
☐ Two months (37 CFR 1.17(a)(2))	$460	$230	$
☒ Three months (37 CFR 1.17(a)(3))	$1050	$525	$ 1050
☐ Four months (37 CFR 1.17(a)(4))	$1640	$820	$
☐ Five months (37 CFR 1.17(a)(5))	$2230	$1115	$

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

☐ A check including 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 additional 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.

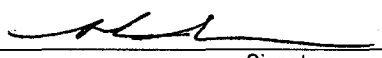
I am the

☐ 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 33,208

☐ attorney or agent under 37 CFR 1.34.
Registration number if acting under 37 CFR 1.34 _____



Signature

8/19/08
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 One forms are submitted.

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
11/141,996	06/01/2005	

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	08/19/2008	(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))	* 12	Minus	** 20	= 0	X $25 =	0	OR	X $ =	
	Independent (37 CFR 1.16(h))	* 1	Minus	***3	= 0	X $105 =	0	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	0	OR	TOTAL ADD'L FEE	

AMENDMENT		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)	OR	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:
/LINDA W. BADIE/

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United States Patent and Trademark Office
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/141,996	06/01/2005	John Fraser Wright	0800-0045	5399

31048 7590 11/18/2008

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

EXAMINER	
SINGH, SATYENDRA K	

ART UNIT	PAPER NUMBER
1657	

MAIL DATE	DELIVERY MODE
11/18/2008	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)	
	11/141,996	WRIGHT ET AL.	
	Examiner	Art Unit	
	SATYENDRA K. 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 *19 August 2008*.

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

3)☐ 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

4)☒ Claim(s) *1,3-9 and 21-24* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,3-9 and 21-24* is/are rejected.

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

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

Application Papers

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

10)☒ The drawing(s) filed on *01 June 2005* 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).

11)☐ 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

12)☐ 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 *8/19/08*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Applicant's response and amendments to claims filed on August 19th 2008 is duly acknowledged.

Claims 2 and 10-20 have been canceled by applicant's previous amendments.

Claims 1, 3-9 and 21-24 (group I, as currently amended) are examined on their merits in this office action.

The following contains new grounds of rejection necessitated by applicant's current amendments to pending claims.

Claim Rejections - 35 USC § 112

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

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

1. Claims 1, 3-9 and 21-24 (as currently amended) are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. Claim 1 now recites the limitation of "wherein the concentration of **purified rAAV virions in said preparation exceeds $1x10^{13}$ vg/ml**", which is not fully supported by the instant disclosure as originally filed by applicants. Applicant's remarks that " *Claim 1 has been amended.....Additionally, the preparation is characterized by having a concentration of purified rAAV virions that exceeds $1x10^{13}$ vg/ml. Support for this recitation can be found throughout the specification at, for example, page 6, line 5 and in the*

examples" is not found to be accurate. For the record, the specification at page 6, 2nd paragraph states the following:

> "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 6.4x 10^{13} vg/mL**, without significant aggregation."

Thus, it is clear that the claimed recitation "wherein the concentration of purified rAAV virions in said preparation exceeds $1x10^{13}$ vg/ml" which is interpreted as a concentration of purified virions exceeding $1x10^{13}$ vg/ml and up to infinity, a broader range than the range actually disclosed in the original disclosure by applicants. There is no specific examples that show or contemplate such a range of concentration for the purified rAAV virions as currently presented in the invention as claimed. This is a matter of written description, not a question of what one of skill in the art would or would not have known. The material within the four corners of the as-filed specification must lead to the generic concept. If it does not, the material is new matter. Declarations and new references cannot demonstrate possession of a concept after the fact. Thus, the insertion of said limitation in claim 1 as currently amended is considered to be the insertion of **new matter** for the above reasons. Appropriate correction is required.

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, 3-9 and 21-24 (as currently amended) are/remain rejected under 35 U.S.C. 103(a) as being unpatentable over Zolotukhin et al (US 6,146,874; [A]) taken with Atkinson et al (US 6,566,118 B1; [C]) and Drittanti et al [U] in view of Qu et al [V], Chen et al (IDS), and Wright et al (US 6,593,123 B1; [A2]).

> Claims are generally directed to **a method of preventing aggregation of rAAV virions** in a preparation of rAAV virions, comprising 1) providing a lysate comprising rAAV virions; 2) purifying rAAV virions from the lysate using ultracentrifugation and/or chromatography, wherein said virions are purified; and 3) adding one or more excipients to said purified virions to produce a preparation of virions with an ionic strength of at least 200 mM, wherein the concentration of purified rAAV virions in said preparation exceeds $1x10^{13}$ vg/ml. (see also specific recitations of the instant claims 3-9, and 21-24)

Zolotukhin et al [A] teach a preparation of virions (rAAV virions; see abstract, summary of the invention, column 9-10, in particular) comprising excipients having ionic strength of at least 200mM and having multivalent ions (such as salt and buffers; see Zolotukhin et al, column 10, 1 M NaCl-PBS-MgCl2 and KCl buffer containing multivalent ions such as magnesium and phosphate; see also applicant's exemplification of multivalent ions at page 20, 2nd paragraph, in particular). The limitations of purifying rAAV virions from the lysate using ultracentrifugation and/or chromatography is met as Zolotukhin et al teach the step of purifying the crude lysate through Iodixanol density gradient which uses ultracentrifugation step, and further purifying the rAAV virions obtained from the above Iodixanol gradient by using a chromatographic step, wherein the "purified" preparation of virions (for example, bound to column such as Heparin-ligand affinity media; see Zolotukhin et al, the entire columns 10 and 11, in particular) was added with one or more excipients (such as PBS-MK with 1M NaCl for elution from the column; see Zolotukhin et al, column 11, lines 36-37, in particular), and the concentrations of purified preparation of virions comprise greater than about 10^{11}, 10^{12}, and even greater than about 10^{13} or 10^{14} particles/ml (taken as vg/ml; see column 6, 1st paragraph, and column 22, section "rAAV Recovery", in particular). In addition, Zolotukhin et al clearly suggest the fact that "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 (see column 3, last paragraph, in particular).

Atkinson et al [C] teach a method for generating high titer helper-free preparations of released recombinant AAV vectors (rAAV; see Atkinson et al, abstract, summary of the invention, Figures 13, 19, 32, 36 and 37, columns 41-42, examples 7, in particular) wherein the preparation of AAV virions is added with one or more excipients to achieve an ionic strength of at least 200 mM (see column 55, last paragraph, in particular) containing multivalent ions (such as NaCl, **Mg**Cl_2, EDTA). The limitations of purifying rAAV virions from the lysate using ultracentrifugation and/or chromatography is met by the method of Atkinson et al because they teach the method steps (see Atkinson et al, example 7, columns 53, 54 and 55, in particular), wherein the crude lysate is subjected to nuclease treatment and CsCl gradient runs, or subjected to anion and/or cation exchange column chromatography before being pooled, and finally concentrated and diafiltered using Modified Ringer's Balanced salt solution having 5% glycerol, which is further concentrated 10-fold (i.e. the step of adding one or more excipients) on a 300K molecular weight cut off membrane (see Atkinson et al, column 54, lines 38-49, in particular), and thus, meets the claimed limitation.

Drittanti et al [U] teach preparation of high quality adeno-associated virus vectors (rAAV virions; see abstract, *Materials & Methods*, in particular) comprising providing a lysate containing rAAV virions; purifying the virions from the nuclease (Benzonase) treated cell lysate using anion and cation exchange column chromatography in tendem (see Drittanti et al, section on Materials & Methods, "Downstream processing", page 61; and figure 4, in particular), wherein the rAAV virions were recovered/eluted by adding 300 mM NaCl through a 0-1M NaCl linear gradient in 100 mM HEPES buffer.

Each of the prior art references cited supra teach a method step for the treatment of rAAV virion with a nuclease (such as Benzonase; see Zolotukhin et al, column 10, 1st paragraph; Atkinson et al, example 7 and 8, in particular; and Drittanti et al, page 61, left column, 4th paragraph, in particular), albeit as a starting step in the purification of the virions. In addition, the process steps such as ultracentrifugation (CsCl gradient), chromatography (anion and cation exchange), and diafilteration (as recited in claims 1 and 21-24) are used and explicitly disclosed by the prior art references cited (see Zolotukhin et al, figure 1, columns 10-12, in particular; see Atkinson et al, example 7, column 53, in particular; and Drittanti et al, page 61, and figure 4,

page 66, in particular) for the process of purification of rAAV virions, and for stabilizing the purified virion preparations obtained.

However, a method of preventing aggregation of preparation of rAAV virions comprising adding one or more excipients to said preparation of virions comprising a multivalent ion, **citrate**, is not explicitly disclosed by the inventions of Zolotukhin et al when taken with Atkinson et al and Drittanti et al.

Qu et al [V] disclose the problems associated with the concentration-induced aggregation of recombinant AAV virions, and highlight the role of ionic interactions (along with other types of interactions, presumably hydrophobic and other inter-particle interactions) in the preparation of concentrated stocks of vectors used for human gene therapy (see Qu et al, abstract, entire document). Qu et al demonstrate (using dynamic light scattering, size-exclusion chromatography, and by quantification of loss of titer following 0.2 micron filtration of the virions) that the aggregation was concentration dependent, and typically occurred when the concentrations of virions exceeded the range 0.5×10^{14} cp/ml for column purified preparations (see Qu et al, abstract). More importantly, Qu et al also demonstrate that changes in buffer pH values resulted in reversal of this aggregation phenomenon, and thus suggested that ionic bridges between charged amino acids (Glu, Asp, Lys, presumably of the viral capsid protein) on the surface of vector particles contribute to the inter-particle interactions, and work in concert with hydrophobic and other types of inter-particle interactions (as the virions were found to be stable in a solution of 3M CsCl at neutral pH) to result in such concentration-dependent aggregation of the viral particles.

