

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

SAREPTA THERAPEUTICS, INC.,
Petitioner

v.

GENZYME CORPORATION,
Patent Owner.

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

**DECLARATION OF BARRIE J. CARTER, PH.D.
IN SUPPORT OF PATENT OWNER'S RESPONSE**

TABLE OF CONTENTS

I.	Introduction.....	1
II.	Professional Qualifications	1
III.	Materials Considered	4
IV.	Person of Skill in the Art (POSA)	6
V.	Background Scientific Concepts.....	7
A.	Adeno-Associated Virus (AAV)	7
B.	Recombinant Adeno-Associated Virus (rAAV)	10
C.	AAV and rAAV Particle Structure.....	18
VI.	Analysis of Dr. Berkowitz’s Opinions on Viral Particle Shape and Size	19
A.	The POSA Would Not Have Assumed that Empty, Fragmented Genome, and Complete Genome rAAVs Are Approximately the Same Size and Shape Based on My Laboratory’s Publications	20
B.	A POSA Would Not Have Understood Our Unpublished Observations that “particle density has an approximately direct linear relationship to the strand length” to Indicate a Quantitative Linear Relationship.....	27
C.	Other Publications Cited by Dr. Berkowitz Are Consistent with My Publications Regarding Variations in Viral Particle Shape and Size	31
VII.	Conclusion	37

I. INTRODUCTION

1. I submit this declaration in connection with the Patent Owner's Response in the *Inter Partes* Review IPR2026-00150 (the "IPR") of claims 1-7 and 10-30 ("challenged claims") of U.S. Patent No. 12,031,894 (Ex. 1001, the "'894 Patent"). I have been retained by Dechert LLP, counsel for Patent Owner Genzyme Corporation in this matter. I am being compensated for my time at my standard rate of \$400 per hour plus actual expenses. The opinions in this declaration are my own, and my compensation is not dependent in any way upon the outcome of the IPR of the '894 Patent.

II. PROFESSIONAL QUALIFICATIONS

2. I attended the University of Otago in Dunedin, New Zealand from 1963-1969 and received a Bachelor of Science with First Class Honors in 1967 and a Ph.D. in Biochemistry in 1969. My research was conducted under the supervision of Mervyn G. Smith Ph.D., who had just returned to New Zealand from working at Cold Spring Harbor, NY in the laboratory of the Nobel Laureate Dr. Alfred Hershey, and initially was based on work from the Hershey lab. The title of my dissertation was "Replication of Bacteriophage Lambda DNA" and primarily involved analysis of the structure of intracellular phage DNA species using ultracentrifuge velocity sedimentation in sucrose density gradients and isotopic pulse-chase labeling to establish precursor relationships.

3. From 1969-1970, I spent a year of postdoctoral study with Dr. Lionel V. Crawford at the Imperial Cancer Research Fund laboratories in London, UK. There I learned about mammalian viruses and in particular parvoviruses. Late in 1970, I moved to the National Institutes of Health (NIH) in Bethesda, MD to work on the then very recently discovered parvovirus, adeno-associated virus (AAV). My initial studies on AAV were conducted with James A. Rose, M.D., and in 1973 I established my own laboratory at NIH to continue AAV studies and ultimately spent 22 years at NIH.

4. In the 1970s at NIH, my laboratory established much of the basic structure of AAV DNA and the properties of various AAV virus particle species. In 1980, the availability of the DNA sequence of AAV2 and a modification of the NIH Recombinant DNA Guidelines, which permitted construction of molecular clones of complete AAV DNA genomes, facilitated a detailed analysis of AAV gene expression and also enabled the construction of the first AAV gene therapy vectors in 1984. By the end of the 1980s, we began development of AAV vectors for the clinical indication of cystic fibrosis.

5. In 1992, I left NIH and entered the biotech arena with the objective of developing gene therapy for human diseases using AAV vectors. That same year I moved to Seattle to join Targeted Genetics Corporation (TGC) as Executive Vice President of R&D and Chief Scientific Officer, where I remained until 2008.

During that time at TGC, we initiated the first AAV vector human clinical trials (in patients with cystic fibrosis), which required the first regulatory discussion of AAV vectors with the FDA and very extensive preclinical studies, as well as developing AAV vector manufacturing to a scale of 250 L in stirred tanks.

6. In 2009, I became a consultant (Carter Bioconsulting) for several years. There I worked with a variety of companies including start-ups and larger companies, as well as non-profits, to provide advice on development (vector construction and design, preclinical studies and manufacturing) of gene therapy for human clinical trials.

7. From 2013 to 2018, I worked at Biomarin Pharmaceutical Inc (BMRN) to initiate and lead a gene therapy program to develop AAV gene therapy for hemophilia A. When I retired from BMRN in Dec 2018, this program was well into final phase III trials, and the AAV vector (named Roctavian[®]) was approved as a gene therapy for treating hemophilia in Europe in 2022 and the United States in 2023.

8. Through my work, I have been recognized as one of the “fathers of rAAV vectors.” Ex. 1040, 491. I received the Special Achievement Award, National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), NIH, the GEN Excellence in Biotechnology Award for Outstanding Achievement in

Drug Delivery, and the Exemplary Service Award from the American Society of Gene and Cell Therapy.

9. During my career, I served on several high-level advisory committees for the NIH, Department of Health and Human Services (HHS), and the Food and Drug Administration (FDA). I also served on numerous journal advisory boards and as an Officer and Director of the American Society of Gene and Cell Therapy, and I am a Past-President of that organization.

10. Since my retirement, I have continued to be involved in the field of gene therapy, particularly with attendance and participation in the annual meetings of the American Society of Gene and Cell Therapy and attendance at the International Parvovirus Meeting. I have also done some additional consulting work. A summary of my professional qualifications is provided in my *curriculum vitae*. Ex. 2034.

III. MATERIALS CONSIDERED

11. My analysis is based on my years of education, research, experience, and background, as well as my investigation and study of relevant materials for this declaration. When developing the opinions set forth in this declaration, I was asked by counsel to assume the perspective of a person having ordinary skill in the art (“POSA”), provided in the Petition and Dr. Steven Berkowitz’s declaration (Ex. 1004, “Berkowitz Declaration”). Petition, 32-33; Berkowitz Declaration, ¶¶ 83-86.

