Filed on behalf of: Novartis Gene Therapies, Inc. and Novartis Pharmaceuticals Corporation

UNITED STATES PATENT AND TRADEMARK OFFICE

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BEFORE THE PATENT TRIAL AND APPEAL BOARD

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NOVARTIS GENE THERAPIES, INC., and NOVARTIS PHARMACEUTICALS CORPORATION
Petitioners,

v.

GENZYME CORPORATION,
Patent Owner.

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Case No. IPR-2023-01044 and IPR-2023-01045
U.S. Patent No. 10,429,288

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DECLARATION OF STEVEN A. BERKOWITZ., PH.D.

## TABLE OF CONTENTS

I. INTRODUCTION ..... 1

II. QUALIFICATIONS ..... 1

III. BACKGROUND AND STATE OF THE ART ..... 4

A. Analytical Ultracentrifugation ("AUC") has been used for nearly 100 years to study biological macromolecules ..... 4

B. Basic Principles ..... 7

C. The Analytical Ultracentrifuge ..... 12

D. Boundary Sedimentation Velocity Analytical Ultracentrifugation provides data on the size, shape, and density of macromolecules ..... 14

E. Demonstration of the SEDFIT Software ..... 16

a. Loading a dataset ..... 18

b. Selecting a model ..... 20

c. Set analysis parameters ..... 21

d. Specify the type of regularization ..... 22

e. Run a sedimentation analysis ..... 23

f. Peak integration ..... 25

F. Viral vectors in gene therapy ..... 27

1. Viral vectors, including rAAVs, were known to be promising avenues for gene therapy modalities ..... 27

2. Like all pharmaceutical products, gene therapy compositions must be monitored for homogeneity, purity and consistency of manufacturing along with other quality attributes to gain and maintain marketing approval ..... 27

IV. MATERIALS CONSIDERED .....29

V. THE ’288 PATENT .....29

A. The Disclosure of the ’288 Patent .....29

B. The Claims of the ’288 Patent .....31

C. The Prosecution History .....32

VI. LEVEL OF ORDINARY SKILL IN THE ART .....36

VII. ANTICIPATION ANALYSIS .....37

A. Legal Standard .....37

B. PCT Publication of International Application Number PCT/EP2014/052978, entitled “Methods for the Production of Double-Stranded AAV Viral Particles” (“Le Bec”) .....38

C. Claim 1 anticipated by Le Bec .....41

1. “A method of characterizing a preparation of recombinant adeno-associated viral (AAV) particles comprising the steps of…” .....41

2. “a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant AAV particles is monitored at time intervals” .....42

3. “b) plotting the differential sedimentation coefficient distribution values (C(s)) versus the sedimentation coefficient in Svedberg units (S)” .....46

4. “c) integrating the area under each peak in the C(s) distribution to determine the relative concentration of each peak, wherein each peak represents a species of recombinant AAV particle” .....48

D. Claim 4 is anticipated by Le Bec .....49

1. "A method to determine the presence of empty capsids or capsid particles comprising variant sized recombinant AAV genomes in a preparation of recombinant AAV particles comprising the steps of" ..... 49

2. "a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant AAV particles is monitored at time intervals" ..... 50

3. "b) plotting the differential sedimentation coefficient distribution value (C(s)) versus the sedimentation coefficient in Svedberg units (S)," ..... 50

4. "wherein the presence of one or more peaks other than the peak for full capsid particles comprising intact recombinant AAV genomes indicates that presence of capsid particles comprising variant sized genomes and/or empty capsids" ..... 50

E. Claim 5 is anticipated by Le Bec ..... 51

1. "A method of measuring the relative amount empty capsids in a preparation of recombinant AAV particles comprising the steps of" ..... 51

2. "a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant AAV particles is monitored at time intervals," ..... 52

3. "b) plotting the differential sedimentation coefficient distribution value (C(s)) versus the sedimentation coefficient in Svedberg units (S)," ..... 52

4. "c) integrating the area under each peak in the C(s) distribution to determine the relative concentration of each species of recombinant AAV particles," ..... 52

5. "d) comparing the amount of recombinant AAV particles having an S value corresponding to empty capsid particles to the amount of recombinant AAV particles having an S value corresponding to recombinant AAV particles comprising intact AAV genomes or the total amount of recombinant AAV particles in the preparation." ..... 52

F. Claim 6 is anticipated by Le Bec ..... 53

1. "A method of measuring the relative amount of capsid particles comprising variant recombinant AAV genomes or empty AAV capsid particles in a preparation of recombinant AAV particles comprising the steps of" ..... 53

2. "a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant AAV particles is monitored at time intervals," ..... 54

3. "b) plotting the differential sedimentation coefficient distribution value (C(s)) versus the sedimentation coefficient in Svedberg units (S)," ..... 54

4. "c) integrating the area under each peak in the C(s) distribution to determine the relative concentration of each species of recombinant AAV particles," ..... 54

5. "d) comparing the amount of recombinant AAV particles having an S value[] that do[es] not correspond to recombinant AAV particles comprising intact AAV genomes to the amount of recombinant AAV particles having an S value that corresponds to recombinant AV particles comprising intact AAV genomes or to the total amount of recombinant AAV particles in the preparation." ..... 55

G. Claim 7 is anticipated by Le Bec ..... 55

1. "A method of measuring the relative amount of capsid particles comprising variant recombinant AAV genomes

in a preparation of recombinant AAV particles comprising the steps of" ..... 55

2. "a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant AAV particles is monitored at time intervals," ..... 56

3. "b) plotting the differential sedimentation coefficient distribution value (C(s)) versus the sedimentation coefficient in Svedberg units (S)," ..... 56

4. "c) integrating the area under each peak in the C(s) distribution to determine the relative concentration of each species of recombinant AAV particles," ..... 56

5. "d) comparing the amount of recombinant AAV particles having [] S values that do not correspond to recombinant AAV particles comprising intact AAV genomes or empty capsid particles to the total amount of recombinant AAV particles in the preparation." ..... 57

H. Claim 8 is anticipated by Le Bec ..... 58

1. "A method of measuring the relative amount of recombinant AAV particles comprising intact AAV genomes in a preparation of recombinant AAV particles comprising the steps of" ..... 58

2. "a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant AAV particles is monitored at time intervals," ..... 59

3. "b) plotting the differential sedimentation coefficient distribution value (C(s)) versus the sedimentation coefficient in Svedberg units (S)," ..... 59

4. "c) integrating the area under each peak in the C(s) distribution to determine the relative concentration of each species of recombinant AAV particles," .....59

5. "d) comparing the amount of recombinant AAV particles having an S value[] corresponding to recombinant AAV particles comprising intact AAV genomes to the amount of recombinant AAV particles having an S value corresponding to empty capsid particles, to capsid particles comprising variant recombinant AAV genomes, and/or to the total amount of recombinant AAV particles in the preparation".....59

I. Claim 10 is anticipated by Le Bec .....60

J. Claim 11 is anticipated by Le Bec .....60

1. "wherein the presence of one or more peaks other than the peak for full capsid particles comprising intact recombinant AAV genomes or empty capsid particles indicates [the] presence of capsid particles comprising variant sized genomes, and "wherein the capsid particles comprising variant sized genomes comprises truncated genomes, aggregates, recombinants and/or DNA impurities compared to the intact recombinant AAV genome" .....61

K. Claim 12 is anticipated by Le Bec .....62

1. "A method of determining the heterogeneity of recombinant AAV particles in a preparation of recombinant AAV particles comprising the steps of…" .....62

2. "a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant AAV particles is monitored at time intervals," .....63

3. "b) plotting the differential sedimentation coefficient distribution value (C(s)) versus the sedimentation coefficient in Svedberg units (S),".....63

4. "wherein the presence of peaks in addition to the peak representing capsids comprising an intact AAV genome indicates heterogeneity of recombinant particles in the preparation." ..... 63

L. Claim 13 is anticipated by Le Bec ..... 64

M. Claim 14 is anticipated by Le Bec ..... 64

N. Claim 15 is anticipated by Le Bec ..... 65

O. Claim 17 is anticipated by Le Bec ..... 66

P. Claim 18 is anticipated by Le Bec. ..... 66

Q. Claim 20 is anticipated by Le Bec ..... 67

R. Claim 21 is anticipated by Le Bec ..... 67

S. Claim 23 is anticipated by Le Bec ..... 68

T. Claim 32 is anticipated by Le Bec ..... 69

U. Claim 33 is anticipated by Le Bec ..... 70

1. "A method of evaluating a process for the production of recombinant AAV particles comprising" ..... 70

2. "the method of claim 1," ..... 70

3. "wherein an increase in the relative amount of recombinant AAV particles comprising intact AAV genomes compared to the relative amount of empty capsid particles and/or recombinant AAV capsid particles with variant recombinant AAV genomes compared to a reference preparation of recombinant AAV particles indicates an improvement in the production of recombinant AAV particles." ..... 71

VIII. OBVIOUSNESS ANALYSIS ..... 71

A. Legal Standard ..... 71

B. Summary of Prior Art References ..... 74

1. PCT Publication of International Application Number PCT/EP2014/052978, entitled "Methods for the Production of Double-Stranded AAV Viral Particles" ("Le Bec") ....74