Chen et al (IDS) 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).

Therefore, it would have been obvious to a person of ordinary skill in the art at the time this invention was made to modify the process of preparation of virions as taught by Drittanti et al or Atkinson et al or Zolotukhin et al, such that the preparation of virions is added with one or more excipients such as multivalent ion (citrate salt) as explicitly suggested by the combined

disclosures of Qu et al and Chen et al, in order to prevent the aggregation of virions which are similarly protein containing, by providing higher ionic strength preparations.

One of ordinary skill in the art would have been motivated to modify the virion preparations of Zolotukhin et al, or Atkinson et al, or Drittanti et al by adding sodium citrate to the preparation of virions because (1) Qu et al explicitly identify the problem of particle aggregation in standard preparations of AAV virions, and suggests the role of ionic interactions (among other type of interactions involved) in the concentration-induced aggregation of virions, and (2) Chen et al demonstrate that using multivalent ions such as citrate salts can effectively reduce/suppress aggregation of fairly unstable proteins (such as rhKGF; see discussion above), thus providing a conceptual as well as practical basis for such modification of the preparations obtained by the invention of Zolotukhin et al, or Atkinson et al, or Drittanti et al.

The person of ordinary skill in the art would have had a reasonable expectation of success when adding citrate as a multivalent ion in order to prevent the aggregation of virions as obtained by the process taught by Zolotukhin et al, or Atkinson et al, or Drittanti et al, because Chen et al explicitly demonstrates the use of salts of **citrate** in the stabilization of highly unstable protein formulations, such as rhKGF. Since, the viral capsid (outer coat) proteins have been shown to undergo similar concentration-induced aggregation phenomenon (as disclosed/suggested by Qu et al; see discussion above), and since Qu et al suggest the role of ionic inter-particle interactions in such aggregation, one of ordinary skill in the art would have been motivated to adapt the strategies taught by Chen et al (i.e. incorporation of multivalent ion such as citrate), and would have had a reasonable expectation of success in avoiding such aggregation in the preparations of virions.

The limitation of claim 7 (wherein the osmolarity of the preparation of virions after addition of the one or more excipients is no greater than about 280 mOsm) would have been a matter of routine optimization to an artisan of ordinary skill in the art as evidenced by the fact that each of the prior art references have used Lactated or modified Ringer's balanced salt solution to concentrate the final purified rAAV product (i.e. using the step of concentration and diafilteration against Ringer's solution; see Zolotukhin et al, example 1, column 12, lines 49-54, in particular; Atkinson et al, column 54, lines 42-44, in particular; and Drittanti et al, page 61, right column, 4th paragraph, in particular) in order to match the physiological osmolarity (for example, of plasma about 300 mOsm, for use in *in vivo* applications in animal models).

The limitations of claim 9 (wherein the recovery of the virions is at least about 90% after filtration through a 0.22 micron filter) would have been a matter of routine optimization for an artisan of ordinary skill in the art as evident by the fact that Drittanti et al (see, page 61 right column), Atkinson, et al (see column 54, 2nd paragraph, in particular), or Zolotukhin et al (see column 5-6, in particular) disclose the step of filtration and recovery of the virions through 0.22 micron filter, and provide the basis for optimization of such method steps.

The limitations of claim 8 (wherein the average particle radius of the virions is less than about 20 nm as measured by dynamic light scattering) would have been a matter of routine optimization for an artisan of ordinary skill in the art (as evident by the fact that Qu et al disclose the process of using dynamic light scattering as one of the techniques for measuring/assessing the size of AAV virion aggregates and thus quantifying the loss of virions following 0.2 micron filtration step; see Qu et al, abstract), and the skilled artisan recognizing the fact that such optimization are routine part of the process for development of such stable clinical formulations

for gene therapy (as disclosed by the inventions of Zolotukhin et al taken with Atkinson et al and Drittanti et al).

However, a method of preventing aggregation of a preparation of rAAV virions, further comprising treating said purified virions (i.e. after step 2) as recited in instant claim 1 as currently amended) with a nuclease, such as Benzonase (see instant claims 3 and 4), is not explicitly disclosed by the inventions of Zolotukhin et al when taken with Atkinson et al and Drittanti et al (in view of Qu et al and Chen et al).

Wright et al [A2] teach a method for large scale rAAV production and purification (see abstract, summary of the invention, columns 3-4, and 11-15), and also disclose the fact that "typically DNA is digested with nuclease, such as DNase I or Benzonase before the purification process", however, they also suggest an alternative embodiment wherein, "the digestion may occur **before, after, or during** the first chromatographic purification step" (see column 15, section "*Nucleic Acid Digestion*", in particular).

Thus, given the detailed disclosure of the method steps in the cited prior art references (as discussed above), and the fact that prior art (i.e. Wright et al) discloses the fact that "nuclease treatment" can be used to prevent aggregation induced by contaminating DNA fragments at any stage of the purification of rAAV virions including after purification, it would have been clearly obvious to a person of ordinary skill in the art to further modify the method as disclosed by Zolotukhin et al when taken with Atkinson et al and Drittanti et al to include a step of nuclease digestion with Benzonase after the ultracentrifugation and/or chromatographic step as explicitly suggested by the disclosure of Wright et al with a reasonable expectation of success, as evidenced by the fact that such method steps have routinely been used in the cited prior art (see teachings of Zolotukhin et al when taken with Atkinson et al and Drittanti et al) in the removal of contaminating DNA fragments from the viral preparations, albeit during cell lysis stage. In the absence of any evidence to the contrary, an artisan of ordinary skill in the clinical art would have been motivated to modify the method of Zolotukhin et al when taken with Atkinson et al and

Drittanti et al by further incorporating such a nuclease treatment step after the virions have been for example, semi-purified using a chromatographic and/or ultracentrifugation steps, with a reasonable expectation of success.

Similarly, the limitations of claims 21-24 (arrangement of method steps well known in the prior art for the purification of rAAV virions; see teachings of cited references as discussed above) would have been a matter arrangement of method steps, which have been explicitly disclosed and employed by the prior art references relied upon in the rejection (see teachings of the primary references, supra), and therefore, in the absence of evidence to the contrary, one of ordinary skill in the art can further incorporate a nuclease treatment step followed by an ultracentrifugation and/or chromatographic step(s) used for purification of rAAV virions from crude cell lysates containing rAAV virions, under conditions that are already known to an artisan of ordinary skill in the art by the disclosures provided in the prior art, as discussed supra.

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

> *As per MPEP 2144.04 (Arrangement of process steps), Ex parte Rubin, 128 USPQ 440 (Bd. App. 1959) (Prior art reference disclosing a process of making a laminated sheet wherein a base sheet is first coated with a metallic film and thereafter impregnated with a thermosetting material was held to render prima facie obvious claims directed to a process of making a laminated sheet by reversing the order of the prior art process steps.). See also In re Burhans, 154 F.2d 690, 69 USPQ 330 (CCPA 1946) (selection of any order of performing process steps is prima facie obvious in the absence of new or unexpected results); In re Gibson, 39 F.2d 975, 5 USPQ 230 (CCPA 1930) (Selection of any order of mixing ingredients is prima facie obvious.).*
>
> As per MPEP 2144.05 [R3], II. OPTIMIZATION OF RANGES - A. Optimization Within Prior Art Conditions or Through Routine Experimentation: Generally, differences in concentration or temperature will not support the patentability of subject matter encompassed by the prior art unless there is evidence indicating such concentration or temperature is critical. "[W]here 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, 105 USPQ 233, 235 (CCPA 1955).
>
> *As per MPEP 2144, [T]the rationale to modify or combine the prior art does not have to be expressly stated in the prior art; the rationale may be expressly or impliedly contained in the prior art or it may be reasoned from knowledge generally available to one of ordinary skill in the art, established scientific principles, or legal precedent established by prior case law. 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).*

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 Applicant's Arguments

Applicant's arguments filed August 19th 2008 (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.