In addition to the Petition and Berkowitz Declaration, and the exhibits cited therein and in this declaration, I reviewed and considered the materials identified in the list below.

Exhibit	Description
2019	Burnham et al., Analytical Ultracentrifugation as an Approach to Characterize Recombinant AAV Vectors, 26 Human Gene Therapy Methods 228 (2015) (“Burnham 2015”)
2020	F. Collaud et al., Preclinical Development of an AAV8-hUGT1A1 Vector for the Treatment of Crigler-Najjar Syndrome, 12 Molecular Therapy: Methods & Clinical Development 157, 166-67 (March 2019)
2021	E. Spies, Assessing the Quality of Adeno-Associated Virus Gene Therapy Vectors by Sedimentation Velocity Analysis, AUC Insights (March 2020)
2025	Q. Xie et al., The Atomic Structure of Adeno-Associated Virus (AAV-2), a Vector for Human Gene Therapy, 99 PNAS 10405-410 (Aug. 6, 2002)
2026	T. Matsushita et al., Adeno-Associated Virus Vectors Can Be Efficiently Produced Without Helper Virus, 5 Gene Therapy 938-45 (February 10, 1998)
2027	J.F. Wright, Product-Related Impurities in Clinical-Grade Recombinant AAV Vectors: Characterization and Risk Assessment, 2 Biomedicines 80-97 (March 3, 2014)
2028	D. Curran-Everett, Explorations in Statistics: Hypothesis Tests and P-values, 33 Advances in Physiology Education 81-86 (June 2009)
2029	Oliver, Christopher, Diffusion Coefficient and Molecular Weight of Type 5 Adenovirus by Photon-Correlation Spectroscopy, Biochimica et Biophysica Acta, 437 (1976) 589-598 (October 21, 1975)
2030	Tanford, Charles, Physical Chemistry of Macromolecules (1961)
2038	D.M. Lipinski et al., Clinical Applications of Retinal Gene

Exhibit	Description
	Therapy, 32 Progress in Retinal and Eye Research 22-47 (2013).
2039	Deposition Transcript of Stephen Berkowitz, Ph.D (July 29, 2026)
2040	A. Roy et al., “Antigenic Differences Among AAV Species of Different Densities” in <i>Replication of Mammalian Parvoviruses</i> (Ward, D., and Tattersall, P., eds.), Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 193-204 (1978)

IV. PERSON OF SKILL IN THE ART (POSA)

12. I have been informed that in patent law scientific references are analyzed as they would be understood by the POSA in the relevant field at the time of the effective filing date of the patent-at-issue, which I was informed was January 20, 2015. I understand that the Petition and Berkowitz Declaration applied the following definition for the POSA in their analysis:

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

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

13. A POSA may work as part of a team with someone familiar with the development, formulation, and/or administration of viruses or viral vectors for gene therapy and quality standards required for such products. For this Declaration, I will apply the same POSA definition as applied by Petitioner (“Sarepta”) and Dr. Berkowitz.

V. BACKGROUND SCIENTIFIC CONCEPTS

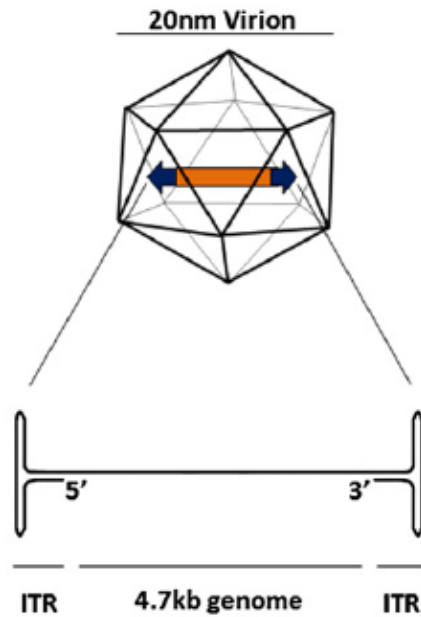
A. Adeno-Associated Virus (AAV)

14. Wild-type adeno-associated virus (“AAV”) was discovered in the 1960s in the laboratories of Bob Atchison at the University of Pittsburgh and Wallace Rowe at the National Institutes of Health (“NIH”). Ex. 1033, 982. Both groups recognized that small particles, which were initially thought to be subunit contaminants of adenovirus preparations, were actually a genus of the parvoviruses, now known as dependoviruses. *Id.* AAV is replication defective, meaning that it is dependent upon helper functions provided by adenovirus or herpesviruses for productive replication in mammalian cells. *Id.* Immediately after its discovery, AAV was isolated from humans, but serological studies indicated that it was not associated with causing any disease, despite appearing to travel in human populations with the helper virus, particularly adenovirus. *Id.*

15. AAV has a single-stranded DNA genome of approximately 5 kilobases (“kb”).¹ *Id.* Individual AAV virions contain a single DNA strand of either plus or minus complementary sense that reassociate to form duplex DNA upon extraction from particles. *Id.*

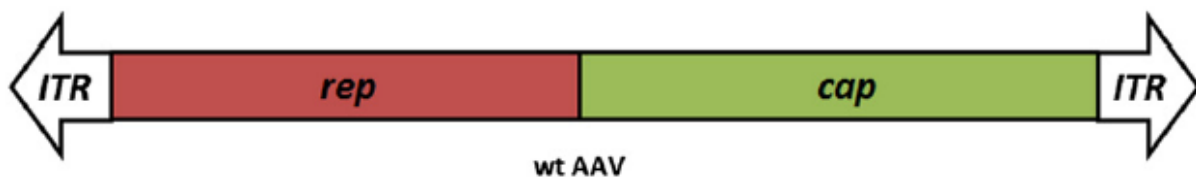
16. AAV DNA contains inverted terminal repeat (“ITR”) sequences at each end of the genome, a defining structural feature of the AAV genome. *Ex.* 1033, 982-83. In 1972, George Khoury and I observed that separated minus or plus single strands of AAV DNA bound and eluted from hydroxylapatite as if they were double stranded. *Id.* at 983. We surmised that the AAV DNA termini contained an inverted repeat. *Id.* This was proven by direct visualization in the electron microscope, which showed that AAV DNA single strands could form hydrogen-bonded single-strand circles and therefore must contain an inverted terminal repeat. *Id.* Direct sequencing of the AAV2 termini in 1980 confirmed that the ITR is a 145-nucleotide sequence of which the first 125 nucleotides form a T-shaped, base-paired hairpin structure. *Id.* The ITRs serve as the origins of DNA replication, with replication involving self-priming from the ITR hairpins. *Id.*

¹ “Kb” is a unit of measurement in molecular biology equal to 1,000 bases of DNA or RNA.



