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 IN SUPPORT OF PATENT OWNER'S PRELIMINARY RESPONSE

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I. INTRODUCTION

1. I submit this declaration in connection with Patent Owner's Preliminary 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 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 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 Johns Hopkins University. I held this position until my promotion to Full Professor with tenure in the T.C. Jenkins Department of Biophysics at 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 their 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.

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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

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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. Berkowitz's declaration (Ex. 1004, "Berkowitz Declaration"). Petition, 32-33; Berkowitz Declaration, ¶¶ 83-86. In addition to the Petition and Berkowitz Declaration, I reviewed and considered the materials identified in the list below.

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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 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)
2032	Curriculum Vitae of Karen G. Fleming

IV. SUMMARY OF MY OPINIONS

12. First, considering Ground 1, a POSA as of January 2015 would not have been motivated to combine Le Bec and de la Maza in view of Sommer to determine fragmented genome sizes from sedimentation coefficients and determine the quantity of one or more species of the variant viral particles, nor would a POSA have had a reasonable expectation of success in doing so. This opinion is

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based on 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) the prior art, including Dr. Berkowitz's own 2007 publication, warned that viral particle friction varies unpredictably, and (3) de la Maza's density measurements using sedimentation equilibrium-AUC ("SE-AUC") cannot predict sedimentation velocity-AUC ("SV-AUC") behavior because SE-AUC eliminates friction effects. 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.

13. Second, it is my opinion that Ground 2, alleging that claims 12-19 are obvious over Le Bec and de la Maza, in view of Sommer and Cole, does not establish the obviousness of any claim. 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 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.

14. The Petition and Berkowitz Declaration argue that, prior to the 2015 priority date of the '894 Patent, a POSA would have been able to combine information from the Le Bec and de la Maza references in view of Sommer to

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conclude that a sedimentation coefficient would have a linear relationship with the number of nucleotides enclosed within a rAAV particle in SV-AUC experiments. They further argue that a POSA's knowledge of this relationship would have allowed the 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 heterogenous mixture.

15. In my opinion, as of January 2015, a POSA would not have expected a linear relationship between sedimentation coefficient and genome size in rAAV particles. This opinion is based on fundamental biophysical principles: 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 may differ between rAAV particles containing different genome contents. The prior art, including Berkowitz (2007), documented that viral particles can lose surface features like fiber spikes, causing unpredictable changes in friction and sedimentation behavior. Simply put, friction had been observed to vary unpredictably between two viral particles of the same type. In my 25+ years of experience in biophysics and analytical ultracentrifugation, I have never encountered a situation where a POSA would assume uniform frictional properties for a heterogeneous mixture of protein

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particles. The Petition's assumption to the contrary reflects hindsight knowledge of the teaching of '894 Patent's disclosure.

16. Le Bec's SV-AUC data indicate the absence of a linear relationship. A standard linear regression analysis on the three data points from Le Bec Figure 2 (the same data plotted in the Petition at page 54) results in a p-value for the slope of 0.091258, which fails the universally accepted 95% confidence threshold ($p \leq 0.05$). This means there is greater than a 9% probability that the observed pattern occurred by random chance alone—far too high to conclude a linear relationship exists. In my role as a reviewer for multiple biophysics journals and as President of the Biophysical Society, I can state unequivocally that no competent scientist would claim a linear relationship exists based on three data points with a p-value of 0.091258. This would not be accepted in any peer-reviewed scientific publication as an established fact. Dr. Berkowitz's failure to report this p-value in his declaration is a significant omission that undermines the Petition's central argument.

17. The addition of de la Maza, a reference published 36 years before Le Bec and 37 years before the '894 Patent was filed, does not support the argument that a POSA would have expected a linear relationship between sedimentation coefficient and genome size. In fact, de la Maza uses SE-AUC to study AAV particles, a different method from SV-AUC for which friction is not a factor.

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Additionally, de la Maza's publication using a completely different AUC methodology, does not make Le Bec's SV-AUC data statistically linear.

18. The Berkowitz Declaration presupposes that all rAAV particles are the same shape and size resulting in the same frictional drag using poor TEM images with no statistical analyses. The POSA would not have made this assumption. High resolution rAAV structural data from Xie et. al. shows spike proteins for AAV2 particles. From this, a POSA would have expected that rAAVs with varied capsid compositions would be of different shapes and/or sizes. Additionally, different capsid protein stoichiometries are part of the same rAAV preparations which would lead to different surface polarities and thus solvent interactions leading to changes in frictional coefficients. The Berkowitz Declaration also fails to account for the fact that hydration could impact the frictional coefficient. 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 and thus the frictional coefficient.

19. Notably, the Berkowitz Declaration contradicts Dr. Berkowitz's own published scientific work. In 2007, Dr. Berkowitz published a detailed study of adenovirus particles analyzed by AUC, in which he explicitly warned that loss of fiber spikes causes "significantly reduced hydrodynamic drag and therefore a higher sedimentation coefficient." Ex. 1023, 31. Dr. Berkowitz's 2007

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publication makes clear that a POSA could not assume uniform frictional properties for viral particles. Yet in his declaration for this proceeding, Dr. Berkowitz assumes exactly that—claiming that all rAAV particles have "approximately the same" size and shape regardless of genome content. Berkowitz Declaration, ¶ 168. This assumption is inconsistent with Dr. Berkowitz's own prior scientific conclusions and does not reflect what a POSA would have understood in 2015. Moreover, Dr. Berkowitz bases this assumption on electron microscopy images, despite the fact that the cited references (Gao (Ex. 1026), Lock (Ex. 1041)) explain the limitations of electron microscopy as a quantitative tool, with Lock explicitly stating that EM "is highly dependent on the procedure used" and "quantification of electron micrographs can be problematic and lead to inconsistent results." Ex. 1041, 57.

V. LEGAL STANDARDS

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

A. Obviousness

21. 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

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

- 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."

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

- combining prior-art elements according to known methods to yield predictable results;
- simple substitution of one known element for another to obtain predictable

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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.

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

24. I have been informed that for a combination of references to render the claimed invention obvious, the POSA must have been able to arrive at the claimed invention by altering or combining the applied references and must have had a reasonable expectation of success in doing so. I understand that the claim is

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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.

25. 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.

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

VI. PERSON OF SKILL IN THE ART

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

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> 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.

28. 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.

VII. BACKGROUND SCIENTIFIC CONCEPTS RELEVANT TO THE ’894 PATENT

29. I provide a background of the relevant scientific concepts relevant to the ’894 Patent below.

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

30. Wild-type (“wt”) adeno-associated viral (“AAV”) vectors contain a single-stranded DNA genome that encodes replication (“rep”) and encapsidation (“cap”) genes flanked by inverted terminal repeats (“ITRs”). Ex. 2027, 87; Ex. 2019, 229.

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31. Recombinant AAV ("rAAV") vectors can be produced by a transient transfection method using a "gutless" vector approach. Ex. 2019, 229; Ex. 2026, 938. Such an approach may employ three plasmids, often referred to as "triple transfection." Ex. 1001, 7:8-11. Encoded on a first plasmid is the therapeutic genes of interest and regulatory elements inserted between the 5' and 3' viral ITRs, encoded on a second plasmid are genes containing the rep and cap functions, and encoded on a third plasmid are the required adenoviral helper genes, E4, E2A, and Va RNA. Ex. 2026, 939-40; *see also* Ex. 1001, 7:8-11, FIG. 4. "When th[e] adenoviral helper plasmid was cotransfected into 293 cells, along with plasmids encoding the AAV vector and *rep* and *cap* genes, AAV vector production occurred at levels equal to those achieved by the use of adenovirus infection as a source of help." Ex. 2026, 939.

32. The transfection of the three plasmids was understood to result in fully assembled viral vectors, as well as populations of viral vectors containing both non-genome-containing particles, including empty capsids, and vectors containing incomplete genomes. Ex. 2027, 81; Ex. 2019, 229. Genome containing vectors can include the complete genome (*i.e.*, fully intact rAAV particles) as well as particles containing incomplete portions of the rAAV genome or nongenomic nucleic acid contaminants, for example, partial or fragmented genomes. Ex. 2027, 81, 86-89. For shorthand, the '894 Patent sometimes describes such incomplete

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genome-containing rAAV particles as variant species. *See, e.g.*, Ex. 1001, Abstract.