Applicant's essentially seem to argue (see remarks, pages 5-9, in particular) that "*None of the cited art, either alone or in combination, teaches or suggests adding one or more excipients to a purified rAAV virion preparation to achieve an ionic strength of at least 200 mM wherein the preparation includes more than $1x10^{13}$ vg/ml, as claimed. For this reason alone, the cited combination fails to render the claims obvious*", which is not found to be persuasive because the combined teachings in the cited prior art references of record disclose all the elements, including the recognition of the problem of "self aggregation" of rAAV virions associated with purified preparations of said virions. The process as claimed includes the steps of "providing a lysate comprising rAAV virions"; and "purifying rAAV virions from the lysate using ultracentrifugation and/or chromatography" to obtain a preparation of purified virions (including other method steps as claimed in the dependent claims), which are explicitly disclosed by the references of Zolotukhin et al taken with Atkinson et al and Drittani et al (see rejection above). The cited prior art of Qu et al (in view of Chen et al) provides the motivation for using high salt concentration (see results with 3M CsCl at neutral pH) in the purified vector stocks (see Qu et al, abstract) of rAAV virions, as Chen et al disclose the benefits of having high salt concentration

(such as 0.5 M sodium citrate; see Chen et al, abstract, in particular) that helps in reducing aggregation of proteins such as rhKGF. Since, viral capsid proteins from the purified rAAV virions will under go similar interactions during "self aggregation", an artisan of ordinary skill in the art would be motivated to use a high salt stock solution (such as 0.5 M sodium citrate suggested by Chen et al to reduce protein aggregation; i.e. "at least 200 mM" ionic strangth) in order to avoid or prevent aggregation of purified stock of rAAV virions. The argument regarding the titer of viral preparation that "exceeds $1x10^{13}$ vg/ml" is not disclosed by the cited prior art is also found to be unpersuasive because Zolotukhin et al explicitly discloses such concentrations (see teachings of Zolotukhin et al above; and column 6, 1st paragraph, and column 22, section "rAAV Recovery", in particular) that can be routinely obtained by the method steps as disclosed in the prior art. Thus, addition of excipients such as multivalent ions to achieve ionic strength of at least 200 mM for a stock of purified rAAV virions as recited in instant claim 1 would have been obvious to a person of ordinary skill in the art at the time the claimed invention was made.

The argument (see page 8, 2nd paragraph) that "*Chen also does not make up for the failures of the primary references and Qu. Chen does not even relate to viruses…….of rAAV virions*" is not found to be persuasive because Chen et al provides the conceptual basis for using multivalent ions such as sodium citrate (see Chen et al, table 2, in particular) in stock solutions that comprise macromolecular components such as proteins, and viral particles in a high titer viral stock solution would be presumed by an artisan of ordinary skill in the art to interact via viral capsid proteins with each other to induce chances of aggregation, as implied by Chen et al. Thus, an artisan of ordinary skill in the macromolecular purification art would have considered

and would be motivated to use such multivalent ions as one of the stabilizing factors when trying to prevent aggregation of high titer stock solutions as claimed in the instant invention.

Regarding the arguments for the teachings of Wright et al (see remarks, page 8, 3rd paragraph), it is to be noted that Wright et al was relied upon in the obviousness rejection above to show the fact that nuclease treatment (see instant claim 4) can be performed before, after, or during the purification steps that are generally used by an artisan of ordinary skill in the art such as chromatographic steps and/or other steps that have been explicitly disclosed by the cited prior art references of record.

Applicant's argument (see remarks, page 9) that "*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*" is not found to be persuasive because no such evidentiary data has been provided by applicants that contradicts the logical outcome from the combined teachings of the prior art references as relied upon in the obviousness rejection of record. Furthermore, in response to applicant's argument that the examiner's conclusion of obviousness is based upon improper hindsight reasoning (see page 9, 4rth paragraph), it must be recognized that any judgment on obviousness is in a sense necessarily a reconstruction based upon hindsight reasoning. But so long as it takes into account only knowledge which was within the level of ordinary skill at the time the claimed invention was made, and does not include knowledge gleaned only from the applicant's disclosure, such a reconstruction is proper. See In re McLaughlin, 443 F.2d 1392, 170 USPQ 209 (CCPA 1971). The fact that factors such as ionic and hydrophobic interactions that have already been disclosed

to play important role in the stability of rAAV viral preparations in the prior art (see Qu et al in view of Chen et al and Wright et al, as discussed above) clearly suggests that the entire invention as claimed would have been obvious to an artisan of ordinary skill in the art, at the time the claimed invention was made.

Thus, the 103(a) rejection of record over the cited prior art references is deemed to be proper, and is therefore, maintained.

Pertinent Prior art not relied upon in the rejections

1. Wu et al. (US 6,689,600 B1), Formulation of adenovirus for gene therapy (see abstract, columns 6, 12-14, 19-20, in particular).

2. Croyle et al. (US 6,399,385 B1), Method for rapid PEG-modification of viral vectors, compositions for enhanced gene transduction, compositions with enhanced physical stability, and uses therefor (see abstract, summary of the invention, table 3, in particular).

3. Orlov et al. Macroscopic aggregation of Tobacco Mosaic Virus coat protein, *Biochemistry* (Moscow), 2001, 66(2): 154-162 (especially, abstract, Materials & Methods, page 157, in particular).

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 K. 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

/Irene Marx/
Primary Examiner
Art Unit 1651

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	11141996	WRIGHT ET AL.
	Examiner	Art Unit
	SATYENDRA K SINGH	1657

SEARCHED

Class	Subclass	Date	Examiner

SEARCH NOTES

Search Notes	Date	Examiner
EAST: USPAT, USOCR, US-PGPUB, JPO, EPO, DERWENT- UPDATED & ATTACHED.	2/12/08	SKS
INVENTOR SEARCH: PALM & EAST- UPDATED	2/13/08	SKS
EAST: USPAT, USOCR, US-PGPUB, JPO, EPO, DERWENT- UPDATED & ATTACHED	11/14/08	SKS
INVENTOR SEARCH: PALM & eDAN & EAST	11/14/08	SKS

INTERFERENCE SEARCH

Class	Subclass	Date	Examiner

/SATYENDRA K SINGH/ Examiner.Art Unit 1657	

EAST Search History

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
S57	11	guang near3 qu	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2008/02/13 14:45
S58	1764	wright near3 john	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2008/11/14 17:12
S59	11	guang near3 qu	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2008/11/14 17:12
S60	2	(S58 or S59) and (citrate or (multivalent near3 ion)) and (aggregat $6 near3 (prevent or avoid or reduc$6 or decreas$6))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2008/11/14 17:16
S61	57938	(vector or AAV) and aggregat$6 and (prevent$6 or reduc $6 or decreas$6 or avoid$6)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2008/11/14 17:19
S62	946	(vector or AAV) same aggregat$6 same (prevent$6 or reduc$6 or decreas $6 or avoid$6)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2008/11/14 17:19
S63	502	S62 and (viral or virion or virus)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2008/11/14 17:20
S64	257	S62 and ((viral or virion or virus) near3 (genome or particle))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2008/11/14 17:20

S65	34	S62 and ((viral or virion or virus) near3 (genome or particle)).clm.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2008/11/14 17:20
S66	16	S62 and ((viral or virion or virus) near3 (genome or particle)) and (ion$6 near3 strength).clm.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2008/11/14 17:22
S67	4	S62 and ((viral or virion or virus) near3 (genome or particle)) and (ion$6 near3 strength) and (multivalent or citrate or citric).clm.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2008/11/14 17:23

11/14/2008 5:26:47 PM
C:\Documents and Settings\ssingh3\My Documents\EAST\Workspaces\11141996-all-dbs.wsp

Please type a plus sign (+) inside this box ➔ [+]

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	11/141,996
				Filing Date	June 1, 2005
				First Named Inventor	John Fraser Wright
				Group Art Unit	1657
				Examiner Name	Satyendra K. Singh
Sheet	1	of	1	Attorney Docket Number	0800-0045

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	U.S. Patent Document Number	Kind Code[2] (*if known*)	Name of Patentee or Applicant of Cited Document	Date of Publication of Cited Document MM-DD-YYYY

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[3]	Foreign Patent Document Office	Number[6]	Kind Code[4] (if known)	Name of Patentee or Applicant of Cited Document	Date of Publication of Cited Document MM-DD-YYYY	T[7]
	B1	WO	99/61643	A	University of Florida	12-02-1999	

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	QU, *et al.*, "Scaling Up Production of Recombinant AAV Vectors for Clinical Applications," *Curr Opin Drug Disc Dev* 3(6):750-755 (2000)	
	C2	STEINBACH, *et al.*, "Assembly of Adeno-Associated Virus Type 2 Capsids In Vitro," *J Gen Virol* 78(6):1453-1462 (1997)	
	C3	SOMMER, *et al.*, "Quantification of Adeno-Associated Virus Particles and Empty Capsids By Optical Density Measurement," *Mol Ther* 7(1):122-128 (2003)	
	C4	WRIGHT, *et al.*, "425. Formulation Development for AAV2 Vectors: Identification of Excipients that Inhibit Vector Aggregation," *Mol Ther* [Online] 9(S1):S163-S163 (2004)	
	C5	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)	

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /SS/

Examiner Signature	/Satyendra Singh/	Date Considered	11/10/2008

*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.

TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Application Number	11/141,996
Filing Date	June 1, 2005
First Named Inventor	John Fraser Wright et al.
Art Unit	1657
Examiner Name	S. K. Singh
Total Number of Pages in This Submission: 3	Attorney Docket Number: 0800-0045

ENCLOSURES *(Check all that apply)*

- ☐ Fee Transmittal Form
  - ☐ Fee Attached
- ☐ Amendment/Reply
  - ☐ After Final
  - ☐ Affidavits/declaration(s)
- ☒ Extension of Time Request (1 page)
- ☐ 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):

Notice of Appeal from the Examiner to the Board of Patent Appeals and Interferences (1 page)

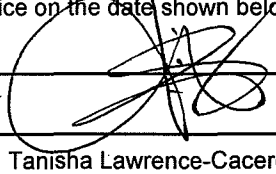
Remarks The Commissioner is authorized to charge any additional fees to Deposit Account 18-1648.

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	Robins & Pasternak LLP		
Signature			
Printed name	Roberta L. Robins		
Date	May 8, 2009	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	Tanisha Lawrence-Caceres	Date	May 8, 2009

PTO/SB/31 (09-04)

NOTICE OF APPEAL FROM THE EXAMINER TO THE BOARD OF PATENT APPEALS AND INTERFERENCES

Docket Number (Optional): 0800-0045

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on May 8, 2009

Signature

Typed or printed name Tanisha Lawrence-Caceres

In re Application of John Fraser Wright et al.

