

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

SAREPTA THERAPEUTICS, INC.,
Petitioner

v.

GENZYME CORPORATION,
Patent Owner.

Case: IPR2026-00150
Patent No. 12,031,894

**DECLARATION OF KAREN G. FLEMING, PH.D.
IN SUPPORT OF PATENT OWNER'S RESPONSE**

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I. INTRODUCTION

1. I submit this declaration in connection with Patent Owner's Response in the *Inter Partes* Review IPR2026-00150 (the "IPR") of claims 1-7 and 10-30 ("challenged claims") of U.S. Patent No. 12,031,894 (the "'894 Patent"; Ex. 1001). 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 '894 Patent.

II. PROFESSIONAL QUALIFICATIONS

2. I am a Full Professor of Biophysics with tenure in the T.C. Jenkins Department of Biophysics at The Johns Hopkins University, a position I have held since 2013. My research is motivated by the power that a deep understanding of the physics/biology intersection can bring to disease, evolution and biological design.

3. I obtained my Ph.D. in Biochemistry & Molecular Biology from Georgetown University in 1993. From 1993-1994, I served as a Postdoctoral Associate in the Department of Molecular Biophysics & Biochemistry at Yale University. In 1996, I completed a two-year NIH Postdoctoral Fellowship in the Department of Molecular Biophysics & Biochemistry at Yale University. From

1997-1998, I was an Associate Research Scientist in the Department of Molecular Biophysics & Biochemistry at Yale University.

4. From 1999-2000, I was a Research Scientist in the Department of Molecular Biophysics & Biochemistry at Yale University. After leaving Yale University, I served as an Assistant Professor in the T.C. Jenkins Department of Biophysics at The Johns Hopkins University. In the Spring of 2007, I was a Visiting Academic with Professor Carol Robinson in the Department of Chemistry at the University of Cambridge. In 2007, I was promoted to an Associate Professor with Tenure in the T.C. Jenkins Department of Biophysics at The Johns Hopkins University. I held this position until my promotion to Full Professor with tenure in the T.C. Jenkins Department of Biophysics at The Johns Hopkins University in 2013.

5. For many years, I have studied the energetics of transmembrane helix-helix interactions. I developed theory to describe their association reactions, defined conditions for “forced cohabitation” of helices in micelles; and discovered thermodynamic coupling in transmembrane helix-helix dimerization. Recently, my laboratory has turned its focus to the water-to-bilayer protein-folding problem. My group also uses molecular simulations to address questions of membrane protein structure in phospholipid bilayers and has applied ODE methods to develop a

quantitative flux model that describes the sorting of membrane proteins in the periplasms of bacterial envelopes.

6. The experimental tools used in my laboratory include an array of biophysical methods including analytical ultracentrifugation (“AUC”), fluorescence spectroscopy, circular dichroism, calorimetry, NMR, and small angle scattering.

7. I teach numerous courses, including Biophysics, Macromolecular Binding, Methods in Molecular Biophysics, and an Advanced Seminar on Membrane Protein Structure Function & Pharmacology.

8. I have published over 90 scientific articles, and I am frequently invited as a guest lecturer. I was the inaugural recipient of the Arne Tiselius Young Investigator Award. I have received a Department of Defense Career Development Award, the 2016 Thomas E. Thompson Award from the Biophysical Society, the 2020 Carl Brändén Award from the Protein Society, and the 2023 Avanti Award from the Biophysical Society.

9. I am the current President of the Biophysical Society. I am a Fellow of the Biophysical Society, the American Society for Biochemistry & Molecular Biology, and the American Association for the Advancement of Science. I have also served on the Executive Councils of the Biophysical Society and the American Society for Biochemistry and Molecular Biology, and have co-chaired

the national meeting of the Biophysical Society (2015) as well as Gordon Research Conferences on Biomolecular Interactions & Methods (2010) and Membrane Protein Folding (2015).

10. Attached as Exhibit 2032 is my curriculum vitae, which summarizes my background, credentials, and includes a list of my publications between 1990 and 2026.

III. MATERIALS CONSIDERED

11. 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. Steven Berkowitz’s declaration (Ex. 1004, “Berkowitz Declaration”). Petition, 32-33; Berkowitz Declaration, ¶¶ 83-86. I considered the Petition, Berkowitz Declaration (Ex. 1004), and exhibits cited in those documents. I also reviewed and considered the materials identified in the list below.

Exhibit	Description
2019	Burnham et al., Analytical Ultracentrifugation as an Approach to Characterize Recombinant AAV Vectors, 26 Human Gene Therapy Methods 228 (2015) (“Burnham 2015”)
2020	F. Collaud et al., Preclinical Development of an AAV8-hUGT1A1 Vector for the Treatment of Crigler-Najjar Syndrome, 12 Molecular

Exhibit	Description
	Therapy: Methods & Clinical Development 157, 166-67 (March 2019)
2021	E. Spies, Assessing the Quality of Adeno-Associated Virus Gene Therapy Vectors by Sedimentation Velocity Analysis, AUC Insights (March 2020)
2025	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)
2026	T. Matsushita et al., Adeno-Associated Virus Vectors Can Be Efficiently Produced Without Helper Virus, 5 Gene Therapy 938-45 (February 10, 1998)
2027	J.F. Wright, Product-Related Impurities in Clinical-Grade Recombinant AAV Vectors: Characterization and Risk Assessment, 2 Biomedicines 80-97 (March 3, 2014)
2028	D. Curran-Everett, Explorations in Statistics: Hypothesis Tests and P-values, 33 Advances in Physiology Education 81-86 (June 2009)
2029	Oliver, Christopher, Diffusion Coefficient and Molecular Weight of Type 5 Adenovirus by Photon-Correlation Spectroscopy, Biochimica et Biophysica Acta, 437 (1976) 589-598 (October 21, 1975)
2030	Tanford, Charles, Physical Chemistry of Macromolecules (1961)
2033	Declaration of Barrie J. Carter (“Carter”)
2034	Curriculum Vitae of Barrie J. Carter
2035	Google Scholar Results for Citations to Burnham et al., Analytical Ultracentrifugation as an Approach to Characterize Recombinant AAV Vectors, 26 <i>Human Gene Therapy Methods</i> 228 (2015) (July 27, 2026)
2036	BioAnalysis, “Empty/Full/Partial Capsid Assay Using Sedimentation Velocity Analytical Ultracentrifugation (SV-AUC),” available at https://bioanalysisllc.com/sv-auc-empty-partial-full-capsid-assay-gene-therapy/ .

Exhibit	Description
2037	Sigma-Aldrich, “Strategies for the Separation and Analysis of Empty and Filled Capsids During Viral Vector Production,” available at https://www.sigmaaldrich.com/US/en/technical-documents/technical-article/pharmaceutical-and-biopharmaceutical-manufacturing/gene-therapy-manufacturing/strategies-separation-analysis-empty-filled-capsids?srsId=AfmBOor6raGiKOeAeg-obMoKHCf4pXXje_9H4YFJM1T8BSW0bX_GKNRe
2038	D.M. Lipinski et al., Clinical Applications of Retinal Gene Therapy, 32 Progress in Retinal and Eye Research 22-47 (2013).
2039	Deposition Transcript of Steven Berkowitz, Ph.D. (July 29, 2026)
2040	A. Roy et al., “Antigenic Differences Among AAV Species of Different Densities” in <i>Replication of Mammalian Parvoviruses</i> (Ward, D., and Tattersall, P., eds.), Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 193-204 (1978)
2041	B. Kokona et al., Particle Size Distribution Resolution Limit of AAV High-Speed Sedimentation Velocity Protocol Relative to Low-Speed Sedimentation Velocity Protocol, Mass Photometry, and Charge-Detection Mass Spectrometry, 114 <i>J. Pharm. Sci.</i> 103878 (2025).
2042	S.A. Berkowitz et al., Rapid High-Resolution Size Distribution Protocol for Adeno-Associated Virus Using High Speed SV-AUC, 689 <i>Anal. Biochem.</i> 115482 (2024).
2043	S.A. Berkowitz & T. Laue, Boundary Convection During Sedimentation Velocity in the Optima Analytical Ultracentrifuge, 631 <i>Anal. Biochem.</i> 114306 (2021).

IV. LEGAL STANDARDS

12. 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 obvious.

A. Obviousness

13. 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 a 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:

- 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.”

14. I have been informed that for a claimed invention to be considered obvious over a combination of references, a 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 a 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.*, 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.

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

16. I have been informed that for a combination of references to render the claimed invention obvious, a 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.

17. 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.

18. 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 a POSA would have followed, as evidenced by the pertinent prior art.

V. PERSON OF SKILL IN THE ART

19. I have been informed that anticipation and obviousness are analyzed from the perspective of a POSA in the relevant field at the time of the effective filing date of the '894 Patent, which was January 20, 2015. I understand that the Petition and Berkowitz Declaration applied the following definition for a POSA in their analysis:

A POSA working in the field of the '894 Patent in January 2015 would have had at least a Ph.D. in biology, chemistry, biochemistry, biophysics, pharmaceutical sciences, or a related discipline, with two or more years of industry, laboratory, and/or clinical experience in analyzing or characterizing biomolecules, including viruses or viral vectors. Alternatively, a POSA would have had at least a Master's or Bachelor's Degree in biology, chemistry, biochemistry, biophysics, pharmaceutical sciences, or a related discipline, with a corresponding number of additional years of experience analyzing or characterizing biomolecules, including viruses or viral vectors.

Petition, 32-33; Berkowitz Declaration, ¶ 83-86.

20. I understand that such a person may work as part of a team with someone familiar with the development, formulation, and/or administration of viruses or viral vectors for gene therapy and quality standards required for such products. For this Declaration I will apply the same POSA definition as applied by

Petitioner (“Sarepta”) and Dr. Berkowitz. However, I reserve the right to apply a modified definition as the case proceeds.

VI. SUMMARY OF MY OPINIONS

21. **Sarepta’s Ground 1:** A POSA as of January 2015 would not have been motivated to combine Le Bec (Ex. 1006) and de la Maza (Ex. 1007), in view of Sommer (Ex. 1010), to determine genome size of fragmented genomes from sedimentation coefficients obtained by sedimentation velocity analytical ultracentrifugation (“SV-AUC”) analysis nor to determine the quantity of one or more species of the variant viral particles. A POSA would not have had a reasonable expectation of success in doing either of these things.

22. This opinion is based on at least three key technical facts: (1) Le Bec’s data show no statistically significant linear relationship between sedimentation coefficient and genome size ($p = 0.091258$); (2) Carter laboratory publications, including de la Maza, demonstrate that sedimentation coefficients did not vary linearly with genome size for AAV particles (*see* Ex. 2033); and (3) the prior art as a whole teaches that a POSA must consider the effect of friction on rAAV particle sedimentation rates.

23. For claim 2, a POSA would not have considered Le Bec’s SV-AUC plots to support Sarepta’s contention that “each species of the individual variant viral particles is fully resolved.” Remaining claims 3-7, 10-11, and 20-30 all

depend from claim 1, so Sarepta's inability to demonstrate obviousness means these narrower claims are also not obvious. Thus, it is my opinion that the combination of Le Bec and de la Maza, and in view of Sommer does not establish the obviousness of claims 1-7, 10-11, and 20-30.

24. **Sarepta's Ground 2:** A POSA would not have found claims 12-19 obvious over Le Bec and de la Maza, in view of Sommer and Cole (Ex. 1009). I understand that challenged claims 12-19 depend from claim 1. I further understand that the only challenge to claim 1 is in Ground 1. Because the combination of Le Bec and de la Maza in view of Sommer does not render claim 1 of the '894 Patent obvious as explained in my opinions for Ground 1, it is my opinion that the combination of Le Bec and de la Maza, in view of Sommer and Cole, does not render claims 12-19 of the '894 Patent obvious.

25. **Secondary Nonobviousness Considerations:** The development of a method that relies on a linear relationship between sedimentation coefficient and the size of a genome contained within a particle, which allows a POSA to determine the quantity of rAAV species in a heterogeneous mixture of rAAV particles in a single experiment, was surprising and unexpected. Based on prior art references, including Le Bec, de la Maza and other Carter laboratory publications, and other references related to SV-AUC, a POSA would not have expected there to have been a linear relationship between sedimentation coefficient and genome size.

The discovery and application of the claimed method to quantify rAAV particle concentration in a single experiment was surprising and unexpected. Further, the claimed invention has been acknowledged by those in the industry as an important advance in quantifying rAAV particles in a gene therapy composition. Finally, the claimed method satisfies a long-felt need in the field of gene therapy for a single experiment capable of characterizing and quantifying rAAV species in heterogeneous mixtures of rAAV particles. This is critical to ensuring the safety and efficacy of gene therapy compositions. For example, fragmented genome-containing rAAV particles had been observed to cause immunotoxic effects. Thus, their accurate detection and quantification are important for the safety and efficacy of gene therapy.

VII. BACKGROUND SCIENTIFIC CONCEPTS RELEVANT TO THE '894 PATENT

26. The following sections describe the scientific concepts directly relevant to this declaration and the '894 Patent.

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

27. A 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. 2033, ¶ 26; Ex. 2027, 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. 2027, 86-87.

28. For clinical applications, the presence of rAAV particles containing variant genomes poses risks; Dr. Carter details those risks in his declaration. Ex. 2033, ¶¶ 25-29. 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. 2027, 87; Ex. 2033, ¶ 27. 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. 1029, 15, 18, 19-20; Ex. 2033, ¶ 27. The EMA raised concerns about the assays used to identify those impurities in particular, stating that the “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 during treatment ... specifications were considered unacceptably high.” Ex. 1029, 18.

29. Indeed, the scientific literature documented how “excessive empty capsids are immunotoxic” and “AAV virions packaged with full or partial viral genomes, are more immunotoxic.” Ex. 1026, 6. Thus, a POSA would have been

concerned with monitoring empty capsids and partially full capsids, because of their potential elevated immunotoxicity. Ex. 2033, ¶ 28. A 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. *Id.* It was not enough to merely identify heterogeneity, rather it needed to be quantitatively analyzed. Ex. 2033, ¶ 29. Because of limitations with existing analytical assays, this was a difficult challenge as of the effective filing date. *Infra*, § XII.C.

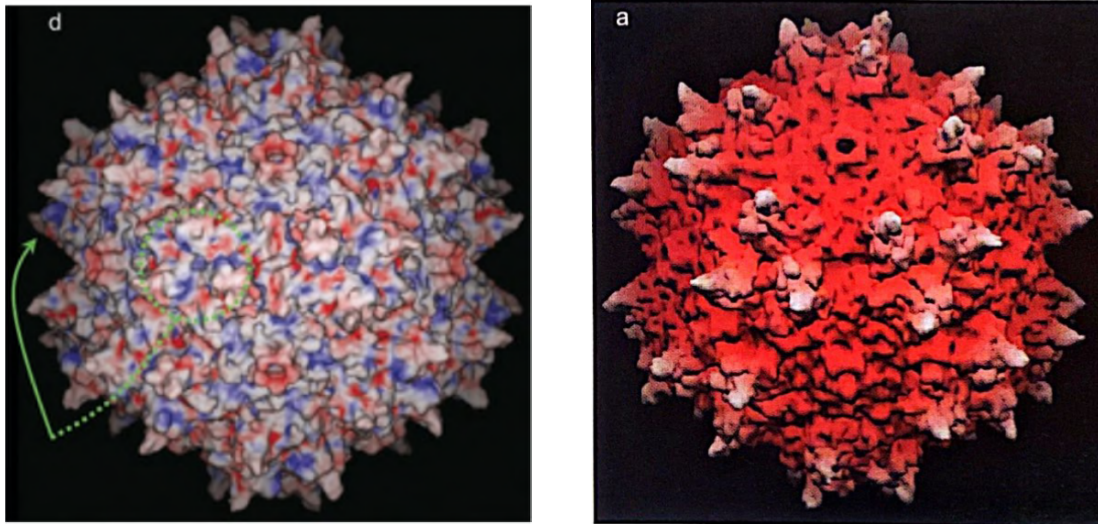
B. rAAV Structural Considerations

30. rAAV vectors are “composed of an icosahedral assembly of 60 capsid proteins (VP1, -2, -3) assembled together in a defined stoichiometry.” Ex. 2027, 81. Despite this expected single structure, “AAV product-related substances may include individual particles with minor variation in the stoichiometry of VP1, -2 and -3 that still retain infectivity or limited heterogeneity in the 5' ends of expression cassettes packaged within AAV [31].” Ex. 2027, 84. A detailed crystallographic analysis of AAV-2 published in 2002 by Xie et al. 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. 2025, 10409 (internal citations omitted). The “threefold-proximal spikes” can be seen in Figure 3d, which shows the electrostatic surface potential of AAV-2 calculated with SPOCK running from -10 (red) to +10 (blue). Ex. 2025, 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 3 equivalent patches join at each threefold (circled).”).



Ex. 2025, Fig. 3d; *see also* Ex. 2025, Fig. 3a. Since the differences between the VP-1, -2, and -3 capsid proteins include protein lengths and functional group polarities, any variation in capsid stoichiometry would lead to differences in the

rAAV shape and surface potentials leading to differences in frictional drag in solution.

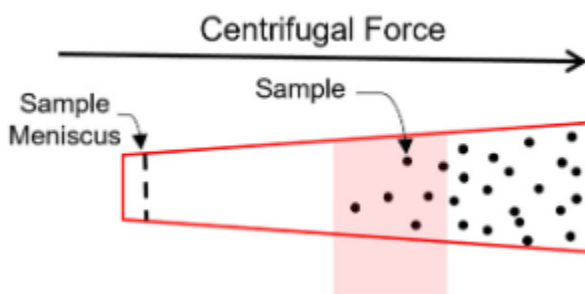
31. Notably, spike features (fiber proteins) on another type of viral particle, adenovirus particles, were observed to impact their AUC analysis in Berkowitz's 2007 publication. Ex. 1023, 18 (Adenoviral "spike projections must impart a significant amount of frictional drag on the overall adenovirus particle."). Some particles were observed to lose these spikes, which impacted their sedimentation coefficient. *Id.*, 31-32. Compositional variations in the stoichiometric capsid proteins would likely lead to changes in the frictional drag for rAAV particles as well.

