

IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

GENZYME CORPORATION,)
)
Plaintiff,)
)
v.) C.A. No. 24-882 (RGA)
)
SAREPTA THERAPEUTICS, INC. and)
SAREPTA THERAPEUTICS THREE, LLC,)
)
Defendants.)

**SAREPTA THERAPEUTICS, INC. AND SAREPTA THERAPEUTICS THREE, LLC’S
SUPPLEMENTAL INITIAL INVALIDITY CONTENTIONS**

Pursuant to the Amended Scheduling Order (D.I. 78) and Paragraph 4.d. of the Default Standard for Discovery, Defendants Sarepta Therapeutics, Inc. and Sarepta Therapeutics Three, LLC (collectively, “Defendants” or “Sarepta”) hereby provide the following Supplemental Initial Invalidity Contentions for each asserted claim of U.S. Patent Nos. 12,031,894 (“the ’894 patent”), 12,013,326 (“the ’326 patent”), 11,698,377 (“the ’377 patent”), 12,123,880 (“the ’880 patent”), and 12,298,313 (“the ’313 patent”) (collectively, the “Additional Patents”), as well as the related invalidating references (*e.g.*, publications, manuals, and patents).¹

I. PRELIMINARY STATEMENT

In its Supplemental Initial Infringement Contentions, Plaintiff Genzyme Corporation (“Plaintiff” or “Genzyme”) alleges infringement of the following claims (collectively, “the Asserted Claims”):

- ’894 patent: claims 1-5, 10-23, and 30;
- ’326 patent: claims 1-3, 7-9, 12-16, 20-22, 25-27, and 30;

¹ Sarepta served initial invalidity contentions for U.S. Patent Nos. 7,704,721 and 9,051,542 on May 16, 2025, which are hereby incorporated by reference.

- '377 patent: claims 1-8, 11, 13-17, and 20;
- '880 patent: claims 1-5, 7, 10-15, and 21; and
- '313 patent: claims 1-6 and 11-27.

For the reasons set forth in the following sections, each of the Asserted Claims is invalid pursuant to at least 35 U.S.C. §§ 101, 103, and/or 112.

These Supplemental Initial Invalidity Contentions are preliminary and are based on Sarepta's current knowledge, understanding, and belief as to the facts and information available as of the date of these contentions. Sarepta does not concede the validity of any un-asserted claim of the Additional Patents and reserves the right to supplement and/or amend these contentions to include all bases of invalidity of any other claims that Genzyme may be permitted to assert as this case progresses.

Sarepta has not yet completed its investigation, discovery, or analysis of information related to this case, and additional discovery may require Sarepta to update these Supplemental Initial Invalidity Contentions. Sarepta has also not yet received relevant discovery, including documents from Genzyme relating to the claims or defenses in this action. For example, Genzyme has not produced documents evidencing the conception or reduction to practice of any purported invention claimed in the Additional Patents. Likewise, Sarepta has not had the opportunity to take any deposition in this action, including the depositions of the named inventors. Accordingly, Sarepta reserves the right to supplement, modify, or amend these contentions as additional facts are ascertained, analyses are made, research is completed, contentions are made, and claims are construed. Sarepta also reserves the right to supplement, modify, or amend these contentions based on any position Genzyme may take, including but not limited to, its infringement contentions, responses to written discovery, and statements made to the U.S. Patent and Trademark Office,

including in connection with any proceeding involving the Additional Patents, or the prosecution of any related patents and applications. Sarepta further reserves the right to supplement, modify, or amend these contentions as permitted by the Federal Rules of Civil Procedure, the Default Standard for Discovery, or pursuant to any Order of the Court.

The Asserted Claims have not yet been construed in this case. In the absence of a claim construction order, Sarepta has generally relied upon the plain and ordinary meaning of claim terms, definitions in the Additional Patents, and Genzyme's apparent claim construction positions from its Supplemental Initial Infringement Contentions, served on September 15, 2025, to the extent any such constructions can be determined or understood. Sarepta's reliance on Genzyme's apparent constructions of the claims should not be taken to mean that Sarepta in any way agrees with Genzyme's contentions or that Sarepta is precluded from propounding alternative claim constructions or requesting more detailed contentions in connection with claim construction proceedings in this case.

Sarepta reserves the right to supplement, modify, or amend these Supplemental Initial Invalidity Contentions in response to any claim construction positions that Genzyme may take in this case or after the claims have been construed by the Court. For example, Sarepta reserves the right to assert that a claim is indefinite, not enabled, and/or fails to meet the written description requirement in light of any claim construction positions Genzyme may assert or based on any claim constructions from the Court. Likewise, prior art not included in these disclosures, whether or not known to Sarepta, may be relevant depending upon the claim constructions that Genzyme asserts or that the Court may adopt. Thus, Sarepta reserves the right to supplement these disclosures in light of newly discovered art or changes in claim constructions.

These contentions are based, in part, on the limited information provided by Genzyme in its Supplemental Initial Infringement Contentions, which fail to show how Sarepta is liable for direct or indirect infringement of the Asserted Claims. Despite obligations under the Local Rules and the Joint Scheduling Order, Genzyme's Supplemental Initial Infringement Contentions fail to provide sufficient detail concerning the meaning and scope of the Asserted Claims and how the claim language in fact applies to Sarepta's activities. Genzyme's Supplemental Initial Infringement Contentions identify as a purported basis for infringement only 35 U.S.C. §§ 271(a) and/or (b), and only literal infringement, to the exclusion of any separate reasoning or rationale regarding infringement under the doctrine of equivalents. Accordingly, Sarepta reserves the right to amend and/or supplement these contentions, including the right to identify additional information concerning the prior art identified here, as well as additional prior art if Genzyme changes or supplements its Supplemental Initial Infringement Contentions, advocates particular claim construction positions, identifies additional theories of infringement, including theories under the doctrine of equivalents or other sections of Title 35, and/or the Court construes the claims.

In addition to these Supplemental Initial Invalidation Contentions, and any supplement to these contentions, Sarepta may rely on the Additional Patents; the prosecution histories of the Additional Patents and all related patents and applications; all references listed in the References Cited portion of the Additional Patents and all related patents and applications; documents and exhibits in IPR2023-01044 and IPR2023-01045; and fact and expert testimony in this case.

Sarepta reserves the right to supplement, modify, or amend these Supplemental Initial Invalidation Contentions in light of further investigation, fact and expert discovery, or any Order of the Court, including rulings on claim construction.

II. U.S. PATENT NOS. 12,031,894 AND 12,013,326

A. PRIORITY DATE

The '326 patent issued from U.S. Patent Application No. 18/506,853 (“the '853 application”) on June 18, 2024. The '894 patent issued from U.S. Patent Application No. 18/513,970 (“the '970 application”) on July 9, 2024. The '326 and '894 patents claim priority to the same provisional and non-provisional applications, and will be referred to collectively as “the AUC Patents.”

The AUC Patents claim priority to the same series of non-provisional applications: U.S. Patent Application No. 18/188,176, filed on March 22, 2023, which is a continuation of application No. 16/547,144, filed on August 21, 2019, which is a continuation of application No. 15/544,498, filed as application No. PCT/US2016/013947 on January 19, 2016. *See* '326 patent at 1 (63); '894 patent at 1 (63). The AUC Patents share the same specification.

The AUC Patents also claim priority to U.S. Provisional Application No. 62/105,714 (“the '714 provisional application”), filed on January 20, 2015. *See* '326 patent at 1 (60); '894 patent at 1 (60).

The earliest possible priority date for the AUC Patents is January 20, 2015.

B. OBVIOUSNESS

Under 35 U.S.C. § 103, a patent claim is invalid “if the differences between the claimed invention and the prior art are such that the claimed invention as a whole would have been obvious before the effective filing date of the claimed invention to a person having ordinary skill in the art to which the claimed invention pertains.” The analysis of obviousness includes consideration of the following factors: (1) the level of ordinary skill in the art; (2) the scope and content of the prior art; (3) the differences between the prior art and the claimed subject matter; and (4) any

objective evidence of non-obviousness, also known as secondary considerations. *Graham v. John Deere Co.*, 383 U.S. 1, 17-18 (1966).

To “determine whether there was an apparent reason to combine the known elements in the fashion claimed by the patent at issue,” a court should “look to interrelated teachings of multiple patents; the effects of demands known to the design community or present in the marketplace; and the background knowledge possessed by a person having ordinary skill in the art.” *KSR Int’l Co. v. Teleflex Inc.*, 550 U.S. 398, 418 (2007). A patent may be obvious if, *inter alia*, there was a “known problem for which there was an obvious solution encompassed by the patent’s claims” or the combination of the patent’s claims was “obvious to try.” *Id.* at 419-421.

Sarepta identifies the prior art references set forth in the table below, which alone or in combination, render the Asserted Claims of the AUC Patents invalid under 35 U.S.C. § 103. The identified prior art is also relevant to show the state of the art and reasons and motivations for making improvements, additions, and combinations of the disclosures in these references. Sarepta reserves the right to identify additional prior art as discovery progresses.

The references listed below are all prior art under AIA 35 U.S.C. § 102(a). The earliest possible priority date for the Asserted Claims of the AUC Patents is the filing date of the ’714 provisional application on January 20, 2015.

Reference	Year
Berkowitz and Philo, “Monitoring the homogeneity of adenovirus preparations (a gene therapy delivery system) using analytical ultracentrifugation” <i>Anal. Biochem.</i> 362:16-37 (2007) (“Berkowitz”)	2007
Carter <i>et al.</i> , “Adeno-Associated Virus Autointerference” <i>Virology</i> 92:449-462 (1979) (“Carter”)	1979
Carter <i>et al.</i> , “Genome Localization of Adeno-Associated Virus RNA” <i>J. Virology</i> 19:1044-1053 (1976) (“Carter (1976)”)	1976

Reference	Year
Carter <i>et al.</i> , “Specific Cleavage of Adenovirus-Associated Virus DNA by Restriction Endonuclease R·EcoRI – Characterization of Cleavage Products” <i>Virology</i> 63:523-528 (1975) (“Carter (1975)”)	1975
Carter, “Adeno-associated virus and the development of adeno-associated virus vectors: a historical perspective” <i>Molecular Therapy</i> 10:981-89 (2004) (“Carter (2004)”)	2004
Cole and Hansen, “Analytical Ultracentrifugation as a Contemporary Biomolecular Research Tool” <i>J. Biomolecular Techniques</i> 10:163-176 (1999) (“Cole (1999)”)	1999
Cole <i>et al.</i> , “Analytical Ultracentrifugation: Sedimentation Velocity and Sedimentation Equilibrium” in <i>Methods in Cell Biology</i> , Vol. 84, 143-179 (2008) (“Cole”)	2008
de la Maza and Carter, “DNA Structure of Incomplete Adeno-Associated-Virus Particles” in <i>Replication of Mammalian Parvoviruses</i> (Ward, D., and Tattersall, P., eds.), Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 193-204 (1978) (“de la Maza”)	1978
de la Maza and Carter, “Heavy and Light Particles of Adeno-Associated Virus” <i>J. Virol.</i> 33:1129-1137 (1980) (“de la Maza (March 1980)”)	1980
de la Maza and Carter, “Molecular Structure of Adeno-Associated Virus Variant DNA” <i>J. Biol. Chem.</i> 255:3194-3203 (1980) (“de la Maza (April 1980)”)	1980
European Medicines Agency, Committee for Medicinal Products for Human Use, Glybera® Assessment Report (July 19, 2012)	2012
Gao <i>et al.</i> , “Empty virions in AAV8 vector preparations reduce transduction efficiency and may cause total viral particle dose-limiting side effects” <i>Molecular Therapy – Methods & Clinical Development</i> 9:1-8 (2014) (“Gao”)	2014
Gombold <i>et al.</i> , “Lot Release and Characterization Testing of Live-Virus-Based Vaccines and Gene Therapy Products, Part 2” <i>BioProcess International</i> 4:56-65 (2006) (“Gombold”)	2006
Hermonat and Muzyczka, “Use of adeno-associated virus as a mammalian DNA cloning vector: transduction of neomycin resistance into mammalian tissue culture cells” <i>Proc. Natl. Acad. Sci. USA</i> 81:6466-70 (1984) (“Hermonat”)	1984

Reference	Year
Hoque <i>et al.</i> , “Chimeric Virus-like Particle Formation of Adeno-Associated Virus” <i>Biochemical and Biophysical Research Communications</i> 266:371–376 (1999) (“Hoque”)	1999
Myers and Carter, “Assembly of Adeno-Associated Virus” <i>Virology</i> 102:71-82 (1980) (“Myers”)	1980
Ralston, "Introduction to Analytical Ultracentrifugation" (Beckman Coulter Life Sciences) (“Ralston”)	1993
Schuck, “Size-Distribution Analysis of Macromolecules by Sedimentation Velocity Ultracentrifugation and Lamm Equation Modeling” <i>Biophysical Journal</i> 78:1606-1619 (2000) (“Schuck”)	2000
Schuck <i>et al.</i> , “Size-Distribution Analysis of Proteins by Analytical Ultracentrifugation: Strategies and Application to Model Systems” <i>Biophysical Journal</i> 82:1096-1111 (2002) (“Schuck (2002)”)	2002
Sommer <i>et al.</i> , “Quantification of Adeno-Associated Virus Particles and Empty Capsids by Optical Density Measurement” <i>Molecular Therapy</i> 7:122-28 (2003) (“Sommer”)	2003
Steinbach <i>et al.</i> , “Assembly of adeno-associated virus type 2 capsids in vitro” <i>J. of Gen. Virology</i> 78:1453-62 (1997) (“Steinbach”)	1997
Tratschin <i>et al.</i> , “A human parvovirus, adeno-associated virus as a eukaryotic vector: transient expression and encapsidation of the prokaryotic gene for chloramphenicol acetyl transferase” <i>Molecular Cell Biology</i> 4:2072–81 (1984) (“Tratschin”)	1984
U.S. Patent Application Publication No. US 2009/0017542 A1 (“Colosi”)	2009
Ward and Tattersall, Preface, in <i>Replication of Mammalian Parvoviruses</i> (Ward, D., and Tattersall, P., eds.), Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, ix-x (1978) (“Ward”)	1978
WO2014/125101 A1 (PCT/EP2014/052978) and certified English translation, “Methods for the Production of Double-Stranded AAV Viral Particles,” published on August 21, 2014 (“Le Bec”)	2014
Zhao <i>et al.</i> , “Overview of Current Methods in Sedimentation Velocity and Sedimentation Equilibrium Analytical Ultracentrifugation” <i>Current Protocols in Protein Science Supplement</i> 71:20.12.1-20.12.49 (2013) (“Zhao”)	2013

The patents, publications, and combinations of references, listed in the table above, disclose, teach, or suggest every element of the Asserted Claims of the AUC Patents. These prior art disclosures, teachings, and suggestions are discussed further in the following sections. In addition, Sarepta incorporates by reference each and every prior art reference of record in the prosecution of the AUC Patents and related patents and applications, including the statements made by the applicants during prosecution and the prior art discussed and/or cited in the specification.

Sarepta may also rely on expert testimony, inventor admissions, and any additional prior art located or developed during the course of discovery. Sarepta may rely on any of the materials in these contentions to demonstrate a motivation to combine and/or a reasonable expectation of success. Sarepta may also rely on expert testimony to further explain the prior art and to, for example, demonstrate a motivation to combine and a reasonable expectation of success. Further, Sarepta may rely on uncited portions of the identified prior art, references cited within the identified prior art, other references (irrespective of whether such references themselves qualify as prior art) to show the state of the art, and/or expert testimony to provide context to, or aid in, understanding the cited portions of the identified prior art.

In the sections below, references identified as rendering a claim obvious are representative and are not intended to be exhaustive. Other references disclosing the same or similar elements may be substituted for the cited references. Likewise, additional obviousness combinations of the references identified below are possible, and Sarepta reserves the right to use any such combination(s) in this litigation. Motivation to combine can be inferred generally for all references within the field of art of the AUC Patents. Furthermore, where references refer to or cite one another, motivation to combine may be inferred, whether or not specifically called out in these

contentions. Finally, Sarepta's identification of motivation to combine in the cited references should not be taken as an admission or a representation that Sarepta will not rely upon other tests for obviousness in view of *KSR*, 550 U.S. at 401. This includes showing any of the following: (1) that the combination of elements was obvious to try; (2) that the combination of elements according to known methods yielded predictable results; (3) that the substitution of one known element for another obtained predictable results; (4) that the application of a known technique to a known device, method, or product ready for improvement yielded predictable results; or (5) that known work in one field of endeavor prompted variations of such work for use in either the same field or a different one based on design incentives or other market forces because the variations are predictable to a person of ordinary skill in the art ("POSA").

The identification of prior art that discloses and/or renders obvious a particular claim element in these contentions is not an admission that the claim element satisfies the requirements of 35 U.S.C. § 112. Where Sarepta asserts that a claim is invalid under 35 U.S.C. § 112 (such as because of a failure to particularly point out and distinctly claim the alleged invention, failure to provide written description support, and/or failure to enable a person of ordinary skill in the art to make and use the claimed invention), Sarepta has also provided prior art that renders the claim invalid for obviousness.

Documents produced by Genzyme to date do not support any secondary considerations of non-obviousness that would lead to a conclusion that the Asserted Claims of the AUC Patents would not have been obvious to a person of ordinary skill. In the event that Genzyme contends that there are any secondary considerations of non-obviousness, Sarepta will respond. Further, Genzyme must establish the requisite nexus between any alleged secondary considerations and the Asserted Claims of the AUC Patents. Sarepta is not aware of any evidence that would establish

the required nexus between any alleged secondary considerations and the Asserted Claims of the AUC Patents. Nevertheless, even if there were any evidence of a nexus between any alleged secondary considerations and the Asserted Claims of the AUC Patents, any such alleged secondary considerations would not rebut the strong *prima facie* evidence of obviousness here.

The methods recited in the Asserted Claims of the AUC Patents are obvious over at least the following combinations of prior art references, discussed in further detail in the sections below.

1. Knowledge in the Art

(a) Analytical Ultracentrifugation

Analytical ultracentrifugation (“AUC”) is an analytical technique for the characterization of macromolecules dating to the early twentieth century. *See* Berkowitz and Philo, “Characterizing Biopharmaceuticals using Analytical Ultracentrifugation” in *Biophysical Characterization of Proteins in Developing Biopharmaceuticals* (Houde, D., and Berkowitz, S., eds.), Elsevier, 211-260 (2015) (“Berkowitz (2015)”) at 211; Cole at 174. AUC analysis is highly versatile and relies upon first principle information to determine properties of different types of particles across a ranges of concentrations and sizes. ’326 patent at 19:37-43 (“AUC analysis has been well characterized over many decades and is highly versatile. Because AUC analysis relies upon first-principle hydrodynamic and thermodynamic information, AUC may be applied to determine the biophysical properties of many types of particles across a wide range of particle concentrations and sizes.”).

Sedimentation velocity analytical ultracentrifugation (“SV-AUC”) is a technique used to characterize macromolecules and nanoparticles in solution, in which the experimenter spins the ultracentrifuge rotor at a fast enough speed to force suspended particles in a sample to migrate to the bottom of the sample cell while collecting data on their differing rates of migration as they transit the cell. Cole (1999) at 165 (“In a sedimentation velocity experiment, a moving boundary

is formed on application of a strong centrifugal field... A series of scans (ie, measurements of sample concentration, c , as a function of radial distance, r) are recorded at regular intervals to determine the rate of movement and broadening of the boundary as a function of time.”); Berkowitz (2015) at 214-215 (“In the case of classical boundary SV-AUC, which is by far the most common form of AUC conducted today in the biopharmaceutical industry, the centrifugal field has sufficient strength to cause the protein molecules of interest to migrate from the meniscus to the bottom (or base) of the AUC cell.”).

Before the earliest priority date for the AUC patents, it was known that the most common form of AUC conducted in the biopharmaceutical industry is SV-AUC. Berkowitz (2015) at 219 (“Today in the biopharmaceutical industry, especially in the process development area, by far the most common form of AUC conducted is SV-AUC in the form of boundary SV-AUC.”).

It was known that sedimentation velocity is a function of the mass, shape, and density of a particle, and is reported as an experimental molecular parameter called the sedimentation coefficient (s). *See, e.g.*, Cole at 146; Cole (1999) at 165; Berkowitz (2015) at 215. Those skilled in the art were aware that the rate of sedimentation in a centrifugal field is described by the Svedberg equation:

$$s = M (1 - \bar{v}\rho) / N_A f$$

where s is the sedimentation coefficient of the particle, M is the molar mass of the particle, \bar{v} is the partial specific volume of the particle, ρ is the density of the solvent, N_A is Avogadro’s number, and f is the frictional coefficient of the particle. *See, e.g.*, Cole (1999) at 165-66; Cole at 146; Berkowitz (2015) at 216.

The Lamm equation has been long known as the equation that describes the sedimentation of a particle under ultracentrifugation, and is used in a variety of publicly available computer

software programs to generate sedimentation coefficient distribution profiles and determine sedimentation coefficients from SV-AUC data. Berkowitz (2015) at 228-233; Cole at 146-147. One example is the SEDFIT program, created and freely distributed by Peter Schuck since 2000. *See, e.g.*, Schuck; Schuck (2002). Multiple other software programs for analyzing SV-AUC data were also known. *See, e.g.*, Cole at 159, 166; Berkowitz (2015) at 228, 230-232.

(b) Adeno-Associated Virus

Adeno-associated virus (“AAV”) is a replication-defective human parvovirus discovered over 60 years ago that has been studied for decades as a potential gene therapy vector. *See, e.g.*, Carter (2004) at 981; de la Maza (April 1980) at 3194; Le Bec at 1:23-29.

By the 1980s, the general structure and mode of replication of AAV DNA was known. Carter (2004) at 983. For example, researchers were aware that AAV assembly occurs by packaging DNA into preformed capsids. Carter (2004) at 983-84; Myers at 71-72, 79-82; de la Maza (April 1980) at 3203.

Recombinant AAVs (“rAAVs”) are AAVs in which the wild-type AAV genes have been replaced by other DNA sequences. Le Bec at 1:33-2:3 (“In rAAV vectors, the rep and cap genes flanked by the ITR (inverted terminal repeat) sequences respectively coding for the replication-regulating proteins and the structural proteins of the capsid are excised and replaced by the transgene (therapeutic gene).”).

By the early 2000s, companies were developing gene therapy products comprising viral-based vectors. *See, e.g.*, Carter (2004) at 981. By 2015, researchers were aware that the “main types of vectors used today in gene therapy are viral vectors, including recombinant adeno-associated virus or rAAV vectors.” Le Bec at 1:23-29; *see also* Berkowitz at 16 (describing “the use of specifically engineered viruses that can carry the desired genetic material into the target

cell.”); *see also* ’326 patent at 1:32-34 (“ Recombinant viruses show great promise and utility as a vehicle to deliver therapeutic nucleic acids for gene therapy applications.”)

By 2015, researchers had used SV-AUC methods to characterize rAAV particles developed for gene therapy applications. *See, e.g.*, Gao at 6 (“[A]nalytic ultracentrifugation technology is a powerful tool for quantitative characterization of structural heterogeneity of rAAV preparations, allowing precise and selective observation of viral capsid sedimentation in real time.”); Glybera[®] EMA Assessment Report at 15 (AUC identified as a method used to “determine mass, density and distribution profiles” for AAV-based gene therapy Glybera[®]); *see also* Berkowitz at 16 (“We demonstrate that a single sedimentation velocity run on an adenovirus sample can detect and accurately quantify a number of different forms of virus particles and subvirus particles” including “a) intact virus monomer particles, (b) virus aggregates, (c) empty capsids (ECs), and (d) smaller assembly intermediates.”); Cole at 144-145 (“For over 75 years, analytical ultracentrifugation (AUC) has proven to be a powerful method for characterizing solutions of macromolecules and an indispensable tool for the quantitative analysis of macromolecular interactions. [...] The range of molecular weights suitable for AUC exceeds that of any other solution technique from a few hundred Daltons (e.g., peptides, dyes, oligosaccharides) to several hundred-million Daltons (e.g., viruses, organelles).”).