Application Number	Filed
11/141,996	June 1, 2005

For COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION

Art Unit	Examiner
1657	S. K. Singh

Applicant hereby **appeals** to the Board of Patent Appeals and Interferences from the last decision of the examiner.

The fee for this Notice of Appeal is (37 CFR 41.20(b)(1)) $ 540.00

☐ Applicant claims small entity status. See 37 CFR 1.27. Therefore, the fee shown above is reduced by half, and the resulting fee is: $ _____

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

☒ Payment by credit card. Form PTO-2038 is attached.

☐ The Director has already been authorized to charge fees in this application to a Deposit Account. I have enclosed a duplicate copy of this sheet.

☒ The Director is hereby authorized to charge any **additional** fees which may be required, or credit any overpayment to Deposit Account No. 18-1648. I have enclosed a duplicate copy of this sheet.

☒ A petition for an extension of time under 37 CFR 1.136(a) (PTO/SB/22) is enclosed.

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 acting under 37 CFR 1.34. Registration number if acting under 37 CFR 1.34. _____

Signature

Roberta L. Robins
Typed or printed name

650.493.3400
Telephone number

May 8, 2009
Date

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.

PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a) **FY 2009** *(Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).)*	Docket Number (Optional) 0800-0045
Application Number: 11/141,996	Filed: June 1, 2005
For COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION	
Art Unit: 1657	Examiner: S. 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))	$130	$65	$
☐ Two months (37 CFR 1.17(a)(2))	$490	$245	$
☒ Three months (37 CFR 1.17(a)(3))	$1110	$555	$ 1,110.00
☐ Four months (37 CFR 1.17(a)(4))	$1730	$865	$
☐ Five months (37 CFR 1.17(a)(5))	$2350	$1175	$

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

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

☒ Payment by credit card. Form PTO-2038 is attached.

☐ 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. I have enclosed a duplicate copy of this sheet.

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	May 8, 2009 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.

Electronic Patent Application Fee Transmittal

Application Number:	11141996
Filing Date:	01-Jun-2005
Title of Invention:	Compositions and methods to prevent AAV vector aggregation
First Named Inventor/Applicant Name:	John Fraser Wright
Filer:	Roberta L. Robins/Tanisha Lawrence-Caceres
Attorney Docket Number:	0800-0045

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:				
Notice of appeal	1401	1	540	540
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension - 3 months with $0 paid	1253	1	1110	1110
Miscellaneous:				
			Total in USD ($)	**1650**

Electronic Acknowledgement Receipt

EFS ID:	5305709
Application Number:	11141996
International Application Number:	
Confirmation Number:	5399
Title of Invention:	Compositions and methods to prevent AAV vector aggregation
First Named Inventor/Applicant Name:	John Fraser Wright
Customer Number:	31048
Filer:	Roberta L. Robins/Tanisha Lawrence-Caceres
Filer Authorized By:	Roberta L. Robins
Attorney Docket Number:	0800-0045
Receipt Date:	08-MAY-2009
Filing Date:	01-JUN-2005
Time Stamp:	19:19:37
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Credit Card
Payment was successfully received in RAM	$1650
RAM confirmation Number	4509
Deposit Account	
Authorized User	

File Listing:

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

1		NOA_20090508161712.pdf	146845 cd0d7cbd002a72f4b0f05d62e517ca745f1f93b6	yes	3

Multipart Description/PDF files in .zip description		
Document Description	**Start**	**End**
Miscellaneous Incoming Letter	1	1
Notice of Appeal Filed	2	2
Extension of Time	3	3

Warnings:

Information:

2	Fee Worksheet (PTO-875)	fee-info.pdf	31897 bdc9aa0537b59e13e5baf006fcdaff4d22a7f8a5	no	2

Warnings:

Information:

Total Files Size (in bytes):	178742

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.

<u>New Applications Under 35 U.S.C. 111</u>
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/21 (09-04)

TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

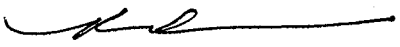
Application Number	11/141,996
Filing Date	June 1, 2005
First Named Inventor	John Fraser Wright et al.
Art Unit	1657
Examiner Name	S. K. Singh
Total Number of Pages in This Submission: 2	Attorney Docket Number: 0800-0045

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
- ☒ Change of Correspondence Address Application
- ☐ 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 & Pasternak LLP		
Signature			
Printed name	Roberta L. Robins		
Date	May 11, 2009	Reg. No.	33,208

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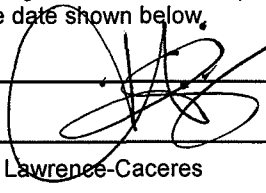
Signature			
Typed or printed name	Tanisha Lawrence-Caceres	Date	May 11, 2009

PTO/SB/122 (11-08)
Approved for use through 11/30/2011. OMB 0651-0035
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

CHANGE OF CORRESPONDENCE ADDRESS
Application

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

Application Number	11/141,996
Filing Date	June 1, 2005
First Named Inventor	John Fraser Wright et al.
Art Unit	1657
Examiner Name	S. K. Singh
Attorney Docket Number	0800-0045

Please change the Correspondence Address for the above-identified patent application to:

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OR

☐ Firm or Individual Name

Address

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Country

Telephone | Email

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I am the:

☐ Applicant/Inventor

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

☑ Attorney or agent of record. Registration Number 33,208.

☐ Registered practitioner named in the application transmittal letter in an application without an executed oath or declaration. See 37 CFR 1.33(a)(1). Registration Number_____.

Signature

Typed or Printed Name Roberta L. Robins

Date May 11, 2009 | Telephone (650) 493-3400

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.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Electronic Acknowledgement Receipt

EFS ID:	5310047
Application Number:	11141996
International Application Number:	
Confirmation Number:	5399
Title of Invention:	Compositions and methods to prevent AAV vector aggregation
First Named Inventor/Applicant Name:	John Fraser Wright
Customer Number:	31048
Filer:	Roberta L. Robins/Tanisha Lawrence-Caceres
Filer Authorized By:	Roberta L. Robins
Attorney Docket Number:	0800-0045
Receipt Date:	11-MAY-2009
Filing Date:	01-JUN-2005
Time Stamp:	14:48:55
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		ChangeofCorrsAdd_20090511114749.pdf	105034 1b4f45517c406d43f583ba5e9a95792414cd3d76	yes	2

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Miscellaneous Incoming Letter	1	1
	Change of Address	2	2

Warnings:	
Information:	
Total Files Size (in bytes):	105034

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.

Request for Continued Examination (RCE) Transmittal

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

Application Number	11/141,996
Filing Date	June 1, 2005
First Named Inventor	John Fraser Wright et al.
Art Unit	1657
Examiner Name	Satyendra K. Singh
Attorney Docket Number	0800-0045

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. 18-1648. **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 in the attached check**

   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	December 8, 2009
Name (Print /Type)	Roberta L. Robins	Registration No.	33,208

CERTIFICATE OF MAILING OR TRANSMISSION

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CERTIFICATE OF TRANSMISSION PURSUANT TO 37 CFR § 1.8

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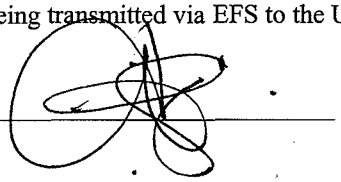
Date 12/08/2009 Signature 

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:

JOHN FRASER WRIGHT et al.	Confirmation No.: 5399
Application No.: 11/141,996	Art Unit: 1657
Filing Date: June 1, 2005	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 November 18, 2008. In lieu of filing an Appeal Brief, a Request for Continued Examination is being filed concurrently herewith. A request for an extension of time, as well as the requisite fee accompanies this submission. 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 method of preventing aggregation of recombinant adeno-associated virus (rAAV) virions in a preparation of rAAV virions, comprising:

1) providing a lysate comprising rAAV virions;

2) purifying rAAV virions from the lysate using ultracentrifugation and/or chromatography, wherein said virions are purified; and

3) adding one or more excipients to said purified virions to produce a preparation of virions with an ionic strength of at least 200 mM, wherein the concentration of purified rAAV virions in said preparation exceeds $1x10^{13}$ vg/ml up to $6.4x10^{13}$ vg/ml.

2. (Canceled)

3. (Previously presented) The method of claim 1, further comprising treating said purified virions with a nuclease.

4. (Previously presented) The method of claim 3, wherein the nuclease is an endonuclease from *Serratia marcescens* (Benzonase®).

5. (Original) The method of claim 1, wherein one or more of the excipients comprises a multivalent ion.

6. (Previously presented) The method of claim 5, wherein the multivalent ion is citrate.

7. (Previously presented) The method of claim 1, wherein the osmolarity of the preparation of virions after addition of the one or more excipients is no greater than about 280mOsm.

8. (Original) The method of claim 1, wherein, after addition of the one or more excipients, the average particle radius (Rh) of the virions in the preparation of virions is less than about 20nm as measured by dynamic light scattering.

9. (Previously presented) The method of claim 1, wherein, after addition of the one or more excipients, recovery of the virions is at least about 90% following filtration of the preparation of virions through a 0.22μm filter.

