

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

PATENT OWNER'S PRELIMINARY RESPONSE

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2001	Declaration of Katherine A. Helm
2002	<i>Genzyme Corporation v. Sarepta Therapeutics Inc.</i> (1:24-cv-00882) D.I. 78 – Joint Stipulation to Amend Scheduling Order
2003	<i>Genzyme Corporation v. Sarepta Therapeutics Inc.</i> (1:24-cv-00882) D.I. 59 – Notice of Service of Plaintiff’s Initial Claim Charts on Infringement
2004	<i>Genzyme Corporation v. Sarepta Therapeutics Inc.</i> (1:24-cv-00882) D.I. 68 – Notice of Service of Defendant’s Initial Invalidity Contentions
2005	<i>Genzyme Corporation v. Sarepta Therapeutics Inc.</i> (1:24-cv-00882) D.I. 28 - Notice of Service of Plaintiff’s First Set of Requests for Production of Documents and Things
2006	<i>Genzyme Corporation v. Sarepta Therapeutics Inc.</i> (1:24-cv-00882) D.I. 35 - Notice of Service of Defendant’s First Set of Requests of Production of Documents and Things to Plaintiff and First Set of Interrogatories and Initial Disclosures
2007	<i>Genzyme Corporation v. Sarepta Therapeutics Inc.</i> (1:24-cv-00882) D.I. 37 - Notice of Service of Plaintiff’s Second Set of Interrogatories and Second Set of Requests for Production of Documents and Things
2008	<i>Genzyme Corporation v. Sarepta Therapeutics Inc.</i> (1:24-cv-00882) D.I. 45 – Notice of Service of Plaintiff’s First Set of Interrogatories and Third Set of Requests for Production of Documents and Things
2009	<i>Genzyme Corporation v. Sarepta Therapeutics Inc.</i> (1:24-cv-00882) D.I. 67 - Notice of Service of Defendant’s Second Set of Requests of Production of Documents and Things to Plaintiff and First Set of Interrogatories
2010	<i>Genzyme Corporation v. Sarepta Therapeutics Inc.</i> (1:24-cv-00882) D.I. 83 - Notice of Service of Defendant’s Amended Second Set of Requests of Production of Documents and Things to Plaintiff and First Set of Interrogatories
2011	<i>Genzyme Corporation v. Sarepta Therapeutics Inc.</i> (1:24-cv-00882) D.I. 87 - Notice of Service of Defendant’s Third Set of Requests of Production of Documents and Things to Plaintiff

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2012	<i>Genzyme Corporation v. Sarepta Therapeutics Inc.</i> (1:24-cv-00882) D.I. 91 – Notice of Service of Plaintiff’s Responses to Defendants Third Set of Requests for Production of Documents and Things
2013	<i>Genzyme Corporation v. Sarepta Therapeutics Inc.</i> (1:24-cv-00882) D.I. 92 - Notice of Service of Plaintiff’s Fourth Set of Requests for Documents and Things
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2015	<i>Genzyme Corporation v. Sarepta Therapeutics Inc.</i> (1:24-cv-00882) D.I. 102 – Notice of Service of Defendant’s Fourth Set of Requests of Production of Documents and Things to Plaintiff
2016	<i>Genzyme Corporation v. Sarepta Therapeutics Inc.</i> (1:24-cv-00882) D.I. 110 – Notice of Service of Plaintiff’s Sixth Set of Requests for Production of Documents and Things
2017	<i>Genzyme Corporation v. Sarepta Therapeutics Inc.</i> (1:24-cv-00882) D.I. 76 – Second Amended Complaint
2018	<i>Genzyme Corporation v. Sarepta Therapeutics Inc.</i> (1:24-cv-00882) - Defendant’s Supplemental Initial Invalidity Contentions
2019	Burnham et al., Analytical Ultracentrifugation as an Approach to Characterize Recombinant AAV Vectors, 26 <i>Human Gene Therapy Methods</i> 228 (2015) (“Burnham 2015”)
2020	F. Collaud et al., Preclinical Development of an AAV8-hUGT1A1 Vector for the Treatment of Crigler-Najjar Syndrome, 12 <i>Molecular Therapy: Methods & Clinical Development</i> 157, 166-67 (March 2019)
2021	E. Spies, Assessing the Quality of Adeno-Associated Virus Gene Therapy Vectors by Sedimentation Velocity Analysis, <i>AUC Insights</i> (March 2020)
2022	<i>Novartis Gene Therapies, Inc. v. Genzyme Corp.</i> , IPR2023-01044, Paper 12 (Jan. 23, 2024), Patent Owner’s Request for Rehearing by the Director
2023	US 2017/0362608 to McLaughlin et al. (“McLaughlin”)
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2028	D. Curran-Everett, Explorations in Statistics: Hypothesis Tests and P-values, 33 Advances in Physiology Education 81-86 (June 2009)
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2031	Declaration of Karen G. Fleming (“Fleming”)
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I. Introduction

Gene therapy using recombinant adeno-associated virus (“rAAV”) vectors holds significant promise for treating genetic diseases. The clinical application of rAAV vector-based gene therapy requires quantifying species of rAAV particles of differing genome sizes. The preparation of rAAV vectors often results in heterogenous mixtures containing “empty viral particles, full genome-containing viral vectors, and vectors with fragmented genomes or non-transgene-related DNA contaminants.” Ex. 2019, 228; *see also, e.g.*, Ex. 2027, 81, 86-89; Ex. 1001, 48:37-44; Fleming, ¶¶ 31-35. As of the 2015 effective filing date of U.S. Patent No. 12,031,894 (Ex. 1001, the “’894 Patent”), there was no single analytical method that permitted the reliable quantification of rAAV particle species. The ’894 Patent provided the first.

This quantification is critical because in therapeutic applications rAAV particles containing incomplete genome fragments can cause dangerous side effects, including immunotoxicity. Ex. 2027, 87; Ex. 1026, 6. Thus, the “generation of AAV vector products requires an analytical method that monitors product quality with regard to homogeneity, purity, and consistency of manufacturing.” Ex. 1001, 43:49-59. Prior to the ’894 Patent methods, “no method to support AAV vector characterization ha[d] been established.” *Id.*

The claimed invention is a breakthrough application of sedimentation velocity analytical ultracentrifugation (“SV-AUC” or “AUC”)¹ to quantitatively characterize recombinant rAAV particle preparations. Prior to this invention, there was no adequate technique that, in a single experiment, could achieve these necessary quantitative analytical results. This unsolved need presented significant impediments to the characterization and overall viability of gene therapies.

Although AUC had been used for decades with viral particle compositions (Ex. 1007), scientists confronted a major obstacle to applying AUC to quantitatively measuring rAAV species within a heterogeneous mixture of rAAV particles. Prior to this invention, it was not possible to determine the concentration of rAAV particles containing fragmented genomes of unknown size. Ex. 1001, 24:56-59. The inventors developed a specific and non-obvious method that solved this problem: by subjecting rAAV preparations to SV-AUC under precisely defined boundary sedimentation velocity conditions—including specific rpm ranges, temperatures, and detection parameters—they were able, for the first time, to resolve and quantify rAAV species in a heterogeneous mixture, including particles containing fragmented genomes of previously unknown size. This technological advance enabled the development of methods for determining the

¹ Unless otherwise specified, AUC without the “SV” prefix refers to SV-AUC.

size of fragmented genomes as a function of empirically measured sedimentation coefficients under those specific conditions and for determining the quantity of one or more species of the variant viral particles in the heterogeneous mixture of viral particles.

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 rAAV species in a heterogeneous mixture. Sarepta Therapeutics Inc. (“Sarepta” or “Petitioner”), however, filed *Inter Partes* Review (“IPR”) petition IPR2026-00150 (the “Petition”) alleging that claims 1-7 and 10-30 (the “challenged claims”) of the ’894 Patent are obvious. Sarepta’s Petition fails to demonstrate by a reasonable likelihood that the prior art disclosed or rendered obvious the challenged claims.

Sarepta’s principal failure is its heavy reliance on impermissible hindsight. Indeed, Sarepta’s 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. Sarepta’s argument that a POSA would have expected a linear relationship between genome size and sedimentation coefficient for AUC is premised on a decades-old publication, de la Maza (Ex.

1007). Petition, 2. Sarepta alleges that de la Maza identified a linear relationship between genome size and particle density and that this linear relationship predicted a linear relationship between genome size and sedimentation coefficient. *Id.*, 7, 20-25. Thirty-seven years elapsed between de la Maza's 1978 density observations and the '894 Patent's 2015 filing date, yet no one made this connection or attempted to apply de la Maza to the problem of determining the size of the fragmented genomes in a preparation of rAAV particles. This temporal gap is the hallmark of hindsight. *Leo Pharm. Prods., Ltd. v. Rea*, 726 F.3d 1346, 1354 (Fed. Cir. 2013).