2. Le Bec and de la Maza

Claims 1-5, 10-11, 20-23, and 30 of the ’894 patent and claims 1-3, 7, and 12-16 of the ’326 patent are obvious over the combination of Le Bec and de la Maza.

Le Bec. Le Bec is a PCT Publication of International Application Number PCT/EP2014/052978, titled “Methods for the Production of Double-Stranded AAV Viral Particles.” Le Bec at (21), (54). Le Bec was published on August 21, 2014. Thus, Le Bec is prior art under AIA 35 U.S.C. § 102(a)(1).

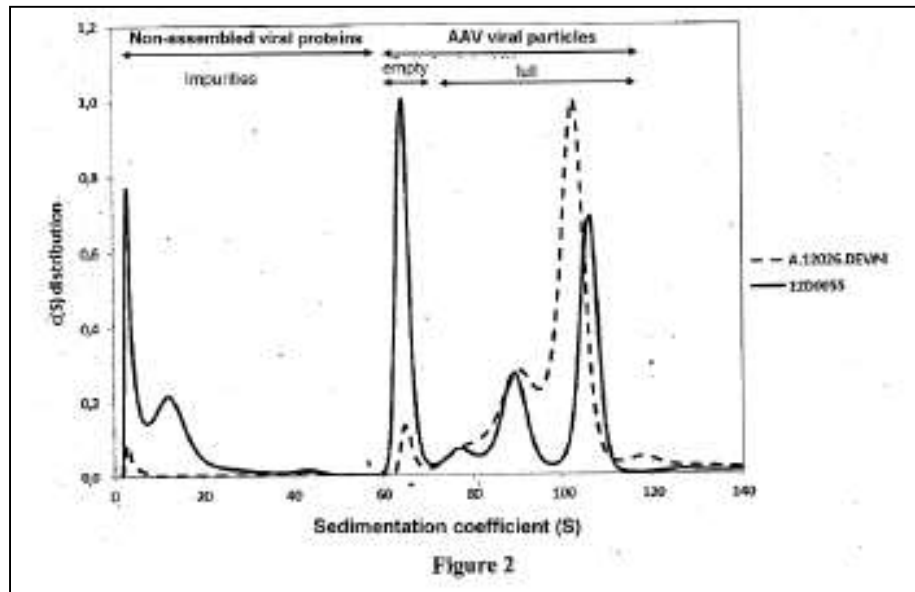
Le Bec discloses methods to produce double stranded/“self-complementary” AAV (“scAAV”) particles, a type of rAAV particle, for gene therapy applications. *Id.* at 3:4-4:10. Le Bec describes the production of scAAV compositions using two methods, one involving insect derived sf9 cells, and another employing human embryonic kidney (HEK293) cells. *Id.* at 6:14-34.

Le Bec discloses characterization data for scAAV preparations using sf9 cells and HEK293 cells. *Id.* at 14:21-15:28. This characterization data includes “[a]nalysis of empty and full AAV viral particles” using “analytical ultracentrifugation.” *Id.* at 15:17-18. Le Bec reports that “[t]he sedimentation coefficient of the various AAV viral particles (empty, full, aggregate) and other present populations (subparticles, contaminant proteins, aggregate) in the purified products was determined by real-time centrifugation” and that “[c]entrifugation of the samples was carried out at a speed of 16,000 rpm using 100 μ l or 400 μ l of undiluted pure vectors, sedimentation was followed by absorbance at the wavelength of 276 nm, and the sedimentation coefficient of the various populations was obtained using the software SEDFIT.” *Id.* at 15:23-28.

Le Bec notes that the densities of empty and full AAV viral particles had been reported in the literature: “It has been reported in the literature that the density is approximately 1.32 g/cm³ (equivalent to 60S) for empty AAV viral particles, i.e. vectors lacking a viral genome, and 1.45 g/cm³ (equivalent to 110S) for full AAV viral particles, i.e. those containing a viral genome with a maximum size of 4800 bases.” *Id.* at 17:29-18:1. Le Bec cites a March 1980 article by Carter and de la Maza, which reports these densities and S-values for empty and full AAV particles. *Id.* at 18:1, n.15 (citing de la Maza (March 1980)). As stated in Le Bec, “[k]nowing that the difference in density is sufficiently significant to distinguish them by centrifugation, we have implemented a

method today for analytically separating by ultracentrifugation and quantifying the different species present in an AAV viral preparation.” Le Bec at 18:1-6.

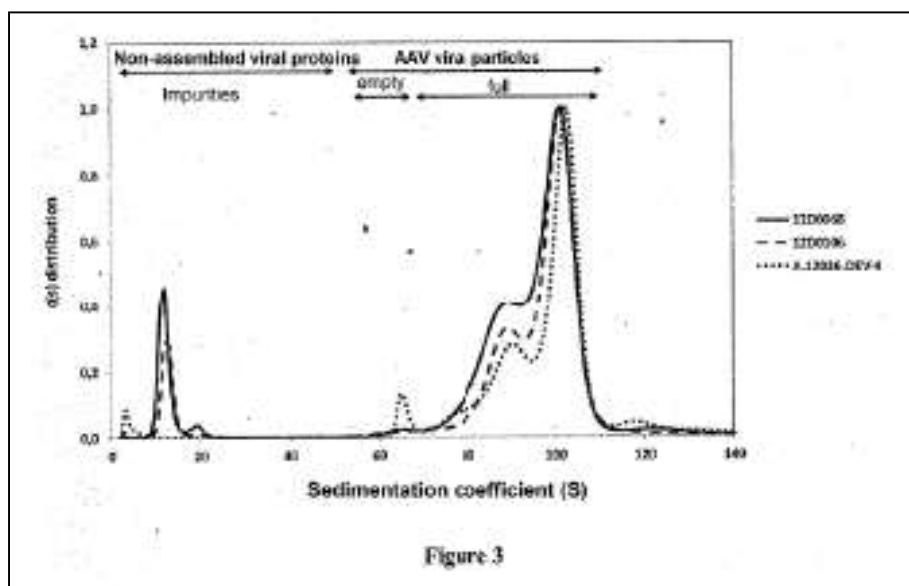
Figures 2 and 3 of Le Bec depict C(s) v. S plots from the AUC analyses. Figure 2 is a C(s) v. S plot from the AUC analysis comparing batches of scAAV produced in sf9 cells (A.12026.DEV-4, dotted line) and HEK293 cells (12D0055, solid line). *Id.* at 18:8-11. Figure 2 is reproduced below:



Le Bec explains that the distribution profile in Figure 2 contains “two categories of populations”: (a) <60S population corresponding to non-assembled viral proteins and/or contaminants, and (b) 60S-110S population corresponding to intact AAV viral particles. *Id.* at 18:11-20. Le Bec notes that the <60S population is “virtually absent in batch A.12026.DEV-4, while it is present in a non-negligible amount in batch 12D0055.” *Id.* at 18:15-17. For the 60S-110S population, Le Bec identifies “two subpopulations,” with “empty AAV viral particles at 65S and full AAV viral particles between 80S and 110S.” *Id.* at 18:21-23. Included in the population of “full” particles, Le Bec identifies AAVs “with the viral genome in single-stranded form at 90S and double stranded form at 105S.” *Id.* at 18:21-25.

Le Bec concludes that “[h]ighly unexpectedly, we detected very few empty AAV viral particles with the sf9 system compared to the HEK293 system.” *Id.* at 18:27-29. Furthermore, the “full” AAV viral particles were “significantly more enriched in the genome in double-stranded form (105S) with the sf9 system compared to the HEK293 system.” *Id.* at 18:29-32.

Figure 3 of Le Bec is a C(s) vs. S. plot of the AUC distribution profiles for three batches of scAAV9 vectors produced in the sf9 system (A.12026.DEV-4, 12D0106, 12D0068). *Id.* at 19:7-10. Figure 3 is reproduced below:



Le Bec states that “these three batches all show a similar distribution and confirm that the sf9 system makes it possible to produce AAV viral particles essentially composed of full AAV particles (> 80%), with a very high percentage of viral genome in double-stranded form (> 50%).” *Id.* at 19:11-15.

Nothing in Le Bec suggests that the use of AUC to characterize rAAV particle compositions was considered unexpected or required more than routine optimization or experimentation.

De la Maza. De la Maza is a book chapter titled “DNA Structure of Incomplete Adeno-Associated-Virus Particles” in the book “Replication of Mammalian Parvoviruses.” See de la Maza at cover, 193. De la Maza was published in 1978 and is prior art under AIA 35 U.S.C. § 102(a)(1). *Id.*

De la Maza discloses experiments characterizing the physical properties of incomplete AAV genomes. De la Maza at 193 (“We have examined the AAV DNA present in both ‘full’ and ‘empty’ particles. These studies revealed that all density classes of AAV virions appear to contain varying proportions of incomplete genomes. ... We summarize here the physical properties of the incomplete AAV genomes.”).

De la Maza discloses that there is a linear relationship between the density of AAV particles with “incomplete” genomes and the length of the DNA packaged inside the capsid. De la Maza at 203 (“The density of the incomplete virus particles appears to be correlated with the size of the incomplete DNA.”); see also de la Maza (April 1980) at 3194 (“The density of the virus particle is generally correlated with the size of the variant DNA.”); Carter at 449 (“Elsewhere we have reported an analysis of the physical structure of DNA in each class of AAV particle (de la Maza and Carter, 1978). Briefly, the densities of the AAV particles which are lighter than 1.41 g/cm³ are proportional to the size of the encapsidated DNA molecule.”). As de la Maza reiterates in a follow-on article describing the earlier results, “[t]hese molecules appear to be encapsidated in AAV virions with usually only one strand per particle; thus, the particle density has an approximately direct linear relationship to the strand length.” De la Maza (April 1980) at 3202.

In de la Maza, the authors grew AAV2 in KB3 spinner cells with Ad2 as the helper. De la Maza at 194. Virus particles present in lysates of KB cells infected with AAV2 and Ad2 were fractionated via equilibrium density centrifugation in CsCl. *Id.* at 194. The authors identified and

analyzed particle components which banded in CsCl at densities of 1.45 g/cm³, 1.41 g/cm³, 1.35 g/cm³, and 1.32 g/cm³. *Id.* De la Maza describes releasing the DNA from virions by sedimentation in alkaline sucrose gradients, reannealing in formamide, and further fractionation in neutral sucrose gradients. *Id.* at 194. The authors defined several classes of incomplete DNA molecules (Types I-IV) that were obtained from AAV virions of different densities, as detailed in Table 1:

DNA	Density of virion ^a (g/cm ³)	Sedimentation coefficient and molecular weight of isolated DNA ^b			
		alkaline sucrose		neutral sucrose	
		S	m.w. × 10 ⁻⁶	S	m.w. × 10 ⁻⁶
Intact	1.41	15.5	1.4	14.5	2.8
Type I	1.41	15.5	1.4	11.5	1.4
Type II	1.41	12.0	0.8	10.0	0.8
Type III (oligomer)	1.35	8.5	0.3	8-15	~1.4
Type III	1.35	8.5	0.3	(1) 8.2 (2) 7.0	0.6 0.3
Type IV	1.32	5.5	~0.13	5.0	~0.13

De la Maza teaches that the molecular weights and sedimentation coefficients in Table 1 were computed from sucrose gradient profiles using molecular-weight markers. *Id.* at 195, Table 1. All gradients contained 3H-labeled duplex monomer AAV2 DNA or fragmented genomes representing 38% (EcoRI B), 22% (Bam B), and 4.5% (EcoRI C) of the AAV2 genome, respectively. *Id.* at 196, Figure 1. From these standards, de la Maza computed the molecular weights and sedimentation coefficients of the isolated incomplete AAV DNA components, as detailed in Table 1.

De la Maza further teaches that the “density of the incomplete virus particles appears to be correlated with the size of the incomplete DNA.” *Id.* at 203. This correlation “implies that virus assembly occurs by encapsidation of DNA into preformed capsids, since any model for

crystallization of viral capsids around the DNA might be expected to yield particles with aberrant morphology with smaller DNA.” *Id.*

Motivation to Combine. A POSA would have been motivated to combine Le Bec with de la Maza because both Le Bec and de la Maza are related to the separation, identification, and characterization of viral particles found in preparations of AAV. Le Bec discloses SV-AUC methods for evaluating the composition of preparations of rAAV particles for use in gene therapy applications. Le Bec at 1:5-19, 3:24-4:10. De la Maza teaches that there is a linear relationship between the density of an AAV particle and the length of the DNA inside the capsid. De la Maza at 203; de la Maza (April 1980) at 3194, 3202; Carter at 449. A POSA would have been motivated to apply de la Maza’s teaching to the analysis of SV-AUC distribution profiles for rAAV preparations, obtained using the methods in Le Bec, to identify and characterize the various species present in a preparation of rAAV particles, including AAV particles with partial or fragmented genomes.

Le Bec teaches that SV-AUC may be used to evaluate the quality of high-titer batches of rAAV during “the large-scale production of human-grade gene therapy vectors in compliance with good manufacturing practice.” Le Bec at 3:32-4:9. Le Bec thus teaches that SV-AUC is a useful method for quality control testing that may be applied to the industrial-scale production of rAAV vectors for gene therapy. *Id.* at 4:9-10, 15:17-28, 17:29-19:15.

Given the importance of drug product quality to achieve FDA approval (Berkowitz at 16; Gao at 1, 6; Gombold at 4-5), and the recognized benefits of using SV-AUC methods for analyzing such aspects of biological samples (Le Bec at 15:17-28, 17:29-19:15; Cole at 145, 149, 161-168), a POSA would have been motivated to select SV-AUC to characterize preparations of rAAV

particles and quantify the presence of different rAAV species during the manufacture of rAAV vectors, particularly rAAV vectors for therapeutic applications.

Indeed, Le Bec includes an express motivation to combine the disclosed SV-AUC methods with the teachings in de la Maza. Le Bec cites an article published by de la Maza and Carter in March 1980 reporting the separation and characterization of empty and full AAV particles using centrifugation in a CsCl gradient. Le Bec at 17:30-18:1 (citing de la Maza (March 1980)). Le Bec notes that “[i]t has been reported in the literature that the density is approximately 1.32 g/cm³ (equivalent to 60S) for empty AAV viral particles, i.e. vectors lacking a viral genome, and 1.45 g/cm³ (equivalent to 110S) for full AAV viral particles, i.e. those containing a viral genome with a maximum size of 4800 bases.” *Id.* Le Bec applies these teachings of de la Maza and Carter to the analysis of SV-AUC profiles for rAAVs, using the density measurements (and S-values) reported by de la Maza and Carter as benchmarks for analyzing the AUC results for rAAVs shown in Le Bec Figures 2 and 3. Le Bec at 18:8-32. Specifically, Le Bec uses the density measurements (and equivalent S-values) reported by de la Maza and Carter as benchmarks for analyzing the AUC results for scAAVs in Figures 2 and 3. *Id.* at 17:33-18:1, 18:21-25.

Thus, a POSA would have been motivated to combine Le Bec with other teachings from the Carter lab analyzing the physical properties of AAV, namely de la Maza (first published in 1978 and reported again in a journal article published in April 1980) which discloses the linear relationship between AAV particle density and the size of the encapsidated DNA.

Reasonable Expectation of Success. A POSA would have had a reasonable expectation of success in combining Le Bec and de la Maza to determine the sedimentation coefficients of rAAVs with fragmented genomes. The techniques required to arrive at the claimed SV-AUC methods were well known to a POSA.

A POSA would have reasonably expected success because the SV-AUC method was well-known and within the skill of an ordinary skilled artisan to optimize, and SV-AUC had already been applied to rAAV particles. '326 patent at 19:37-43. The sedimentation coefficients of empty and full rAAV particles were known and reported in the literature, and Le Bec taught that rAAV particles could be effectively separated, characterized, and quantified by AUC. Le Bec at 15:17-28, 18:8-32.

In particular, Le Bec teaches that AAV particles that can be separated using equilibrium centrifugation in a CsCl gradient, as taught in de la Maza, may also be separated and quantified using SV-AUC methods. Le Bec at 18:1-6 (“Knowing that the difference in density is sufficiently significant to distinguish them by centrifugation [using a CsCl gradient], we have implemented a method today for analytically separating by ultracentrifugation and quantifying the different species present in an AAV viral preparation.”). A POSA would have had a reasonable expectation of success that AAV particles with fragmented genomes that could be separated using a CsCl gradient may also be separated and quantified using the SV-AUC methods in Le Bec.

Further, a POSA would have had a reasonable expectation of success in applying the teachings of de la Maza to analyze sedimentation coefficients obtained using the SV-AUC methods of Le Bec and determine the size of the DNA in rAAVs with fragmented genomes. De la Maza teaches the linear relationship between AAV particle density and the length of the genome. De la Maza at 203; de la Maza (April 1980) at 3194, 3202; Carter at 449. A POSA would have had a reasonable expectation of success in applying the teaching of de la Maza to the SV-AUC methods of Le Bec and determining the size of the DNA in rAAVs with fragmented genomes by comparison to the S-values for rAAVs with genomes of known length.

Finally, a POSA would have had a reasonable expectation of success in using publicly available software programs, such as SEDFIT, to generate distribution profiles from SV-AUC data and to integrate the area under the observed peaks to determine the relative concentrations of the rAAV species in the preparation, including rAAV particles with fragmented genomes. *See, e.g.*, Le Bec at 15:19-28, 19:1-15.

3. Le Bec, de la Maza, and Cole

Claims 12-19 of the '894 patent and claims 8-9 of the '326 patent are obvious over Le Bec and de la Maza, in view of Cole.

Cole. Cole is a chapter in *Methods in Cell Biology*, titled “Analytical Ultracentrifugation: Sedimentation Velocity and Sedimentation Equilibrium.” Cole at 143. Cole was published in 2008 and qualifies as prior art under AIA 35 U.S.C. § 102(a)(1). *Id.*

Cole provides a general overview of various analytical ultracentrifugation techniques, including SV-AUC, and explains the parameters to be adjusted depending on the experimental system and samples being tested. Cole states that AUC is considered “a versatile and powerful method,” with “broad applications for the study of biomacromolecules in a wide range of solvents and over a wide range of solvent concentrations.” *Id.* at 144.

Cole teaches that a common application of SV-AUC is to determine the distribution of sedimentation coefficients for different macromolecules, e.g., by their $C(s)$ values, explaining that “often a simple relationship between s [sedimentation coefficient] and M [effective particle mass] may be used to identify particular peaks as belonging to certain oligomers (e.g., dimer, trimer, etc.) or certain fragments of the monomer.” *Id.* at 149. In particular, “[s]edimentation coefficient distributions are used widely in the pharmaceutical industry to assess the stability of protein formulations and to characterize preparations of inherently heterogeneous samples (e.g., vaccines based on bacterial cell wall preparations).” *Id.*

Cole describes the instrumentation routinely used in SV-AUC analysis. Analytical ultracentrifuges are distinguished from other high-speed centrifuges by “the specialized rotors, sample holders and optical systems that permit the observation of samples during sedimentation.” *Id.* at 150. Samples are loaded into cells that permit the passage of light allowing for optical assessments during centrifugation. These “fundamental measurements” are known as “scans” and “are acquired at intervals ranging from minutes (for velocity sedimentation) to hours (for equilibrium sedimentation).” *Id.* at 151.

Cole instructs how to determine and optimize parameters for an SV-AUC experiment and how to perform the analytical steps necessary to interpret the data. *Id.* at 161-168. For example, Cole teaches that most SV-AUC experiments “are conducted at 20°C.” *Id.* at 163. Cole further teaches that for SV-AUC experiments, “[i]t should take a boundary at least 2 h to sediment the full length of the cell (1.5 cm maximum), to ensure sufficient scans will be acquired.” *Id.* at 164. Cole discloses “maximum recommended rotor speeds for various sedimentation coefficients” to enable “[a]bout 2 h of data acquisition” at the listed centrifuge rotor speed. *Id.* at 164, Table III, note c.

Motivation to Combine. A POSA would have been motivated to combine Cole with Le Bec and de la Maza because Cole discloses the instrumentation used for SV-AUC analysis, and provides guidance for preparing samples and selecting the optimum parameters to run SV-AUC experiments. As stated in the specification of the AUC Patents, “[i]t is within the purview of the skilled artisan to optimize the parameters of AUC for different types of viral particles.” ’326 patent at 26:19:21. Such parameters include “any conditions related to the rotor speed, distance between sample and rotor center, temperature, solvent, sample, buffer, ultracentrifugation time, time interval for detection, sector and optical window characteristics, AUC instrumentation (including ultracentrifuge and detection apparatus), equilibrium dialysis of reference solvent, and data

analysis algorithms.” *Id.* at 17:44-51. A POSA would have been motivated to apply Cole in order to optimize the separation of rAAV particles using SV-AUC according to Le Bec.

Reasonable Expectation of Success. A POSA would have had a reasonable expectation of success in combining Cole with Le Bec and de la Maza to arrive at the claimed methods. As the AUC Patents acknowledge, the selection of optimal conditions for running SV-AUC was a matter of routine and well within the level of ordinary skill. *Id.* at 26:19-21.

4. Le Bec, de la Maza, and Sommer

Claims 20-22, 26-27, and 30 of the '326 patent are obvious over Le Bec and de la Maza, in view of Sommer.

Sommer. Sommer is an article titled “Quantification of Adeno-Associated Virus Particles and Empty Capsids by Optical Density Measurement.” Sommer at 122. Sommer was published in 2003 and is prior art under AIA 35 U.S.C. § 102(a)(1). *Id.*

Sommer discloses “a simple, rapid, and direct method for quantifying vector genomes and capsid proteins in solution.” Sommer at Abstract. Sommer notes that “[m]ethods for largescale production of highly purified AAV for clinical use have improved considerably in recent years.” *Id.* at 122. However, as Sommer explains, “[e]mpty capsids that are typically formed during AAV vector production copurify with genome-containing vector particles during chromatographic purification, and the excess of empty capsids confounds simple determinations of vector genome concentration by absorbance.” *Id.* Sommer discloses a method that “takes into account the capsid ratio, deduced from the A260/A280 ratio, to determine vector genome (vg) concentrations.” *Id.* This approach results in “a simple, rapid, and accurate quantification method for vector genomes at concentrations above 5×10^{11} vg/ml in the presence of up to 40-fold excess empty capsids.” *Id.* at 122-23.