Ex. 2038, 29, Fig. 1B.

17. The wild-type AAV genome encodes two major gene functions. The right half of the genome contains the cap gene, which codes for the three overlapping AAV capsid proteins VP1, VP2, and VP3 and is transcribed from the p40 promoter (“cap”). Ex. 1033, 983-84. The left half of the genome contains the rep gene, which codes for the replication (“Rep”) proteins and is transcribed from the p5 and p19 promoters. *Id.* These two gene functions—rep and cap—are flanked by the ITRs. Ex. 1033, 985; *see also* Ex. 2027, 87; Ex. 2019, 229.



Ex. 2038, 29, Fig. 1C.

18. A key property of AAV is its ability to establish a latent state in host cells. In 1972, David Hoggan at the National Institute of Allergy and Infectious Diseases (“NIAID”) demonstrated that when human cell lines were infected with AAV at a high virion to cell ratio in the absence of any helper virus, infectious AAV was no longer detectable after growth through at least 10 cell passages, but superinfection of the cultures with adenovirus resulted in rescue of infectious AAV. Ex. 1033, 983. This was the first indication that AAV may persist in a cell in the absence of helper virus when conditions are not permissive for productive replication. *Id.* Further analysis showed that a relatively low number of AAV genomes were integrated into the host cell chromosome mostly as head-to-tail tandem repeats. *Id.* The idea that AAV could establish a latent state was an early impetus to thinking about using AAV as a gene delivery vector for gene therapy. *Id.*

B. Recombinant Adeno-Associated Virus (rAAV)

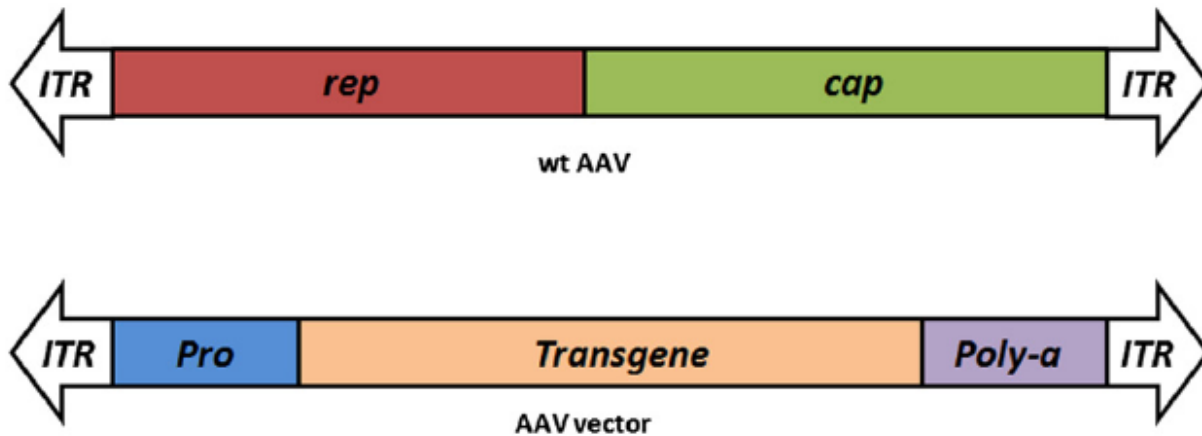
19. The first recombinant AAV (“rAAV”) vectors were described in 1984 and were generated using a two-plasmid (vector plasmid and complementing plasmid) transfection system in transformed human cell lines that were also infected with adenovirus. *Id.* at 985. Tratschin, West, Sandbank, and Carter generated an AAV2 vector, AVHiCAT, with the bacterial gene CAT (chloramphenicol acetyltransferase) inserted so that CAT was expressed from the

AAV p40 promoter. *Id.* Hermonat and Muzyczka constructed a vector, dl52-91/neo, with the bacterial gene neo as a selective marker. *Id.* at 986. Transfection of these vectors into cells led to expression of foreign transgene activity (*i.e.*, CAT or neo), providing definitive proof-of-concept that AAV virions having recombinant genomes could be generated and could transduce mammalian cells to express foreign transgenes. *Id.*

20. Genetic analysis enabled by these molecular clones showed that mutations in the ITRs had a cis-acting (regulatory sequences that control the expression of genes on the same molecule) Ori⁻ phenotype² consistent with their function as replication origins. *Id.* at 985. The right half gene had a cap⁻ phenotype and thus coded for the AAV capsid proteins, while the left half gene had a rep⁻ phenotype and coded for the replication function. *Id.* These studies delineated the basic approach to making rAAV vectors: because the ITRs are cis-acting, they must be retained in rAAV vector genomes, but the trans-acting (sequences that act on a target gene to control its expression regardless of the location of the gene (*i.e.*, on the same or different molecules)) rep and cap functions can be provided by

² An Ori⁻ phenotype refers to a genetic or plasmid state where a DNA molecule has a defective or missing origin of replication (ori), leading to a failure to initiate DNA replication.

complementation. *Id.* Thus, most of the AAV genome, except the ITRs, can be deleted, and the maximum payload for an AAV vector is approximately 4.5 kb. *Id.*



Ex. 2038, 29, Fig. 1C (“Removal of the viral Rep and Cap genes allows the insertion of an expression cassette with a maximum size of 4.7 kb; note the 146 bp inverted terminal repeats (TR) which form t-shaped hairpin loops at both the 5' and 3' termini which facilitates genome packaging, second strand synthesis and genome replication. The transgene cassette minimally contains a promoter, a transgene and poly-A tail: regulatory elements which are required for gene expression.”)