Sarepta's obviousness theory rests on two fundamental scientific errors:

First, Sarepta conflates density with sedimentation coefficient. While related, they are not the same thing. Sedimentation coefficient depends on both buoyant mass (density) and friction: $s \propto Mb/f$. Ex. 1009, 146. De la Maza studied density using sedimentation equilibrium AUC (SE-AUC), which is friction-independent. The '894 Patent uses sedimentation velocity AUC (SV-AUC), where friction matters. At the time of filing of the '894 Patent and in the absence of hindsight reasoning, a POSA would not have assumed that a linear density/genome-size relationship predicts a linear sedimentation-coefficient/genome-size relationship—particularly given warnings in the literature,

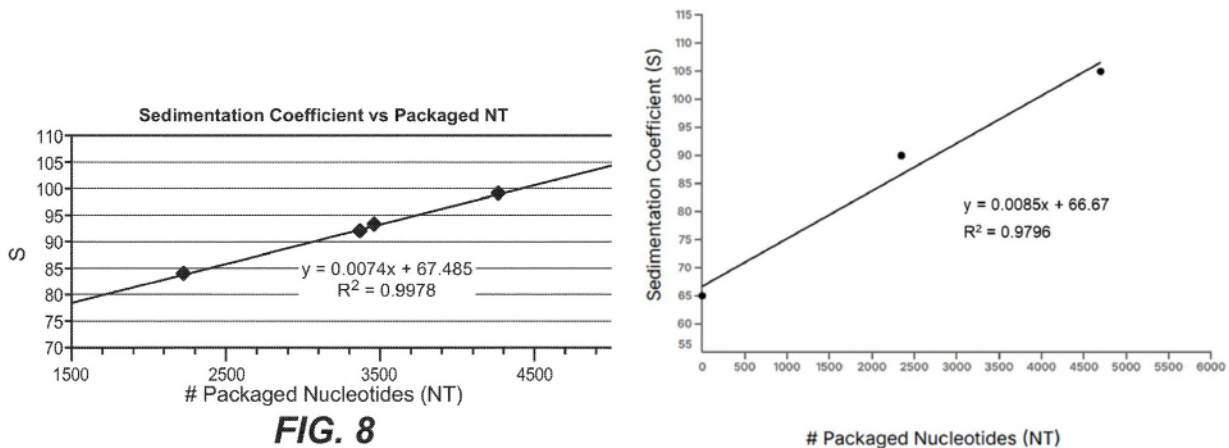
including from Sarepta’s expert, that viral particle friction varies unpredictably.

Ex. 1023 (Berkowitz 2007), 31.

Second, Petitioner misrepresents the data in Le Bec, its primary reference. The Petition claims Le Bec’s Figure 2 shows a linear relationship between sedimentation coefficient and genome size. Petition, 27-28. It does not. Petitioner drew a trendline through three data points but failed to report the p-value—the critical statistical measure a POSA would have calculated. That p-value is 0.091258, which fails the standard 95% confidence threshold ($p \leq 0.05$). Fleming, ¶ 16. Even assuming, *arguendo*, that a POSA would attempt such an exercise, a POSA would have concluded that Le Bec’s data show no statistically significant linear relationship. Drawing a trendline is a mathematical operation; establishing a linear relationship requires statistical validation. Petitioner did not provide it because the statistics refute its argument. Compare Le Bec’s data to the ’894 Patent’s Figure 8:

Reference	Data Points	p-value	R ²	Conclusion
Le Bec Fig. 2	3	0.091258 (fails 95% threshold)	Not Reported by Berkowitz	No linear relationship
’894 Patent Fig. 8	4	0.00118 (passes 99% threshold)	0.9978	Strong linear relationship

Even visual inspection confirms the difference. In Figure 8, all four points cluster tightly on or near the regression line, whereas none of Le Bec's three points fall on the trendline Sarepta drew.



Ex. 1001, Figure 8 (left); Petition, 54 (right). The visual difference in the plots reflects the underlying statistical difference ($p = 0.00118$ (highly significant) vs. $p = 0.091258$ (not significant)).

Sarepta's failure to address the entirety of the art and its mistaken regression analysis of Le Bec's data are fatal to its obviousness grounds. It is a "longstanding principle that the prior art must be considered for all its teachings, not selectively." *Henny Penny Corp. v. Frymaster LLC*, 938 F.3d 1324, 1332 (Fed. Cir. 2019). Sarepta, however, "selectively culled from the prior art to fit the parameters of the patented invention." *ATD Corp. v. Lydall, Inc.*, 159 F.3d 534, 546 (Fed. Cir. 1998). The Board "must avoid 'hindsight bias and must be cautious of arguments

reliant upon ex post reasoning.” *Arendi S.A.R.L. v. Apple Inc.*, 832 F.3d 1355, 1361 (Fed. Cir. 2016). Thus, the Board should find that Sarepta fails to establish a reasonable likelihood of succeeding in proving the challenged claims obvious.

II. Background

A. The '894 Patent

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. Fleming, ¶¶ 55-56. The claimed method remains the industry standard for detection of rAAV species and a practical and useful release assay. *Infra* § II.B.

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 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.

B. The Importance of Genzyme's Innovative AUC Method

A 2015 peer-reviewed publication co-authored in-part by the inventors of the '894 Patent, Burnham and O'Riordan, and colleagues (Ex. 2019), publicized experiments disclosed in the '894 Patent, including Examples 1-6. *See, e.g.*, Ex. 2019, 232, Figure 1 (Ex. 1001, Example 1 (FIGS. 1A, 1B, 2A, 2B)), 233, Figure 2 (Ex. 1001, Example 2, Table 4, FIGS. 3A-3B), 234, Figures 3A, 3B (Ex. 1001, Example 3, FIGS. 6B, 6C), 235, Figure 4 (Ex. 1001, Example 4, FIG. 7A-7C), 236, Figure 5 (Ex. 1001, Example 5, FIGS. 8, 9A-9C), 237, Figure 6D (Ex. 1001, Example 6, FIGS. 11A); Fleming, ¶ 52. 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 tool to analyze rAAV vectors notwithstanding the composition and length of the transgene or the viral serotype.

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

Citing the inventors' work, a 2018 publication co-authored by an inventor of the Petitions' 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. Fleming, ¶ 53. 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.

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; Fleming, ¶ 54. Sarepta 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.

C. Disclosed Innovation of the '894 Patent

"[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.

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. Fleming, ¶ 56.

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

quantification of each species present—including capsids comprising partial or fragmented genomes. Fleming, ¶ 57.

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

The POSA understood, however, that merely integrating peak signal area would not accurately quantify the concentration of each rAAV species. Fleming, ¶ 58. 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.”); *see also* Fleming, ¶ 58.

Extinction coefficients had been determined for empty capsids and fully intact rAAV particles at an absorbance wavelength of 260 nm. Ex. 1001, 24:44-47 (citing Ex. 1010, 127) (“Molar absorbance extinction coefficients for both empty capsids ($\epsilon_{260/\text{capsid}}=3.72\text{e}6$) and intact vector ($\epsilon_{260/\text{vector}}=3.00\text{e}7$) can be calculated based on published formulae.”). However, “it [was] not possible to determine

empirically the extinction coefficient of fragmented genomes,” because they are “of unknown size and sequence” *Id.*, 24:56-59; 45:2-5; 49:57-61; Fleming,

¶ 59. 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.

Ex. 1001, 24:59-67; *see also id.*, 45:2-8.

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; Fleming, ¶ 60. 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.” Ex. 1001, 49:57-67.

TABLE 5

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

Ex. 1001, 50:1-19.

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

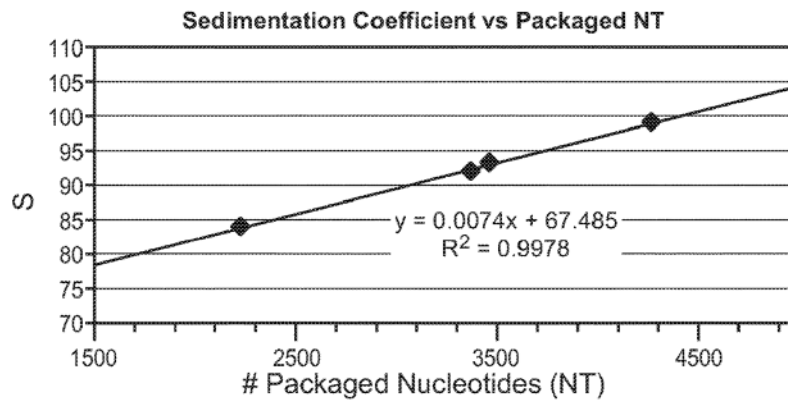


FIG. 8

Id., Fig. 8.

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; Fleming, ¶¶ 61-62.

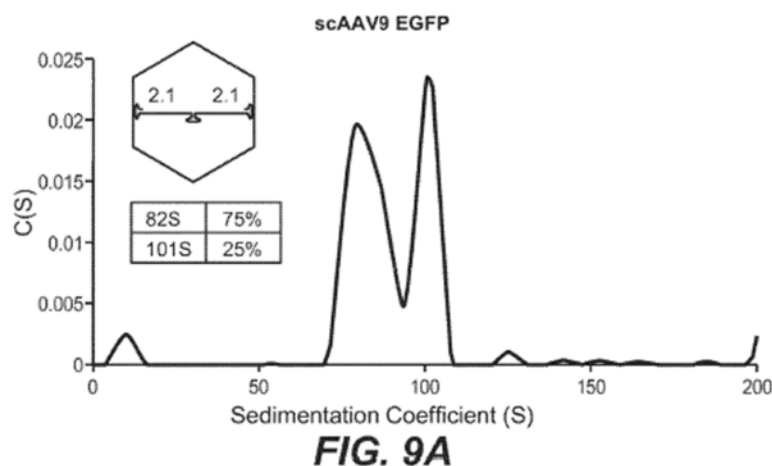
2. One-Step rAAV Preparation Analysis by AUC

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. Fleming, ¶¶ 63-76.

a. Example 5

“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.

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-complimentary] scAAV9 EGFP vector preparation. Single stranded monomeric (82S) and double stranded dimeric (101S) species are labeled with the corresponding sedimentation coefficients and relative abundance values (%).”



Ex. 1001, 9:41-50; 50:21-31; FIG. 9A.² 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 both the single-stranded monomeric species (***) and dimeric double stranded species (*) in lane 1, shown below.

² 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. Fleming, ¶ 65; 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....”).

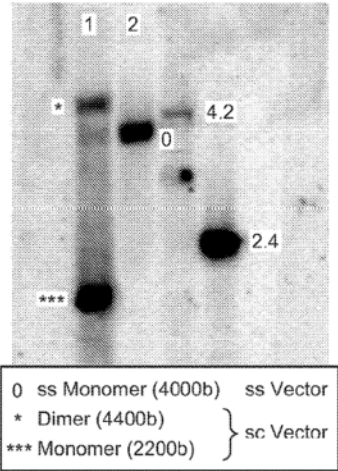


FIG. 9B

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

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 99 S, corresponding to approximately 4.1 kb by the standard curve and 84% of capsid abundance” and a second peak single stranded rAAV vector peak at 85 S having a relative concentration of 16%.