33. The POSA would have understood that the production method influences the composition of an rAAV preparation, particularly with respect to the amounts of empty capsid particles, capsids containing fragmented rAAV genomes, and capsids containing nongenomic nucleic acid contaminants. Ex. 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.

34. For clinical applications, the presence of rAAV particles containing variant genomes poses risks. For example, "[d]epending on the origin of the cells used for AAV generation, i.e., human or non-human origin, genotoxicity and immunotoxicity are two theoretical risks." Ex. 2027, 87. Concerning the gene therapy Glybera, the European Medicine Agency ("EMA") licensure assessment raised quality objections over residual baculovirus genetic sequences encapsidated in AAV1 capsids and co-purified with the product. Ex. 1029, 15, 18, 19-20. The EMA also raised concerns relating to residual Rep and Cap genes packaged in AAV1 capsids. Ex. 1029, 142. More generally, the EMA raised concerns that

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"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.

35. Indeed, the scientific literature documented how "excessive empty capsids are immunotoxic" and "AV virions packaged with full or partial viral genomes, are more immunotoxic." Ex. 1026, 6. Thus, the POSA would have been concerned with monitoring empty capsids ***and*** especially partially full capsids, because of their potential elevated immunotoxicity. The POSA would have understood that given the difficulty with the purification of viral compositions, the ability to properly identify and quantify partially full capsids was essential for the clinical applications of rAAV-based gene therapies. It was not enough to merely identify heterogeneity, rather it needed to be quantitatively analyzed. This was a difficult challenge as of the effective filing date.

B. rAAV Structural Considerations

36. AAV 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

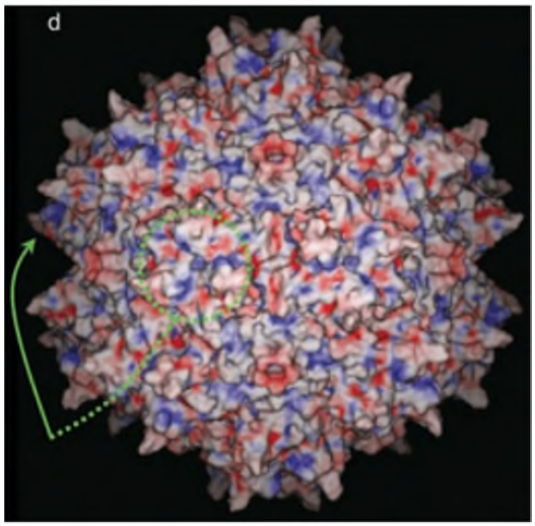
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expression cassettes packaged within AAV [31]." Ex. 2027, 84. A detailed crystallographic analysis of AAV-2 published in 2002 by Xie et.al. describes the outer surface of the particle:

> 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.

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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.

37. 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. Thus, compositional variations in the stoichiometric capsid proteins would likely lead to changes in the frictional drag for rAAV particles as well.

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38. 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*.

39. 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. Several forces impact the sedimentation of a species in an AUC sedimentation velocity experiment. Balancing these forces, results in a relationship where sedimentation, "*s* is proportional to the buoyant molar mass, M_b, and inversely proportional to the frictional coefficient, *f*" as shown in Equation (1) reproduced from Cole 2008 (Ex. 1009):

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

Ex. 1009, 146.

40. The 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, $V_{h:}$

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

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 I 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

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translational frictional coefficient is a variable in the translational diffusion coefficient, which a POSA would also know.

41. The POSA would have been aware of the possibility that not all rAAV particles would have been assumed to have identical surfaces. Berkowitz 2007 observed that some "adenovirus monomers [may] have lost their fiber spikes" resulting in "significantly reduced hydrodynamic drag" decreasing friction, therefore leading to "a higher sedimentation coefficient than the intact adenovirus particle." Ex. 1023, 31. The POSA would have understood that changes to the rAAV surface (*e.g.*, the spikes on the AAV surface) would have been expected to result in frictional changes to the rAAV surface and thus impacted its sedimentation. Additionally, Berkowitz 2007 dismissed the possibility 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.

42. In addition, the changes to the surface of an AAV particle would alter the protein mass. Berkowitz 2007 explained that changes to the spike proteins on the outside of adenovirus capsids have implications not only for frictional coefficient, but also for the mass ratio of genome to protein. Ex. 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

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adenovirus particle."). Higher buoyant density of a particle would likewise increase the *s* value for that particle. Thus, from Berkowitz 2007, the POSA would have understood that changes to particle mass would not be limited to genome size but could also be attributed to changes in the capsid protein. The Petition and Berkowitz Declaration ignored these issues and made assumptions about the uniformity of the structure of rAAV particles that the POSA would not have made. *See infra*, § X; Petition, 45; Berkowitz Declaration, ¶¶ 144-181.

C. Absorbance Detection for AUC

43. 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.*

44. Absorbance at a wavelength of 276 nm was the method employed by the Le Bec reference for analyzing AAV 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 extinction coefficient of that material; c is the molar concentration of those species; ℓ is the path length.

$$A = \varepsilon c \ell$$

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

45. Absorbance data requires the use of extinction coefficients to calculate the molar concentration and the percent value of the empty and genome-containing capsids. The impact of differing absorbance between species is starkly illustrated by Figure 1B of the ’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 concentration of empty capsids and full genome capsids from an 80%/20% mixture).” Ex. 1001, 8:24-35.

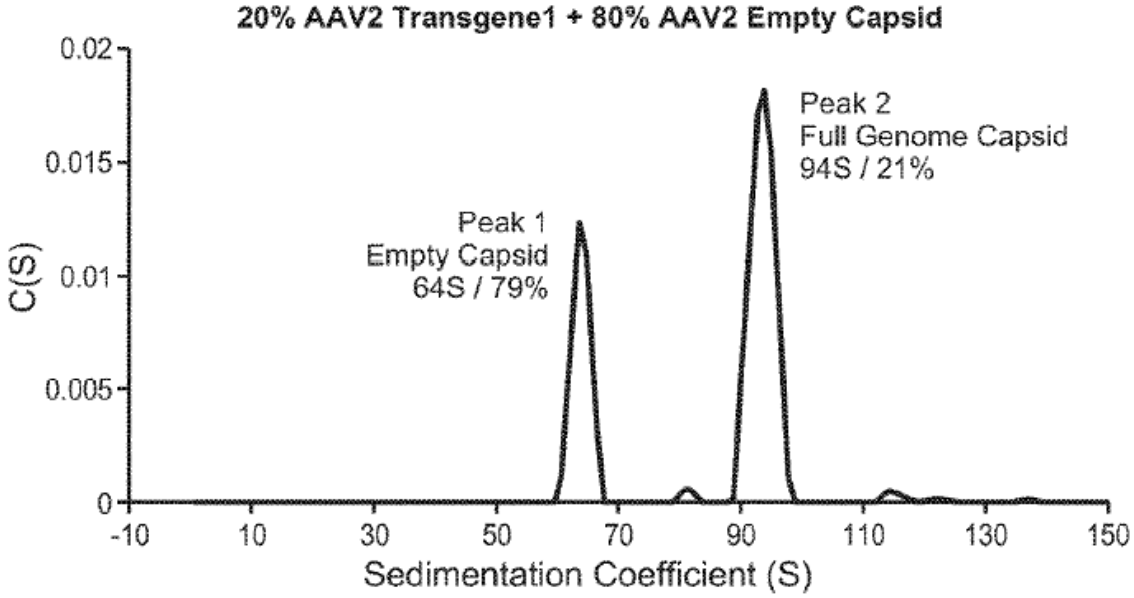


FIG. 1B

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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 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 absorbances of empty capsid and full rAAV particle at the absorbance detection wavelength. The application of the extinction coefficient at this wavelength adjusts for this difference and thus is essential for determining the relative concentrations of the two particles in the sample.