32. 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. 1026, 6. 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. 1026, 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.*

33. Changes to the capsid, including variations in the stoichiometry of the proteins in the capsid, are relevant to AUC analysis because they impact the sedimentation coefficient in a $c(s)$ distribution.

C. Analytical Ultracentrifugation

34. SV-AUC measures the sedimentation rate of particles migrating through solution under centrifugal force generated at high rotational speed. The image below captures a snapshot of the sedimentation process as particles migrate within the chamber.¹



When centrifugal force is applied, the particles begin to sediment towards the bottom of the cell. Buoyant and frictional forces oppose this sedimentation. Different particles experience different buoyant and/or frictional forces. The process of sedimentation and diffusion creates a concentration boundary near the

¹ See <https://www.beckman.com/resources/technologies/analytical-ultracentrifugation/experiments>

meniscus, which moves in the direction of the centrifugal force along with the particles in the sample.

35. Balancing the forces acting on a particle 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), known as the Svedberg equation, reproduced from Cole 2008 (Ex. 1009):

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

Ex. 1009, 146.

36. A POSA would have been aware that the frictional coefficient in this equation is the translational frictional coefficient, f_T , which depends on two unknown factors: (1) the deviation of the shape of the hydrodynamic particle of the sphere; and (2) the extent of hydration, where:

$$f_T = 6\pi n \frac{f}{f_o} \left[\frac{3M(\bar{v}_2 + \delta_1 \bar{v}_1)}{4\pi N_A} \right]$$

which is often discussed in an equivalent form as the radius of hydrated sphere of volume, V_h :

$$V_h = \frac{4\pi R_o^3}{3} \text{ where } R_o = \left[\frac{3M(\bar{v}_2 + \delta_1 \bar{v}_1)}{4\pi N_A} \right]^{1/3}$$

where $\frac{f}{f_o}$ represents the shape factor (deviation from a sphere) and the δ_1 term represents bound water. Ex. 2030. Even if the overall shapes of the empty, partially filled and full viruses were not distinguishable by EM, the hydration unknown remains. Oliver et al. measured the hydrodynamic radius of Type 5 adenovirus and noticed it was quite large and noted that “it seems reasonable to assume that the greater part of the discrepancy must lie in the association of water with the virion.” Ex. 2029, 597. Note that the same translational frictional coefficient is a variable in the translational diffusion coefficient, which a POSA would also know.

37. A POSA would have been aware of the possibility that not all rAAV particles would have been assumed to have identical surfaces. Berkowitz 2007 studied another type of viral particles, adenovirus particles. Berkowitz 2007 observed that some “adenovirus monomers [may] have lost their fiber spikes” resulting in “significantly reduced hydrodynamic drag” decreasing friction, therefore leading to “a higher sedimentation coefficient than the intact adenovirus particle.” Ex. 1023, 31. A 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. Additionally, Berkowitz 2007 indicated that hydration could impact the frictional coefficient. Ex. 1023, 18. A POSA would have been aware that any change to the

molecular structure or shape of the viral surface, such as the loss of fiber spikes, can also impact the hydration. In testimony, Dr. Berkowitz explained the complex interactions between particle and solvent, including an “adhesion” force from “dragging additional solvent with the particle as it migrates” and “charge” effects. Ex. 2039, 44:16-47:8. A POSA would understand that such chemical interactions could impact a particle’s frictional coefficient.

38. In addition, the changes to the surface of an rAAV 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. 1023, 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 of a particle would likewise increase the *s* value for that particle. Thus, from Berkowitz 2007, a 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. The Petition and Berkowitz Declaration ignored these issues and made assumptions about the uniformity of the structure of rAAV particles that a POSA would not have made. *See infra*, § X.B; Petition, 45; Berkowitz Declaration, ¶¶ 144-181.

D. Absorbance Detection for AUC

39. AUC instruments at the time of the '894 Patent's effective date often used absorbance optical detection systems to detect particles. Ex. 1025, 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.*

40. Absorbance at a wavelength of 276 nm was the method employed by the Le Bec reference for analyzing rAAV particles by AUC. Ex. 1006, 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 (or molar) extinction coefficient of that material; c is the weight (or molar) concentration of those species; ℓ is the path length.

$$A = \epsilon c \ell$$

Ex. 1009, 152. Thus, where the Beer–Lambert law holds, the absorbance signal is directly proportional to solute concentration. Ex. 1009, 152.

41. Absorbance data is typically used with molar 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 '894 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 [detected signal] of empty capsids and full genome capsids from an 80%/20% mixture," which can be used to determine concentration. Ex. 1001, 8:24-35.

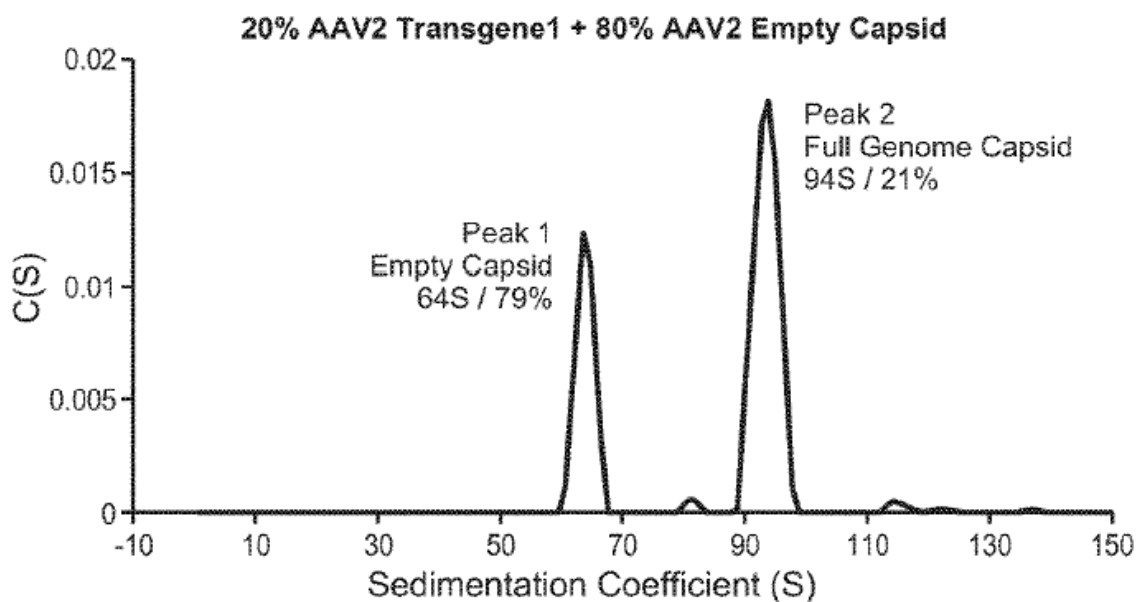


FIG. 1B

Ex. 1001, Fig. 1B. What stands out about this figure is that the peak corresponding to empty capsids, present in four-fold excess to the full genome capsids, has a visibly smaller area than the full genome capsid peak. If the peak area reflected actual molar concentration, the empty capsid peak at 64 S would have been four times the area as the full genome capsid peak at 94 S. However, this is not the case with the 94 S peak having what appears to be a larger area than the 64 S peak. This

discrepancy is attributable to the different capacities to absorb light (*e.g.*, molar extinction coefficients) of empty capsid and full rAAV particles at the absorbance detection wavelength. The application of the extinction coefficient at this wavelength adjusts for this difference and thus is useful for determining the relative concentrations of the two particles in the sample.

42. For genome-containing rAAV particles, which are a combination of protein (from the capsid) and DNA (genome), the extinction coefficient depends on the amounts of protein and genome in a given particle. Ex. 1010, 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 $6.31 \times 10^6 \text{ M}^{-1} \text{ cm}^{-1}$, respectively.”); Ex. 1001, 24:44-47. To estimate extinction coefficients for variant genomes (*e.g.*, rAAV particles containing partial or fragmented genomes), a POSA would have needed to know the respective molecular weights of both the protein and encapsidated genome. Ex. 1010, 127. At the time of the effective filing date, there was no known way to make such a determination. As explained below, the inventors of the ’894 Patent identified how to use AUC to determine the genomic molecular weight of rAAV particles.

E. Assessing Linearity from Experimental Data

43. Assessing linearity in experimental data requires both visual inspection and quantitative analysis. A scatter plot provides the initial indication of whether a linear trend may exist, allowing identification of the general trend and potential outliers. Linear regression analysis then fits a line through the data, yielding the slope, intercept, and R^2 value (coefficient of determination), which quantifies the fraction of variance in the dependent variable explained by the independent variable. R^2 ranges from 0 to 1, where values closer to 1 indicate stronger linear associations. For small data sets, R^2 is particularly uninformative regarding the statistical significance of the fit.

44. The p-value quantifies the probability of observing the experimental result under the null hypothesis, that is, the probability that the observed pattern arose from random chance rather than a genuine relationship. Ex. 2028, 82. A low p-value constitutes strong evidence against the null hypothesis; a high p-value indicates insufficient evidence to reject it. In linear regression, the null hypothesis posits that the slope is zero, and the alternative hypothesis posits that the slope is significantly different from zero, confirming linearity of the data. The standard significance threshold is $p \leq 0.05$, corresponding to less than a 5% probability that the relationship is attributable to chance (95% confidence level). Ex. 2028, 82. This 95% threshold is the universally accepted standard for statistical significance

across the sciences. More stringent thresholds— $p \leq 0.01$ (99% confidence) and $p \leq 0.001$ (99.9% confidence)—apply in situations demanding stronger evidence to reject the null hypothesis.

45. A linear relationship is generally considered statistically significant when the p-value of the slope of the linear regression is 0.05 or less. Non-linear models can be hypothesized in cases when the linear regression analysis is not significant, and non-linear regression methods are used to confirm the statistical significance of those models as well by relying on the resulting p-values for the parameters in those fits.

VIII. THE '894 PATENT

46. The '894 Patent (Ex. 1001), titled “Analytical Ultracentrifugation for Characterization of Recombinant Viral Particles,” represents an important advance in the manufacture and purification of rAAV preparations, by providing the gene therapy field with a method to reliably identify and quantify concentrations of each species (*e.g.*, empty capsids, capsids containing partial or fragmented genomes, and fully intact capsids) present in an rAAV preparation. The claimed method remains the industry standard for detection of rAAV species and is a practical and useful release assay. *See infra*, § XII.B.

47. The prior method for detecting rAAV species, Southern blot, used a specific complementary DNA probe sequence to detect rAAV species that could

miss the presence of key fragmented genome species. Ex. 1001, 1:44-58. In contrast, the claimed AUC method provides a single experiment to detect and quantify multiple rAAV species, including species containing incomplete genomes, that either evaded detection or required multiple experiments for accurate detection. *Id.* Because of the inventors' innovative application of AUC to analyze rAAV preparations, AUC has become a critically important tool for the development of rAAV-based gene therapies.

A. Disclosed Innovation of the '894 Patent

48. “[A] major problem in producing pure AAV vector preparations is the presence of capsids with ***partial or fragmented genomes***.” Ex. 1001, 48:38-40 (emphasis added). “[T]he variant genomes” include partial or fragmented genomes that “are smaller than the intact recombinant viral genome (e.g., truncated genomes).” *Id.*, 25:19-21.

49. The '894 Patent provides a single method, using AUC boundary sedimentation under velocity conditions, which can analyze a sample in a matter of hours and provide quantitative characterization of rAAV preparations. *Id.*, 43:49-59. During rAAV production, for example, AUC facilitates “monitor[ing] [rAAV preparation] at time intervals (e.g., one or more times).” *Id.*, 11:45-51. Multiple innovations contributed to the inventors' success.

50. The '894 Patent discloses and claims “measuring the rate of movement or migration of the sedimenting boundaries, wherein movement or migration of each species of individual viral particles in the heterogeneous mixture of viral particles results in distinct sedimenting boundaries, each distinct sedimenting boundary corresponding to a resolvable species of viral particle.” *See, e.g.*, Ex. 1001, claim 1. The result can be fitted by the “Lamm equation, and the resulting size distribution is a ‘distribution of sedimentation coefficients’ that looks like a chromatogram.” *Id.*, 24:25-55. The inventors identified sedimentation velocity conditions that resulted in a plot where each individual rAAV species was present as a separate and distinct peak, each containing varying levels of genome encapsidation. *Id.*, 54:9-18. This peak separation allowed for integration of the peaks to determine each peak’s area (*id.*, 12:25-40), enabling relative quantification of each species present, including capsids comprising partial or fragmented genomes.

1. Overcoming the Challenge of Determining rAAV Species Concentration by AUC

51. A POSA understood, however, that merely integrating peak signal area would not accurately quantify the molar concentration of each rAAV species. To determine the relative concentrations of species within a heterogeneous rAAV preparation using AUC, a POSA must correlate peak area (determined by peak signal integration) to concentration. Ex. 1001, 24:27-31. When absorbance

spectroscopy at a given wavelength (*e.g.*, 280 nm) is applied in AUC methods to monitor sedimentation of viral particles, knowing the proportion of peak area to concentration makes use of additional information about each species, for example, the extinction coefficient (ϵ), because different rAAV species will absorb certain wavelengths of light to different degrees. Ex. 1001, 24:41-44 (“[E]xtinction coefficients are used to calculate molar concentration and the actual percent value of the intact vector peak from absorbance data.”).

52. Extinction coefficients had been determined for empty capsids and fully intact rAAV particles at an absorbance wavelength of 260 nm. Ex. 1001, 24:44-47 (citing Ex. 1010, 127) (“Molar absorbance extinction coefficients for both empty capsids ($\epsilon_{260/\text{capsid}}=3.72\text{e}6$) and intact vector ($\epsilon_{260/\text{vector}}=3.00\text{e}7$) 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” *Id.*, 24:56-59; 45:2-5; 49:57-61. To overcome this obstacle, the inventors determined that:

[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., 24:59-67; *see also id.*, 45:2-8.

53. The application of this innovative method is illustrated in Example 5, which demonstrates the implementation of “AUC ... as a tool to quantify the aberrant packaging of fragmented genomes in rAAV vector preps.” Ex. 1001, 49:39-50:67. To generate an exemplary standard curve, “rAAV vector preps with encapsidated viral genomes of known size were analyzed by AUC.” *Id.*, 49:57-67. Table 5 below shows how to calculate genome size based on the trend line of Figure 8. *Id.*

TABLE 5

S values for rAAV vectors with known genome size.			
Predicted Trend Line			
Sedimentation		Calculated values	
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

Ex. 1001, 50:1-19.

54. Figure 8, which “shows the linear relationship between sedimentation coefficient and vector genome size,” provides the standard curve of Example 5, correlating genome size and S value. *Id.*, 9:36-40. Data plotted and fit in Figure 8 surprisingly “demonstrated a highly linear relationship ($R^2=0.9978$) between sedimentation coefficient and genome size.” *Id.*, 49:66-67.

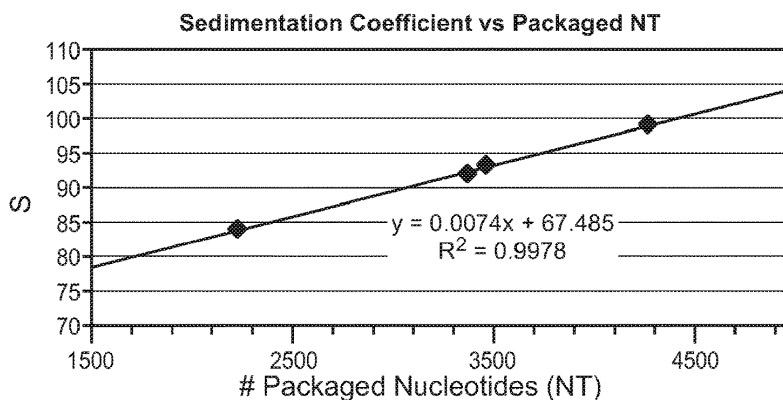


FIG. 8

Id., Fig. 8.

55. Generating the standard curve allowed the inventors to determine the molecular weight, genome size, and extinction coefficient at 260 nm of unknown rAAV species, for example, partial or fragmented species. Ex. 1001, 49:42-50:67 (Example 5, Table 5); Figs. 8, 9A. Once the standard curve was generated, a study was conducted to show “that assessment of capsid genome size using AUC data correlates with assessment of genome size by Southern blot.” *Id.*, 9:41-43; 50:21-42.

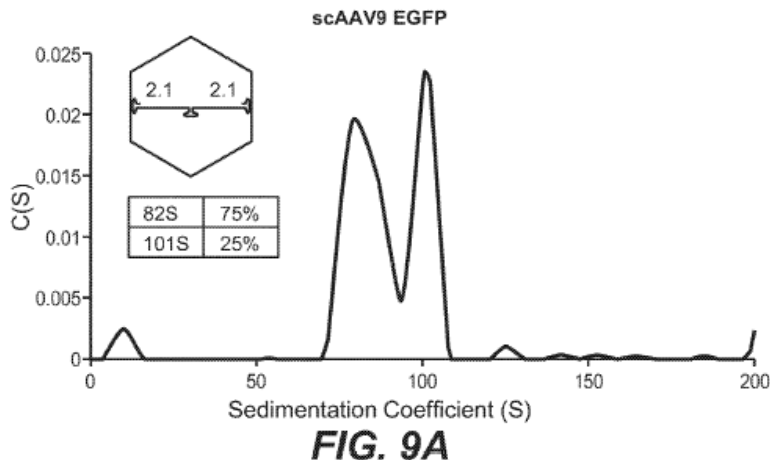
2. One-Step rAAV Preparation Analysis by AUC

56. The inventors applied their method to quantitatively analyze the compositions of several samples to demonstrate how the method identified peaks corresponding to rAAV species containing partial or fragmented genomes and could determine their concentrations.

a. Example 5

57. “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, 50:21-24. 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.*, 50:27-31.

58. In Figure 9A, 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-complementary] 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 (%).”