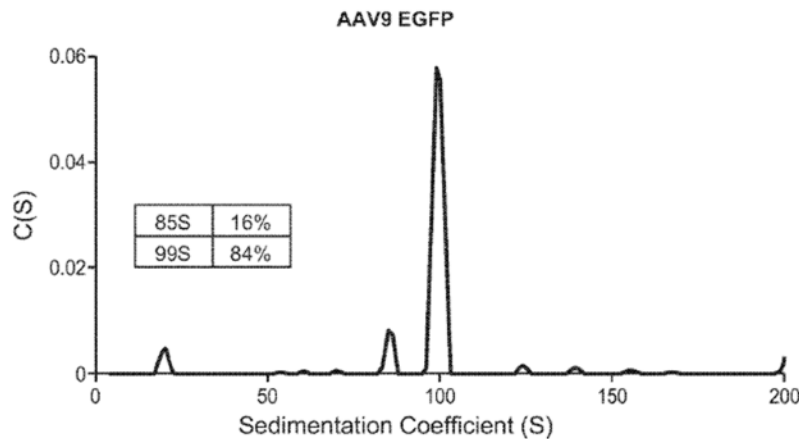
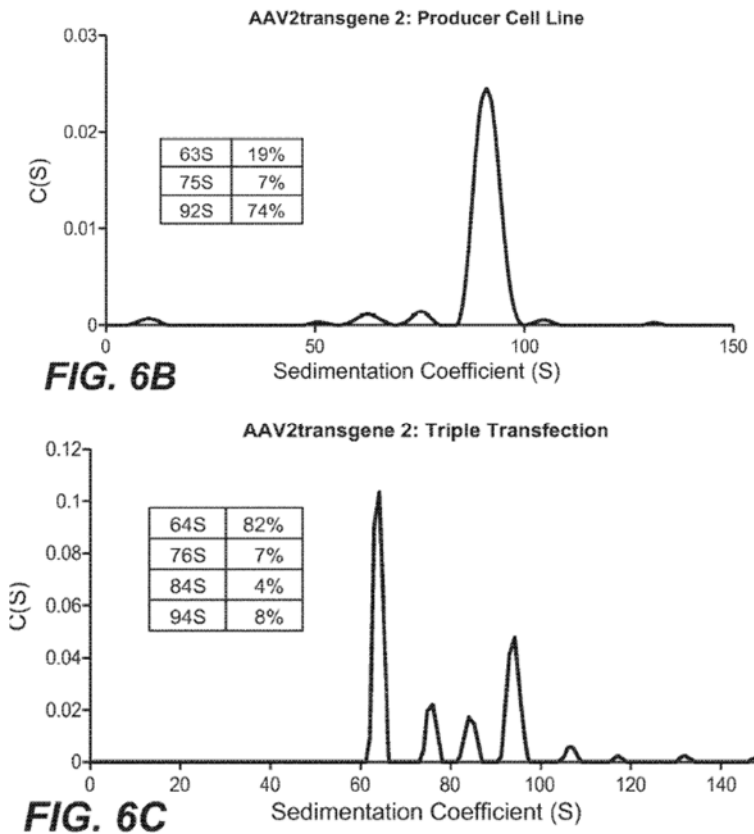


FIG. 9C

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

b. Example 3

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).



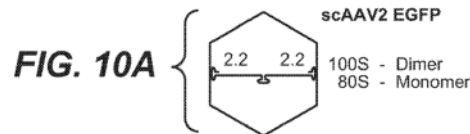
The fragmented genomes were identified to have peaks at 75 S (FIG. 6B) 76 S and 84 S (FIG. 6C). Ex. 1001, 48:51-60. The inventors determined the concentrations of the fragmented species identified in their experiments, observing

that “[t]he AUC profiles of vector preps produced by these methods were remarkably different.” *Id.*, 48:51-52. “Using the producer cell line method, 74% of capsids contained a full genome, represented by the 92 S species (FIG. 6B). 19% were empty capsids, with the remainder containing a fragmented genome species (75 S species, 7%).” *Id.*, 48:52-56. The “triple transfection method” resulted in a less successful outcome: “82% of the capsids ... were empty, 64 S species (FIG. 6C), with 11% of capsids containing a fragmented genome (76 S and 84 S species) and only 8% of capsids having a full genome (94 S).” *Id.*, 48:56-60. The identification and quantification of each species illustrate the value of the inventive method and its utility for determining the relative advantage of rAAV preparation and manufacturing procedures.

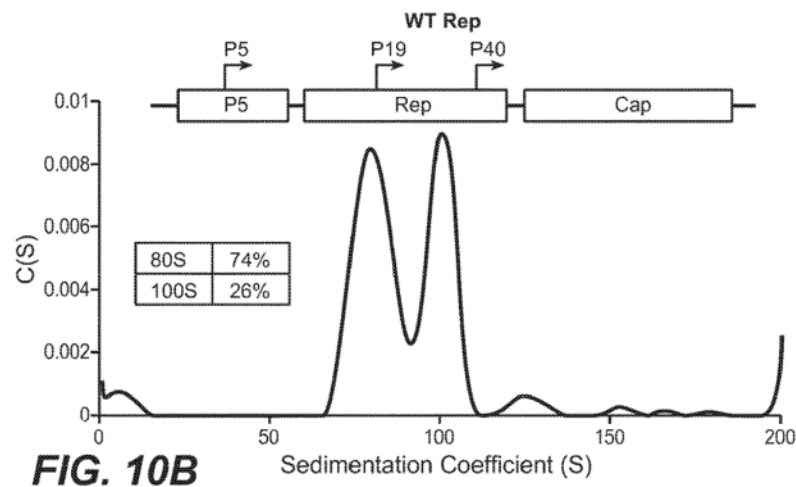
c. Example 6

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

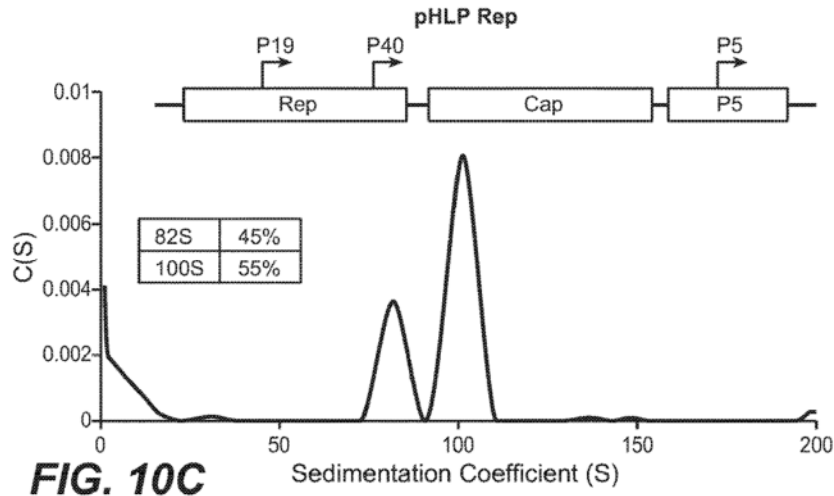
(FIG. 10C)” where “the helper was modified” (the “pHLP Rep” construct). *Id.*, 51:15-21. “The full scAAV2 EFGP capsid was predicted to have a sedimentation coefficient of 100 S in dimeric genome form and 80 S in monomeric genome form (FIG. 10A).” *Id.*, 51:21-24.



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



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

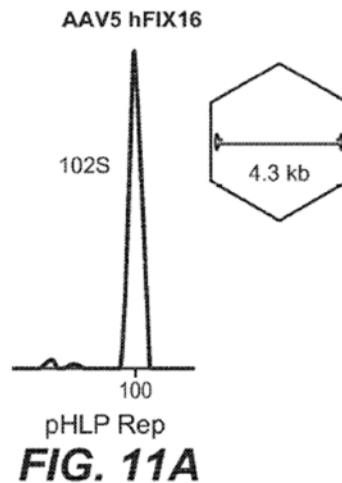


Id., 51:25-29. “These results uncovered a significant difference in genome packaging induced by” modifying “the helper plasmid.” *Id.*, 51:35-38. Detection of this result was facilitated by the AUC analysis differentiating between rAAV species containing different levels of genome encapsidation.

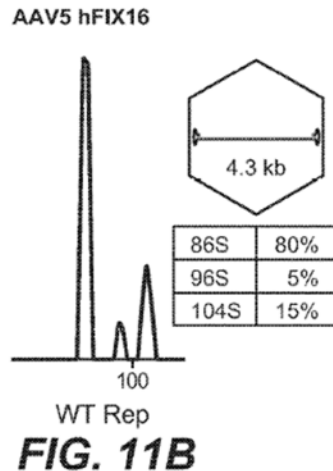
d. The Power of the Innovative AUC Method Compared to Southern Blot

The power of the disclosed AUC method was further revealed through a comparison of AUC with Southern blot analysis. Fleming, ¶¶ 71-76. In another experiment of Example 6, a “single stranded AAV5 Factor IX vector, AAV5FIX, (FIG. 11A-B) and a single stranded AAV5hSMN vector (FIGS. 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.

“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.”

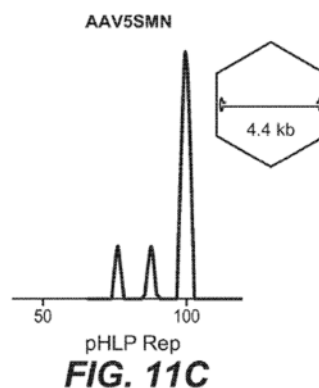


Id., 51:44-50, FIG. 11A. “In contrast, AAV5 FIX vector generated using [the unmodified wild-type, WT Rep,] generated a strikingly different AUC profile....Instead of a predominant peak at 101 S, this profile revealed more capsid heterogeneity, with the majority of the AAV5 FIX (80%) sedimenting at a lower S value of 86 S, likely representing packaging of a fragmented genome” whereas “only 15% of the AAV5 FIX vector capsids sedimented at the correct S value of ~104 S.”