2. Berkowitz, S.A. & Philo, J.S. "Monitoring the homogeneity of adenovirus preparations (a gene therapy delivery system) using analytical ultracentrifugation" 362 ANAL BIOCHEM. 16-37 (2007) ("Berkowitz") ....74

3. Cole, J.L. et al., "Analytical Ultracentrifugation: Sedimentation Velocity and Sedimentation Equilibrium", *in* METHODS IN CELL BIOLOGY, VOL. 84 (2008) ("Cole") ....76

4. Schuck, P., "Size-Distribution Analysis of Macromolecules by Sedimentation Velocity Ultracentrifugation and Lamm Equation Modeling" 78 BIOPHYS. J. 1606-1619 (2000) ....78

5. Zhao, H. et al., "Overview of Current Methods in Sedimentation Velocity and Sedimentation Equilibrium Analytical Ultracentrifugation" 71 Curr. Protocols in Protein Sci. 20.12.1-20.12.49 (2013) ("Zhao") ....80

C. Claims 1, 4-8, 10-15, 17-18, 20-21, 23 and 32-33 are Obvious in View of Le Bec ....82

1. Claim 1 ....82

2. Claims 4-8 and 10-15 ....85

3. Claims 17-18, 20-21, 23, and 32-33 ....87

D. Claims 1-26 and 31-33 Are Obvious Over Le Bec In View Of Berkowitz and/or Cole ....90

1. Claim 1 ....90

2. Claim 2 is Obvious Over Le Bec In View of Berkowitz, Cole and the Knowledge of a POSA ....93

a. "A method to assess vector genome integrity of recombinant AAV particles in a preparation of recombinant AAV particles comprising" .....93

b. "a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant AAV particles is monitored at time intervals," .....94

c. "b) plotting the differential sedimentation coefficient distribution value C(s) versus the sedimentation coefficient in Svedberg units (S), and" .....94

d. "c) identifying species of recombinant AAV particles in the preparation by presence of peaks on the plot corresponding to an S value," .....95

e. "wherein the genome size of a particular species of recombinant AAV particles is calculated by comparing the S value of the species to a standard curve generated by S values of recombinant AAV particles comprising encapsidated AAV genomes of known nucleotide sizes." .....95

3. Claim 3 .....98

4. Claims 4-8 and 10-15 .....99

5. Claims 9 and 16 .....100

6. Claims 17-25 .....103

7. Claim 26 .....108

8. Claim 31 .....109

9. Claim 32 .....109

10. Claim 33 .....110

E. Claims 27-30 are Obvious Over Le Bec In View Of Berkowitz and Schuck and/or Zhao ..... 111

1. Claim 27 ..... 111

2. Claims 28-29 ..... 113

3. Claim 30 ..... 114

a. "resolution of about 200 to about 5000," ..... 114

b. "$S_{min}$ is about 1 S to about 100 S", ..... 115

c. "$S_{max}$ is about 100 S to about 5000 S," ..... 116

d. "and frictional ratio is about 1.0 or is left to float to a value determined by centrifugation software" ..... 117

F. Claim 34 is Obvious Over Colosi In View Of Le Bec ..... 118

IX. DECLARATION ..... 120

## I. INTRODUCTION

1. I have been retained by Finnegan, Henderson, Farabow, Garrett & Dunner, LLP on behalf of Petitioners Novartis Gene Therapies, Inc. and Novartis Pharmaceuticals Corporation as an independent expert consultant in the above referenced *inter partes* review ("IPR") proceedings to provide information and opinions on the teachings of the prior art and the state of the art, as relevant to the issued claims of U.S. Patent No. 10,429,288 ("the '288 patent") (Ex.1001). It is my understanding that the '288 patent identifies Genzyme Corporation as its assignee.

## II. QUALIFICATIONS

2. In 1968, I graduated from Fairleigh Dickinson University and received a Bachelor of Science degree in biology. In 1975, I received a Ph.D. in Biochemistry from New York University, School of Medicine, under the supervision of Dr. Loren Day. My dissertation work focused on applying biophysical tools (predominately analytical ultracentrifugation and light scattering techniques) to characterize the structure and physicochemical properties of a group of filamentous bacteriophages and its DNA.

3. I then spent 1975-1980 as a post-doctoral fellow at Yale University in the biology department under Dr. Robley Williams, Jr., the chemistry department under Dr. Julian Sturtevant and at the NIH under Dr. Jan Wolff. During

my post-doctoral work, I was involved in research focused on studying the biochemical and biophysical structure and properties of microtubules.

4. After my post-doctoral work, I held various positions at Celanese Research Company (conducting analytical work on characterizing synthetic polymers), J.T. Baker (developing chromatography materials and automating analytical instruments and pilot plant equipment) and Lederle Laboratories (developing analytical and purification methods relating to monitoring and making of polysaccharide vaccines and pursuing efforts to automate these areas) before taking a position at Biogen where I spent more than twenty years working in the areas of analytical and biophysical characterization, purification and formulation development on a wide range of biopharmaceutical products (involving proteins and complex drugs such as virus like particles and viral vectors for potential gene therapy applications) before retiring as a senior principal investigator at the end of 2013.

5. Since retiring I have continued to participate in science in helping other companies on a part time basis as a consultant in the biophysical characterization of biopharmaceuticals where I have continued to be active in publishing novel and original work and occasionally served as an expert scientific reviewer for various scientific journals. Much of the scientific work I have been involved with in my retirement has focused on the application of analytical

ultracentrifugation to gene therapy products, specifically the characterization of adeno-associated virus (AAV), which started as early as 2014 when I did consulting work regarding how to do boundary SV-AUC analysis on AAV using the software program SEDFIT and CsCl density gradient sedimentation equilibrium (a very common technique used for purifying viruses), but in an analytical mode, as described in Berkowitz & Philo[1] for Adenovirus.

6. Overall, my technical areas of expertise are concentrated in the separation and physical sciences associated with the characterization of a wide range of biopolymers and synthetic polymers. Much of my work has centered on assessing the physicochemical properties, micro-heterogeneity, aggregation properties and higher-order structure of biopharmaceuticals using a range of biochemical and biophysical techniques that has predominately involved the use of dynamic and static (or intensity) light scattering, analytical ultracentrifugation, chromatography, electrophoresis, microcalorimetry and various forms of spectroscopy.

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[1] Berkowitz SA, Philo JS., Monitoring the homogeneity of adenovirus preparations (a gene therapy delivery system) using analytical ultracentrifugation, Anal Biochem. 2007 Mar 1;362(1):16-37. doi: 10.1016/j.ab.2006.11.031. Epub 2006 Dec 20.PMID: 17223062. (Ex.1004).

7. In applying these techniques, I have published approximately 35 peer reviewed papers in the scientific literature, book chapters and co-edited a book entitled "Biophysical Characterization of Proteins in Developing Biopharmaceuticals" and have given a number of invited scientific talks that demonstrate novel extension of those techniques mentioned in paragraph 5 above.

8. Based on my education, training, and over 24 years of experience in the biopharmaceutical industry, which includes the January 2015 time frame, in pharmaceutical product development, which involved drug purification, formulation and analytical characterization and release testing, I am qualified to provide useful technical information regarding the subject matter of this case.

## III. BACKGROUND AND STATE OF THE ART

### A. Analytical Ultracentrifugation ("AUC") has been used for nearly 100 years to study biological macromolecules

9. The development of AUC-based techniques dates to the early twentieth century. It initially played a key role in demonstrating the existence of macromolecules via the pioneering studies carried out by Svedberg and his colleagues. In the years following this ground-breaking work, the analytical ultracentrifuge was heavily used to characterize the macromolecular properties of biological and synthetic polymers, with a significant focus in characterizing homogeneity, paucidispersity, or polydispersity of these macromolecular materials via size-distribution distribution analysis (by assessing the sedimentation

coefficient distributions, c(s) vs S.[2] Overall, however, the analytical ultracentrifuge has found its most effective application in the biological sciences where it has been employed to study the physical and chemical properties of the enormous range of macromolecules and other materials present in the biological world, e.g., proteins, nucleic acids, polysaccharides, and supramolecular structures (such as ribosomes, multi-subunit enzymes, and viruses) by assessing the presence/absence and the amounts or distribution of the different components present in a biological sample and studying their sedimentation behavior. Indeed, as pointed by Fujita, in his book

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[2] Howard K. Schachman, Ultracentrifugation in Biochemistry, Academic Press, New York, 1959, pp. 128-138; Stafford WF 3rd., Boundary analysis in sedimentation transport experiments: a procedure for obtaining sedimentation coefficient distributions using the time derivative of the concentration profile, Anal Biochem. 1992 Jun;203(2):295-301. doi: 10.1016/0003-2697(92)90316-y.PMID: 1416025; Schuck P., Size-distribution analysis of macromolecules by sedimentation velocity ultracentrifugation and lamm equation modeling, Biophys J. 2000 Mar;78(3):1606-19. doi: 10.1016/S0006-3495(00)76713-0.PMID: 10692345 (Ex.1007.)