46. For genome-containing rAAV particles, which are a combination of protein (from the capsid) and DNA (genome), the extinction coefficient will depend on the amounts of protein and genome in a given particle. Ex. 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 $6.31 \times 10^6\ M^{-1}\ cm^{-1}$, respectively."); Ex. 1001, 24:44-47. To estimate extinction

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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.

D. Assessing Linearity from Experimental Data

47. Assessing linearity in experimental data begins with visual and quantitative analysis. A scatter plot provides the first indication of whether a linear relationship exists by displaying data points graphically, allowing the POSA to observe the general trend and identify potential outliers. Linear regression analysis then fits a line through the data, producing key metrics including the slope, intercept, and R^2 value (coefficient of determination), which indicates how well the linear model explains the variance in the data. The coefficient of determination (r) estimates the fraction of the variation in the dependent variable, y, that is explained by the independent variable, x, in a simple linear regression, ranging from -1 to +1, where values closer to ±1 indicate stronger linear associations. However, there is no statistical significance associated with R^2 in terms of the significance of the slope and intercept for the linear regression. Thus, R^2 is not useful for determining the linearity of the regression analysis beyond a qualitative metric. The

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appropriate statistical confirmation of a linear relationship is the significance testing of the slope and intercept resulting from the linear regression analysis. In his declaration, Berkowitz confounds R^2 values with the appropriate statistical significance of the slope and intercept, at the universally accepted 95% confidence limits. For small data sets, the value of R^2 is especially irrelevant to statistical significance of the linearity of the fit.

48. The p-value is a critical statistical measure that indicates the probability of observing research results that occurred by random chance under the null hypothesis. In essence, it tells you how likely your observed pattern occurred by random chance alone rather than reflecting a genuine relationship. A low p-value suggests strong evidence against the null hypothesis, while a high p-value indicates insufficient evidence to reject the null hypothesis. In linear regression analysis, the null hypothesis is that the resulting slope is zero, and the alternative hypothesis is that the regression slope is significant, confirming the linearity of the data. The standard significance threshold is $p \leq 0.05$, meaning there is less than a 5% probability the relationship is due to chance, which corresponds to a 95% confidence level. This 95% confidence limit is used widely throughout science as the gold standard for significance. More stringent thresholds include $p \leq 0.01$ (99% confidence) and $p \leq 0.001$ (99.9% confidence) for special situations requiring stronger evidence to reject the null hypothesis.

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49. 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

50. 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 a practical and useful release assay. *See infra*, §§ VIII.A-B.

51. The prior method for detecting rAAV species, Southern blot, used a specific complimentary 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

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genomes, that either evaded detection or required multiple experiments for accurate detection. *Id.* Because of the inventors' innovative application of AUC to analyzing rAAV preparations, AUC has become a critically important tool for the development of rAAV-based gene therapies.

A. The Importance of Genzyme's Innovative AUC Method

52. 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 much of 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). A 2020 white paper by Beckman Coulter GmbH, the manufacturer of instruments for 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

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> tool to analyze rAAV vectors notwithstanding the composition and length of the transgene or the viral serotype.

Ex. 2021 (citing Ex. 2019) (emphasis added).

53. A 2019 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. If Le Bec's own method had provided a reliable way to quantify rAAV species, she would not have needed to cite the inventors' work in 2019. 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 rAAV species within an rAAV preparation.

54. Indeed, 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 "the ratio between empty, partially filled and filled viral particles." Ex. 2021. The Petition neither demonstrates that AUC was used in the prior art to identify the size of each species present in a rAAV composition, nor that it could quantify the concentrations of those rAAV species, particularly partially filled rAAV species.

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B. Disclosed Innovation of the ’894 Patent

55. “[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.

56. 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.

57. 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

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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

58. The POSA understood, however, that merely integrating peak signal area would not accurately quantify the concentration of each rAAV species. To determine the relative concentrations of species within a heterogeneous AAV preparation using AUC, the POSA must correlate peak area (determined by peak 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 requires knowing the extinction coefficient (ε) of each species, 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.").

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59. 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 ($\varepsilon_{260/capsid}$=3.72e6) and intact vector ($\varepsilon_{260/vector}$=3.00e7) can be calculated based on published formulae."). However, "it [was] not possible to determine empirically the extinction coefficient of [] fragmented genomes," because they are "of unknown size and sequence …." *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.

60. 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, and their corresponding S values were determined, as shown in Table 5." *Id.*, 49:57-67.

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TABLE 5

S values for rAAV vectors with known genome size.

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

Ex. 1001, 50:1-19.

61. 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.

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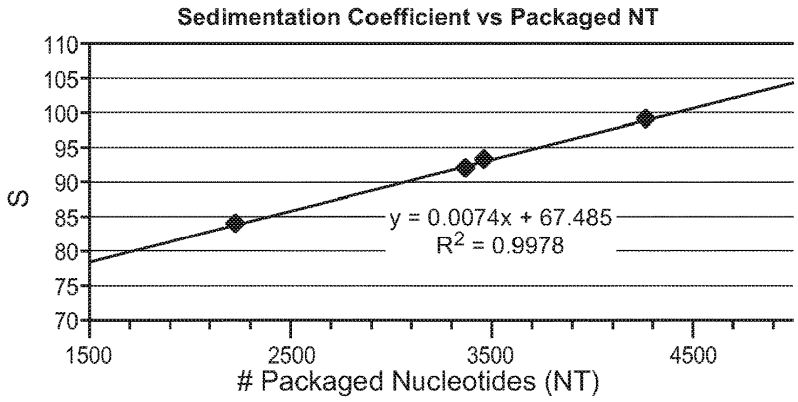


FIG. 8

Id., Fig. 8.

62. 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

63. 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.

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a. Example 5

64. "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.

65. 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 (%)."

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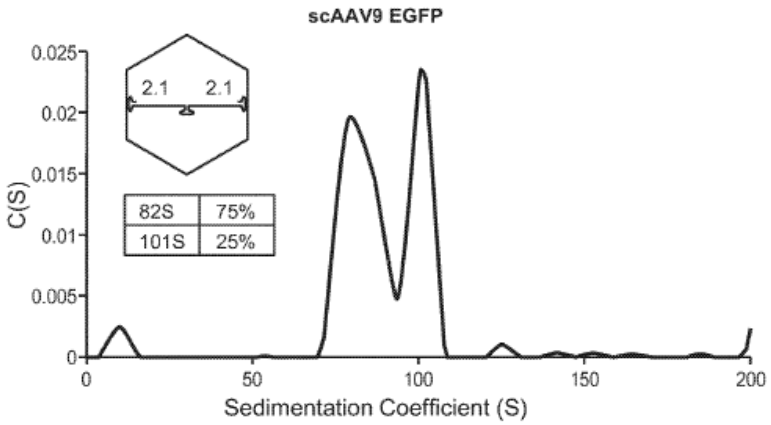


FIG. 9A

Id. at 9:41-50; 50:21-31; FIG. 9A.[1] Applying the standard curve, the inventors determined that the 101 S peak corresponded to an rAAV vector of ~4.3 kb (kb=kilobases), while the 82 S peak corresponded to a vector genome size of ~ 2 kb. *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

[1] The 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….").

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both the single-stranded monomeric species (***) and dimeric double stranded species (*) in lane 1, shown below.

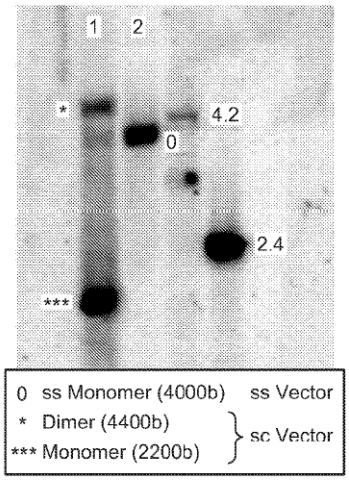


FIG. 9B

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

66. 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 peak single stranded rAAV vector peak at 85 S having a relative concentration of 16%.