To calculate capsid ratio and vector genome concentration, Sommer discloses a formula for determining the extinction coefficient for a recombinant AAV vector. *Id.* at 127. In particular, Sommer discloses that the extinction coefficient for recombinant AAV can be calculated by adding the extinction coefficient of the vector DNA to the extinction coefficient for the AAV capsid. *Id.* at, Abstract, 127.

$$\epsilon_{(\text{rAAV vector})} = \epsilon_{(\text{DNA})} + \epsilon_{(\text{capsid})}$$

Sommer discloses the formulas for the extinction coefficients for an AAV2 vector at 260 nm and 280 nm. *Id.* at 127. First, the molar extinction coefficients of AAV2 capsids at 260 and 280 nm were determined to be 3.72×10^6 and $6.31 \times 10^6 \text{ M}^{-1} \text{ cm}^{-1}$, respectively. *Id.* Second, the molar extinction coefficients for the vector genome at 260 and 280 nm were determined to be $20.0 \text{ g}^{-1} \text{ cm}^{-1} \times \text{MW}_{\text{DNA}}$ and $11.1 \text{ g}^{-1} \text{ cm}^{-1} \times \text{MW}_{\text{DNA}}$, respectively. *Id.*

Finally, Sommer teaches that “the individual absorbance of capsid protein and vector genome can be added to arrive at the extinction coefficient for a vector particle. *Id.* The formulas for calculating the molar extinction coefficients for AAV2-based vector particles at 260 nm and 280 nm are set forth below. *Id.*

$$\epsilon_{260(\text{rAAV vector})} = 20.0 \times \text{MW}_{\text{DNA}} + 3.72 \times 10^6$$

$$\epsilon_{280(\text{rAAV vector})} = 11.1 \times \text{MW}_{\text{DNA}} + 6.31 \times 10^6$$

Using the formulas disclosed in Sommer, the extinction coefficient for a particular recombinant AAV particle may be calculated if the molecular weight of the encapsidated DNA is known. *Id.* Applying Beer’s Law, the extinction coefficient and absorbance at a given wavelength may then be used to calculate the concentration of a particular recombinant AAV particle in a preparation of AAV particles. *See* ’326 patent at 24:41-47, 44:58-61, 46:42-50; Cole at 152.

Motivation to Combine. A POSA would have been motivated to combine Le Bec and de la Maza with Sommer. Le Bec, de la Maza, and Sommer all relate to the identification and characterization of viral particles in preparations of AAV, such as rAAV vectors for use in gene therapy applications. Sommer at 122. A POSA would have been motivated to combine Le Bec and de la Maza with Sommer in order to obtain additional information regarding the composition and quality of an rAAV preparation.

Le Bec and de la Maza teach methods for determining the size of the DNA in rAAVs with fragmented genomes. Sommer discloses a mathematical formula for calculating the extinction coefficient of an rAAV particle based on the size of the DNA packaged in the capsid. *Id.* at 127. The AUC patents report using the formulas in Sommer to calculate the molar concentrations of empty and full rAAV particles, where the size of the DNA is known. '326 patent at 24:35-49, 44:49-61, 46:27-47 (Table 2).

A POSA would have been further motivated to use the formula in Sommer to calculate the molar concentrations of rAAV particles with fragmented genomes. Specifically, a POSA would have been motivated to use the DNA size measured for each identified species, according to the methods of Le Bec and de la Maza, to calculate the extinction coefficients for rAAV particles with fragmented genomes, according to the formula in Sommer. *See* Sommer at 127. A POSA would have been motivated to then use the extinction coefficients to calculate the molar concentration of each species of AAV virion, according to Beer's Law, in order to evaluate the composition and quality of the rAAV preparation. '326 patent at 24:41-47, 44:58-61, 46:42-50; Cole at 152.

Reasonable Expectation of Success. A POSA would have had a reasonable expectation of success in applying the mathematical formula in Sommer to the methods disclosed in Le Bec and de la Maza to calculate the extinction coefficients for rAAV particles with fragmented

genomes and the molar concentrations of each species according to Beer's Law. Cole at 152. These known mathematical formulas involve straightforward calculations routinely performed by those skilled in the art.

5. Le Bec, de la Maza, Sommer, and Cole

Claim 25 of the '326 patent is obvious over Le Bec and de la Maza, in view of Sommer and Cole. As noted above, Cole teaches that "[i]t should take a boundary at least 2 h to sediment the full length of the cell." Cole at 164.

Motivation to Combine. A POSA would have been motivated to combine Le Bec, de la Maza, and Sommer with Cole because Cole discloses the instrumentation used for SV-AUC analysis, and provides guidance for preparing samples and selecting the optimum parameters to run SV-AUC experiments. As stated in the specification of the AUC Patents, "[i]t is within the purview of the skilled artisan to optimize the parameters of AUC for different types of viral particles." '326 patent at 26:19:21. Such parameters include "any conditions related to the rotor speed, distance between sample and rotor center, temperature, solvent, sample, buffer, ultracentrifugation time, time interval for detection, sector and optical window characteristics, AUC instrumentation (including ultracentrifuge and detection apparatus), equilibrium dialysis of reference solvent, and data analysis algorithms." *Id.* at 17:44-51. A POSA would have been motivated to apply Cole in order to optimize the separation of rAAV particles using SV-AUC according to Le Bec.

Reasonable Expectation of Success. A POSA would have had a reasonable expectation of success in combining Cole with Le Bec, de la Maza, and Sommer to arrive at the claimed method. As the AUC Patents acknowledge, the selection of optimal conditions for running SV-AUC was a matter of routine and well within the level of ordinary skill. *Id.* at 26:19-21.

C. NON-PATENTABLE SUBJECT MATTER

The Asserted Claims of the AUC Patents are directed to a patent-ineligible natural phenomenon and/or law of nature. *See Alice Corp. Pty. Ltd. v. CLS Bank Intern.*, 573 U.S. 208 (2014). During prosecution, the applicants identified the allegedly distinguishing feature of the Asserted Claims. In particular, the applicants argued that they were the first to “discover” that there is a linear relationship between the sedimentation coefficients measured for AAV particles and the molecular weights of the DNA packaged inside the AAV capsids. ’326 patent prosecution history, 04/09/2024 Amendment and Response at 12. The applicants used this mathematical relationship to calculate the size of each of the individual genomes in a population of rAAV particles, including particles with fragmented genomes. *Id.* As the applicants characterized the alleged invention:

The inventors of the present application discovered that sedimentation coefficients can be used to accurately calculate the molecular weight of the genomes in every viral particle of the preparation, including the molecular weights of the fragmented genomes. ***The linear relationship between sedimentation coefficients and molecular weights of genomes was not appreciated in the art prior to the present invention***, and it was not disclosed or suggested in the art applied in the Office Action, as discussed below. Therefore, the present application provides for the first time a means of calculating the size of each of the individual genomes in the population of viral particles.

Id. (emphasis added).

However, it is well established that method claims directed to measuring a natural phenomenon or law of nature – like the Asserted Claims of the AUC Patents – are not patent eligible subject matter. *See, e.g., Ariosa Diagnostics, Inc. v. Sequenom, Inc.*, 788 F.3d 1371 (Fed. Cir. 2015); *Athena Diagnostics, Inc. v. Mayo Collaborative Servs.*, 915 F.3d 743 (Fed. Cir. 2019); *In re BRCA1- & BRCA2-Based Hereditary Cancer Test*, 774 F.3d 755 (Fed. Cir. 2014); *CareDx, Inc. v. Natera, Inc.*, 40 F.4th 1371 (Fed. Cir. 2022); *Genetic Techs. Ltd. v. Merial L.L.C.*, 818 F.3d 1369 (Fed. Cir. 2016); *Roche Molecular Sys. v. Cepheid*, 905 F.3d 1363 (Fed. Cir. 2018).

Here, the linear relationship between sedimentation coefficients and the molecular weights of the genomes packaged in rAAV capsids is a natural phenomenon and/or law of nature regarding the physical properties of AAV particles. The additional elements of the Asserted Claims of the AUC Patents are all well-known features in the prior art that reflect well-understood, routine, and conventional activity engaged in by the scientific community as of the earliest priority date for the AUC Patents and add nothing to transform the natural phenomenon and/or law of nature into patent-eligible subject matter. *See Mayo Collaborative Services v. Prometheus Labs., Inc.*, 566 U.S. 66, 80 (2012). Thus, the Asserted Claims are invalid under 35 U.S.C. § 101.

The Supreme Court has “set forth a [two-step] framework for distinguishing patents that claim laws of nature, natural phenomena, and abstract ideas from those that claim patent-eligible applications of those concepts.” *Alice*, 573 U.S. at 217 (citing *Mayo*, 566 U.S. at 77-78).

First, in step one, the claims at issue are evaluated to determine whether they are “directed to” a patent-ineligible concept. *Id.* This inquiry includes an evaluation of the claims themselves and the description of the “invention” in the specification. The inventors’ description of their “discovery” as a natural phenomenon or law of nature is compelling evidence that the claims are directed to ineligible subject matter. *See, e.g., Athena Diagnostics*, 915 F.3d at 751, 754 (specification states that the inventors “surprisingly found” a natural law); *Roche*, 905 F.3d at 1371-72 (alleged invention based on the “undiscovered presence” of a natural phenomenon); *Cleveland Clinic Found. v. True Health Diagnostics LLC*, 859 F.3d 1352, 1360-61 (Fed. Cir. 2017) (alleged invention “based on the discovery” of a natural law); *Genetic Techs.*, 818 F.3d at 1375 (same); *Ariosa Diagnostics*, 788 F.3d at 1376 (natural phenomenon was a “surprising and unexpected finding”); *see also CareDx, Inc. v. Natera, Inc.*, 563 F. Supp. 3d 329, 339-347 (D. Del.

2021), *aff'd*, 40 F.4th 1371 (Fed. Cir. 2022); *ChromaDex, Inc. v. Elysium Health, Inc.*, 561 F. Supp. 3d 460 (D. Del. 2021), *aff'd*, 59 F.4th 1280 (Fed. Cir. 2023).

Second, in step two, the claims are evaluated to determine whether the additional elements add enough to the concept to qualify as patent eligible. *Mayo*, 566 U.S. at 77. In making this assessment, the claim elements should be considered individually and “as an ordered combination” to determine whether the additional elements “transform the nature of the claim” into something patent-eligible. *Id.* at 78-80. However, the “inventive concept” necessary to satisfy the second step cannot be furnished by the unpatentable natural phenomena or law of nature itself. *See, e.g., Athena Diagnostics*, 915 F.3d at 754 (citing *Genetic Techs.*, 818 F.3d at 1376). Concessions in the intrinsic record that the additional claim elements (other than the natural phenomenon or law of nature itself) were in the prior art are generally conclusive as to patent-ineligibility. *See, e.g., Genetic Techs.*, 818 F.3d at 1377 (specification and arguments during prosecution made clear that no new techniques for PCR amplification or DNA analysis disclosed); *Ariosa Diagnostics*, 788 F.3d at 1377 (specification confirmed “that the preparation and amplification of DNA sequences in plasma or serum were well-understood, routine, conventional activities performed by doctors in 1997”); *see also CareDx*, 563 F. Supp. 3d at 342-43; *ChromaDex*, 561 F. Supp. 3d at 466 (concluding that there were no additional features sufficient “to ensure that the claim is more than a drafting effort designed to monopolize the [natural product]”).

Step 1: The Asserted Claims of the AUC Patents are directed to a non-patentable natural phenomenon and/or law of nature. At the most basic level, the Asserted Claims purport to claim methods of determining the size, quantity, and molar concentration of fragmented rAAV genomes via AUC, based on the relationship between sedimentation coefficients and genome size.

For example, claim 1 of the '326 patent recites:

A method of determining the size of one or more fragmented genomes in a preparation of viral particles comprising recombinant adeno-associated viral (rAAV) vectors encapsidated into viral capsids, said method comprising:

(i) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions to generate one or more sedimenting boundaries, wherein the boundary sedimentation velocity is from about 3,000 rpm to about 20,000 rpm;

(ii) measuring the rate of movement or migration of the one or more sedimenting boundaries, wherein movement or migration of the viral particles results in distinct sedimenting boundaries, each distinct sedimenting boundary corresponding to a resolvable viral particle, and wherein one or more of the viral particles comprise a fragmented genome, and determining the sedimentation coefficients of the viral particles comprising one or more of the fragmented genomes in the preparation; and

(iii) determining the size of the one or more fragmented genomes as a function of the sedimentation coefficients of the viral particles comprising the one or more fragmented genomes.

'326 patent at 55:13:36.

The central element of claim 1 is determining the size of the one or more fragmented genomes *as a function of the sedimentation coefficients* of the viral particles comprising the one or more fragmented genomes.

Similarly, claim 20 of the '326 patent recites:

A method of determining the molar concentrations of each species of individual viral particles in a heterogeneous mixture of viral particles comprising recombinant adeno-associated viral (rAAV) vectors encapsidated into viral capsids, said method comprising:

(i) subjecting the heterogeneous mixture of viral particles to analytical ultracentrifugation under boundary sedimentation velocity conditions to generate sedimenting boundaries, wherein the boundary sedimentation velocity is from about 3,000 rpm to about 20,000 rpm;

(ii) measuring the rate of movement or migration of the sedimenting boundaries, wherein movement or migration of each species of the individual viral particles in the heterogeneous mixture of viral

particles results in distinct sedimenting boundaries, each distinct sedimenting boundary corresponding to a resolvable species of viral particle, and wherein the heterogeneous mixture of viral particles comprises full genomes, fragmented genomes and empty capsids without genome;

(iii) determining the sedimentation coefficients of each species of the individual viral particles in the heterogeneous mixture of viral particles; and

(iv) quantifying the molar concentration of each species of the individual viral particles in the heterogeneous mixture of viral particles.

Id. at 56:17-42.

Similar to claim 1, the central elements of claim 20 involve determining the sedimentation coefficients of species of viral particles and quantifying the molar concentration of each species of the individual viral particles in the heterogeneous mixture of viral particles.

Claim 1 of the '894 patent recites:

A method of quantifying one or more species of individual variant viral particles comprising fragmented recombinant adeno-associated viral (rAAV) genomes in a heterogeneous mixture of viral particles, said method comprising:

(i) subjecting the heterogeneous mixture of viral particles to analytical ultracentrifugation under boundary sedimentation velocity conditions to generate sedimenting boundaries, wherein the boundary sedimentation velocity is from about 3,000 rpm to about 20,000 rpm;

(ii) measuring the rate of movement or migration of the sedimenting boundaries, wherein movement or migration of each species of individual viral particles in the heterogeneous mixture of viral particles results in distinct sedimenting boundaries, each distinct sedimenting boundary corresponding to a resolvable species of viral particle, and wherein the individual viral particles comprise empty particles without genome, particles with full genomes and particles with fragmented genomes;

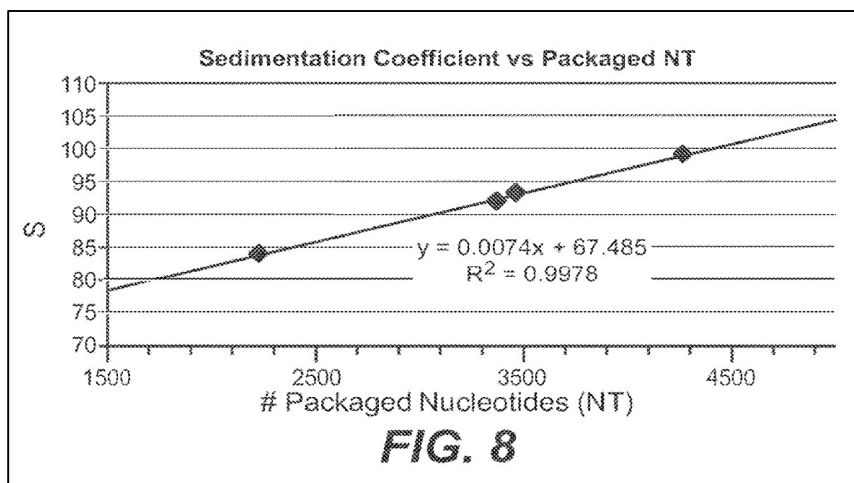
(iii) determining the genome size of one or more species of the individual variant viral particles in the heterogeneous mixture of viral particles; and

(iv) determining the quantity of one or more species of the variant viral particles in the heterogeneous mixture of viral particles.

'894 patent at 55:2-26.

Again, the central elements are determining the genome size of one or more species of variant (i.e. fragmented) viral particles and determining the quantity of one or more species of the variant (i.e. fragmented) viral particles.

The specification of the AUC Patents confirms that the claims are directed to non-patentable subject matter. The specification states that “[b]ecause it is not possible to determine empirically the extinction coefficient of fragmented genomes of unknown size and sequence, *a relationship between S value and genome size was established.*” To achieve this, rAAV vector preps with encapsidated viral genomes of known size were analyzed by AUC, and their corresponding S values were determined.” *See, e.g.*, '326 patent at 49:58-64 (emphasis added); *see also id.* at 44:64-45:5. “A standard curve was then generated to correlate genome size and S value (FIG. 8).” *Id.* at 49:64-67. Figure 8 of the AUC Patents is reproduced below:



This “standard curve was then used to assign genome size to each of the resolved genome containing capsids” of a preparation of AAV2 genomes packaged into AAV9 capsids, including full and fragmented genomes. *Id.* at 50:20-35. To calculate molar concentrations, the AUC Patents

describe using the known formulas from Sommer (discussed above) to first calculate molar absorbance extinction coefficients using genome size, and then calculate molar concentrations using Beer's Law. *Id.* at 46:42-47.

During prosecution of the AUC Patents, the applicants made representations to the United States Patent and Trademark Office confirming that the Asserted Claims of the AUC Patents are directed to a non-patentable natural phenomenon and/or law of nature. According to the applicants, “[t]he inventors of the present application ***discovered*** that sedimentation coefficients can be used to accurately calculate the molecular weight of the genomes in every viral particle of the preparation, including the molecular weights of the fragmented genomes.” ’326 patent prosecution history, 04/09/2024 Amendment and Response at 12 (emphasis added). “As described in the present application, AUC is used to separate all of the viral particles, including viral particles with fragmented genomes,” and “[t]he sedimentation coefficients of the fragmented genomes are used to determine the molecular weight of the fragmented genomes.” *Id.* In particular, applicants argued:

The linear relationship between sedimentation coefficients and molecular weights of genomes was not appreciated in the art prior to the present invention, and it was not disclosed or suggested in the art applied in the Office Action, as discussed below. Therefore, the present application provides for the first time a means of calculating the size of each of the individual genomes in the population of viral particles.

Id. (emphasis added). Applicants emphasized that “nothing in the prior art disclosed or suggested the ability to determine the molar concentrations of genomes of unknown molecular weights (e.g., fragmented genomes) in a population of viral particles.” *Id.*

Applicants made similar arguments during prosecution of the ’894 patent. *See, e.g.*, ’894 patent prosecution history, 04/18/2024 Amendment and Response at 10-13 (“The inventors of the present application discovered that sedimentation coefficients can be used to accurately calculate

the molecular weight of the genomes in every viral particle of the preparation, including the molecular weights of the fragmented genomes” and “determining the size of fragmented genomes based on sedimentation coefficients was discovered by the inventors.”).

Thus, according to applicants, the purported invention of the AUC Patents is based on the “discover[y]” that sedimentation coefficients can be used to calculate the molecular weight of the genomes in viral particles, including the molecular weights of fragmented genomes. Applicants further highlighted that this discovery was made possible by the “linear relationship between sedimentation coefficients and molecular weights of genomes.” ’326 patent prosecution history, 04/09/2024 Amendment and Response at 12.

Sedimentation velocity is a function of the mass, shape, and density of a particle. Cole (1999) at 165-66; Cole at 146; Berkowitz (2015) at 215-16. The relationship between sedimentation coefficients and molecular weights is a patent ineligible natural phenomenon/law of nature that reflects the physical properties of AAV particles. The claimed methods involve nothing more than measuring this natural phenomenon/law of nature to determine the size of fragmented genomes and using known mathematical formulas to calculate the molar concentrations of the different species of rAAV particles in a heterogenous mixture. As the applicants argued during prosecution, the allegedly distinguishing feature of the Asserted Claims is the linear relationship between sedimentation coefficients and molecular weights of rAAV genomes – which is a natural phenomenon/law of nature. Thus, the Asserted Claims of the AUC Patents are directed to non-patentable subject matter, and Step 1 is satisfied.

Step 2. The additional elements do not transform the Asserted Claims into patent eligible subject matter. Instead, the specification and prosecution histories of the AUC Patents describe these additional elements as directed to well-understood, routine, and conventional activities that

were well within the capabilities of a person of ordinary skill in the art. Whether considered individually or as a whole, the additional elements in the Asserted Claims do not add anything unconventional or inventive to the patent-ineligible relationship between sedimentation coefficients and molecular weights of genomes.

The AUC Patents state that the “techniques and procedures described or referenced herein are generally well understood and commonly employed using conventional methodology by those skilled in the art.” ’326 patent at 13:4-9. And as applicants conceded during prosecution, Le Bec “teaches analyzing capsids that are empty (i.e., contain no genome) and capsids that contain full genomes” via SV-AUC. ’326 patent prosecution history, 04/09/2024 Amendment and Response at 14; *see also* ’894 patent prosecution history, 04/18/2024 Amendment and Response at 12. The claim elements of subjecting heterogeneous mixtures or preparations of rAAV particles to AUC under boundary sedimentation velocity conditions at specified sedimentation velocities, measuring the rate of migration of the sedimenting boundaries, and determining the sedimentation coefficients of viral particles (including fragmented genomes) in the mixture or preparation are all routine and conventional activities that were well within the capabilities of a person of ordinary skill in the art, and were explicitly taught by Le Bec.

Likewise, the specification of the AUC Patents acknowledges that “[i]t is *within the purview of the skilled artisan* to optimize the parameters of AUC for different types of viral particles.” ’326 patent at 26:19:21 (emphasis added). Such parameters include “any conditions related to the rotor speed, distance between sample and rotor center, temperature, solvent, sample, buffer, ultracentrifugation time, time interval for detection, sector and optical window characteristics, AUC instrumentation (including ultracentrifuge and detection apparatus), equilibrium dialysis of reference solvent, and data analysis algorithms.” *Id.* at 17:44-51.

The specification of the AUC Patents further acknowledges that use of software programs such as the SEDFIT algorithm to analyze SV-AUC data was routine. *See, e.g.*, '326 patent at 18:1-4 (“As used herein, *the ‘SEDFIT algorithm’ is an algorithm that allows one to analyze hydrodynamic data such as sedimentation velocity* (Schuck (2000) Biophys. J., 78: 1606-19)”) (emphasis added); 21:5-18 (“*Several programs known in the art, such as SEDFIT* (Schuck (2000) Biophys. J., 78:1606-19), *may be used to model the Lamm equation to AUC data*. These programs are also able to apply the Lamm equation to solutions containing multiple solutes or multiple sedimenting boundaries.”) (emphasis added).

The additional elements in the dependent claims of the AUC Patents are also directed to conventional subject matter. By way of example, the dependent claims add conventional elements such as AUC parameters (e.g. centrifugation speed, time, temperature) and specify aspects of the rAAV preparations (e.g. capsid serotypes and particle concentrations). Again, “[i]t is *within the purview of the skilled artisan* to optimize the parameters of AUC for different types of viral particles.” '326 patent at 26:19:21 (emphasis added).

Even when taken together, the elements of methods recited in the Asserted Claims of the AUC Patents do not provide anything inventive over the prior art. *See, e.g.*, '326 patent at 13:4-9 (“The techniques and procedures described or referenced herein are generally well understood and commonly employed using conventional methodology by those skilled in the art.”).

In sum, the Asserted Claims of the AUC Patents are directed to methods of determining sedimentation coefficients, genome size, and molar concentrations of fragmented AAV genomes using conventional methods that were well within the capabilities of a person of ordinary skill in the art. In particular, the sedimentation coefficients recited in the Asserted Claims are obtained using conventional SV-AUC methods. The data generated using these SV-AUC methods are

interpreted using conventional software programs such as SEDFIT. The mathematical calculations carried out to determine genome size and molar concentration involve no more than the measurement of a natural phenomenon/law of nature – *i.e.*, the linear relationship between sedimentation coefficients and genome size. None of the additional elements recited in the Asserted Claims, either individually or in combination, transform the claimed methods into patent eligible subject matter. Thus, Step 2 is satisfied, and the Asserted Claims of the AUC Patents are invalid under 35 U.S.C. § 101.