FOUNDATIONS OF ULTRACENTRIFUGATION ANALYSIS,[3] "biochemists and molecular biologists are primarily interested in utilizing the ultracentrifuge for separating paucidisperse macromolecular solutions into components solutes and estimating the concentrations and sedimentation rates of the separated components." Thematically, such work is very much analogous to purity assessment work. Of the several analytical ultracentrifuges that were commercially developed over these early years, it was Beckman's Model E that was the workhorse instrument employed to power the many experiments that drove the developments of molecular biology and biochemistry.

10. Fujita noted in his book[4] that the ultracentrifuge "has become an almost indispensable instrument for macromolecular research," but in 1973 when Fujita's book appeared, the advent of simple, inexpensive and high throughput techniques such as chromatography/electrophoresis, along with the eventual developments in mass spectroscopy, lead to the temporary phasing out of interest in AUC.

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[3] Hiroshi Fujita, Foundations of ultracentrifugation analysis, Chemical Analysis, Vol. 42, John Wiley & Sons, New York, 1973, p. 142.

[4] Hiroshi Fujita, Foundations of ultracentrifugation analysis, Chemical Analysis, Vol. 42, John Wiley & Sons, New York, 1973, p. vii (preface).

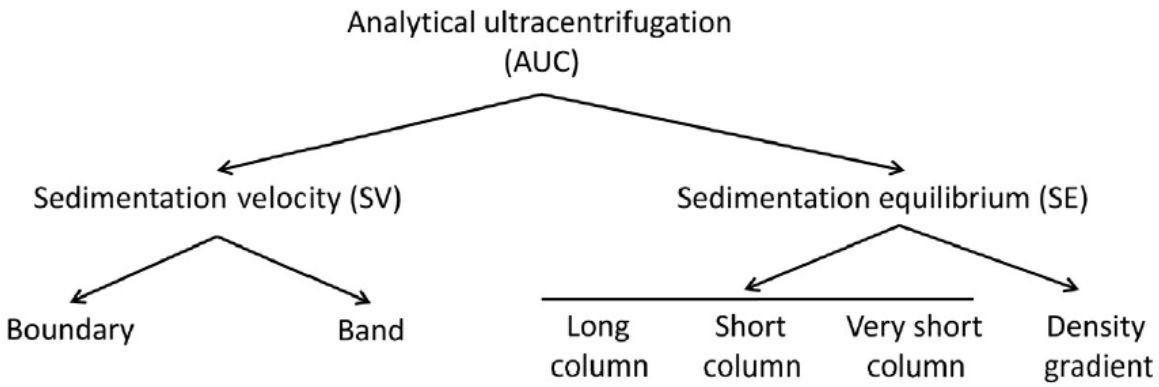
11. However, development of Beckman's 2nd generation automated analytical ultracentrifuge, the ready availability of computational power via inexpensive personal computers, the associated development of powerful computer software, and the renewed interest and need in applying biophysics to understand biological and biochemical processes all converged in the early to mid-1990s to drive AUC's reemergence during the first decade of the twenty-first century.

12. A particularly important attribute of AUC that made it highly valued in the biotechnology area was the ability to place virtually any biological sample in the ultracentrifuge, with little to no sample preparation, to acquire characterization information about its structure, physicochemical properties and assess its purity in its native environment. Avoiding sample processing steps, commonly needed when employing other techniques, greatly reduces the exposure of these delicate biological materials to hostile environments that can lead to altered data.

**B. Basic Principles**

13. Despite the technological and analytical advances that have occurred over the many years, the core physics underlying the AUC have remained constant. Simply put, a sample is loaded into an optically accessible cell in a centrifuge and spun to induce a centrifugal (or gravitational) field and the movement of any solute particles (or molecular species) is monitored as a function of time. AUC

experiments can be broadly classified as either sedimentation equilibrium ("SE-AUC") or sedimentation velocity ("SV-AUC"):




14. The key difference between SV-AUC and SE-AUC experiments is rotor speed. In the case of SV-AUC, the experimenter spins the rotor at a fast enough speed to force suspended particles or dissolved molecular species present in a sample to migrate (due to the net interaction of three forces, *see* ¶19 below) to the bottom of the cell while collecting data on their differing rates of movement (velocities) as they transit the cell. In the case of SE-AUC, the experimenter spins the rotor at a much slower speed, which allows each suspended particle or dissolved molecular species present to establish its own unique static exponential concentration distribution, which is a function of the molecular weight (actually its buoyant molecular weight, *see* ¶19-21 below), after it has reached an equilibrium balance between two opposing forces (diffusion and centrifugal, or gravitational forces).

15. The strength of a centrifugal field generated in any AUC experiment (the centrifugal acceleration) is equal to the product of the angular velocity, ω (expressed in radians/s), squared times the radial distance from the center of rotation, *r* (expressed in cm). As a result, the strength of the centrifugal field in an AUC experiment is not a constant, but rather increases linearly with radial distance:

$$\textbf{Centrifugal field} = \omega^2 r = [(\text{rpm})(2\pi)/60]^2 r \ \textbf{(expressed as cm/s}^2\textbf{)}$$

16. For SV-AUC experiments, this field is usually strong enough that all particle or molecular species of interest will transit the length of the cell over a unique period of time (typically in a few hours) depending to the buoyant mass (*see* ¶¶19-21 below) and size and shape of the particle and the viscosity of the solution through which it moves through. This migration rate as a function of time is of key importance in these experiments and is characterized by an experimental molecular parameter called the sedimentation coefficient, s. The value of s for a given particle, macromolecule or molecule is a unique parameter that is specific to each particular species in a particular solvent run under other specific experimental conditions (e.g., temperature). Experimentally, s is simply defined as the ratio of the velocity, ν, with which a molecular entity moves with respect to the strength of the centrifugal field present:

$$\textbf{Sedimentation coefficient (s)} = \textbf{velocity/centrifugal field} = v/[\omega^2 r] = [dr/dt]/[\omega^2 r]$$

17. In practice, sedimentation coefficients of many important biological molecules range from roughly $10^{-13}$ to $10^{-11}$ sec. Therefore, s-values are usually reported in Svedberg units (using the symbol S), where 1 S $=10^{-13}$ sec. Hence a molecule with a sedimentation coefficient value of 5.3 S actually equals 5.3 x $10^{-13}$ sec.

18. A molecule's velocity, and therefore its s value in an SV-AUC experiment, is determined by the summation of three forces (F), that are influenced by the physical and chemical properties of a particle or molecular entity in a specific formulation buffer in combination with the centrifugal field and other defined experimental conditions, e.g., solvent composition and temperature.

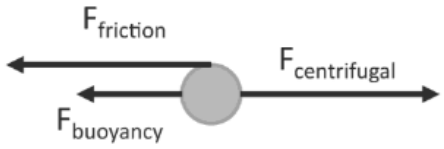
19. These three forces include:

- The centrifugal force ($F_c$) that drives molecules to the base of the AUC cell, which is equal to the product of the centrifugal acceleration ($\omega^2 r$) times the unique mass of the particle or molecular entity. If $M$ is the molar mass of the particle or molecular entity, dividing it by Avogadro's number ($N_a$) yields the mass of one particle or molecule, and hence we get $F_c = (M/N_a)\ \omega^2 r$.
- The buoyant force ($F_b$), which opposes $F_c$, is the force which the solvent molecules put on the solute molecules placed into the solvent solution which is equal to mass of solvent displaced by the

particle or molecular entity when it is placed into the solvent ($M_o$), which depends on its chemical composition, size and shape, times the strength of the centrifugal field, $F_b = -M_0(\omega^2 r)$, where the negative sign indicates that this force is in the opposite direction of $F_c$. Note, $M_0$ is related to the product of the mass of particle or molecular entity times its partial specific volume, $\bar{\upsilon}_2$, times the solvent density, $\rho_0$. As a result $M_0$ is equal to the following: $M_0 = (M/N_a)(\bar{\upsilon}_2 \rho_0)$.

- The frictional force ($F_f$) expressed as $-fv$, also opposes $F_c$ (hence again the negative sign), where $f$ is the frictional coefficient of the particle or molecular entity which uniquely characterizes its hydrodynamic frictional properties (which depends on the particle or molecular entity's size, shape, and chemical composition) as it moves through a solution under a net driving force.