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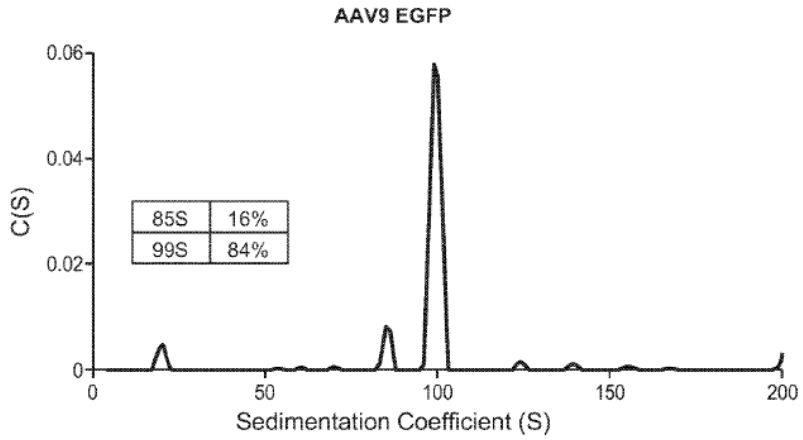


FIG. 9C

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

b. Example 3

67. The inventors compared the heterogenous rAAV preparations produced by two AAV 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).

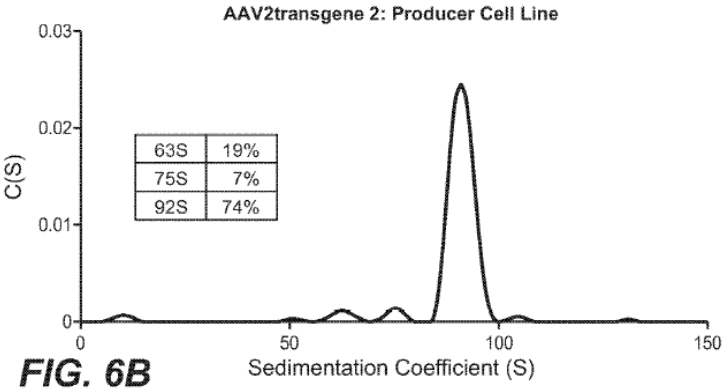


FIG. 6B

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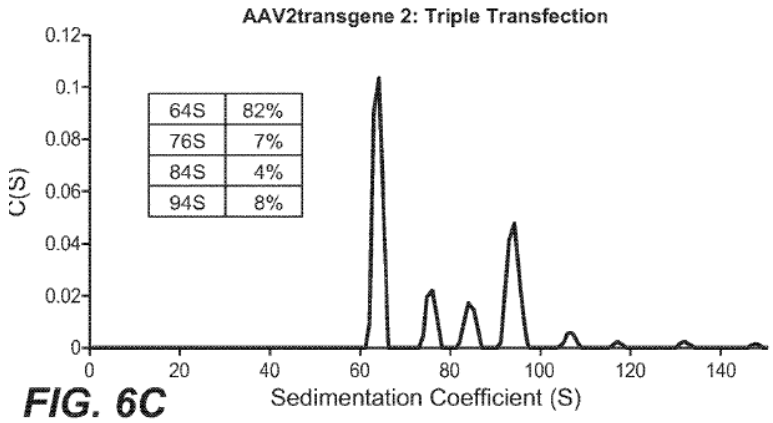


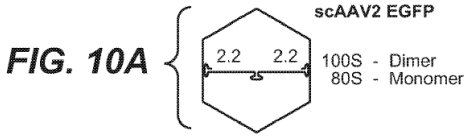
FIG. 6C

68. 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.

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c. Example 6

69. 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.



70. 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%.

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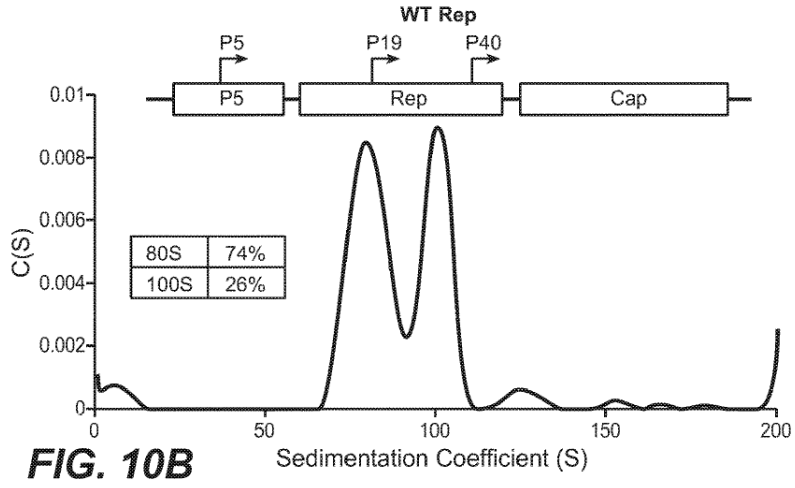


FIG. 10B

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%.

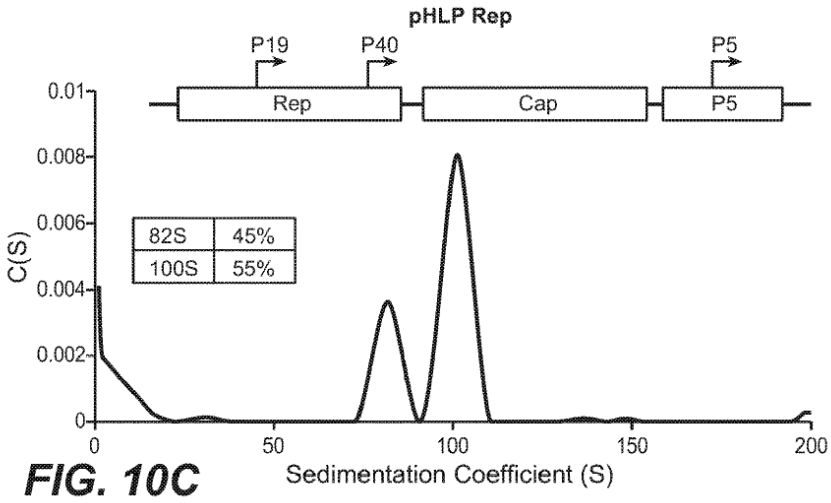


FIG. 10C

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.

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d. The Power of the Innovative AUC Method Compared to Southern Blot

71. 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.

72. "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/SFIX 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."

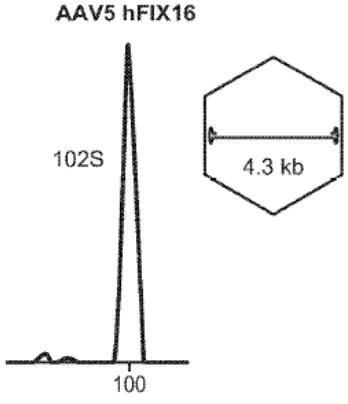


FIG. 11A

Id., 51:44-50, FIG. 11A. "In contrast, AAV5 FIX vector generated using [the unmodified wild-type, WT Rep,] generated a strikingly different AUC

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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."

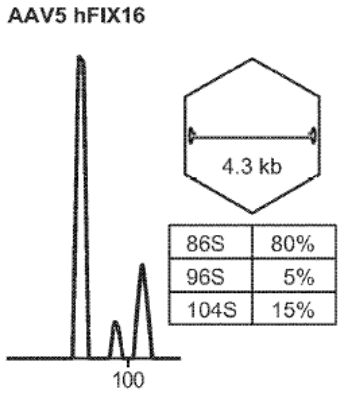


FIG. 11B

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

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

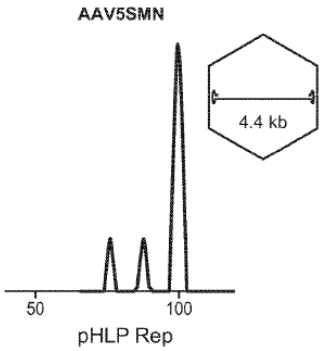


FIG. 11C

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)."