D. LACK OF WRITTEN DESCRIPTION

The Asserted Claims of the AUC Patents are invalid for lack of written description under 35 U.S.C. § 112(a). To satisfy the written description requirement, the description must “clearly allow persons of ordinary skill in the art to recognize that [the inventor] invented what is claimed,” such that “the disclosure of the application relied upon reasonably conveys to those skilled in the art that the inventor had possession of the claimed subject matter as of the filing date.” *Ariad Pharm., Inc. v. Eli Lilly and Co.*, 598 F.3d 1336, 1351 (Fed. Cir. 2010) (en banc) (citation omitted).

Demonstrating adequate written description “requires a precise definition” of the invention. *Id.* at 1350. In particular, for written description of a claimed genus, the specification must disclose “a representative number of species falling within the scope of the genus or structural features common to the members of the genus so that one of skill in the art can ‘visualize or recognize’ the members of the genus.” *Id.* (citation omitted). “[F]unctional claim language can meet the written description requirement when the art has established a correlation between structure and function.” *Id.* (citation omitted). “But merely drawing a fence around the outer limits of a purported genus is not an adequate substitute for describing a variety of materials constituting the genus and showing that one has invented a genus and not just a species.” *Id.*

“[G]eneric claim language appearing *in ipsius verbis* in the original specification does not satisfy the written description requirement if it fails to support the scope of the genus claimed.” *Ariad*, 598 F.3d at 1350 (citing *Enzo Biochem, Inc. v. Gen-Probe Inc.*, 323 F.3d 956, 968 (Fed. Cir. 2002)). A “mere wish or plan for obtaining the claimed invention is not adequate written description.” *Juno Therapeutics, Inc. v. Kite Pharma, Inc.*, 10 F.4th 1330, 1335 (Fed. Cir. 2021) (citing *Centocor Ortho Biotech, Inc. v. Abbott Labs.*, 636 F.3d 1341, 1348 (Fed. Cir. 2011)) (internal quotation omitted).

The Asserted Claims of the AUC Patents are invalid under 35 U.S.C. § 112(a) because the specification lacks an adequate written description of the “boundary sedimentation velocity conditions” recited in the Asserted Claims. The Asserted Claims of the AUC Patents recite the step of subjecting a mixture of viral particles “to analytical ultracentrifugation under boundary sedimentation velocity conditions.” The specification defines the term “boundary sedimentation velocity conditions” to “refer to *any experimental conditions* under which a sample solution is *subjected to sedimentation velocity analysis*.” ’326 patent, 17:29-32 (emphasis added). Thus, the claims of the AUC Patents purport to cover all types of experiments and/or experimental conditions in which a sample is subjected to sedimentation velocity analysis – including already existing and later developed methods – which are not disclosed by the inventors. Examples of such methods include band sedimentation-velocity, synthetic boundary, and gravitational sweep sedimentation velocity. The disclosure of the AUC Patents does not demonstrate that the inventors were in possession of all experimental conditions under which an rAAV sample is subjected to sedimentation velocity analysis, and thus the inventors have not met the written description requirement for the “entire scope of the claimed invention.”

For at least these reasons, the Asserted Claims of the AUC Patents are invalid under 35 U.S.C. § 112(a) for failing to satisfy the written description requirement.

E. LACK OF ENABLEMENT

The Asserted Claims are invalid for lack of enablement under 35 U.S.C. § 112(a). To satisfy the enablement requirement, the disclosure “must teach those skilled in the art how to make and use the full scope of the claimed invention without ‘undue experimentation.’” *Genentech, Inc. v. Novo Nordisk A/S*, 108 F.3d 1361, 1365 (Fed. Cir. 1997) (internal citation omitted).

Specifically, if a patent claim recites an entire class of processes, machines, manufactures, or compositions of matter – such as the compositions and methods recited in the Asserted Claims – the specification must enable a person skilled in the art to make and use the entire class. *Amgen Inc. v. Sanofi*, 598 U.S. 594, 610 (2023). At the same time, an instruction to attempt “random trial-and-error discovery” is not sufficient to enable others to make and use an entire class of claimed subject matter. *Id.* at 614-15. In other words, “[t]he more one claims, the more one must enable.” *Id.* at 610.

The Federal Circuit has enumerated several factors to consider in determining whether a disclosure would require “undue experimentation”: (1) the quantity of experimentation necessary; (2) the amount of direction or guidance presented; (3) the presence or absence of working examples; (4) the nature of the invention; (5) the state of the prior art; (6) the relative skill of those in the art; (7) the predictability or unpredictability of the art; and (8) the breadth of the claims. *In re Wands*, 858 F.2d 731, 737 (Fed. Cir. 1988).

In determining whether the full scope of a claim is enabled or not, it is also appropriate to consider whether categories of embodiments within the claim are enabled. *See, e.g., Trs. of Boston Univ. v. Everlight Elecs. Co.*, 896 F.3d 1357, 1364 (Fed. Cir. 2018) (holding a claim invalid for not enabling one of six embodiments falling within the scope of the claim); *Chiron Corp. v.*

Genentech, Inc., 363 F.3d 1247, 1257 (Fed. Cir. 2004) (claims encompassing undisclosed chimeric antibodies required undue experimentation). Additionally, “[w]hile functional claim limitations are not necessarily precluded in claims that meet the enablement requirement, such limitations pose high hurdles in fulfilling the enablement requirement for claims with broad functional language.” *Amgen Inc. v. Sanofi*, 987 F.3d 1080, 1087 (2021).

The Asserted Claims of the AUC Patents are invalid under 35 U.S.C. § 112(a) because the specification fails to enable the full scope of the “boundary sedimentation velocity conditions” recited in the Asserted Claims. The Asserted Claims of the AUC Patents recite the step of subjecting a mixture of viral particles “to analytical ultracentrifugation under boundary sedimentation velocity conditions.” The specification defines the term “boundary sedimentation velocity conditions” to “refer to *any experimental conditions* under which a sample solution is *subjected to sedimentation velocity analysis*.” ’326 patent, 17:29-32 (emphasis added). Thus, the claims of the AUC Patents purport to cover all types of experiments and/or experimental conditions in which a sample is subjected to sedimentation velocity analysis – including already existing and later developed methods – which are not disclosed by the inventors. Examples of such methods include band sedimentation-velocity, synthetic boundary, and gravitational sweep sedimentation velocity. The specification does not enable all possible experimental conditions under which an rAAV sample is subjected to sedimentation velocity analysis. Thus, the inventors have not met the enablement requirement for the “entire scope of the claimed invention.”

For at least these reasons, the Asserted Claims of the AUC Patents are invalid under 35 U.S.C. § 112(a) for failing to satisfy the enablement requirement.

F. INDEFINITENESS

The Asserted Claims are invalid for indefiniteness. Section 112 provides that the specification must “conclude with one or more claims particularly pointing out and distinctly

claiming the subject matter which the [applicant] regards as the invention.” 35 U.S.C. § 112(b). A patent is indefinite “if its claims, read in light of the specification delineating the patent, and the prosecution history, fail to inform, with reasonable certainty, those skilled in the art about the scope of the invention.” *Nautilus, Inc. v. Biosig Instruments, Inc.*, 572 U.S. 898, 901 (2014).

Patent claims are invalid for indefiniteness when a claim term may be determined by multiple test methods, the results of which “will necessarily fall within or outside the claim scope depending on the . . . method chosen.” *Honeywell Int’l, Inc. v. Int’l Trade Comm’n*, 341 F.3d 1332, 1341 (Fed. Cir. 2003). Where a patent fails to specify which of available alternative tests to use for measuring compliance with a claim limitation, and different tests can have different results, the claims are indefinite. *HZNP Meds. LLC v. Actavis Labs. UT, Inc.*, 940 F.3d 680, 698 (Fed. Cir. 2019).

All Asserted Claims of the AUC Patents recite the step of subjecting a mixture of viral particles “to analytical ultracentrifugation under boundary sedimentation velocity conditions.” The specification defines the term “boundary sedimentation velocity conditions” to “refer to **any experimental conditions** under which a sample solution is **subjected to sedimentation velocity analysis.**” ’326 patent, 17:29-32 (emphasis added). To the extent that the claims of AUC Patents purport to cover all types of experiments in which a sample is subjected to sedimentation velocity analysis, they fail to delineate the scope of the claims with reasonable certainty. Different experimental conditions or sedimentation velocity methods may yield different results. The AUC Patents provide insufficient clarity regarding the scope of the claimed methods. *See, e.g., Nautilus*, 572 U.S. at 901 (patent is invalid for indefiniteness if it “fail[s] to inform, with reasonable certainty, those skilled in the art about the scope of the invention.”).

III. U.S. PATENT NOS. 11,698,377, 12,123,880, AND 12,298,313

A. PRIORITY DATE

The '377 patent issued from U.S. Patent Application No. 16/325,653 (“the '653 application”) on July 11, 2023. The '880 patent issued from U.S. Patent Application No. 18/321,542 (“the '542 application”) on October 22, 2024. The '313 patent issued from U.S. Patent Application No. 19/013,863 (“the '863 application”) on May 13, 2025. The '377, '880, and '313 patents claim priority to the same provisional and non-provisional applications, and will be referred to collectively as “the LC-MS Patents.”

The LC-MS Patents claim priority to the same non-provisional application: application No. PCT/US2017/046814, filed on August 14, 2017. *See* '377 patent at 1 (22), (86); '880 patent at 1 (62); '313 patent at 1 (60). The LC-MS Patents share the same specification.

The LC-MS Patents also claim priority to U.S. Provisional Application No. 62/375,314 (“the '314 provisional application”), filed on August 15, 2016. *See* '377 patent at 1 (60); '880 patent at 2 (60); '313 patent at 2 (60).

The earliest possible priority date for the LC-MS Patents is August 15, 2016.

B. OBVIOUSNESS

Under 35 U.S.C. § 103, a patent claim is invalid “if the differences between the claimed invention and the prior art are such that the claimed invention as a whole would have been obvious before the effective filing date of the claimed invention to a person having ordinary skill in the art to which the claimed invention pertains.” The analysis of obviousness includes consideration of the following factors: (1) the level of ordinary skill in the art; (2) the scope and content of the prior art; (3) the differences between the prior art and the claimed subject matter; and (4) any objective evidence of non-obviousness, also known as secondary considerations. *Graham v. John Deere Co.*, 383 U.S. 1, 17-18 (1966).

To “determine whether there was an apparent reason to combine the known elements in the fashion claimed by the patent at issue,” a court should “look to interrelated teachings of multiple patents; the effects of demands known to the design community or present in the marketplace; and the background knowledge possessed by a person having ordinary skill in the art.” *KSR Int’l Co. v. Teleflex Inc.*, 550 U.S. 398, 418 (2007). A patent may be obvious if, *inter alia*, there was a “known problem for which there was an obvious solution encompassed by the patent’s claims” or the combination of the patent’s claims was “obvious to try.” *Id.* at 419-421.

Sarepta identifies the prior art references set forth in the table below, which alone or in combination, render the Asserted Claims of the LC-MS Patents invalid under 35 U.S.C. § 103. The identified prior art is also relevant to show the state of the art and reasons and motivations for making improvements, additions, and combinations of the disclosures in these references. Sarepta reserves the right to identify additional prior art as discovery progresses.

The references listed below are all prior art under AIA 35 U.S.C. § 102(a). The earliest possible priority date for the Asserted Claims of the LC-MS Patents is the filing date of the ’314 provisional application on August 15, 2016.

Reference	Year
Alqahtani, “Analysis of purified wild type and mutant adenovirus particles by SILAC based quantitative proteomics,” <i>Journal of General Virology</i> 95.11 (2014): 2504-2511 (“Alqahtani”)	2014
Anacleto and Boyd, “Calibration of ion spray mass spectra using cluster ions,” <i>Organic Mass Spectrometry</i> 27.6 (1992): 660-666 (“Anacleto”)	1992
Ansong <i>et al.</i> , “Top-down proteomics reveals a unique protein S-thiolation switch in <i>Salmonella</i> Typhimurium in response to infection-like conditions,” <i>Proceedings of the National Academy of Sciences</i> 110.25 (2013): 10153-10158 (“Ansong”)	2013

Reference	Year
Ayuso <i>et al.</i> , “Manufacturing and Characterization of a Recombinant Adeno-Associated Virus Type 8 Reference Standard Material,” <i>Human Gene Therapy</i> 25.11 (2014): 977-987 (“Ayuso”)	2014
Becerra <i>et al.</i> , “Direct mapping of adeno-associated virus capsid proteins B and C: a possible ACG initiation codon,” <i>Proceedings of the National Academy of Sciences</i> 82.23 (1985): 7919-7923 (“Becerra”)	1985
Bondarenko <i>et al.</i> , “Mass Measurement and Top-Down HPLC-MS Analysis of Intact Monoclonal Antibodies on a Hybrid Linear Quadrupole Ion Trap-Orbitrap Mass Spectrometer,” <i>Journal of the American Society for Mass Spectrometry</i> 20.8 (2009): 1415-1424 (“Bondarenko”)	2009
Burova and Ioffe, “Chromatographic purification of recombinant adenoviral and adeno-associated viral vectors: methods and implications,” <i>Gene Therapy</i> 12.1 (2005): S5-S17 (“Burova”)	2005
Byeon <i>et al.</i> , “Structural Identification of a Non-Glycosylated Variant at Ser126 for O-Glycosylation Site from EPO BRP, Human Recombinant Erythropoietin by LC-MS Analysis,” <i>Molecules and Cells</i> 38.6 (2015): 496-505 (“Byeon”)	2015
Cecchini <i>et al.</i> , “Toward exascale production of recombinant adeno-associated virus for gene transfer applications,” <i>Gene Therapy</i> 15.11 (2008): 823-830 (“Cecchini”)	2008
Chelius <i>et al.</i> , “Analysis of the adenovirus type 5 proteome by liquid chromatography and tandem mass spectrometry methods,” <i>Journal of Proteome Research</i> 1.6 (2002): 501-513 (“Chelius”)	2002
Chen <i>et al.</i> , “Molecular characterization of adeno-associated viruses infecting children,” <i>Journal of Virology</i> 79.23 (2005): 14781-14792 (“Chen”)	2005
Coon <i>et al.</i> , “Tandem mass spectrometry for peptide and protein sequence analysis.” <i>Biotechniques</i> 38.4 (2005): 519-523 (“Coon 2005”)	2005
Coon, “Collisions or electrons? Protein Sequence Analysis in the 21st Century,” <i>Anal. Chem.</i> (2009): 3208-3215 (“Coon 2009”)	2009
Craig and Beavis, “TANDEM: matching proteins with tandem mass spectra,” <i>Bioinformatics</i> 20.9 (2004): 1466-1467 (“Craig”)	2004
Cueto-Rojas, “Interferon- α 2b quantification in inclusion bodies using Reversed Phase-Ultra Performance Liquid Chromatography (RP-UPLC),” <i>Journal of Chromatography B</i> 878.13-14 (2010): 1019-1023 (“Cueto-Rojas”)	2010

Reference	Year
Davis <i>et al.</i> , “Rational Design and Engineering of a Modified Adeno-Associated Virus (AAV1)-Based Vector System for Enhanced Retrograde Gene Delivery,” <i>Neurosurgery</i> 76.2 (2015): 216-225 (“Davis”)	2015
Dong <i>et al.</i> , “Proteomics analysis of co-purifying cellular proteins associated with rAAV vectors,” <i>PLoS One</i> 9.2 (2014): e86453 (“Dong”)	2014
Glish and Vachet, “The Basics of Mass Spectrometry in the Twenty-First Century,” <i>Nature Reviews Drug Discovery</i> 2.2 (2003): 140-150 (“Glish”)	2003
Good and Coon, “Advancing proteomics with ion/ion chemistry,” <i>Mass Spectrometry For Proteomics Analysis Review, Biotechniques</i> 40.6 (2006): 783-789 (“Good”)	2006
Grimm and Kay, “From Virus Evolution to Vector Revolution: Use of Naturally Occurring Serotypes of Adeno-Associated Virus (AAV) as Novel Vectors for Human Gene Therapy,” <i>Current Gene Therapy</i> 3.4 (2003): 281-304 (“Grimm and Kay”)	2003
Han, <i>et al.</i> “Extending top-down mass spectrometry to proteins with masses greater than 200 kilodaltons,” <i>Science</i> 314.5796 (2006): 109-112 (“Han”)	2006
Huang <i>et al.</i> , “In Vivo Deamidation Characterization of Monoclonal Antibody by LC-MS/MS,” <i>Analytical Chemistry</i> 77.5 (2005): 1432-1439 (“Huang”)	2005
Lock <i>et al.</i> , “Analysis of Particle Content of Recombinant Adeno-Associated Virus Serotype 8 Vectors by Ion-Exchange Chromatography,” <i>Human Gene Therapy, Part B: Methods</i> 23.1 (2012): 56-64 (“Lock”)	2012
Mahoney and Hermodson, “Separation of Large Denatured Peptides by Reverse Phase High Performance Liquid Chromatography; Trifluoroacetic Acid as a Peptide Solvent,” <i>Journal of Biological Chemistry</i> 255.23 (1980): 11199-11203 (“Mahoney”)	1980
Merten and Al-Rubeai, <i>Viral Vectors for Gene Therapy</i> , Vol. 737, Totowa, NJ: Humana Press, 2011 (“Merten”)	2011
Rayaprolu <i>et al.</i> , “Comparative analysis of adeno-associated virus capsid stability and dynamics,” <i>Journal of Virology</i> 87.24 (2013): 13150-13160 (“Rayaprolu”)	2013
Rhoads <i>et al.</i> , “Neutron-Encoded Mass Signatures for Quantitative Top-Down Proteomics,” <i>Analytical Chemistry</i> 86.5 (2014): 2314-2319 (“Rhoads”)	2014

Reference	Year
Richards <i>et al.</i> , “Proteome sequencing goes deep,” <i>Current Opinion in Chemical Biology</i> 24 (2015): 11-17 (“Richards”)	2015
Riley <i>et al.</i> , “Activated Ion Electron Transfer Dissociation for Improved Fragmentation of Intact Proteins,” <i>Analytical Chemistry</i> 87.14 (2015): 7109-7116 (“Riley”)	2015
Rouse <i>et al.</i> , “Top-down characterization of protein pharmaceuticals by liquid chromatography/mass spectrometry: Application to recombinant factor IX comparability – A case study,” <i>Therapeutic proteins: Methods and Protocols</i> , Totowa, NJ: Humana Press, 2005, 435-460 (“Rouse”)	2005
Satkunanathan <i>et al.</i> , “Establishment of a Novel Cell Line for the Enhanced Production of Recombinant Adeno-Associated Virus Vectors for Gene Therapy,” <i>Human Gene Therapy</i> 25.11 (2014): 929-941 (“Satkunanathan”)	2014
Shytuhina <i>et al.</i> , “Development and application of a reversed-phase high-performance liquid chromatographic method for quantitation and characterization of a Chikungunya virus-like particle vaccine,” <i>Journal of Chromatography A</i> 1364 (2014): 192-197 (“Shytuhina”)	2014
Siuzdak, “Probing Viruses with Mass Spectrometry,” <i>Journal of Mass Spectrometry</i> 33.3 (1998): 203-211 (“Siuzdak”)	1998
Venkatakrishnan <i>et al.</i> , “Structure and Dynamics of Adeno-Associated Virus Serotype 1 VP1-Unique N-Terminal Domain and Its Role in Capsid Trafficking,” <i>Journal of Virology</i> 87.9 (2013): 4974-4984 (“Venkatakrishnan”)	2013
Yost and Fetterolf, “Tandem mass spectrometry (MS/MS) instrumentation,” <i>Mass Spectrometry Reviews</i> 2.1 (1983): 1-45 (“Yost”)	1983
Yuan <i>et al.</i> , “Reversed-phase high-performance liquid chromatography of virus-like particles,” <i>Journal of Chromatography A</i> 816.1 (1998): 21-28 (“Yuan”)	1998
Zabrouskov <i>et al.</i> , “Stepwise deamidation of ribonuclease A at five sites determined by top-down mass spectrometry,” <i>Biochemistry</i> 45.3 (2006): 987-992 (“Zabrouskov”)	2006

The patents, publications, and combinations of references, listed in the table above, disclose, teach, or suggest every element of the Asserted Claims of the LC-MS Patents. These prior art disclosures, teachings, and suggestions are discussed further in the following sections. In

addition, Sarepta incorporates by reference each and every prior art reference of record in the prosecution of the LC-MS Patents and related patents and applications, including the statements made by the applicants during prosecution and the prior art discussed and/or cited in the specification.

Sarepta may also rely on expert testimony, inventor admissions, and any additional prior art located or developed during the course of discovery. Sarepta may rely on any of the materials in these contentions to demonstrate a motivation to combine and/or a reasonable expectation of success. Sarepta may also rely on expert testimony to further explain the prior art and to, for example, demonstrate a motivation to combine and a reasonable expectation of success. Further, Sarepta may rely on uncited portions of the identified prior art, references cited within the identified prior art, other references (irrespective of whether such references themselves qualify as prior art) to show the state of the art, and/or expert testimony to provide context to, or aid in, understanding the cited portions of the identified prior art.

In the sections below, references identified as rendering a claim obvious are representative and are not intended to be exhaustive. Other references disclosing the same or similar elements may be substituted for the cited references. Likewise, additional obviousness combinations of the references identified below are possible, and Sarepta reserves the right to use any such combination(s) in this litigation. Motivation to combine can be inferred generally for all references within the field of art of the LC-MS Patents. Furthermore, where references refer to or cite one another, motivation to combine may be inferred, whether or not specifically called out in these contentions. Finally, Sarepta's identification of motivation to combine in the cited references should not be taken as an admission or a representation that Sarepta will not rely upon other tests for obviousness in view of *KSR*, 550 U.S. at 401. This includes showing any of the following: (1)

that the combination of elements was obvious to try; (2) that the combination of elements according to known methods yielded predictable results; (3) that the substitution of one known element for another obtained predictable results; (4) that the application of a known technique to a known device, method, or product ready for improvement yielded predictable results; or (5) that known work in one field of endeavor prompted variations of such work for use in either the same field or a different one based on design incentives or other market forces because the variations are predictable to a person of ordinary skill in the art (“POSA”).

The identification of prior art that discloses and/or renders obvious a particular claim element in these contentions is not an admission that the claim element satisfies the requirements of 35 U.S.C. § 112. Where Sarepta asserts that a claim is invalid under 35 U.S.C. § 112 (such as because of a failure to particularly point out and distinctly claim the alleged invention, failure to provide written description support, and/or failure to enable a person of ordinary skill in the art to make and use the claimed invention), Sarepta has also provided prior art that renders the claim invalid for obviousness.

Documents produced by Genzyme to date do not support any secondary considerations of non-obviousness that would lead to a conclusion that the Asserted Claims of the LC-MS Patents would not have been obvious to a person of ordinary skill. In the event that Genzyme contends that there are any secondary considerations of non-obviousness, Sarepta will respond. Further, Genzyme must establish the requisite nexus between any alleged secondary considerations and the Asserted Claims of the LC-MS Patents. Sarepta is not aware of any evidence that would establish the required nexus between any alleged secondary considerations and the Asserted Claims of the LC-MS Patents. Nevertheless, even if there were any evidence of a nexus between any alleged

secondary considerations and the Asserted Claims of the LC-MS Patents, any such alleged secondary considerations would not rebut the strong *prima facie* evidence of obviousness here.

The methods recited in the Asserted Claims of the LC-MS Patents are obvious over at least the following combinations of prior art references, discussed in further detail in the sections below.

1. Knowledge in the Art

Well over a decade before the earliest possible priority date for the LC-MS Patents, researchers were using liquid chromatography, RP-HPLC in particular, to detect and quantify viral capsid proteins for purification process development. Yuan, Abstract.