Id. at 9:41-50; 50:21-31; FIG. 9A. A POSA would have understood that C(s) distribution peaks having lower sedimentation values than peaks for empty capsids do not correspond to rAAV species. Ex. 1023, 27 (The “slow-migrating material most likely corresponds to smaller fragments of [empty capsid or 'EC'] and virus particles and possibly aggregates of viral proteins....”). The inventors applied the standard curve to approximate the size of the genomes of the rAAV species corresponding to the 101S and 82S peaks. *Id.*, 50:28-36. 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 101 S peak double-stranded species and 75% for the 82 S peak single stranded species. *Id.*, 50:28-36; FIG. 9A. 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.

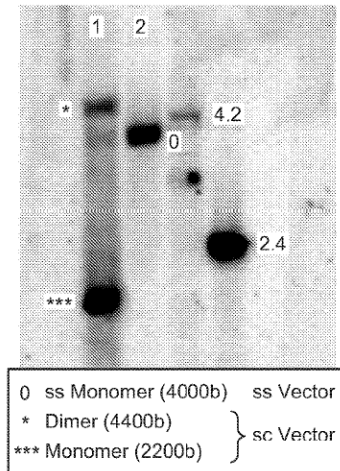


FIG. 9B

Id., 50:43-46; FIG. 9B.

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

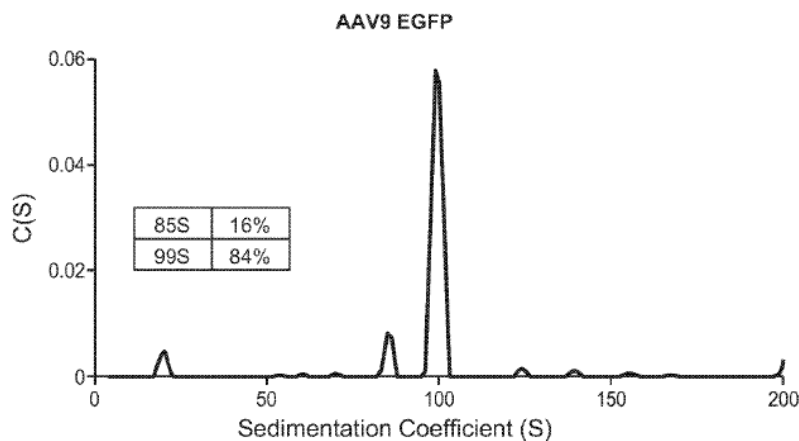
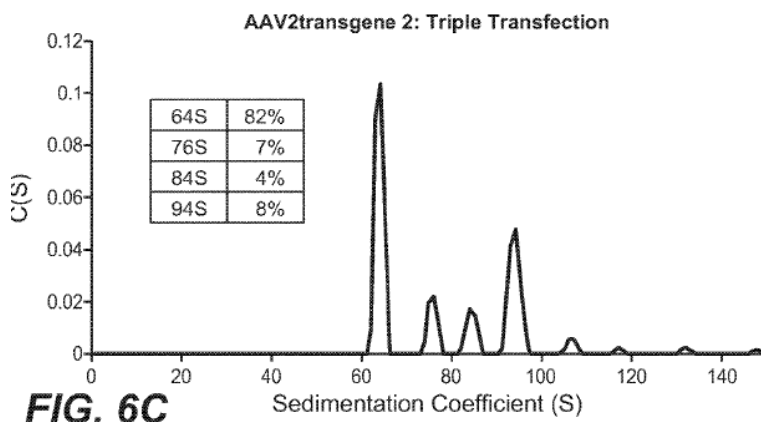
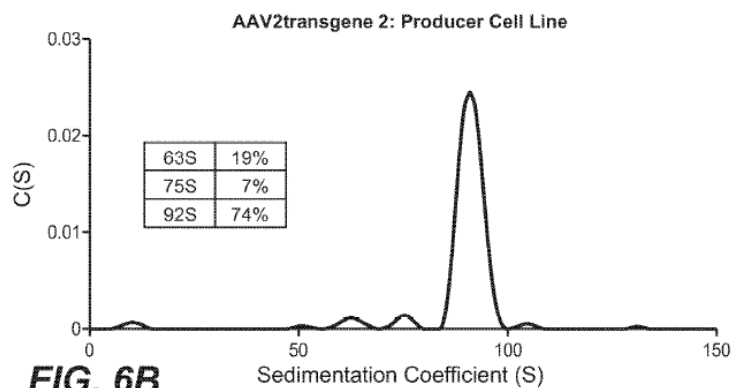


FIG. 9C

Ex. 1001, 50:48-52; FIG. 9C. This result also agreed with the Southern blot analysis, showing this predominant species in lane 2 (0) of FIG. 9B. *Id.*, 50:56-59; FIG. 9B. Absent from the Southern blot, however, is the second single stranded rAAV species.

b. Example 3

60. The inventors compared the heterogeneous rAAV preparations produced by two rAAV production methods, which both resulted in the production of empty capsids, partially and/or fragmented capsids, and fully intact capsids. *Id.*, 48:30-49:4; FIGs. 6B-C (reproduced below).

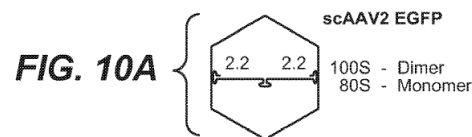


61. The fragmented genomes were identified to have peaks at 75 S (FIG. 6B), 76 S and 84 S (FIG. 6C). Ex. 1001, 48:51-60. The inventors determined the concentrations of the fragmented species identified in their experiments, observing that “[t]he AUC profiles of vector preps produced by these methods were remarkably different.” *Id.*, 48:51-52. “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 (75 S species, 7%).” *Id.*, 48:52-56. The “triple transfection method” resulted in a less successful outcome: “82% of the capsids ... were empty, 64 S species (FIG. 6C), with 11% of capsids containing a fragmented genome (76 S and 84 S species) and only 8% of capsids having a full genome (94 S).” *Id.*, 48:56-60. The identification and quantification of each species illustrate the value of the inventive method and its utility for determining the relative advantage of rAAV preparation and manufacturing procedures.

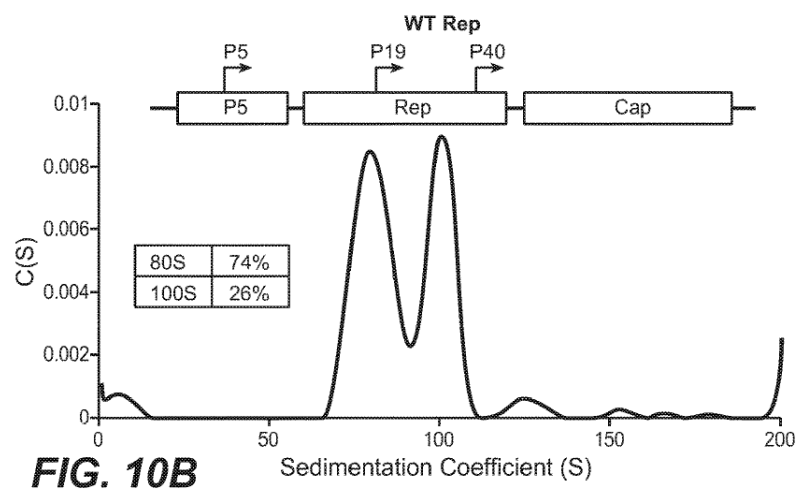
c. Example 6

62. Example 6 demonstrates the application of the AUC 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 method was used to analyze how modification to the helper factor in a vector preparation for the “self-complementary AAV vector harboring an EGFP

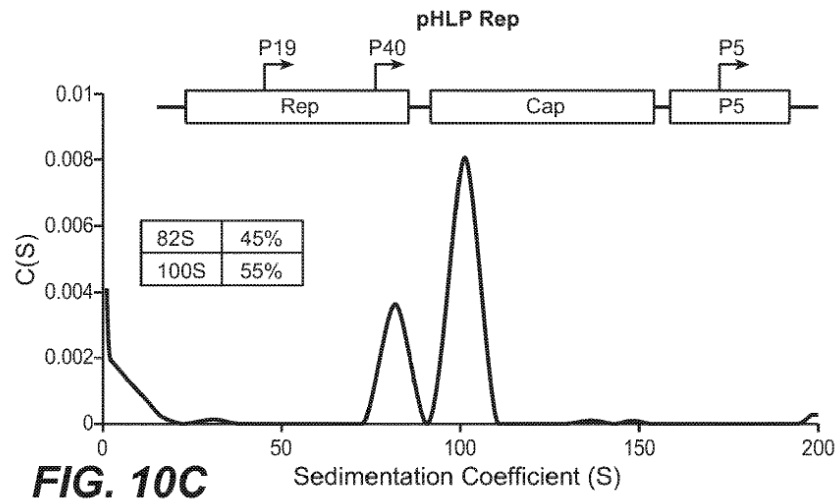
transgene” impacted rAAV production. Ex. 1001, 51:13-24. The experiments compared a “first method (FIG. 10B)” for rAAV preparation employing an unmodified “helper plasmid” (the “WT Rep” construct) and a “second method (FIG. 10C)” where “the helper was modified” (the “pHLP Rep” construct). *Id.*, 51:15-21. “The full scAAV2 EFGP capsid was predicted to have a sedimentation coefficient of 100 S in dimeric genome form and 80 S in monomeric genome form (FIG. 10A).” *Id.*, 51:21-24.



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



Ex. 1001, 51:31-35, Fig. 10B. In contrast, the modified helper of the pHLP Rep construct resulted in a ratio of rAAV particles containing dimeric genomes (~100 S peak) at 55% versus monomeric particles (~82 S) at 45%.



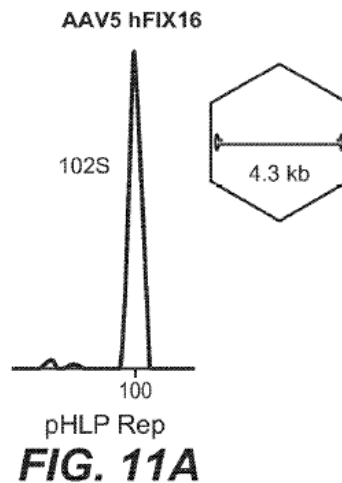
Id., 51:25-29. “These results uncovered a significant difference in genome packaging induced by” modifying “the helper plasmid.” *Id.*, 51:35-38. Detection of this result was facilitated by the AUC analysis differentiating between rAAV species containing different levels of genome encapsidation.

d. Proof of Concept: Single Step SV-AUC Analyses for rAAV Particle Concentrations

64. The power of the disclosed AUC method 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, (FIGS. 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, 51:39-44.

65. “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 101 S. AUC analysis of AAV2/5FIX made in the presence of” the modified pHLP Rep helper “revealed a homogenous profile with the majority of the vector (90%) sedimenting with an S value of the expected size.”



Id., 51:44-50, FIG. 11A. “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 101 S, 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 ~104 S.”

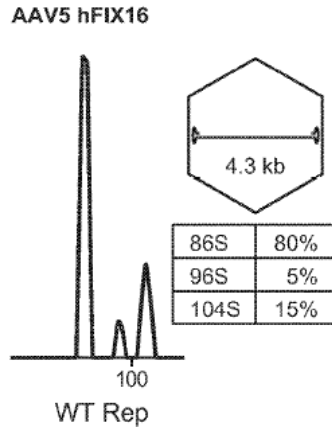


FIG. 11B

Id., 51:50-60, FIG. 11B.

66. AAV5SMN vectors made using the 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 101 S consistent with packaging of a genome of the predicted size of ~4.4 kb.”

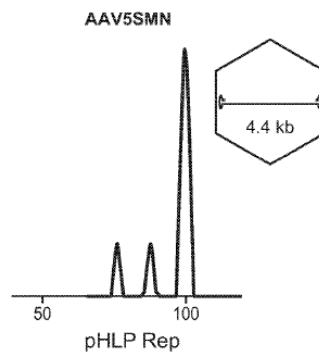
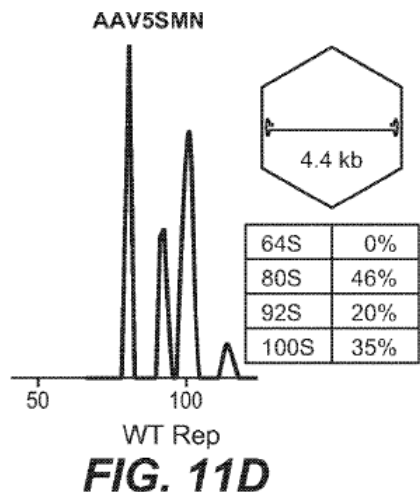


FIG. 11C

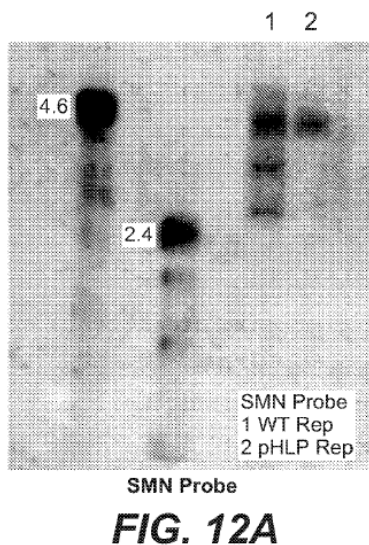
Ex. 1001, 51:61-52:1, FIG. 11C. “In contrast, the AUC profile for the same vector genome packaged using ‘wild-type’” WT Rep helper “revealed three distinct AAV vector species, with sedimentation coefficients of 100 S..., 92 S (representing a

fragmented genome of approximately 3300 [nucleotides]) and 80 S (representing a fragmented genome of 2000 nucleotides).”

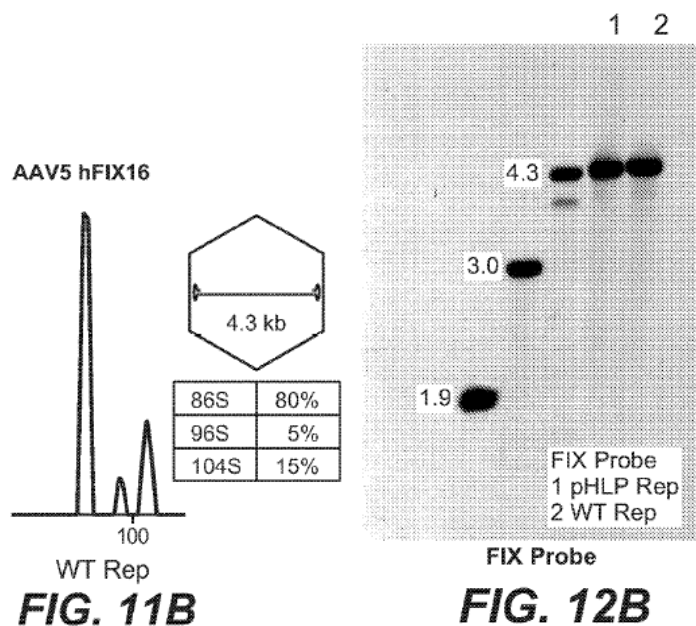


Id., 52:1-10, FIG. 11D.

67. Southern blot analysis of the AAV5SMN preparations was consistent with the AUC results for the pHLP helper (FIG. 11C) 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). Ex. 1001, 52:11-27.



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.*, 52:24-27 (emphasis added).



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

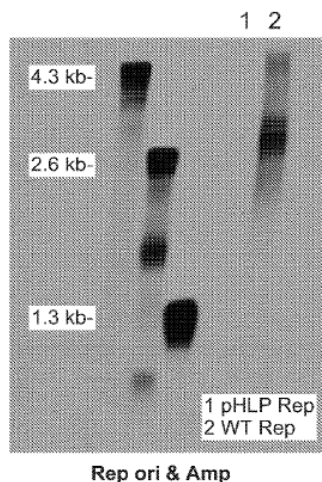


FIG. 14B

Ex. 1001, 52:36-45, FIG. 14B. 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. *Id.* 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 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.

69. The '894 Patent also applies the AUC method to analyze “the heterogeneity of AAV vectors produced using oversized genomes.” Ex. 1001, 52:60-63. “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.*, 52:63-67. Oversized rAAV genomes “have been shown to be fragmented into subgenomic-length DNA.” *Id.*, 52:56-60. 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] 93 S 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 105 S. There was no evidence of packaging of a full-length 5.4 kb genome, which was predicted to sediment at 108-109 S.”

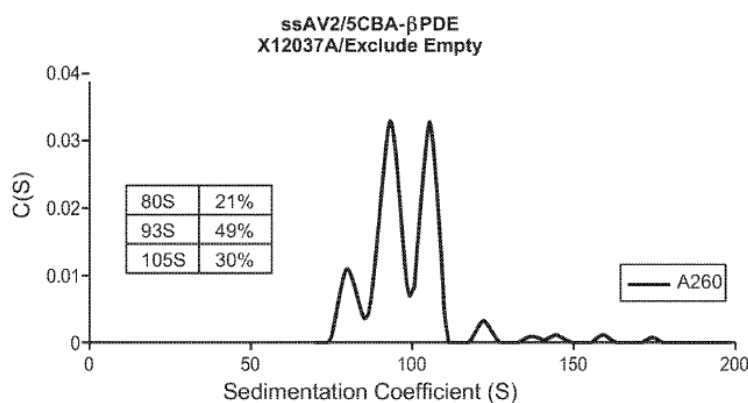
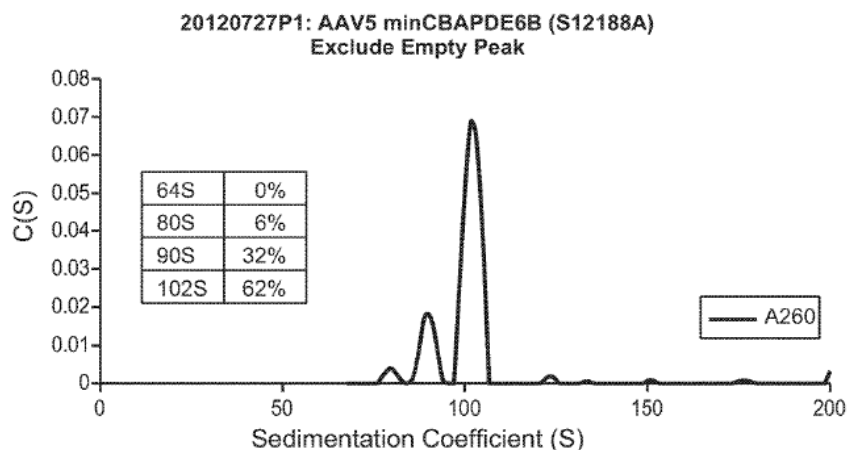


FIG. 15A

Id., 53:4-12; FIG. 15A. “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., 53:12-20; FIG. 15B. “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.*, 53:17-20.