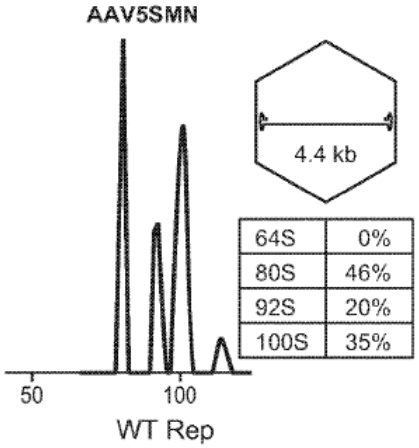


FIG. 11D

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

74. Southern blot analysis of the AAV5SMN preparations were consistent with the AUC results for the pHLP helper (FIG. 11A) showing a homogenous

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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.

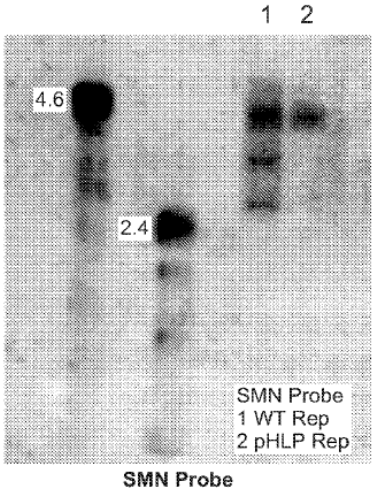


FIG. 12A

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).

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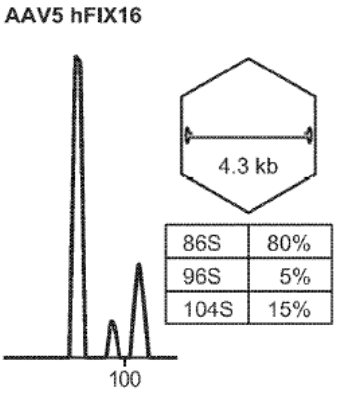


FIG. 11B

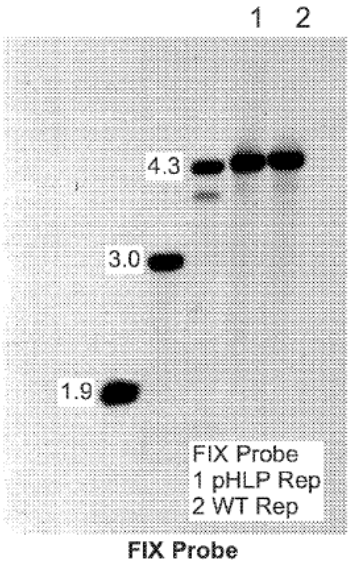


FIG. 12B

75. 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.

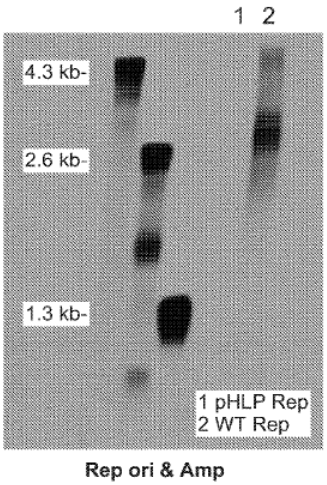


FIG. 14B

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

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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.

76. 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."

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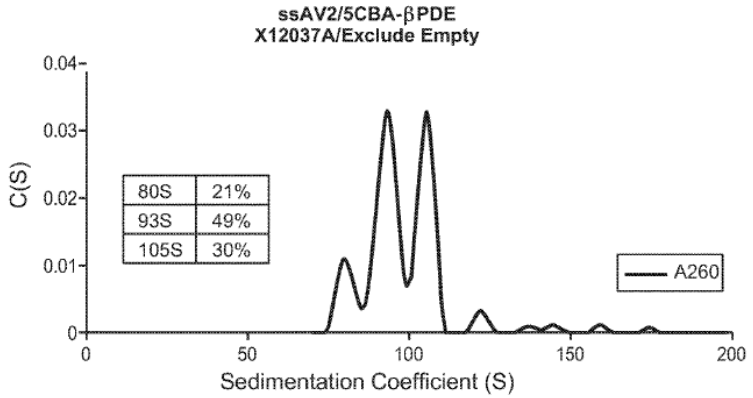


FIG. 15A

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."

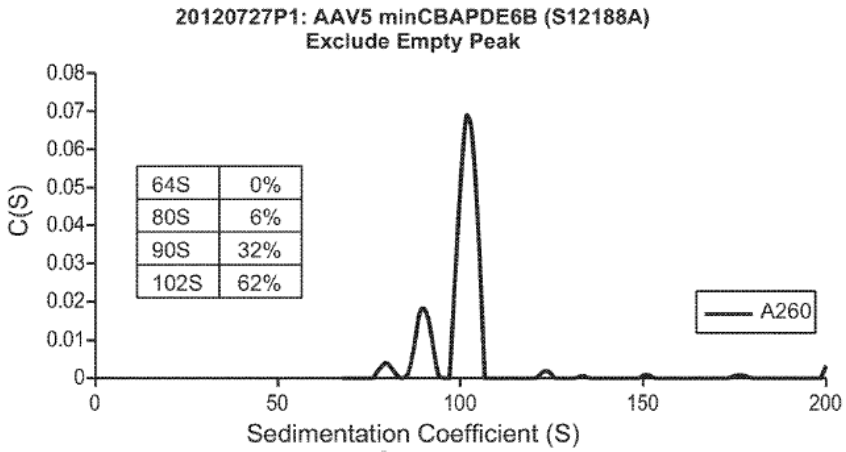


FIG. 15B

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.

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C. Prosecution History

77. 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 heterogenous 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.

78. 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,

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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.

79. 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," the 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.

D. Challenged Claims

80. 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:

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(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. Element 1(iii) requires determining the size of the one or more species of the individual variant viral particles in the heterogeneous mixture of viral particles and element 1(iv) requires determining the quantity of one or more species of the variant viral particles in the heterogeneous mixture of viral particles.

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Challenged claims 2-7 and 10-30 depend from claim 1. Dependent claim 3, recites that this can include "***comparing the sedimentation coefficients of the variant viral particles*** with a standard curve generated by sedimentation coefficients of viral particles comprising recombinant AAV genomes of known nucleotide sizes." *Id.*, 55:30-36.

IX. CLAIM CONSTRUCTION

81. 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. I reserve the right to address specific claim construction issues should the Board institute review.

X. A POSA WOULD NOT HAVE EXPECTED A PREDICTIVE RELATIONSHIP BETWEEN SEDIMENTATION COEFFICIENT AND GENOME SIZE BASED ON THE PRIOR ART

82. The specific method developed by the inventors—applying SV-AUC under defined boundary sedimentation velocity conditions to rAAV preparations—defied the reasonable expectations of a person of ordinary skill in the art. This innovative 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 all rAAV species in a heterogeneous mixture. A holistic assessment of the state of the art as of the effective filing date demonstrates why a POSA would not have expected a linear relationship between genome size and

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sedimentation coefficient. The relationship between genome size and sedimentation coefficient was unknown prior to the effective filing date. It is only through the inventors' innovative method that those in the field can now both appreciate and utilize the linear relationship between the size of encapsidated genome and sedimentation coefficient in AUC. *Supra* § VIII.B.

83. De la Maza's discussion of genome size versus particle density published in 1978, thirty-seven years before the effective filing date. During this time, AUC technology was widely available, rAAV gene therapy was an active area of research, and the need to characterize rAAV preparations was well known. Yet no one discovered or even hypothesized the linear relationship between sedimentation coefficient and genome size for intact rAAV particles, nor applied that relationship to determine the size of one or more fragmented genomes in a preparation of viral particles or determine the molar concentrations of each species of individual viral particles in a heterogeneous mixture of such particles. This long time gap is strong evidence that the invention was not obvious.

