UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

NOVARTIS GENE THERAPIES, INC. &
NOVARTIS PHARMACEUTICALS CORPORATION,
Petitioner

v.

GENZYME CORPORATION,
Patent Owner.

CASE: IPR2023-01045
PATENT NO. 10,429,288

DECLARATION OF JEFFREY C. HANSEN, PH.D.

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I. INTRODUCTION

1. I submit this declaration in connection with Patent Owner's Preliminary Response in the *Inter Partes* Review IPR2023-01045 (the "IPR") of claims 5-9 and 15-16 ("challenged claims") of U.S. Patent No. 10,429,288 (the "'288 patent"; Ex. 1001).[1] I have been retained by Dechert LLP, counsel for Patent Owner Genzyme Corporation in this matter. I am being compensated for my time at my standard rate of $600 per hour plus actual expenses. The opinions in this declaration are my own, and my compensation is not dependent in any way upon the outcome of the IPR of the '288 patent.

II. PROFESSIONAL QUALIFICATIONS

2. I received an A.S. degree in Biology in 1978 from Delta College, a B.S. degree in Biological Sciences in 1980 from Oakland University, and a Ph.D. in Biochemistry in 1986 from University of Wisconsin, where I researched estrogen receptor structural dynamics.

3. After completing my Ph.D., I was a postdoctoral fellow in the Department of Biochemistry and Biophysics at Oregon State University from 1986 to 1990 under K.E. van Holde, a pioneer of using Analytical Ultracentrifugation

[1] Although the Petition challenges claims 4-16, I have been informed that claims 4 and 10-14 are no longer part of this proceeding.

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("AUC") for biochemical research. My research at Oregon State focused on the physical biochemistry of nucleosome positioning and chromatin assembly, and the application of AUC was central to my postdoctoral research, and featured in several of my publications during this period.

4. In 1990, I became a faculty member at The University of Texas Health Science Center at San Antonio (UTHSCSA) in 1990. The focus of my research was chromatin structure and function relationships. While at UTHSCA I was promoted from Assistant Professor (1990-1996) to Associate Professor (1996-2001), and then to Professor (2001-2003).

5. AUC has been an important component of my research for decades. From 1995 to 2003, I was the Director of the UTHSCSA Analytical Ultracentrifugation Laboratory (now CAUMA Facility). The laboratory was one of the few analytical AUC laboratories in the world, with access to three AUC instruments: Beckman Optima XLA, XLI and XLF (equipped with fluorescence optics). The laboratory provided researchers and industry professionals with access to AUC technology, training, support, and consulting services on AUC instruments and data analysis. The laboratory further enabled the application of AUC analysis on a wide-range of molecules, including proteins, nucleic acids, carbohydrates, quantum dots, virus particles, capsids, networks, cages, nanotubes,

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assemblies, and nanoparticles. The focus of my work at the laboratory at this time was on analytical ultracentrifugation of complex macromolecular assemblies.

6. In 1999, during my directorship, I co-authored, with James L. Cole, the publication titled *Analytical Ultracentrifugation as a Contemporary Biomolecular Research Tool*, J. of Biomolecular Techniques 10(4):163-76 (1999), which was cited in the Petition as Exhibit 1006.

7. From 2003 to present, I have been a Professor in the Department of Biochemistry and Molecular Biology at Colorado State University (CSU). Since 2016, I have also held the position of Associate Chair for Undergraduate Studies. The focus of my research at CSU has been on chromatin structure and function relationships, chromatin architectural proteins, and intrinsic disorder in proteins.

8. I have served as a member of several Study Sections and Review Panels at the National Institute of Health ("NIH"), National Science Foundation ("NSF"), and several non-governmental funding organizations including the American Heart Association.

9. I was a member of the AUC Special Study Section 4 from 1995-1996 and AUC Special Study Section 2 in 1997. NIH Special Study Sessions are formed for specific research topics or when grant applications require a unique combination of expertise that is not available in the standing sections. Members of these special sections are chosen based on their expertise in the field and are tasked

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with deciding which research is qualified to receive millions of dollars in grant funding.

10. I was also a member of the Advisory Panel on Biological Instrumentation & Instrument at the NSF. The Advisory Panel comprises experts in analytical instrumentation to review grant proposals directed to the development of novel and groundbreaking improvements to instrumentation for biological research needs.

11. I was also a member of the Genetic Mechanisms Panel at NSF, which supports research on the fundamental mechanisms involving the organization, dynamics, processing, expression, regulation and evolution of genetic and epigenetic information.

12. My research has yielded more than 100 peer-reviewed publications, including analytical ultracentrifugation of complex molecular systems, and the structure and dynamics of chromatic fiber. A significant number of my published works employ AUC analytical techniques. Overall, my research publications have been cited more than 14,000 times by the scientific community.

13. I have also served on the editorial board for renowned scientific journals, including the JOURNAL OF BIOLOGICAL CHEMISTRY, CHEMTRACTS: BIOCHEMISTRY & MOLECULAR BIOLOGY, CHEMTRACTS: Analytical Ultracentrifugation special issue, and CHEMTRACTS: BIOCHEMISTRY AND

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MOLECULAR BIOLOGY. Additionally, for the last decade, I have been an ad hoc journal referee for several of the leading scientific journals in the world, including Nature, Science, and Molecular Cell, for which I have reviewed over 100 papers.

14. My work in the above-mentioned special sections and advisory panel, as well as the editorial boards of renowned scientific journals, has given me a unique perspective on the state of the art and advancements in the field of AUC. It has also been influential in shaping the research in the field of AUC and molecular genetics.

15. I have also given national talks related to AUC technology including the 1996 Annual Meeting for the Biophysical Society's Advances in Sedimentation Velocity Workshop and the 1998 Protein Society's Analytical Ultracentrifugation Workshop. I attended several invite-only, Gordon Research Conferences for the sections on Chromatin Structure and Function, and Reversible Associations in Structural and Molecular Biology. Notably, I was the session organizer and chair of the 2008 Gordon Research Conference on Biomolecular Interactions and Methods. Additionally, I was invited to give department seminars at the NIH Technology Transfer Series on AUC in 1994, and the University of Connecticut National Analytical Ultracentrifuge Facility in 1995 and 1996.

16. My research and teaching have been recognized by several organizations, and I have been awarded the National Research Service Award and

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the First Independent Research Transition Award from the National Institute of General Medicinal Sciences, Distinguished Alumni Achievement Award from Oakland University, Provost's R. Preston Davis Award for Instructional Innovation and the Faculty Excellence Undergraduate Teaching and Mentoring Award from CSU. In 2022, I was named Professor Laureate in the College of Natural Science at CSU.

17. Attached as Exhibit 2002 is my curriculum vitae, which summarizes my background, credentials, and includes a list of my publications between 2010-2021.

III. MATERIALS CONSIDERED

18. My analysis is based on my years of education, research, experience, and background, as well as my investigation and study of relevant materials for this declaration. When developing the opinions set forth in this declaration, I was asked by counsel to assume the perspective of a person having ordinary skill in the art ("POSA"), provided in the Petition and Dr. Berkowitz's declaration (Ex. 1020, "Berkowitz Declaration"). Petition, 20-21; Berkowitz, ¶¶ 57-59. In addition to the Petition and Berkowitz Declaration, I reviewed and considered the materials identified in the list below.

Exhibit	Description
1001	U.S. Patent No. 10,429,288 ("the '288 patent")
1002	File History of the '288 patent
1003	WO2014/125101 A1 and certified English translation ("Le Bec")

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Exhibit	Description
1004	Steven A. Berkowitz & John S. Philo, *Monitoring the homogeneity of adenovirus preparations (a gene therapy delivery system) using analytical ultracentrifugation*, Analytical Biochemistry 362:16-37 (2007)
1005	James L. Cole et al., *Analytical Ultracentrifugation: Sedimentation Velocity and Sedimentation Equilibrium*, Methods in Cell Biology 84:143-79 (2008)
1006	James L. Cole & Jeffrey C. Hansen, *Analytical Ultracentrifugation as a Contemporary Biomolecular Research Tool*, J. of Biomolecular Techniques 10(4):163-76 (1999)
1007	Peter Schuck, *Size-Distribution Analysis of Macromolecules by Sedimentation Velocity Ultracentrifugation and Lamm Equation Modeling*, Biophysical J. 78(3):1606-19 (2000)
1008	Kai Gao et al., *Empty virions in AAV8 vector preparations reduce transduction efficiency and may cause total viral particle dose-limiting side effects*, Molecular Therapy – Methods & Clinical Development 1(9):1-8 (2014)
1009	Mainul Hoque et al., *Chimeric Virus-like Particle Formation of Adeno-Associated Virus*, Biochemical & Biophysical Research Communications 266:371-76 (1999)
1010	European Medicines Agency, Committee for Medicinal Products for Human Use, Glybera Assessment Report (July 19, 2012)
1011	Steven A. Berkowitz & John S. Philo, *Characterizing Biopharmaceuticals using Analytical Ultracentrifugation, in* Biophysical Characterization of Proteins in Developing Biopharmaceuticals 211-60 (2015)
1012	European Patent Office, Third Party Observation for application Number EP20160703205 (July 30, 2020)
1013	Greg Ralston, *Introduction to Analytical Ultracentrifugation* (Beckman Coulter Life Sciences)
1014	Silke Steinbach et al., *Assembly of adeno-associated virus type 2 capsids* in vitro, J. of Gen. Virology 78:1453-62 (1997)
1015	U.S. Patent Application Publication No. US 2009/0017542 A1
1016	Huaying Zhao et al., *Overview of Current Methods in Sedimentation Velocity and Sedimentation Equilibrium Analytical Ultracentrifugation*, Current Protocols in Protein Science Supplement 71:20.12.1-20.12.49 (2013)
1017	Douglas M. McCarty, *Self-complementary AAV Vectors; Advances*

Exhibit	Description
	and Applications, Molecular Therapy 16(10): 1648-56 (2008)
1018	Timothy R. Dafforn, *So how do you know you have a macromolecular complex?*, Acta Crystallographica D63:17-25 (2007)
1019	Jim Gombold et al., *Lot Release and Characterization Testing of Live-Virus-Based Vaccines and Gene Therapy Products, Part 2*, BioProcess Int'l 4(5): 56-65 (2006)
1020	Declaration of Dr. Steven Berkowitz
2003	Burnham et al., Analytical Ultracentrifugation as an Approach to Characterize Recombinant AAV Vectors, 26 Human Gene Therapy Methods 228 (2015) ("Burnham 2015")
2004	F. Collaud et al., Preclinical Development of an AAV8-hUGT1A1 Vector for the Treatment of Crigler-Najjar Syndrome, 12 Molecular Therapy: Methods & Clinical Development 157, 166-67 (March 2019)
2005	E. Spies, Assessing the Quality of Adeno-Associated Virus Gene Therapy Vectors by Sedimentation Velocity Analysis, AUC Insights (March 2020)
2006	Q. Xie et al., The Atomic Structure of Adeno-Associated Virus (AAV-2), a Vector for Human Gene Therapy, 99 PNAS 10405-410 (Aug. 6, 2002)
2007	J.M. Sommer et al., Quantification of Adeno-Associated Virus Particles and Empty Capsids by Optical Density Measurement, 7 Molecular Therapy 122-28 (January 2003)
2008	T. Matsushita et al., Adeno-Associated Virus Vectors Can Be Efficiently Produced Without Helper Virus, 5 Gene Therapy 938-45 (February 10, 1998)
2009	J.F. Wright, Product-Related Impurities in Clinical-Grade Recombinant AAV Vectors: Characterization and Risk Assessment, 2 Biomedicines 80-97 (March 3, 2014)

IV. SUMMARY OF MY OPINIONS

19. First, it is my opinion that Ground 1, alleging that certain claims, including claims 5-8 and 15, are anticipated by Le Bec (Ex. 1003), does not

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establish the anticipation, particularly of claim elements 5[c], 6[c], 7[c], 8[c], and claim 15.

20. Second, it is my opinion that Ground 2, alleging that claims 5-8 and 15 are obvious over Le Bec (Ex. 1003) alone, does not establish the obviousness of claim elements 5[c], 6[c], 7[c], 8[c], and claim 15.

21. Third, it is my opinion that Ground 3, alleging that claims, are obvious over Le Bec (Ex. 1003) and Berkowitz 2007 (Ex. 1004) does not establish the obviousness of claims 5-9 and 15-16, particularly claim elements 5[c], 6[c], 7[c], 8[c], and claim 15. I have been informed a dependent claim incorporates all the elements of the claims from which it depends. Because claim 9 depends from claim 5 and claim 16 depends from claim 15, these claims are also not obvious.

V. LEGAL STANDARDS FOR ANTICIPATION AND OBVIOUSNESS

22. I understand that the Petitioner has the burden of proving the challenged claims anticipated or obvious by a preponderance of evidence, which means proving that the challenged claims are more likely than not anticipated and/or obvious.