10-20. (Canceled)

21. (Previously presented) The method of claim 3, wherein rAAV virions are purified from the lysate using cesium chloride gradient ultracentrifugation.

22. (Previously presented) The method of claim 3, wherein rAAV virions are purified from the lysate using cation exchange chromatography.

23. (Previously presented) The method of claim 3, wherein rAAV virions are purified from the lysate using cation exchange chromatography and cesium chloride gradient ultracentrifugation.

24. (Previously presented) The method of claim 3, further comprising diafiltering the purified rAAV virions to achieve an ionic strength of at least 200 mM.

II. REMARKS

Introductory Comments

Claims 1, 3-9 and 21-24 were examined in the Final Office Action dated November 18, 2008 and stand rejected under 35 U.S.C. §112, first paragraph and 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

Claim 1 has been amended to recite that the concentration of purified rAAV virions in the preparation 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.

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. §112, First Paragraph

Claims 1, 3-9 and 21-24 were rejected under 35 U.S.C. §112, first paragraph as failing to comply with the written description requirement. In particular, the Office asserts the concentration of purified AAV virions in the preparation can exceed "$1x10^{13}$ vg/ml and up to infinity." Office Action, page 3. Solely in an effort to advance prosecution, claim 1 has been amended to recite that the concentration of purified rAAV virions in the preparation exceeds $1x10^{13}$ vg/ml up to $6.4x10^{13}$ vg/ml. Thus, this basis for rejection has been overcome and withdrawal thereof is respectfully requested.

Rejections Under 35 U.S.C. §103(a)

Claims 1, 3-9 and 21-24 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. 6,566,118 to Atkinson et al. ("Atkinson") and Drittanti et al, *J. Gene Med.* (2001) 3:59-71

("Drittanti'); in view of Qu et al., *Molec. Ther.* (2003) 5:S348, Abstract 901 ("Qu") and Chen et al., *J. Pharm. Sci.* (1994) 83:1657-1661 ("Chen"), and further in view of U.S. Patent No. 6,593,123 to Wright et al. ("Wright").

The Office argues each of the references of Zolotukhin, Atkinson and Drittanti teach a method step for the treatment of rAAV virions with a nuclease and that process steps such as ultracentrifugation, chromatography and diafiltration are used and disclosed by each of these references. Office Action, page 5. The Examiner acknowledges methods of preventing aggregation by adding a citrate are not disclosed by the primary references. Office Action, page 6. Qu is cited for disclosing problems associated with concentration-induced aggregation of rAAV virions and Chen is cited for describing strategies to suppress aggregation of proteins. Office Action, page 6. Finally, Wright is cited for teaching a method for large scale production of rAAV virions and the use of a nuclease. Office Action, page 9. However, Applicants submit the cited combination does not render the claimed invention obvious.

The Office disputes Applicants' previous arguments alleging "the combined teachings in the cited prior art references of record disclose all the elements, including the recognition of the problem of 'self aggregation' of rAAV virions associated with purified preparations of said virions." Office Action, page 11. However, this statement is in error. 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 methods of preventing aggregation of rAAV virions in a **purified** preparation. The rAAV virions are purified from a lysate using ultracentrifugation and/or chromatography. One or more excipients are then added to achieve an ionic strength of at least 200 mM and the concentration of purified rAAV virions in the preparation exceeds $1x10^{13}$ up to $6.4x10^{13}$ vg/ml. In certain embodiments, the purified preparation is treated with a nuclease. None of the cited art, either alone or in combination, teaches or suggests adding one or more excipients to a **purified** rAAV virion preparation to achieve an ionic strength of at least 200 mM wherein the preparation includes more than $1x10^{13}$ up to $6.4x10^{13}$ vg/ml, as claimed. For this reason alone, the cited combination fails to render the claims obvious.

As previously explained, Zolotukhin does not recognize that rAAV virions self-aggregate and therefore does not provide any suggestions regarding a method 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 Applicants' 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, the reference does not teach or suggest that self-aggregation of rAAV virions is a problem at all, let alone a problem in **purified**, concentrated AAV preparations. 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, in each of the Zolotukhin passages 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 in the previous responses, 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.

Similarly, Atkinson fails to provide the requisite teaching or suggestion to arrive at the instant invention. The Office points to Figures 13, 19, 32, 36 and 37 to evidence that excipients are added to a preparation of AAV virions to achieve an ionic strength of at least 200 mM. Office Action, page 5. However, these figures are not relevant to the present invention. These Figures pertain to the adjustment of ionic strength in cell culture media, well before purification,

in order to promote release of the virus into the cell culture without cell lysis. See, columns 41-42 of Atkinson. Additionally, as in Zolotukhin, there is no disclosure in Atkinson regarding adding excipients to a **purified** preparation of rAAV virions to achieve an ionic strength of at least 200 mM where the concentration of virions in the purified preparation is more than $1x10^{13}$ vg/ml up to $6.4x10^{13}$. Indeed, as with Zolotukhin, **all** of the steps cited by the Office where the buffers utilized may have an ionic strength of greater than 200mM, are steps mid-purification. Furthermore, as with all of the references cited by the Office, the purified AAV preparations disclosed in Atkinson are formulated in a final buffer consisting of Modified Ringer's Solution with 5% glycerol (for example, see Atkinson at column 54, lines 33-44). As noted before, Modified Ringer's Solution does not have an ionic strength of at least 200 mM.

Thus, Atkinson, as with Zolotukhin, fails to teach or suggest a method where excipients are added to a **purified** preparation of rAAV virions to achieve an ionic strength of at least 200 mM in order to prevent aggregation and to result in a purified preparation with more than $1x10^{13}$ up to $6.4x10^{13}$ vg/ml.

Drittanti also fails to teach or suggest these elements of the claimed invention. The Office argues rAAV virions are recovered/eluted by adding 300 mM NaCl through a 0-1M NaCl linear gradient in 100 mM HEPES buffer. Office Action, page 5. However, as with Zolotukhin and Atkinson, this step is not a final purification step. Rather, as explained at page 61, second column, third full paragraph, the final preparation is in Ringer-lactate buffer, and as explained above, this buffer does not have an ionic strength of at least 200 mM. Drittanti, as with Zolotukhin and Atkinson, does not teach or suggest a method where excipients are added to a **purified** preparation of rAAV virions to achieve an ionic strength of at least 200 mM in order to prevent aggregation and to result in a purified preparation with more than $1x10^{13}$ up to $6.4x10^{13}$ vg/ml. Thus, none of the primary references teaches or suggests the claimed invention.

The secondary references of Qu and Chen fail to cure the defects of the primary references. The Examiner states that Qu in view of Chen provides the motivation for using high salt concentration in purified vector stocks. Office Action, page 11. However, Qu merely proposes a mechanism for AAV vector aggregation. The Examiner further asserts Qu suggests that ion bridges between charged amino acids on the surface of vector particles contribute to inter-particle interactions. Office Action, page 6. At best, this teaching might lead one of skill in

the art to adjust the pH of the final formulation, which Qu expressly teaches results "in reversal of concentration-induced vector aggregation". Alternatively, the skilled artisan might postulate that high concentrations of free amino acids could block vector particle interactions. However, as explained at page 9, lines 18-20 of the instant specification, 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 states that further work is needed in order to provide a viable purification procedure.

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 carry out a purification protocol as claimed in order to prevent aggregation of rAAV virions and to result in a purified preparation with more than $1x10^{13}$ up to $6.4x10^{13}$ vg/ml of rAAV virions.

Finally, although Wright discusses nuclease treatment, Wright does not provide the other elements missing from Zolotukhin, Atkinson, Drittanti, Qu and Chen. Wright nowhere mentions that self-aggregation of rAAV virions occurs and hence does not teach or suggest methods for preventing aggregation of rAAA virions as claimed. Wright uses high salt concentrations initially to promote release of rAAV virions from cells and in some mid-purification steps. The only discussion in Wright regarding adding excipients to the final purified product is in Example 9. However, unlike Applicants' purification method, the excipient added is phosphate-buffered saline, pH 7.1, containing 5% sorbitol. This buffer does not have an ionic strength of at least 200 mM.

Thus, Wright, as with all of the references discussed above, does not teach or suggest a method for preventing aggregation of rAAV virions where excipients are added to a purified preparation of rAAV virions to achieve an ionic strength of at least 200 mM and to result in a purified preparation with more than $1x10^{13}$ up to $6.4x10^{13}$ vg/ml .

Applicants' process, on the other hand, provides a commercially viable method for producing high amounts of rAAV virions. As explained at pages 16-17 of the specification and in Table 2, vector recovery using Applicants' claimed methods results in yields of more than 90%.

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; *In re Lee*, 61 USPQ2d 1430 (Fed. Cir. 2002), affirming that common knowledge and common sense are not the specialized knowledge and expertise necessary to establish a motivation to arrive at the claimed invention.

Thus, the requirement is not whether each claimed element can be identified individually in a reference but, rather, whether the Examiner can show "reasons that the skilled artisan, confronted with the same problem as the inventor, and with no knowledge of the claimed invention, would select the elements from the cited prior art reference for combination in the

manner claimed." *In re Rouffet*, 47 USPQ2d at 1458. In the pending case, the Office has not met this burden.

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 method for preventing 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.