MS had also been used to explore the properties of viruses for more than a decade before the earliest priority date for the LC-MS Patents. Siuzdak, Abstract. A 1998 review, “Probing Viruses with Mass Spectrometry,” stated, “[m]ass measuring viral proteins is now routine and since viruses are typically well characterized, in that the capsid proteins and DNA (or RNA) sequences are known, identifying a virus based on the mass of the protein and enzymatic digestion fragments is relatively straightforward.” Siuzdak, Abstract, p. 205. This review (Siuzdak), discusses the use of MS to identify capsid proteins, including in cases in which “more than one type of protein inhabits the capsid.” Siuzdak, p. 205. Siuzdak also describes the use of MS to identify viral protein post-translational modifications (“PTMs”) such as myristoylation, phosphorylation, and disulfide bridging, including the use of MS to identify a previously uncharacterized site of a particular PTM in a viral capsid protein. Siuzdak, Abstract, pp. 205-206. It also had been known since at least 1985 that AAV vp3 is acetylated *in vivo*. Becerra, p. 7920.

At least as early as 2002, LC-MS had been used to analyze viral composition. *See, e.g.*, Chelius. Chelius disclosed the use of LC-MS/MS to analyze the composition of adenovirus particles. Chelius, Abstract. Chelius separated adenoviral proteins by RP-HPLC, and then carried

out tryptic digestion and analysis by LC-MS/MS. Chelius, Abstract. All of the major adenoviral proteins were found through this method. Chelius, Abstract.

LC-MS/MS was also used before the priority date for process development for rAAV. Dong, Abstract; Satkunanathan, Abstract. Satkunanathan used LC-MS/MS to explore cellular components associated with three different serotypes of rAAV, specifically, AAV2, AAV5, and AAV8. Satkunanathan, Abstract. Satkunanathan identified capsid proteins unique to each serotype, and cellular components associated with one or more of the serotypes tested. Satkunanathan, Supplementary Table S1.

Satkunanathan cites Dong, an earlier proteomics analysis of co-purifying cellular proteins associated with rAAV2 vectors. Satkunanathan, p. 930, citing Dong. Using LC-MS, Dong identified two PTMs, carbamidomethylation of cysteine and oxidation of methionine, in AAV capsid proteins. Dong, Abstract, p. 2, Figure S1. Dong commented that “[p]rotein identification has benefited from advancements in sensitivity and accuracy of mass spectrometry (MS) that led to its broad use in proteomics.” Dong, p. 1. Unlike Satkunanathan, Dong studied only AAV2-based vectors and did not explore differences in co-purifying proteins among different serotypes. Dong; Satkunanathan. Also unlike Satkunanathan, Dong used gel electrophoresis as part of its LC-MS method. Dong; Satkunanathan.

Before the priority date, researchers had also used MS techniques to study mutant viral proteins in proteomics studies. Alqahtani, Abstract, p. 2509. Alqahtani used SILAC (stable isotope labeling of amino acids in cell culture) and high-throughput quantitative MS mass spectrometry to analyze the protein composition of highly purified wild-type (WT) adenoviruses, mutant adenoviruses lacking an internal protein component (protein V), and recombinant adenoviruses of the type commonly used in gene therapy, including one virus that had been used

in a clinical trial. Alqahtani, Abstract. Alqahtani concluded that their approach would be a valuable way to analyze the composition and batch-to-batch variation of WT, mutant, and genetically engineered viruses, VLPs, or nanoparticles. Alqahtani, p. 2510.

Also before the priority date, researchers were using intact LC-MS to analyze viral structural proteins for process development. *See, e.g.,* Shytuhina, Abstract. Shytuhina applied intact LC-MS to analyze undigested viral structural proteins, including to identify post-translational modifications. Shytuhina, Abstract, pp. 193-94.

AAV researchers were well aware of the importance of studying and comparing different AAV serotypes for process development and for understanding the different functions and capabilities of the different serotypes, which influence their suitability for use as therapeutic gene delivery vectors in different kinds of gene therapies. Satkunanathan. LC-MS techniques, moreover, were well known to be powerful tools for analyzing differences among various AAV serotypes. Satkunanathan.

2. Satkunanathan and Shytuhina

Satkunanathan. Satkunanathan was published in November 2014, more than one year before the earliest possible filing date for the LC-MS Patents. Satkunanathan. Therefore, Satkunanathan is AIA 35 U.S.C. § 102(a)(1) prior art.

Satkunanathan studied three different AAV serotypes (AAV2, AAV5, and AAV8), focusing in particular on analyzing the protein composition present in purified AAV vectors of each serotype. Satkunanathan, Abstract. Satkunanathan used LC-MS/MS to identify the proteins present in preparations of these purified AAV vectors. Satkunanathan, Abstract. Satkunanathan states that they sought to identify differences in protein composition among the three AAV serotypes AAV2, AAV5, and AAV8 by analyzing host cellular proteins coproduced and copurified with AAV vectors. Satkunanathan, p. 930.

Satkunanathan states that their work is directed towards improving the production of AAV vectors for gene therapy. Satkunanathan, Abstract, p. 930. Satkunanathan discusses the problem of pre-existing immune responses in patients, requiring administration of higher titers and, presumably, the design of vectors based on different AAV serotypes. Satkunanathan, Abstract.

Data analysis including mass spectra processing and database searching was carried out using Thermo Proteome Discoverer 1.2 with built-in Sequest. Satkunanathan, p. 931. Initial mass tolerances were set to 10 ppm. Satkunanathan, p. 931. Up to two missed tryptic cleavages were considered and methionine oxidation was set as a dynamic modification. Satkunanathan, p. 931. Notably, Dong had identified oxidation of methionine as a PTM found in the AAV2 capsid proteins. Dong, Abstract, p. 2.

Peptide sequences by MS/MS were only included when Xcorrelation scores were greater than 1.5, 2, or 2.2 for charge states 1, 2, and 3, respectively. Satkunanathan, p. 931. An unambiguous identification was considered when at least two peptides matched to the protein. Satkunanathan, p. 931. The protein FASTA databases were downloaded from www.uniprot.org, release 2012-07, including the complete entries from homo sapiens (taxon identifier 9606) bos taurus (9913); complete genome of AAV2, AAV5, and AAV8; and green fluorescent protein (GFP; P42212). Satkunanathan, p. 931.

Satkunanathan's results showed that 44 proteins were detected in at least two out of three runs of three batches of samples. Satkunanathan, p. 932, Supplementary Table S1. These proteins were considered to be significant components and further studied. Satkunanathan, p. 932, Supplementary Table S1. Among the significant proteins, ten were common to three of the AAV serotypes, including 70 kDa protein 1A/1B, alpha-enolase, GapDH, heat shock, histone H2A type

1H, histone H2B, nucleolin, nucleophosmin, RuvB2, and YB1. Satkunanathan, p. 932, Supplementary Table S1.

Out of eight proteins shared by two serotypes, five were shared by AAV2 and AAV5, indicating a relative similarity between AAV2 and AAV5 vectors. Satkunanathan, p. 932, Supplementary Table S1. Twenty-six were unique to individual serotypes of vectors. Satkunanathan, p. 932, Supplementary Table S1.

257. As shown in the excerpt from Supplementary Table S1 below, among the unique proteins for each serotype, Satkunanathan identified AAV2, AAV5, and AAV8 capsid proteins:

SUPPLEMENTARY TABLE S1. (CONTINUED)

<i>Protein ID</i>	AAV2	AAV5	AAV8
Actin, gamma 1 OS=Homo sapiens GN=ACTG1 PE=3 SV=1 - [F5H0N0_HUMAN]		+	
Annexin A2 (Fragment) OS=Homo sapiens GN=ANXA2 PE=4 SV=1 - [H0YKZ7_HUMAN]		+	
ATP synthase subunit alpha OS=Homo sapiens PE=2 SV=1 - [B4DY56_HUMAN]		+	
ATP synthase-coupling factor 6, mitochondrial OS=Homo sapiens GN=ATP5J PE=1 SV=1 - [ATP5J_HUMAN]		+	
Capsid protein VP1 OS=Adeno-associated virus 2 (isolate Srivastava/1982) PE=1 SV=2 - [CAPSD_AAV2S]	+		
Capsid protein OS=Adeno-associated virus - 5 GN=cap PE=1 SV=1 - [Q9YIJ1_9VIRU]		+	
Capsid protein OS=Adeno-associated virus - 8 PE=1 SV=1 - [Q8JQF8_9VIRU]			+

Satkunanathan, Supplementary Table S1 (excerpt) (yellow highlights added).

In the table above, a POSA would have understood OS to indicate the name of the organism or species. A POSA would also have understood that PE, which stands for “protein existence,” is a metric of certainty in the existence of a protein. Where PE=1, there is credible experimental evidence for the existence of the protein. A POSA would have understood that that SV indicates the version of the sequence in the UniProt database of protein sequences. Lastly a POSA would have understood GN to stand for “gene name.”

Satkunanathan found a serotype-specific role for an AAV-associated cellular protein, YB1. Satkunanathan, Abstract, p. 938. Satkunanathan found that knockdown of YB1 improved AAV2

and AAV8 production by 45- and 9-fold, respectively, but had no significant effect on AAV5 production. Satkunanathan, p. 938. In considering differences that could account for this result, Satkunanathan notes that the *cap* gene sequences are clearly different among the three serotypes tested (AAV2, AAV5, and AAV8). Satkunanathan, p. 938. Satkunanathan points out that AAV5 is one of the most divergent AAV serotypes, sharing only about 55% sequence homology with AAV2 and AAV8, which, in contrast, share about 82% homology with one another in their primary sequence. Satkunanathan, p. 938.

Satkunanathan therefore teaches the importance of identifying and characterizing different AAV serotypes accurately for rAAV vector purification and production. Satkunanathan's discussion of the problem of pre-existing immunity among patients to various AAV serotypes further underscores the need to ensure the serotypic purity of any preparation of rAAV for possible clinical use. Satkunanathan, p. 929. Satkunanathan also teaches that as of 2014, it was routine to identify capsid proteins of different AAV serotypes using LC-MS/MS. Satkunanathan, Supplementary Table S1.

Satkunanathan concludes that the precise mechanism of YB1 effects in AAV production required further investigation and that “[u]nderstanding the role of YB1 in the AAV life cycle greatly facilitates future production of high titer AAV vectors and, ultimately, improves the quality and safety of AAV vectors for clinical use.” Satkunanathan, p. 939.

Shytuhina. Shytuhina was published in 2014, more than a year before the earliest possible priority date for the LC-MS Patents (August 15, 2016), and is therefore AIA 35 U.S.C. § 102(a)(1) prior art.

Shytuhina discloses the development and application of an RP-HPLC-MS method for analysis of intact viral structural proteins for process development for a vaccine. Shytuhina,

Abstract. In particular, Shytuhina disclosed using their HPLC-MS method to identify post-translational modifications on intact viral structural proteins. Shytuhina, Abstract.

Shytuhina is directed towards development of a vaccine against Chikungunya virus, a mosquito-borne virus that causes acute illness including fever, rash, and severe arthralgia. Shytuhina, Abstract, p. 192. Chikungunya virus causes incapacitating and prolonged joint pain. Shytuhina, p. 192. Proof of concept experiments had shown that Chikungunya virus like particles (VLPs) were sufficient to elicit a protective humoral response against Chikungunya infection. Shytuhina, p. 192. Researchers therefore sought further characterization of Chikungunya VLPs. Shytuhina, p. 192.

Shytuhina explains that, traditionally, vaccines composed of VLPs were characterized by SDS-PAGE for purity and quantified by colorimetric protein assays such as Bradford, bicinchoninic acid (BCA) or Lowry assay. Shytuhina, p. 192. Shytuhina describes the disadvantages of these approaches. Shytuhina, p. 192. Specifically, Shytuhina states that SDS-PAGE is labor and time intensive. Shytuhina, p. 192. Colorimetric protein assays, according to Shytuhina, can be sensitive to detergents, reducing agents or certain salts. Shytuhina, p. 192. Moreover, Shytuhina explains that the colorimetric protein assays measure total protein concentration, and are therefore not specific for the antigenic components of the vaccine product. Shytuhina, p. 192.

Shytuhina states that to support process and formulation development effectively, it is highly desirable to have a sensitive and robust method available that can be automated to measure both vaccine purity and antigen specific vaccine mass. Shytuhina, p. 192. Shytuhina discloses that HPLC was an attractive analytical tool, in light of its high sensitivity and reproducibility. Shytuhina, p. 192. Shytuhina notes that HPLC had been applied for the identification and

quantitation of viral proteins and VLPs from a variety of other viruses, including serotypes of adenovirus (types 3 and 5), influenza, lentivirus, Sendai virus, poliovirus, human papillomavirus VLP, and Hepatitis B VLP. Shytuhina, pp. 192-93. Shytuhina explains that as a result of the hydrophobic nature of most viral glycoproteins and the presence of lipids with enveloped virus (such as Sendai virus and lentivirus), it had been challenging to achieve good resolution and recovery for all the viral components. Shytuhina, p. 193.

The Chikungunya VLP has three structural proteins – E1 (envelope protein 1), E2 (envelope protein 2), and a capsid protein. Shytuhina, p. 193. The capsid and envelope are organized as follows: an outer surface composed of 240 copies of glycoproteins E1 and E2 embedded in a lipid bilayer surrounding a nucleocapsid made of 240 copies of capsid protein. Shytuhina, p. 193.

Shytuhina states that their goal was to develop an RP-HPLC assay that would separate E1, E2, and capsid proteins of Chikungunya VLPs. Shytuhina, p. 193. This assay would evaluate and quantitate the mass and purity of the vaccine product. Shytuhina, p. 193. This method would be a tool to assess both protein degradation and post-translational modifications for formulation and process development. Shytuhina, p. 193.

Shytuhina used LC-MS intact protein analysis to validate their RP-HPLC method, and to identify specific PTMs on the E1 and E2 proteins. Shytuhina, pp. 193-96. Shytuhina discusses monitoring PTMs as a key element of process development. Shytuhina, pp. 196-97.

Shytuhina describes the RP-HPLC method they used to separate the Chikungunya capsid and envelope proteins. Shytuhina, p. 193. They analyzed Chikungunya VLPs on an XBridge BEH300 C4 column (3.5 μm , 4.6 \times 150 mm, 300 \AA , Part # 186004504, from Waters), held at 60 $^{\circ}\text{C}$, with a linear AB gradient elution. Shytuhina, p. 193. Their mobile phases were as follows: mobile

phase A contained 0.1% TFA in water; mobile phase B contained 30% ACN, 70% 2-propanol and 0.1% TFA. Shytuhina, p. 193. The separation was carried out with a 60-min gradient ranging from 0% to 100% mobile phase B followed by a 7-min re-equilibration with mobile phase A at a flow rate of 1 mL/min. Shytuhina, p. 193. Eluted proteins were detected by fluorescence at excitation at 280 nm and emission at 350 nm. Shytuhina, p. 193.

Shytuhina discloses the steps they took to optimize their RP-HPLC-MS method. Shytuhina, p. 194. Shytuhina screened several HPLC columns with different combinations of mobile phases. Shytuhina, p. 194. They found that while capsid protein could be readily eluted off of the HPLC column with 0.1% TFA in acetonitrile, E1 tended to stick to the column and required a strong organic solvent such as 2-propanol to elute it. Shytuhina, p. 194. In addition, TFA was necessary in the mobile phase to promote VLP interaction with the stationary phase. Shytuhina, p. 194. The lack of TFA in the mobile phases led to the elution of VLPs in the void volume of the column. Shytuhina, p. 194. Shytuhina notes that TFA, at the concentration used, did not cause aggregation of the Chikungunya proteins. Shytuhina, p. 194. Instead, Shytuhina discloses that TFA appeared to help disassemble the VLPs and facilitate the binding of proteins to the stationary phase. Shytuhina, p. 194.

Shytuhina found that a Waters XBridge BEH300 C4 column with 70% 2-propanol in the elution mobile phase had the best recovery for all three Chikungunya proteins, while column temperature of 60°C improved peak sharpness. Shytuhina, p. 194.

Shytuhina also screened different sample pre-treatment methods to optimize their separation. Shytuhina, p. 194. In particular, they initially observed carry-over of E1 and E2 between HPLC runs. Shytuhina, p. 194. Shytuhina discovered that pre-incubation of sample with 5% Zwittergent 3-12 detergent increased the peak area of E1 by 130% and E2 by 60% and

decreased the total carry-over to 4%. Shytuhina, p. 194. Shytuhina discloses that Zwittergent 3-12 detergent was believed to solubilize and stabilize the glycoproteins and prevent non-specific binding during chromatography. Shytuhina, p. 194. Shytuhina explains that incubation with 5% Zwittergent 3-12 detergent was therefore routinely used as a sample pre-treatment method. Shytuhina, p. 194.

To characterize the RP-HPLC chromatogram and identify which antigen protein was present in the individual peak, fractions were collected, concentrated and analyzed by SDS-PAGE and MALDI-ToF MS along with the corresponding unfractionated sample. Shytuhina, p. 194.

Shytuhina used a Waters Acquity LC system coupled to a Synapt G2 mass spectrometer (Waters, Milford, MA) to separate and analyze the proteins in the VLP samples by LC-MS. Shytuhina, p. 193. The mobile phases were as follows: mobile phase A was 0.1% formic acid (v/v) in water, and mobile phase B was 0.1% formic acid (v/v) in 30% ACN/70% isopropanol. Shytuhina, p. 193. Shytuhina explains that formic acid was used as the mobile phase modifier because TFA caused ion suppression. Shytuhina, p. 193. Shytuhina used 10 consecutive injections of 25 μ L of the sample to increase the signal intensity for intact protein accurate mass measurement. Shytuhina, p. 193. These injections were made with a short 2 min isocratic flow of 5% mobile B. Shytuhina, p. 193. Following the last injection, the proteins were eluted using a linear gradient of 5-80% of mobile phase B in 18 min at a flow rate of 0.2 mL/min. Shytuhina, p. 193.

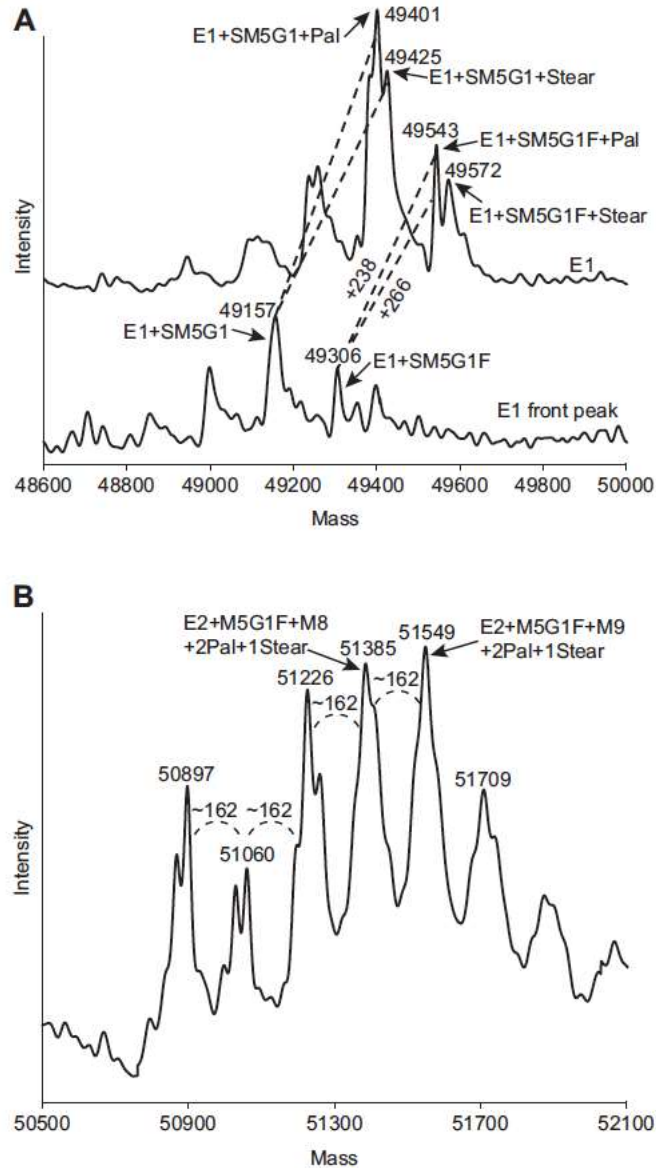
Mass spectra were obtained in positive mode by spraying the eluent into the mass spectrometer using an ESI source. Shytuhina, p. 193. The capillary, source cone, and extraction cone voltages were set at 3 kV, 20 V, and 4 V, respectively. Shytuhina, p. 193. Nitrogen was used as a desolvation gas at a flow rate of 800 L/h. Shytuhina, p. 193. The source and desolvation

temperatures were set at 110 and 450 °C, respectively. Shytuhina, p. 193. The instrument was operated in Sensitivity mode and spectra were acquired in an m/z range of 1000–2500. Shytuhina, p. 193.

Data acquisition and analysis (deconvolution) were performed with Waters MassLynx 4.1 software. Shytuhina, p. 193. Protein spectra were deconvoluted to obtain the observed intact protein masses. Shytuhina, p. 193. MaxEnt deconvolution parameters were set with output mass range of 40,000-60,000 and resolution of 0.1 Da/channel. Shytuhina, p. 193. Minimum intensity ratios were 33% for both the left and right parameters. Shytuhina, p. 193. A uniform Gaussian model was used with width at half height of either 1 or 0.8 Da. Shytuhina, p. 193. For spectra with width at half height of 1 Da, a maximum of 10 iterations were used. Shytuhina, p. 193. For spectra with width at half height of 0.8 Da, a maximum of 11 iterations were used. Shytuhina, pp. 193-94.

The ability to separate E1 and E2 by RP-HPLC chromatography allowed Shytuhina to characterize post-translational modifications of these viral glycoproteins by mass spectrometry. Shytuhina, p. 194. Both E1 and E2 of Chikungunya were known to contain N-glycosylation, and both glycoproteins were also expected to contain acylation based on sequence similarity with another virus, Semliki Forest virus (SFV). Shytuhina, p. 194.

Shytuhina identified post-translational modifications present on Chikungunya VLP proteins by LC-MS using accurate mass measurements of the intact protein antigens. Shytuhina, p. 194. Fig. 2, reproduced below, shows the deconvoluted spectra for E1 (Fig. 2A) and E2 (Fig. 2B).



Shytuhina, pp. 194-95, Fig. 2.

As shown in the figures above, multiple masses were observed, with each representing different glycosylation and acylation modifications. Shytuhina, pp. 194-95, Fig. 2. Based on known glycoprotein acylation and expected N-linked glycans, Shytuhina identified glycoprotein modifications in several major peaks by matching the observed mass to the theoretical molecular weight. Shytuhina, pp. 194-95, Fig. 2.

Shytuhina detected one N-glycosylation and one acylation – either palmitoylation (Pal) or stearoylation (Stear) – for the majority of E1 (Fig. 2A, top trace). Shytuhina, pp. 194-95, Fig. 2. A small portion of glycosylated but deacylated E1 eluted slightly before the main population (E1 front peak in Figs. 1A and 2A bottom trace). Shytuhina, pp. 194-95, Fig. 2. Shytuhina explains that this result was not surprising, given that protein acylation is a reversible process, and enzymatic depalmitoylation of viral glycoprotein had been demonstrated. Shytuhina, pp. 194-95, Fig. 2. Fig. 2B shows that E2 contained two N-glycosylations and three acylations. Shytuhina, pp. 194-95, Fig. 2.