A. Anticipation

23. I have been informed by counsel that a claim is anticipated when a single prior art reference discloses, either expressly or inherently, each and every claim element arranged in the order specified by the claim. I understand that if the

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claim covers a genus, the claim is deemed anticipated if any species falling within the genus were known in the prior art. I have been informed that a claim limitation is disclosed for the purpose of anticipation if a person of ordinary skill in the art would have understood the reference to disclose the limitation based on inferences that a POSA would reasonably be expected to draw from the explicit teachings in the reference when read in light of the POSA's knowledge and experience.

24. I have been informed that a claim limitation is inherent in a prior art reference if that limitation is necessarily present when practicing the teachings of the reference, regardless of whether a POSA recognized the presence of that limitation in the prior art. I also have been informed that if a prior art reference must be modified to meet a disputed claim limitation that reference does not anticipate the claim.

B. Obviousness

25. I have been informed by counsel that a patent claim may be unpatentable if it had been obvious in view of a combination of prior-art references. I have further been informed that a patent claim is obvious if the differences between the subject matter of the claim and the prior art are such that the subject matter as a whole was obvious to the POSA in the relevant field at the time the invention was made. Specifically, I understand that the obviousness question involves a consideration of the following factors:

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- the scope and content of the prior art;
- the differences between the prior art and the claims at issue;
- the knowledge of a person of ordinary skill in the pertinent art; and
- if present, objective factors indicative of non-obviousness, sometimes referred to as "secondary considerations."

26. I have been informed that for a claimed invention to be considered obvious over a combination of references, the POSA must have had a motivation and/or reason for combining teachings from multiple prior-art references in the manner proposed to arrive at the claim. I further understand that in determining whether a prior-art reference would have been combined with other prior art or with other information within the knowledge of the POSA, the following are examples of approaches and rationales that may be considered:

- combining prior-art elements according to known methods to yield predictable results;
- simple substitution of one known element for another to obtain predictable results;
- use of a known technique to improve similar devices in the same way;
- applying a known technique to a known device ready for improvement to yield predictable results;
- applying a technique or approach that would have been "obvious to try," i.e.,

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choosing from a finite number of identified, predictable solutions, with a reasonable expectation of success;

- known work in one field of endeavor may prompt variations of it for use in either the same field or a different one based on design incentives or other market forces if the variations would have been predictable to one of ordinary skill in the art;
- some teaching, suggestion, or motivation in the prior art that would have led one of ordinary skill to modify the prior-art reference or to combine prior-art reference teachings to arrive at the claimed invention.

27. I understand that this motivation or reason to combine may come from a prior-art reference or based on the POSA's knowledge or common sense.

28. I have been informed that for a combination of references to render the claimed invention obvious, the POSA must have been able to arrive at the claimed invention by altering or combining the applied references, and must have had a reasonable expectation of success in doing so. I understand that the claim is considered as a whole, so the obviousness analysis requires consideration of whether the claimed combination of elements was rendered obvious by the cited prior art.

29. I also have been informed that a claim can be obvious if it is the product of routine experimentation or optimization. For ranges recited in a claim, I

have been informed that a particular parameter must first be recognized as a result-effective variable (i.e., a variable which achieves a recognized result), before the determination of the optimum or workable ranges of said variable might be characterized as routine experimentation.

30. Finally, I understand that it is important to guard against slipping into the use of hindsight and to resist the temptation to read into the prior art the teachings of the invention in issue. I have been informed it is improper to rely on the inventor's own path to support a conclusion of obviousness; what matters is the path that the POSA would have followed, as evidenced by the pertinent prior art.

VI. THE LEVEL OF ORDINARY SKILL IN THE ART

31. I have been informed that anticipation and obviousness are analyzed from the perspective of the POSA in the relevant field at the time of the effective filing date of the '288 patent, which was January 20, 2015. I understand that the Petition and Berkowitz Declaration applied the following definition for the POSA in their analyses:

> A POSA working in the field of the '288 patent on January 20, 2015 would have possessed at least a B.S. in biology, chemistry, chemical engineering, biochemistry, biophysics, pharmaceutical science, or a related discipline, with more than two years of industry, laboratory, and/or clinical experience in analyzing or characterizing biomolecules, including viruses or viral vectors. Such a person may be familiar with, or consult with someone familiar with, the development, formulation,

> and/or administration of viral vectors for gene therapy and quality standards required to market such products.

Petition, 17 (citing Berkowitz, ¶ 58).

32. For this Declaration I will apply the same POSA definition as applied by Petitioners and Dr. Berkowitz. However, I reserve the right to apply a modified definition for future opinions in this proceeding.

VII. BACKGROUND SCIENTIFIC CONCEPTS RELAVENT TO THE ’288 PATENT

33. In my opinion, certain scientific concepts relevant to the issue of the patentability of the claims were inadequately addressed, or not addressed at all, by the Petition and the Berkowitz Declaration. In particular, the Petition and Berkowitz Declaration did not substantively address issues relating to the identification and quantification of partial- or fragmented-genome containing rAAV particles, which the ’288 patent refers to alternatively as variant particles, despite the identification and quantification of these particles by AUC being an important part of the ’288 patent. Section VIII.B, below. For any biological system that is heterogenous, this problem is not trivial and have been rate-limiting for as long as AUC has been used as an analytical technique. The challenges are significant.

34. Finally, the Petition and Berkowitz Declaration did not explain how the method of optical detection, including the wavelength, impacted the way in

which the POSA determines the concentration of variant rAAV particles. Section VII.C, below. For example, Le Bec describes its AUC method as using absorbance detection (Ex. 1003, 15:19-26), the most common way to detect AAV particles as of the effective filing date. Ex. 1005, 152. To determine the concentration of AAV species using absorbance, the POSA generally needed to apply the extinction coefficient of that species at most UV wavelengths. Ex. 1004, 17; Ex. 1005, 152. As the ’288 patent explains, as of the effective filing date of the ’288 patent “it [was] not possible to determine empirically the extinction coefficient of particular species of recombinant viral particles (e.g., viral particles with fragmented genomes of unknown size and sequence).” Ex. 1001, 24:35-38. Despite being a critical concept to determining the concentration of an AAV species by absorbance detection, neither the Petition nor Berkowitz Declaration addressed the extinction coefficient issue.

A. Variant Recombinant Adeno-Associated Viral (rAAV) Particles

35. Wild-type (“wt”) adeno-associated viral (“AAV”) vectors contain a single-stranded DNA genome that encodes replication (“rep”) and encapsidation (“cap”) genes flanked by inverted terminal repeats (“ITRs”). Ex. 2009, 87; Ex. 2003, 229.

36. Recombinant AAV (“rAAV”) vectors can be produced by a transient transfection method using a “gutless” vector approach. Ex. 2003, 229; Ex. 2008,

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938. Such an approach may employ three plasmids, often referred to as "triple transfection." Ex. 1001, 7:1-4. Encoded on a first plasmid is the therapeutic genes of interest and regulatory elements inserted between the 5' and 3' viral ITRs, encoded on a second plasmid are genes containing the rep and cap functions, and encoded on a third plasmid are the required adenoviral helper genes, E4, E2A, and Va RNA. Ex. 2008, 939-40; *see also* Ex. 1001, 7:1-4, FIG. 4. "When th[e] adenoviral helper plasmid was cotransfected into 293 cells, along with plasmids encoding the AAV vector and *rep* and *cap* genes, AAV vector production occurred at levels equal to those achieved by the use of adenovirus infection as a source of help." Ex. 2008, 939.

37. The transfection of the three plasmids was understood to result in fully assembled viral vectors, as well as populations of viral vectors containing both non-genome-containing particles, including empty capsids, and vectors containing incomplete genomes. Ex. 2009, 81; Ex. 2003, 229. Genome containing vectors can include the complete genome (i.e., fully intact rAAV particles) as well as particles containing incomplete portions of the rAAV genome or nongenomic nucleic acid contaminants, for example, partial or fragmented genomes. Ex. 2009, 81, 86-89. For shorthand, the '288 patent sometimes describes such incomplete genome-containing rAAV particles as variant species. *See, e.g.*, Ex. 1001, Abstract, 2:24-27.

38. The POSA would have understood that the production method influences the composition of an rAAV preparation, particularly with respect to the amounts of empty capsid particles, capsids containing fragmented rAAV genomes, and capsids containing nongenomic nucleic acid contaminants. Ex. 2009, 81 ("AAV capsid particles that are empty or that encapsidate fragments of nucleic acids other than the intended therapeutic expression cassette are examples of vector product-related impurities."). "[E]ven inefficient packaging of host cell DNA into AAV particles can result in formation of a significant amount of this product-related impurity during AAV vector generation." Ex. 2009, 86-87.

39. For clinical applications, the presence of rAAV particles containing variant genomes poses risks. For example, "[d]epending on the origin of the cells used for AAV generation, *i.e.*, human or non-human origin genotoxicity and immunotoxicity are two theoretical risks." Ex. 2009, 87. Concerning the gene therapy Glybera, the European Medicine Agency ("EMA") licensure assessment raised quality objections over residual baculovirus genetic sequences encapsidated in AAV1 capsids and co-purified with the product. Ex. 1010, 15, 18, 19-20. The EMA also raised concerns relating to residual Rep and Cap genes packaged in AAV1 capsids. Ex. 1010, 142. More generally, the EMA raised concerns that "assays used to measure residual cellular DNA and protein did not have particularly low [limits of detection] and given the amount of product that is used

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during treatment … specifications were considered unacceptably high." Ex. 1010, 18.

40. Indeed, the scientific literature documented how "excessive empty capsids are immunotoxic" and "AV virions packaged with full or partial viral genomes, are more immunotoxic." Ex. 1008, 6. Thus, the POSA would have been concerned with monitoring empty capsids ***and*** especially partially full capsids, because of their potential elevated immunotoxicity. The POSA would have understood that given the difficulty with the purification of viral compositions, the ability to properly identify and quantify partially full capsids was essential for the clinical applications of rAAV-based gene therapies. It was not enough to merely identify heterogeneity, rather it needed to be quantitatively analyzed. This was a difficult challenge as of the effective filing date.

41. Notably, in its consideration of Gao (Ex. 1008), the Berkowitz Declaration focuses on the effect that "removal of empty particles from rAAV preparation may improve efficacy and safety of AAV in clinical applications." Berkowitz, ¶ 42 (citing Ex. 1008, 1, 6); *see also id.* at ¶ 218 ("A POSA would have been motivated to assess homogeneity of rAAV preparations in pursuit of a drug product of high quality (with regard to homogeneity, purity, and drug product consistency) and determine the percentage of empty rAAV particles.") (citing Ex. 1008, 1, 6); *id.* at ¶ 221 ("The prior art, including Le Bec, teaches that empty

capsids are undesirable.") (citing Ex. 1008, 1, 6). However, although Gao explained that partially full rAAV particles presented a greater of side effects and immunotoxcity (Ex. 1008, 6), none of the prior art identified by the Petition or Berkowitz Declaration taught how to quantify such variant genome particles with AUC. This was a critical issue for which the '288 patent posed a viable solution, as explained below in Section VIII.B.

B. rAAV Structural Considerations

42. AAV vectors are "composed of an icosahedral assembly of 60 capsid proteins (VP1, -2, -3) assembled together in a defined stoichiometry." Ex. 2009, 81. A detailed crystallographic analysis of AAV-2 published in 2002 describes the outer surface of the particle:

> Overall, the outside surface is positively charged with a prominent ring of symmetry-related patches in a depression surrounding the fivefold axes (Fig. 3d). Similarity to the rhinoviral canyon, the major group receptor-binding site, seems to be coincidental. The AAV depression is wider and more accessible to antibodies, and mutational data (previous paragraph) implicate not this region, but the side of the threefold-proximal spikes, which is the other prominently positively charged region, extending from the valley between peaks to merge at the threefold axis (Fig. 3d).

Ex. 2006, 10409. The "threefold-proximal spikes" can be seen in Figure 3d, which shows the electrostatic surface potential of AAV-2 calculated with SPOCK

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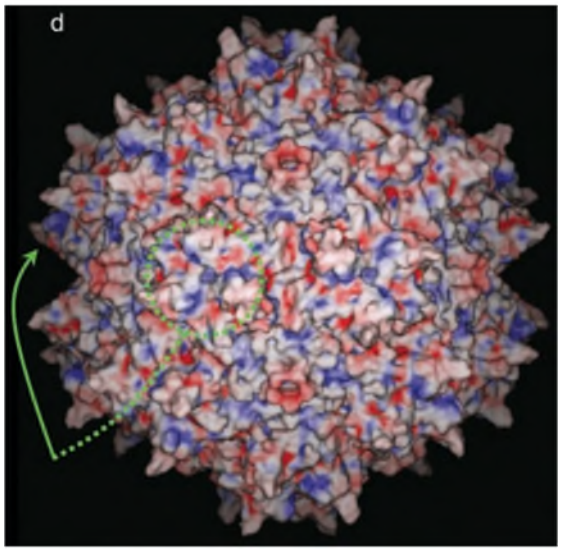
running from -10 (red) to +10 (blue). Ex. 2006, 10408 ("The putative receptor-binding sites are positively charged patches on the side of each threefold-proximal peak, viewed edge-on (arrowed) or head-on where equivalent patches join at each threefold (circled).").