A. The Petition Conflates "Density" with "Sedimentation Coefficient"

84. Density alone does not determine a viral particle's sedimentation coefficient. Density is one factor and can be important, but friction as a particle migrates in the AUC cell also influences that particle's sedimentation coefficient. The equation below mathematically describes the forces that influence the

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sedimentation coefficient, *s*. Balancing these forces, results in a relationship where sedimentation, "*s* is proportional to the buoyant molar mass, M_b, and inversely proportional to the frictional coefficient, *f* " as shown in Equation (1) reproduced from Cole 2008:

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

Ex. 1009, 146.

85. Friction, *f*, is impacted by particle size and shape, 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

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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 the POSA would not have expected such a relationship. *Infra* § X.B.

87. The de la Maza analyses on AAV particles were conducted with sedimentation equilibrium or SE-AUC, which is different from sedimentation velocity, or SV-AUC. The Berkowitz Declaration admits that the distribution of particles in SE-AUC "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), shortly after the earliest priority date of the '894 Patent, contrasts the claimed method of SV-AUC with SE-AUC. 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.

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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.

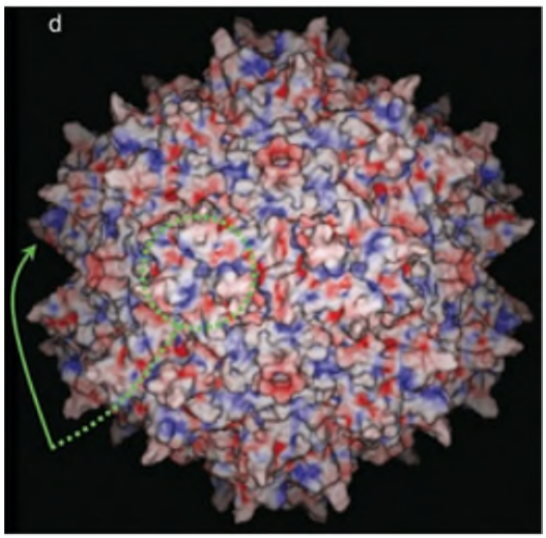
B. A POSA Would Have Expected rAAV Particle Friction to Impact Sedimentation Coefficients for Different Particles

89. It was understood before 2015 that AAV 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:

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> 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).").



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Ex. 2025, Fig. 3d.

90. 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.").

91. The POSA would have understood that any 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. Indeed, such changes to the viral particle surfaces were observed for adenovirus particles. Ex. 1023, 31.

92. 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

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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, the POSA would have understood that changes to particle mass would not be limited to genome size but could also be attributed to changes in the capsid protein stoichiometry since VP-1, -2, and -3 capsid proteins have different sizes and molecular weights.

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

93. 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* § VII.B), 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.

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94. 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 is solely attributed to differences in their densities could lead to incorrect genome size calculations.

95. 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

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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*. The POSA would have understood that such changes to the capsid are relevant to AUC analysis because they impact the sedimentation coefficient.

96. 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

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> 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 AAV virions. Thus, a POSA would not have had any basis to rely on electron microscopy to establish that all AAV particle species have the same size and shape.

97. The Petition's obviousness argument is premised on the assumption 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). 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

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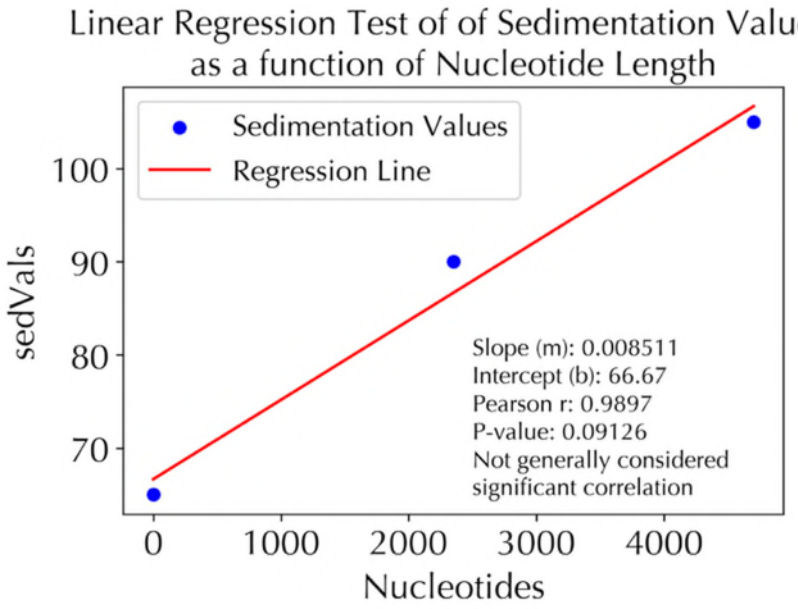
art. Because of this failure to consider the totality of the prior art's teachings, the Petition fails to establish the challenged claims as obvious.

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

98. 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). Both cursory visual inspection and a mathematical analysis of the Petition's plot refute a linear relationship between sedimentation coefficient and genome size.

99. 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.

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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. $p = 0.091258$).

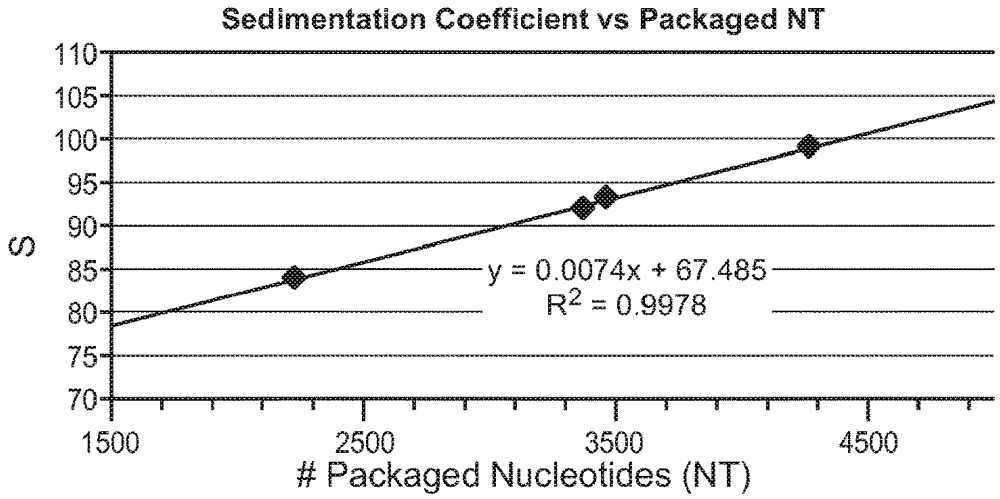


FIG. 8

Ex. 1001, Fig. 8.

100. A best-fit line can always be computed through any set of points—that is a mathematical operation, not a scientific conclusion. The meaning of a linear relationship requires statistical evidence. The standard piece of evidence is the p-value of the slope, which tests whether the null hypothesis of no relationship is disproven or not. The Petition drew a line; it did not establish statistical significance or linearity.

101. 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.

102. 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

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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* §§ X.A-C) validates the claimed method's nonobviousness.

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

103. I understand that the Petitioner bears the burden of proving that there is a reasonable likelihood that the challenged claims are unpatentable as anticipated and/or obvious based on the art and arguments set forth in its Petition. The inadequacies in the Petition's proofs identified herein are so significant, the merits failures cannot be remedied. The Petition fails to establish that it would have been obvious for a POSA to determine the size of a viral particle, *i.e.*, the amount of encapsidated genome, or its molecular weight, from its sedimentation coefficient.

A. Ground 1: The Petition Fails to Establish That Le Bec and de la Maza in View of Sommer Rendered Claims 1-7, 10-11 and 20-30 Obvious

104. In my opinion, Ground 1, alleging that claims 1-7, 10-11 and 20-30 are obvious over Le Bec and de la Maza in view of Sommer does not establish the obviousness of any claim.