Shytuhina states that they likely observed deamidation of one of the viral structural proteins they studied, the E2 protein of chikungunya virus. Shytuhina, p. 196. Specifically, in carrying out RP-HPLC, they observed an E2 degradant peak that eluted slightly earlier than the regular E2, suggesting that the degradant was more hydrophilic. Shytuhina, pp. 194-196, Figure 1A. Regarding this degradant peak, Shytuhina states: “Proteins are prone to deamidation at high pH and deamidation introduces a net increase in surface charge. Thus, we speculate at pH 9.0 E2 undergoes deamidation and that this chemical modification affects CHIKV VLP antigenicity.” Shytuhina, p. 196.

Shytuhina explains that Chikungunya VLPs produced in different cell lines exhibit different post-translational modifications. Shytuhina, pp. 196-97. Shytuhina states that it is therefore important to monitor post-translational modifications to ensure product lot-to-lot consistency throughout the vaccine development cycle. Shytuhina, p. 197.

Motivation to Combine. A POSA would have been motivated to combine Satkunanathan and Shytuhina because both are directed towards efficient and precise methods for analysis of viral particle composition for the purpose of process development. Both use LC-MS to characterize

purified viral preparations, identifying properties such as differences among different viral serotypes, or post-translational modifications.

Satkunanathan is expressly directed towards enhanced production of AAV vectors for gene therapy. Satkunanathan, Abstract. Satkunanathan states: “The significance of our findings should be considered in the context of future development of production methods for AAV-based gene therapy products.” Satkunanathan, p. 930.

Shytuhina is similarly expressly directed towards an analytical method for monitoring viral particles for process development of pharmaceuticals, including vaccines. Shytuhina, Abstract. Shytuhina states: “We developed a RP-HPLC method that separates capsid, E1 and E2, and allowed the characterization and quantitation of CHIKV VLP components. . . . This method can be applied as a release test for dose of CHIKV VLP vaccine product. Because this method provides good separation, we are able to characterize the post-translational modifications of the two viral glycoproteins. We demonstrated that this RP-HPLC method could support process development by monitoring product purity, and support formulation development by monitoring the product protein degradation.” Shytuhina, p. 197.

Satkunanathan’s method, however, involves digesting proteins before subjecting them to LC-MS. Satkunanathan sought to identify cellular proteins that co-purified with vectors based on different AAV serotypes. Satkunanathan studied differences in co-purifying proteins among three different AAV serotypes (AAV2, AAV5, and AAV8). Using LC-MS/MS, Satkunanathan identified capsid proteins from each of the three serotypes tested, AAV2, AAV5, and AAV8. Satkunanathan, Supplementary Table S1. A POSA at the time would have understood that Satkunanathan’s methods would likely have distinguished the three serotypes from one another in a mixture.

A POSA would have understood, however, that, given the structure of the AAV capsid proteins, vp1, vp2, and vp3, it would have been difficult, perhaps impossible, to determine using Satkunanathan's method, whether a fragment towards the C terminus of a given capsid protein originated from vp1, vp2, or vp3. Specifically, a POSA would have understood that AAV vp1, vp2, and vp3 are alternate transcripts from a single gene that differ at their N termini but share a common C terminus. Cecchini, p. 824, Figure 1. As a result, as a POSA at the time would have understood that Satkunanathan's method likely could not have identified vp3, for example, separate from vp1 or vp2. A POSA would similarly have understood that Satkunanathan's method could not, in most cases, have identified vp2 separate from vp1. A POSA would further have understood that enzymatic digestion is a laborious, time-consuming process, which can introduce artificial modifications, such as cyclization of N-terminal glutamine, and deamidation. Bondarenko, p. 1415. A POSA at the time would have further been motivated to improve methods for characterizing AAV proteins for process development, as Satkunanathan teaches.

A POSA would have understood that more precise, accurate characterization to improve process development would involve the capability to distinguish AAV serotypes, identify PTMs, and monitor degradation products. A POSA would have understood that the intact LC-MS method of Shytuhina would have been an efficient, cost-effective way to optimize AAV production and purification. Shytuhina discloses the use of intact LC-MS of viral structural proteins to identify and monitor PTMs to improve process development. In particular, a POSA would have understood that the intact LC-MS method of Shytuhina would have allowed efficient, reliable, accurate analysis of AAV capsid proteins, and assessing PTMs and other variations and truncations.

Shytuhina discloses LC-MS intact protein analysis of viral structural proteins. Shytuhina, pp. 193-94. Using intact LC-MS, Shytuhina separated different post-translationally modified

forms of viral structural proteins E1 and E2 from one another. Shytuhina, pp. 194-95, Fig. 2. Shytuhina characterized post-translational modifications for each of the two viral structural proteins, E1 and E2, using intact LC-MS. Shytuhina, pp. 194-95, Fig. 2. Using intact LC-MS, for example, Shytuhina separated and identified two different PTMs for the E1 structural protein. Shytuhina, pp. 194-95, Fig. 2A. Specifically, a POSA would have understood that Shytuhina's intact LC-MS method involved, as discussed above, deconvoluting the peaks obtained using software and identifying within the deconvoluted peaks modified forms of E1 and E2. Shytuhina, pp. 194-95, Fig. 2.

A POSA would have understood that the AAV capsid protein masses differ from one another by substantially more than the masses of the post-translationally modified viral structural proteins in Shytuhina differ from one another or from the unmodified proteins. A POSA would therefore have also understood that the intact LC-MS method of Shytuhina, applied to the capsid proteins identified by LC-MS/MS in Satkunanathan, would have identified the three different capsid proteins of serotypes such as those studied in Satkunanathan. A POSA would further have understood, particularly in light of Shytuhina's identification and separation of glycosylated but deacylated E1 from glycosylated and acylated E1, that Shytuhina's intact LC-MS method had sufficiently high resolution to separate varying forms of capsid proteins such as those studied in Satkunanathan from one another.

Satkunanathan discloses that the capsid proteins of different AAV serotypes have different sequences, can uniquely identify a particular AAV serotype, and determine key properties of the particular serotype. Satkunanathan, pp. 930, 938. A POSA would further have understood at the relevant time that the different capsid protein sequences would be reflected in different masses

identified through LC-MS and that therefore the determined masses of the capsid proteins would be indicative of at least the three AAV serotypes Satkunanathan studied.

Reasonable expectation of success. A POSA would have had a reasonable expectation of success in combining Satkunanathan with Shytuhina. The techniques required, namely, RP-HPLC, or RP-UPLC, intact LC-MS, and application of software to deconvolute and interpret MS data, were well known to people of skill in the art at the time and would have required nothing more than routine experimentation. Shytuhina successfully separated glycoproteins by liquid chromatography, specifically RP-HPLC, and analyzed them by intact LC-MS, successfully identifying several different post-translational modifications.

Intact LC-MS analysis of AAV capsid proteins would have been even more straightforward for a POSA than working with the glycoproteins Shytuhina analyzed. In particular, a POSA would have had a reasonable expectation of success in determining the serotype of different AAV serotypes such as those studied in Satkunanathan. Glycoproteins are more variable and therefore more difficult to analyze than AAV capsid proteins by intact LC-MS.

A POSA furthermore, using nothing more than routine experimentation, would have been able to select the best column, C4 or C8, for example, to optimize the separation by RP-HPLC, and then the analysis by MS. Shytuhina discloses that the specific RP-HPLC column they used was a C4 column. Shytuhina, p. 193. Although Shytuhina does not expressly disclose the column used for the RP-HPLC coupled to the mass spectrometer, a POSA would have understood it was likely that they used the same C4 column that had successfully separated E1 and E2 in the prior RP-HPLC step.

In any event, a POSA at the time would have understood that it was generally necessary to test a few different columns to optimize the desired separation. Yuan, pp. 22-23. Yuan discloses

a RP-HPLC method to detect a capsid protein of HPV, the L1 protein. Yuan, Abstract. Yuan tested both C4 and C8 columns, finding that either column could be used with equal efficiency to purify the L1 capsid protein. Yuan, p. 23. Satkunanathan used a reversed phase C18 column for LC-MS/MS. Satkunanathan, p. 931. Mahoney used a C8 column for LC-MS of large peptides. Mahoney, pp. 11199-200, Table I, Table II (testing various different organic solvents including acetonitrile and 2-propanol on a C8 column, and comparing their ability to separate large denatured peptides with and without TFA).

A POSA would have understood that these columns, C4, C8, and C18, were commonly used columns for separation of viral structural proteins, including AAV capsid proteins. A POSA would therefore have likely tested all of them, in applying Shytuhina's intact LC-MS method to the separation and identification of AAV capsid proteins.

A POSA furthermore, using nothing more than routine experimentation, would have tested and been able to optimize the separation by RP-UPLC, and then the analysis by MS. Ansong. A POSA would have been aware that researchers were using intact LC-MS with UPLC to study mixtures of proteins and to identify PTMs. Ansong. A POSA would have been aware of the advantages of UPLC, such as the efficient separation and increased sampling of proteins in the mixture and identification of PTMs. Ansong, p. 10154.

Satkunanathan successfully identified and distinguished three different AAV serotypes, and Shytuhina successfully separated viral structural glycoproteins with multiple different PTMs. Moreover, researchers in the field for years before the relevant date had been successfully separating reduced monoclonal antibodies and identifying differences among them, including PTMs. Applying intact LC-MS to AAV capsid proteins at the relevant date would have been considerably more routine and straightforward than applying the technique to monoclonal

antibodies. A POSA would have a reasonable chance of success in separating AAV capsid proteins and identifying them, including PTMs, by intact LC-MS.

3. Satkunanathan, Shytuhina, and Ansong

Satkunanathan and Shytuhina, the motivation to combine them, and the reasonable expectation of success are discussed above, and that discussion is incorporated by reference in its entirety here.

Ansong. Ansong was published on June 18, 2013, more than a year before the earliest possible priority date for the LC-MS Patents (August 15, 2016), and is therefore AIA 35 U.S.C. § 102(a)(1) prior art. Ansong.

Ansong used intact LC-MS with a UPLC column to study the proteome of *Salmonella typhimurium*. Ansong, Abstract, pp. 10153-54. Specifically, Ansong used a high-throughput top-down proteomics pipeline consisting of a single-dimension Waters UPLC system augmented with long (80-cm) nanocapillary LC columns and an extended (250-min) gradient interfaced with a Velos Orbitrap mass spectrometer to profile the intact proteome of *Salmonella typhimurium* and comprehensively characterize endogenous PTMs and their modulation in response to physiologically relevant conditions. Ansong, Abstract, p. 10154.

Ansong discloses that in high-resolution (MS and MS/MS), high-throughput mode, the ultrahigh-pressure single-dimension top-down proteomics platform consumed ~5 µg of intact protein extract sample per analysis. Ansong, Abstract, p. 10154. Ansong analyzed intact mass spectra using MS-Align+, an algorithm for intact protein identification that enables searches for unexpected PTMs. Ansong, Abstract, p. 10154.

Ansong states that a number of factors contributed to the improved intact proteome coverage using their single-dimension intact platform compared with prior studies. Ansong, Abstract, p. 10154. Ansong states that these factors included the use of a UPLC system with long

columns (80 cm) and long gradients (250 min) that afforded efficient protein separation, as well as enough time to limit MS/MS undersampling, a well-recognized issue for bottom-up proteomics that can be relaxed using long columns and gradients. Ansong, Abstract, p. 10154. Ansong says that an additional factor was application of the software tool MS-Align+ for top-down protein identification based on spectral alignment, which had been shown to increase the number of identified spectra compared with other available tools. Ansong, Abstract, p. 10154.

Using intact (top down) LC-MS with UPLC, Ansong identified 563 unique proteins including 1665 proteoforms generated by PTMs. Ansong, Abstract. They found 12 proteins with an N-terminal acetylation modification, among other PTMs. Ansong, Abstract, pp. 10154-55.

Motivation to Combine. A POSA would further have been motivated to combine Ansong's use of UPLC with the methods of Satkunanathan and Shytuhina. Ansong carried out intact LC-MS using UPLC to analyze the proteome of *Salmonella typhimurium* and identify multiple different PTMs. Ansong, Abstract.

A POSA would have understood that UPLC is a chromatographic technique that, for example, can use sub-2 μm particles, mobile phases at high linear velocities, and instrumentation that operates at high pressure. Cueto-Rojas, p. 1019. A POSA would have been aware that it was well known in the art that UPLC could offer certain benefits as compared with HPLC, including increased resolution, sensitivity and speed of the chromatographic analysis, which are desirable for a chromatographic method to analyze proteins for in-process control. Cueto-Rojas, p. 1019. A POSA would have been aware of the advantages of UPLC, for example, those set out in Ansong, such as the efficient separation and increased sampling of proteins in the mixture and identification of PTMs. Ansong, p. 10154.

A POSA would therefore have likely tested Ansong's UPLC approach, in applying Shytuhina's intact LC-MS method to the separation and identification of AAV capsid proteins.

Reasonable Expectation of Success. A POSA would have had a reasonable expectation of success in combining Satkunanathan with Shytuhina and Ansong. The techniques required to make the claimed combinations, namely, RP-UPLC, intact LC-MS, and application of software to deconvolute and interpret MS data, were well known to people of skill in the art at the time and would have required nothing more than routine experimentation.

Shytuhina successfully separated glycoproteins by liquid chromatography, specifically RP-HPLC, and analyzed them by intact LC-MS, successfully identifying several different post-translational modifications. Intact LC-MS analysis of AAV capsid proteins would have been even more straightforward for a POSA than working with the glycoproteins Shytuhina analyzed. Glycoproteins are more variable and therefore more difficult to analyze than AAV capsid proteins by intact LC-MS.

A POSA furthermore, using nothing more than routine experimentation, would have been able to optimize the separation by UPLC, and then the analysis by MS, as Ansong disclosed. Moreover, a POSA would have a reasonable chance of success in separating AAV capsid proteins and identifying them, including PTMs, by intact LC-MS.

Satkunanathan successfully identified and distinguished three different AAV serotypes, and Shytuhina successfully separated viral structural glycoproteins with multiple different PTMs.

Moreover, researchers in the field for years before the relevant date had been successfully separating reduced monoclonal antibodies and identifying PTMs. *See, e.g.,* Bondarenko. Applying intact LC-MS to AAV capsid proteins at the relevant date would have been considerably more routine and straightforward than applying the technique to monoclonal antibodies.

4. Satkunanathan, Shytuhina, and Yuan

Satkunanathan and Shytuhina, the motivation to combine them, and the reasonable expectation of success are discussed above, and that discussion is incorporated by reference in its entirety here.

Yuan. Yuan was published in 1998, more than a year before the earliest possible priority date for the LC-MS Patents (August 15, 2016), and is therefore AIA 35 U.S.C. § 102(a)(1) prior art. Yuan.

Yuan discloses an RP-HPLC method for analysis of virus-like particles (VLPs) of human papillomavirus (HPV) for process development. Yuan, Abstract. In particular, Yuan's method is directed towards identifying the L1 capsid protein that comprises 90-95% of HPV capsids. Yuan, p. 21. Yuan explains that the HPV capsid is typically composed of 72 pentameric capsomers of L1 arranged on a skewed icosahedral lattice. Yuan, p. 21. A second structural protein, L2, represents the remaining 5-10% of the capsid. Yuan, p. 21. Yuan states that their method is quantitative and can be used to facilitate HPV purification process development. Yuan, p. 21.

The first step in Yuan's analysis was denaturation of the HPV samples using a dissociation buffer of 8 M guanidine-HCl/50 mM Tris, pH 8.0. Yuan, p. 22. Then, 10% 2-mercaptoethanol was added to the samples, which were incubated at 55°C for 15 minutes. Yuan, p. 22. The second step, HPLC, was then performed on a Hewlett-Packard 1100 Series liquid chromatography system equipped with a G1322A vacuum degasser, a G1311A quaternary pump, a G1315A diode array detection (DAD) system, a G1313A autosampler and a G1316A thermostatted column compartment. Yuan, p. 22.

Yuan states that its HPLC analyses were carried out on C8 or C4 reversed-phase columns (25 cm x 4.6 mm I.D., 5 µm particle size, 300 Å pore size) purchased from Vydac (Hesperia, CA, USA). Yuan, p. 22. Column temperature was set at 37°C. Yuan, p. 22. Yuan discloses that the

solvents used were as follows: solvent A was 0.1% TFA in water and solvent B was 0.075% TFA in acetonitrile. Yuan, p. 22. Gradient conditions used in the analysis are shown in the table below:

Table 1
Slow and fast gradient methods used for RP-HPLC

Slow gradient method		Fast gradient method	
Time (min)	% Solvent B	Time (min)	% Solvent B
0	5	0	40
10	5	5	40
15	40	15	55
30	60	17	100
32	100	20	100
37	100		

Yuan, p. 22, Table 1. SDS-PAGE analysis was performed on the peak fractions. Yuan, p. 22.

Yuan discusses the importance of the first step of dissociating the VLPs before placing them on the HPLC column. Yuan, p. 22. Yuan explains that the VLPs they used in the analysis were approximately 55 nm in diameter and contained a single capsid protein, L1, with a molecular mass of about 55,000. Yuan, p. 22. According to Yuan, the VLPs are typically composed of 72 pentameric capsomers of L1 arranged on a skewed icosahedral lattice. Yuan, p. 22. The molecular mass of the intact recombinant particle is approximately 20×10^6 . Yuan, p. 22. Yuan explains that to quantitate the L1 capsid protein, it was necessary to dissociate the VLPs prior to RP-HPLC analysis. Yuan, p. 22.

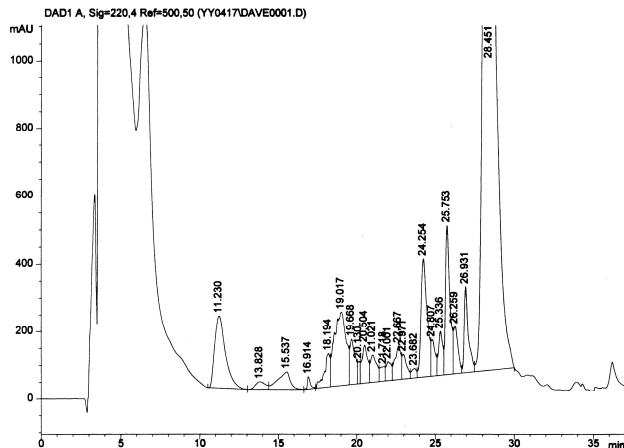
Yuan found that a buffer composed of 50 mM Tris, pH 8.0, containing 8 M guanidine-HCl and 10% 2-mercaptoethanol was effective in reducing disulfide bonds and destabilizing the VLPs to facilitate dissociation. Yuan, p. 22. The VLPs were incubated in this buffer at 55°C for 15 minutes before being loaded onto the column. Yuan, p. 22. After incubation, the VLP sample containing monomeric L1 capsid protein was injected onto a Vydac C4 or C8 column. Yuan, p. 22. The L1 capsid protein was eluted using an acetonitrile-water/0.1% TFA gradient, as discussed above. Yuan, pp. 22-23. A single, clearly resolved peak was obtained. Yuan, pp. 23-24, Fig. 1.

Yuan tested both C4 and C8 columns, noting that “either the C4 or C8 column can be used with equal efficiency of separation providing calibration is carried out with purified reference standard material.” Yuan, p. 23. Yuan noted that the retention time of L1 capsid protein on a C4 column was 0.2 minutes shorter than the retention time on a C8 column under fast gradient conditions. Yuan, p. 23. Yuan confirmed the identity of the L1 capsid protein in the L1 containing peak fractions using SDS-PAGE. Yuan, p. 23. For quantitation, Yuan generated a calibration curve using HPV-18 VLPs purified by sucrose gradient centrifugation. Yuan, p. 23. Two C8 columns and one C4 column were used for analysis. Yuan, p. 23.

Yuan analyzed and quantitated HPV-18 in-process samples by both their RP-HPLC method and by Western blot. Yuan, p. 23. They found that the L1 concentration for all samples was higher by RP-HPLC than by semi-quantitative Western blot. Yuan, p. 23.

Yuan also conducted spiking studies to evaluate whether their RP-HPLC method could be used for to quantitate partially purified samples for in-process development. Yuan, p. 23. They recovered from 92% to 99% of the spiked protein, indicating that their method was suitable for quantitation of partially purified samples for process development. Yuan, p. 23.

Yuan applied their method to quantitate HPV produced in bioreactors (insect cells). Yuan, pp. 23, 25. They diluted the sample in dissociation buffer, then injected it onto a C8 column using a slow gradient method. Yuan, pp. 23, 25. The chromatogram is shown below:



Yuan, p. 26, Fig. 3. The L1 capsid protein eluted in fractions from 23.8 to 25.0, as confirmed by SDS-PAGE. Yuan, p. 27.

Motivation to Combine. As discussed above, a POSA would have been motivated to apply Shytuhina’s efficient and precise intact LC-MS method to improve on Satkunanathan’s use of LC-MS/MS to optimize process development for AAV vectors, particularly in light of differences among the different AAV serotypes.

Shytuhina’s method involves separation of the viral proteins by RP-HPLC before analysis by mass spectrometry. Shytuhina, pp. 193-195. Shytuhina states, “The ability to separate E1 and E2 by RP-HPLC chromatography allows us to characterize post-translational modifications of these viral glycoproteins by mass spectrometry.” Shytuhina, p. 194.

Shytuhina discloses that the specific RP-HPLC column they used was a C4 column. Shytuhina, p. 193. Although Shytuhina does not expressly disclose the column used for the RP-HPLC coupled to the mass spectrometer, a POSA would have understood it was likely that they used the same C4 column that had successfully separated E1 and E2 in the prior RP-HPLC step.

In any event, a POSA at the time would have understood that it was generally necessary to test a few different columns to optimize the desired separation. Yuan, pp. 22-23. Yuan discloses a RP-HPLC method to detect a capsid protein of HPV, the L1 protein. Yuan, Abstract. Yuan

tested both C4 and C8 columns, finding that either column could be used with equal efficiency to purify the L1 capsid protein. Yuan, p. 23. Satkunanathan used a reversed phase C18 column for LC-MS/MS. Satkunanathan, p. 931.

A POSA would have understood that these columns, C4, C8, and C18, were commonly used columns for separation of viral structural proteins, including AAV capsid proteins. A POSA would therefore have likely tested all of them, in applying Shytuhina's intact LC-MS method to the separation and identification of AAV capsid proteins, in combination with Yuan's method of testing columns such as C4 and C8, to optimize the LC-MS separation and protein identification.

Reasonable Expectation of Success. A POSA would have had a reasonable expectation of success in combining Satkunanathan and Shytuhina with Yuan to arrive at the claimed combination. The techniques required to make the claimed combination, namely, RP-HPLC, intact LC-MS, and application of software to deconvolute and interpret MS data, were well known to people of skill in the art at the time and would have required nothing more than routine experimentation.

In particular, optimizing RP-HPLC for a particular separation would have required only routine experimentation. As Yuan demonstrates, researchers at the time routinely tested columns such as C4 and C8 columns, including for separation of viral structural proteins, to determine which column separated most effectively and efficiently. Yuan successfully used both C4 and C8 columns to detect the L1 capsid protein of HPV. Yuan expressly found that either the C4 or C8 column could be used with equal efficiency of separation.

Shytuhina successfully separated glycoproteins by liquid chromatography, specifically RP-HPLC, and analyzed them by intact LC-MS, successfully identifying several different post-translational modifications. A POSA would have understood that intact LC-MS analysis of AAV

capsid proteins would have been even more straightforward for a POSA than working with the glycoproteins Shytuhina analyzed. Glycoproteins are more variable and therefore more difficult to analyze than AAV capsid proteins by intact LC-MS.