Ex. 2006, Fig. 3d.

43. Notably, spike features on another type of viral particle, adenovirus particles, were observed to impact their AUC analysis in Berkowitz's 2007 publication. Ex. 1004, 18 (Adenoviral "spike projections must impart a significant amount of frictional drag on the overall adenovirus particle."). Some particles were observed to lose these spikes, which impacted their sedimentation coefficient. *Id*., 31-32.

44. Additionally, the presence of partial genomes encapsidated in rAAV particles was also observed to impact the conformation of the capsid and change its biological interactions, including infectability and immunotoxicity. Ex. 1008, 6.

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Gao explains that "encapsidation of full or partial AAV genomes may lead to capsid conformational changes … on capsid structure expos[ing] both phospholipase A2 domain and nuclear localization signal domains and facilitates endosome escape and viral infection." Ex. 1008, 6. Gao further explains that "partial viral genomes may also contribute to the side effects of the [partially empty] virions due to their different capsid conformations as compared with [completely empty] particles." *Id.*

45. Changes to the capsid are relevant to AUC analysis because they impact the sedimentation coefficient in a c(s) distribution. Several forces impact the sedimentation of a species in an AUC sedimentation velocity experiment. Balancing these forces, results in a relationship where sedimentation, "*s* is proportional to the buoyant molar mass, M_b, and inversely proportional to the frictional coefficient, *f*" as shown in Equation (1) reproduced from Cole 2008 (Ex. 1005):

$$s \equiv \frac{v}{\omega^2 r} = \frac{M_b}{f}$$

Ex. 1005, 146.

46. The POSA would have been aware of the possibility that not all rAAV particles would have been assumed to have identical surfaces. Berkowitz 2007 observed that some "adenovirus monomers [may] have lost their fiber spikes"

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resulting in "significantly reduced hydrodynamic drag" decreasing friction, therefore leading to "a higher sedimentation coefficient than the intact adenovirus particle." Ex. 1004, 31. The POSA would have understood that changes to the rAAV surface (e.g., the spikes on the AAV surface) would have been expected to result in frictional changes to the rAAV surface, and thus impacted its sedimentation.

47. In addition, the changes to the surface of an AAV particle would alter the protein mass. Berkowitz 2007 explained that changes to the spike proteins on the outside of adenovirus capsids have implications not only for frictional coefficient, but also for the mass ratio of genome to protein. Ex. 1004, 31-32 ("[P]articles with missing spikes would also have a slightly higher DNA to protein ratio and therefore have a slightly higher buoyant density than the intact adenovirus particle."). Higher buoyant density would likewise increase the *s* value. Thus, from Berkowitz 2007, the POSA would have understood that changes to particle mass would not be limited to genome size, but could also be attributed to changes in the capsid protein.

C. Optical Detection for AUC

48. A common AUC instrument at the time of the '288 patent's effective date, the Beckman Coulter XL-I— the instrument used in the '288 patent's experiments—contains both absorbance and interference optical detection systems.

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Ex. 1006, 164. The absorbance optical system is based on a xenon flashlamp and a scanning monochromator that allows measurement of sample concentration at wavelengths ranging from 200 to 800 nm. *Id*. The Rayleigh interference optical system measures sample concentration based on refractive index changes. *Id*.

49. "Absorption optics are particularly sensitive for detection of macromolecules containing strong chromophores." *Id*. Absorbance detection is particularly suitable for the detection of proteins and nucleic acids, the building blocks of DNA, "which can be studied … by following their absorbance at 260 nm." *Id*. Thus, the POSA would have understood for a particle like rAAV, which is a mixture of protein and DNA, absorbance would have been considered the preferred detection technique.

50. In contrast, the "Rayleigh interference optical system is used to analyze macromolecules lacking intense chromophores (eg, polysaccharides) and samples that contain strongly absorbing buffer components." *Id*. It is also used for characterizing "very concentrated samples." *Id*. However, "since any path length difference between the sample and reference beams contributes to the fringe displacement, even tiny optical imperfections (dust, oil, dirt, scratches on the lenses and mirrors) are visible in the signal. Consequently, there is significant time-independent systematic noise." Ex. 1005, 154.

51. In addition, interference had the disadvantage of requiring a higher sample concentration than absorbance. Ex. 1004, 26; Ex. 1001, 47:31-35. The interference optics method was affected by a mismatch between the reference and the rAAV vector sample buffer. Ex. 1005, 160 ("Buffer matching is most critical when using interference optics, where any mismatch of salts or other buffer components contributes to the fringe displacement."); Ex. 1001, 47:31-35. In general, the POSA understood that interference spectroscopy was often impractical for obtaining accurate and reproduceable data, and it is a method that does not lend itself to high-throughput analysis.

52. Absorbance was the preferred optical detection method at the effective date of the '288 patent. Ex. 1005, 152. Absorbance at a wavelength of 276 nm was the method employed by the Le Bec reference for analyzing AAV particles by AUC. Ex. 1003, 15:19-28. The absorbance of a chemical or biological species depends on the pathlength and the concentration of the species, according to the Beer–Lambert law where ε is the weight extinction coefficient of that material; c is the molar concentration of those species; ℓ is the path length.

$$A = \varepsilon c \ell$$

Ex. 1005, 153. Thus, where the Beer–Lambert law holds, the absorbance signal is directly proportional to solute concentration. Ex. 1005, 153.

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53. Absorbance data most often requires the use of extinction coefficients to calculate the molar concentration and the percent value of the empty and genome-containing capsids. The impact of differing absorbance between species is starkly illustrated by Figure 1B of the ’288 patent. Figure 1B shows an “AAV2 mixture contain[ing] empty capsids (‘Empty Cap’) and full genome capsids (‘Intact Vector’) …. A plot of concentration in units of detection, C(s), versus sedimentation coefficient (Svedberg units, S) showing that AUC can be used to measure the concentration of empty capsids and full genome capsids from an 80%/20% mixture).” Ex. 1001, 8:19-27.

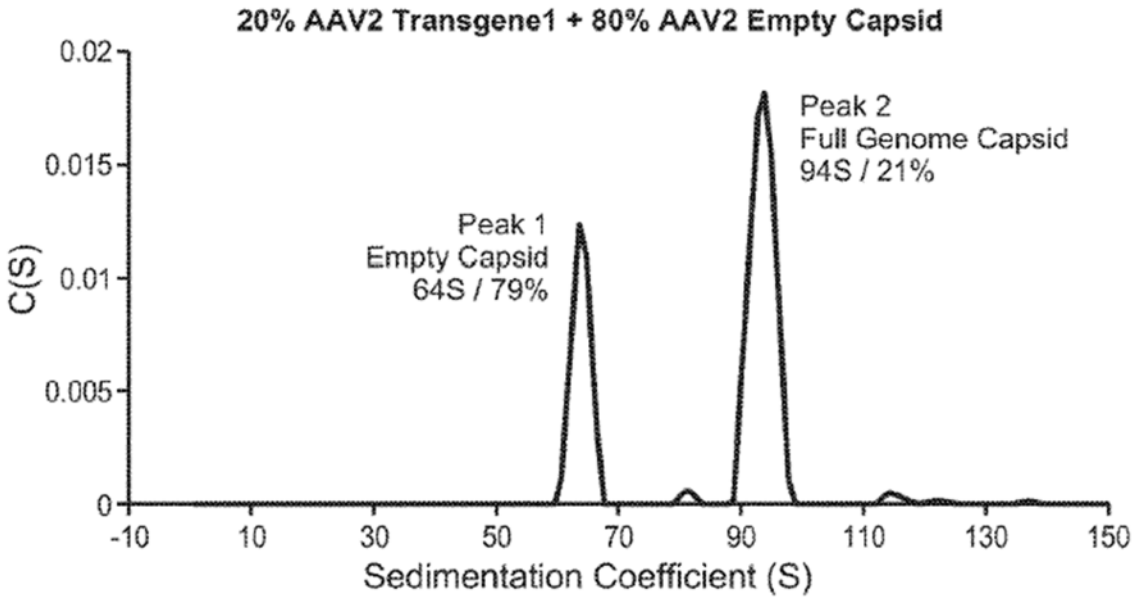

FIG. 1B

Ex. 1001, Fig. 1B. What stands out about this figure is that the peak corresponding empty capsids, present in four-fold excess to the full genome capsids, has a visibly

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smaller area than the full genome capsid peak. If the peak area reflected actual concentration, the empty capsid peak at 64S would have been four-times the area as the full genome capsid peak at 94S. However, this is not the case with the 94S peak having what appears to be a larger area than the 64S peak. This discrepancy is attributable to the different absorbances of empty capsid and full rAAV particle at the absorbance detection wavelength. The application of the extinction coefficient at this wavelength adjusts for this difference, and thus is essential for determining the relative concentrations of the two particles in the sample.

54. For genome-containing rAAV particles, which are a combination of protein (from the capsid) and DNA (genome), the extinction coefficient will depend on the amounts of protein and genome in a given particle. Ex. 2007, 127. Extinction coefficients of empty and fully intact rAAV particles had been estimated. *Id*. ("Based on the known molecular weight, the total protein concentration determined by quantitative amino acid analysis of the highly purified AAV empty capsids, and the measured absorbance values, the molar extinction coefficients of capsids at 260 and 280 nm were estimated at 3.72×10^6 and 6.31×10^6 $M^{-1}cm^{-1}$, respectively."); Ex. 1001, 24:23-26. To estimate extinction coefficients for variant genomes (e.g., rAAV particles containing partial or fragmented genomes) would have needed to know the respective molecular weights of both the protein and encapsidated genome. Ex. 2007, 127. At the time of the effective

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filing date, there was no known way to make such a determination. As explained below, the inventors of the ’288 patent identified how to use AUC to determine the genomic molecular weight of rAAV particles.

VIII. THE ’288 PATENT

55. In my opinion, the ’288 patent describes and claims a significant invention for the characterization of rAAV preparations. The ’288 patent describes the detection, identification, and quantification of rAAV particles by AUC that was not previously possible. For that reason, the methods in the ’288 patent was recognized as important advancements in the field of rAAV characterization.

56. As part of my analysis, I reviewed the ’288 patent and I also reviewed a 2015 peer-reviewed publication co-authored in-part by the inventors of the ’288 patent, Burnham and O’Riordan, and colleagues. Ex. 2003 (“Burnham 2015”). My review of both documents found that many of the publicized experiments described in the ’288 patent were also published in Burnham 2015, including many of the experiments included in Examples 1-6 of the ’288 patent. *See, e.g.*, Ex. 2003, 232, Figure 1 (Ex. 1001, Example 1 (FIGS. 1A, 1B, 2A, 2B)), 233, Figure 2 (Ex. 1001, Example 2, Table 4, FIGS. 3A-3B), 234, Figures 3A, 3B (Ex. 1001, Example 3, FIGS. 6B, 6C), 235, Figure 4 (Ex, 1001, Example 4, FIG. 7A-7C), 236, Figure 5 (Ex. 1001, Example 5, FIGS. 8, 9A-9C), 237, Figure 6D (Ex. 1001, Example 6, FIGS. 11A) .

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57. Burnham 2015 was recognized by the industry as providing an important tool for the characterization of rAAV particles. Notably, a 2020 white paper by Beckman Coulter GmbH, the manufacturer of instruments for AUC analysis highlighted the importance of the Burnham's contribution, stating:

> Burnham and colleagues showed that sedimentation velocity … [AUC] experiments are able to characterize the quality of viral vector productions. A ***single AUC experiment provides insights into the composition of the encapsulated DNA, the success of the purification, the presence of aggregates and the ratio between empty, partially filled and filled viral particles***….Taken together, the AUC is a valuable tool to analyze rAAV vectors notwithstanding the composition and length of the transgene or the viral serotype.

Ex. 2005 (citing Burnham 2015) (emphasis added).

58. A 2018 publication co-authored by an inventor of the Petitioners' primary prior art reference (Ex. 1003), Christine Le Bec, and others, stated that "for the determination of empty-to-full capsid ratio, here we used AUC, a method that has been demonstrated to provide a reliable readout." Ex. 2004, 166-67 (citing Ex. Burnham 2015). It is not surprising that the rAAV field, including Le Bec, would rely on the inventors' AUC method. As explained herein, the Le Bec prior art reference relied on by the Petition (Ex. 1003) did not provide a method that could quantify the ratio of different rAAV species within an rAAV preparation. Le Bec and co-authors referenced Burnham 2015, and not their own work, including

the Le Bec patent application publication (Ex. 1003). This indicates that Le Bec and co-workers did not view the capabilities of their method on par with the inventors' method that was also described and claimed in the '288 patent. As I explain below in my report, the claimed methods of the '288 patent have capabilities that prior methods, including the Le Bec reference (Ex. 1003) did not have.