20. The figure below summarizes the interplay between these forces:




21. Upon reaching the desired rotor speed in an SV-AUC experiment, these three forces will very quickly equilibrate ($F_{friction} + F_{bouyancy} = F_{centrifugal}$), and the particle will travel at a unique constant velocity defined by its sedimentation

coefficient. Thus, by substituting the relationship defined in paragraph 19 for each force and rearranging the resulting equation it can be simply shown that the sedimentation coefficient of a particle or molecular entity is directly equal to its buoyant mass divided by its friction coefficient: **s = buoyant mass /frictional coefficient = $M_b$/f.**

### C. The Analytical Ultracentrifuge

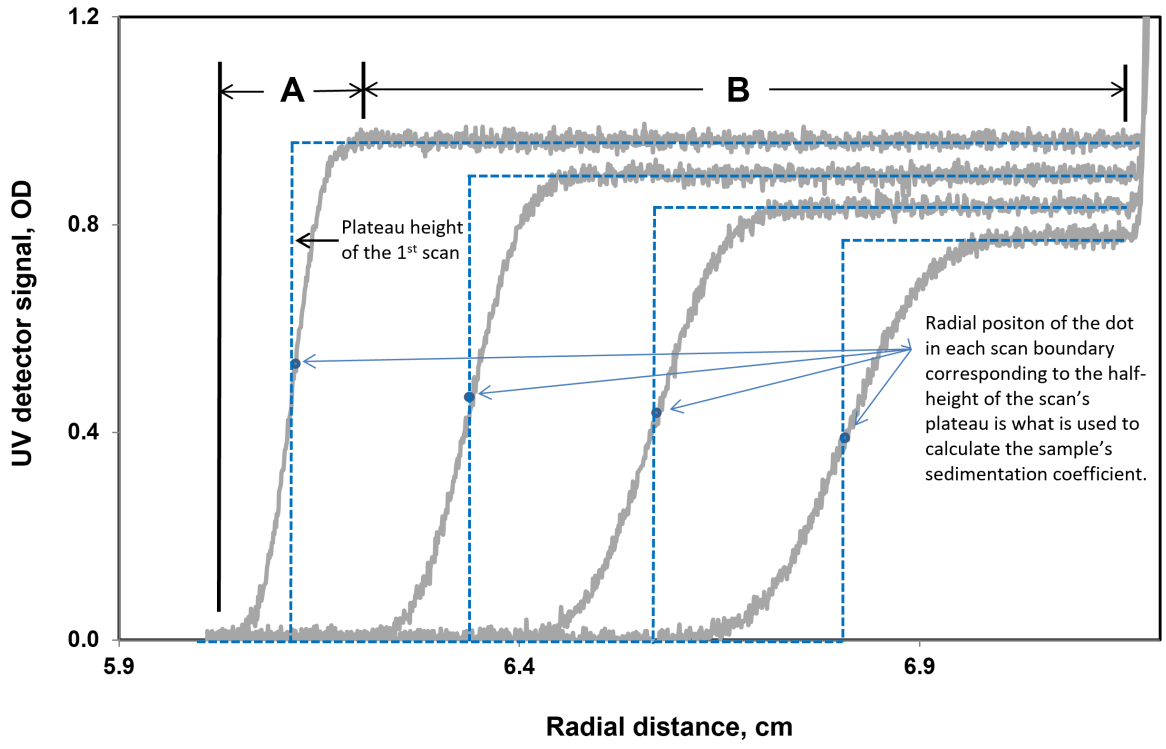
22. Analytical ultracentrifuges differ from other centrifuges that might be found in a laboratory setting by the ability to allow the operator to watch and monitor quantitatively the migration of particles or molecules of interest during the centrifugation process. Depending on the nature of the sample and data sought, this monitoring can be performed using a range of detectors that include UV absorbance, refractometeric (Raleigh interference, or refractometry), or fluorescence detection. At present, Beckman Coulter is the only commercial supplier of AUC instruments on the market. An exemplary instrument is shown below:

23. For a typical AUC experiment, a sample and reference (reference buffer or formulation buffer) solution are loaded into separate chambers or channels within a cell's centerpiece that is sandwiched between two window assemblies. Different centerpieces are used depending on the application. For instance, SV-AUC experiments typically use a cell containing a double sector cell centerpiece as shown below on left. A cell containing a six channel (that is typically not sector shape) centerpiece on the right are typically used for SE-AUC experiments:

24. In typical AUC experiments, to account for any optical properties of the buffer solution, a reference buffer solution is appropriately loaded into one channel while the sample solution is appropriately loaded into the other channel of the centerpiece. The cells are then sealed and precisely aligned into a titanium rotor so that the optical detection systems scan both sample and reference buffer solutions while rotating at speeds of up to 60,000 rpm and under very high vacuum at a defined and controlled temperature.

### D. Boundary Sedimentation Velocity Analytical Ultracentrifugation provides data on the size, shape, and density of macromolecules

25. For the past nearly twenty years in the biopharmaceutical industry, especially in the process development area, by far the most common form of AUC conducted is boundary SV-AUC. The primary reasons for this are the high information content of this mode, as well as the typically much shorter time required to conduct these experiments. In conducting a boundary SV-AUC experiment on a homogeneous biopharmaceutical sample the resulting data output consists of a series of data scans, which typically are separated by a constant time interval over the course of the SV-AUC run.




26. Generally, in the early days of AUC (i.e. in the 1980s and before), scientists would simply monitor the change in the radial position of the boundary (e.g., area "A" shown in the above Figure for the 1st scan) of each data scan, which was simply evaluated by locating the radial position in the boundary area that corresponded to the scan's half-height of its associated plateau (e.g., area "B", in

the above Figure for the 1st scan) shown as a dot in the above Figure, with time.[5] Using this data and plotting the logarithm of the radial boundary position with time allows one to determine the particle or molecular entity's sedimentation coefficient from the resulting slope of the best line through these data points. However, given the computational bandwidth and developed software available in the past twenty years (e.g., SEDFIT) much greater detailed analysis of the recorded data scans can now be carried out to extract the rich source of information present in the moving boundary of these collected data scans.

### E. Demonstration of the SEDFIT Software

27. SEDFIT is a versatile software tool for the analysis of SV-AUC data that was first released and described by Peter Schuck in 2000. I have included screenshots below that I captured for purposes of this declaration to demonstrate the use and capabilities of the SEDFIT software. The specific version of SEDFIT used to generate these screenshots was Version 14.4d, which was publicly available as of October 2013, and which I understand is prior to the filing date of

[5] c(r.t) is the detector's signal readouts (e.g., UV absorbance readings), of all the macromolecular species present in a sample in an AUC cell as a function of radial distance from the center of rotation, r, for a given time, t, since the rotor started rotating.

the ’288 patent.[6] A POSA would be well aware of the existence of the SEDFIT software and similar tools used for analyzing SV-AUC data.

28. In a typical SV-AUC experiment using SEDFIT, data is obtained on an analytical ultracentrifuge on one or more samples, and the resulting scan data is exported to the SEDFIT software. For a c(s) analysis as described in the Le Bec reference discussed below, the user will specify certain parameters depending on what is already known about the sample, and SEDFIT will attempt to find the best fit a c(s) distribution that would fit (or model) the experimental data. Once a

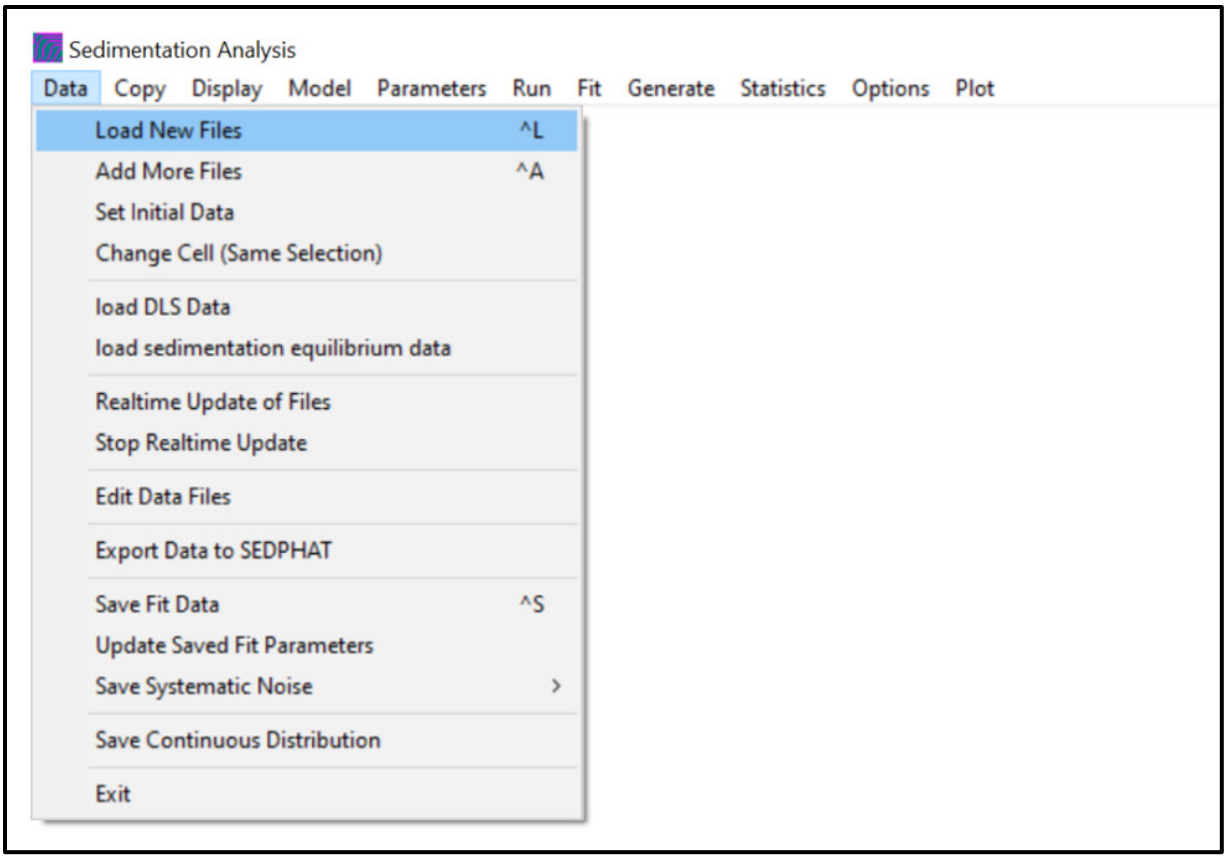
[6] This particular version—as well as others—of the SEDFIT software is available at https://spsrch.cit.nih.gov/software/default.aspx.