A POSA therefore, using nothing more than routine experimentation, would have been able to select the best column, C4 or C8, for example, to optimize the separation by RP-HPLC, and then the analysis by MS. A POSA would have had a reasonable chance of success that a C4 or a C8 column would have successfully separated the AAV capsid proteins. The HPV L1 capsid protein is approximately the same size as the AAV capsid proteins (55 kDa), further demonstrating that a POSA would have had a reasonable chance of success in separating the AAV capsid proteins on a C8 column. Similarly, the Chikungunya structural proteins are approximately the same size as the AAV capsid proteins (55 kDa), further demonstrating that a POSA would have had a reasonable chance of separating the AAV capsid proteins on a C4 column, similar to the one Shytuhina used.

Moreover, a POSA would have a reasonable chance of success in separating AAV capsid proteins and identifying them, including any PTMs, by intact LC-MS. Satkunanathan successfully identified and distinguished three different AAV serotypes, and Shytuhina successfully separated viral structural glycoproteins with multiple different PTMs.

Moreover, researchers in the field for years before the relevant date had been successfully separating reduced monoclonal antibodies and identifying PTMs. Applying intact LC-MS to AAV capsid proteins at the relevant date would have been considerably more routine and straightforward than applying the technique to monoclonal antibodies.

C. NON-PATENTABLE SUBJECT MATTER

The Asserted Claims of the LC-MS Patents are directed to patent-ineligible natural phenomena and/or laws of nature. *See Alice Corp. Pty. Ltd. v. CLS Bank Intern.*, 573 U.S. 208

(2014). Specifically, the Asserted Claims are directed to determining the masses of naturally occurring viral proteins, and comparing those masses with theoretical masses, to derive information about the proteins and about viral particles containing them, such as serotype and/or the presence of modifications. The additional elements of the Asserted Claims of the LC-MS Patents are all well-known features in the prior art that reflect well-understood, routine, and conventional activity engaged in by the scientific community as of the earliest priority date for the LC-MS Patents and add nothing to transform the natural phenomena and/or laws of nature into patent-eligible subject matter. Thus, the Asserted Claims are invalid under 35 U.S.C. § 101. *See Mayo Collaborative Services v. Prometheus Labs., Inc.*, 566 U.S. 66, 80 (2012).

The Supreme Court has “set forth a [two-step] framework for distinguishing patents that claim laws of nature, natural phenomena, and abstract ideas from those that claim patent-eligible applications of those concepts.” *Alice*, 573 U.S. at 217 (citing *Mayo*, 566 U.S. at 77-78).

First, in step one, the claims at issue are evaluated to determine whether they are “directed to” a patent-ineligible concept. *Id.* This inquiry includes an evaluation of the claims themselves and the description of the “invention” in the specification. The inventors’ description of their “discovery” as a natural phenomenon or law of nature is compelling evidence that the claims are directed to ineligible subject matter. *See, e.g., Athena Diagnostics, Inc. v. Mayo Collaborative Svcs., LLC*, 915 F.3d 743, 751, 754 (Fed. Cir. 2019) (specification states that the inventors “surprisingly found” a natural law); *Roche Molecular Sys. v. Cepheid*, 905 F.3d 1363, 1371-72 (Fed. Cir. 2018) (alleged invention based on the “undiscovered presence” of a natural phenomenon); *Cleveland Clinic Foundation v. True Health Diagnostics LLC*, 859 F.3d 1352, 1360-61 (Fed. Cir. 2017) (alleged invention “based on the discovery” of a natural law); *Genetic Techs. Ltd. v. Meril L.L.C.*, 818 F.3d 1369, 1375 (Fed. Cir. 2016) (same); *Ariosa Diagnostics,*

Inc. v. Sequenom, Inc., 788 F.3d 1371, 1376 (Fed. Cir. 2015) (natural phenomenon was a “surprising and unexpected finding”); *see also CareDx, Inc. v. Natera, Inc.*, 563 F. Supp. 3d 329, 339-347 (D. Del. 2021), *aff’d*, 40 F.4th 1371 (Fed. Cir. 2022); *ChromaDex, Inc. v. Elysium Health, Inc.*, 561 F. Supp. 3d 460 (D. Del. 2021), *aff’d*, 59 F.4th 1280 (Fed. Cir. 2023).

Second, in step two, the claims are evaluated to determine whether the additional elements add enough to the concept to qualify as patent eligible. *Mayo*, 566 U.S. at 77. In making this assessment, the claim elements should be considered individually and “as an ordered combination” to determine whether the additional elements “transform the nature of the claim” into something patent-eligible. *Id.* at 78-80. However, the “inventive concept” necessary to satisfy the second step cannot be furnished by the unpatentable natural phenomena or law of nature itself. *See, e.g., Athena Diagnostics*, 915 F.3d at 754 (citing *Genetic Techs.*, 818 F.3d at 1376). Concessions in the intrinsic record that the additional claim elements (other than the natural phenomenon or law of nature itself) were in the prior art are generally conclusive as to patent-ineligibility. *See, e.g., Genetic Techs.*, 818 F.3d at 1377 (specification and arguments during prosecution made clear that no new techniques for PCR amplification or DNA analysis disclosed); *Ariosa Diagnostics*, 788 F.3d at 1377 (specification confirmed “that the preparation and amplification of DNA sequences in plasma or serum were well-understood, routine, conventional activities performed by doctors in 1997”); *see also CareDX*, 563 F. Supp. 3d at 342-43; *ChromaDex*, 561 F. Supp. 3d at 466 (concluding that there were no additional features sufficient “to ensure that the claim is more than a drafting effort designed to monopolize the [natural product]”).

Step 1: The Asserted Claims of the LC-MS Patents are directed to non-patentable natural phenomena and/or laws of nature. At the most basic level, the Asserted Claims purport to claim methods of determining the masses of naturally occurring viral proteins, and comparing those

masses with theoretical masses, to derive information about the proteins and about viral particles containing them, such as serotype and/or the presence of modifications. The relationship between the mass of a protein and characteristics of that protein is a non-patentable natural phenomenon/law of nature.

For example, claim 1 of the '377 patent recites:

1. A method to determine the serotype of an adeno-associated virus (AAV) particle comprising:

a) denaturing the AAV particle,

b) directly subjecting the denatured AAV particle to liquid chromatography/mass spectrometry (LC/MS) intact protein analysis, and

c) determining the masses of VP1, VP2 and VP3 of the AAV particle;

wherein the specific combination of masses of VP1 VP2 and VP3 are indicative of the AAV serotype,

and wherein the method is performed in the absence of a gel separation step.

'377 patent, 109:19-30.

The central element of claim 1 is determining the masses of naturally occurring viral proteins, and comparing those masses with theoretical masses, to derive information about the proteins and about viral particles containing them, specifically, the serotype of the viral particle.

Similarly, claim 1 of the '880 patent recites:

A method of analyzing a preparation of AAV particles, the method comprising:

a) denaturing the AAV particles,

b) subjecting the denatured AAV particles to liquid chromatography/mass (LC/MS) intact protein analysis, and

c) determining the masses of one or more viral proteins (VPs) of the particles in the preparation,

wherein the method is performed in the absence of a gel separation step.

'880 patent, 83:10-20.

Similar to claim 1 of the '377 patent, the central element of claim 1 of the '880 patent involves determining the masses of naturally occurring viral proteins to “analyze” them, *i.e.*, to derive information about the proteins and about viral particles containing them from the protein masses.

Claim 1 of the '313 patent recites:

1. A method of detecting post-translational modifications of one or more viral proteins (VPs) in a preparation of adeno-associated virus (AAV) particles, the method comprising:

a) denaturing the AAV particles;

b) subjecting the denatured AAV particles to liquid chromatography/mass spectrometry (LC/MS) intact protein analysis;

c) determining the masses of the one or more VPs; and

d) determining any deviation of the determined masses of the one or more VPs from the theoretical masses of corresponding VPs that have not undergone post-translational modifications to detect a deviation in the compared masses,

wherein the VPs comprise VP1, VP2 and VP3 capsid proteins, and wherein the method is performed in the absence of a gel separation step.

'313 patent, 83:1-18.

Similar to claim 1 of the '377 patent and claim 1 of the '880 patent, the central element of claim 1 of the '313 patent involves determining the masses of naturally occurring viral proteins to derive information about those proteins – specifically, information about post-translational modifications. The obtained masses are compared to theoretical masses of the unmodified proteins.

The specification of the LC-MS Patents shows that the claims are directed to non-patentable subject matter, specifically, the natural phenomenon of the relationship between the masses of viral proteins and viral serotype and/or the presence of modifications, as determined through comparison with theoretical protein masses. The specification states, in the Brief Summary of the Invention: “Using rAAV as an example, described herein is the use of LC/MS as an analytical tool to specifically identify different 5 viral capsid serotypes (e.g., rAAV capsid serotypes). As part of viral characterization, LC/MS can be used to augment the molecular identification methods. . . . ***This method can be used e.g., as an AAV serotype identity test or to monitor viral capsid protein heterogeneity*** in recombinant AAV gene therapy development.” See, e.g., ’377 patent, 2:3-13 (emphasis added). The specification further states: “The accurate masses of VP1, VP2 and VP3 of each serotype are unique and therefore ***intact protein analysis can be used as an identity test*** to differentiate AAV capsid serotypes.” See, e.g., ’377 patent, 57:19-22 (emphasis added). The specification also states that “intact protein measurement of VP1, VP2 and VP3 is highly specific as an identity test.” See, e.g., ’377 patent, 59:16-17.

Moreover, the specification defines “heterogeneity” of AAV capsids as determined by comparison of observed masses with “reference” or “theoretical” masses: “‘Heterogeneity’ when used in reference to an AAV capsid refers to an AAV capsid ***characterized by one or more capsid polypeptides observed to deviate from a reference mass of a VP1, VP2, and/or VP3 polypeptide, or fragment thereof. A reference mass may include, without limitation, a theoretical, predicted, or expected mass of a VP1, VP2, and/or VP3 polypeptide, e.g., of a known AAV serotype.*** For example, an AAV capsid may be said to display heterogeneity if it demonstrates one or more of the following properties (without limitation): a mixed serotype, a variant capsid, a capsid amino

acid substitution, a truncated capsid, or a modified capsid.” *See, e.g.,* ’377 patent, 19:49-60 (emphasis added).

The specification further discloses data showing “Theoretical Mass vs. Experimental Mass for AAV VPs.” *See, e.g.,* ’377 patent, 55:55-56:25, Table 2. Table 2 is reproduced below:

TABLE 2

Theoretical Mass vs Experimental Mass for AAV VPs

Serotype	Isoform	Predicted amino acid sequence	Actual amino acid sequence	Theoretical Ms.(Da)	Experimental Ms.(Da)
AAV1	VP1	1-736	2(ac)-736	81286	81291
	VP2	138-736	139-736	66093	66098
	VP3	203-736	204(ac)-736	59517	59520
AAV2	VP1	1-735	2(ac)-735	81856	81856
	VP2	138-735	139-735	66488	66488
	VP3	203-735	204(ac)-735	59974	59974

TABLE 2-continued

Theoretical Mass vs Experimental Mass for AAV VPs

Serotype	Isoform	Predicted amino acid sequence	Actual amino acid sequence	Theoretical Ms.(Da)	Experimental Ms.(Da)
AAV5	VP1	1-724	2(ac)-724	80336	80336
	VP2	137-724	138-724	65283	65284
	VP3	193-724	194(ac)-724	59463	59463
AAV7	VP1	1-737	2(ac)-737	81564	81567
	VP2	138-737	139-737	66372	66374
	VP3	204-737	213(ac)-737	59101	59103
AAV9	VP1	1-736	2(ac)-736	81291	81288
	VP2	138-736	139-736	66210	66209
	VP3	203-736	204(ac)-736	59733	59733
AAVRh10	VP1	1-738	2(ac)-738	81455	81455
	VP2	138-738	139-738	66253	66252
	VP3	204-738	205(ac)-738	59634	59634

’377 patent, 55:55-56:25, Table 2.

As Table 2 shows, the inventors relied on the difference between the observed mass of each viral protein (VP1, VP2, VP3), as determined by intact mass analysis, and the theoretical mass, to

hypothesize which observed masses corresponded to which modifications. For example, the AAV1 VP1 protein was hypothesized to be modified by N-terminal truncation and acetylation. Based on an observed mass of 81291 as compared with a theoretical mass of 81286, the inventors concluded that the “Actual amino acid sequence” of VP1 was (2(ac)-736), rather than the “Predicted amino acid sequence” of 1-736. ’377 patent, 55:55-56:25, Table 2.

Step 2. The additional elements do not transform the Asserted Claims into patent eligible subject matter. Instead, the LC-MS patents describe these additional elements as directed to well-understood, routine, and conventional activities that were well within the capabilities of a person of ordinary skill in the art. Whether considered individually or as a whole, the additional elements in the Asserted Claims do not add anything unconventional or inventive to the naturally-occurring masses of viral proteins, their naturally occurring modifications, and the combination of these masses in a given viral strain.

The specification concedes that LC-MS methods were known in the art. *See, e.g.,* ’377 patent, 20:48-59 (“In some embodiments, the methods include subjecting a denatured viral particle of the present disclosure to liquid chromatography/mass spectrometry (LC/MS). ***As is known in the art,*** LC/MS utilizes liquid chromatography for physical separation of ions and mass spectrometry for generation of mass spectral data from the ions. Such mass spectral data may be used to determine, e.g., molecular weight or structure, identification of particles by mass, quantity, purity, and so forth. These data may represent properties of the detected ions such as signal strength (e.g., abundance) over time (e.g., retention time), or relative abundance over mass-to-charge ratio.”). The specification further concedes that reversed phase liquid chromatography and UPLC, specific types of liquid chromatography recited in some Asserted Claims, were also known in the art. *See, e.g.,* ’377 patent, 20:60-21:9; 22:44-23:32.

The specification further concedes that mass analyzers suitable for LC-MS were known in the art. *See, e.g.*, '377 patent, 24:22-39.

The specification further concedes that methods to determine protein mass and/or identity from MS data were known in the art, including software to use in interpreting mass spectrometry data. *See, e.g.*, '377 patent, 24:51-25:9 (“In some embodiments, masses of viral capsid proteins may be determined, e.g., based on LC/MS and/or LC/MS/MS data. In some embodiments, masses of VP1, VP2 and VP3 of an AAV particle, or of fragments of VP1, VP2 and VP3 of the AAV particle, may be determined, e.g., based on LC/MS and/or LC/MS/MS data. ***Various methods to determine protein mass and/or identity from MS data are known in the art.*** For example, peptide mass fingerprinting may be used to determine protein sequence based on MS data, or proteins may be identified based on MS/MS data related to one or more constituent peptides. When using tandem MS, product ion scanning may be used to analyze m/z data related to one or more peptides of a protein of interest. ***Software known in the art*** may then be used, e.g., to match identified peaks to reference or known peaks, to group peaks into isotopomer envelopes, and so forth. Peptide mass values may be compared to a database of known peptide sequences. For example, Mascot may be used to match observed peptides with theoretical database peptides, e.g., resulting from application of a particular digest pattern to an in silica protein database. Other suitable software may include without limitation Proteome Discoverer, ProteinProspector, X!Tandem, PeptideFinder, Borrius, or MassLynx™ (Waters). Other software suitable for various steps of MS data analysis may be found, e.g., at www.ms-utils.org/wiki/pmwiki.php/Main/SoftwareList.”) (emphases added).

The specification of the LC-MS patents further concedes that methods of denaturing viral particles were well known in the art. *See, e.g.*, '377 patent, 20:32-47 (“In some embodiments, the

methods include denaturing a viral particle. In some embodiments, a viral particle such as an AAV particle may be denatured using detergent, heat, high salt, or buffering with a low or high pH. In certain embodiments, an AAV particle may be denatured using acetic acid or guanidine hydrochloride. *The skilled artisan will recognize that a variety of methods useful for promoting and/or monitoring protein denaturation are available in the art and may suitably select a denaturation method compatible with liquid chromatography/mass spectrometry.* For example, if heat denaturation is used, care may be applied to avoid protein precipitation and reverse phase column clogging. Similarly, high salt denaturation may be coupled with a desalting step prior to LC/MS or LC/MS/MS. In other embodiments, high pH denaturation, low pH denaturation, or denaturation using organic solvents is used.”) (emphasis added).

In sum, the Asserted Claims of the LC-MS Patents are directed to methods of determining masses of viral proteins to derive information about the proteins such as serotype and/or the presence of modifications using conventional methods that were well within the capabilities of a person of ordinary skill in the art. Nothing in the additional elements of the Asserted Claims of the LC-MS Patents, either individually or taken together, transforms the claimed methods into patent eligible subject matter. As such, the Asserted Claims of the LC-MS Patents purport to claim a natural phenomenon and/or law of nature, and are therefore invalid under 35 U.S.C. § 101.

D. LACK OF WRITTEN DESCRIPTION

The Asserted Claims of the LC-MS Patents are invalid for lack of written description under 35 U.S.C. § 112(a). To satisfy the written description requirement, the description must “clearly allow persons of ordinary skill in the art to recognize that [the inventor] invented what is claimed,” such that “the disclosure of the application relied upon reasonably conveys to those skilled in the art that the inventor had possession of the claimed subject matter as of the filing date.” *Ariad Pharm., Inc. v. Eli Lilly and Co.*, 598 F.3d 1336, 1351 (Fed. Cir. 2010) (en banc) (citation omitted).

Demonstrating adequate written description “requires a precise definition” of the invention. *Id.* at 1350. In particular, for written description of a claimed genus, the specification must disclose “a representative number of species falling within the scope of the genus or structural features common to the members of the genus so that one of skill in the art can ‘visualize or recognize’ the members of the genus.” *Id.* (citation omitted). “[F]unctional claim language can meet the written description requirement when the art has established a correlation between structure and function.” *Id.* (citation omitted). “But merely drawing a fence around the outer limits of a purported genus is not an adequate substitute for describing a variety of materials constituting the genus and showing that one has invented a genus and not just a species.” *Id.*

“[G]eneric claim language appearing *in ipsius verbis* in the original specification does not satisfy the written description requirement if it fails to support the scope of the genus claimed.” *Ariad*, 598 F.3d at 1350 (citing *Enzo Biochem, Inc. v. Gen-Probe Inc.*, 323 F.3d 956, 968 (Fed. Cir. 2002)). A “mere wish or plan for obtaining the claimed invention is not adequate written description.” *Juno Therapeutics, Inc. v. Kite Pharma, Inc.*, 10 F.4th 1330, 1335 (Fed. Cir. 2021) (citing *Centocor Ortho Biotech, Inc. v. Abbott Labs.*, 636 F.3d 1341, 1348 (Fed. Cir. 2011)) (internal quotation omitted).

The Asserted Claims of the LC-MS Patents are invalid under 35 U.S.C. § 112(a) because the specification lacks an adequate written description of multiple limitations in the asserted claims, including the following terms:

- “A method to determine the serotype of an adeno-associated virus (AAV) particle” [’377 patent, claim 1, 109:19-20]
- “determining the masses of VP1, VP2 and VP3 of the AAV particle” [’377 patent, claim 1, 109:25-26]
- “wherein the specific combination of masses of VP1, VP2 and VP3 are indicative of the AAV serotype” [’377 patent, claim 1, 109:27-28]

- “A method to determine the serotype of a viral particle” [’377 patent, claim 2, 109:31]
- “determining the masses of one or more capsid proteins of the viral particle” [’377 patent, claim 2, 109:37-38]
- “wherein the specific combination of masses of the one or more capsid proteins are indicative of the virus serotype” [’377 patent, claim 2, 109:39-41]
- “determining the masses of one or more viral proteins (VPs) of the particles in the preparation” [’880 patent, claim 1, 83:16-17]
- “A method of determining post-translational modifications of viral proteins (VPs) in a preparation of viral particles” [’880 patent, claim 10, 83:41-43]
- “wherein a deviation of one or more of the masses of the one or more VPs from the theoretical masses of VPs that have not undergone post-translational modifications is indicative of post-translational modifications of the VPs” [’880 patent, claim 10, 83:50-54]
- “A method of detecting post-translational modifications of one or more viral proteins (VPs) in a preparation of adeno-associated virus (AAV) particles” [’313 patent, claim 1, 83:2-4]
- “determining the masses of the one or more VPs” [’313 patent, claim 1, 83:10]
- “determining any deviation of the determined masses of the one or more VPs from the theoretical masses of corresponding VPs that have not undergone post-translational modifications to detect a deviation in the compared masses” [’313 patent, claim 1, 83:11-15]
- “wherein the post-translational modifications are selected from the group consisting of acetylation, deacetylation, deamidation, glycosylation, truncation and ubiquitination” [’313 patent, claim 2, 83:19-22]
- “wherein the post-translational modification is N-terminal acetylation” [’313 patent, claim 2, 83:23-24]
- “A method of determining the heterogeneity of viral particles in a preparation of adeno-associated virus (AAV) particles comprising VP1, VP2 and VP3 capsid proteins” [’313 patent, claim 11, 83:43-45]
- “determining the masses of one or more of the VP1, VP2 and VP3 capsid proteins and additional capsid proteins within one or more of the deconvoluted peaks” [’313 patent, claim 11, 83:54-56]

- “wherein the additional capsid proteins within one or more of the deconvoluted peaks are variant capsids” [’313 patent, claim 12, 83:59-61]
- “wherein the additional capsid proteins within one or more of the deconvoluted peaks are capsid amino acid substitutions” [’313 patent, claim 13, 83:62-64]
- “wherein the additional capsid proteins within one or more of the deconvoluted peaks are truncated capsids” [’313 patent, claim 14, 83:65-67]
- “wherein the additional capsid proteins within one or more of the deconvoluted peaks are modified capsids” [’313 patent, claim 15, 84:1-3]
- “wherein the modifications of the modified capsids are selected from the group consisting of acetylation, deacetylation, deamidation, glycosylation, truncation, and ubiquitination” [’313 patent, claim 16, 84:4-7]
- “wherein the modification is N-terminal acetylation” [’313 patent, claim 17, 84:8-9]
- “determining the masses of one or more VPs of the AAV particles” [’313 patent, claim 20, 84:29-30]
- “comparing the determined masses of the one or more VPs to theoretical masses of corresponding VPs, wherein the theoretical masses of corresponding VPs are those VPs of known AAV serotypes and/or those that have not undergone undesired post-translational modifications” [’313 patent, claim 20, 84:31-36]
- “determining if there is any deviation of the determined masses of the one or more VPs from the theoretical masses of the corresponding VPs” [’313 patent, claim 20, 84:37-39]
- “wherein the determination of any deviation of the determined masses of the one or more VPs from the theoretical masses of corresponding VPs thereby monitors the AAV particles for consistency and/or identity” [’313 patent, claim 20, 84:40-43]
- “wherein the monitoring of the AAV particles for consistency and/or identity includes determining the serotype of the AAV particles based on the comparison of the determined masses of the VPs to the theoretical masses of the corresponding VPs” [’313 patent, claim 21, 84:51-55]
- “wherein a determination of any actual deviation in masses reflects heterogeneity in the AAV particle preparation” [’313 patent, claim 22, 84:56-58]
- “wherein the heterogeneity in the AAV particle preparation is due to mixed AAV capsid serotypes, variant AAV capsid proteins, AAV capsid protein amino acid

substitutions, truncated AAV capsid proteins or modified AAV capsid proteins” [’313 patent, claim 23, 84:59-63]

- wherein the undesired post-translational modifications are selected from the group consisting of acetylation, deacetylation, deamidation, glycosylation, truncation and ubiquitination [’313 patent, claim 24, 84:64-67]

The specification states that “[a]t least 13 AAV serotypes and ~150 gene sequences have been isolated from human and non-human primate tissues; AAV serotypes differ in the amino acid sequence of viral capsid proteins and their corresponding cellular receptors and co-receptors for targeting.” *See, e.g.*, ’313 patent, 51:58-62. The specification lists an indeterminate number of AAV serotypes, and modified serotypes. *See, e.g.*, ’313 patent, 9:28-36; 45:47-57 (“Non-limiting examples of AAV capsid proteins of the invention include VP1 and/or VP3 of any of the following AAV serotypes: AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAVS, AAVrh8, AAVrh8R, AAV9, AAV10, AAVrh10, AAV11, AAV12, AAV LK03, AAV2R471A, AAV2/2-7m8, AAV DJ, AAV DJ8, AAV2 N587A, AAV2 E548A, AAV2 N708A, AAV V708K, goat AAV, AAV1/AAV2 chimeric, bovine AAV, mouse AAV, or rAAV2/HBoV1 serotype capsid. In some embodiments, the AAV capsid further comprises a tyrosine mutation or a heparin binding mutation.”).