59. The method described and claimed in the '288 patent made AUC a valuable tool to identify the species present in rAAV compositions and measure the "the ratio between empty, partially filled and filled viral particles." Ex. 2005. The Petition neither demonstrates that AUC was used in the prior art to identify each species present in a rAAV preparation, nor could quantify those rAAV species, particularly rAAV species containing partial or fragmented genomes.

60. The '288 patent describes a method of using AUC to identify and quantify the many species resulting from the preparation of rAAV vectors, for which "***a major problem***" was "the presence of capsids with ***partial or fragmented genomes***." Ex. 1001 at 47:49-52 (emphasis added). Partial and fragmented genomes are examples of what the '288 patent describes as "variant genomes." *Id.* at 24:65-25:2. My review of the cited art in the Petition led me to the same conclusion as the inventors regarding the magnitude of this unsolved issue.

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61. A significant innovation of the ’288 patent is the application of AUC under boundary sedimentation velocity conditions for the quantitative analysis of rAAV species in a preparation, including variant species. Ex. 1001, 43:42-47. One valuable application of this method was “monitor[ing] [rAAV preparation] at time intervals (e.g., one or more times),” during, for example, rAAV production to determine whether a process is meeting requirements for potency and purity. *Id*., 11:7-12. The application of AUC to identifying species present in a heterogenous mixtures and determining their concentration is not straightforward. There were several issues that the inventors had to overcome to in order to develop the method of the claims.

62. The ’288 patent describes and claims “plotting the differential sedimentation coefficient distribution value (C(s)) versus the sedimentation coefficient in Svedberg units (S).” Ex. 1001, 1:64-67; claims 5-8. As the ’288 patent describes, the “‘distribution of sedimentation coefficients” plot, c(s), “looks like a chromatogram.” Ex. 1001, 24:6-10. A first challenge in identifying and quantifying species present in an rAAV preparation was resolving the peaks of the species present. The inventors identified sedimentation velocity conditions that resulted in a plot where each individual rAAV species was present as a separate and distinct peak, where each peak contains varying levels of genome encapsidation. *Id*., 53:27-36. The resolution of each peak allowed for the

separation needed to integrate the peaks and determine each peak's area. *Id*., 12:16-30. Adequately resolving the peaks is a first step in determining the concentration of the rAAV species present, but it is not the only step.

A. The '288 Patent Describes the Inventors' Method of Determining Concentration for Variant Genome-Containing rAAV Species

63. As explained above in Section VII.C, determining the relative concentrations of species within a heterogeneous rAAV preparation using AUC, requires correlating peak area, determined by peak integration, to concentration. Ex. 1001, 24:6-10. Consistent with my analysis, the '288 patent recognizes that when absorbance spectroscopy is used as the optical detection method for AUC methods, at certain wavelengths correlating peak area to concentration may require accounting for the extinction coefficient (ε) of each species, because different rAAV species will absorb light to a different degree. *Id*., 24:20-23 ("[E]xtinction coefficients are used to calculate molar concentration and the actual percent value of the intact vector peak from absorbance data.").

64. The '288 patent explains that extinction coefficients had been estimated for empty capsids and fully intact rAAV particles. *Id*., 24:23-26 (citing Ex. 2007, 127) ("Molar absorbance extinction coefficients for both empty capsids ($\varepsilon_{260/capsid}$=3.72e6) and intact vector ($\varepsilon_{260/vector}$=3.00e7) can be calculated based on published formulae."). However, "it [was] ***not possible to determine empirically***

the extinction coefficient of fragmented genomes" because they are "of ***unknown size and sequence*** …." Ex. 1001, 24:35-38; 44:25-32; 49:9-12 (emphasis added).

65. As I also explained above in Section VII.C, an "alternative optical detection method for AUC" to absorbance spectroscopy was Rayleigh Interference Optics, which "measures the sample concentration based on refractive index differences between a reference solution and the AAV containing sample." Ex. 1001, 46:58-67 (Example 2). Although "interference detection yields integrated peaks that are directly proportional to concentration" (*id*.), a POSA would have understood that there were potential limitations to its application. For example, interference detection required "a high concentration of sample" and its results could "be affected by a mismatch between the reference and AAV sample buffer." Ex. 1001, 47:31-35. For studying rAAV preparations, the sample concentration issue would have been understood by a POSA to have been particularly problematic because "AAV samples … typically contain a low protein concentration." *Id*., 47:35-38. This low protein concentration would have been further compounded by the fact that certain rAAV species were expected to be present at low concentrations relative to other species, so a POSA would have had concerns with the viability of interference detection for rAAV preparations. *See* Section VII.C, above.

66. Additionally, the need to "completely match the AAV sample and reference buffers" presented another drawback to interference optics that was not presented by absorbance spectroscopy. Ex. 1001, 47:35-38. Thus, absorbance spectroscopy was understood to be a preferred and generally more practical method for the detection of rAAV species, particularly partial or fragmented species that may be less abundant than other species. Thus, absorbance detection was applied to most examples of the '288 patent.

67. Detection by absorbance, therefore, provided certain practical advantages over interference for rAAV preparations. But the inventors were faced with the challenge that "it [was] not possible to determine empirically the extinction coefficient of particular species of recombinant viral particles," particularly variant species. *Id*., 24:35-38. The inventors, however, developed an innovative approach to addressing this issue, determining:

> [A] relationship between S value and genome size may be established by analyzing recombinant viral vector preps with encapsidated viral genomes of known nucleotide size and a corresponding S value…. The calculated S values can be plotted to generate a ***standard curve*** to which recombinant viral species of unknown molecular weight or genome size can be compared to determine the molecular weight of the unknown species.

Id., 24:38-46; 44:25-32.

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68. Example 5 illustrates the applicability of "AUC … as a tool to quantify the aberrant packaging of fragmented genomes in rAAV vector preps." *Id*., 48:60-50:17. The inventors generated a standard curve by taking "rAAV vector preps with encapsidated viral genomes of known size" analyzing those vector preps by AUC so "their corresponding S values were determined, as shown in Table 5," (*id.*, 49:15-39), which is reproduced below:

TABLE 5

S values for rAAV vectors with known genome size.

Predicted Trend Line		Calculated values	
Sedimentation coefficient (S) (y)	Genome size (# NT) (x)	MW	Extinction coefficient (260 nm)
Empty capsid	N/A		3.72E+06
74	880	2.7E+05	9.17E+06
78	1421	4.4E+05	1.25E+07
82	1961	6.1E+05	1.59E+07
84	2232	6.9E+05	1.75E+07
88	2772	8.6E+05	2.09E+07
92	3313	1.0E+06	2.42E+07
96	3853	1.2E+06	2.76E+07
100	4394	1.4E+06	3.09E+07
104	4934	1.5E+06	3.43E+07
108	5475	1.7E+06	3.76E+07

Id., Table 5.

69. From this data, the inventors plotted "Sedimentation Coefficient vs Packaged NT [nucleotides]" thereby correlating the sedimentation coefficient with the amount of encapsidated genome in the rAAV particles. Ex. 1001, 9:28-32, 49:9-19 (Fig. 8). The plotted data "demonstrated a highly linear relationship (R^2=0.9978) between sedimentation coefficient and genome size," which can be seen in the excerpted figure below. *Id.*, 49:9-19.

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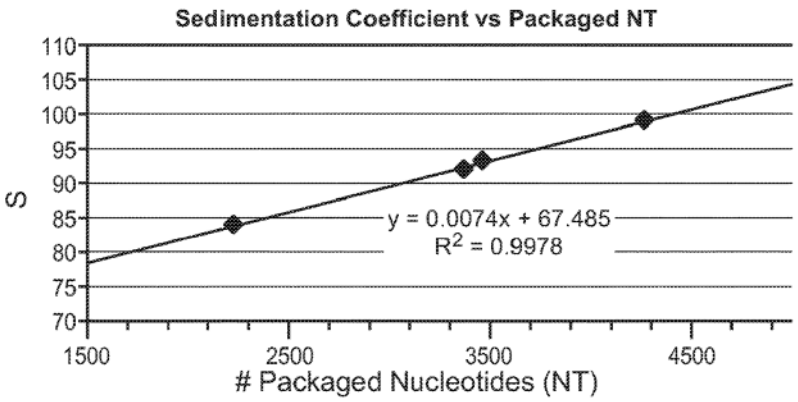

FIG. 8

Id., Fig. 8. The inventors could use the standard curve to determine the molecular weights, genome sizes, and extinction coefficients at 260 nm of variant rAAV species, for example, rAAV particles containing partial or fragmented genomes.

B. Application of the Standard Curve to Determine the Genome Size of Variant rAAV Species

70. As a proof of concept, the inventors ran several experiments, documented in Examples 3, 5, and 6 showing how C(s) plots and sedimentation coefficients could be applied to determining genome size.

1. Example 5

71. "To demonstrate the utility of AUC to detect genome fragments a self-complementary vector comprising AAV2 ITRS, a minimal CBA promoter, and an EGFP transgene was packaged into an AAV9 capsid." Ex. 1001, 49:40-44. After purifying the preparation to eliminate empty capsids, the remaining vector particles were "analyzed by AUC" and "[t]he standard curve was then used to

assign genome size to each of the resolved genome containing capsids." *Id*., 49:44-47.

72. Figure 9A is a "plot of differential sedimentation coefficient distribution value, c(s), vs sedimentation coefficient in Svedberg units (S), yields the distribution of sedimentation coefficients for a [self-complimentary] scAAV9 EGFP vector preparation. Single stranded monomeric (82S) and double stranded dimeric (101S) species are labeled with the corresponding sedimentation coefficients and relative abundance values (%)."

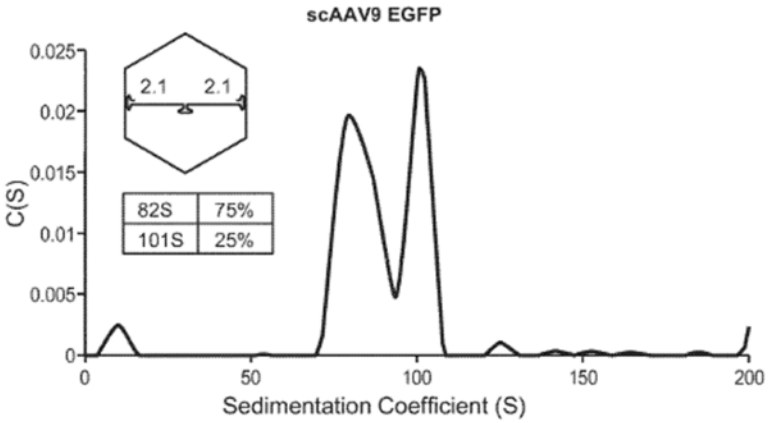

FIG. 9A

Id., 9:35-42; 49:40-49; FIG. 9A.

73. Applying the standard curve, the inventors determined that 101S peak corresponded to an rAAV vector of ~4.3 kb (kb=kilobases), while the 82S peak corresponded to a vector genome size of ~ 2kb. *Id*., 49:47-55. With the molecular weights of the two components determined, it was possible to calculate their extinction coefficients at 260 nm and, therefore, their relative concentrations of

25% for the 101S peak double-stranded species and 75% for the 82S peak single stranded species. Ex. 1001, 49:47-55; FIG. 9A. In note that the POSA would have understood that C(s) distribution peaks having lower sedimentation values than the *s* values for empty capsids do not correspond to rAAV species. As an example, for adenovirus particles, Berkowitz 2007 described how the "slow-migrating material" (i.e., the material with the lowest *s* values) "most likely corresponds to fragments of [empty capsid or 'EC'] and virus particles and possibly aggregates of viral proteins…for EC particles since this material is known to be unstable." Ex. 1004, 27.

74. The AUC results showed good agreement with Southern blot results, which identified both the single-stranded monomeric species (***) and dimeric double stranded species (*) in lane 1, shown below.

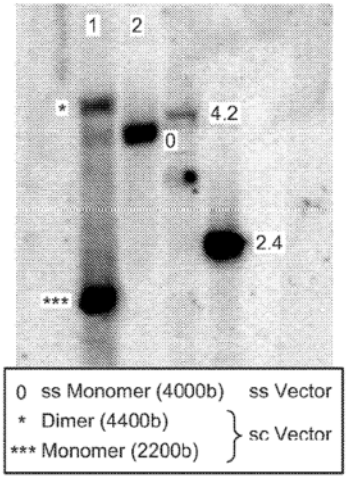

FIG. 9B

Ex. 1001, 49:61-64; FIG. 9B.

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75. Applying the inventor's AUC method, Example 5 further described "AUC analysis of a single stranded AAV9 EGFP vector" preparation which resulted in a "predominant peak with a measured S value of 99 S, corresponding to approximately 4.1 kb by the standard curve and 84% of capsid abundance" and a second peak single stranded rAAV vector peak at 85S having a relative concentration of 16%.