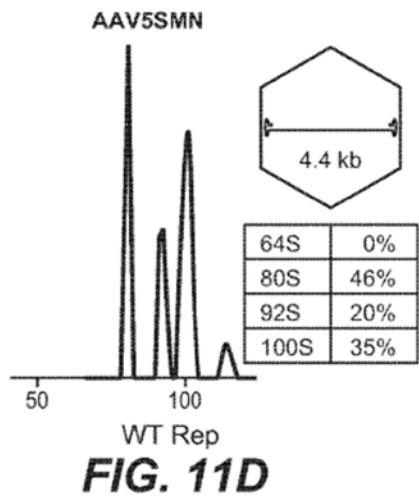
Id., 51:50-60, FIG. 11B.

AAV5SMN vectors made using these same wild-type and mutated helpers also had different profiles. Fleming, ¶ 73. “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.”



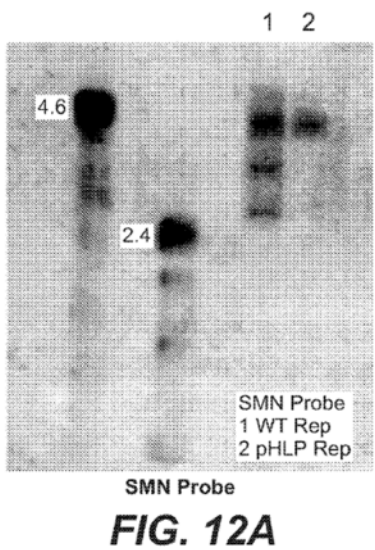
Ex. 1001, 51:61-52:1, FIG. 11C. “In contrast, the AUC profile for the same vector genome packaged using ‘wild-type’” WT Rep helper “revealed three distinct AAV vector species, with sedimentation coefficients of 100 S...92 S (representing a

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

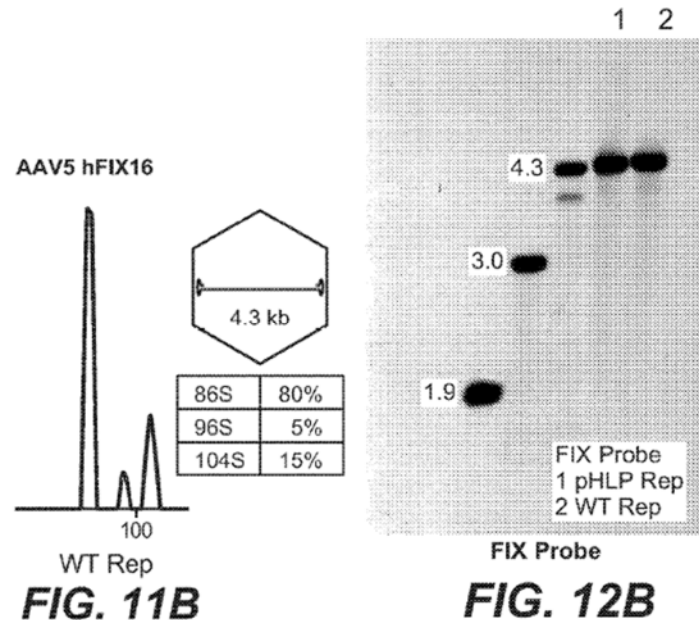


Id., 52:1-10, FIG. 11D.

Southern blot analysis of the AAV5SMN preparations were consistent with the AUC results for the pHLP helper (FIG. 11A) showing a homogenous profile of the full-length vector (FIG. 12A, lane 2) compared with packaging of full-length and fragmented SMN genomes (FIG. 12A, lane 1). Ex. 1001, 52:11-27.



Southern blot analysis of the FIX vector preparation made with the pHLP modified helper agreed with the AUC result (FIG. 11A), showing predominantly full-length AAV (FIG. 12B, lane 1). In contrast, “AUC analysis of this AAV5FIX vector (FIG. 11B)” made with the WT-Rep helper “showed that 80% of the capsids contained a fragmented genome (~3000 nucleotides)” but these fragments “were *undetected* by the FIX probe” in a Southern blot experiment. *Id.*, 52:24-27 (emphasis added).



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

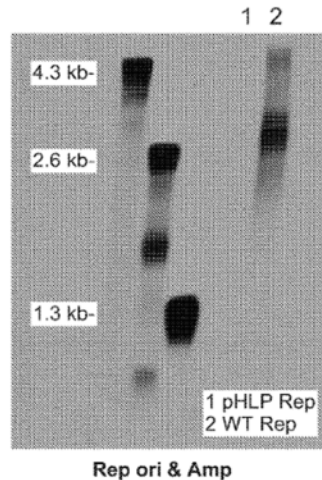


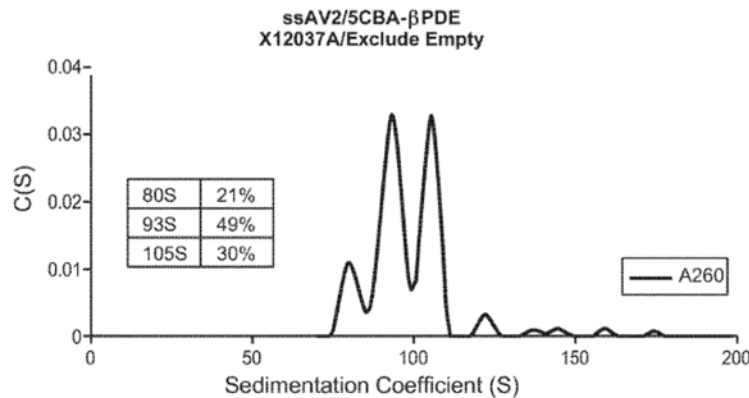
FIG. 14B

Ex. 1001, 52:36-45, FIG. 14B. But the Amp^R probe did **not detect** the fully intact genome in the rAAV5 FIX vector prep generated with the modified pHLP Rep helper. *Id.* Thus, multiple experiments were required to identify the species in the preparation by Southern blot.

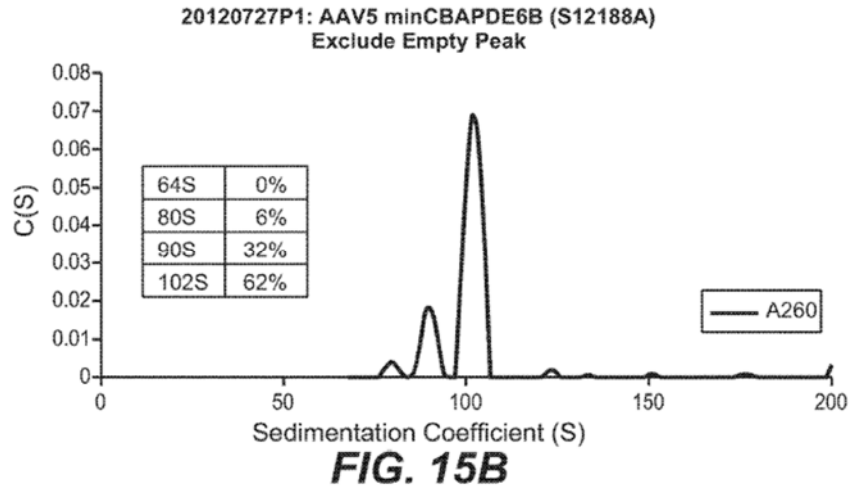
These comparative experiments illustrate the power of the inventors' AUC method. Unlike Southern blot, AUC is not sequence dependent, so the claimed AUC method allows for the detection and quantification of rAAV species, including partial or fragmented genomes, in a single experiment. Fleming, ¶ 75.

The '894 Patent also applies the AUC method to analyze "the heterogeneity of AAV vectors produced using oversized genomes." Ex. 1001, 52:60-63; Fleming, ¶ 76. "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.”



Id., 53:4-12; FIG. 15A. “In contrast, AUC analysis revealed that the same transgene under control of the abbreviated CBA promoter” of wild-type size “sedimented predominantly as a vector species of 102 S, consistent with packaging of the predicted, full-length vector genome of 4.6 kb.”



Id., 53:12-20; FIG. 15B. “These results demonstrate the utility of AUC analysis in profiling AAV vectors with oversized genomes, and this profiling is critical, given the observed incidence of genome fragmentation.” *Id.*, 53:17-20.

D. Prosecution History

The Examiner allowed the '894 Patent (Ex. 1002, 27-36) after Genzyme explained that the claimed 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 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.

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

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.

E. Person of Skill in the Art

Petitioner asserts that 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, according to Petitioner, 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.

For purposes of this Preliminary Response, Patent Owner agrees with this general framework, but reserves the right to apply a modified definition as the case proceeds.

F. Challenged Claims

Claim 1 of the '894 Patent is the only independent claim. Therefore, all claims share the elements recited by this claim. Claim 1 recites, in part,

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

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

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

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

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

Ex. 1001, 55:1-26. 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. 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.

III. Claim Construction

For this Preliminary Response only, Genzyme does not challenge the Petition’s alleged “plain and ordinary meaning” constructions. Petition, 33. Patent Owner reserves the right to address specific claim construction issues should the Board institute review.

IV. A POSA Would Not Have Expected a Predictive Relationship Between Sedimentation Coefficient and Genome Size Based on the Prior Art

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 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* § II.

De la Maza's discussion of genome size versus particle density published in 1978, thirty seven years before the effective filing date. If the inventors' "discoveries and advances were routine and relatively easy," as Sarepta alleges "the record would undoubtedly have shown that some ordinary artisan would have achieved this invention within months Instead this invention does not appear for more than" three decades. *Leo*, 726 F.3d at 1354 (Fed. Cir. 2013). "Indeed this considerable time lapse suggests instead that [the obviousness argument] only traverses the obstacles to this inventive enterprise with a resort to hindsight." *Id.*, 1356.