21. In wild-type AAV, the ~4.7-kb genome retains its native rep and cap open reading frames, and transcription is driven from the virus’s own three endogenous promoters, p5 and p19 (which produce the large Rep78/68 and small Rep52/40 non-structural proteins, respectively) and p40 (which produces the VP1/VP2/VP3 capsid proteins and the assembly-activating protein), with the

inverted terminal repeats (ITRs) flanking the genome serving primarily as replication and packaging elements rather than the transcriptional machinery itself.

22. By contrast, an rAAV vector has had the rep and cap genes removed and replaced with a transgene expression cassette, so transcription no longer relies on the viral p5/p19/p40 promoters at all. Instead, it is driven by a heterologous promoter (for example a CMV promoter) supplied within the vector cassette between the two retained ITRs. Expression depends on host cell transcription machinery recruited to that exogenous promoter rather than on virally-encoded Rep-regulated promoters.

23. In contrast, wild-type AAV transcription (of p5 and p19 in particular) is normally activated only in the presence of a helper virus (typically adenovirus, whose E1a protein relieves cellular repression of p5) and is further subject to Rep-mediated autoregulation. rAAV transgene transcription proceeds independently of any helper virus once the vector genome is delivered to the target cell. Because the rAAV genome carries no Rep protein of its own, it also lacks the Rep-mediated positive and negative transcriptional feedback that governs wild-type AAV gene expression, so rAAV transgene transcription is instead influenced by other cis-acting and cellular factors, such as low-level residual promoter/enhancer activity intrinsic to the ITRs and capsid- or host-factor-dependent effects (*e.g.*, the

ubiquitin ligase RNF121) that specifically affect transcription from vector-delivered genomes.

24. One common rAAV production method uses a transient transfection approach with three plasmids, often referred to as “triple transfection.” Ex. 1001, 7:8-11; Ex. 2019, 229; Ex. 2026, 938. A first plasmid encodes the therapeutic gene of interest and regulatory elements inserted between the 5’ and 3’ viral ITRs; a second plasmid encodes the rep and cap functions; and a third plasmid provides the required adenoviral helper genes, E4, E2A, and VA RNA. Ex. 2026, 939-40; *see also* Ex. 1001, 7:8-11, FIG. 4. Matsushita et al. reported that “[w]hen th[e] adenoviral helper plasmid was cotransfected into 293 cells, along with plasmids encoding the AAV vector and *rep* and *cap* genes, AAV vector production occurred at levels equal to those achieved by the use of adenovirus infection as a source of help.” Ex. 2026, 939.

C. The Long-Felt and Unmet Need to Characterize rAAV Compositions Containing a Mixture of rAAV Species

25. I was asked to provide my assessment, based on my own experience and my review of the art, of the need for characterizing the rAAV particle species in gene therapy compositions as of 2015. Transfection was understood to produce fully assembled viral vectors, as well as populations of particles that do not contain the intended genome. Ex. 2027, 81; Ex. 2019, 229. These populations include non-genome-containing particles, such as empty capsids, and particles containing

incomplete portions of the rAAV genome or nongenomic nucleic acid contaminants, for example, partial or fragmented genomes. Ex. 2027, 81, 86-89.

26. The production method influences the composition of an rAAV preparation, particularly with respect to the amounts of empty capsid particles, capsids containing fragmented rAAV genomes, and capsids containing nongenomic nucleic acid contaminants. Ex. 2027, 81-82. As Wright explained, “AAV capsid particles that are empty or that encapsidate fragments of nucleic acids other than the intended therapeutic expression cassette are examples of vector product-related impurities.” *Id.* Indeed, “even inefficient packaging of host cell DNA into AAV particles can result in formation of a significant amount of this product-related impurity during AAV vector generation.” *Id.* at 86-87. The environment affecting assembly of AAV and rAAV particles is different. A POSA would not assume that products of viral particle assembly for wild type AAV and rAAV are the same, contrary to Dr. Berkowitz’s assumption. Ex. 1004, ¶ 41.

27. For clinical applications, the presence of rAAV particles containing variant genomes poses significant risks. As Wright noted, “[d]epending on the origin of the cells used for AAV generation, *i.e.*, human or non-human origin, genotoxicity and immunotoxicity are two theoretical risks.” Ex. 2027, 87. The European Medicines Agency (“EMA”) licensure assessment of the gene therapy Glybera® illustrates these concerns: the EMA raised quality objections over

residual baculovirus genetic sequences encapsidated in AAV1 capsids and co-purified with the product. Ex. 1029, 15, 18, 19-20. The EMA also raised concerns relating to residual Rep and Cap genes packaged in AAV1 capsids. *Id.* at 142. More generally, the EMA noted that “assays used to measure residual cellular DNA and protein did not have particularly low [limits of detection], and given the amount of product that is used during treatment ... specifications were considered unacceptably high.” *Id.* at 18.

28. The scientific literature further documented that “excessive empty capsids are immunotoxic” and that “AAV virions packaged with full or partial viral genomes, are more immunotoxic.” Ex. 1026, 6. A POSA would therefore have been concerned with monitoring empty capsids **and** especially partially full capsids, given their potential for elevated immunotoxicity. The difficulty of purifying viral compositions made the ability to properly identify and quantify partially full capsids essential for the clinical applications of rAAV-based gene therapies.

29. Dr. Berkowitz acknowledged the risks of overlooking the presence of rAAV particle impurities in his deposition transcript.

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

[Objection]

A. Right.

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

A. Yes.

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

A. Yes.

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

[Objection]

A. Absolutely. Yes.

Ex. 2039, 119:9-120:7. I agree with Dr. Berkowitz's opinion. Safe and effective gene therapy required a practical and accurate analytical method to analyze the products of rAAV vector production. This was critical to ensuring that an excessive amount of potentially dangerous impurities were not present in compositions administered to patients. Merely identifying heterogeneity was insufficient; it needed to be quantitatively analyzed—a difficult challenge as of the 2015 effective filing date.