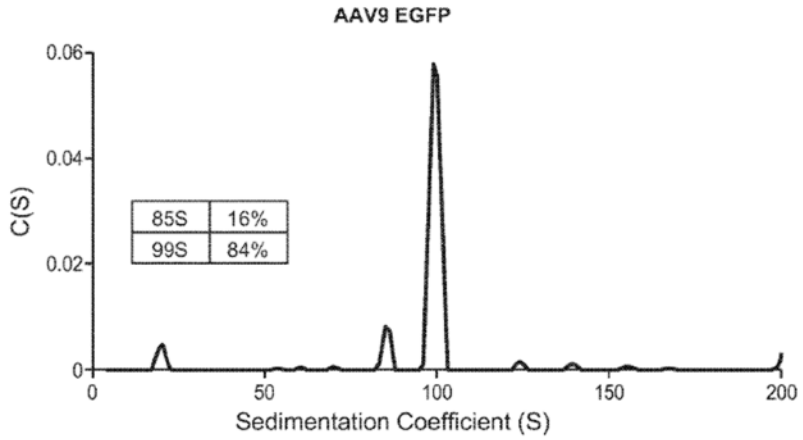

FIG. 9C

Id., 49:66-50:3; FIG. 9C. This result also agreed with the Southern blot analysis, showing this species—ss Monomer (4000b) in lane 2 (0)—of FIG. 9B. *Id*., 50:6-9; FIG. 9B.

2. Example 3

76. The '288 patent describes a comparison of two heterogenous rAAV preparations produced by two AAV production methods. Both production processes resulted in the production rAAV preparations containing empty capsids,

partially and/or fragmented capsids, and fully intact capsids. Ex. 1001, 47:40-48:16; Figs. 6B-C (reproduced below).

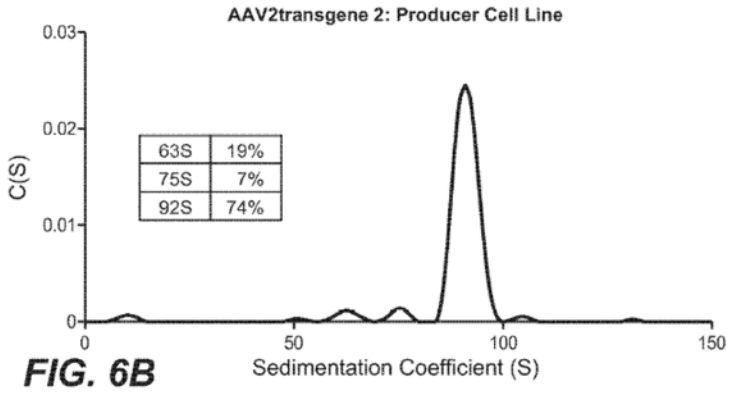

FIG. 6B

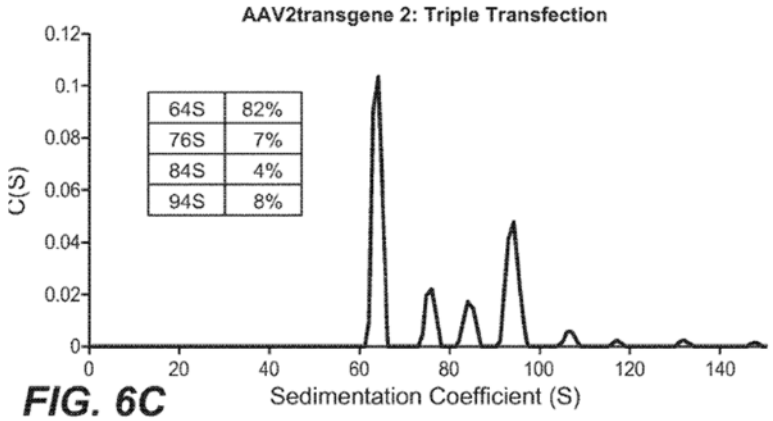

FIG. 6C

Id., Figs. 6B-C.

77. The fragmented genomes were identified to have peaks at 75S (FIG. 6B) 76S and 84S (FIG. 6C). *Id*., 47:64-48:5. The inventors determined the concentrations of the fragmented species identified in their experiments, observing that "[t]he AUC profiles of vector preps produced by these methods were remarkably different." *Id*., 47:63-64. "Using the producer cell line method, 74% of capsids contained a full genome, represented by the 92 S species (FIG. 6B).

19% were empty capsids, with the remainder containing a fragmented genome species (75S species, 7%)." *Id*., 47:64-48:1. The "triple transfection method" resulted in a far less successful outcome: "82% of the capsids … were empty, 64S species (FIG. 6C), with 11% of capsids containing a fragmented genome (76S and 84S species) and only 8% of capsids having a full genome (94S)." *Id*., 48:1-5. The identification and quantification of each species, particularly fragmented species, illustrates the value of the inventive method and its utility for determining the quality of rAAV preparation and manufacturing procedures by facilitating the quantification of empty capsids, fully intact rAAV particles, and partial/fragmented genome-containing rAAV particles.

3. Example 6

78. Example 6 further illustrates the application of the inventors' method to identify and quantify unknown rAAV species containing partial or fragmented genomes that eluded the detection of the established Southern blot analytical method. The inventors' method was used to analyze how modification to the helper factor in a vector prep for the "self-complementary AAV vector harboring an EGFP transgene" impacted rAAV production. Ex. 1001, 50:29-42. The experiments compared a "first method (FIG. 10B)" for rAAV preparation employing an unmodified "helper plasmid" (the "WT Rep" construct) and a "second method (FIG. 10C)" where "the helper was modified" (the "pHLP Rep"

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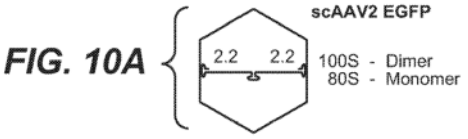
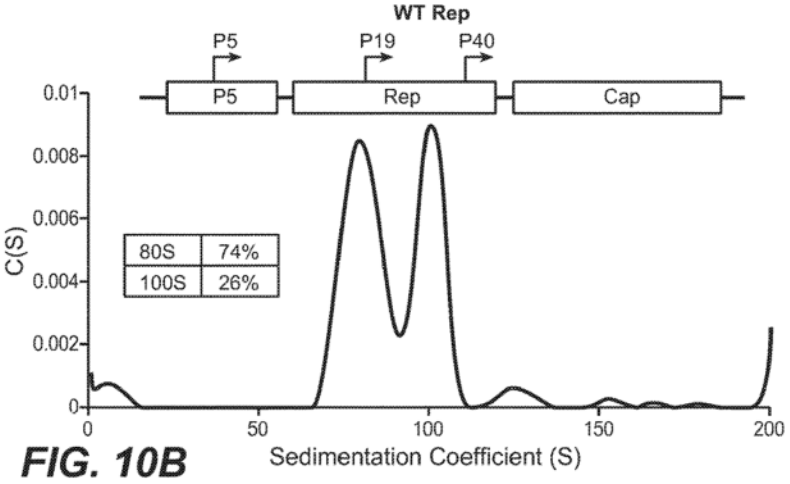
construct). *Id*., 50:33-39. "The full scAAV2 EFGP capsid was predicted to have a sedimentation coefficient of 100S in dimeric genome form and 80S in monomeric genome form (FIG. 10A)." *Id*., 50:39-42.



79. For the unmodified helper of the WT Rep construct, the resulting packaging of the rAAV genome dimeric genomes (~100S peak) unexpectedly decreased to 26% relative to those containing monomeric genomes (~80S peak) at 74%.



Id., 50:49-53, Fig. 10B. In contrast, the modified helper of the pHLP Rep construct resulted in a ratio of rAAV particles containing dimeric genomes (~100S peak) at 55% versus monomeric particles (~82S) at 45%.

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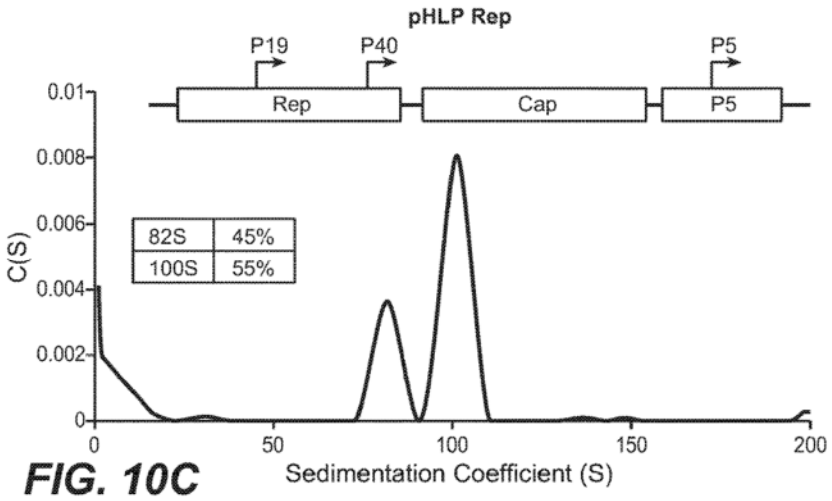

FIG. 10C

Id., 50:43-47. "These results uncovered a significant difference in genome packaging induced by" modifying "the helper plasmid." *Id.*, 50:53-56. Detection of this result was facilitated by the AUC analysis differentiating between rAAV species containing different levels of genome encapsidation.

80. The power of the AUC method of the '288 patent was further revealed through a comparison of AUC with Southern blot analysis. In another experiment of Example 6, a "single stranded AAV5 Factor IX vector, AAV5FIX, (FIG. 11A-B) and a single stranded AAV5hSMN vector (FIG. 11C-D) were generated using" the modified helpers, pHLP Rep, "as described above, but cap sequences of AAV2 were replaced by cap sequences of AAV5." Ex. 1001, 50:57-62. "Based on the nucleotide size of the FIX expression cassette (4.3 kb), the predicted sedimentation coefficient for the AAV5 FIX vector capsid was approximately 101S. AUC analysis of AAV2/SFIX made in the presence of" the modified pHLP Rep helper

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"revealed a homogenous profile with the majority of the vector (90%) sedimenting with an S value of the expected size."

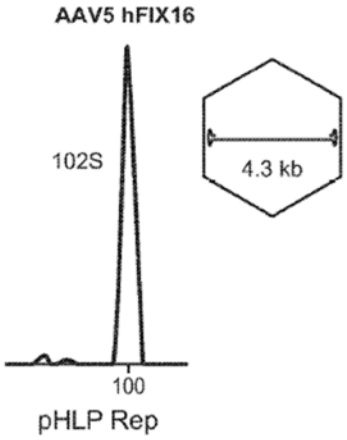

FIG. 11A

Id., FIG. 11A.

81. "In contrast, AAV5 FIX vector generated using [the unmodified wild-type, WT Rep,] generated a strikingly different AUC profile….Instead of a predominant peak at 101S, this profile revealed more capsid heterogeneity, with the majority of the AAV5 FIX (80%) sedimenting at a lower S value of 86 S, likely representing packaging of a fragmented genome" whereas "only 15% of the AAV5 FIX vector capsids sedimented at the correct S value of ~104S." *Id*., 51:1-11.

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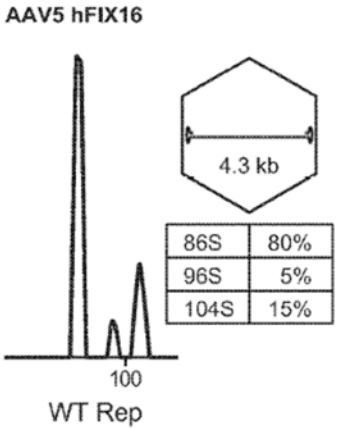

FIG. 11B

Id., FIG. 11B.

82. AAV5SMN vectors made using these same wild-type and mutated helpers also had different profiles. "AAV5 SMN vectors generated in the presence of" the modified pHLP Rep helper "showed less heterogeneity by AUC analysis, with a single capsid species sedimenting at the S value of 101S consistent with packaging of a genome of the predicted size of ˜4.4 kb." *Id*., 51:12-19.

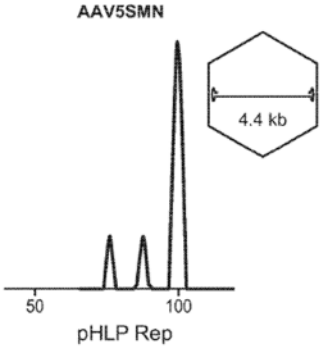

FIG. 11C

Id., FIG. 11C.

83. "In contrast, the AUC profile for the same vector genome packaged using 'wild-type'" WT Rep helper "revealed three distinct AAV vector species,

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with sedimentation coefficients of 100 S…92 S (representing a fragmented genome of approximately 3300 [nucleotides]) and 80 S (representing a fragmented genome of 2000 nucleotides)." *Id*., 51:19-28.

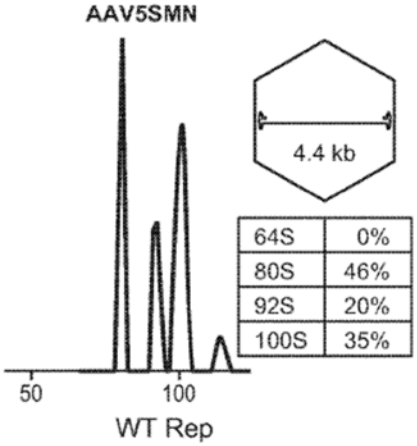

FIG. 11D

Id., FIG. 11D.