B. Prosecution History

70. The Examiner allowed the '894 Patent (Ex. 1002, 21-30) after Genzyme explained that the claimed method, when applied under the specific conditions set forth in the claims, demonstrated that SV-AUC could in fact be used to resolve and characterize disparate rAAV species, including those with fragmented genomes, in a heterogeneous mixture:

The inventors ... discovered that sedimentation coefficients can be used to accurately calculate the molecular weight of the genomes in every viral particle of the preparation, including the molecular weights of the fragmented genomes. The linear relationship between sedimentation coefficients and molecular weights of genomes was not disclosed in Le Bec.

Ex. 1002, 146; *see also* Ex. 1001, 9:36-40. Independent claim element 1(iii) (claim element 96(iii)) recites “determining the genome size of one or more species of the individual variant viral particles in the heterogeneous mixture of viral particles.”

Ex. 1002, 144.

71. In a January 18, 2024 non-final rejection, the Examiner rejected pending claim 96 (claim 1 of the '894 Patent) as anticipated and obvious. The Examiner explained that Le Bec “fails to teach generation of a standard curve to determine the size of AAV particles with genomes in unknown AAV samples” but that “all of these steps would be routine optimization steps to one of skill in the art, as evidenced by the teachings of McLaughlin, Cole, and [Berkowitz (2007)].” Ex. 1002, 177. The Examiner explained that the prior art “teach[es] expected sedimentation coefficients for empty and full AAV were known, thus giving a reasonable expectation of success to a skilled artisan in generating a standard curve of other viral particles based on sedimentation coefficients, thus rendering the limitations ... obvious.” Ex. 1002, 180.

72. In its April 9, 2024 response, Genzyme responded that the cited references do not teach ““determining the genome size of one or more species of the individual variant viral particles in the heterogeneous mixture of viral particles.”” Ex. 1002, 141. Moreover, because the sedimentation coefficient depends on multiple factors including “molecular weight ... and also on molecular

shape,” a POSA would not have expected a linear correlation between sedimentation and molecular weight. Ex. 1002, 144-146. After Genzyme’s response, the claims were allowed on May 2, 2024. Ex. 1002, 21.

C. Challenged Claims

73. Claim 1 of the ’894 Patent is an independent claim. Therefore, all claims share the elements recited by claim 1. Claim 1 recites, in part,

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

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

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

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

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

Ex. 1001, 55:1-26. Challenged claims 2-7 and 10-30 depend from claim 1.

74. Dependent claim 2 recites “wherein each species of the individual variant viral particles in a heterogeneous mixture of viral particles is fully resolved.”

75. The remaining Challenged Claims depend from claim 1.

76. Sarepta’s Grounds do not establish the unpatentability of claim 1 and 2. Because all claims depend either directly, or indirectly, from claim 1, all Challenged Claims are not unpatentable.

IX. CLAIM CONSTRUCTION

77. 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.

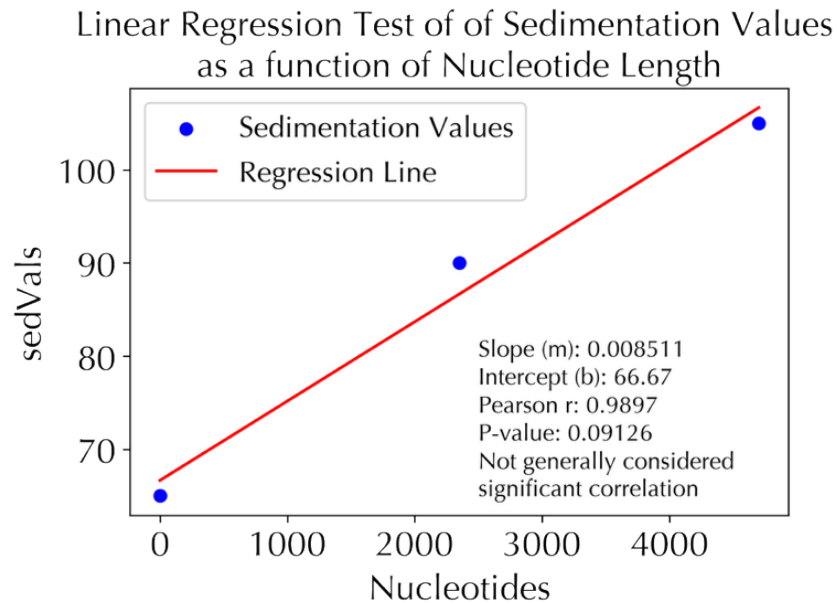
X. A POSA WOULD NOT HAVE UNDERSTOOD OR EXPECTED A LINEAR RELATIONSHIP BETWEEN SEDIMENTATION COEFFICIENT AND GENOME SIZE

78. The method developed by the inventors: applying SV-AUC under defined boundary sedimentation velocity conditions to rAAV preparations, defied the reasonable expectations of a POSA with expertise in SV-AUC. When applied under the specific conditions recited in the claims, this method demonstrated that SV-AUC resolves and characterizes all rAAV species in a heterogeneous mixture. A comprehensive assessment of the state of the art as of the effective filing date establishes why a POSA would not have expected a linear relationship between genome size and sedimentation coefficient. No such relationship was known prior to the effective filing date. Only through the inventors' method can those in the field now appreciate and utilize the linear relationship between encapsidated genome size and sedimentation coefficient in AUC. *Supra* § VIII.A.1.

A. Le Bec's Data Does Not Support a Linear Relationship Between Sedimentation Coefficient and Genome Size

79. The contention in the Petition and Dr. Berkowitz's declaration that plotting sedimentation coefficient and packaged nucleotide data in Figure 2 shows a "linear relationship between the sedimentation coefficient and the length" is mistaken. Petition, 53-54 (citing Berkowitz Declaration, ¶ 194). Visual inspection and a mathematical analysis of the Petition's plot refute a linear relationship between sedimentation coefficient and genome size.

80. As an initial matter, with only three data points spanning a limited range, the deviation of each point from the line represents a large percentage of the total variation. A POSA would recognize this as a poor fit, not a confirmed linear relationship.



Petition, 53-54 (citing Berkowitz Declaration, ¶ 194). In contrast, Figure 8 shows all four data points close to the line, spanning a wider range, with consistent residuals. The visual difference in the plots reflects the underlying statistical difference ($p = 0.00118$ vs. $p = 0.091258$).

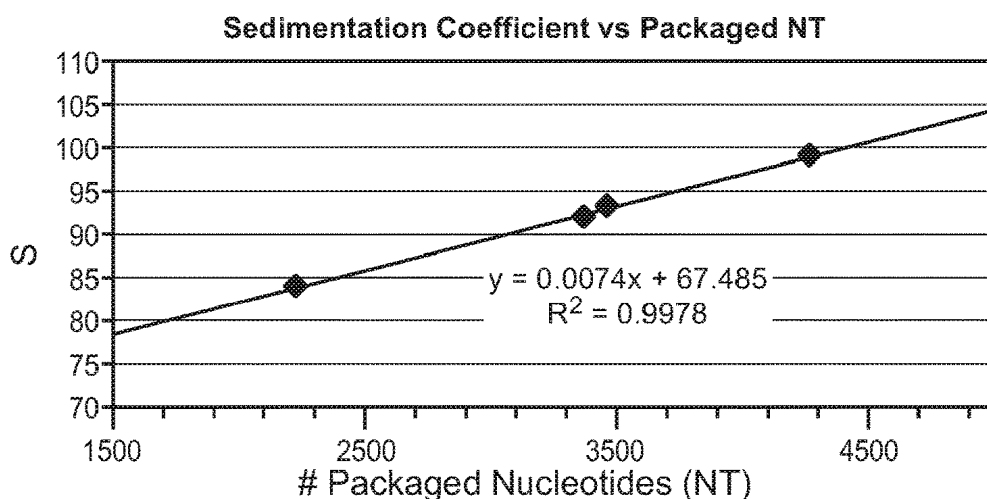


FIG. 8

Ex. 1001, Fig. 8.

81. A best-fit line can always be computed through any set of data points. This is a mathematical operation, not a scientific conclusion. Establishing a linear relationship requires statistical evidence. The standard test is the p-value of the slope, which determines whether the null hypothesis of no relationship can be rejected. The Petition computed a best-fit line through Le Bec's data but did not test for statistical significance and therefore did not establish linearity.

82. A statistical analysis of Le Bec's Figure 2 data confirms a clear lack of linearity. The p-value, a critical statistical measure that indicates the probability of observing the results if no true linear relationship exists between the variables, does not support the linearity of the plotted data. A low p-value suggests strong evidence against the null hypothesis (no relationship), while a high p-value indicates insufficient evidence to conclude a linear relationship exists. The standard significance threshold is $p \leq 0.05$, meaning there's less than a 5%

probability the relationship is due to chance, which corresponds to a 95% confidence level. More stringent thresholds include $p \leq 0.01$ (99% confidence) and $p \leq 0.001$ (99.9% confidence) for situations requiring stronger evidence.

83. The p-value for Le Bec's data is over 0.09, which is well above the standard significance threshold, indicating an acceptance of the null hypothesis that the data do not have a statistically significant linear slope at the 95% confidence level. The lack of the significance of the slope of the linear regression in Le Bec's data and a POSA's expectation that genome size and sedimentation coefficient would not have a linear relationship due to the impact of friction (*supra* §§ VII.B-C) validate the claimed method's nonobviousness.

B. A POSA Would Have Been Aware of the Frictional Forces Experienced by rAAV Particles

84. Density alone does not determine a viral particle's sedimentation coefficient. The sedimentation coefficient depends on both density and friction as the particle migrates through the AUC cell. The Svedberg equation describes these forces: "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:

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

Ex. 1009, 146.

85. Friction, f , is impacted by particle size, shape, and chemical properties (*e.g.*, hydration and solvation) so particles with identical densities but different shapes or different hydration from surface-solvent interactions will have different frictional coefficients. Assuming for the sake of argument that “the relationship between the density of an AAV particle and the size of the genome had been disclosed decades before the earliest priority date for the ’894 Patent” by de la Maza (Petition, 14), this would not mean a POSA expected a linear relationship between sedimentation coefficient and genome size. Cole states that “[a]lthough the $c(s)$ distribution can be converted to a distribution of molar masses [$c(M)$ distribution], the derived masses will only be accurate if there is one dominant species present or if all the species have equal frictional ratios.” Ex. 1009, 166. For heterogeneous mixtures of rAAV particles, it was understood that there was not one dominant species.

86. A particle that loses surface spikes would sediment faster than expected based on its genome size alone due to the decreased frictional drag discussed in Berkowitz 2007. These friction-induced variations would completely obscure any attempt to infer genome size from sedimentation coefficient. A POSA would recognize this as a fundamental obstacle to the approach claimed in the ’894 Patent. Only with hindsight gained from the knowledge supplied by the ’894 Patent’s disclosure is it known that differences in friction between rAAV particles

were insufficient to disrupt the linear relationship between sedimentation coefficient and genome size. But a POSA would not have expected such a relationship. *Infra* §§ X.C-E.

87. De la Maza (1978) used preparative sedimentation equilibrium to isolate individual AAV particle species. Ex. 1007, 195; Ex. 2033, ¶ 37. The Berkowitz Declaration admits that the distribution of particles in sedimentation equilibrium “is a function of its buoyant mass” and is not impacted by friction. Berkowitz Declaration, ¶ 31 (citing Ex. 1024, 241). Dr. Berkowitz’s own publication from 2015 (Ex. 1024, “Berkowitz 2015”), shortly after the earliest priority date of the ’894 Patent, contrasts the claimed method of SV-AUC with SE-AUC. For example, Berkowitz 2015 explains that SE-AUC “was this mode of sedimentation analysis that was most commonly used many years ago to assess the [molecular weight] MW of proteins and their complexes since it was totally independent of the frictional properties of the protein molecule, which made ***MW assessment via SV-AUC much more difficult.***” Ex. 1024, 241.

88. Berkowitz 2015 further explains the complicated picture of forces impacting sedimentation coefficient that Dr. Berkowitz’s declaration minimizes here:

From these simple equations it can be seen that these three forces acting on the sedimenting molecule depend on several factors that are unique properties of the protein being studied. Those factors include (1) the

protein's buoyant mass (which depends on its amino acid composition, glycosylation, and the density of the formulation buffer) (2) its frictional properties, which depend on its overall size and shape (conformation or three-dimensional structure), (3) its hydration (the amount of water bound on its surface) and (4) solvation or preferential solvation effects (exclusion of excipients, or binding of excipients at the protein surface).

Ex. 1024, 216. Dr. Berkowitz's Declaration contradicts his own publication. The Petition and Berkowitz Declaration fail to establish that a POSA working with heterogeneous rAAV particle mixtures would have ignored this collection of factors known to impact protein sedimentation.

C. Carter Laboratory References, Including de la Maza, Indicated a Non-Linear Relationship Between Genome Size and Sedimentation Coefficient

89. Density measurements using sedimentation equilibrium, like those in de la Maza (1978), cannot predict SV-AUC behavior. Sedimentation equilibrium eliminates friction effects, which can be impacted by the size, shape, and chemical interactions of a particle subjected to sedimentation conditions. *Supra* § X.B. The de la Maza (1978) publication does not describe any sedimentation velocity experiments on AAV particles. Rather, its sucrose sedimentation studies are conducted on non-encapsidated DNA. Ex. 1007, 194-95 ("DNA was released from virions by sedimentation in alkaline sucrose gradients,"). But other references

from the Carter laboratory did discuss sedimentation velocity experiment results on intact AAV particles. *E.g.*, Ex. 1013, 1129.

90. Genzyme submits a declaration from Dr. Barrie Carter (Ex. 2033) in support of its Response to address Sarepta’s characterization of his publications. Sarepta describes Dr. Carter’s laboratory as having done “groundbreaking work.” Petition, 2. Sarepta and Dr. Berkowitz highlight that Dr. Carter has been recognized as one of the “fathers of rAAV vectors.” Petition, 11-12; Ex. 1004, ¶¶ 53, 135 (citing Ex. 1040, 491). Several of the references that Sarepta and Dr. Berkowitz have cited in their filings are from Dr. Carter’s research. Ex. 1004, pp. 5-7 (citing Exs. 1007-1008, 1011-1013, 1033-1038). Dr. Carter’s opinion, and several of his references, contradict Dr. Berkowitz’s opinion that “in 2015 would have known that intact, non-aggregated AAV particles have approximately uniform size and shape.” Ex. 1004, ¶¶ 41, 168, *see also id.*, ¶ 170 (“The size and shape of intact, non-aggregated rAAV particles are about the same, regardless of the size of the genome packaged in the capsid.”).

91. I have considered Dr. Carter’s declaration in developing my opinion for this declaration. Dr. Carter’s declaration confirms that AAV particles were understood to assume different structural conformations. Ex. 2033, ¶¶ 35-42. Based on my assessment of Dr. Carter’s opinion and the references I considered, it is clear that a POSA would not have assumed in 2015 “that intact, non-aggregated

AAV particles have approximately uniform size and shape.” AAV particles having both identical and different genomes were observed to vary in size and shape.

92. For this reason, I also disagree with Dr. Berkowitz’s contention that “the frictional coefficient for AAV particles is approximately constant for empty particles, particle containing full genomes, and particles containing fragmented genomes.” Ex. 1004, ¶¶ 41, 170. The fact that rAAV particles would not have been expected to have been the same size and shape means that frictional coefficients would affect their sedimentation coefficients.

93. For example, de la Maza’s March 1980 publication (Ex. 1013) observed two AAV particle species having different densities, AAV (1.41 g/cm^3) and (1.45 g/cm^3), for which “the major components had an identical DNA content and DNA/protein ratio.” Ex. 1013, 1129; Ex. 2033, ¶ 40. The fact that two particles having the same “DNA content and DNA/protein ratio” (Ex. 1013, 1129), but different densities, indicates that density is not exclusively determined by genome size. If genome size alone determined rAAV density, then the Carter laboratory would not have observed two rAAV particle species with the same “DNA content and DNA/protein ratio,” but different densities. Ex. 1013, 1129; Ex. 2033, ¶ 40.

94. The de la Maza (March 1980) publication shows that a POSA would not have assumed that there was a “direct linear relationship” or “known linear

relationship” between the density of an AAV particle and the length of the encapsidated DNA for AAV particles. This contradicts a foundational assumption of Dr. Berkowitz’s opinion repeated throughout his declaration. Ex. 1004, ¶¶ 59-60, 102-103, 132, 138-139, 147, 165, 168, 170, 172, 184, 187, 193, 244. As Dr. Carter explains, a POSA would have understood that the statement “particle density has an approximately direct linear relationship to the strand length” (Ex. 1008, 3202) relates to unpublished results and was meant as a qualitative observation consistent with the proposed mechanism for AAV particle assembly researched by Dr. Carter. Ex. 2033, ¶ 48. A POSA would not have considered an observation made based on unpublished data in the context of a biological mechanism study to support its application to a quantitative SV-AUC assay used to quantify potentially deleterious impurities in a therapy.