D. AAV and rAAV Particle Structure

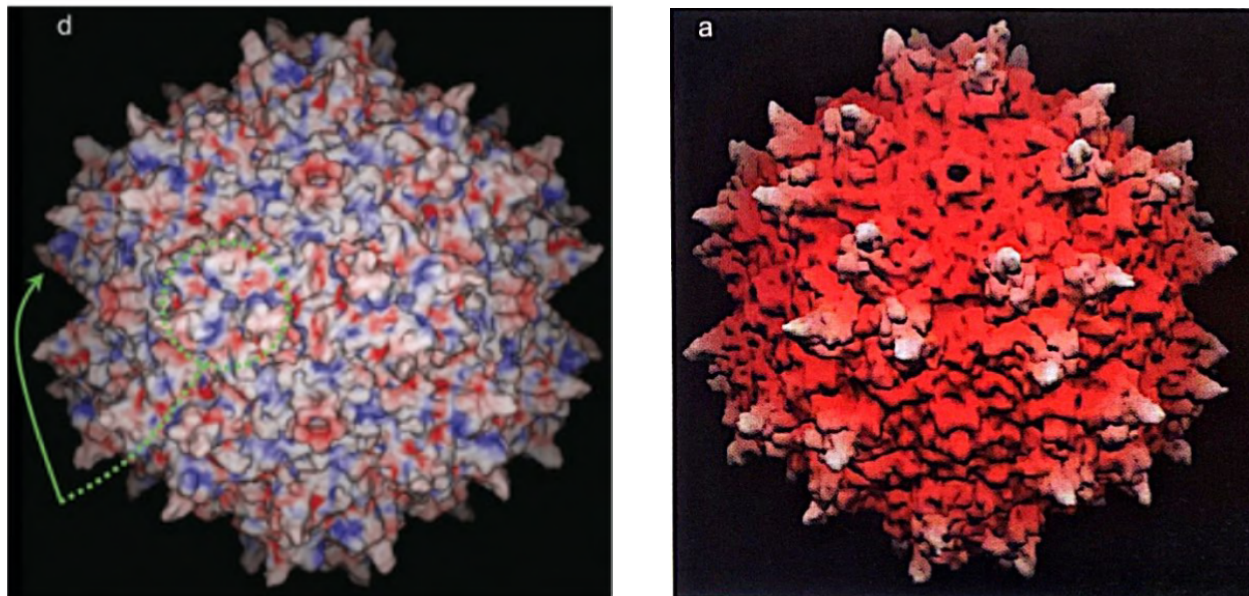
30. rAAV vectors are “composed of an icosahedral assembly of 60 capsid proteins (VP1, -2, -3) assembled together in a defined stoichiometry.” Ex. 2027, 81. Despite this expected single structure, Wright observed that “AAV product-related substances may include individual particles with minor variation in the stoichiometry of VP1, -2 and -3 that still retain infectivity or limited heterogeneity in the 5’ ends of expression cassettes packaged within AAV.” *Id.* at 84. A detailed crystallographic analysis of AAV-2 published in 2002 by Xie et al. described 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).

31. 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 202610 (blue). *Id.* at 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).”). *Id.* at Fig. 3d.; *see also id.* at Fig. 3a.



32. Gao et al. further observed that the presence of partial genomes encapsidated in rAAV particles impacts the conformation of the capsid and alters its biological interactions, including infectivity and immunotoxicity. Ex. 1026, 6.

VI. ANALYSIS OF DR. BERKOWITZ’S OPINIONS ON VIRAL PARTICLE SHAPE AND SIZE

33. I do not agree with Dr. Berkowitz’s opinion that a POSA “would understand that empty AAV particles, particles containing fragmented genomes, and particles containing complete genomes are all approximately the same size and shape.” Ex. 1004, ¶ 168. Dr. Berkowitz purports to have derived his opinion, in

part, from publications that I co-authored with members of my laboratory. My publications, however, do not support his opinion.

34. The POSA, in fact, would draw the opposite conclusion from my laboratory's publications and from other publications relating to viral particle size and shape. The POSA would not have assumed or expected that "that empty AAV particles, particles containing fragmented genomes, and particles containing complete genomes are all approximately the same size and shape."

A. The POSA Would Not Have Assumed that Empty, Fragmented Genome, and Complete Genome rAAVs Are Approximately the Same Size and Shape Based on My Laboratory's Publications

35. The Petition and Dr. Berkowitz allege that because the genomes are packaged into preformed capsids, the capsid surface is the same regardless of DNA content. Petition, 12 (citing Ex. 1033, 983-84, Ex. 1034, 495; Ex. 1012, 71-72, 79-82), 24 (Ex. 1007, 203), 43-44; Ex. 1004, ¶¶ 61, 100, 166-168. This conclusion disregards the teachings of my references showing that not all particles are identical and indicating that viral vector assembly was more complex.

36. De la Maza (1978) explains that the preformed-capsid inference for particle assembly may explain why AAV particles do not have "aberrant morphology with smaller DNA." Ex. 1007, 202. Contrary to Dr. Berkowitz's opinion (Ex. 1004, ¶¶ 100, 166), this statement regarding "aberrant morphology" would not be understood by a POSA to mean that an empty capsid, a capsid

containing a fragmented DNA strand, and a capsid containing a fully intact DNA strand would have the same shape and size. Rather the POSA would understand that the reference to “aberrant morphology” refers to the observation that capsids do not appear to be constructed around DNA. Such a mechanism would be expected to potentially produce malformed capsids for DNA fragments that are smaller than intact DNA strands. Our laboratory observed that capsids can still vary in size and shape without being deformed.