1. Claim 1

105. 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 its Petition and Dr. Berkowitz's declaration, which is that a "POSA would understand that the size and shape of intact, non-

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aggregated AAV particles are approximately the same, regardless of the size of the genome packaged in the capsid." Petition, 45; Berkowitz Declaration, ¶¶ 164-170. The art as of the effective date, including Berkowitz 2007, demonstrates this is not true. A POSA would have understood that viral particles vary in unpredictable ways that impact particle shape and/or size, which impact 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. The lack of such a linear relationship in the Le Bec data would have further discouraged a POSA from seeking to identify such a relationship. *Supra* § X.D. The Petition reasons backward from the information in the patent itself. Thirty-seven years passed between de la Maza 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

106. The Petition fails to establish that a POSA would have had a reason to combine the teachings of Le Bec and de la Maza in view of Sommer in a way that would result in the method of claim 1. Even if a POSA was familiar with both Le

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Bec's AUC methodology, de la Maza's density-genome size observations, and Sommer's extinction coefficient calculations, the references I considered do not support that a POSA would have been motivated to combine their teachings in a way that would have resulted in at the claimed method.

107. 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… (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, claim 1. is inconsistent with a POSA's thinking in at least four ways.

108. 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. 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. Le Bec's

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method 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.

109. ***A***proper linear regression analysis conducted according to accepted techniques refutes the contention (Petition, 53-54) that Le Bec supports a linear relationship between sedimentation coefficients and genome size. *Infra*, § X.D. 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.

110. A POSA would not have understood de la Maza's discussion (Petition, 34-37) of the linear relationship between rAAV particle density and genome size as applicable to sedimentation coefficient. 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. Based on this knowledge, the POSA would not have had any reason to think there would have been a reliable relationship between genome size and sedimentation coefficient.

111. Sommer merely supplies extinction coefficient equations, which require the molecular weight of the encapsidated genome as an input. Ex. 1010,

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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 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 (*supra* Section X), a POSA would not have been motivated to develop the method of claim 1.

112. \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 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.

b. Le Bec's Method Does Not Resolve Viral Particles Comprising Fragmented Genomes

113. The Petition does not address that Le Bec's AUC studies fail to resolve peaks associated with rAAV particles containing fragmented genomes. Petition, 42. A POSA would have considered this a significant deficiency in Le Bec's disclosure. Thus, a POSA would not have relied on Le Bec for developing

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claim 1's method of determining the size of fragmented genomes in a heterogeneous mixture of viral particles.

114. Le Bec does not attempt to identify rAAV particles containing fragmented genomes. Le Bec's category of "full AAV viral particles" includes multiple peaks as shown in Le Bec's Figure 2.

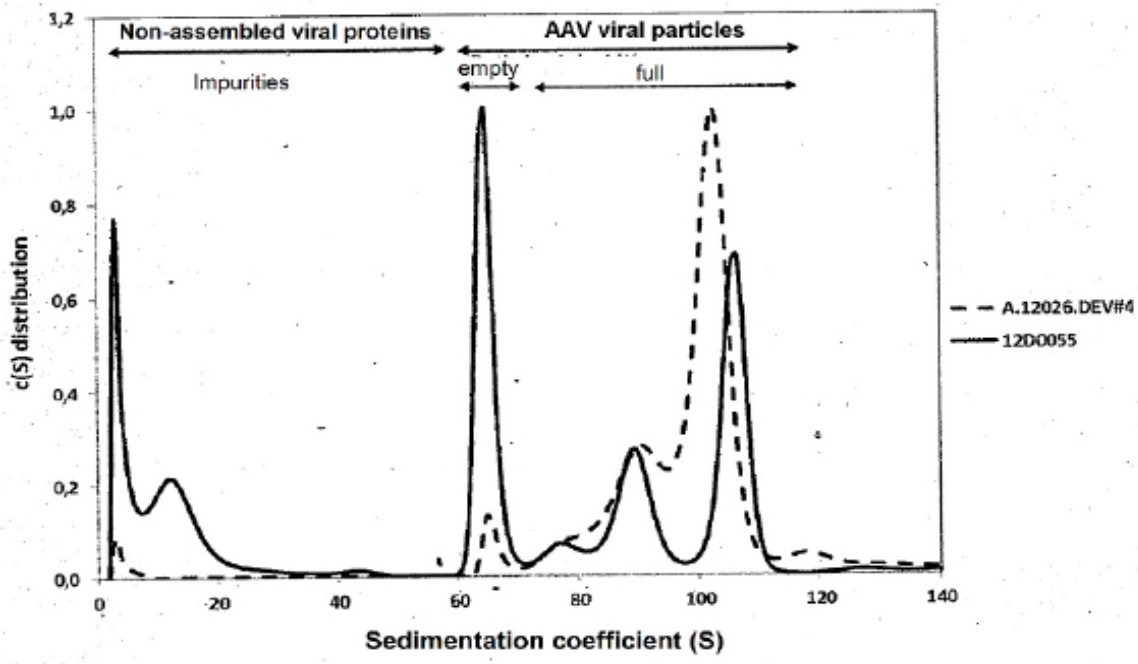


Figure 2

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 15 proteins and/or contaminants" and "b) 60S-110S corresponding to intact AAV viral particles" and "two subpopulations with empty AAV viral particles at 65S and full AAV viral particles between 80S and 110S, with the viral genome in single-stranded form at 90S and double-

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stranded form at 105S." *Id.*, 18:11-25. Given the poor resolution of peaks between 60S and 110S, a POSA would not have considered Le Bec useful for characterizing fragmented genomes.

115. 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 75 S to about 110 S in Figure 3 of Le Bec.

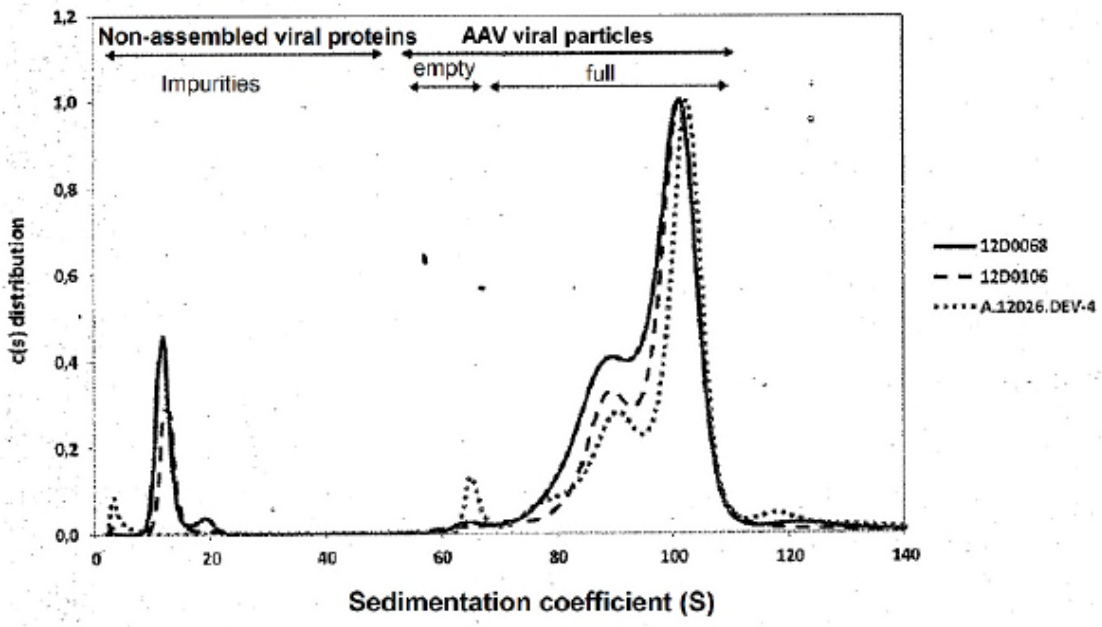


Figure 3

Ex. 1006, Fig. 3. This commingling 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. *See* Ex. 1025, 169 ("This method can be used to study … heterogeneous noninteracting and interacting systems ***provided that***, in

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the latter cases, the ***multiple components separate sufficiently during sedimentation to form distinct peaks*** in the g(*s**) versus *s** plots.") (emphasis added); Ex. 1031, 1606-08 (When "diffusion broadens the sedimentation boundary, [it] makes it more difficult to resolve subpopulations of the distribution").