84. Southern blot analysis of the AAV5SMN preparations were consistent with the AUC results for the pHLP helper (FIG. 11A) showing a homogenous profile of the full-length vector (FIG. 12A, lane 2) compared with packaging of full-length and fragmented SMN genomes (FIG. 12A, lane 1). *Id*., 51:29-37.

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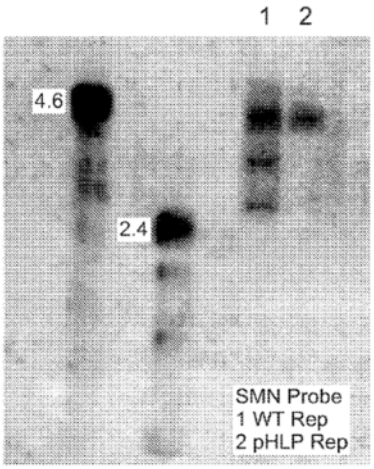

SMN Probe

FIG. 12A

Id., Fig. 12A.

85. For example, Southern blot analysis of the FIX vector preparation made with the pHLP modified helper agreed with the AUC result (FIG. 11A), showing predominantly full-length AAV (FIG. 12B, lane 1). In contrast, "AUC analysis of this AAV5FIX vector (FIG. 11B)" made with the WT-Rep helper "showed that 80% of the capsids contained a fragmented genome (~3000 nucleotides)" but these fragments "were ***undetected*** by the FIX probe" in a Southern blot experiment. *Id*., 51:42-45 (emphasis added).

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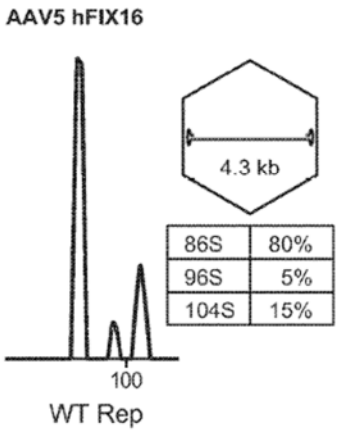

FIG. 11B

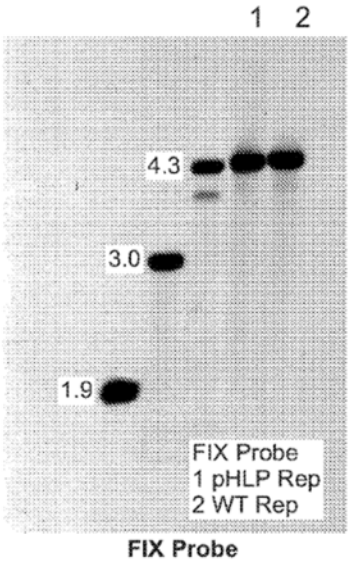

FIG. 12B

Id., Figs. 11B, 12B.

86. When an Amp^R specific probe was used in a Southern blot experiment, the vector genome species from the FIX vector preparation sedimenting at 86S containing a ~3 kb fragment (FIG. 11B) was detected. *Id*., 51:54-63.

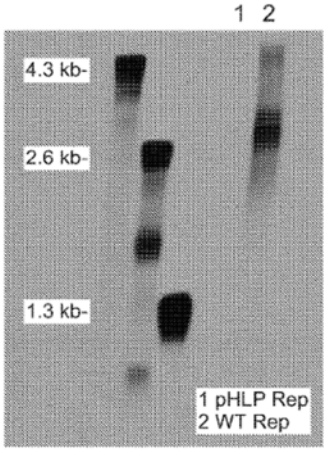

FIG. 14B

Id., FIG. 14B.

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87. But the Amp^R probe did ***not detect*** the fully intact genome in the rAAV5 FIX vector prep generated with the modified pHLP Rep helper. Ex. 1001, 51:54-63. Thus, multiple experiments were required to identify the species in the preparation by Southern blot. These comparative experiments illustrate the power of the inventors' AUC analysis method. Unlike Southern blot AUC is not sequence dependent, so the claimed AUC method allows for the detection and quantification of rAAV species, including partial or fragmented genomes, in a single experiment.

88. Example 6 of the '288 patent also applies the AUC method to analyze "the heterogeneity of AAV vectors produced using oversized genomes." Ex. 1001, 52:11-14. "An expression cassette harboring the full length CBA promoter driving expression of the β-phosphodiesterase transgene was packaged as an oversized genome of 5.4 kb (FIG. 15A) or as a wild type size genome of 4.6 kb (FIG. 15B)." *Id*., 52:14-18. Oversized rAAV genomes "have been shown to be fragmented into subgenomic-length DNA." *Id*., 52:7-11. Indeed, "the AUC profile of the AAV vector prep generated using the oversized vector genome demonstrated that nearly half of the vector prep sedimented as a [fragmented] 93S species … of approximately 3.5 kb. 30% of the preparation was represented by another sub-genomic vector species of approximately 4.9 kb sedimenting at 105S. There was

no evidence of packaging of a full-length 5.4 kb genome, which was predicted to sediment at 108-109 S." *Id*., 52:22-30.

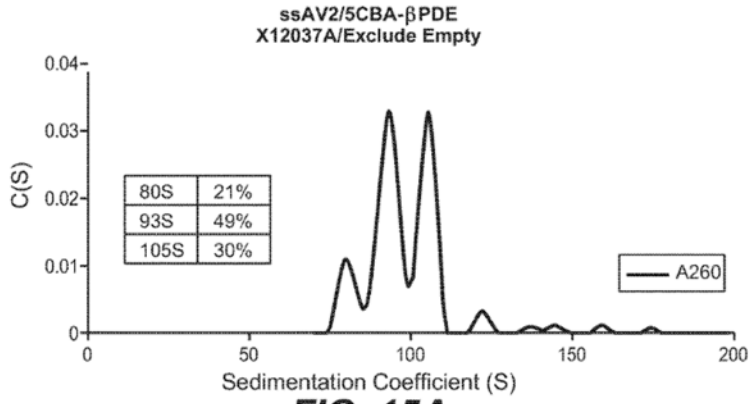

FIG. 15A

Id., FIG. 15A.

89. "In contrast, AUC analysis revealed that the same transgene under control of the abbreviated CBA promoter" of wild-type size "sedimented predominantly as a vector species of 102 S, consistent with packaging of the predicted, full-length vector genome of 4.6 kb." *Id*., 52:30-34.

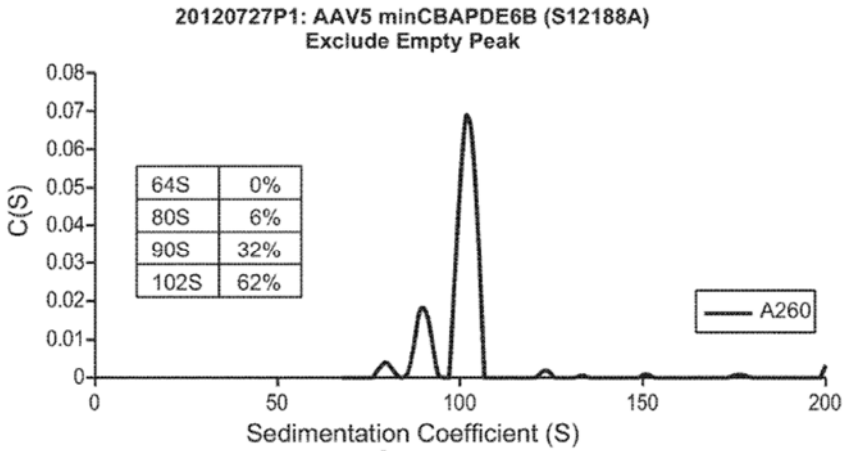

FIG. 15B

Id., FIG. 15B.

90. "These results demonstrate the utility of AUC analysis in profiling AAV vectors with oversized genomes, and this profiling is critical, given the observed incidence of genome fragmentation." *Id*., 52:35-38.

C. Claims at Issue

91. Claims 5-8 recite:

5. A method of measuring the relative amount empty capsids in a preparation of recombinant AAV particles comprising the steps of

a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant AAV particles is monitored at time intervals,

b) plotting the differential sedimentation coefficient distribution value (C(s)) versus the sedimentation coefficient in Svedberg units (S),

c) integrating the area under ***each peak*** in the C(s) distribution to determine the ***relative concentration of each species*** of recombinant AAV particles, and

d) comparing the amount of recombinant AAV particles having an S value corresponding to empty capsid particles to the amount of recombinant AAV particles having an S value corresponding to recombinant AAV particles comprising intact AAV genomes or the total amount of recombinant AAV particles in the preparation.

6. A method of measuring the relative amount of capsid particles comprising variant recombinant AAV genomes or empty AAV capsid

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particles in a preparation of recombinant AAV particles comprising the steps of

a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant AAV particles is monitored at time intervals,

b) plotting the differential sedimentation coefficient distribution value (C(s)) versus the sedimentation coefficient in Svedberg units (S),

c) integrating the area under ***each peak*** in the C(s) distribution to determine the ***relative concentration of each species*** of recombinant AAV particles,

d) comparing the amount of recombinant AAV particles having an S values that do not correspond to recombinant AAV particles comprising intact AAV genomes to the amount of recombinant AAV particles having an S value that corresponds to recombinant AAV particles comprising intact AAV genomes or to the total amount of recombinant AAV particles in the preparation.

7. A method of measuring the relative amount of capsid particles comprising variant recombinant AAV genomes in a preparation of recombinant AAV particles comprising the steps of

a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant AAV particles is monitored at time intervals,

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b) plotting the differential sedimentation coefficient distribution value (C(s)) versus the sedimentation coefficient in Svedberg units (S),

c) integrating the area under ***each peak*** in the C(s) distribution to determine the ***relative concentration of each species*** of recombinant AAV particles,

d) comparing the amount of recombinant AAV particles having an S values that do not correspond to recombinant AAV particles comprising intact AAV genomes or empty capsid particles to the total amount of recombinant AAV particles in the preparation.

8. A method of measuring the relative amount of recombinant AAV particles comprising intact AAV genomes in a preparation of recombinant AAV particles comprising the steps of

a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant AAV particles is monitored at time intervals,

b) plotting the differential sedimentation coefficient distribution value (C(s)) versus the sedimentation coefficient in Svedberg units (S),

c) integrating the area under ***each peak*** in the C(s) distribution to determine the ***relative concentration of each species*** of recombinant AAV particles,

d) comparing the amount of recombinant AAV particles having an S values corresponding to recombinant AAV particles comprising intact AAV genomes to the amount of recombinant AAV particles having an

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> S value corresponding to empty capsid particles, to capsid particles comprising variant recombinant AAV genomes, and/or to the total amount of recombinant AAV particles in the preparation.

Ex. 1001, 55:4-56:18.

92. I understand that claim elements 5[c], 6[c], 7[c], 8[c] require integrating the area under ***each peak*** in the C(s) distribution to determine the ***relative concentration of each species*** of recombinant AAV particles—not only peaks corresponding to empty capsids and fully intact viral particles, but also partial or fragmented species that may be present in the rAAV preparation. Claim 9 depends from claim 5, and thereby incorporate claim element 5[c].

93. Claim 15, which depends from claim 12, recites:

> 15. The method of claim 12, further comprising integrating the area under ***each peak*** in the C(s) distribution to ***determine the relative concentration of each species*** of recombinant AAV particles.

Ex. 1001, 57:1-4.

94. I understand that claim 15, like claims 5-8, requires integrating the area under ***each peak*** in the C(s) distribution to determine the ***relative concentration of each species*** of recombinant AAV particles—not only peaks corresponding to empty capsids and fully intact viral particles, but also partial or fragmented species that may be present in the rAAV preparation. Claim 16 depends from claim 15, so it incorporates claim 15's requirements.

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IX. CLAIM CONSTRUCTION

95. I have been informed by counsel to interpret the language of the claims according to their plain and ordinary meaning as understood by a person of ordinary skill in the art.

X. GROUND 1: ALLEGED ANTICIPATION OF CLAIMS 5-8 AND 15 BY LE BEC

A. Element 5[c], 6[c], 7[c], 8[c], and Claim 15 Were Not Anticipated by Le Bec

96. Elements 5[c], 6[c], 7[c], 8[c], and claim 15 state "integrating the area under ***each peak*** in the C(s) distribution to determine the ***relative concentration of each species*** of recombinant AAV particles." Ex. 1001, 55:4-56:18, 57:1-4. In my opinion, Le Bec does not disclose the information required by claim elements 5[c], 6[c], 7[c], 8[c], and claim 15, and therefore Le Bec does not anticipate claims 5-8 and 15.