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: 12/4/09

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

ROBINS & PASTERNAK LLP
1731 Embarcadero Road, Suite 230
Palo Alto, CA 94303
Telephone: (650) 493-3400
Facsimile: (650) 493-3440

Electronic Patent Application Fee Transmittal

Application Number:	11141996
Filing Date:	01-Jun-2005
Title of Invention:	Compositions and methods to prevent AAV vector aggregation
First Named Inventor/Applicant Name:	John Fraser Wright
Filer:	Roberta L. Robins/Tanisha Lawrence-Caceres
Attorney Docket Number:	0800-0045

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	1110	1110

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
Request for continued examination	1801	1	810	810
		Total in USD ($)		**1920**

Electronic Acknowledgement Receipt

EFS ID:	6597395
Application Number:	11141996
International Application Number:	
Confirmation Number:	5399
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/Tanisha Lawrence-Caceres
Filer Authorized By:	Roberta L. Robins
Attorney Docket Number:	0800-0045
Receipt Date:	08-DEC-2009
Filing Date:	01-JUN-2005
Time Stamp:	18:03:35
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Credit Card
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RAM confirmation Number	4939
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File Listing:

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

1		Respto11182008FOA_20091208150423.pdf	701356 24b7e83bf929afbfa2430ea52edc7f9c635f59a9	yes	13

Multipart Description/PDF files in .zip description		
Document Description	**Start**	**End**
Miscellaneous Incoming Letter	1	1
Extension of Time	2	2
Request for Continued Examination (RCE)	3	3
Amendment After Final	4	4
Claims	5	6
Applicant Arguments/Remarks Made in an Amendment	7	13

Warnings:

Information:

2	Fee Worksheet (PTO-875)	fee-info.pdf	31920 57ae362c72e685bbac01b9da88379db794efbf7f	no	2

Warnings:

Information:

Total Files Size (in bytes):	733276

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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/21 (09-04)

TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Application Number	11/141,996
Filing Date	June 1, 2005
First Named Inventor	John Fraser Wright et al.
Art Unit	1657
Examiner Name	S. K. Singh
Total Number of Pages in This Submission: 13	Attorney Docket Number: 0800-0045

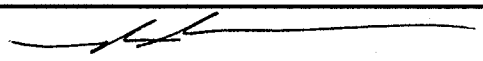
ENCLOSURES *(Check all that apply)*

- ☐ Fee Transmittal Form
  - ☐ Fee Attached
- ☒ Amendment/Reply to 11/18/2008 Final Office Action (10 pages)
  - ☐ After Final
  - ☐ Affidavits/declaration(s)
- ☒ Extension of Time Request (1 page)
- ☐ 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
- ☐ Change of Correspondence Address Application
- ☐ 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):

Request for Continued Examination (RCE) Transmittal (1 page)

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	December 8, 2009	Reg. No.	33,208

CERTIFICATE OF TRANSMISSION/MAILING

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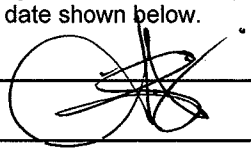
Signature			
Typed or printed name	Tanisha Lawrence-Caceres	Date	December 8, 2009

PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a) **FY 2009** *(Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).)*	Docket Number (Optional) 0800-0045
Application Number: 11/141,996	Filed: June 1, 2005
For COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION	
Art Unit: 1657	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))	$130	$65	$
☐ Two months (37 CFR 1.17(a)(2))	$490	$245	$
☒ Three months (37 CFR 1.17(a)(3))	$1110	$555	$ 1110
☐ Four months (37 CFR 1.17(a)(4))	$1730	$865	$
☐ Five months (37 CFR 1.17(a)(5))	$2350	$1175	$

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

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

☒ Payment by credit card. Form PTO-2038 is attached.

☐ 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. I have enclosed a duplicate copy of this sheet.

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.

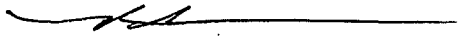
I am the

☐ 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 — December 8, 2009 (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 (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
11/141,996	06/01/2005	

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	12/08/2009	(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))	* 12	Minus	** 20	= 0	X $26 =	0	OR	X $ =	
	Independent (37 CFR 1.16(h))	* 1	Minus	***3	= 0	X $110 =	0	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	0	OR	TOTAL ADD'L FEE	

AMENDMENT		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)	OR	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:
/DORIS m. BURNS/

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

NOTICE OF ALLOWANCE AND FEE(S) DUE

20855 7590 12/18/2009

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

EXAMINER	
SINGH, SATYENDRA K	
ART UNIT	PAPER NUMBER
1657	

DATE MAILED: 12/18/2009

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/141,996	06/01/2005	John Fraser Wright	0800-0045	5399

TITLE OF INVENTION: COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION

APPLN. TYPE	SMALL ENTITY	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	YES	$755	$300	$0	$1055	03/18/2010

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 SMALL ENTITY status shown above.

If the SMALL ENTITY is shown as YES, verify your current SMALL ENTITY status:

A. If the status is the same, pay the TOTAL FEE(S) DUE shown above.

B. If the status above is to be removed, check box 5b on Part B - Fee(s) Transmittal and pay the PUBLICATION FEE (if required) and twice the amount of the ISSUE FEE shown above, or

If the SMALL ENTITY is shown as NO:

A. Pay TOTAL FEE(S) DUE shown above, or

B. If applicant claimed SMALL ENTITY status before, or is now claiming SMALL ENTITY status, check box 5a on Part B - Fee(s) Transmittal and pay the PUBLICATION FEE (if required) and 1/2 the ISSUE FEE shown above.

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 12/18/2009

ROBINS & PASTERNAK
1731 EMBARCADERO ROAD
SUITE 230
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.
11/141,996	06/01/2005	John Fraser Wright	0800-0045	5399

TITLE OF INVENTION: COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION

APPLN. TYPE	SMALL ENTITY	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	YES	$755	$300	$0	$1055	03/18/2010

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, 1_____

(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. 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 credit any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)

❑ a. Applicant claims SMALL ENTITY status. See 37 CFR 1.27.

❑ b. Applicant is no longer claiming SMALL ENTITY status. See 37 CFR 1.27(g)(2).

NOTE: The Issue Fee and Publication Fee (if required) will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the United States Patent and Trademark Office.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____

This collection of information 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.

UNITED STATES PATENT AND TRADEMARK OFFICE

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/141,996	06/01/2005	John Fraser Wright	0800-0045	5399

20855 7590 12/18/2009

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

EXAMINER	
SINGH, SATYENDRA K	
ART UNIT	PAPER NUMBER
1657	

DATE MAILED: 12/18/2009

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(application filed on or after May 29, 2000)

The Patent Term Adjustment to date is 0 day(s). If the issue fee is paid on the date that is three months after the mailing date of this notice and the patent issues on the Tuesday before the date that is 28 weeks (six and a half months) after the mailing date of this notice, the Patent Term Adjustment will be 0 day(s).

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 Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

Notice of Allowability	Application No.	Applicant(s)	
	11/141,996	WRIGHT ET AL.	
	Examiner	Art Unit	
	SATYENDRA K. SINGH	1657	

-- 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 *12/8/09*.

2. ☒ The allowed claim(s) is/are *1,3,4,6-9 and 21-24*.

3. ☐ 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 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.

4. ☐ A SUBSTITUTE OATH OR DECLARATION must be submitted. Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL PATENT APPLICATION (PTO-152) which gives reason(s) why the oath or declaration is deficient.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   (a) ☐ including changes required by the Notice of Draftsperson's Patent Drawing Review (PTO-948) attached
      1) ☐ hereto or 2) ☐ to Paper No./Mail Date _____.
   (b) ☐ 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. ☐ Notice of Draftperson's Patent Drawing Review (PTO-948)
3. ☐ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____
4. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
5. ☐ Notice of Informal Patent Application
6. ☒ Interview Summary (PTO-413), Paper No./Mail Date *12/14/09* .
7. ☒ Examiner's Amendment/Comment
8. ☒ Examiner's Statement of Reasons for Allowance
9. ☒ Other *See Continuation Sheet*.

Continuation of Attachment(s) 9. Other: A DRAFT copy of the faxed Exam. Amend. is attached..

Examiner-Initiated Interview Summary	Application No.	Applicant(s)	
	11/141,996	WRIGHT ET AL.	
	Examiner	Art Unit	
	SATYENDRA K. SINGH	1657	

All Participants:

(1) *SATYENDRA K. SINGH*.

(2) *ROBERTA L. ROBINS (ATTORNEY OF RECORD)*.

Status of Application: *AFTER RCE*

(3) _____.

(4) _____.

Date of Interview: *10 December 2009*

Time: *4:45 PM*

Type of Interview:
☒ Telephonic
☐ Video Conference
☐ Personal (Copy given to: ☐ Applicant ☐ Applicant's representative)

Exhibit Shown or Demonstrated: ☒ Yes ☐ No
If Yes, provide a brief description: *see attached DRAFT exam. amendment faxed to applicants on 12/10/09*.

Part I.

Rejection(s) discussed:
N/A

Claims discussed:
1,5,6

Prior art documents discussed:
N/A

Part II.

SUBSTANCE OF INTERVIEW DESCRIBING THE GENERAL NATURE OF WHAT WAS DISCUSSED:
See Continuation Sheet

Part III.

☒ It is not necessary for applicant to provide a separate record of the substance of the interview, since the interview directly resulted in the allowance of the application. The examiner will provide a written summary of the substance of the interview in the Notice of Allowability.
☐ It is not necessary for applicant to provide a separate record of the substance of the interview, since the interview did not result in resolution of all issues. A brief summary by the examiner appears in Part II above.