37. De la Maza (1978) contradicts Dr. Berkowitz’s conclusion regarding AAV particle size and shape. We isolated two virions of different densities from a CsCl gradient in a preparative sedimentation equilibrium AUC experiment, AAV (1.41 g/cm³) and AAV (1.45 g/cm³). Ex. 1007, 195. Once isolated, the encapsidated “DNA was released from [both] virions.” *Id.* at 194. The released DNA was subjected to sedimentation velocity AUC (“SV-AUC”) to assess the genome content that was present in those virions. Both virions had the same genomic content:

When virions from the 1.41- or the 1.45-g/cm³ band of a CsCl gradient were sedimented in alkaline sucrose, about 90% of the DNA formed a single 15.5S peak and the remainder sedimented as a shoulder at about 12S. This 12S shoulder was pooled, neutralized, and reassociated, and is designated as incomplete DNA type II (see Table 1). The 15.5S AAV DNA component from the alkaline sucrose gradient was reassociated and sedimented in a neutral sucrose gradient to yield a major 14.5S

component of intact AAV duplex. In the neutral sucrose gradient there was also a small amount of an 11.5S component, which was collected and designated as incomplete DNA type I (Table 1).

Id. at 194-95 From this passage, the POSA would understand the genomic content of two particles having different densities to be effectively identical. The data indicate that the capsids had to be different in some way.

38. A subsequent publication in March 1980 from my laboratory explains the reason for the different densities between these two particles with identical genomes. Ex. 1013. The denser particle AAV (1.45) had a smaller diameter than the less dense particle (1.41). Ex. 1013, 1129 (“Electron microscope studies revealed that the more dense, infectious particles were 10 to 20% smaller in diameter than the 1.41 particles.”).

39. A prior analysis of AAV(1.45) and AAV(1.41) by the laboratory of David Hoggan’s group at the National Institute of Allergy and Infectious Disease (NIAID) of the NIH referred to these AAV species as the high (“H”) and low (“L”) density particles. Ex. 2040. Roy’s electron micrograph analysis estimated that “the average diameter of H particles was 12% less than the diameter of the L particles ... and suggested that these findings might reflect a lack of, or configurational alteration of, a protein at the capsid surface.” *Id.* at 427. These studies “found that H particles have a higher DNA/protein ratio than L particles,” suggesting a lack of capsid protein in the H particles. *Id.* at 428.

40. Later and more comprehensive studies by my laboratory, however, reached a slightly different conclusion than the Hoggan group regarding these particles. My laboratory applied the techniques of “density centrifugation, velocity sedimentation, and restriction endonuclease analysis in order to further characterize the infectious 1.45 and 1.41 AAV constituents.” Ex. 1013, 1129. In contrast to the Hoggan research, our studies revealed that “[f]or both AAV (1.41) and (1.45), the major components had an **identical DNA content and DNA/protein ratio.**” *Id.* (emphasis added). Spectrophotometric analysis confirmed identical UV spectra for the purified 111S (1.41) and 109S (1.45) particles, with each showing an A280/A260 ratio of 0.72 ± 0.02 . *Id.* at 1132. The fact that both the AAV (1.41) and AAV (1.45) particles had the same DNA content, the same capsid protein/DNA ratio, and identical UV spectra indicated that the difference in diameters between the two particles was attributed to some type of conformational change in the capsid proteins, rather than an absence of protein. *Id.* at 1136.

41. SV-AUC assays supported our theory of conformational change. Unlike the preparative sedimentation studies my laboratory used to separate the AAV species according to their density, SV-AUC considers the rate of sedimentation. The rate of sedimentation depends on both the buoyant density of the particle and its “frictional coefficient (which is directly related to macromolecular shape and size).” Ex. 1025, 165-66. Notably, the AAV (1.41) and

AAV (1.45) particles also “differed slightly in their sedimentation rates.” Ex. 1013, 1129.

42. The sedimentation coefficient results are particularly instructive regarding how AAV particles can differ in size and shape. Because AAV (1.45) has a higher density than AAV (1.41), and because sedimentation coefficient is proportionate to density, if the size and shape of the particles were identical, then AAV (1.45) would sediment at a higher rate, and have a higher S value, than AAV (1.41). But the opposite is true:

We found that the 1.41 component has a slightly higher S value (111) than the 1.45 particle (109) Assuming that the masses of the two types of particles are equivalent, the data suggest that ***a change in structural conformation accounts for these differences between the two classes of particles.*** This may account for the antigenic differences between the 1.45 and 1.41 particles.

Id. at 1136 (emphasis added). This observation, made decades before the 2015 effective filing date of the '894 patent, contradicts Dr. Berkowitz's opinion that “particles containing complete genomes are all approximately the same size and shape.” Ex. 1004, ¶ 168.

43. In his deposition testimony, Dr. Berkowitz agreed that the data demonstrate to a POSA that rAAV particles do not all have “approximately the same size and shape.”

Q. Now you say in this first sentence: “Because AAV assembly occurs via packaging of DNA into preformed capsids, a POSA would understand that empty AA particles, particles containing fragmented genomes, and particles containing complete genomes are all approximately the same size and shape.”

A. Correct.

Q. Now we just discussed this Exhibit 1013 of de la Maza and Carter, showing that particles appear like they can have different sizes and shape? Right?

[Objection]

A. That appears to be the case. Yes.

Ex. 2039, 116:4-117:15. The observation by our group demonstrates that the POSA would not have assumed that sedimentation coefficient was solely a function of rAAV particle density. *Id.* at 117:16-118:8.

44. In another publication from my laboratory, which I co-authored with Maureen Myers (Ex. 1012), we proposed that AAV (1.45) may have been a precursor to AAV (1.41) made during rAAV assembly. Ex. 1012. The Myers publication undermines Dr. Berkowitz’s contention that the mechanism of assembly “in which progeny DNA strands are packaged into preformed capsids” supports his opinion that the POSA would have assumed that rAAV particles are “of approximately the same size, regardless of the length of the DNA.” Ex. 1004, ¶

166; Petition 12, (citing Ex. 1012, 71-72, 79-82). Myers discusses the same viral particles as the de la Maza publications, AAV(1.41) and AAV(1.45), having the same DNA content and protein to DNA ratio. Ex. 1012, 73, 81.