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

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. Fleming, ¶ 84.

The equation below mathematically describes the forces that influence the 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.

Friction, f , is impacted by particle size and shape, so particles with identical densities but different shapes will have different frictional coefficients. Fleming, ¶¶ 85-86. 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.

Only with hindsight from 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* §§ IV.B-D.

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. Fleming, ¶ 87. 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. Ex. 1004, ¶ 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.

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. Fleming, ¶ 88.

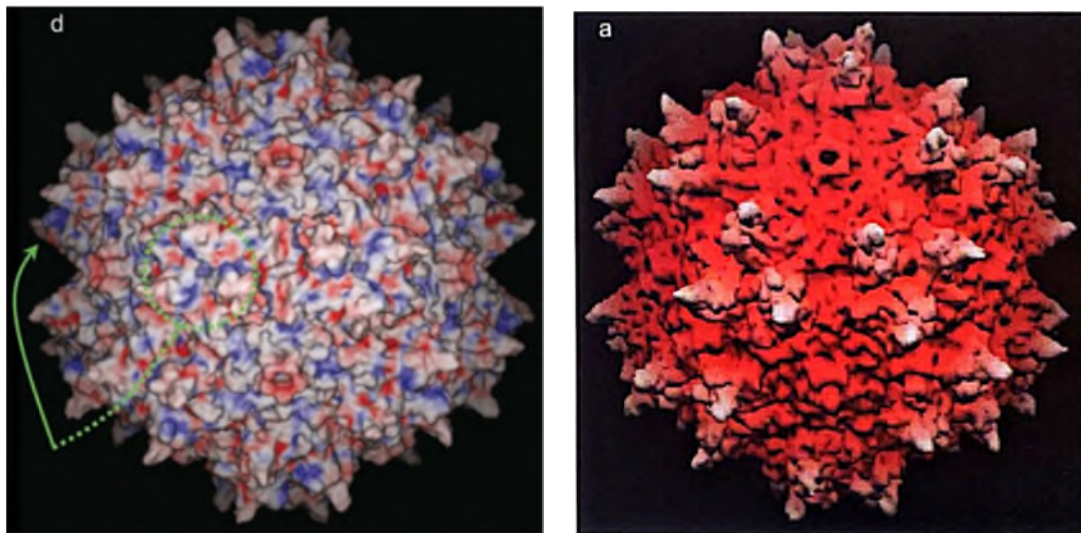
B. A POSA Would Have Expected rAAV Particle Friction to Impact Sedimentation Coefficients for Different Particles

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:

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).”), Fig. 3d.; *see also id.*, Fig. 3a.



Since the differences between the VP-1, -2, and -3 capsid proteins include protein lengths and functional group polarities, any variation in capsid stoichiometry would lead to differences in the rAAV shape and surface potentials leading to differences in frictional drag in solution. Fleming, ¶ 89.

Spike features on another type of viral particle, adenovirus particles, were observed to impact their AUC analysis. Ex. 1023; Fleming, ¶ 90. Berkowitz 2007

explained that “spike projections must impart a significant amount of frictional drag on the overall adenovirus particle.” Ex. 1023, 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.”).

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. Fleming, ¶ 91.

In addition, changes to the surface of an rAAV particle would alter the protein mass. Berkowitz 2007 explained that changes to the spike proteins on the outside of adenovirus capsids have implications not only for frictional coefficient, but also for the mass ratio of genome to protein. “[P]articles with missing spikes would also have a slightly higher DNA to protein ratio and

therefore have a slightly higher buoyant density than the intact adenovirus particle.” Ex. 1023, 31-32. Higher buoyant density would likewise increase the *s* value. Thus, from Berkowitz 2007, 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. Fleming, ¶ 92.

C. Berkowitz 2007 Explains why a POSA Would Not Have Discounted Frictional Coefficient Variation for Viral Particles

Sarepta’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” and 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; Ex. 1004, ¶ 170). As explained (*supra* §§ IV.A-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 Sarepta’s contention. Fleming, ¶ 93.

Sarepta 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), 43-44.

Sarepta 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 attributable to differences in their densities could lead to incorrect genome size calculations. Fleming, ¶ 94.

The second category of information that Sarepta 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 Sarepta represents as "showing transmission electron microscopy images of full and empty AAV particles with the same size and shape," explain the limitations of electron microscopy ("EM") as a quantitative tool. Petition, 44.

Gao, for example, states:

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

Ex. 1026, 6. Gao explains that “encapsidation of full or partial AAV genomes may lead to capsid conformational changes ... on capsid structure expos[ing] both phospholipase A2 domain and nuclear localization signal domains and facilitates endosome escape and viral infection.” Ex. 1026, 6. Gao further explains that “partial viral genomes may also contribute to the side effects of the [partially empty] virions due to their different capsid conformations as compared with [completely empty] particles.” *Id.* The POSA would have understood that such changes to the capsid are relevant to AUC analysis because they impact the sedimentation coefficient. Fleming, ¶ 95.

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

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

quantification of electron micrographs can be problematic and lead to inconsistent results.

Ex. 1041, 57. Sarepta's reliance on electron microscopy to establish that all AAV particle species have the same size and shape is flawed because a POSA would have understood that electron microscopy could not accurately distinguish empty, partially empty, and full AAV virions. Fleming, ¶¶ 96-97.

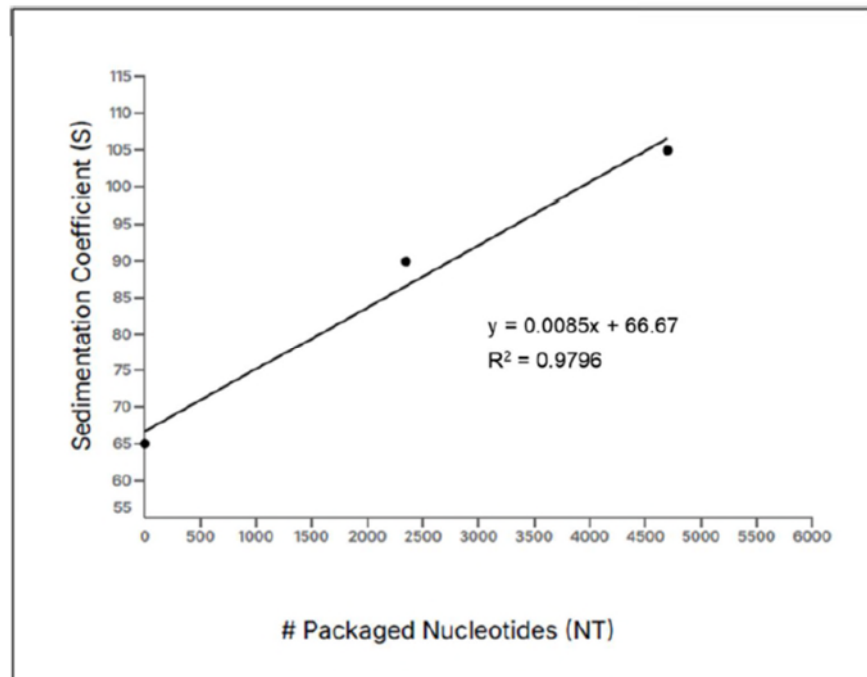
Had Sarepta considered the prior art "for all its teachings, not selectively," it would not have concluded that a POSA would have assumed all rAAV particles had approximately the same size and shape regardless of the genome size. *Henny Penny*, 938 F.3d at 1332. Sarepta arrives at its conclusion by "pick[ing] and choos[ing] from any one reference only so much of it as will support a given position, to the exclusion of other parts necessary to the full appreciation of what such reference fairly suggests to one of ordinary skill in the art." *In re Wesslau*, 353 F.2d 238, 241 (CCPA 1965). 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

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 Ex. 1004, ¶¶ 194). Both cursory visual inspection and a mathematical analysis of Sarepta's plot refute a linear relationship between sedimentation coefficient and genome size. Fleming, ¶ 98.

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. Fleming, ¶ 99.



Petition, 54 (citing Ex. 1004, ¶¶ 194). In contrast, Figure 8 of the '894 Patent 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. $p = 0.091258$). Fleming, ¶ 99.

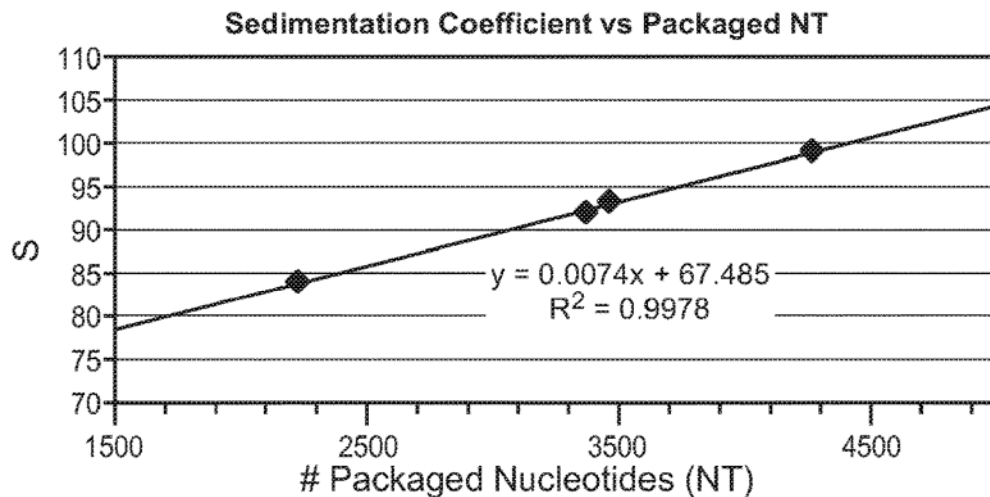


FIG. 8

Ex. 1001, Fig. 8.