116. Berkowitz 2015 explains that "diffusional broadening results in low resolution (fairly poor separation of different species), making detection and quantitation of minor species rather limited, especially for protein species that migrate with sedimentation coefficient values that are close to the other peaks and/or are present at low levels." Ex. 1024, 230. The '894 Patent explains that "if multiple species of solute are present, this may lead to the formation of multiple sedimenting boundaries, each corresponding to a resolvable species." Ex. 1001, 20:10-13. But "the presence of multiple species with similar sedimentation coefficients will cause the boundary to be broader than expected on the basis of diffusion alone." *Id.*, 17:41-43. The record indicates that the POSA would have considered the broad peaks in Figure 3 to have been concealing peaks that correspond to partial or fragmented species. Thus, the POSA would not have attempted to apply Le Bec's method to determine the size of a fragmented genome in an rAAV particle. The POSA would not have considered Le Bec's peaks sufficiently resolved for this application.

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117. The contrast between Le Bec's poorly resolved peaks and the well-resolved peaks in the '894 Patent is stark. Compare Le Bec Figure 3 (Ex. 1006) with Figures 6B-C of the '894 Patent (Ex. 1001). The '894 Patent clearly shows distinct, well-separated peaks, allowing precise identification and quantification of each species. Le Bec's Figure 3 shows a broad, poorly resolved region from approximately 75S to 110 S that obscures the presence of individual species. A POSA would immediately recognize that Le Bec's method was inadequate for the task of identifying and quantifying fragmented genome species.

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

118. In my opinion, 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.D. Drawing a "best fit" line is different from conducting a meaningful linear regression.

119. 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

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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. More stringent thresholds include $p \leq 0.01$ (99% confidence) and $p \leq 0.001$ (99.9% confidence) for situations requiring stronger evidence.

120. The Petition fails to apply the accepted linear regression analysis that a POSA would have applied. Petition, 53-54. Analytical analyses require linear regression to be statistically significant at the 95% confidence level. 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.

121. 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.

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

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

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

	Coefficients	*Standard Error*	*t Stat*	*p-value*
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 (.9976 vs .9978). In this linear regression, the p-value for the slope of the linear regression is 0.00118, which is highly significant ($p << 0.05$) 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

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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.

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

Metric	**Le Be (Ex. 1006)**	**'894 Patent Fig. 8 (Ex. 1001)**
Data Points	3	4
Slope p-value	0.091258 (NOT significant)	0.00118 (highly significant)
R2	Not reported by Berkowitz	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 data pass even the more stringent 99% confidence threshold ($p \leq 0.01$). Le Bec's data fail the basic 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.

124. 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

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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.

d. A POSA Would Not Have Considered de la Maza's Relationship Between AAV Particle Density and Genome Size to Apply to Sedimentation Coefficients

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

126. De la Maza describes the use of SV-AUC to study the fractionation of incomplete genomes but not in the context of intact viral particles. To conduct its SV-AUC experiment "DNA was released from virions by sedimentation in alkaline sucrose gradients." Ex. 1007, 194. This means that de la Maza did not conduct SV-AUC with intact viral particles but instead used SV-AUC 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.

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127. Likewise, a POSA would not have been 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.C. 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.

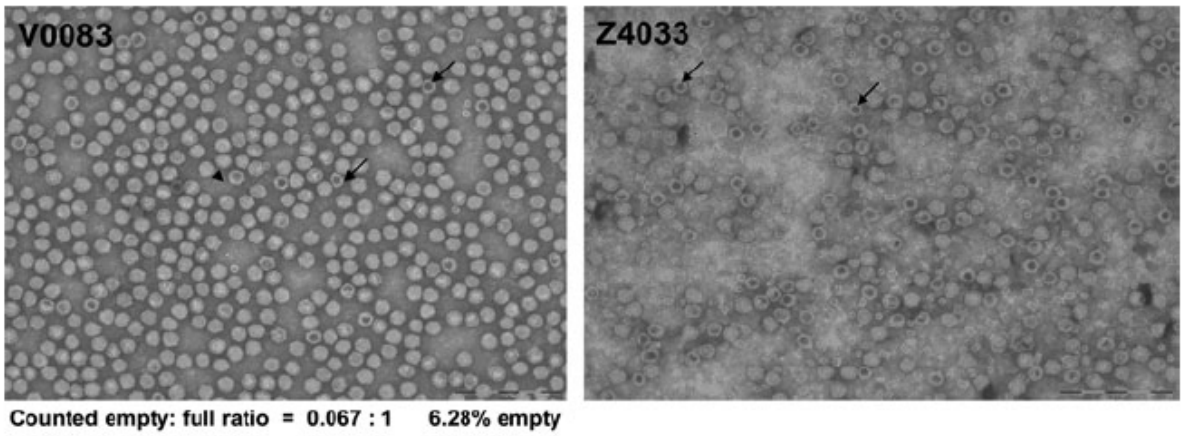
128. The Petition attempts to bridge its failure of proof with a conclusory characterization of publications from 1978 and 1980 relating to the mechanism of genome packaging. Petition, 45 (citing Ex. 1007, 203; Ex. 1008, 3202; Ex. 1004, ¶170). Decades later, Berkowitz 2007 explained that viral particles 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.

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129. The Petition contends 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. One of the references that the Petition cites, however, Gao (Ex. 1026), indicates that partially empty particles, in fact, do have different confirmations 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.

130. 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. Lock's images indicate a qualitative difference in particle shapes and sizes.

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

131. 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.

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132. 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.

133. 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 the POSA would not have expected a linear relationship between *S*-values and genome molecular weight.

e. 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

134. 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 above. 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 the extinction coefficient of the particle at

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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. 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).

135. Quantification of the different species in a heterogenous composition can be determined by integrating the areas under the curve in a sedimentation coefficient distribution, but only if one knows the extinction coefficient of each species. This is because the degree of absorbance depends on the extinction coefficient as expressed by Beer's Law:

$$A = \varepsilon c l$$

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.

136. 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 requires knowing the extinction coefficient (ε) of each species, because different rAAV species will absorb certain wavelengths of light to

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different degrees. *Id.*, 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.").

137. 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 adjusts for this difference between species. Ex. 1009, 152 (explaining that "absorbance signal is directly proportional to solute concentration: $A=\varepsilon cl$, where ε is the solute's weight extinction coefficient, c is the weight concentration, and l is the sample path length."); *see also* Ex. 1010, 126-127.

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

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

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

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

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

Id.

139. 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.

f. A POSA Would Not Have Reasonably Expected that Sedimentation Coefficients Could Be Used to Determine Fragmented Genome Size in rAAV Particles and to Successfully Quantify the rAAV Particles Containing Fragmented Genomes

140. It is my opinion that the reasonable expectation of success argument in the Petition presents the same problem as its motivation argument. 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.

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141. A POSA would not have considered de la Maza's disclosure of the linear relationship between particle density and genome size to suggest that a linear relationship exists between sedimentation coefficient and genome size. The Petition's argument relies on the assumption that a POSA would have ignored the effects of friction. As Dr. Berkowitz's own publications emphasize, a POSA would not have done so. The prior art, including Berkowitz 2007, described viral particles having differing shapes and sizes, which impacts 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.

142. 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

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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 the size of the encapsidated genome as the Petition alleges. Petition, 63.

143. 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

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establish 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.

144. I understand that it is Petitioner's burden to demonstrate that the 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.D. 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. Claims 2-7, 10-11 and 20-30

145. 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 2-7, 10-11 and 20-30 all depend from claim 1, it is my opinion

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that the combination of Le Bec and de la Maza in view of Sommer does not render claims 2-7, 10-11 and 20-30 of the ’894 Patent obvious.

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

146. 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. CONCLUSION

147. The challenged claims of the ’894 Patent are not obvious in view of the Grounds set forth in the Petition and the Berkowitz Declaration.

148. 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: March 4, 2026

Dr. Karen G. Fleming

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