95. Also significant, de la Maza’s March 1980 publication shows that the lower density particle AAV (1.41) sediments at a higher rate ($S=111$) than the higher density particle (1.45, $S=109$). Ex. 1013, 1129; Ex. 2033, ¶ 42. This is the opposite of what would be expected if the two particles experienced identical friction. The higher density of the AAV (1.45) particle would have been expected to sediment faster than the lower density particle, because sedimentation coefficient is proportional to buoyant density. *Supra* § VII.C. But the opposite happens. A POSA would have understood that the denser particle is subjected to a

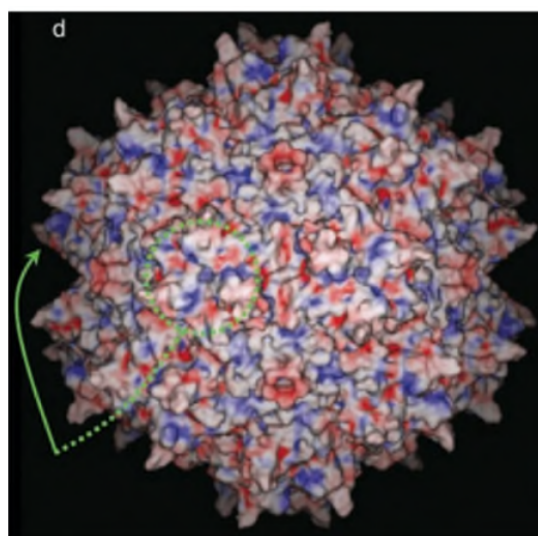
higher frictional coefficient, which offsets its higher density and causes it to sediment at a slower rate than the less dense particle. Dr. Carter and his laboratory attributed this effect to conformational differences between the AAV (1.41) and AAV (1.45) particles. Ex. 1013, 1136; Ex. 2033, ¶ 42. This shows why a POSA would not have assumed constant friction for all rAAV particles, or that all rAAV particles have the same size and shape.

D. Other Prior Art References Indicate that a POSA Would Have Expected rAAV Particle Friction to Impact Sedimentation Coefficients for Different Particles

96. It was understood before 2015 that rAAV vectors are “composed of an icosahedral assembly of 60 capsid proteins (VP1, -2, -3) assembled together in a defined stoichiometry.” Ex. 2027, 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. 2025, 10409. The “threefold-proximal spikes” can be seen in Figure 3d, which shows the electrostatic surface potential of AAV-2 calculated with SPOCK running from -10 (red) to +10 (blue). Ex. 2025, 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. 2025, Fig. 3d.

97. Spike features on another type of viral particle, adenovirus particles, were observed to impact their AUC analysis. Ex. 1023. Berkowitz 2007 explained that “spike projections must impart a significant amount of frictional drag on the overall adenovirus particle.” *Id.*, 18. Some particles, however, were observed to lose these spikes, which impacted their sedimentation coefficient. *Id.*, 31-32. Berkowitz 2007 observed that some “adenovirus monomers [may] have lost their

fiber spikes” resulting in “significantly reduced hydrodynamic drag” decreasing friction, therefore leading to “a higher sedimentation coefficient than the intact adenovirus particle.” *Id.*, 31. This was a phenomenon observed for other viral particles as well. *Id.* (“Similar effects have been observed for the bacterial virus T2 with regard to an extended versus compact geometric arrangement of its tail spikes or fibers.”).

98. A POSA would have understood that any changes to the rAAV surface (*e.g.*, the spikes on the rAAV surface) would have been expected to result in frictional changes to the rAAV surface and thus impacted its sedimentation. Indeed, such changes to the viral particle surfaces were observed for adenovirus particles. Ex. 1023, 31.

99. In addition, changes to the surface of an rAAV 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. “[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.” Ex. 1023, 31-32. Higher buoyant density would likewise increase the *s* value. Thus, from Berkowitz 2007, a 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 stoichiometry since VP-1, -2, and -3 capsid proteins have different sizes and molecular weights.

E. Berkowitz 2007 Explains Why a POSA Would Have Considered Frictional Coefficient Variation for Viral Particles of Different Genome Contents

100. The Petition contends that a “POSA would understand that the size and shape of intact, non-aggregated AAV particles are approximately the same, regardless of the size of the genome packaged in the capsid.” Petition, 45 (citing Ex. 1007, 203; Ex. 1008, 3202; Berkowitz Declaration, ¶ 170). As explained (*supra* §§ X.B-D), however, the prior art, including Berkowitz 2007, indicates that a POSA would not have expected rAAV particles of variable genome content to have approximately the same size and shape. And a POSA would not have considered the cited publications to support the Petition’s contention.

101. The Petition relies on two categories of information to conclude that rAAV shape is unaffected by genome size. The first category consists of references that describe the packaging of genomes into preformed capsids. Petition, 12 (citing Ex. 1033, 983-84, Ex. 1034, 495; Ex. 1012, 71-72, 79-82), 24 (Ex. 1007, 203), 42-43. The Petition argues that because the genomes are packaged into preformed capsids, the capsid surface is the same regardless of DNA content. This theory fails to address Dr. Berkowitz’s observations that viral particles with spike projections, a feature shared by rAAV particles, could lose these spikes. Ex. 1023, 31. “Such a

particle would have nearly the same molecular mass as an intact virus monomer but significantly reduced hydrodynamic drag and therefore a higher sedimentation coefficient than the intact adenovirus particle.” *Id.* From this, a POSA would have understood that assuming the differences in sedimentation coefficients between particles are solely attributed to differences in their densities could lead to incorrect genome size calculations.

102. The second category of information that the Petition relies on is electron microscopy studies. A POSA would have understood, however, that electron microscopy would not be suited to detecting the types of surface changes that would impact a particle’s frictional coefficient, like the loss of fibrous spikes on viral particle surfaces. Gao (Ex. 1026) and Lock (Ex. 1041), references that the Petition represents as “showing transmission electron microscopy images of full and empty AAV particles with the same size and shape,” explain the limitations of electron microscopy (“EM”) as a quantitative tool. Petition, 44.

Gao, for example, states:

EM technology is very useful and informative for qualitatively studying the structures of virions but not ideal for precise quantitative assessment of empty and full virions in a viral preparation. In addition, EM cannot distinguish full virions packaged with full-length genomes from those with partial viral genomes.

Ex. 1026, 6. 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. 1026, 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.* A POSA would have understood that such changes to the capsid are relevant to AUC analysis because they impact the sedimentation coefficient.

103. Lock (Ex. 1041) confirms Gao’s characterization of electron microscopy.

[Electron microscopy] is highly dependent on the procedure used and also on the purity of the preparation. In the wrong hands, images can be produced where it is difficult to distinguish empty and full particles and this situation is greatly exacerbated if contaminating protein or other material is present. Even in the sharpest images, some particles appear only partially stained, and this creates ambiguity in characterizing them as empty or full. In general, therefore, quantification of electron micrographs can be problematic and lead to inconsistent results.

Ex. 1041, 57. A POSA would have understood that electron microscopy could not accurately distinguish empty, partially empty, and full rAAV virions. Thus, a

POSA would not have had any basis to rely on electron microscopy to establish that all rAAV particle species are the same size and shape.

104. The Petition's obviousness argument is premised on the assumption that "the size and shape of intact, non-aggregated rAAV particles are approximately the same, regardless of the size of the genome packaged in the capsid." Petition, 45 (citing Ex.1007, 203; Ex. 1008, 3202; Berkowitz Declaration, ¶ 170). A POSA considering the complete state of the art would not have assumed all rAAV particles had approximately the same size and shape regardless of the genome size. In fact, the art indicated the opposite was likely to be true. The Petition arrives at its conclusion by picking and choosing from any one reference only so much of it as will support a given position, to the exclusion of other parts necessary to the full appreciation of what such reference fairly suggests to one of ordinary skill in the art. Because of this failure to consider the totality of the prior art's teachings, the Petition fails to establish the challenged claims as obvious.

XI. GROUND 1 OF THE PETITION HAS NOT DEMONSTRATED THAT THE CHALLENGED CLAIMS ARE OBVIOUS

105. The Petition has failed to establish that Le Bec and de la Maza (1978) in view of Sommer rendered claims 1-7, 10-11 and 20-30 obvious as of January 2015.

106. The Petition and Berkowitz Declaration argue that, prior to the 2015 priority date of the '894 Patent, a POSA would have combined information from

the Le Bec and de la Maza (1978) references to conclude that a sedimentation coefficient would have a linear relationship with the number of nucleotides enclosed within an rAAV particle in SV-AUC experiments. They further argue that a POSA's knowledge of this relationship would have allowed a POSA to determine the size of fragmented DNA trapped within the rAAV particle by determining its sedimentation coefficient and determine the quantity of variant viral particles in a heterogeneous mixture.

107. As of January 2015, a POSA would not have understood or expected a linear relationship between sedimentation coefficient and genome size in rAAV particles. This opinion rests on fundamental biophysical principles: the sedimentation coefficient depends on both buoyant mass and frictional coefficient (Svedberg equation), and the frictional coefficient varies with particle shape, surface properties, and hydration—all of which differ among rAAV particles containing different genome contents. The prior art, including Berkowitz (2007), documented that viral particles lose surface features such as fiber spikes, producing unpredictable changes in friction and sedimentation behavior. Friction was observed to vary unpredictably between viral particles of the same type.

108. In my over 25 years of research in biophysics and analytical ultracentrifugation, I have never encountered a basis for assuming uniform frictional properties across a heterogeneous mixture of macromolecular particles.

The Petition's contrary assumption reflects hindsight drawn from the '894 Patent's disclosure.

A. Claim 1

109. Le Bec and de la Maza, in view of Sommer, fail to render obvious at least two elements of claim 1's "method of quantifying one or more species of individual variant viral particles comprising fragmented recombinant adeno-associated viral (rAAV) genomes in a heterogeneous mixture of viral particles."

Those elements are:

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

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

1. Le Bec and de la Maza, in View of Sommer, Do Not Teach or Suggest "determining the genome size of one or more species of the individual variant viral particles" with SV-AUC

110. Neither Le Bec nor de la Maza teach or suggest that it would have been possible to determine genome size from sedimentation coefficients generated by SV-AUC. Le Bec groups together as "full" rAAV species having different sizes. Le Bec does not differentiate the sedimentation peaks corresponding to different rAAV particles as containing fragmented versus fully intact genomes.

111. Le Bec's AUC studies also fail to resolve peaks associated with rAAV particles containing fragmented genomes. This failure to resolve the peaks would have made it difficult, if not impossible, to accurately quantify rAAV species containing fragmented genomes. A POSA would have considered the usefulness of SV-AUC to depend on the POSA's "ability to resolve [] distinct peaks for each species" in a heterogeneous mixture, because "if for some reason they overlap, you are missing information." Ex. 2039, 54:16-56:8. A POSA, therefore, would not, and could not, have relied on Le Bec's SV-AUC experiments to develop claim 1's method of determining the size of fragmented genomes in a heterogeneous mixture of viral particles.

112. As Dr. Berkowitz testified, Le Bec does not attempt to identify or quantify rAAV particles containing fragmented genomes. Ex. 2039, 76:7-77:14; 143:23-146:15; *see also id.*, 143:23-146:15. A POSA would have understood that Le Bec's concentration analyses were qualitative. For example, Le Bec did not account for the different extinction coefficients of each particle or make any quantitative concentration measurements. As Dr. Berkowitz explained in his deposition, Le Bec's use of SV-AUC analyses were directed to "try[ing] to get optimum performance in terms of reducing the amount of empty capsids." Ex. 2039, 64:23-66:6. Le Bec would not have been helpful to a POSA trying to develop a quantitative assay for determining the amounts of different particle

species within a heterogeneous mixture. Le Bec's category of "full AAV viral particles" includes multiple peaks as shown in Le Bec's Figure 2. Le Bec, however, does not attempt to identify rAAV particles containing fragmented genomes.

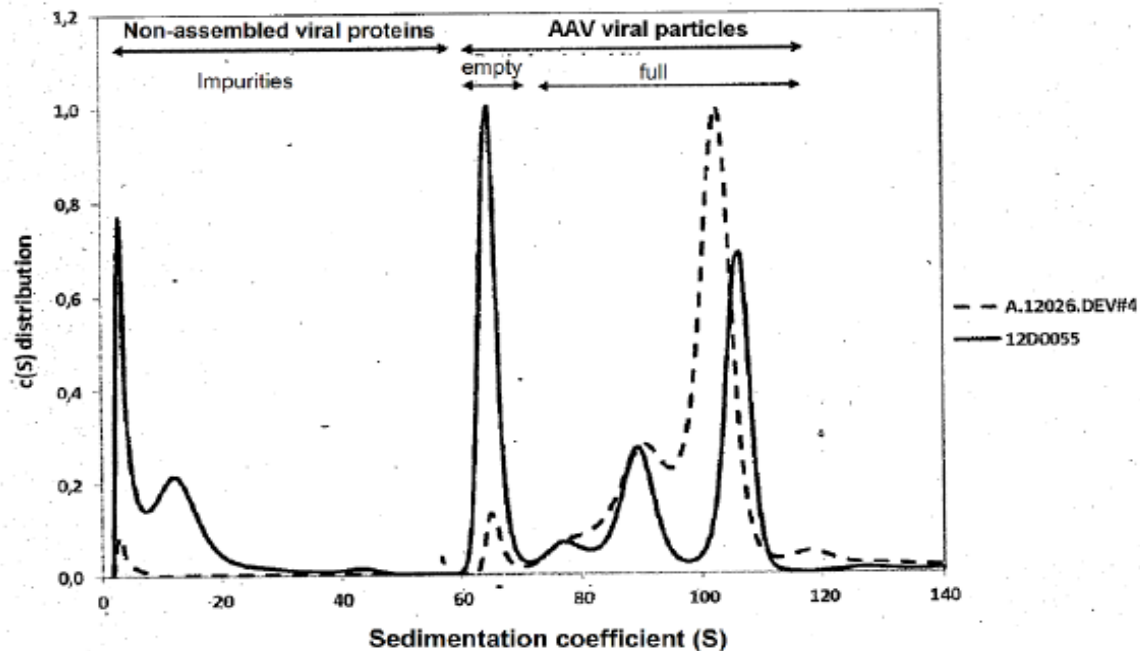


Figure 2

Ex. 1006, 6:3-7; 18:8-11; Fig. 2. 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 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." Ex. 1006, 18:11-25 (emphasis added). In contrast to Le Bec, the

method of claim 1 requires “quantifying one or more species of individual variant viral particles comprising fragmented recombinant adeno-associated viral (rAAV) genomes,” which is a species Le Bec does not address.

113. The Petition and Dr. Berkowitz concede that “Le Bec reports that the peak at 65S corresponds to empty rAAV particles and that the peak at 105S corresponds to an rAAV particle with a complete double-stranded, self-complementary genome.” Petition, 43; Ex. 1004, ¶ 162 (citing Ex. 1006, 18:21-25). Le Bec itself, however, does not identify specific S values for peaks having partial or fragmented genomes, which claim 1 requires. Dr. Berkowitz approximates S values for other rAAV particle species from Le Bec’s poorly resolved distribution profiles. Petition, 43; Ex. 1004, ¶ 162 (citing Ex. 1006, 18:23-25, Figures 2 and 3). But given the poor resolution of peaks between 60S and 110S, a POSA would not have considered Le Bec useful for quantifying fragmented genomes, because accurate peak area determinations would not have been readily feasible.

114. Dr. Berkowitz also conceded that beneath the broad peaks of Figure 2 may lie peaks corresponding to unidentified genome species. Ex. 2039, 75:12-76:5. Dr. Berkowitz testified that because Le Bec was not concerned with characterizing all peaks in its c(s) distribution, its authors made no effort to sharpen the peaks to identify different species of genome-containing rAAV particles. Ex. 2039, 64:23-

65:12. Because of this deficiency, the Petition guesses at approximate *S* values for other rAAV particle species from Le Bec's poorly resolved distribution profiles.

Petition, 43 (citing Ex. 1006, 18:23-25, Figures 2 and 3).

115. Le Bec's Figure 3 is even worse. The Petition and Berkowitz Declaration never address the unresolved agglomeration of what appears to be multiple peaks ranging from about 75S to about 110S in Figure 3 of Le Bec.

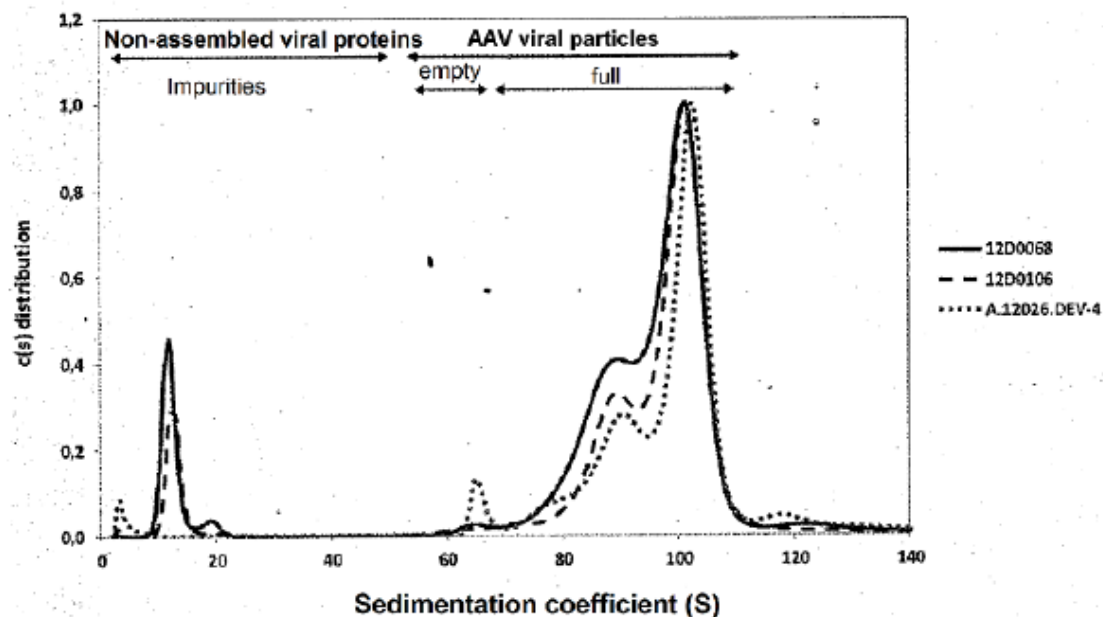


Figure 3

Ex. 1006, Fig. 3. The distribution plot of Figure 3 masks the presence of multiple peaks. A POSA would have understood that for heterogeneous mixtures of particles, the use of AUC under boundary sedimentation conditions depends on resolving peaks of different species.