A best-fit line can always be drawn through any set of points—that is a mathematical operation, not a scientific conclusion. A linear relationship requires statistical evidence. A POSA would rely on the p-value of the slope as the standard piece of evidence, which tests whether the null hypothesis of no relationship is disproven or not. Petitioner drew a line; it did not establish statistical significance or linearity. Fleming, ¶ 100.

A statistical analysis of Le Bec's Figure 2 data confirms a clear lack of linearity. Fleming, ¶ 101. The p-value, a critical statistical measure that indicates the probability of observing the results if no true linear relationship exists between the variables, shows that the plotted data do not support a linear relationship. A low p-value suggests strong evidence against the null hypothesis (in this case, no linear relationship), while a high p-value indicates insufficient evidence to conclude a linear relationship exists. The standard scientific 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. More stringent thresholds include $p \leq 0.01$ (99% confidence) and $p \leq 0.001$ (99.9% confidence) for situations requiring stronger evidence.

The p-value for Le Bec's data is 0.09, well above the standard significance threshold, indicating a lack of linearity. Fleming, ¶ 102. The lack of linearity in Le Bec's data and a POSA's expectation that genome size and sedimentation coefficient would not have a linear relationship due to the impact of friction (*supra* §§ IV.B-D) validates the claimed method's nonobviousness.

V. Sarepta Has Not Demonstrated a Reasonable Likelihood that the Challenged Claims Are Obvious

Sarepta 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. *In re Magnum Oil Tools Int'l Ltd.*, 829 F.3d 1364, 1381 (Fed. Cir. 2016) (“[T]he Board must base its decision on arguments that were advanced by a party ...”). The inadequacies in the Petition's proofs identified herein are so significant that the merits failures cannot be remedied. These failures warrant denying institution. *Id.* at 1380 (To meet its burden the “petitioner must instead articulate specific reasoning, based on evidence of record, to support the legal conclusion of obviousness.”); *see also* Consolidated

Trial Practice Guide, Section II.I³ (“Petitioner may not submit new evidence or argument in reply that it could have presented earlier, e.g. to make out a prima facie case of unpatentability.”). 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 size of the encapsidated genome, or its molecular weight, from its sedimentation coefficient.

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

Petitioner’s 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 fails.

1. Claim 1

Sarepta’s argument that a POSA would have understood that sedimentation coefficient depends only on particle density is premised on an assumption it repeats throughout its Petition and Dr. Berkowitz’s declaration: 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; Ex. 1004, ¶ 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

³ Available at <https://www.uspto.gov/patents/ptab/trial-practice-guide>.

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* §§ IV.B-C. 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* § IV.D; Fleming, ¶ 105.

Sarepta falls prey to “hindsight syndrome by reasoning backward from the teaching of the patent itself.” *Creo Prod., Inc. v. Presstek, Inc.*, 166 F. Supp. 2d 944, 966 (D. Del. 2001) (internal quotation marks omitted), *aff’d*, 305 F.3d 1337 (Fed. Cir. 2002); *see also Rockwell Int’l Corp. v. U.S.*, 147 F.3d 1358, 1364 (Fed. Cir. 1998); *Jones v. Hardy*, 727 F.2d 1524, 1529 (Fed. Cir. 1984); *W.L. Gore & Assocs., Inc. v. Garlock, Inc.*, 721 F.2d 1540, 1553 (Fed. Cir. 1983). Thirty-seven years passed between de la Maza and the ’894 Patent without anyone discovering the linear relationship or its claimed application—and Petitioner can construct its obviousness theory only by ignoring the statistical failure of Le Bec’s data and the friction variability that its expert acknowledged. Fleming, ¶ 105. The Board should reject Sarepta’s hindsight driven arguments for claim 1.

**a. A POSA Would Not Have Been Motivated to Combine
Le Bec and de la Maza, in view of Sommer to Arrive at
Claim 1's Method**

The Board should reject Sarepta's hindsight-driven theory that a POSA would have found it obvious to successfully determine the size of fragmented genomes as a function of the sedimentation coefficient of the viral particles in which they are contained. Petition, 38-50. The Petition's obviousness challenge to claim 1 fails at the threshold motivation requirement. While a POSA may have been familiar with both Le Bec's AUC methodology, de la Maza's density-genome size observations, and Sommer's extinction coefficient calculations, the record does not support that a POSA would have been motivated to combine their teachings to arrive at the claimed method. Fleming, ¶ 106-112.

Specifically, a POSA would not have been motivated 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. Sarepta's motivation argument suffers from at least four significant failures.

First, 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, as Sarepta alleges. Petition, 33-37. Le Bec was focused on determining the approximate amount of empty versus filled capsids in an rAAV mixture. Ex. 1006, 15:17-20:3. But Le Bec did not differentiate rAAV particles containing full genomes from rAAV particles containing genome fragments. *Id.*, 17:29-20:3. Le Bec's 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. *Infra*, § V.A.1.b.

Second, a proper linear regression analysis refutes Sarepta's contention (Petition, 53-54) that Le Bec supports a linear relationship between sedimentation coefficients and genome size. *Infra*, § V.A.1.c. A POSA would have considered Sarepta'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.

Third, the POSA would not have understood de la Maza's disclosure (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 variations, which a

POSA would have understood could impact frictional coefficients. Ex. 1023, 18, 31. Therefore, the POSA would not have had any reason to think there would have been a reliable relationship between genome size and sedimentation coefficient. *Infra*, § V.A.1.d.

Fourth, Sommer merely supplies extinction coefficient equations, which require the molecular weight of the encapsidated genome as an input. Ex. 1010, 127. The Petition relies on Le Bec and de la Maza to supply a POSA's motivation to determine the size of fragmented genomes by determining sedimentation coefficients. Petition, 34-37, 51. But because the combination of Le Bec and de la Maza would not have motivated a POSA to determine genome size by determining the sedimentation coefficients of fragmented genomes (*supra* § V.A.1), a POSA would not have been motivated to develop the method of claim 1. Fleming, ¶ 111.

“[O]bviousness concerns whether a skilled artisan not only *could have made* but *would have been motivated to make* the combinations or modifications of prior art to arrive at the claimed invention.” *Belden Inc. v. Berk-Tek LLC*, 805 F.3d 1064, 1073 (Fed. Cir. 2015)) (emphasis original). 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 the nonobviousness of the sedimentation and genome size relationship and the claimed method. *Leo*, 726 F.3d at 1354.

**b. Le Bec's Method Fails to Resolve Viral Particles
Comprising Fragmented Genomes**

The Petition does not address that Le Bec's AUC studies fail to resolve peaks associated with rAAV particles containing fragmented genomes. Petition, 38-47. A POSA would have considered this a significant deficiency in Le Bec's disclosure. Fleming, ¶ 113. Thus, a POSA would not have relied on Le Bec for developing claim 1's method of determining the size of fragmented genomes in a heterogeneous mixture of viral particles.

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.

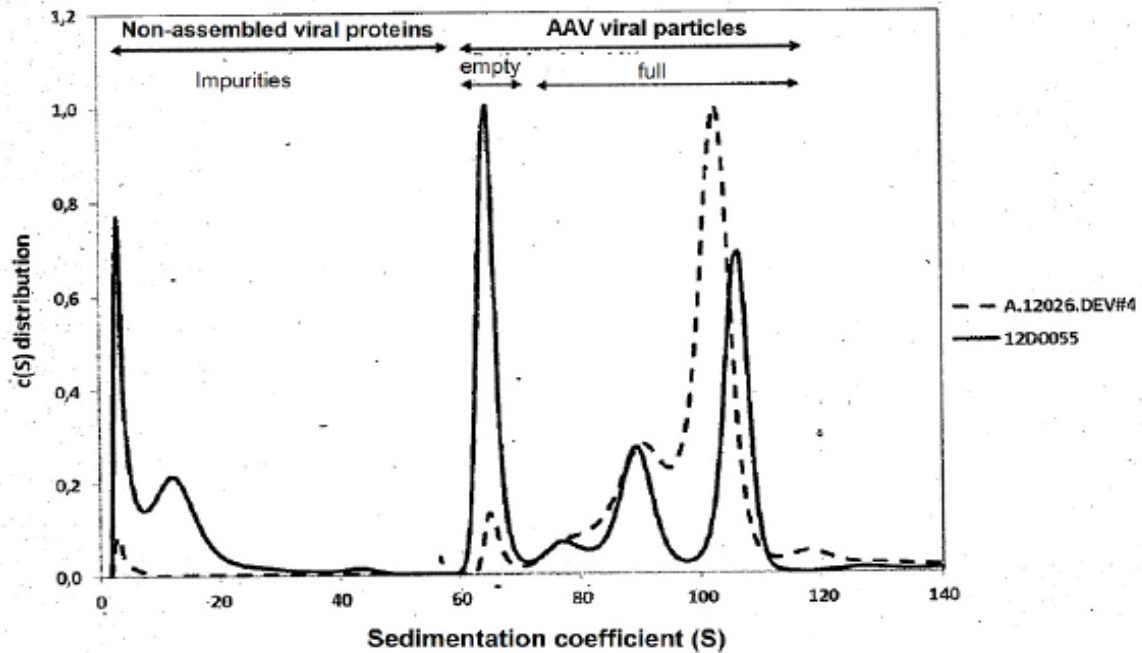


Figure 2

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-stranded form at 105S.” Ex. 1006, 18:11-25. Given the poor resolution of peaks between 60 S and 110 S, a POSA would not have considered Le Bec useful for characterizing fragmented genomes. Fleming, ¶ 114.

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. Fleming, ¶ 115.