97. The Petition states that "a POSA would understand that obtaining the percentages/relative concentrations disclosed in Le Bec using a distribution profile involves integrating the area under the curve for the peaks of interest." Petition, 27 (citing Ex 1020, ¶ 84). The Petition and Berkowitz Declaration argue that "the SEDFIT software referenced in Le Bec and available prior to January 2015 calculated relative concentrations by integrating the area under the peaks of a sedimentation coefficient distribution profile." Ex. 1020, ¶ 83; *see also* Petition 27. Even accepting this argument, the POSA would not have considered Le Bec to

have provided any indication that the area under ***each peak*** was determined nor that the area under ***each peak*** was converted to concentrations by applying the extinction coefficients for the rAAV species corresponding to each peak.

1. Le Bec Does Not Describe How It Determined Rough Percent Concentration Values

98. Le Bec states "the HEK293 system essentially produced very few full AAV particles (< 25%), with only 10% of viral particles containing the genome in double-stranded form" and "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%)." Ex. 1003, 19:1-15. Le Bec never explains how it arrived at those percentages, which from the POSA's perspective is a critical missing piece of Le Bec's experimental description.

99. Le Bec does not describe integrating the area under peaks to arrive at those percentages. As described below, Le Bec also does not describe the use of extinction coefficients at 276 nm to arrive at those percentages. The POSA would not have considered the rough percentages that Le Bec provides for its experimental results ("<25%," 10%, ">80%" and ">50%") to have been anything more than an imprecise estimate of the concentrations of each species. Le Bec ***only refers to SEDFIT software in the context of obtaining the sedimentation coefficient***. Ex. 1003, 15:26-28 ("the *sedimentation coefficient* of the various populations was obtained using the software SEDFIT.") (emphasis added). With

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such scant detail, the POSA would not have assumed that Le Bec determined the relative concentration of each species by integrating the area under each peak.

2. Le Bec Does Not Disclose Determining Concentrations for Each Peak in Its C(s) Distributions

100. Neither the Petition nor the Berkowitz Declaration address Le Bec's omissions of concentration values for ***each peak*** in its C(s) distributions in Figures 2 and 3 that correlate to, for example, partial or fragmented genomes. Le Bec is directed to "alleviate problems" that cause the production of an excess of empty capsids when "producing double-stranded rAAV particles." Ex. 1003, 3:24-29. Le Bec uses AAV to monitor "improvement in the ratio of particles containing viral DNA to empty particles and an increased percentage of particles containing a double-stranded genome." *Id*., 3:24-29; 17:29-18:6. Le Bec only reports on the known densities for "empty AAV viral particles, i.e. vectors lacking a viral genome, and … for full AAV viral particles, i.e. those containing a viral genome with a maximum size of 4800 bases." Ex. 1003, 17:30-18:1.

101. Although Le Bec reports rough concentrations for empty and fully intact AAV particles (Ex. 1003, 19:1-15), Le Bec does not provide the concentrations of partial or fragmented rAAV species. The Petition and Berkowitz Declaration do not address this part of claim elements 5[c], 6[c], 7[c], 8[c], and claim 15 — "integrating the area under ***each peak*** in the C(s) distribution to determine the ***relative concentration of each species*** of recombinant AAV

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particles" — that Le Bec does not report. It is my opinion that Le Bec does not disclose the elements of claims 5-8 and 15 that state "integrating the area under ***each peak*** in the C(s) distribution to determine the ***relative concentration of each species*** of recombinant AAV particles." Thus, Le Bec does not anticipate claims 5-8 and 15.

102. In arguing that Le Bec anticipates ***integrating each peak***, the Petition states that Le Bec "teaches that the 'distribution profile of the species allows the identification of two categories of populations' and differentiates peaks as corresponding to empty or full particles." Petition, 27 (citing Ex. 1003, 18:11-27; Figs. 2 and 3.). But the POSA would have clearly seen that Le Bec experimental figures and explanations do not differentiate ***each*** peak in order to determine their concentrations. For example, Le Bec's categorizes "full AAV viral particles" includes multiple peaks as shown in Le Bec's Figure 2:

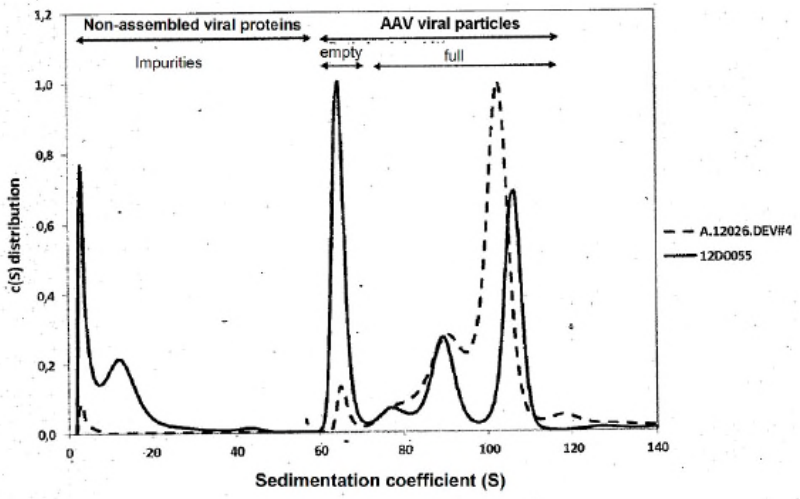

Figure 2

Ex. 1003, 6:3-7; 18:8-11; Fig. 2.

103. Le Bec explains that "[t]he distribution profile of the species allows the identification of two categories of population: a) <60S corresponding to non-assembled viral 15 proteins and/or contaminants" and "b) 60S-110S corresponding to intact AAV viral particles" and "two subpopulations with empty AAV viral particles at 65S and full AAV viral particles between 80S and 110S, with the viral genome in single-stranded form at 90S and double-stranded form at 105S." *Id.*, 18:11-25.

104. The Petition also quotes Le Bec's statement that Le Bec's C(s) "distribution profile…confirms that the HEK293 system essentially produced very few full AAV particles (< 25%)" (Petition, 27 (quoting Ex. 1003, 19:1-5), with "only 10% of viral particles containing the genome in double-stranded form." Ex. 1003, 19:1-5, *see also* Petition at 15. The Petition and Berkowitz Declaration ignores that within Le Bec's "full" viral particles subpopulation were multiple peaks corresponding to species of rAAV particles, including at the peaks at 90S and 105S. Ex. 1003, 18:21-25. The Berkowitz Declaration highlighted another "peak at approximately 77S that does not correspond to the peak for empty particle." Ex. 1020, ¶¶ 108, 122, 130, 132, 211 (citing Ex. 1003, Fig. 2). The Petition, however, does not identify where Le Bec describes "integrating the area under ***each peak*** in the C(s) distribution to determine the ***relative concentration of***

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each species of recombinant AAV particles" specifically those species at 77S and 90S in Le Bec's Figure 2. In my opinion, this is a critical missing piece of Le Bec.

105. Le Bec's Figure 3, which the Petition also cites to (Petition, 13-15, 27) does not show anticipation either.

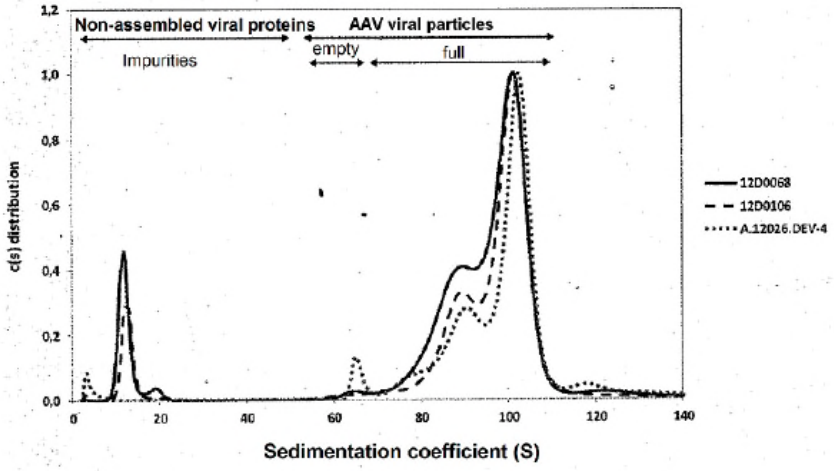

Figure 3

Ex. 1003, Fig. 3.

106. The Petition and Berkowitz Declaration never address the agglomeration of what appears to be multiple peaks ranging from about 75S to about 110S in Figure 3. The non-technical term my lab likes to use for figures like Figure 3 is a "hot mess." Figure 3 embodies the issue of diffusional broadening, which indicates, but masks, the presence of multiple peaks and the POSA would have understood that for heterogenous mixtures of particles, the use of AUC under boundary sedimentation conditions depends on resolving peaks of different species. *See* Ex. 1006, 169 ("This method can be used to study … heterogeneous

noninteracting and interacting systems provided that, in the latter cases, the ***multiple components separate sufficiently during sedimentation to form distinct peaks*** in the g(*s**) versus *s** plots.") (emphasis added); Ex. 1007, 1606-08 (When "diffusion broadens the sedimentation boundary, [it] makes it more difficult to resolve subpopulations of the distribution").

107. After the 2015 priority date of the '288 patent, Dr. Berkowitz co-authored a publication on AUC that explained that "diffusional broadening results in low resolution (fairly poor separation of different species), making detection and quantitation of minor species rather limited, especially for protein species that migrate with sedimentation coefficient values that are close to the other peaks and/or are present at low levels." Ex. 1011, 230. Consistent with the references filed with this Petition, the '288 patent explains that "if multiple species of solute are present, this may lead to the formation of multiple sedimenting boundaries, each corresponding to a resolvable species." Ex. 1001, 19:57-60. But "the presence of multiple species with similar sedimentation coefficients will cause the boundary to be broader than expected on the basis of diffusion alone." *Id.*, 17:29-31. Neither the Petition nor Berkowitz Declaration address the poor quality of Le Bec's c(s) plots, and the likely masking of peaks.

108. The Petition also does not address Le Bec's description of the C(s) distribution in Figure 3, and never accounts for the single stranded AAV peak at

about 90S. At most, Le Bec provides a rough qualitative assessment of the relative amounts "of full AAV particles (>80%)," and "viral genome in double-stranded form (>50%)." Ex. 1003, 19:11-15. These concentration values are insufficient to meet the requirement in claim elements 5[c], 6[c], 7[c], 8[c], and claim 15 of "integrating the area under ***each peak*** in the C(s) distribution to determine the ***relative concentration of each species*** of recombinant AAV particles."

109. Le Bec states 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." Ex. 1003, 15:19-28; Petition 13. The Petition does not provide any evidence that Le Bec was "integrating the area under ***each peak*** in the C(s) distribution to determine the ***relative concentration of each species*** of recombinant AAV particles." Specifically, I did not see any evidence of integrating peaks for partial or fragmented species.

3. Le Bec Is Missing Information Required to Determine Concentrations from Absorbance Detection

110. Le Bec states that it used absorbance detection to analyze its AAV preparations by AUC and generate its C(s) plots. Ex. 1003, 15:23-28 ("sedimentation was followed by absorbance at the wavelength of 276 nm."). As I note above in Section VII.C, absorbance detection has several notable advantages for AUC. In order to determine the relative concentration of a particular peak

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obtained with absorbance detection according to Le Bec's method, however, the POSA would have needed to know or determine the extinction coefficient for each rAAV species represented by a peak. Thus, determining the concentration for peaks corresponding to partial and fragmented rAAV species in Le Bec's C(s) distributions (Ex. 1003, Figures 2-3, showing peaks at 77S and 90S) would have required applying extinction coefficients for each species at the wavelength of 276 nm. Section VII.C, above.

111. The '288 patent details the challenge extinction coefficients had posed for POSAs for determining the concentrations of partial or fragmented species and identified an innovative way to overcome this challenge. Section VIII.B, above (citing Ex. 1001, 24:35-38); *see also* Ex. 1005, 152 (explaining absorbance is dependent upon a solute's extinction coefficient.). Berkowitz 2007 explains that "AUC experiments can provide accurate and useful results ***when appropriate steps are taken*** to deal with the variability in signal response (***extinction coefficient***) for different adenovirus species." Ex. 1004, 17 (emphasis added). In the studies documented in Berkowitz 2007, however, the authors "ha[d] not attempted to apply corrections to account for extinction coefficient differences." Ex. 1004, 21. Berkowitz 2007 further raised "concerns about the accuracy of [] reported adenovirus UV extinction coefficient." *Id*. at 34.

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112. Cole (Ex. 1005), states that "[u]nder conditions where the Beer–Lambert law holds, the absorbance signal is directly proportional to solute concentration: $A=\varepsilon cl$, where ε is the solute's weight extinction coefficient, c is the weight concentration, and l is the sample path length." Ex. 1005, 152; *see also* Section VII.C, above. Thus, to compare relative concentrations among species at wavelengths where the extinction coefficients vary between species, the POSA needed to determine the extinction coefficient for each species.

113. It is noteworthy, therefore, that Le Bec, does not contain any statements regarding the extinction coefficients of the partial and fragmented species in its C(s) distributions. Thus, the Petition never establishes how Le Bec alone would have permitted the POSA to determine concentrations of partial and fragmented rAAV species without relying on additional sources of information to modify its AUC analysis.