45. Additionally, our pulse-chase labeling experiments also identified an assembly intermediate, AAV(SP), having an approximately 60S sedimentation rate with DNA associated in a “DNase-sensitive conformation (in contrast to the complete DNase resistance of DNA in mature particles).” *Id.* at 71. The existence of this intermediate—an open capsid-DNA complex distinct from both empty capsids and fully assembled virions—demonstrates that the assembly process generates particles with **different** conformational states. In Myers, we theorized that AAV(1.45) may have been a precursor to AAV(1.41), indicating additional complexity in the rAAV virion assembly process that may impact shape and size. *Id.* at 81. Alternatively, the smaller and denser AAV(1.45) product may have been an end product. *Id.*

46. Under either theory, a POSA would have understood that the rAAV assembly process resulted in different shaped particles produced as a precursor or final product, a fact to which Dr. Berkowitz agreed. Ex. 2039, 155:5-158:21. Dr. Berkowitz also speculated that the AAV (1.45) product could be a degradant. *Id.* at 158:23-159:9. This theory is inconsistent with the observation that all of the DNA and capsid protein was present in AAV (1.45). Ex. 1013, 1129 (“For both AAV

(1.41) and (1.45), the major components had an identical DNA content and DNA/protein ratio”). Regardless, even if AAV (1.45) was a degradant, it still was present in the particle mixture. Ex. 2039, 158:23-159:23. Thus, it would not change a POSA’s understanding that not all AAV particles have identical size and shape.

47. Later publications from other research groups confirm our group’s observations regarding variation in capsid conformations. For example, 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.

B. A POSA Would Not Have Understood Our Unpublished Observations that “particle density has an approximately direct linear relationship to the strand length” to Indicate a Quantitative Linear Relationship

48. Dr. Berkowitz’s opinion is premised on the existence of “a linear relationship between the density of an AAV particle and the size of the DNA inside the capsid.” Ex. 1004, ¶¶ 103, 132, 165. To support his opinion, he cites a quote from one of my publications out of context. Ex. 1004, ¶¶ 59, 60, 102 (quoting Ex. 1008, 3202). The statement that “particle density has an

approximately direct linear relationship to the strand length” was based on “unpublished observations” (Ex. 1008, 3202 n.3), which we never published. Ex. 2039, 105:2-6. The purpose of providing this general observation was to qualitatively support our theory that DNA is inserted into preformed capsids, which Dr. Berkowitz recognized. *Id.* at 151:3-6.

49. A POSA would have understood that the omission of data underlying the “unpublished observations” signaled that this statement was intended as only a qualitative observation. In his deposition testimony, Dr. Berkowitz acknowledged the purpose of this statement to support our mechanistic proposal and that there are exceptions to this general trend. *Id.* at 106:24-107:20. A POSA would have recognized that these exceptions, which are described in our publications, meant that this density trend was not intended to guide the development of a quantitative assay.

50. Table 1 of de la Maza (1978), reproduced below, shows three isolated AAV particles having identical densities, 1.41 g/cm^3 , but containing genomes of different DNA: Intact, Type I, and Type II.

TABLE 1
Sedimentation Properties of Incomplete AAV-DNA Molecules

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

Ex. 1007, 195 (highlighting added). The highlighted entries show that Intact, Type I, and Type II DNA appear to be different. In the alkaline sucrose SV-AUC experiment, Intact and Type I DNA had the same molecular weights, with Type II having a lower molecular weight. In the neutral sucrose SV-AUC experiment, Intact, Type I, and Type II DNA all had different molecular weights. The data demonstrate that AAV particles can have the same density, but contain different molecular weight DNA.

51. De la Maza (1978) contradicts Dr. Berkowitz's assertion that "a POSA would understand that there is a linear relationship between the density of AAV particles and the size of the DNA fragment contained therein." Dr. Berkowitz admitted this in his deposition testimony:

Q. So the three particles corresponding to the intact Type 1 and Type 2 all have identical density? Right?

A. Right.

Q. But they have different amounts of genome that has been encapsidated [inside]? Right?

[Objection]

A. That's correct.

Q. So from this, would this data tend to indicate that the density of an AAV particle is not solely determined by the size of the genome inside?

[Objection]

A. It would look like that's the case. Yes.

Ex. 2039, 99:9-100:3.

Q. So the data in the exhibit that we were just discussing [Ex. 1007] doesn't seem to indicate a direct linear relationship between density and DNA strand length? Right?

A. I guess you would have to say that basically. Yes.

Id. at 101:3-11.

52. The isolation of the AAV(1.41) and AAV(1.45) particles also undercuts Dr. Berkowitz's assertion that "there is a linear relationship between the

density of an AAV particle and the size of the nucleic acid packaged inside the capsid.” Ex. 1013, 1129. The two particles contain the same amount of capsid protein and DNA, but have different densities.

53. In summary, my laboratory’s observations on linearity were only intended to qualitatively support our research on AAV packaging. For preparing a quantitative assay, however, a POSA would not have considered there to have been a sufficiently reliable linear relationship between genome size and AAV particle density. Dr. Berkowitz agreed on this point. Ex. 2039, 104:12-107:20.

C. Other Publications Cited by Dr. Berkowitz Are Consistent with My Publications Regarding Variations in Viral Particle Shape and Size

54. The POSA would not have considered the transmission electron microscopy (“EM”) images that Dr. Berkowitz cites in his declaration to indicate that all particles would have the same size and shape regardless of genome content. Ex. 1004, ¶¶ 41, 168. Qualitative visual inspection of images of bulk particles in a publication, as Dr. Berkowitz appears to do, is not suited to detecting the types of surface changes that would impact a particle’s frictional coefficient, the types of changes observed in the AAV particles that my laboratory studied. Quantitative higher resolution images, for example X-ray crystal structures, are better suited to identifying structural changes on the surface of the rAAV particle. Ex. 2025, 10405-10406 (describing the surface topology of AAV particles as analyzed by “3-Å resolution by x-ray crystallography”)

55. The POSA would not have concluded that the Gao (Ex. 1026) and Lock (Ex. 1041) “show[] transmission electron microscopy images of full and empty AAV particles with the same size and shape.” Petition, 44; Ex. 1004, ¶¶ 41, 168. It is not clear how Dr. Berkowitz even reached that conclusion, as he does not provide the methods he used to analyze the similarities between the particles. Dr. Berkowitz also admits that neither he nor Gao conducted a quantitative analysis of the EM images. Ex. 2039, 122:2-123:1. Dr. Berkowitz, however, acknowledged that frictional coefficient is a quantitative measure.