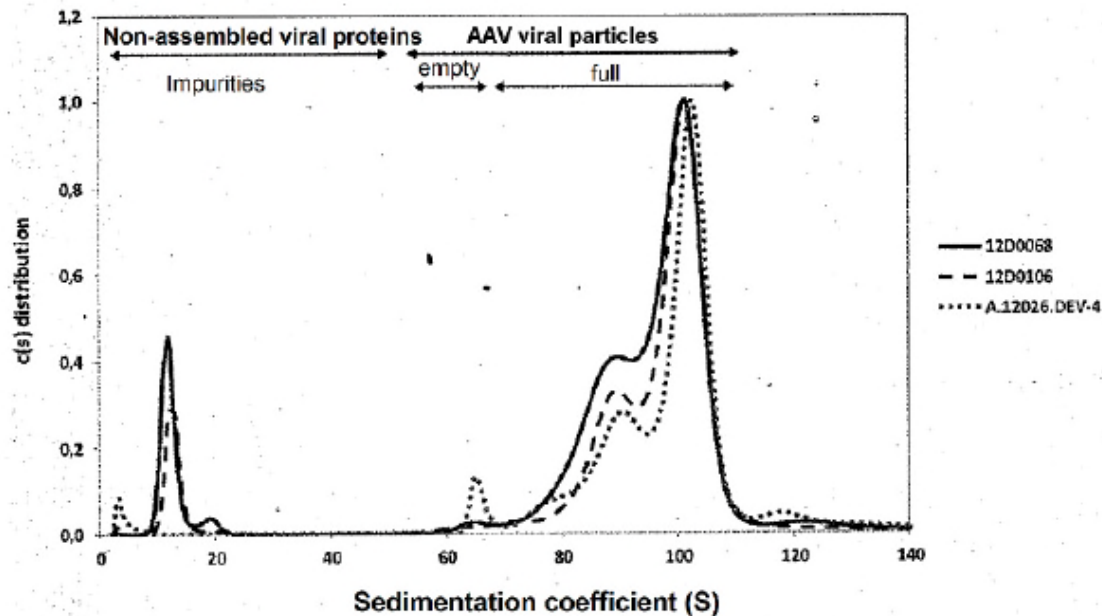


Figure 3

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 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”).

Indeed, 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. Fleming, ¶¶ 117-118. Thus, the POSA would not have been motivated to apply Le Bec’s method to determine the size of a fragmented genome in an rAAV particle, because these peaks are not sufficiently resolved.

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

Sarepta’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, 54 (citing Ex. 1004, ¶¶ 194); *supra* § IV.D. Drawing a “best fit” line is different from conducting a meaningful linear regression. Fleming, ¶ 118.

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. Fleming, ¶ 119. In this case, a low p-value suggests strong evidence against the null hypothesis (no linear relationship), while a high p-value indicates insufficient evidence to conclude a linear relationship exists. Ex. 2028, 82. The standard significance threshold is $p \leq 0.05$, meaning there is less than a 5% probability the observed relationship is due to chance, which corresponds to a 95% confidence level. More stringent thresholds include $p \leq 0.01$ (99% confidence) and $p \leq 0.001$ (99.9% confidence) for situations requiring stronger evidence.

Sarepta 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 Sarepta’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 0.05. Fleming, ¶ 121. 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.

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. Fleming, ¶ 121.

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

The linear regression output are summarized in the table below:

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

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

This summary agrees with the R^2 value in the patent (.9976 vs .9978). In this linear regression, the p-value for the slope of the linear regression is 0.00118, which is highly significant ($p \ll 0.05$) and passes even the more stringent threshold of $p \leq 0.01$ (99% confidence). This confirms that the linear relationship between sedimentation coefficient and genome size in the '894 Patent data is statistically robust. The p-value for the intercept (0.000151) is also highly significant, passing the $p \leq 0.001$ threshold (99.9% confidence). This analysis confirms the inventors' representation that the '894 Patent provides the first documentation of the linear relationship between sedimentation coefficient and genome size for rAAV particles. Fleming, ¶¶ 122-123.

Sarepta's failure to conduct a proper regression analysis of Le Bec—which a POSA would have done—represents a failure of its obviousness analysis.

Fleming, ¶ 124. "It is impermissible within the framework of section 103 to pick and choose from any one reference only so much of it as will support a given position, to the exclusion of other parts necessary to the full appreciation of what such reference fairly suggests to one of ordinary skill in the art." *Wesslau*, 353 F.2d at 241; *see also Henny Penny*, 938 F.3d at 1332; *W.L. Gore*, 721 F.2d at 1551.

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. Fleming, ¶ 124. This validates the claimed method's nonobviousness.

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

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. Fleming, ¶ 125.

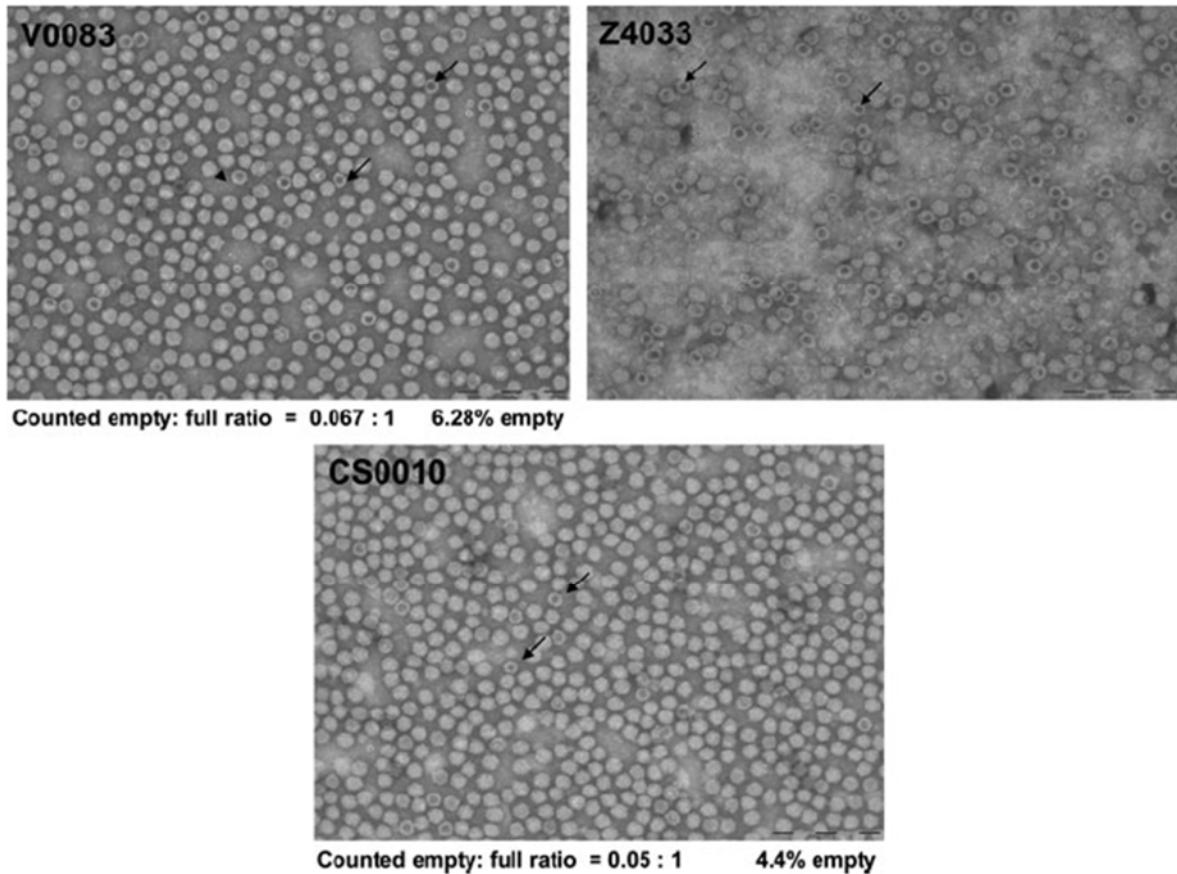
De la Maza describes the use of SV-AUC to study the fractionation of incomplete genomes—but not in the context of intact viral particles. To conduct its SV-AUC experiment "DNA was released from virions by sedimentation in alkaline sucrose gradients." Ex. 1007, 194. This means that de la Maza did not conduct SV-AUC with intact viral particles but instead used SV-AUC only to determine the sedimentation coefficients of DNA released from viral particles. Fleming, ¶ 126. De la Maza did not attempt to identify a relationship between the size of genome present in an intact AAV particle and sedimentation coefficient.

Likewise, a POSA would not have been motivated 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; Fleming, ¶ 127. 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* §§ IV.B-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.

Sarepta 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, 43-44 (citing Ex. 1007, 203; Ex. 1008, 3202; Ex. 1004, ¶ 165). But 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.

Sarepta 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 Sarepta 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. Fleming, ¶ 129.

The other reference that Sarepta 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 Sarepta’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. Fleming, ¶ 130.



Ex. 1041, 59. Moreover, a POSA would have considered the frictional coefficient to have required a quantitative assessment of particle shape. Fleming, ¶ 130. Neither Lock nor Gao supports Sarepta's reliance on their qualitative images for assessing quantifiable differences in particle frictional coefficients. Fleming, ¶ 130.

Sarepta 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.

There is no dispute that nearly forty years elapsed from de la Maza's 1978 publication to the effective filing date. Sarepta 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. "[T]his considerable time lapse" is an indicator that Sarepta "only traverses the obstacles to this inventive enterprise with a resort to hindsight." *Leo*, 726 F.3d at 1356; Fleming, ¶ 132.

Sarepta uses the challenged claims as a roadmap to "selectively cull[] from the prior art to fit the parameters of the patented invention." *ATD Corp.*, 159 F.3d at 546. In so doing, Sarepta ignores that the teachings of Berkowitz 2007 and Berkowitz 2015 in their entirety fail to support Sarepta'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. Fleming, ¶ 133.