116. Consistent with my opinion regarding the poor resolution of individual peaks in Le Bec's figures, Dr. Berkowitz testified:

Q. Now under this complex of peaks in figure 3, is it possible that there are other species that are present that we don't see separate peaks for?

[Objection]

A. That is possible.

Ex. 2039, 62:18-24. Dr. Berkowitz further explained in his testimony that the inability to separate peaks would have made the quantitation of species difficult:

Q. So would an issue like the peaks overlapping that you see in figure 3, would that impact the accuracy by which a person of ordinary skill in the art could determine the concentration of the different species?

[Objection]

A. Yes.

[Objection]

A. It is like chromatography. If you have peaks overlapping, it is more challenging. If it is really a side shoulder and you can't even see the maximum, it is really very difficult to quantitate.

Q. So would you say a prerequisite to getting an accurate determination of the quantity of an AAV particle species would be to have well resolved peaks?

[Objection]

A. Yes. Sure.

Ex. 2039, 69:1-24.

117. The '894 Patent further 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, 20:10-13. But the presence of multiple species with similar sedimentation coefficients will cause the boundary to be broader than expected.” *Id.*, 17:41-43. A POSA would have considered the broad peaks in Figures 2 and 3 of Le Bec to conceal peaks corresponding to partial or fragmented species. Because these peaks are not resolved, a POSA would not have been motivated to apply Le Bec’s method to determine the size of a fragmented genome in an rAAV particle. Ex. 2039, 75:4-76:5. A POSA would have considered the broad peaks in Figures 2 and 3 to conceal peaks corresponding to partial or fragmented species. Ex. 2039, 75:12-21 (Figure 2); 69:1-24 (Figure 3).

118. De la Maza (1978) does not even use sedimentation analysis on intact viral particles. Its studies are conducted on non-encapsidated DNA. Ex. 1007, 195; Ex. 2033, ¶¶ 37, 50-51.

119. In summary, Le Bec and de la Maza, the two references the Petition and Dr. Berkowitz rely on to teach or suggest “determining the genome size of one or more species of the individual variant viral particles in the heterogeneous mixture of viral particles” (Petition, 43-47; Ex. 1004, ¶¶ 164-174), fail to do so.

120. The Petition’s argument that a POSA would have understood that sedimentation coefficient depends only on particle density is premised on one assumption it repeats throughout the Petition and Dr. Berkowitz’s declaration, which is that “[b]ecause all particles are approximately the same size and shape, the density of an AAV particle—and thus its sedimentation coefficient, which is a function of particle density—is directly proportional to the size of the encapsidated DNA, as explicitly taught by de la Maza.” Petition, 45; Ex. 1004, ¶ 168.

121. The art as of the effective date, including the Carter laboratory references (*e.g.*, Exs. 1007, 1008, 1012, 1013), Gao (Ex. 1026), Berkowitz 2007 (Ex. 1023), demonstrates this is not true. A POSA would have understood that viral particles vary in unpredictable ways that impact their shape and/or size, which in turn impacts the frictional coefficient. The effect of this variability would have been understood to exclude a consistent, linear relationship—or potentially any predictive correlation—between genome size and sedimentation coefficient. *Supra* §§ X.B-E.

122. The lack of such a linear relationship in the Le Bec data would have discouraged a POSA from seeking to identify such a relationship. *Supra* § X.A. The Petition reasons backward from the information in the patent itself. Thirty-seven years passed between de la Maza (1978) and the ’894 Patent without anyone discovering the linear relationship or its claimed application—and the Petition can

construct its obviousness theory only by ignoring the statistical failure of Le Bec's data and the friction variability that its expert acknowledged.

a. A POSA Would Not Have Had a Reason to Combine Le Bec and de la Maza in View of Sommer to Arrive at Claim 1's Method

123. Specifically, a POSA would not have had a reason to develop a “method of quantifying one or more species of individual variant viral particles comprising fragmented recombinant adeno-associated viral (rAAV) genomes in a heterogeneous mixture of viral particles, said method comprising ... determining the genome size of one or more species of the individual variant viral particles in the heterogeneous mixture of viral particles” for at least five reasons. **First**, a POSA would have avoided using SV-AUC for molecular weight calculations because of the complexity introduced by friction. Ex. 1024, 241; Ex. 2039, 52:16-53:6; *supra* § X.B.

124. **Second**, Le Bec would not have given a POSA a reason to use its AUC method to identify the size of one or more species of the individual variant viral particles in the heterogeneous mixture of viral particles. Petition, 43-47; Ex. 1004, ¶¶ 164-174. Le Bec focused on determining the approximate amount of empty versus filled capsids in an rAAV mixture. Ex. 1006, 15:17-20:3. But Le Bec's analysis did not differentiate rAAV particles containing full genomes from rAAV particles containing genome fragments—they were all characterized as

“full.” *Id.*, 17:29-20:3; *supra* § XI.A.1. A POSA would have understood that Le Bec was not attempting to differentiate rAAV particles containing full genomes from rAAV particles containing genome fragments (*id.*, 17:29-20:3), attempt to determine the molecular weights of genomes, or quantify rAAV species. Ex. 2039, 144:3-15. Le Bec’s method also failed to achieve the degree of peak resolution that a POSA would have considered necessary to determine the size of the one or more fragmented genomes.

125. Furthermore, a linear regression analysis conducted according to accepted techniques refutes the contention (Petition, 53-54; Ex. 1004, ¶¶ 188-193) that Le Bec supports a linear relationship between sedimentation coefficients and genome size. *Supra*, § X.A. A POSA would have considered the Petition’s linear regression analysis incomplete, which leads to an erroneous conclusion of linearity. A POSA analyzing Le Bec’s data via a linear regression would have concluded that there was no cognizable relationship between sedimentation coefficient and genome size.

126. **Third**, a POSA would not have understood the Carter laboratory publications to support the assumption in the Petition that rAAV particle density is linearly related to genome size. Ex. 2033, ¶¶ 35-40; Ex. 2039, 101:3-11; 105:19-106:9. But even if a POSA assumed a linear relationship between rAAV particle density and genome size (*supra* § X.C), a POSA would not assume a linear

relationship between sedimentation coefficient and genome size. A POSA's understanding of rAAV particles was that their capsids were vulnerable to surface changes, which a POSA would have understood to potentially impact frictional coefficients. Ex. 1023, 18, 31; *supra* §§ X.B-E; Ex. 2033, ¶ 54. Based on this knowledge, a POSA would not have had any reason to think there would have been a reliable relationship (linear, or otherwise) between genome size and sedimentation coefficient.

127. **Fourth**, de la Maza's March 1980 publication refutes Sarepta's reliance on de la Maza (1978) to argue that the alleged linear relationship between particle density and genome size also applied to sedimentation coefficient. *Supra* § X.C. The de la Maza March 1980 publication discloses rAAV particles having identical DNA content but different densities and sedimentation coefficients. Ex. 1013, 1136; Ex. 2033, ¶ 42. The authors attribute this to conformational differences in the capsids. *Id.* Further, a POSA would not have understood de la Maza's disclosure (Petition, 34-37) as applicable to the sedimentation coefficient of intact rAAV particles. *Supra* § X.C, Ex. 2033, ¶¶ 37, 50-51.

128. **Fifth**, a POSA understood generally that rAAV particles varied in shape and size, which impacted their frictional coefficients. Ex. 1023, 18, 31. A POSA would not have had any reason to expect a reliable relationship (linear, or otherwise) between genome size and sedimentation coefficient. *Supra*, §§ X.B-E;

Ex. 2033, ¶ 42. A POSA also understood that shape and size were not the only factors for friction. Chemical interactions, including hydration, could affect friction. Neither Sarepta nor Dr. Berkowitz addressed certain forces, including hydration and other chemical properties, that were understood by a POSA to affect rAAV particle friction in SV-AUC. Ex. 2039, 82:14-83:7; 78:18-21; 130:12-131:1; 134:16-135:11. The POSA would not have had any reason to expect a reliable relationship between genome size and sedimentation coefficient.

129. The other prior art reference in Ground 1, Sommer, merely supplies extinction coefficient equations, which require the molecular weight of the encapsidated genome as an input. Ex. 1010, 127. The Petition relies on Le Bec and de la Maza to supply the reason why a POSA would have sought to determine the size of fragmented genomes by determining sedimentation coefficients. Petition, 43-47 (citing Section IX.A), 51 (citing Section IX.A.1.d). But because the combination of Le Bec and de la Maza would not have given a POSA a reason to determine genome size by determining the sedimentation coefficients of fragmented genomes, a POSA would not have been motivated to develop the method of claim 1.

130. In view of their technical limitations, the combination of Le Bec and de la Maza in view of Sommer would not have motivated a POSA to develop an AUC method to determine the genome size of fragmented genomes and accurately

quantify them in a heterogeneous mixture of viral particles. The nearly four decades that elapsed between de la Maza's publication and the '894 Patent's effective filing date reflects that the sedimentation and genome size relationship and the claimed method were not obvious.

i. The POSA Would Have Chosen SE-AUC, Not SV-AUC, to Determine Genome Size

131. In his deposition testimony, Dr. Berkowitz explains that a POSA would use SE-AUC because it is not impacted by friction.

Q. If you had a particle and you don't know what its molecular weight was, which of the AUC techniques would a person of ordinary skill in the art find as most useful for determining the molecular weight of that particle?

[Objection]

A. Equilibrium tends to be the best.

Q. And is equilibrium the best technique because you don't have to concern yourself with the friction that different particles would experience?

[Objection]

A. Yes.

Ex. 2039, 52:16-53:6. Dr. Berkowitz further testified:

Q. So just generally in AUC analyses, determining the molecular weight of a particle is -- was generally done using sedimentation equilibrium AUC? Right?

[Objection]

A. Yes. That made it easier.

Ex. 2039, 146:3-10.

132. This testimony is consistent with Berkowitz 2015, which states that SE-AUC “was totally independent of the frictional properties of the protein molecule, which made MW assessment via SV-AUC much more difficult.” Ex. 1024, 241. Thus, SE-AUC “was this mode of sedimentation analysis that was most commonly used many years ago to assess the MW of proteins and their complexes.” Ex. 1024, 241. Based on this prior art knowledge, a POSA would not have been motivated to use SV-AUC to determine genome size.

ii. Le Bec’s Data Directs a POSA Away from Using Sedimentation Coefficient to Determine Genome Size

133. The Petition’s argument that plotting sedimentation coefficient and packaged nucleotide data in Le Bec’s Figure 2 shows a “linear relationship between the sedimentation coefficient and the length” is based on an erroneous linear regression analysis. Petition, 53-54 (citing Ex. 1004, ¶ 194). The plot contains only three data points, and none of them fall on the line drawn. Petition,

53-54 (citing Ex. 1004, ¶ 194); *supra* § X.A. Drawing a “best fit” line is different from conducting a meaningful linear regression analysis.

134. A statistical analysis of Le Bec’s Figure 2 data confirms a lack of linearity. The p-value, a critical statistical measure that indicates the probability of observing the results if no true linear relationship exists between the variables, does not support the linearity of the plotted data. In this case, a low p-value suggests strong evidence against the null hypothesis (no linear relationship), while a high p-value indicates insufficient evidence to conclude a linear relationship exists. Ex. 2028, 82. The standard significance threshold is $p \leq 0.05$, meaning there is less than a 5% probability the observed relationship is due to chance, which corresponds to a 95% confidence level. *Id.* More stringent thresholds include $p \leq 0.01$ (99% confidence) and $p \leq 0.001$ (99.9% confidence) for situations requiring stronger evidence. *Id.*

135. The Petition and Dr. Berkowitz fail to apply the accepted linear regression analysis that a POSA would have applied. Petition, 53-54; Ex. 1004, ¶¶ 191-192. Analytical analyses require linear regression to be statistically significant at the 95% confidence level. Ex. 2028, 82. The slope in the Petition’s linear regression is not statistically significant at the 95% confidence level. The p-value is 0.091258, which significantly exceeds the standard significance threshold of $p \leq 0.05$. Thus, a POSA would not have considered Le Bec’s data to be consistent with

a statistically significant linear regression or to establish a linear relationship between genome size and sedimentation coefficient.

136. In contrast, the data in Figure 8 of the '894 Patent (*see also* Ex. 2019, Figure 5A) confirmed a linear relationship between sedimentation coefficient and genome size. The nucleotide values were taken from page 16 of the Petition and are reproduced below for clarity.

Nucleotides	Sedimentation Coefficients (S)
2200	84
3400	92
3450	93
4300	99

137. The linear regression output is summarized in the table below:

SUMMARY OUTPUT	
<i>Regression Statistics</i>	
Multiple R	0.99882
R Square	0.997642
Adjusted R Square	0.996463
Standard Error	0.366599
Observations	4

	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>p-value</i>
Intercept	68.20201174	0.838356933	81.352	0.000151
X Variable 1	0.007130483	0.000245116	29.0903	0.00118

This summary agrees with the R^2 value in the patent (0.9976 vs. 0.9978). In this linear regression, the p-value for the slope of the linear regression is 0.00118, which is highly significant ($p \ll 0.05$) and passes even the more stringent threshold of $p \leq 0.01$ (99% confidence). This confirms that the linear relationship between sedimentation coefficient and genome size in the '894 Patent data is statistically robust. The p-value for the intercept (0.000151) is also highly significant, passing the $p \leq 0.001$ threshold (99.9% confidence). This analysis confirms the inventors' representation that the '894 Patent provides the first documentation of the linear relationship between sedimentation coefficient and genome size for rAAV particles.

138. The contrast between Le Bec's data and the '894 Patent data is stark:

Metric	Le Bec (Ex. 1006)	'894 Patent Fig. 8 (Ex. 1001)
Data Points	3	4
Slope p-value	0.091258 (NOT significant)	0.00118 (highly significant)
R^2	0.9796	0.9978
Confidence Level	Fails 95% threshold	Passes 99% threshold

Visual Fit	Points deviate from line	Points fall on/near line
POSA Conclusion	No linear relationship	Strong linear relationship

The '894 Patent's data pass the even more stringent 99% confidence threshold ($p \leq 0.01$). Le Bec's data fail the baseline 95% threshold. This is not a close call—it is a clear, objective, quantitative demonstration that Le Bec's data do not support linearity while the '894 Patent data strongly do.

139. The Petition's failure to conduct a proper regression analysis of Le Bec—which a POSA would have done—represents a failure of its obviousness analysis. Based on Le Bec's data, a POSA would have concluded a nonlinear relationship, and possibly no consistent relationship at all, between sedimentation coefficient and genome size. This validates the claimed method's nonobviousness.

iii. A POSA Would Not Have Considered a Linear Relationship Between rAAV Particle Density and Genome Size to Apply to Sedimentation Coefficients

140. Based on my review of de la Maza (1978), de la Maza (April 1980), the Carter declaration, and Dr. Berkowitz's testimony, a POSA would not have considered the data in de la Maza (1978) to indicate a sufficiently reliable linear relationship between genome size and particle density to be the basis for a quantitative assay. Ex. 2033, ¶¶ 48-53; Ex. 1007, 195 (Table 1); Ex. 1008, 3202 n.3; Ex. 2039, 99:9-100:3; 101:3-11; 105:2-107:20; 151:3-6. Even if such a linear

relationship existed, however, it would not support a linear relationship between sedimentation coefficient and genome size.

141. A POSA also would not have understood de la Maza's "sedimentation equilibrium" study to provide useful information about sedimentation coefficients for AAV particles. *Supra* § XI.A.1.a.i. Because sedimentation equilibrium separates particles by density alone, and does not account for the effects of friction, a POSA would not have found de la Maza's sedimentation equilibrium studies as predictive of "sedimentation velocity," SV-AUC.

142. De la Maza describes the use of velocity sedimentation to study the fractionation of incomplete genomes but not in the context of intact viral particles. To conduct its experiment "DNA was released from virions by sedimentation in alkaline sucrose gradients." Ex. 1007, 194. This means that de la Maza did not conduct sedimentation velocity experiments with intact viral particles but instead used velocity sedimentation only to determine the sedimentation coefficients of DNA released from viral particles. De la Maza did not attempt to identify a relationship between the size of genome present in an intact AAV particle and sedimentation coefficient.

143. Likewise, a POSA would not have had a reason to conduct SV-AUC studies with de la Maza's incomplete genome-containing particles because "MW assessment via SV-AUC [is] much more difficult." Ex. 1024, 241. The literature,

including Dr. Berkowitz's own publications, indicated that changes in viral particle shape were known to be an issue impacting sedimentation coefficient that could not be disregarded. *Supra* §§ X.B-E. In contrast, SE-AUC was the "mode of sedimentation analysis that was most commonly used many years ago to assess the MW of proteins and their complexes since it was totally independent of the frictional properties of the protein molecule." Ex. 1024, 241.

144. The Petition and Dr. Berkowitz attempt to bridge the failure of proof with a conclusory characterization of publications from 1978 and 1980 relating to the mechanism of genome packaging. Petition, 45 (citing Ex. 1007, 203; Ex. 1008, 3202; Ex. 1004, ¶ 170). The Carter laboratory's publications make clear that density was not always linearly related to genome size. *Supra* § X.C; Ex. 2033, ¶¶ 35-47; Ex. 1012, 81; Ex. 1013, 1129, 1136. De la Maza (March 1980) further makes clear that AAV particle conformation can change in size and shape in ways that unpredictably affect the sedimentation coefficient. *Supra* § X.C; Ex. 2033, ¶ 35-53; Ex. 1013, 1129, 1136; Ex. 2039, 92:3-10; 110:18-112:16. As a result, a denser particle can sediment slower than a less dense particle, which demonstrates the power of friction in SV-AUC. *Id.*

145. Based on the Carter laboratories publications, a POSA never would have assumed a linear relationship between sedimentation coefficient and genome size. Only with the benefit of the '894 Patent, a POSA is aware that such a

relationship is sufficiently reliable for determining rAAV particle genome size with SV-AUC.

iv. A POSA Would Not Have Ignored the Effect of Friction on Sedimentation Rate

146. Several other references, including one by Sarepta’s expert, Dr. Berkowitz, further describe changes in the size and shape of viral particles.