Q. So in your opinion, there is sufficient resolution at the surface level to see differences in particle shape or size that would impact frictional coefficients?

A. Qualitatively, yes.

Q. Is frictional coefficient qualitative, though?

[Objection]

A. No. It can be quantitative. So could these pictures. You could quantitate these.

Q. You did not quantitate these?

A. No, I did not.

Q. And the authors did not quantitate these?

A. That’s correct.

Id. at 123:13-124:7.

56. The Gao reference even states that “EM cannot distinguish full virions packaged with full-length genomes from those with partial viral genomes.” Ex. 1026, 6. Unable to ascertain which particles correspond to each species, the POSA could not have possibly used EM to conclude that all particle species had approximately the same size and shape. Gao explains that electron micrographs are acceptable merely for “qualitatively studying the structures of virions” (*id.*), a fact that Dr. Berkowitz acknowledged in his testimony.

Q. So would you agree that Gao is stating here that it wasn’t possible to accurately distinguish whether or not you had a particle with a fully intact DNA genome from those that have a partial DNA genome?

A. Yes. It was difficult. Yes.

Q. And would the inability to distinguish between which particle was which hinder the confirmation of whether or not these particles actually were all identical sizes?

[Objection]

A. It is a general -- it is a general statement within its limitations basically.

Q. So Gao is saying that electron microscope is limited application in terms of assessing the composition of a mixture, heterogeneous mixture of AAV particles?

[Objection]

A. For its time. I mean at this given date, yes.

Ex. 2039, 125:18-126:19.

57. In fact, Gao (Ex. 1026) indicates that partially empty particles have different conformations than empty particles. Ex. 1026, 6 (“particles with partial viral genomes may also contribute to the side effects of the [partially empty] virions due to their different capsid conformations as compared with [completely empty] particles”). Gao states that “encapsidation of full or partial AAV genomes may lead to capsid conformational changes” *Id.* The POSA would understand Gao to refute relying on EM to evaluate quantitative issues, including whether differences in particle size and shape could impact friction in SV-AUC experiments. Dr. Berkowitz acknowledged in his testimony relating to Gao’s discussion of conformational changes (*id.*) that such changes would be understood to potentially impact frictional coefficients of particles.

Q. But would a change in capsid formation potentially change how the particle [is] interacting chemically with the environment?

[Objection]

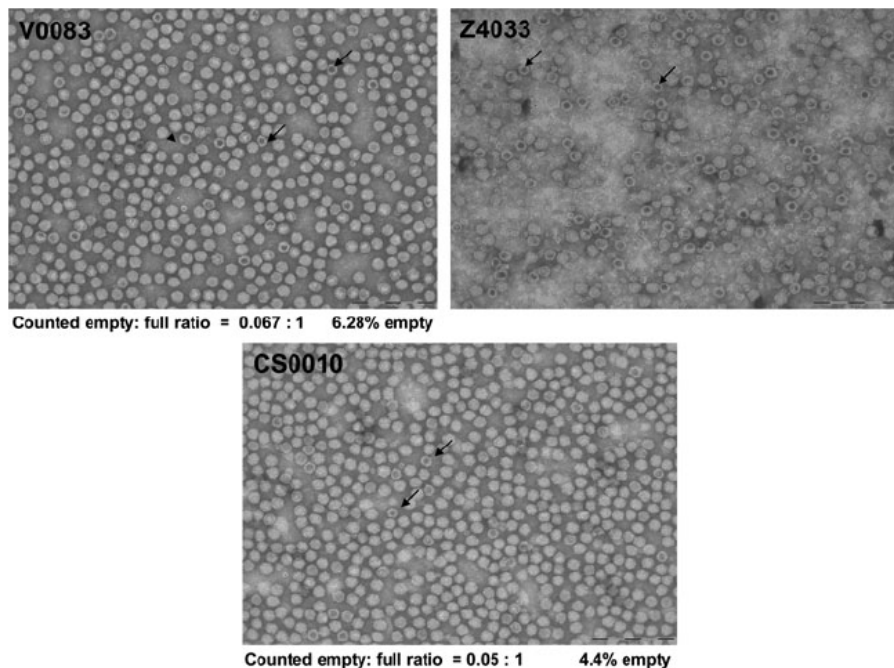
A. Then you are speaking of hydration of some sort or solvation effects.

Q. And hydration and solvation effects do matter for frictional coefficients? Right?

A. Yes, they could.

Ex. 2039, 129:20-131:1.

58. Lock (Ex. 1041) confirms Gao's characterization of EM, stating that "[e]ven in the sharpest images, some particles appear only partially stained, and this creates ambiguity in characterizing them as empty or full." Ex. 1041, 57. The 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; Ex. 1004, ¶¶ 41, 168. Lock's images, reproduced below, indicate a qualitative difference in particle shapes and sizes.



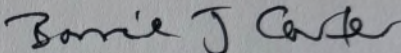
Ex. 1041, 59. To the extent they even can be observed, the shapes in the above images appear irregular and highly varied. Regardless, nothing in these images would give the POSA a reason to conclude that rAAV particles share identical shapes. Moreover, Lock does not even analyze particles containing partial genomes, as Dr. Berkowitz acknowledged. Ex. 2039, 132:16-133:2.

59. Quantitative studies, like those conducted in my laboratory, disprove Dr. Berkowitz's assumption that rAAV particles, regardless of genome content, have the same size and shape. Neither Lock nor Gao supports Dr. Berkowitz's reliance on their qualitative images for assessing quantifiable differences in particle frictional coefficients that can be caused by variations in capsid conformation.

VII. Conclusion

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

Date: August 5, 2026


Dr. Barrie J. Carter