/Satyendra K. Singh/
Examiner, Art Unit 1657

(Applicant/Applicant's Representative Signature – if appropriate)

Continuation of Substance of Interview including description of the general nature of what was discussed: Applicant's representative of record Miss Roberta L. Robins (phone- 650-493-3400; fax no. 650-493-3440) was telephonically contacted by the examiner, and provided with a proposed Examiner's Amendment (sent by fax on 12/10/09; see attached DRAFT Exam. Amend. attached) to the pending claims (that have been found to be allowable through an in house patentability conference with the Primary examiner, Sandy Saucier and SPE, Michael Wityshyn) for applicant's considerations. Applicant's attorney of record called the examiner (on 12/14/09; at 11:45 AM) and informed that applicant's have accepted the proposed Examiner's Amendment to the claims.

/Satyendra K. Singh/
Examiner, AU1657

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 **12/08/2009** has been entered.

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. Robbins (ATTORNEY OF RECORD) on December 14th 2009.

The application has been amended as follows:

In The Claims

Claim 5 has been canceled by this Examiner's amendment.

Claims 1, 3, 4, 6-9 and 21-24 have been allowed by this Examiner's amendment. Claims 1, 4, 6, 7, 8 and 9 have been amended as follows:

1. A method of preventing aggregation of recombinant adeno-associated virus (rAAV) virions in a purified preparation of rAAV virions, comprising:
1) providing a lysate comprising rAAV virions;

2) purifying rAAV virions from the lysate using ultracentrifugation and/or chromatography, wherein said virions are purified; and
3) adding one or more ~~excipients~~ 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, wherein the concentration of purified rAAV virions in said preparation exceeds $1x10^{13}$ vg/ml up to $6.4x10^{13}$ vg/ml; and wherein the pH of the purified preparation of rAAV virions is between 7.5 and 8.0.

4. The method of claim 3, wherein the nuclease is an endonuclease from *Serratia marcescens* ~~(Benzonase®)~~.

6. The method of claim ~~5~~ 1, wherein the multivalent ion is citrate.

7. The method of claim 1, wherein the osmolarity of the preparation of virions after addition of the one or more ~~excipients~~ salts of multivalent ions is no greater than about 280mOsm.

8. The method of claim 1, wherein, after addition of the one or more ~~excipients~~ salts of multivalent ions, the average particle radius (Rh) of the virions in the preparation of virions is less than about 20nm as measured by dynamic light scattering.

9. The method of claim 1, wherein, after addition of the one or more ~~excipients~~ salts of multivalent ions, recovery of the virions is at least about 90% following filtration of the preparation of virions through a 0.22μm filter.

The following is an examiner's statement of reasons for allowance:

The amended claims (see instant claim 1 as amended and allowed) were found to be unobvious over the cited art as the combined teachings of the prior art references do not suggest the use of salts of multivalent ions such as citrate, phosphate, sulfate or magnesium at a pH range of 7.5 to 8.0, and an ionic strength of at least 200 mM for preventing aggregation of purified rAAV vector genome and/or rAAV particle preparations. As also argued by applicants (see remarks, pages 5-6, in particular), the cited prior art wants to reduce the high salt concentrations in purified viral stock solutions, and therefore do not suggest the use of multivalent ions at an ionic strength of

at least 200mM at pH 7.5-8.0, which is the desired pH for normal physiological or clinical use of such viral vector preparations. Applicants have provided superior results and appropriate evidentiary data in the instant specification (see table 1, figures 1-2, in particular) to support the amendments in the instant claims, which are not made obvious by the combined teachings in the cited prior art.

Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SATYENDRA K. 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, Manjunath N. Rao can be reached on 571-272-0939. 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.

/Sandra Saucier/
Primary Examiner, Art Unit 1651

/Satyendra K. Singh/
Examiner, Art Unit 1657

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	11141996	WRIGHT ET AL.
	Examiner	Art Unit
	SATYENDRA K SINGH	1657

SEARCHED

Class	Subclass	Date	Examiner

SEARCH NOTES

Search Notes	Date	Examiner
EAST: USPAT, USOCR, US-PGPUB, JPO, EPO, DERWENT- UPDATED & ATTACHED.	2/12/08	SKS
INVENTOR SEARCH: PALM & EAST- UPDATED	2/13/08	SKS
EAST: USPAT, USOCR, US-PGPUB, JPO, EPO, DERWENT- UPDATED & ATTACHED	11/14/08	SKS
INVENTOR SEARCH: PALM & eDAN & EAST	11/14/08	SKS
EAST: USPAT, USOCR, USPGPUB, JPO, EPO & DERWENT- UPDATED & ATTACHED	12/15/09	SKS
INVENTOR SEARCH: EAST, PALM, EDAN	12/15/09	SKS
APPLICATION: 11607703- ABDN	12/15/09	SKS

INTERFERENCE SEARCH

Class	Subclass	Date	Examiner
435	239- TEXT-LIMITED INTERFERENCE SEARCH PERFORMED	12/15/09	SKS

/SATYENDRA K SINGH/ Examiner.Art Unit 1657	

Issue Classification

Application/Control No.	Applicant(s)/Patent Under Reexamination
11141996	WRIGHT ET AL.
Examiner	**Art Unit**
SATYENDRA K SINGH	1657

ORIGINAL		INTERNATIONAL CLASSIFICATION														
CLASS	SUBCLASS	CLAIMED					NON-CLAIMED									
435	239	C	1	2	N	7 / 02 (2006.01.01)										

CROSS REFERENCE(S)					
CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)				

☒ 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

/SATYENDRA K SINGH/ Examiner.Art Unit 1657 (Assistant Examiner)	12/15/09 (Date)	**Total Claims Allowed:** 11
/Sandra Saucier/ Primary Examiner.Art Unit 1651 (Primary Examiner)	12/16/2009 (Date)	O.G. Print Claim(s) 1 O.G. Print Figure NONE

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

BIB DATA SHEET

CONFIRMATION NO. 5399

SERIAL NUMBER	FILING or 371(c) DATE RULE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
11/141,996	06/01/2005	435	1657	0800-0045

APPLICANTS

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

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

This appln 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 **** ** SMALL ENTITY **
07/06/2005

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	20	3

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		
565	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

EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
S67	4	S62 and ((viral or virion or virus) near3 (genome or particle)) and (ion$6 near3 strength) and (multivalent or citrate or citric).clm.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2008/11/14 17:23
S68	1813	WRIGHT near3 JOHN	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2009/12/10 17:39
S69	11	QU near3 GUANG	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2009/12/10 17:40
S70	1817	S68 or S69	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2009/12/10 17:40
S71	59637	(raav or aav or adenovir$6 or adeno$9) and (citrate or citric)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2009/12/10 17:41
S72	15	S70 and S71	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2009/12/10 17:41
S73	28500	(citrate or citric or magnesium or phosphate or sulfate) same (raav or aav or adenovir$6 or adeno$9 or adeno$1associat $6)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2009/12/10 17:48
S74	2180	(citrate or citric or magnesium or phosphate or sulfate) same (raav or aav or adenovir$6 or adeno$9 or adeno$1associat $6) same (aggregat$6 or stabiliz$6 or stable or (loss near3 titer))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2009/12/10 17:49
S75	34	(citrate or citric or magnesium or phosphate or sulfate) same (raav or aav or adenovir$6 or adeno$9 or adeno$1associat $6) same (aggregat$6 or stabiliz$6 or stable or (loss near3 titer)).clm.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2009/12/10 17:49

S76	0	(citrate or citric or magnesium or phosphate or sulfate) same (raav or aav or adenovir$6 or adeno$1associat$6) same (aggregat$6 or stabiliz$6 or stable or (loss near3 titer)).clm.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2009/12/10 17:51
S77	522	(citrate or citric or magnesium or phosphate or sulfate) same (raav or aav or adenovir$6 or adeno$1associat$6) same (aggregat$6 or stabiliz$6 or stable or (loss near3 titer))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2009/12/10 17:51
S78	375	S77 and ((prevent near3 aggregat$6) or stabiliz$6)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2009/12/10 17:53
S79	99	S77 same ((prevent near3 aggregat$6) or stabiliz$6)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2009/12/10 17:53
S80	29	S77 same raav same ((prevent near3 aggregat$6) or stabiliz$6)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2009/12/10 17:54
S81	2	S77 same raav same ((prevent near3 aggregat$6) or stabiliz$6) same titer	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2009/12/10 18:01
S82	2142	adenovir$6 same ((prevent$6 near3 aggregat$6) or stabil$6 or (loss near3 titer))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2009/12/10 18:04
S83	166	adenovir$6 same ((prevent$6 near3 aggregat$6) or stabil$6 or (loss near3 titer)) same (salt or excipient or (multivalent near3 ions))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2009/12/10 18:05
S84	0	adenovir$6 same ((prevent$6 near3 aggregat$6) or stabil$6 or (loss near3 titer)) same (salt or excipient or (multivalent near3 ions)).clm.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2009/12/10 18:06
S85	46336	(435/235.1,239,320.1,172.3,).CCLS.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2009/12/15 15:22
S86	22966	S85 and (adenovir$6 or AAV or rAAV or (recombin$6 near3 adenovir$6))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2009/12/15 15:24