147. Berkowitz 2007, in the context of adenovirus particles, explained that viral particles can exhibit structural changes that impact their sedimentation coefficients in unforeseen ways. Ex. 1023, 31-32. Neither the Petition nor the Berkowitz Declaration explains why a POSA would have considered decades-old publications on AAV packaging as confirming that “the size and shape of intact, non-aggregated AAV particles are approximately the same, regardless of the size of the genome packaged in the capsid” when publications, like Berkowitz 2007, indicated uniformity in particle size and shape could not be assumed. Petition, 45.

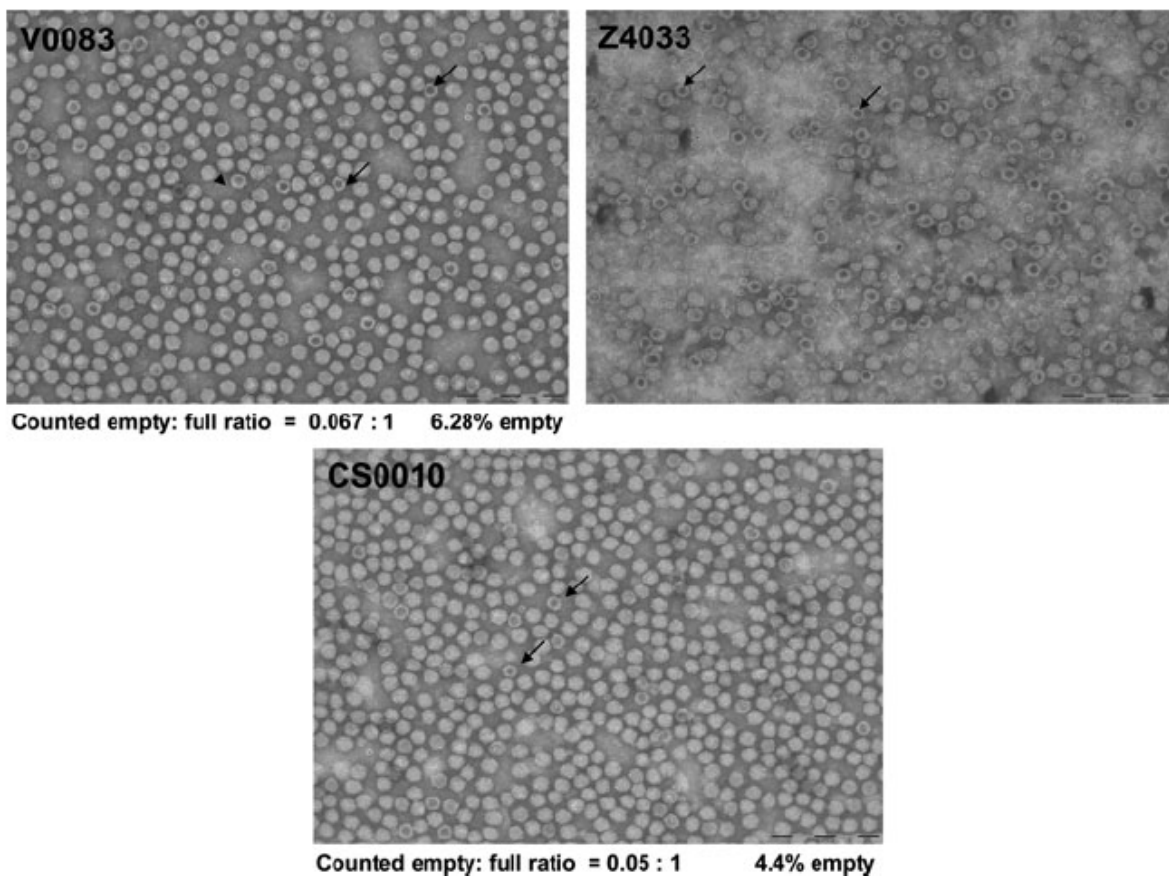
148. The Petition and Dr. Berkowitz contend that certain references “show[] transmission electron microscopy images of full and empty AAV particles with the same size and shape.” Petition, 44; Ex. 1026, 2, Figure 1; Ex. 1041, 59, Figure 2; Ex. 1004, ¶¶ 166-168. A POSA would have understood that visual inspection of EM images would have been insufficient for analyzing the factors that contribute to particle friction. At best, the EM images that Dr. Berkowitz cites are useful for qualitatively observing—“eyeballing”—the physical topology of a

particle. In deposition testimony, Dr. Berkowitz concedes that EM would not detect “chemical differences between particles that could impact the[] hydrodynamic forces” that affect friction in an SV-AUC experiment. Ex. 2039, 47:19-49:15; 50:7-20.

149. One of the references that the Petition cites, however, Gao (Ex. 1026), indicates that partially empty particles, in fact, do have different conformations than empty particles. Ex. 1026, 6 (“particles with 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”). Gao explains that electron micrographs are acceptable for “qualitatively studying the structures of virions” (Ex. 1026, 6) but a POSA would understand Gao to refute relying on electron micrograph images to evaluate quantitative issues. Gao even states that “EM cannot distinguish full virions packaged with full-length genomes from those with partial viral genomes.” Ex. 1026, 6; Ex. 2039, 125:18-126:19 (acknowledging Gao’s inability to distinguish an rAAV particle containing fully intact versus partial genomes). Dr. Berkowitz acknowledged that neither his declaration nor Gao quantified rAAV particle size and shape based on Gao’s EM images. Ex. 2039, 122:2-123:1. He acknowledged that frictional coefficient, in contrast, is quantitative. Ex. 2039, 123:13-124:7.

150. A POSA would have understood that such conformational changes to the capsid are relevant to SV-AUC analysis because they affect the sedimentation coefficient by impacting the frictional coefficient of a particle. *Supra* § X.B; Ex. 2033, ¶ 41. Indeed, Dr. Berkowitz testified that conformational changes could affect the chemical interactions (*e.g.*, hydration and solvation) between rAAV particles and the solution in SV-AUC experiments, which potentially could cause changes to frictional coefficients. Ex. 2039, 128:16-130:10. Dr. Berkowitz admitted that his analysis was limited to topology (*i.e.*, particle size and shape), and did not consider chemical interactions known to impact frictional coefficients. Ex. 2039, 130:12-131:1; 134:16-135:4.

151. The other reference that the Petition points to, Lock (Ex. 1041), makes similar statements to Gao regarding the qualitative nature of electron micrograph images. Ex. 1041, 57. Moreover, a POSA would disagree with the Petition's contention that Lock "show[s] transmission electron microscopy images of full and empty AAV particles with the same size and shape." Petition, 44; Ex. 1004, ¶¶ 41, 168. A POSA would interpret Lock's images as indicating a qualitative difference in particle shapes and sizes. Lock would not have been useful for analyzing the chemical properties of particles that could affect sedimentation coefficient, like solvation and hydration.



Ex. 1041, 59. Moreover, a POSA would have considered the frictional coefficient to have required a quantitative assessment of particle shape that was beyond the capability of visually observing particle shape and size. Neither Lock nor Gao supports the Petition's reliance on their qualitative images for assessing quantifiable differences in particle frictional coefficient.

152. Another issue with the use of EM techniques, like those in Gao and Lock, is the use of negative staining applied to help the user visualize particles and their contents. Negative staining can change the size and shape of particles. Dr. Berkowitz explained that negative staining used in EM experiments dehydrates the

particles so that they are not the same size and shape as in their native environment.

Q. So when you do negative staining, you said you dehydrate the structure? Right?

A. Yes. The processing, the sample processing, potentially involves this.

Q. And would dehydrating the structure impact its size and shape?

[Objection]

A. It is possible.

Q. And the Lock and Gao electron microscopy images that you looked at, those were done through the negative staining technique that you are referring to?

A. Yes.

Q. So it is possible that the particles are not the same size and shape that they would be in a sedimentation velocity AUC experiment?

[Objection]

A. It is possible, yes, because they are not in their native environment.

Ex. 2039, 139:20-140:20.

153. For all of the above reasons, the POSA would not have considered bulk EM images as useful for assessing differences in particle size or shape that could impact the sedimentation rate of different rAAV particle species. Ex. 2033, ¶

54. I agree with Dr. Berkowitz that other techniques, including X-ray crystallography, cryo-EM, and size exclusion chromatography, may provide more high-resolution information about particle size, shape, and chemical properties than the bulk EM images in the Petition. Ex. 2039, 137:15-138:15; 138:21-139:8; 140:19-141:17. Dr. Berkowitz concedes that he did not consider any of these techniques, in part, because “they were more recent” than the ’894 Patent and access to this type of information was “much more limited” before 2015. Ex. 2039, 142:5-22. The absence of such analysis from the art would have hindered a POSA from assuming that all rAAV particles are the same size and shape, especially because there is so much data suggesting otherwise. *Supra* §§ X.B-E.

154. In summary, the Petition does not cite any prior art patents or publications that describe the use of sedimentation coefficients to determine the size of a fragmented genome for viral particles of any type. Indeed, despite evidence of rAAV particles containing partial genomes, Le Bec did not attempt to use sedimentation coefficients to calculate genome size. The art also indicates, as Dr. Berkowitz testified, that rAAV structure would not have been considered predictable prior to 2015.

Q. So I asked whether the fact that the denser particle sediments at a lower rate than the less dense particle –

A. Right.

Q. -- is an indicator that there is unpredictable friction in AAV particles?

[Objection]

A. It is unpredictable structure of the particle, how it is behaving. It plays out in terms of whatever the particle structure is there, then that reflects the physical properties basically. So I am sort of maybe getting when you say unpredictability about friction, you are talking about the unpredictability of the structure.

Ex. 2039, 175:24-176:17; *see also id.* 174:16-175:17; 176:19-177:5.

155. There is no dispute that nearly forty years elapsed from de la Maza's 1978 publication to the effective filing date. The Petition failed to even identify a reference that *hypothesized* that such a linear relationship existed, much less applied it to determine the size of fragmented genomes in viral particles. I understand that this considerable time lapse is an indicator that the claims are not obvious.

156. The Petition uses the challenged claims to find statements in the prior art to fit the parameters of the patented invention. In so doing, the Petition ignores that the teachings of Berkowitz 2007 and Berkowitz 2015 in their entirety fail to support the Petition's conclusions of obviousness. These references make clear that a POSA would not have expected a linear relationship between S-values and genome molecular weight.

b. A POSA Would Not Have Reasonably Expected That Sedimentation Coefficients Could Be Used for “determining the genome size of one or more species of the individual variant viral particles in the heterogeneous mixture of viral particles”

157. The reasonable expectation of success argument in the Petition presents the same problems as its motivation argument. *Supra* §§ XI.A.1.a.i-iv. A POSA, understanding the unpredictable nature of rAAV particles, would not have reasonably expected to be able to reliably determine the size of a fragmented encapsidated genome from a particle’s sedimentation coefficient based on Le Bec and de la Maza. Dr. Berkowitz, in fact, testified to the “unpredictability of the structure” of rAAV particles. *Supra* § XI.A.1.a.iv; Ex. 175:24-176:17; *see also* 174:16-175:17; 176:19-177:5.

158. A POSA would not have considered the Carter laboratories disclosures to support a linear relationship between particle density, or that a linear relationship exists between sedimentation coefficient and genome size. *Supra* § X.B-E. The Petition’s argument relies on the assumption that a POSA would have ignored the effects of friction caused by physical and chemical factors. As Dr. Berkowitz’s own publications emphasize, however, a POSA would not have done so. The prior art, including Berkowitz 2007, described viral particles having differing shapes and sizes, which impact the relative friction of those particles. From this art, a POSA would never have assumed that “AAV particles are

approximately the same, regardless of the size of the genome packaged in the capsid.” Petition, 45. The unpredictability of changes to viral particles would have led a POSA to conclude that there may be no predictive relationship between encapsidated genome size and sedimentation coefficient. In particular, a POSA would not have predicted a linear relationship between encapsidated genome size and sedimentation coefficient. I understand that a reasonable expectation of success argument that fails to account for and rebut this unpredictability cannot prove obviousness.

159. A POSA with knowledge of viral particle structure would not have expected that particles containing genomes of varying sizes would exhibit the same frictional properties. For example, Berkowitz 2007 explained that some particles were observed to lose surface spikes, which impacted their sedimentation coefficient. Ex. 1023, 31-32. Berkowitz 2007 observed that some “adenovirus monomers [may] have lost their fiber spikes” resulting in “significantly reduced hydrodynamic drag,” decreasing friction therefore leading to “a higher sedimentation coefficient than the intact adenovirus particle.” *Id.*, 31. This was a phenomenon observed for other viral particles as well. *Id.* (“Similar effects have been observed for the bacterial virus T2 with regard to an extended versus compact geometric arrangement of its tail spikes or fibers”). A POSA would have expected a similar issue to arise for rAAV particles, which also exhibit spikes projecting

from their capsids. Thus, a POSA would not have expected a predictive correlation between sedimentation coefficient and the size of the encapsidated genome as the Petition alleges. Petition, 63.

160. The Petition also fails to establish a reasonable expectation of success in determining the quantity of one or more species of the variant viral particles in a heterogeneous mixture of viral particles by the claimed method. The Petition relies on Le Bec and de la Maza to supply a POSA's reasonable expectation of success to determine the size of fragmented genomes by determining sedimentation coefficients. Petition, 43-47. But because the combination of Le Bec and de la Maza would not have led a POSA to reasonably expect that it was possible to determine genome size by determining the sedimentation coefficients of fragmented genomes according to the claimed method, the Petition has failed to establish that the combination of Le Bec and de la Maza, in view of Sommer, would have led a POSA to reasonably expect that it was possible to determine the quantity of one or more species of the variant viral particles in a heterogeneous mixture of viral particles.

161. I understand that it is Petitioner's burden to demonstrate that a POSA would have had a reasonable expectation of success in combining teachings to achieve the claimed invention. It is my opinion that the Petition fails to carry its burden. A POSA's lack of reasonable expectation of success is highlighted by the

proper statistical analysis of Le Bec's data. *Supra* § X.A. This analysis indicates a lack of predictive relationship between sedimentation coefficient and genome size in a prior art reference. Because this relied-upon reference refutes the existence of a predictive relationship, the Petition cannot succeed in proving a POSA would have reasonably expected to be able to use sedimentation coefficients to determine genome size and quantify one or more species of the variant viral particles in a heterogeneous mixture of rAAV particles.

2. Without Determining the Molecular Weight of a Variant Genome, A POSA Would Not Have Been Able to Determine the Extinction Coefficients for Variant Particles Needed to Quantify Them

162. The Petition's arguments with respect to element (iv) of claim 1, which requires "determining the quantity of one or more species of the variant viral particles in the heterogeneous mixture of viral particles" (Petition, 47), fail for the same reason as for claim element 1(iii). *Supra* §§ XI.A.1-2. In order to quantify viral particles from a C(s) distribution obtained with absorbance spectroscopy detection, the detection method applied by Le Bec, one must be able to determine additional information about the viral particles, for example the extinction coefficient of the particle at the measured wavelength. Petition, 47. Calculating the extinction coefficient requires determining the molecular weight of DNA packaged in the rAAV particle, *i.e.*, the size of the genome. Petition, 51; *see also id.*, 32.

163. Le Bec's concentration analyses were qualitative and did not account for the different extinction coefficients of each particle or make any quantitative concentration measurements. Ex. 2039, 77:1-14; *see also id.*, 144:23-145:2; *supra* § XI.A.1. To quantify viral particles from a C(s) distribution obtained with absorbance spectroscopy detection, the detection method applied by Le Bec, the POSA requires additional information about the viral particles, for example the extinction coefficient of the particles at the measured wavelength. Petition, 47-50; Ex. 2039, 145:4-146:15 (Dr. Berkowitz testifying that Le Bec does not attempt to obtain the molecular weights or extinction coefficient of its rAAV particles). Thus, the Petition's obviousness argument with respect to element (iv) of claim 1 relies on its Ground 1 argument for claim element 1(iii). Petition, 51 (citing Section IX.A.1.d). It fails for the same reasons. *Supra* § XI.A.1.

164. Quantification of the different species in a heterogeneous composition can be determined by integrating the areas under the curve in a sedimentation coefficient distribution, but only if one accounts for the degree of absorbance as expressed by Beer's Law:

$$A = \epsilon cl$$

Ex. 1009, 152. Beer's law (or the Beer-Lambert law) states that the absorbance (A) of light passing through a composition is directly proportional to its molar

extinction coefficient (ϵ), concentration (c), and the path length (l) through the sample.

165. When absorbance spectroscopy at given wavelengths (*e.g.*, Le Bec follows absorbance at 276 nm) is applied in AUC methods to monitor sedimentation of viral particles, knowing the proportion of peak area to concentration makes use of additional information about each species, for example, the extinction coefficient (ϵ), because different rAAV species will absorb certain wavelengths of light to different degrees. Ex. 1001, 24:41-44 (“[E]xtinction coefficients are used to calculate molar concentration and the actual percent value of the intact vector peak from absorbance data.”).

166. The Berkowitz 2007 publication regarding the application of AUC to adenovirus particles (Ex. 1023) 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. 1023, 17. Restated, depending on the wavelength, different species of viral particles absorb UV light to different degrees, so the correlation between peak area and concentration may be different for each species. The extinction coefficient can adjust for this difference between species. Ex. 1009, 152 (explaining that “absorbance signal is directly proportional to solute concentration: $A = \epsilon cl$, where ϵ

is the solute's weight extinction coefficient, c is the weight concentration, and l is the sample path length.”); *see also* Ex. 1010, 126-127.

167. For rAAV particles, the extinction coefficient is determined by adding the extinction coefficient for the packaged genome DNA and the capsid.

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

Ex. 1010, 127. For the AAV2 serotypes, the equations, depending on the wavelength, are as follows:

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

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

Id.

168. Consistent with Berkowitz 2007, the inventors explained 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, 24:56-59.

By finding a way to determine the genome size, which allows them 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 claim 1.

B. Claim 2

169. A POSA would not have considered either of Le Bec's SV-AUC plots to support Sarepta's contention that "each species of the individual variant viral particles is fully resolved" in Le Bec's Figures 2 and 3. Petition, 50 (citing Ex. 1006, 18:8-32). It is clear that species appearing in the sedimentation plots of both Figures 2 and 3 of Le Bec are not resolved. A POSA would have understood that Le Bec was not attempting to quantify peaks corresponding to different rAAV species containing fragmented genomes, as Dr. Berkowitz himself testified. Ex. 2039, 76:7-77:7. Thus, Le Bec's analysis did not necessitate resolving peaks for fragmented-genome containing rAAV particles.