An obviousness analysis requires examining what the POSA would have done without the guidance from the inventors. *Arendi*, 832 F.3d at 1361 (stating that "[i]n reaching this conclusion, the court must avoid 'hindsight bias and must be cautious of arguments reliant upon ex post reasoning.'"); *Trivascular, Inc. v.*

Samuels, 812 F.3d 1056, 1066 (Fed. Cir. 2016) (the Board must “be careful not to allow hindsight reconstruction of references . . . without any explanation as to how or why the references would be combined to produce the claimed invention”); *InTouch Techs., Inc. v. VGO Commc’ns*, 751 F.3d 1327, 1352 (Fed. Cir. 2014) (finding that the “expert also succumbed to hindsight bias in her obviousness analysis.”).

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

Sarepta’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 determine the extinction coefficient of the particle at the measured wavelength. Petition, 47-50; Fleming, ¶¶ 45-48. Calculating the extinction coefficient requires determining the molecular weight of DNA packaged in the rAAV particle, *i.e.*, the size of the genome. Petition, 51-52; *see also id.*, 32; Fleming, ¶ 134. Thus, Sarepta’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).

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.

Fleming, ¶ 135. This is because the degree of absorbance depends on the extinction coefficient as expressed by Beer's Law:

$$A = \epsilon cl$$

Ex. 1009, 152. Beer's law (or the Beer-Lambert law) states that the absorbance (A) of light passing through a composition is directly proportional to its molar extinction coefficient (ϵ), concentration (c), and the path length (l) through the sample.

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 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.”); Fleming, ¶ 136.

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: $A = \epsilon cl$, where ϵ is the solute’s weight extinction coefficient, c is the weight concentration, and l is the sample path length.”); *see also* Ex. 1010, 126-127; Fleming, ¶ 137.

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

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

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

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

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

Id.; Fleming, ¶ 138.

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. Fleming, ¶ 139. 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

Sarepta’s reasonable expectation of success argument fails for much the same reasons as its motivation argument. As a “touchstone of obviousness,” a reasonable expectation of success requires not only an “expectation that prior art elements are capable of being physically combined, but also that the combination would have *worked for its intended purpose*.” *Depuy Spine, Inc. v. Medtronic Sofamor Danek, Inc.*, 567 F.3d 1314, 1326 (Fed. Cir. 2009) (emphasis added). A POSA 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, in view of Sommer. Fleming, ¶ 140.

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. Sarepta'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. Fleming, ¶ 141. A reasonable expectation of success argument that fails to account for and rebut this unpredictability cannot prove obviousness. *Amneal Pharms. LLC v. Cubist Pharms. LLC*, IPR2020-00193, Paper 7 at 22-32 (PTAB May 29, 2020); *Sandoz Inc. v. AbbVie Biotechnology Ltd.*, IPR2017-01823, Paper 16 at 14 (PTAB Feb. 9, 2018); *Coherus Biosciences Inc. v. AbbVie Biotechnology Ltd.*, IPR2016-01018, Paper 12 at 4 (PTAB Feb. 2, 2017); *Momenta Pharms., Inc. v. Bristol-Myers Squibb Co.*, IPR2015-01537, Paper 37 at 12-13 (PTAB Dec. 22, 2016).

A POSA with knowledge of viral particle structure, would not have expected that particles containing genomes of varying sizes would exhibit the same frictional properties. For example, Berkowitz 2007 explained that some particles were observed to lose surface spikes, which impacted their sedimentation coefficient. Ex. 1023, 31-32. Berkowitz 2007 observed that some “adenovirus monomers [may] have lost their fiber spikes” resulting in “significantly reduced hydrodynamic drag” decreasing friction therefore leading to “a higher sedimentation coefficient than the intact adenovirus particle.” *Id.*, 31. This was a phenomenon observed for other viral particles as well. *Id.* (“Similar effects have been observed for the bacterial virus T2 with regard to an extended versus compact geometric arrangement of its tail spikes or fibers ”). A POSA would have expected a similar issue to arise for rAAV particles, which also exhibit spikes projecting from their capsids. Thus, a POSA would not have expected a predictive correlation between sedimentation coefficient the size of the encapsidated genome as Sarepta alleges. Petition, 52-53; Fleming, ¶ 142.

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, 38-46. 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, Sarepta has failed to 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. Fleming, ¶ 143.

“It was [Petitioner]’s burden to demonstrate” that the POSA “would have had a reasonable expectation of success in” combining teachings “to achieve the claimed invention” and Sarepta fails to carry its burden. *Intelligent Bio-Sys., Inc. v. Illumina Cambridge Ltd.*, 821 F.3d 1359, 1367–68 (Fed. Cir. 2016) (internal quotation marks and citations omitted). A POSA’s lack of reasonable expectation of success is highlighted by the proper statistical analysis of Le Bec’s data. *Supra* §§ V.A.c, f. 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, Sarepta 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.

Fleming, ¶ 144.

2. Claims 2-7, 10-11, and 20-30

Because challenged claims 2-7, 10-11, and 20-30 depend from claim 1, and Sarepta's only challenge to claim 1 is in Ground 1, Sarepta's failure to prove claim 1 obvious also results in a failure of proof for claims 2-7, 10-11, and 20-30 of the '894 Patent. Fleming, ¶ 145.

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

Because challenged claims 12-19 depend from claim 1, and Sarepta's only challenge to claim 1 is in Ground 1, Sarepta's failure to prove claim 1 obvious also results in a failure of proof for claims 12-19 of the '894 Patent.

VI. The IPRs of U.S. Patent No. 10,429,288 Should Have No Bearing on the Instant IPR

The grandparent of the '894 Patent, U.S. Patent No, 10,429,288 (the "'288 Patent") (Ex. 1015) was challenged in IPR2023-01044 and -1045 ("'288 Patent IPRs"). There are several reasons why the institution decisions on those petitions (Exs. 1020, 1021; Petition, 17-18) should have no bearing on this proceeding.

First, the independent claim 1 of the '894 Patent presents different issues than claim 1 of the '288 Patent. Claim 1 of the '894 Patent requires "determining the genome size of one or more species of the individual variant viral particles in the heterogeneous mixture of viral particles" and "determining the quantity of one

or more species of the variant viral particles in the heterogeneous mixture of viral particles.” Sarepta’s obviousness arguments are based on a POSA’s assumed ability to determine genome molecular weight from the sedimentation coefficient. Petition, 1, 32, 51-52. Claim 1 of the ’288 Patent, in contrast, did not require determining the molecular weight of the fragmented particle. Ex. 1015, claim 1. Thus, the institution decision of the ’288 Patent IPRs did not address correlation between the sedimentation coefficient and genome size in its analysis of claim 1 of the ’288 Patent.

Second, the institution decision expressly declined to resolve whether a POSA would have assumed a linear relationship between sedimentation coefficient (S-value) and genome molecular weight when using the prior art references to calculate genome size of recombinant AAV particles. The decisions noted that the parties’ experts provided conflicting testimony on whether all rAAV particle species would have the same frictional coefficient, which was critical to determining whether sedimentation would vary linearly with molecular weight. Ex. 1020, 38-39; Ex. 1021, 26. Because this conflicting testimony created, according to the Board, a genuine issue of material fact, the panel viewed the material fact in the light most favorable to Petitioner solely for purposes of deciding whether to institute inter partes review. Ex. 1020, 39; Ex. 1021, 26.

The Board committed a fundamental legal error by applying the rescinded version of 37 C.F.R. § 42.108(c), explicitly stating it would view “testimonial evidence ... in the light most favorable to the petitioner” when resolving factual disputes regarding the S-value/molecular weight linearity question. Ex. 1020, 25; Ex. 1021, 18. This presumption favoring petitioners was eliminated by the December 9, 2020 rule amendment, which responded to concerns that viewing testimonial evidence in the light most favorable to petitioners “conflicts with the decision of Congress to place the burden of proof on the petitioner” and that such a presumption is “inconsistent with the statutory framework” of 35 U.S.C. § 314(a). ’288 Patent IPRs, Paper 12 at 1-2. The rule change was designed to “remove[] any bias or appearance of bias in favor of petitioner, and provide a balanced approach to ensure that all testimonial evidence submitted by the parties is fairly considered.” ’288 Patent IPRs, Paper 12 at 3-4 (citing PTAB Rules of Practice, 85 FR 79120 at 79125 (Dec. 9, 2020)). Had the Board properly weighed this testimonial evidence under the current totality-of-the-evidence standard without placing a “thumb on the scale” in favor of Petitioner, it would have been compelled to find that Petitioner failed to establish a reasonable likelihood of proving obviousness. ’288 Patent IPRs, Paper 12, 2, 13. The case settled before the Director had the opportunity to correct the Board’s misapplication of the institution standard.

Third, the preliminary response here demonstrates that the data in Le Bec, the primary reference relied upon by Sarepta, does not establish a linear relationship between sedimentation coefficient and genome size. Le Bec's data are definitively nonlinear. When considered in the context of the art, where the shape and size of viral capsids were understood to potentially change based on genome content, Le Bec's data would have raised significant doubts as to whether any predictive relationship exists between sedimentation coefficient and genome size.

Finally, the '288 Patent IPRs were made of record and the Examiner considered them prior to allowing the '894 Patent over the Le Bec reference and other references raised in the '288 Patent IPRs, including Berkowitz 2007 and Cole. Ex. 1002, 38, 188. As explained in Genzyme's discretionary denial request, there is no reason to second-guess the Examiner here.

VII. Conclusion

For the foregoing reasons, the Petition should be denied, and no proceeding instituted.

Respectfully submitted,

Dated: March 4, 2026

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WORD COUNT CERTIFICATION

Pursuant to 37 C.F.R. § 42.24(d), I certify that this Preliminary Response contains 13,692 words (excluding the title page, table of contents, table of exhibits, claim listing, this certificate, and the certificate of service), as determined by Microsoft Word.

Dated: March 4, 2026

/ Blaine Hackman /
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CERTIFICATE OF SERVICE

I certify that today in:

Sarepta Therapeutics, Inc., v. Genzyme Corp., IPR2026-00150
(U.S. Patent 12,013,894)

I caused to be served a copy of:

PATENT OWNER'S PRELIMINARY RESPONSE
EXHIBITS 2025-2032

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