S87	11773	S85 and (adenovir$6 or AAV or rAAV or (recombin$6 near3 adenovir$6)) and (titer or (stock near5 titer))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2009/12/15 15:24
S88	11351	S85 and (adenovir$6 or AAV or rAAV or (recombin$6 near3 adenovir$6)) and (titer or (stock near5 titer)) and (citrate or citric or magnesium or phosphate or sulfate or multival$6)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2009/12/15 15:25
S89	0	S85 same (adenovir$6 or AAV or rAAV or (recombin$6 near3 adenovir$6)) same (titer or (stock near5 titer)) same (citrate or citric or magnesium or phosphate or sulfate or multival$6)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2009/12/15 15:26
S90	0	S85 same (adenovir$6 or AAV or rAAV or (recombin$6 near3 adenovir$6)) same (citrate or citric or magnesium or phosphate or sulfate or multival$6)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2009/12/15 15:26
S91	1113	S85 and (adenovir$6 or AAV or rAAV or (recombin$6 near3 adenovir$6)) and (citrate or citric or magnesium or phosphate or sulfate or multival$6).clm.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2009/12/15 15:26
S92	101	S85 and (adenovir$6 or AAV or rAAV or (recombin$6 near3 adenovir$6)) and (citrate or citric or magnesium or phosphate or sulfate or multival$6) and (titer or (stock near3 titer)).clm.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2009/12/15 15:27
S93	47	S92 and (rAAV or (recombin$6 near3 adenovir$6))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2009/12/15 15:28
S94	35	S92 and (rAAV or (recombin$6 near3 adenovir$6)) and (aggregat$6 or precipitat $6 or agglutin$6)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2009/12/15 15:31
S95	13	S92 and (prevent or avoid or reduce or decrease) same (aggregat$6 or precipitat $6)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2009/12/15 15:33
S96	1	("7094604").PN.	USPAT; USOCR	OR	OFF	2009/12/15 15:51
S97	1	S96 and (vector near3 genome)	USPAT	OR	ON	2009/12/15 15:51
S98	6	(Vg near3 (vector near3 genome))	USPAT	OR	ON	2009/12/15 16:22
S99	1813	WRIGHT near3 JOHN	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2009/12/15 16:25

S100	11	QU near3 GUANG	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2009/12/15 16:25
S101	1817	S99 or S100	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2009/12/15 16:26
S102	26	S101 and (adenovir$6 or vector or AAV) and aggregat$6	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2009/12/15 16:28
S103	1	S101 and (adenovir$6 or vector or AAV) and aggregat$6.clm.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2009/12/15 16:28
S104	4	S101 and ((adenovir$6 or vector or AAV) same aggregat$6)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2009/12/15 16:30

EAST Search History (Interference)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
S105	10620	((adenovir$6 or vector or AAV) same aggregat$6)	US-PGPUB; USPAT; UPAD	OR	ON	2009/12/15 16:31
S106	85	(prevent or reduc$6 or avoid) same ((adenovir$6 or vector or AAV) near3 aggregat$6)	US-PGPUB; USPAT; UPAD	OR	ON	2009/12/15 16:31
S107	3	(prevent or reduc$6 or avoid) same ((adenovir$6 or vector or AAV) near3 aggregat$6).clm.	US-PGPUB; USPAT; UPAD	OR	ON	2009/12/15 16:32
S108	55	(adenovir$6 or AAV or rAAV) and (prevent or reduc$6 or avoid) and ((adenovir$6 or vector or AAV) near3 aggregat $6)	US-PGPUB; USPAT; UPAD	OR	ON	2009/12/15 16:33
S109	2	(adenovir$6 or AAV or rAAV) and (prevent or reduc$6 or avoid) and ((adenovir$6 or vector or AAV) near3 aggregat $6).clm.	US-PGPUB; USPAT; UPAD	OR	ON	2009/12/15 16:33

12/15/2009 5:14:58 PM
C:\Documents and Settings\ssingh3\My Documents\EAST\Workspaces\11141996-all-dbs.wsp

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 12/18/2009

ROBINS & PASTERNAK
1731 EMBARCADERO ROAD
SUITE 230
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 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.

Denise M. Vallancourt (Depositor's name)
(Signature)
3-12-10 (Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/141,996	06/01/2005	John Fraser Wright	0800-0045	5399

TITLE OF INVENTION: COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION

APPLN. TYPE	SMALL ENTITY	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	~~YES~~ NO	~~$755~~ $1510	$300	$0	~~$1055~~ $1810	03/18/2010

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 Robins & Pasternak LLP
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: 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 credit any overpayment, to Deposit Account Number 18-1648 (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)
☐ a. Applicant claims SMALL ENTITY status. See 37 CFR 1.27.
☒ b. Applicant is no longer claiming SMALL ENTITY status. See 37 CFR 1.27(g)(2).

NOTE: The Issue Fee and Publication Fee (if required) will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the United States Patent and Trademark Office.

Authorized Signature _____ Date 3-12-10

Typed or printed name Roberta L. Robins Registration No. 33,208

This collection of information 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.

Electronic Patent Application Fee Transmittal

Application Number:	11141996
Filing Date:	01-Jun-2005
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

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:				
Utility Appl issue fee	1501	1	1510	1510
Publ. Fee- early, voluntary, or normal	1504	1	300	300

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Miscellaneous:				
			Total in USD ($)	**1810**

Electronic Acknowledgement Receipt

EFS ID:	7196086
Application Number:	11141996
International Application Number:	
Confirmation Number:	5399
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
Receipt Date:	12-MAR-2010
Filing Date:	01-JUN-2005
Time Stamp:	10:46:58
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Credit Card
Payment was successfully received in RAM	$1810
RAM confirmation Number	9919
Deposit Account	
Authorized User	

File Listing:

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

1		if_20100312074751.pdf	154015 efe138af2d0f995aaf4e459e70e54c5af25d68b9	yes	2

Multipart Description/PDF files in .zip description		
Document Description	**Start**	**End**
Miscellaneous Incoming Letter	1	1
Issue Fee Payment (PTO-85B)	2	2

Warnings:

Information:

2	Fee Worksheet (PTO-875)	fee-info.pdf	32109 b22cdb93c90ac832f55bb1035f356451ad75f0e1	no	2

Warnings:

Information:

Total Files Size (in bytes):	186124

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	11/141,996
Filing Date	June 1, 2005
First Named Inventor	Wright et al.
Art Unit	1657
Examiner Name	S.K. Singh
Attorney Docket Number	0800-0045

Total Number of Pages in This Submission: 2

ENCLOSURES *(Check all that apply)*

- ☐ Fee Transmittal Form
- ☒ Fee Attached
- ☐ Amendment/Reply (8 pages)
  - ☐ 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):

Part B – Fee(s) Transmittal (1 page)

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	3/12/10	Reg. No.	33,208

CERTIFICATE OF TRANSMISSION/MAILING

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Signature			
Typed or printed name	Denise M. Vallancourt	Date	3/12/10

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United States Patent and Trademark Office
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P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

Bib Data Sheet

CONFIRMATION NO. 5399

SERIAL NUMBER	FILING OR 371(c) DATE / RULE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
11/141,996	06/01/2005	435	1657	0800-0045

APPLICANTS

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

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

This appln 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
** 07/06/2005

Foreign Priority claimed	STATE OR COUNTRY	SHEETS DRAWING	TOTAL CLAIMS	INDEPENDENT CLAIMS
☐ yes ☐ no	NJ	3	20	3

35 USC 119 (a-d) conditions met: ☐ yes ☐ no ☐ Met after Allowance

Verified and Acknowledged: _____ Examiner's Signature Initials

ADDRESS
20855

TITLE

COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION

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

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APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/141,996	04/27/2010	7704721	0800-0045	5399

20855 7590 04/07/2010

ROBINS & PASTERNAK
1731 EMBARCADERO ROAD
SUITE 230
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**
7,704,721	Apr. 27, 2010	COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION	Reel 016972 Frame 0854 Reel 039960 Frame 0117

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 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 previsions 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 LLC** 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.

Date: 06-Sep-2023

Assignee: Genzyme Corporation

Signed: Allyson Hatton

Print Name: Allyson Hatton

Print Title: Principal Counsel, Attorney-in-Fact on behalf of Genzyme Corporation

Electronic Acknowledgement Receipt

EFS ID:	48564330
Application Number:	11141996
International Application Number:	
Confirmation Number:	5399
Title of Invention:	COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION
First Named Inventor/Applicant Name:	John Fraser Wright
Customer Number:	20855
Filer:	David Michael Lee/Kerri Leary
Filer Authorized By:	David Michael Lee
Attorney Docket Number:	0800-0045
Receipt Date:	11-SEP-2023
Filing Date:	01-JUN-2005
Time Stamp:	15:34:13
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	Power of Attorney	199648_Genzyme_POA_signed.pdf	139942 d9f99cc36ed6a9a15665e01c7a659113950d5398	no	1

Warnings:

Information:	
Total Files Size (in bytes):	139942

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.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
11/141,996	06/01/2005	John Fraser Wright	0800-0045

CONFIRMATION NO. 5399

IMPROPER CPOA LETTER

20855
FOLEY & LARDNER LLP
3000 K STREET N.W.
SUITE 600
WASHINGTON, DC 20007-5109

OC000000062309446

Date Mailed: 09/26/2023

NOTICE REGARDING POWER OF ATTORNEY

This is in response to the power of attorney filed 09/11/2023. 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 statement required by 37 CFR 3.73(c) has not been received or is incomplete.

/jfgerrety/

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

37509
DECHERT LLP
Three Byrant Park
1095 Avenue of the Americas
New York, NY 10036-6797
UNITED STATES