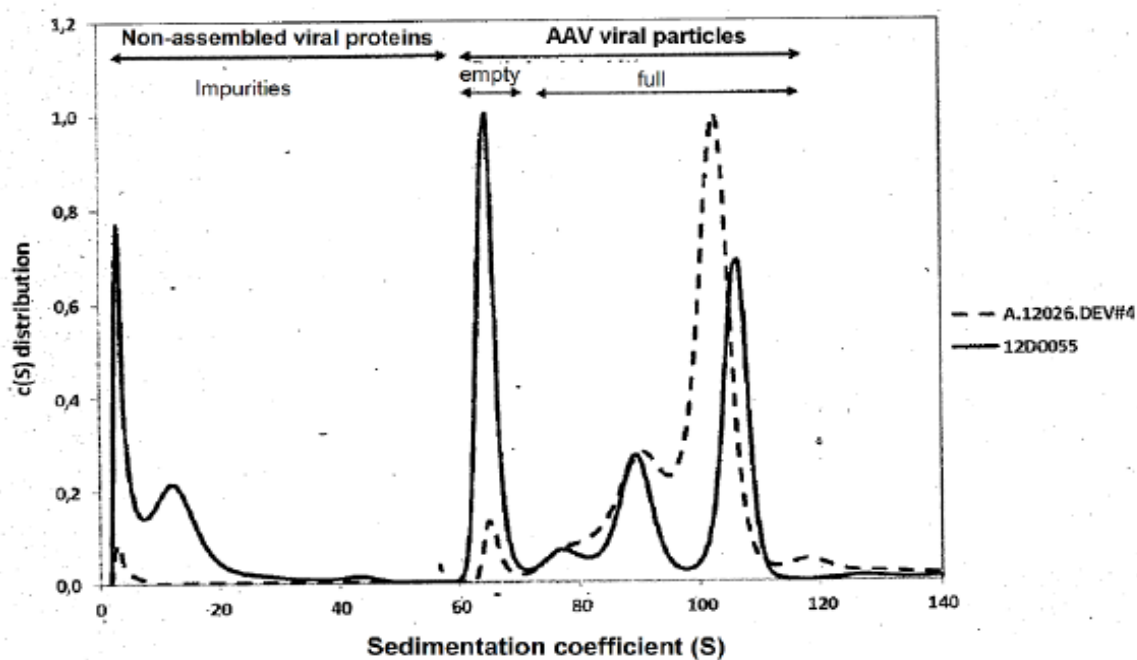


Figure 2

Ex. 1006, Figure 2. The peak corresponding to empty capsids, as well as rAAV particles at approximately 75S and 90S, according to Sarepta (Petition, 50) are not fully resolved from each other.

170. Le Bec's Figure 3 is even worse. 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 of Le Bec.

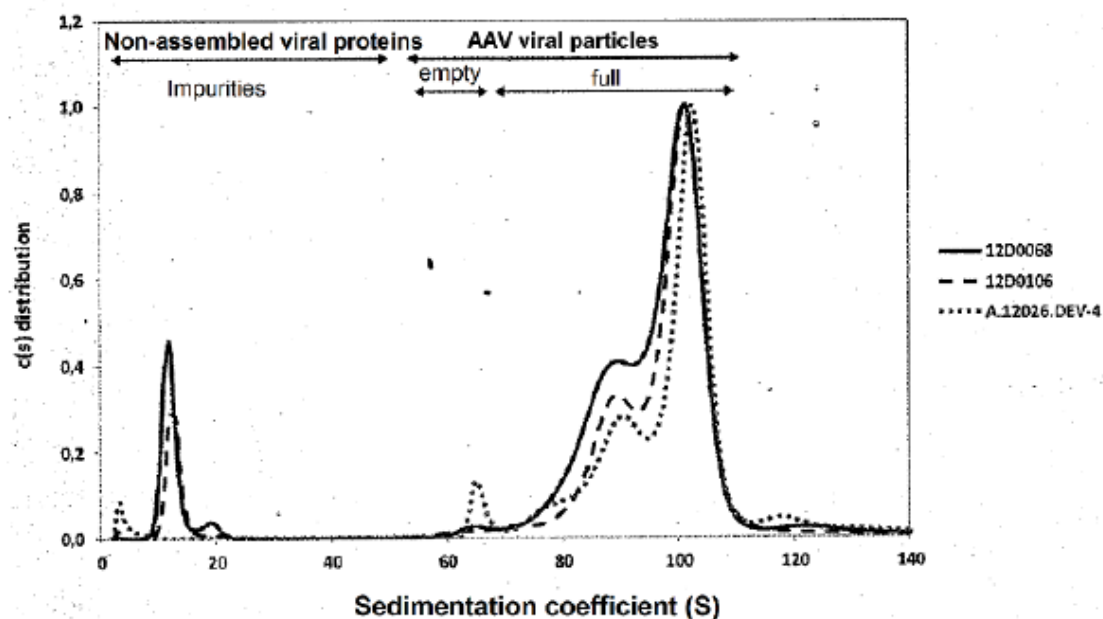
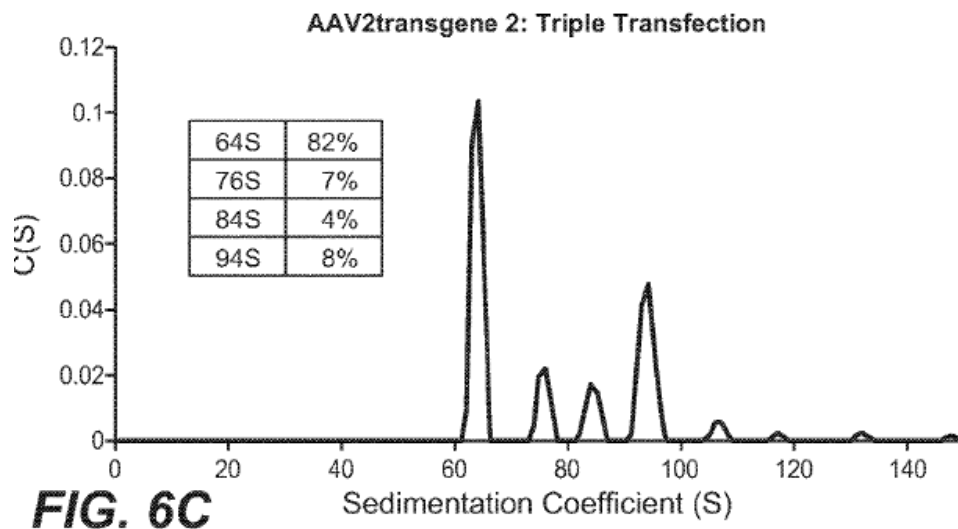


Figure 3

Ex. 1006, Fig. 3. This commingling of species masks the presence of multiple peaks, whereas a POSA would have understood that for heterogeneous mixtures of particles, the use of AUC under boundary sedimentation conditions depends on resolving peaks of different species. *Supra* § XI.A.1.

171. Le Bec's Figures 2 and 3 sharply contrast with the fully resolved peaks of the figures of the '894 Patent. Ex. 1001, FIGs. 1B, 2A, 2B, 3A, 3B, 6B, 6C, 7B, 7C, 9C, 10C, 11A, 11B, 11C, 11D, 15B. For example, Figure 6C illustrates the contrast between Le Bec's experiments and the claimed method with respect to fully resolving peaks.



Ex. 1001, FIG. 6C. In this figure, it is not only possible to identify the empty capsids (64S) and rAAV particles containing full genomes, but also fragmented species. Ex. 1001, 48:51-60. Sarepta fails to point to any reference cited in the Petition presenting with what a POSA would consider sufficiently resolved peaks for reliable quantitative concentration measurements. Accordingly, Sarepta fails to prove claim 2 was obvious.

C. Claims 3-7, 10-11, and 20-30

172. Because the combination of Le Bec and de la Maza in view of Sommer does not render claim 1 of the '894 Patent obvious, and because I understand claims 3-7, 10-11 and 20-30 all depend from claim 1, it is my opinion that the combination of Le Bec and de la Maza in view of Sommer does not render claims 3-7, 10-11 and 20-30 of the '894 Patent obvious.

D. Ground 2: The Petition Fails to Establish That Le Bec and de la Maza in View of Sommer and Cole Renders Claims 12-19 Obvious

173. I understand that challenged claims 12-19 depend from claim 1. I further understand that the only challenge to claim 1 is in Ground 1. Because the combination of Le Bec and de la Maza in view of Sommer does not render claim 1 of the '894 Patent obvious as explained for Ground 1, it is my opinion that the combination of Le Bec and de la Maza, in view of Sommer and Cole, does not render claims 12-19 of the '894 Patent obvious.

XII. OBJECTIVE INDICIA OF NONOBVIOUSNESS

A. The Claimed Method's Capability to Determine Genome Size and Quantify Fragmented Genome rAAV Particles in a Single Experiment Was Surprising and Unexpected

174. Le Bec and de la Maza are the references Sarepta alleges were the closest prior art to the claimed invention. These references, however, indicate to a POSA that it was not possible to determine the size of a fragmented genome from a sedimentation coefficient. Le Bec never attempts to determine genome size from

sedimentation coefficients of viral particles containing fragmented genomes. *Supra* § XI.A.1. Le Bec focuses on the much less ambitious goal of analyzing “empty and full AAV viral particles.” To do so, Le Bec groups rAAV particles containing partial genomes together with those having intact genomes as “full AAV viral particles.” *Supra* § XI.A.1; Ex. 1006, 18:21-25; Figure 2. As such, Le Bec did not need to resolve the species to the degree required by the claimed invention here. Dr. Berkowitz’s attempt to take Le Bec’s data and make it fit a linear model—only after the ’894 Patent described its method of using a previously unknown linear relationship between sedimentation coefficient and genome size—still fails to indicate a linear relationship. *Supra* § X.A. Indeed, Le Bec provides no basis for a POSA to consider there to be any predictive correlation at all between genome size and sedimentation coefficient.

175. The 1978 de la Maza publication does not attempt to subject intact rAAV particles to SV-AUC. *Supra* § X.C; Ex. 2033, ¶ 37. The March 1980 publication by de la Maza and Carter, however, shows that rAAV particles containing the same DNA content can nonetheless have different densities and different sedimentation coefficients, which is attributable to conformational change. Ex. 1013, 1136; *Supra* § X.C. From the Carter laboratory’s disclosures, a POSA would not have considered there to be a sufficiently reliable linear relationship between genome size and rAAV density for quantifying rAAV

particles in a heterogeneous composition. *Supra* § X.C; Ex. 2033, ¶ 35-53. Several other references indicated that rAAV particles, like other viral particles, adopted different structural conformations depending on their genome content. *Supra* § X.B-E; Ex. 2033, ¶ 54.

176. The inventors achieved a result that no POSA would have predicted. Despite the prior art indicating no linear relationship between sedimentation coefficient and genome size, the inventors demonstrated that a statistically significant linear relationship exists. Applying this relationship in the claimed method enables determination of genome size and quantification of rAAV particles containing fragmented genomes in a single experiment. This unexpected capability established AUC as the “gold standard” in rAAV analysis, as discussed below.

B. Industry Praise Is Further Evidence of Nonobviousness

177. A 2015 peer-reviewed publication co-authored in-part by the inventors of the '894 Patent, Burnham and O’Riordan, and colleagues (Ex. 2019), publicized experiments disclosed in the '894 Patent, including Examples 1-6. *See, e.g.*, Ex. 2019, 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).

178. Between its publication in 2015 to July 2026, the Burnham publication has been cited over 200 times according to Google Scholar. Ex. 2035. Citations in peer-reviewed references are a reflection that the work is of importance in the field in which it was published. Additionally, several industry sources have made comments that reflect the value of the claimed AUC method. Dr. Berkowitz is among the authors to have cited the Burnham publication, which he has done in multiple of his own publications. Ex. 2043, 7, 8 (citing Ex. 2019); Ex. 2042, 3 (citing Ex. 2019); Ex. 2041, 3, 5 (citing Ex. 2019).

179. A 2020 white paper by Beckman Coulter GmbH, the leading manufacturer of instruments for SV-AUC analysis, highlighted the importance of the inventors' 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. 2021 (citing Ex. 2019) (emphasis added). Thus, Genzyme's work rendered AUC the "gold standard in the detection and characterization of AAV particles."

Id.

180. BioAnalysis LLC, a contract research organization whose business is built around the AUC method for gene therapy applications, recognizes that SV-AUC “has emerged as the gold standard technique for the empty/partial/full capsid assay in gene therapy product development,” offering “unmatched resolution, accurate quantitation, and regulatory compatibility” and representing “the preferred method for characterizing viral vectors such as AAV, lentivirus, and other delivery systems.” Ex. 2036, 1. Compared to alternative methods, including transmission EM, UV/Vis spectroscopy, charge detection mass spectrometry, mass photometry, and SEC-MALS, SV-AUC is superior for quantifying rAAV species. *Id.*

181. Sigma-Aldrich, a major scientific reagent and instrumentation provider, describes AUC as “widely recognized as a gold standard method for determining the empty/full AAV capsid ratio.” Ex. 2037, 4. It extols the method for “high resolution separation, allowing for quantitation of empty partial and full capsids where partial capsids contain partial or truncated fragments of the gene or capsid with contaminant from the host cell,” and that it is “serotype independent.” *Id.*

182. Citing the inventors’ work, a 2018 publication co-authored by an inventor of the Petition’s primary prior art reference (Ex. 1006), 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.

2020, 166-67 (citing Ex. 2019). It is not surprising the rAAV field, including Le Bec, would rely on the AUC inventors' method. As explained herein, the Le Bec prior art reference relied on by the Petition (Ex. 1006) did not provide a method that could quantify the ratio of different variant rAAV species, including those containing fragmented genomes, within an rAAV preparation. Le Bec's prior methods were much more qualitative and geared more towards tracking the presence of empty capsids. *Supra* § XI.A.1.

183. The method disclosed and claimed in the '894 Patent was the innovation that made AUC a valuable tool to identify the species present in rAAV compositions and measure "the ratio between empty, partially filled and filled viral particles." Ex. 2021. Sarepta and Dr. Berkowitz neither demonstrate that SV-AUC was used in the prior art to determine the size of the genome in each species present in an rAAV composition, nor that SV-AUC could be used to quantify the concentrations of those rAAV species, particularly rAAV species containing genome fragments.

184. The praise is directed to the utility of the claimed method for "determining the genome size of one or more species of the individual variant viral particles in the heterogeneous mixture of viral particles; and [] determining the quantity of one or more species of the variant viral particles" in a single

experiment. Because the praise is tied to the elements expressly recited in the claims, it supports the nonobviousness of that method.

C. The Claimed Method Satisfied a Long-Felt Need for Characterizing rAAV Impurities

185. Prior to the '894 Patent's effective date, I understand from my review of the art published prior to 2015 and Dr. Carter's declaration that there was a long-felt and unsolved need for a method that accurately quantified the concentration of the rAAV particles containing fragmented genomes. Ex. 2033, ¶¶ 25-29; Ex. 2019, 228; EX2044, 123:10-14; *supra* § VII.A. The inventors' contemporaneous peer-reviewed publication, submitted in April 2015 and published in September 2015, after the January 20, 2015 priority date, also explained this issue:

[N]o established approaches exist for assessing the homogeneity of rAAV vector preparations that include measuring both the presence and fractional content of empty viral particles, full genome-containing viral vectors, and vectors with fragmented genomes or non-transgene-related DNA contaminants. Thus, identifying and developing sensitive and accurate methods to assess vector quality are needed.

Ex. 2019, 228. Ensuring the safety and efficacy of rAAV-based gene therapies requires a method to analyze the manufacture of rAAV compositions and ensure the quality of final compositions. Ex. 2033, ¶ 25-29; Ex. 2019, 229. The EMA's concerns regarding the poor detection of rAAV particle species lacking complete

genomes for Glybera® is a clear illustration about the long-felt need for an effective assay to detect these impurities. Ex. 2033, ¶ 27 (citing Ex. 1029); *supra* § VII.A.

186. Dr. Berkowitz also testified to the importance of characterizing all rAAV species in a gene therapy composition, because of risks associated with safety and efficacy.

Q. In characterizing a gene therapy composition, if there is an error in the analysis of the amounts of different species present, that could impact the infectivity of that composition? Right?

[Objection]

A. Right.

Q. And infectivity would be a concern for the efficacy of that composition? Right?

A. Yes.

Q. And infectivity could also be a concern for the safety of that composition? Right?

A. Yes.

Q. So would you agree that the stakes of analyzing an AAV composition accurately are high for its efficacy and safety?

[Objection]

A. Absolutely. Yes.

Ex. 2039, 119:9-120:7.

187. Prior to the inventions claimed in the '894 Patent, determining the DNA content of recombinant viral vectors was typically performed by Southern blot analysis. *Supra* § VIII.A.2.d. Southern blot requires a sequence-specific probe and cannot quantify different species of rAAV in a heterogeneous rAAV mixture. Fragmented genomes of unknown sequence could evade detection. *Id.* Failure to detect and quantify a fragmented genome-containing rAAV impurity could compromise the safety of a gene therapy composition by, for example, causing immunotoxicity effects. Ex. 2033, ¶ 28; Ex. 2027, 87; Ex. 1026, 6.

188. Le Bec demonstrates that even researchers who used AUC as part of their characterization work in the rAAV field did not develop, and could not derive, the quantitative, comprehensive methodology claimed in the '894 Patent. Le Bec used AUC qualitatively to track empty and genome-containing rAAV particles, and never attempted to characterize rAAV particles containing fragmented genomes. *Supra* § XI.A.1. The claimed invention was the first single assay that could distinguish full, properly formed rAAV particles from the empty capsids and fragmented genome-containing rAAV particles, and quantify each component.

XIII. CONCLUSION

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

Date: August 5, 2026



Dr. Karen G. Fleming