The specification defines the term “heterogeneity” as follows: “‘Heterogeneity’ when used in reference to an AAV capsid refers to an AAV capsid characterized by one or more capsid polypeptides observed to deviate from a reference mass of a VP1, VP2, and/or VP3 polypeptide, or fragment thereof. A reference mass may include, without limitation, a theoretical, predicted, or expected mass of a VP1, VP2, and/or VP3 polypeptide, e.g., of a known AAV serotype. For example, an AAV capsid may be said to display heterogeneity if it demonstrates one or more of the following properties (without limitation): a mixed serotype, a variant capsid, a capsid amino acid substitution, a truncated capsid, or a modified capsid.” *See, e.g.*, ’313 patent, 19:54-65.

The specification lists an indeterminate number of modifications, including an indeterminate number of post-translational modifications. *See, e.g.*, '313 patent, 16:23-41 (“The terms ‘polypeptide’ and ‘protein’ are used interchangeably to refer to a polymer of amino acid residues, and are not limited to a minimum length. Such polymers of amino acid residues may contain natural or non-natural amino acid residues, and include, but are not limited to, peptides, oligopeptides, dimers, trimers, and multimers of amino acid residues. Both full-length proteins and fragments thereof are encompassed by the definition. The terms also include post-translational modifications of the polypeptide, for example, glycosylation, sialylation, acetylation, phosphorylation, and the like. Furthermore, for purposes of the present invention, a ‘polypeptide’ refers to a protein which includes modifications, such as deletions, additions, and substitutions (generally conservative in nature), to the native sequence, as long as the protein maintains the desired activity. These modifications may be deliberate, as through site-directed mutagenesis, or may be accidental, such as through mutations of hosts which produce the proteins or errors due to PCR amplification.”); 26:10-29:53; 42:45-43:55.

The specification defines two post-translational modifications as follows: “As used herein ‘N-acetylation’ refers to a process whereby an acetyl group is covalently added to the amino group of the N-terminal amino acid of a protein. Typically, N-terminal acetyltransferases (NATs) transfer an acetyl group from acetyl-coenzyme A (Ac-CoA) to the α -amino group of the first amino acid residue of the protein. As used here in, ‘deamidation’ refers to a chemical reaction in which an amide functional group in the side chain of asparagine or glutamine is removed or converted to another functional group. For example, asparagine may be converted to aspartic acid or isoaspartic acid. In other examples, glutamine is converted to glutamic acid or pyroglutamic acid (5-oxoproline).” *See, e.g.*, '313 patent, 27:55-67.

The specification further states, “[s]ince different production conditions may cause different expression levels of viral capsid proteins, post-translational modifications, and truncations, the viral capsid proteins need to be characterized and monitored to ensure the product consistency in gene therapy development programs.” *See, e.g.*, ’313 patent, 52:21-26.

Thus, the Asserted Claims of the LC-MS Patents purport to cover all serotypes, and all capsid protein modifications. Notably, all Asserted Claims require analysis using LC-MS intact protein analysis.

Representative Species. The inventors of the LC-MS patents, however, disclose experimental data using LC-MS intact protein analysis for only a few serotypes (AAV2, AAV1, AAV5, AAV7, AAV9 and AAVrh10), and an extremely limited number of modifications (N-terminal acetylation combined with a single amino acid truncation, and a single amino acid truncation in the absence of acetylation). *See, e.g.*, ’313 patent, 55:53-56:23, Table 2. Notably, none of the Asserted Claims are limited as to serotype.

Moreover, even those asserted claims that recite a subset of modifications are not limited to a single modification or a single type of modification. For example, based on Table 2, “N-terminal acetylation,” a term that is not defined in the specification, which instead defines the term “N-acetylation,” could refer to acetylation with or without various different truncations of the protein. *See, e.g.*, ’313 patent, 54:53-60 (stating, “As a result, the VP1 and VP2 masses were obtained in this shoulder peak at the signal intensities shown in FIG. 2A. The masses of VP1 and VP3 correspond to a.a. 2-735 (acetylation) and a.a. 204-735 (acetylation), respectively (FIGS. 2A&2B). No acetylation was observed in VP2 (a.a.139-735). In addition, a minor peak with a smaller mass than VP3 was observed, with a mass corresponding to amino acid sequence 212-735 with one acetylation (FIG. 2B))”).

Moreover, intact mass analysis will not provide conclusive information about where an acetylation is present and whether or not it is “N-terminal.” The inventors carried out LC-MS/MS using enzymatic digestion to confirm the location of the acetylations they hypothesized based on their intact protein analysis data. *See, e.g.*, ’313 patent, 55:9-22 (“To further confirm the N-termini and acetylation observed in the intact protein analysis, peptide mapping was performed using multiple enzymes and analyzed using multiple instruments. Various sample preparation methods, including denaturation methods and desalting steps, have been evaluated. The final digestion method, including denaturation with 6M guanidine HCl, reduction and alkylation with 4-vinylpyridine, and dialysis using slide-A-lyzer followed by enzymatic digestion, created clean peptide mapping with low artificial modifications during the digestion process. As low as 5 µg starting material was tested, yielding complete sequence coverage using nano LC/MS/MS and UPLC/MS/MS.”).

Other Asserted Claims are even broader, covering indeterminately large numbers of modifications of indeterminate numbers of serotypes.

The Asserted Claims, moreover, do not recite any limitations as to the accuracy of the claimed LC-MS intact protein analysis, further expanding the scope of Asserted Claims. And the data in the specification show varying discrepancies between the theoretical masses for the capsid proteins containing hypothesized modifications and the experimental masses observed using LC-MS intact mass analysis. *See, e.g.*, ’313 patent, Table 2, 55:57-56:22.

In addition, although the Asserted Claims recite deamidation as a modification detectable using LC-MS intact protein analysis, the specification discloses the detection of deamidation only using LC-MS/MS analysis involving enzymatic digestion. *See, e.g.*, ’313 patent, Example 3, 62:7-64:16; Example 5, 67:1-68:26.

Moreover, the specification discloses that the masses of the capsid proteins of certain serotypes are very close to one another, even in the absence of multiple confounding modifications encompassed by the Asserted Claims. *See, e.g.*, '313 patent, Table 3, Table 4, Table 5, Table 6, 57:1-60:16.

The disclosure of the LC-MS Patents does not demonstrate the inventors to be in possession of all combinations and permutations of serotypes and modifications, and thus the inventors have not met the written description requirement for the “entire scope of the claimed invention.”

For these reasons, the Asserted Claims of the LC-MS Patents are invalid under 35 U.S.C. § 112(a) for failing to satisfy the written description requirement.

E. LACK OF ENABLEMENT

The Asserted Claims of the LC-MS Patents are invalid for lack of enablement under 35 U.S.C. § 112(a). To satisfy the enablement requirement, the disclosure “must teach those skilled in the art how to make and use the full scope of the claimed invention without ‘undue experimentation.’” *Genentech, Inc. v. Novo Nordisk A/S*, 108 F.3d 1361, 1365 (Fed. Cir. 1997) (internal citation omitted).

Specifically, if a patent claim recites an entire class of processes, machines, manufactures, or compositions of matter – such as the compositions and methods recited in the Asserted Claims – the specification must enable a person skilled in the art to make and use the entire class. *Amgen Inc. v. Sanofi*, 598 U.S. 594, 610 (2023). At the same time, an instruction to attempt “random trial-and-error discovery” is not sufficient to enable others to make and use an entire class of claimed subject matter. *Id.* at 614-15. In other words, “[t]he more one claims, the more one must enable.” *Id.* at 610.

The Federal Circuit has enumerated several factors to consider in determining whether a disclosure would require “undue experimentation”: (1) the quantity of experimentation necessary;

(2) the amount of direction or guidance presented; (3) the presence or absence of working examples; (4) the nature of the invention; (5) the state of the prior art; (6) the relative skill of those in the art; (7) the predictability or unpredictability of the art; and (8) the breadth of the claims. *In re Wands*, 858 F.2d 731, 737 (Fed. Cir. 1988).

In determining whether the full scope of a claim is enabled or not, it is also appropriate to consider whether categories of embodiments within the claim are enabled. *See, e.g., Trs. of Boston Univ. v. Everlight Elecs. Co.*, 896 F.3d 1357, 1364 (Fed. Cir. 2018) (holding a claim invalid for not enabling one of six embodiments falling within the scope of the claim); *Chiron Corp. v. Genentech, Inc.*, 363 F.3d 1247, 1257 (Fed. Cir. 2004) (claims encompassing undisclosed chimeric antibodies required undue experimentation). Additionally, “[w]hile functional claim limitations are not necessarily precluded in claims that meet the enablement requirement, such limitations pose high hurdles in fulfilling the enablement requirement for claims with broad functional language.” *Amgen Inc. v. Sanofi*, 987 F.3d 1080, 1087 (2021).

The Asserted Claims of the LC-MS Patents are invalid under 35 U.S.C. § 112(a) because the specification fails to enable the full scope of the following limitations that are recited in the Asserted Claims:

- “A method to determine the serotype of an adeno-associated virus (AAV) particle” [’377 patent, claim 1, 109:19-20]
- “determining the masses of VP1, VP2 and VP3 of the AAV particle” [’377 patent, claim 1, 109:25-26]
- “wherein the specific combination of masses of VP1, VP2 and VP3 are indicative of the AAV serotype” [’377 patent, claim 1, 109:27-28]
- “A method to determine the serotype of a viral particle” [’377 patent, claim 2, 109:31]
- “determining the masses of one or more capsid proteins of the viral particle” [’377 patent, claim 2, 109:37-38]

- “wherein the specific combination of masses of the one or more capsid proteins are indicative of the virus serotype” [’377 patent, claim 2, 109:39-41]
- “determining the masses of one or more viral proteins (VPs) of the particles in the preparation” [’880 patent, claim 1, 83:16-17]
- “A method of determining post-translational modifications of viral proteins (VPs) in a preparation of viral particles” [’880 patent, claim 10, 83:41-43]
- “wherein a deviation of one or more of the masses of the one or more VPs from the theoretical masses of VPs that have not undergone post-translational modifications is indicative of post-translational modifications of the VPs” [’880 patent, claim 10, 83:50-54]
- “A method of detecting post-translational modifications of one or more viral proteins (VPs) in a preparation of adeno-associated virus (AAV) particles” [’313 patent, claim 1, 83:2-4]
- “determining the masses of the one or more VPs” [’313 patent, claim 1, 83:10]
- “determining any deviation of the determined masses of the one or more VPs from the theoretical masses of corresponding VPs that have not undergone post-translational modifications to detect a deviation in the compared masses” [’313 patent, claim 1, 83:11-15]
- “wherein the post-translational modifications are selected from the group consisting of acetylation, deacetylation, deamidation, glycosylation, truncation and ubiquitination” [’313 patent, claim 2, 83:19-22]
- “wherein the post-translational modification is N-terminal acetylation” [’313 patent, claim 2, 83:23-24]
- “A method of determining the heterogeneity of viral particles in a preparation of adeno-associated virus (AAV) particles comprising VP1, VP2 and VP3 capsid proteins” [’313 patent, claim 11, 83:43-45]
- “determining the masses of one or more of the VP1, VP2 and VP3 capsid proteins and additional capsid proteins within one or more of the deconvoluted peaks” [’313 patent, claim 11, 83:54-56]
- “wherein the additional capsid proteins within one or more of the deconvoluted peaks are variant capsids” [’313 patent, claim 12, 83:59-61]
- “wherein the additional capsid proteins within one or more of the deconvoluted peaks are capsid amino acid substitutions” [’313 patent, claim 13, 83:62-64]

- “wherein the additional capsid proteins within one or more of the deconvoluted peaks are truncated capsids” [’313 patent, claim 14, 83:65-67]
- “wherein the additional capsid proteins within one or more of the deconvoluted peaks are modified capsids” [’313 patent, claim 15, 84:1-3]
- “wherein the modifications of the modified capsids are selected from the group consisting of acetylation, deacetylation, deamidation, glycosylation, truncation, and ubiquitination” [’313 patent, claim 16, 84:4-7]
- “wherein the modification is N-terminal acetylation” [’313 patent, claim 17, 84:8-9]
- “determining the masses of one or more VPs of the AAV particles” [’313 patent, claim 20, 84:29-30]
- “comparing the determined masses of the one or more VPs to theoretical masses of corresponding VPs, wherein the theoretical masses of corresponding VPs are those VPs of known AAV serotypes and/or those that have not undergone undesired post-translational modifications” [’313 patent, claim 20, 84:31-36]
- “determining if there is any deviation of the determined masses of the one or more VPs from the theoretical masses of the corresponding VPs” [’313 patent, claim 20, 84:37-39]
- “wherein the determination of any deviation of the determined masses of the one or more VPs from the theoretical masses of corresponding VPs thereby monitors the AAV particles for consistency and/or identity” [’313 patent, claim 20, 84:40-43]
- “wherein the monitoring of the AAV particles for consistency and/or identity includes determining the serotype of the AAV particles based on the comparison of the determined masses of the VPs to the theoretical masses of the corresponding VPs” [’313 patent, claim 21, 84:51-55]
- “wherein a determination of any actual deviation in masses reflects heterogeneity in the AAV particle preparation” [’313 patent, claim 22, 84:56-58]
- “wherein the heterogeneity in the AAV particle preparation is due to mixed AAV capsid serotypes, variant AAV capsid proteins, AAV capsid protein amino acid substitutions, truncated AAV capsid proteins or modified AAV capsid proteins” [’313 patent, claim 23, 84:59-63]
- wherein the undesired post-translational modifications are selected from the group consisting of acetylation, deacetylation, deamidation, glycosylation, truncation and ubiquitination [’313 patent, claim 24, 84:64-67]

The specification states that “[a]t least 13 AAV serotypes and ~150 gene sequences have been isolated from human and non-human primate tissues; AAV serotypes differ in the amino acid sequence of viral capsid proteins and their corresponding cellular receptors and co-receptors for targeting.” *See, e.g.*, ’313 patent, 51:58-62. The specification lists an indeterminate number of AAV serotypes, and modified serotypes. *See, e.g.*, ’313 patent, 9:28-36; 45:47-57 (“Non-limiting examples of AAV capsid proteins of the invention include VP1 and/or VP3 of any of the following AAV serotypes: AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAVS, AAVrh8, AAVrh8R, AAV9, AAV10, AAVrh10, AAV11, AAV12, AAV LK03, AAV2R471A, AAV2/2-7m8, AAV DJ, AAV DJ8, AAV2 N587A, AAV2 E548A, AAV2 N708A, AAV V708K, goat AAV, AAV1/AAV2 chimeric, bovine AAV, mouse AAV, or rAAV2/HBoV1 serotype capsid. In some embodiments, the AAV capsid further comprises a tyrosine mutation or a heparin binding mutation.”).

The specification defines the term “heterogeneity” as follows: “‘Heterogeneity’ when used in reference to an AAV capsid refers to an AAV capsid characterized by one or more capsid polypeptides observed to deviate from a reference mass of a VP1, VP2, and/or VP3 polypeptide, or fragment thereof. A reference mass may include, without limitation, a theoretical, predicted, or expected mass of a VP1, VP2, and/or VP3 polypeptide, e.g., of a known AAV serotype. For example, an AAV capsid may be said to display heterogeneity if it demonstrates one or more of the following properties (without limitation): a mixed serotype, a variant capsid, a capsid amino acid substitution, a truncated capsid, or a modified capsid.” *See, e.g.*, ’313 patent, 19:54-65.

The specification lists an indeterminate number of modifications, including an indeterminate number of post-translational modifications. *See, e.g.*, ’313 patent, 16:23-41 (“The terms ‘polypeptide’ and ‘protein’ are used interchangeably to refer to a polymer of amino acid

residues, and are not limited to a minimum length. Such polymers of amino acid residues may contain natural or non-natural amino acid residues, and include, but are not limited to, peptides, oligopeptides, dimers, trimers, and multimers of amino acid residues. Both full-length proteins and fragments thereof are encompassed by the definition. The terms also include post-translational modifications of the polypeptide, for example, glycosylation, sialylation, acetylation, phosphorylation, and the like. Furthermore, for purposes of the present invention, a ‘polypeptide’ refers to a protein which includes modifications, such as deletions, additions, and substitutions (generally conservative in nature), to the native sequence, as long as the protein maintains the desired activity. These modifications may be deliberate, as through site-directed mutagenesis, or may be accidental, such as through mutations of hosts which produce the proteins or errors due to PCR amplification.”); 26:10-29:53; 42:45-43:55.

The specification defines two post-translational modifications as follows: “As used herein ‘N-acetylation’ refers to a process whereby an acetyl group is covalently added to the amino group of the N-terminal amino acid of a protein. Typically, N-terminal acetyltransferases (NATs) transfer an acetyl group from acetyl-coenzyme A (Ac-CoA) to the α -amino group of the first amino acid residue of the protein. As used here in, ‘deamidation’ refers to a chemical reaction in which an amide functional group in the side chain of asparagine or glutamine is removed or converted to another functional group. For example, asparagine may be converted to aspartic acid or isoaspartic acid. In other examples, glutamine is converted to glutamic acid or pyroglutamic acid (5-oxoproline).” *See, e.g.*, ’313 patent, 27:55-67.

The specification further states, “[s]ince different production conditions may cause different expression levels of viral capsid proteins, post-translational modifications, and

truncations, the viral capsid proteins need to be characterized and monitored to ensure the product consistency in gene therapy development programs.” *See, e.g.*, ’313 patent, 52:21-26.

Thus, the Asserted Claims of the LC-MS Patents purport to cover all serotypes, and all capsid protein modifications. Notably, all Asserted Claims require analysis using LC-MS intact protein analysis.

The inventors of the LC-MS patents, however, disclose experimental data using LC-MS intact protein analysis for only a few serotypes (AAV2, AAV1, AAV5, AAV7, AAV9 and AAVrh10), and an extremely limited number of modifications (N-terminal acetylation combined with a single amino acid truncation, and a single amino acid truncation in the absence of acetylation). *See, e.g.*, ’313 patent, 55:53-56:23, Table 2. Notably, none of the Asserted Claims are limited as to serotype.

Moreover, even those asserted claims that recite a subset of modifications are not limited to a single modification or a single type of modification. For example, based on Table 2, “N-terminal acetylation,” a term that is not defined in the specification, which instead defines the term “N-acetylation,” could refer to acetylation with or without various different truncations of the protein. *See, e.g.*, ’313 patent, 54:53-60 (stating, “As a result, the VP1 and VP2 masses were obtained in this shoulder peak at the signal intensities shown in FIG. 2A. The masses of VP1 and VP3 correspond to a.a. 2-735 (acetylation) and a.a. 204-735 (acetylation), respectively (FIGS. 2A&2B). No acetylation was observed in VP2 (a.a.139-735). In addition, a minor peak with a smaller mass than VP3 was observed, with a mass corresponding to amino acid sequence 212-735 with one acetylation (FIG. 2B)).”).

Moreover, intact mass analysis will not provide conclusive information about where an acetylation is present and whether or not it is “N-terminal.” The inventors carried out LC-MS/MS

using enzymatic digestion to confirm the location of the acetylations they hypothesized based on their intact protein analysis data. *See, e.g.*, '313 patent, 55:9-22 (“To further confirm the N-termini and acetylation observed in the intact protein analysis, peptide mapping was performed using multiple enzymes and analyzed using multiple instruments. Various sample preparation methods, including denaturation methods and desalting steps, have been evaluated. The final digestion method, including denaturation with 6M guanidine HCl, reduction and alkylation with 4-vinylpyridine, and dialysis using slide-A-lyzer followed by enzymatic digestion, created clean peptide mapping with low artificial modifications during the digestion process. As low as 5 µg starting material was tested, yielding complete sequence coverage using nano LC/MS/MS and UPLC/MS/MS.”).

Other Asserted Claims are even broader, covering indeterminately large numbers of modifications of indeterminate numbers of serotypes.

The Asserted Claims, moreover, do not recite any limitations as to the accuracy of the claimed LC-MS intact protein analysis, further expanding the scope of Asserted Claims. And the data in the specification show varying discrepancies between the theoretical masses for the capsid proteins containing hypothesized modifications and the experimental masses observed using LC-MS intact mass analysis. *See, e.g.*, '313 patent, Table 2, 55:57-56:22.

In addition, although the Asserted Claims recite deamidation as a modification detectable using LC-MS intact protein analysis, the specification discloses the detection of deamidation only using LC-MS/MS analysis involving enzymatic digestion. *See, e.g.*, '313 patent, Example 3, 62:7-64:16; Example 5, 67:1-68:26.

Moreover, the specification discloses that the masses of the capsid proteins of certain serotypes are very close to one another, even in the absence of multiple confounding modifications

encompassed by the Asserted Claims. *See, e.g.*, '313 patent, Table 3, Table 4, Table 5, Table 6, 57:1-60:16.

The inventors did not enable all combinations and permutations of serotypes and modifications, and thus the inventors have not met the enablement requirement for the “entire scope of the claimed invention.”

For these reasons, the Asserted Claims of the LC-MS Patents are invalid under 35 U.S.C. § 112(a) for failing to satisfy the enablement requirement.

F. INDEFINITENESS

The Asserted Claims are invalid for indefiniteness. Section 112 provides that the specification must “conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the [applicant] regards as the invention.” 35 U.S.C. § 112(b). A patent is indefinite “if its claims, read in light of the specification delineating the patent, and the prosecution history, fail to inform, with reasonable certainty, those skilled in the art about the scope of the invention.” *Nautilus, Inc. v. Biosig Instruments, Inc.*, 572 U.S. 898, 901 (2014).

Patent claims are invalid for indefiniteness when a claim term may be determined by multiple test methods, the results of which “will necessarily fall within or outside the claim scope depending on the . . . method chosen.” *Honeywell Int’l, Inc. v. Int’l Trade Comm’n*, 341 F.3d 1332, 1341 (Fed. Cir. 2003). Where a patent fails to specify which of available alternative tests to use for measuring compliance with a claim limitation, and different tests can have different results, the claims are indefinite. *HZNP Meds. LLC v. Actavis Labs. UT, Inc.*, 940 F.3d 680, 698 (Fed. Cir. 2019).

To the extent that the Asserted Claims of the LC-MS Patents purport to cover all combinations and permutations of serotypes and modifications, they fail to delineate the scope of the claims with reasonable certainty. Different experimental conditions with different accuracies

of measurement and different mixtures of particles may yield different results. The LC-MS Patents provide insufficient clarity regarding the scope of the claimed methods. *See, e.g., Nautilus*, 572 U.S. at 901 (patent is invalid for indefiniteness if it “fail[s] to inform, with reasonable certainty, those skilled in the art about the scope of the invention”).

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