XI. GROUND 2: ALLEGED OBVIOUSNESS OF CLAIMS 5-8 AND 15 BASED ON LE BEC ALONE

114. The Petition further alleges that Le Bec alone rendered claims 5-8 and 15 obvious because "POSAs were motivated to assess virus preparations for such undesirable contaminants." Petition, 44. For claim elements 5[c], 6[c], 7[c], 8[c], and claim 15, the Petition argues that "[i]ntegrating the area under the peaks generated in an SV-AUC experiment is a standard technique used to determine the relative concentrations of components in the analyzed sample." Petition, 46.

115. As explained above in Section X.A, Le Bec at most describes the use of AUC to obtain approximate concentration comparisons between empty capsids and fully intact capsids. Ex. 1003, 19:1-15. Le Bec, however, does not provide relative concentrations for each peak in its C(s) distribution, including peaks corresponding to partial and fragmented rAAV species present in its rAAV preparations, for example at 90S and 77S in Figure 2. Section X.A.2, above. The Petition does not demonstrate that Le Bec alone would have motivated the POSA to use AUC to determine the concentration of each peak of an rAAV preparation with a reasonable expectation of success in doing so. The POSA would have understood that the need to know the extinction coefficient of each rAAV species in Le Bec's disclosure presented an obstacle to determining the concentrations of rAAV peaks associated with partial or fragmented genome. Extinction coefficients were not readily obtained, and this is reflected in the Berkowitz 2007 reference. Ex. 1004, 17, 21, 24. Therefore, the Le Bec reference would not have made claims 5-8 and 15 obvious to the POSA.

A. The Petition Does Not Provide a Reason for Why a POSA Would Have Been Motivated to Modify Le Bec to Arrive at Elements 5[c], 6[c], 7[c], 8[c], and Claim 15

116. Ground 2 does not explain why the POSA would have been motivated to modify Le Bec to arrive at claim elements 5[c], 6[c], 7[c], 8[c], and claim 15. The Petition's argument that "[i]ntegrating the area under the peaks generated in an

SV-AUC experiment is a standard technique used to determine the relative concentrations of components in the analyzed sample" (Petition, 46) does not support how or why the POSA would have modified Le Bec's method to determine the relative concentrations for each peak, including, those for partial and fragmented genome rAAV species, by integrating the area under each peak in the C(s) distribution.

117. As of the effective date of the '288 patent, the POSA would have understood that to determine the concentration of species within rAAV preparations by AUC using absorbance at 276 nm, the POSA would need to apply the extinction coefficient of each species to correlate peak area to concentration. Sections VII.C; X.A.3, above (citing Exs. 1004, 1005). Scientific literature filed with the Petition, including a peer-reviewed publication by Dr. Berkowitz, explains that obtaining accurate extinction coefficients was often not readily possible. The Berkowitz 2007 publication regarding the application of AUC to adenovirus particles (Ex. 1004) explains that "AUC experiments can provide accurate and useful results when appropriate steps are taken to deal with the variability in signal response (extinction coefficient) for different adenovirus species." Ex. 1004, 17. Restated, because each species absorbs UV light at a specific wavelength (e.g., 276 nm) to a different degree, the peak area per concentration will be different for each species. The extinction coefficient adjusts for this difference between species. Ex.

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1005, 152 ("Explaining that "absorbance signal is directly proportional to solute concentration: $A=\varepsilon cl$, where ε is the solute's weight extinction coefficient, c is the weight concentration, and l is the sample path length.").

118. Because of the need to account for differing extinction coefficients between species in Berkowitz 2007, the authors "ha[d] not attempted to apply corrections to account for extinction coefficient differences" in his publication about applying AUC to adenoviruses. Ex. 1004, 21. The Berkowitz 2007 publication further noted "concerns about the accuracy of [] reported adenovirus UV extinction coefficient" as a reason to avoid using absorbance in AUC experiments. Ex. 1004, 34.

119. Consistent with Berkowitz 2007, the inventors explain that at the time of the invention, "it [was] not possible to determine empirically the extinction coefficient of particular species of recombinant viral particles (e.g., viral particles with fragmented genomes of unknown size and sequence)." Ex. 1001, 24:35-38. By finding a way to calculate extinction coefficients of variant rAAV species, the inventors were able to determine the relative concentrations of these species. This innovation helped lead to the invention of claims 5-8 and 15. Section VIII.B, above. Neither the Petition nor the Berkowitz Declaration mention the need to account for differing extinction coefficients among different rAAV species in Le Bec, particularly unknown partial and/or fragmented genome species at 77S and

90S. It follows, therefore, that neither the Petition nor the Berkowitz Declaration explain how to determine extinction coefficients for unknown partial and/or fragmented genome species. In fact, the Berkowitz 2007 publication indicates that the POSA would have avoided relying on absorbance-based peak detection for AUC applied by Le Bec because of issues with extinction coefficients.

120. The Petition does not establish why the POSA would have been motivated to use Le Bec's AUC method to integrate the area under each peak in the C(s) distribution to determine the relative concentrations of each species present in the rAAV preparation. The peaks for partial and fragmented genome-containing rAAV particles, in particular, presented a specific barrier to AUC methods with respect to quantifying concentrations from peak integration. The POSA would have understood the need to know the extinction coefficient of individual species to accurately determine the relative concentrations of each species present in an rAAV preparation using Le Bec's method. Ex. 1004, 17; Ex. 1005, 152. Given the absence of information regarding extinction coefficients in Le Bec regarding any of the species at 276 nm, the POSA had ***no guidance*** on how to apply Le Bec's AUC method to determine the relative concentration of each species in an rAAV preparation. Without this crucial information, or any indication of where or how to get this crucial information, the POSA would not have been motivated to determine concentrations of certain peaks, including peaks

at 77S and 90S in Le Bec's Figures 2 and 3. It simply was not possible to accurately determine their concentrations without their extinction coefficients at 276 nm.

121. The Petition's argument that "[i]ntegrating the area under the peaks generated in an SV-AUC experiment is a standard technique used to determine the relative concentrations of components in the analyzed sample" (Petition, 46) ignores the reasons why the POSA would not have been motivated to do so. Without extinction coefficients at 276 nm merely integrating peak area in Le Bec's C(s) distributions would not have alone provided a meaningful measure of concentration. Because Le Bec does not disclose information required to accurately determine concentration of partial or fragmented rAAV species—or even how to obtain such information—the POSA would not have been motivated to practice claim elements 5[c], 6[c], 7[c], 8[c], and claim 15.

B. The Petition Does Not Establish that the POSA Would Have Had a Reasonable Expectation of Success in Arriving at Element 1[c]

122. Le Bec lacks guidance on extinction coefficients at 276 nm for any of its peaks, and the Petition does not explain how the POSA would have obtained the required extinction coefficients for partial or fragmented rAAV species, like those at 77S and 90S in Le Bec's Figure 2. Without this information, the Petition cannot establish a reasonable expectation in successfully determining the relative concentrations of each peak, particularly peaks corresponding to partial or

fragmented genomes, based on Le Bec alone. The Petition's obviousness analysis, which merely presents Le Bec while alleging that "[i]ntegrating the area under the peaks generated in an SV-AUC experiment is a standard technique used to determine the relative concentrations of components in the analyzed sample" (Petition, 46, citing Berkowitz, ¶ 182) does not consider all aspects of claim elements 5[c], 6[c], 7[c], 8[c], and claim 15. These claims recite "integrating the area under ***each peak*** in the C(s) distribution to determine the ***relative concentration of each species*** of recombinant AAV particles." The Petition does not provide evidence as to whether the POSA would have reasonably expected to successfully integrate the area of each peak to determine the concentration of each rAAV species, despite lacking the extinction coefficient information needed to make such determinations.

123. The Petition and accompanying Berkowitz Declaration do not provide any explanation for why the POSA would have reasonably expected the claimed method to have worked without the ability to apply extinction coefficient to partial or fragmented genome AAV species peaks. In actuality, the references submitted with the Petition indicate that Le Bec's lack of any hint of information relating to extinction coefficient would have led the POSA to conclude that it was not reasonably possible to determine relative concentrations for each peak. Section

X.A.3, above. Thus, the Petition does not establish a reasonable expectation of success for practicing claim elements 5[c], 6[c], 7[c], 8[c], and claim 15.

XII. GROUND 3: ALLEGED OBVIOUSNESS OF CLAIMS 5-9 AND 15-16 BASED ON LE BEC IN VIEW OF BERKOWITZ 2007 AND/OR COLE

A. The Petition Does Not Establish that Le Bec In View of Berkowitz 2007 Rendered Elements 5[c], 6[c], 7[c], 8[c], and Claim 15 Obvious

124. The addition of the Berkowitz 2007 (Ex. 1004) and Cole (Ex. 1005) references to Le Bec to form the Petition's obviousness Ground 3 does not change my opinion regarding the shortfalls of Ground 2 for claims 5-8 and 15. The Petition contends that "Berkowitz [2007] expressly teaches that 'quantification' of each particle would be accomplished by integration." Petition, 51 (citing Ex. 1004, 20). The Petition cites Berkowitz 2007's statement that "simple peak area integration, taking into account radial dilution effects … allows the percentage of ***EC [empty capsid]*** material present in a virus preparation to be readily calculated." Petition, 17, 51 (citing Ex. 1004, 20) (emphasis added). In my opinion, this Ground 3 argument based on Le Bec in view of Berkowitz does not add anything of substance to the Petition's Ground 2 argument that "[i]ntegrating the area under the peaks generated in an SV-AUC experiment is a standard technique used to determine the relative concentrations of components in the analyzed sample."

Petition, 46. The Petition is merely providing a specific example of integrating an AUC peak by citing to the Berkowitz 2007 reference.

125. Neither argument, however, addresses the absence of any statements in Le Bec relating to determining the concentration of peaks at, for example, 77S and 90S. Sections X.A.2-3, above. The issue is not whether the that the POSA would not have known how to integrate peaks, rather part of the claims not addressed in all of the grounds is the second part of Element 1[c], "integrating the area under ***each peak*** in the C(s) distribution to determine the ***relative concentration of each species*** of recombinant AAV particles." Le Bec indicates to the POSA that it did not determine concentration for certain peaks by any method and Berkowitz 2007 does not address the absence of information in Le Bec.

126. For example, the quoted passage in Berkowitz 2007 (Petition, 51 (citing Ex. 1004, 21)) addresses the quantification of empty capsids for adenovirus compositions. Le Bec discloses approximate relative concentrations of "empty" and "full" capsids, but Le Bec does not address the concentration of peaks for other partial or fragmented rAAV particle species in its C(s) distribution, for example the species at 77S and 90S. Sections X.A.2-3, above.

127. Merely applying Berkowitz's quantification techniques to Le Bec's AUC method for rAAV preparation would not make obvious the requirement of claim elements 5[c], 6[c], 7[c], 8[c], and claim 15 for integrating the area under

each peak in the C(s) distribution to determine the ***relative concentration of each species*** of recombinant AAV particles, like those at 77S and 90S in Le Bec. The Petition never explains how or why the POSA would have modified Le Bec based on Berkowitz 2007. For example, Le Bec uses absorbance detection at 276 nm to obtain its C(s) distribution (Ex. 1003, 15:25-26) whereas Berkowitz 2007 describes avoiding absorbance detection because of issues obtaining accurate extinction coefficients. Ex. 1004, 21 ("At present we have not attempted to apply corrections to account for extinction coefficient differences.").

128. The Petition does not explain why the POSA would have had a reason to combine Berkowitz 2007—which expressly avoids absorbance measurements because of issues obtaining extinction coefficients (Ex. 1004, 21, 34)—with Le Bec, which uses absorbance measurements that require applying extinction coefficients to determine concentration. Moreover, the Petition does not identify teachings in Berkowitz demonstrating the application of AUC to quantify partial and fragmented viral particles. The Petition states that "Berkowitz provides examples of these calculations for empty capsids and aggregates in Table 2." Petition, 16 citing (Ex. 1004, 29 (Table 2)). The Petition, however, never addresses the quantification of partial and/or fragmented virus particles using either Le Bec or Berkowitz.

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129. The Petition's argument that Berkowitz 2007 teaches "'quantification' of each particle would be accomplished by 'simple peak area integration'" benefits from the knowledge that the invention of the '288 patent works. For example, the inventors were able to rely on absorbance spectroscopy, and its incumbent advantages of permitting analysis of smaller sample size and the absence of a requirement to match exactly buffer systems between sample and reference (*see* Section VII.C, above), because they determined a way to calculate extinction coefficients for partial and fragmented genome particles at 260 nm. Section VIII.B, above. In contrast, neither the Petition nor Berkowitz Declaration identify any reference in the art that addresses the extinction coefficient issue associated with the 276 nm absorbance detection applied in Le Bec, nor provide any relevant analysis on this topic. Thus, Ground 3, like Ground 2, does not establish the obviousness of claims 5-8 and 15 in my opinion.

XIII. CONCLUSION

I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and that these statements were made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code.

Date: October 18, 2023

Professor Jeffrey C. Hansen