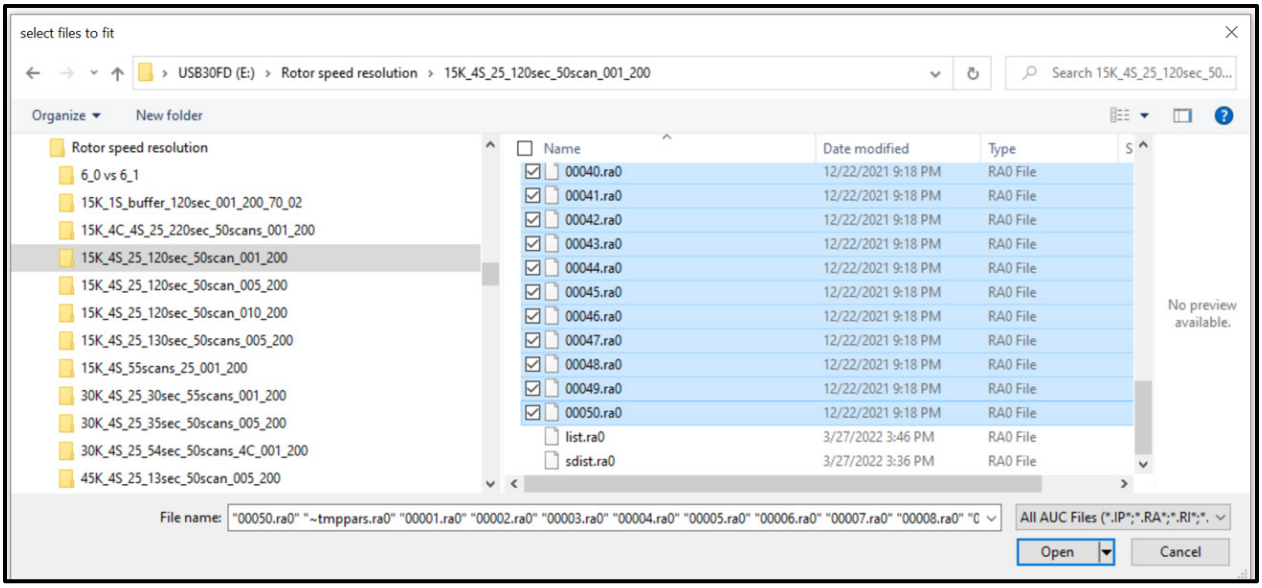
suitably parsimonious distribution is achieved, the user can then choose to integrate certain peaks to calculate their relative amounts in the sample.

29. When the software is first opened, the screen is blank with key commands at the top left:

**a. Loading a dataset**

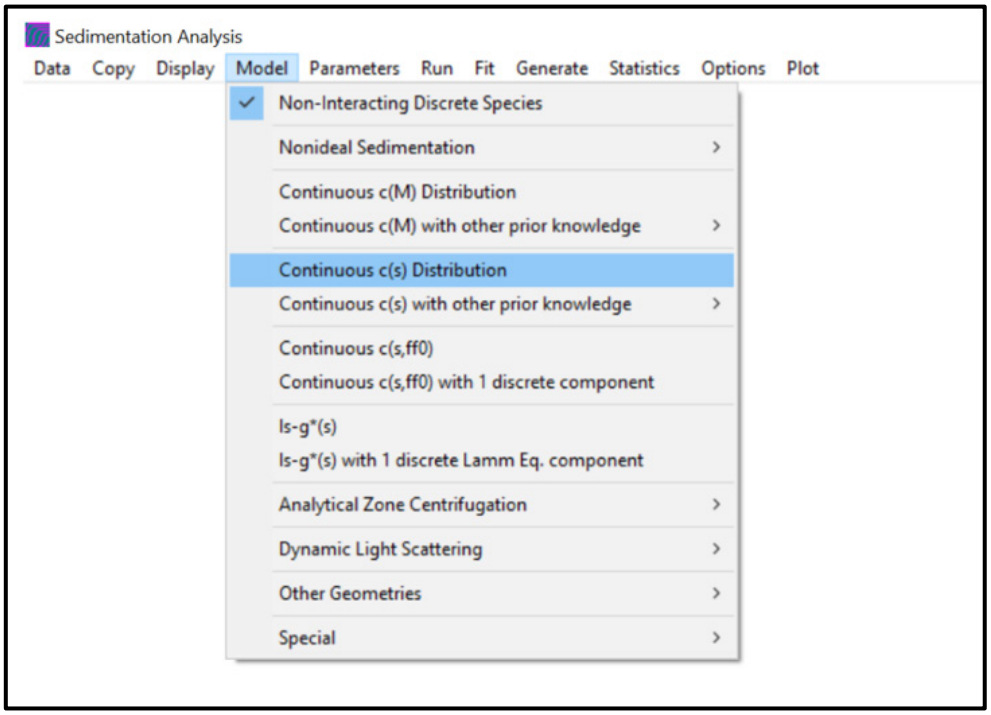
An SV-AUC dataset consists of several (on the order of a few to a few hundred) individual scan files taken at regular time intervals during the ultracentrifugation. The first step is to select the files to be loaded into the program.


Sedimentation Analysis
Data
Copy
Display
Model
Parameters
Run
Fit
Generate
Statistics
Options
Plot
Load New Files ^L
Add More Files ^A
Set Initial Data
Change Cell (Same Selection)
load DLS Data
load sedimentation equilibrium data
Realtime Update of Files
Stop Realtime Update
Edit Data Files
Export Data to SEDPHAT
Save Fit Data ^S
Update Saved Fit Parameters
Save Systematic Noise
Save Continuous Distribution
Exit


select files to fit
USB30FD (E:) › Rotor speed resolution › 15K_4S_25_120sec_50scan_001_200
Organize
New folder
Rotor speed resolution
6_0 vs 6_1
15K_1S_buffer_120sec_001_200_70_02
15K_4C_4S_25_220sec_50scans_001_200
15K_4S_25_120sec_50scan_001_200
15K_4S_25_120sec_50scan_005_200
15K_4S_25_120sec_50scan_010_200
15K_4S_25_130sec_50scans_005_200
15K_4S_55scans_25_001_200
30K_4S_25_30sec_55scans_001_200
30K_4S_25_35sec_50scans_005_200
30K_4S_25_54sec_50scans_4C_001_200
45K_4S_25_13sec_50scan_005_200
Name
Date modified
Type
00040.ra0 12/22/2021 9:18 PM RA0 File
00041.ra0 12/22/2021 9:18 PM RA0 File
00042.ra0 12/22/2021 9:18 PM RA0 File
00043.ra0 12/22/2021 9:18 PM RA0 File
00044.ra0 12/22/2021 9:18 PM RA0 File
00045.ra0 12/22/2021 9:18 PM RA0 File
00046.ra0 12/22/2021 9:18 PM RA0 File
00047.ra0 12/22/2021 9:18 PM RA0 File
00048.ra0 12/22/2021 9:18 PM RA0 File
00049.ra0 12/22/2021 9:18 PM RA0 File
00050.ra0 12/22/2021 9:18 PM RA0 File
list.ra0 3/27/2022 3:46 PM RA0 File
sdist.ra0 3/27/2022 3:36 PM RA0 File
No preview available.
File name: "00050.ra0" "~tmppars.ra0" "00001.ra0" "00002.ra0" "00003.ra0" "00004.ra0" "00005.ra0" "00006.ra0" "00007.ra0" "00008.ra0"
All AUC Files (*.IP*;*.RA*;*.RI*;*.
Open
Cancel

### b. Selecting a model

30. Once the data is loaded, the user selects a model under the 'Model' drop down menu. As demonstrated below, a "continuous c(s) distribution" is one of a few readily available options in the SEDFIT software:

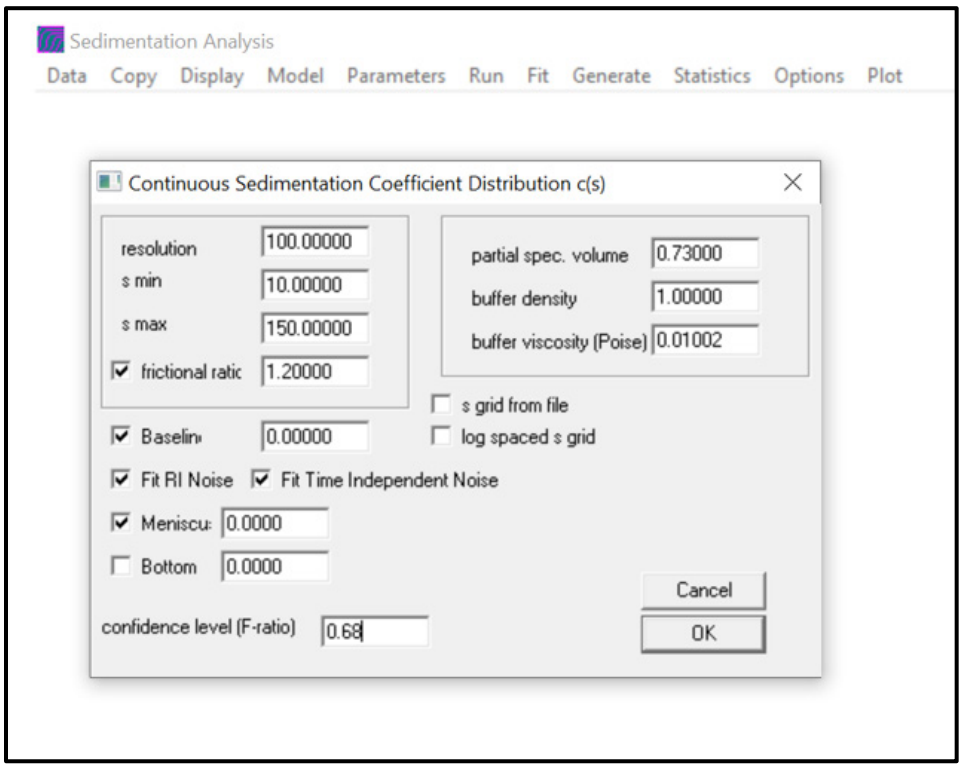



31. C(s) is the differential sedimentation coefficient distribution, which is to say it is a function that quantitates the amount of particles that are present at a certain s value. When c(s) is plotted versus S the resulting plot yields the sedimentation coefficient distribution or c(s) distribution that characterizes the sample. As I explained in my 2007 article, "[t]his c(s) distribution is similar to a chromatogram: each species produces a peak at its characteristic sedimentation coefficient and the area under each peak gives the concentration of that species (in

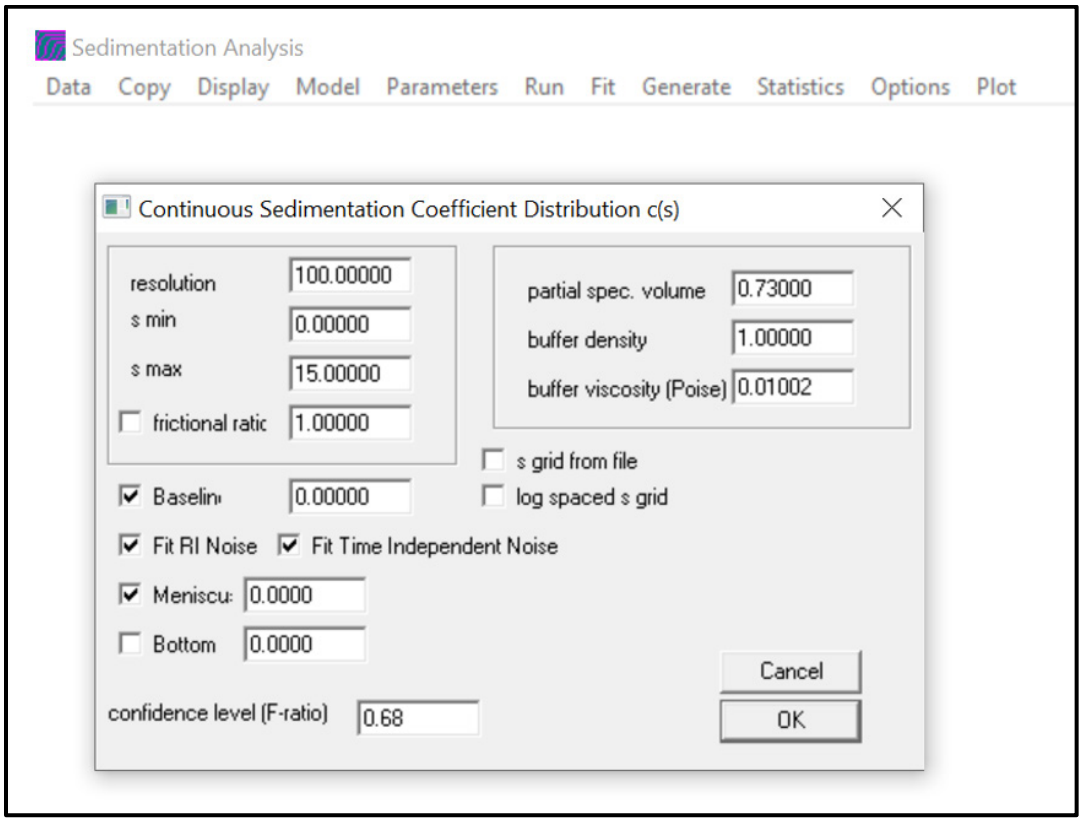
signal units of OD or fringes)." Ex.1004, 25. Furthermore, Le Bec's disclosure of its c(s) v. S plots indicates that it analyzed its rAAV data using this model. Ex.1003, Figs. 2 and 3.

### c. Set analysis parameters

32. The user then has the option to specify certain parameters prior to analyzing the data. For instance, one can define the resolution, the minimum and maximum S-values that will be considered when the data is being analyzed, the confidence level of the regularization used, and the $f/f_0$ ratio used to assess frictional effects. Here, although it is initially set to 1.2, I have checked the toggle box next to it, which directed SEDFIT to allow the value to float, i.e., be adjusted by the software during the data analyze to provide the best fit to the data:

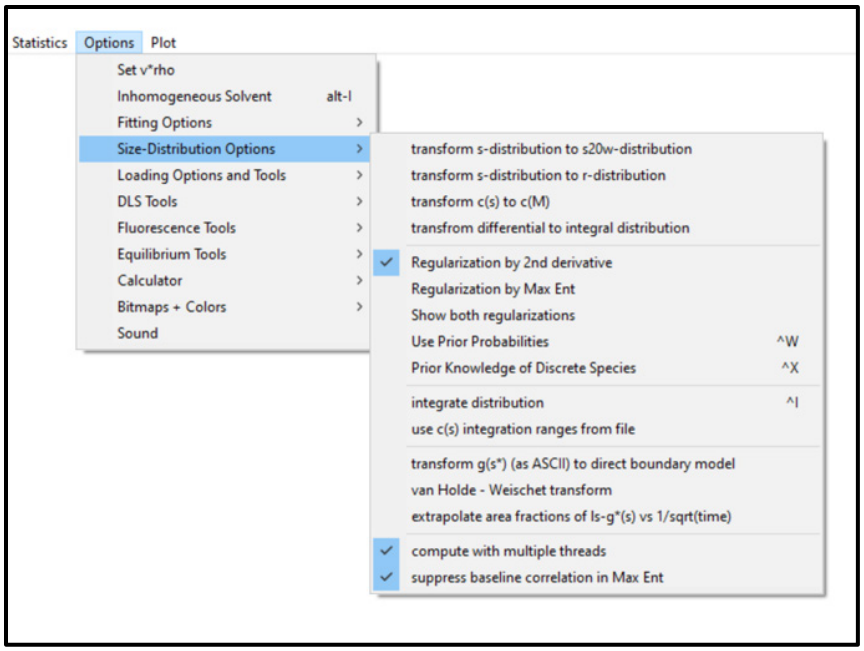


33. Alternatively, by unchecking the box and picking a specific value (set to 1.0 in the below picture), the frictional ratio will just use the set value:



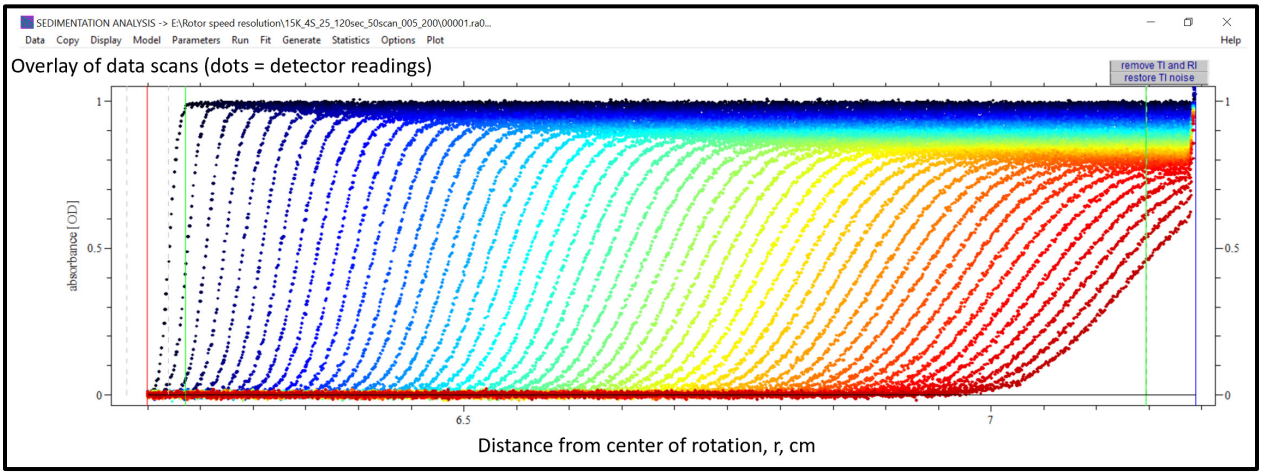

### d. Specify the type of regularization

34. The user may also select a regularization method for analyzing the data. Below, I have selected the default "Regularization by 2nd derivative," but regularization by Maximum Entropy ("Max Ent") or both methods is also an option:



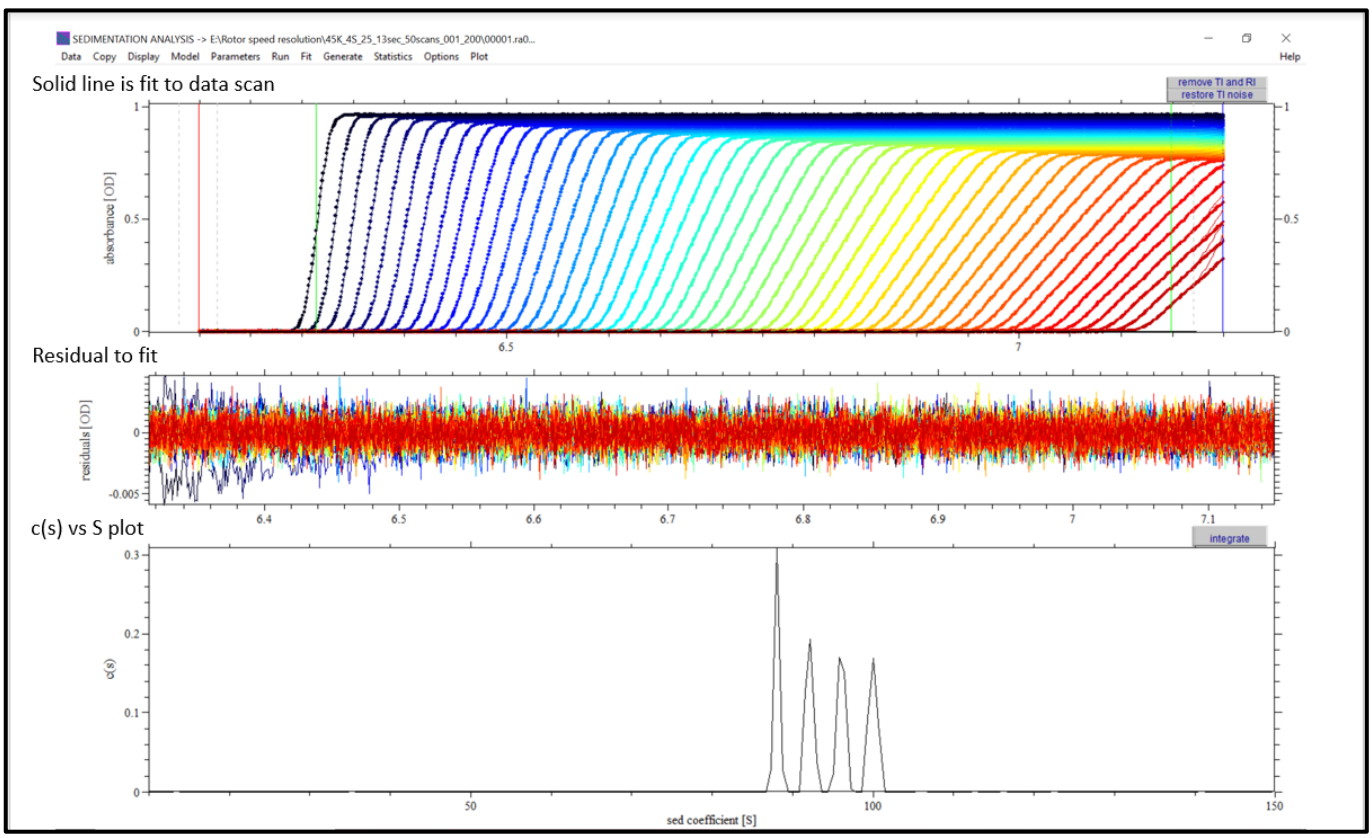

### e. Run a sedimentation analysis

35. When the data file scans are loaded, the user sets the initial meniscus position (red line, which is typically allowed to float during data analysis), the data limits (green lines), and the base position (blue line):



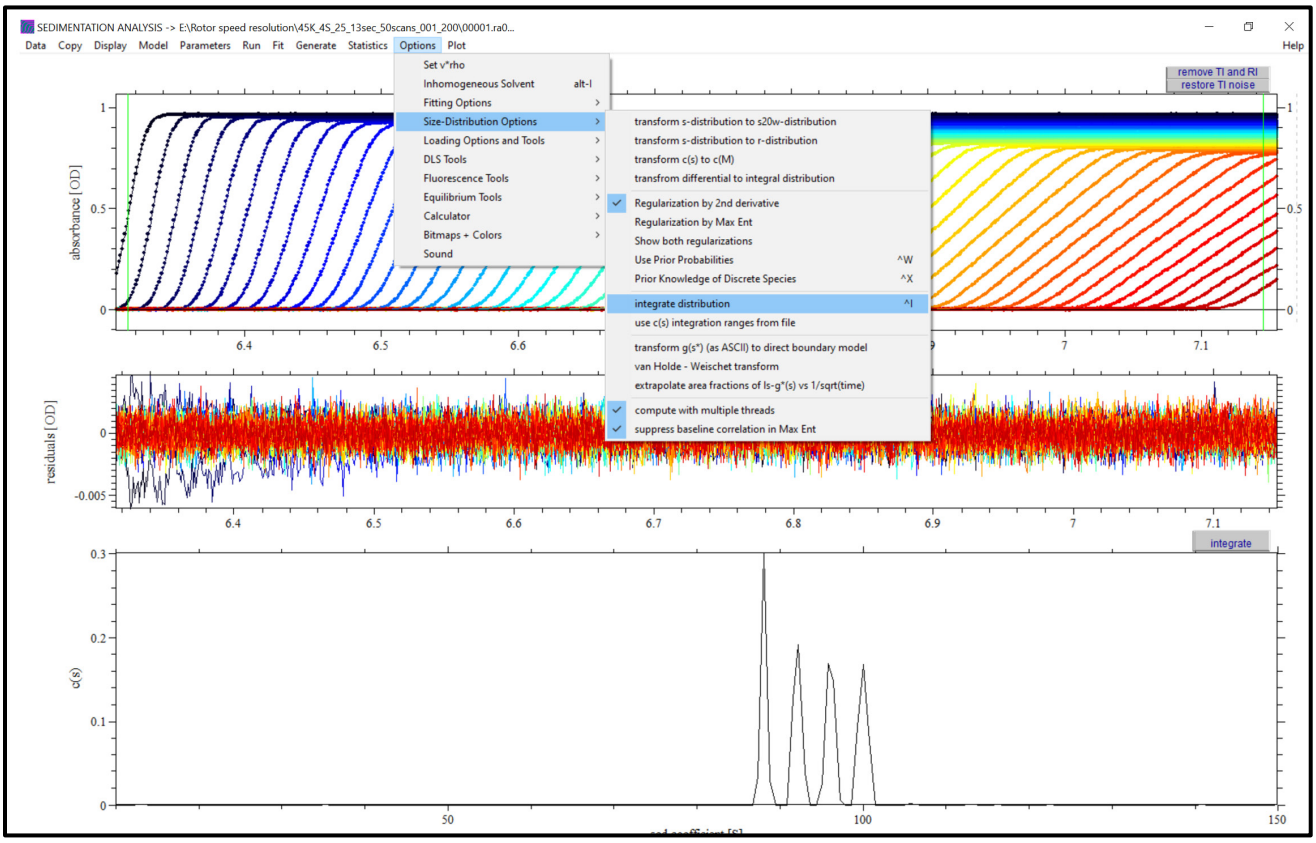

36. The 'Fit' function on the drop-down menu simulates (searches out) the best distribution of S-values, c(s), that matches the experimental recorded

sedimentation run (date scans) based on the provided model and parameters. When the analysis is complete SEDFIT provides an output screen showing the raw scan data (indicated as a series of dots, detector readings at radial position r for time t, for each scan) and the corresponding resulting fit model to each scan (indicated by a continuous line tracing for each scan) in the top part of the screen, as shown in the figure below. A c(s) v. S plot (or c(s) distribution) is displayed at the bottom of the screen while the middle plot shows the overlay of deviations (residuals) of the model's fit to the experimental data for each data scan:

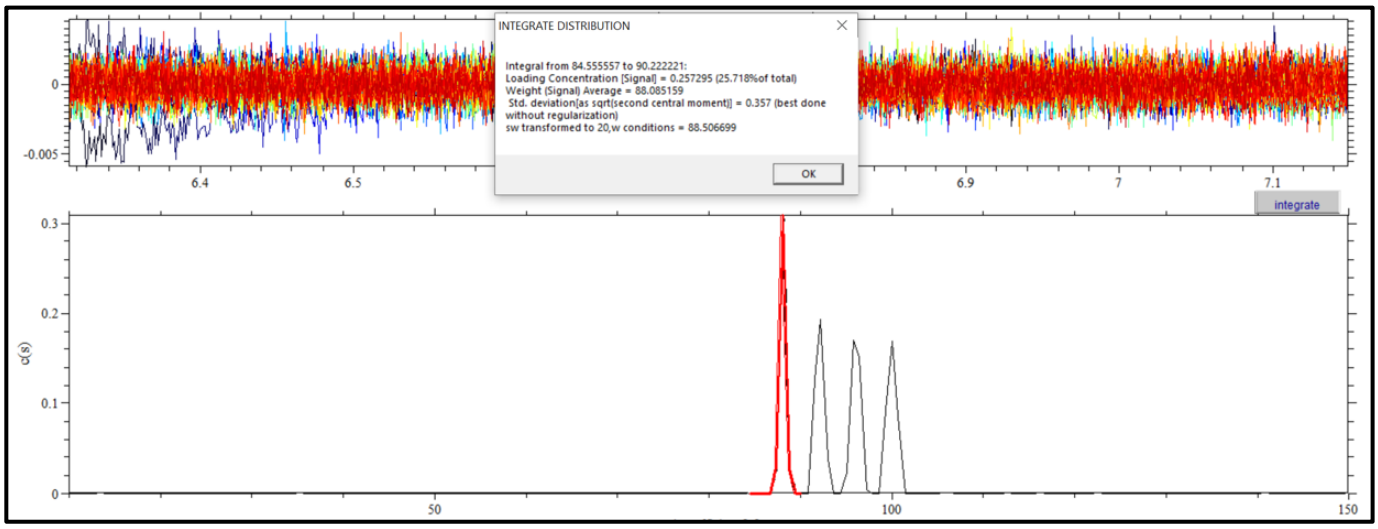


### f. Peak integration

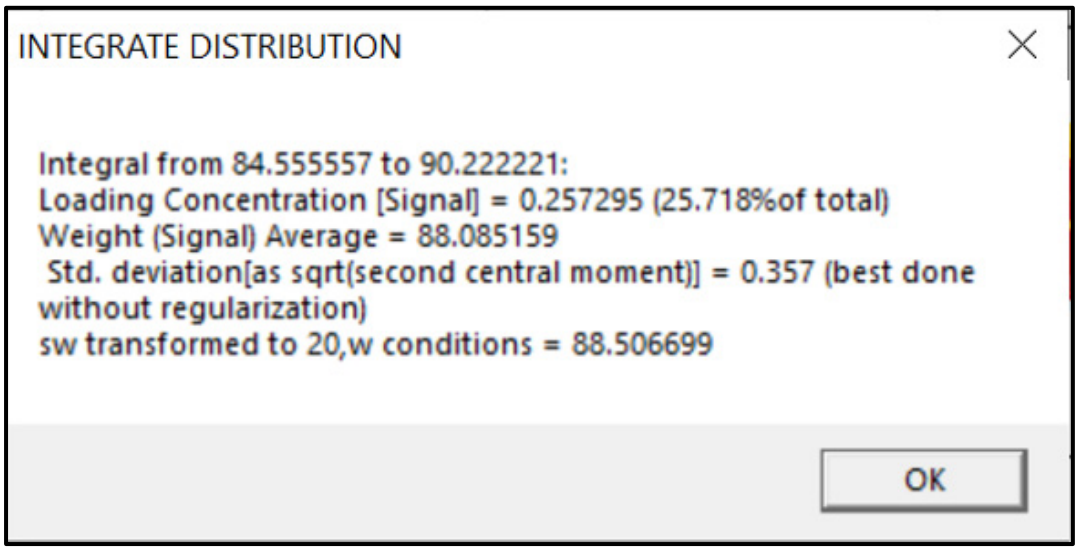
37. The user now has the option to quantify the relative area under the curve of each peak by integration. This can be done by selecting the "integrate distribution" option from the "Size Distribution Options" sub-menu:




38. The user employs their mouse to define a range of interest, and SEDFIT then calculates the area under the curve for the selected peak or range, the percent fraction of this peak area relative to the total area of the plot and the average (weight-average) sedimentation coefficient of this peak. For illustration purpose, I have integrated the peak spanning approximately 84-90S.




39. In this example, SEDFIT reports the area under the curve of that particular peak is shown to be ~25.718% of the total area under the curve for all species:




40. Thus, as demonstrated above, the SEDFIT software allows the determination of relative concentrations of the species in the sample at each peak.

### F. Viral vectors in gene therapy

#### 1. Viral vectors, including rAAVs, were known to be promising avenues for gene therapy modalities

41. By 2015, it had already been known for some time that recombinant viruses show great promise and utility as a vehicle to deliver therapeutic nucleic acids for gene therapy applications. Ex.1001, 1:26-28; *see also* Ex.1004, 16. Multiple viral platforms had been developed, which included "adeno-associated virus (AAV), adenovirus, lentivirus, and herpes simplex virus (HSV)." Ex.1001, 1:34-27.

#### 2. Like all pharmaceutical products, gene therapy compositions must be monitored for homogeneity, purity and consistency of manufacturing along with other quality attributes to gain and maintain marketing approval

42. As the '288 patent discloses, "[t]he generation of recombinant viral vectors for the clinic requires an analytical method that monitors drug product, quality with regard to homogeneity, purity and consistency of manufacturing…" Ex.1001, 1:38-41. This is not a requirement unique to gene therapy, but rather one common to any marketed pharmaceutical. As I explained in my 2007 article, "[t]o achieve drug licensure, drug product quality (with regard to homogeneity and purity) and consistency of manufacturing are of key importance." Ex.1004, 16. Similarly, Gao recognized that "removal of empty particles from rAAV preparation may improve efficacy and safety of AAV in clinical applications,"

(Ex.1008, 1) and that "our results emphasize the necessity to develop and optimize good manufacturing practice-compatible manufacturing process for efficient removal of empty virions from rAAV production/purification process and enhancing therapeutic potency and safety profile of clinical gene therapy vectors." (Ex.1008, 6).

43. However, what is unique to gene therapeutic products is the need to develop purification procedures and analytical methods rigorous enough to produce and validate the purity of these complex and very large gene drug products, to establish that they were being consistently made and were being delivered to the patient in known and in controlled (dosage) amounts. Thus, a POSA would have known of the need to minimize these impurities, as well as the need to prove their absence. Given the unique large size of viral gene therapeutic drugs products, many commonly used analytical methods were unable to provide adequate capability to effectively monitor them.

44. As noted in the prior art, the unique attributes of SV-AUC were known to provide significant capability to overcome this problem long before the '288 patent priority date. *See* Ex.1008, 6 ("[A]nalytical ultracentrifugation technology is a powerful tool for quantitative characterization of structural heterogeneity of rAAV preparations, allowing precise and selective observation of viral capsid sedimentation in real time."); Ex.1005, 16 ("We demonstrate that a

single sedimentation velocity run on an adenovirus sample can detect and accurately quantify a number of different forms of virus particles and subvirus particles" including "a) intact virus monomer particles, (b) virus aggregates, (c) empty capsids (ECs), and (d) smaller assembly intermediates."). Thus, SV-AUC was recognized as a suitable technique for analyzing purity and homogeneity of viral gene therapeutic products.

## IV. MATERIALS CONSIDERED

45. Exhibit A provides a list of the materials I considered in preparing this declaration. I also relied on my experience, education, and knowledge of the art.

## V. THE '288 PATENT

### A. The Disclosure of the '288 Patent

46. The '288 patent is titled "Analytical ultracentrifugation for characterization of recombinant viral particles" and relates to methods of analyzing recombinant viral preparations using analytical ultracentrifugation. Generally, the '288 patent discloses methods of analyzing viral preparations which involve a) subjecting the viral preparations to SV-AUC conditions and monitoring them, b) plotting the resulting data as c(s) v. S plots, c) integrating the area under specific S-values and/or d) making inferences about the preparation and the relative or absolute amounts of different species therein based on the data. Ex.1001, 1:59-2:3.

47. The ’288 patent admits that “[t]he techniques and procedures described or referenced herein are generally well understood and commonly employed using conventional methodology by those skilled in the art.” Ex.1001, 12:62-65; *see also* Ex.1001 at 28:62-64 (“Suitable ultracentrifugation conditions, analysis algorithms, and other parameters may be determined empirically through methods known in the art.”). I agree that optimizing SV-AUC conditions for viruses, including rAAVs, would have been well known to a skilled practitioner as of the January 2015 filing date.

48. The ’288 patent also references other disclosures, including one I co-authored, teaching the use of AUC to evaluate viral preparations for gene therapy. Ex.1001, 19:4-6 (“Use of analytical ultracentrifugation to characterize adenovirus preparations is provided by Berkowitz, S A & Philo J S, (2007) Anal. Biochem., 362:16-37.”). I also note that the ’288 patent specification relies on Cole (2008): “Several methods for optically detecting a sedimenting boundary and measuring its rate of movement or migration are known in the art (for reference, see Cole et al. (2008) Methods Cell Biol., 84:143-79).” Ex.1001 at 19:61-64. I further agree with the ’288 patent that Cole teaches that multiple well-known methods of optically detecting a sedimenting boundary were known in the art as of the filing date.

49. The experimental data in the ’288 patent is consistent with these admissions. In Example 1, the ’288 patent discloses the performance of an SV-

AUC assay on AAV preparation using a Proteome Lab™ XL-1 (Beckman Coulter) analytical ultracentrifuge, at "20,000 RPM, 20C, 0.003 radial step setting with no delay and no replicates" and acquiring data using either optical absorbance or Rayleigh interference. Ex.1001, 43:35-51. In my opinion, these are standard SV-AUC conditions using widely available instrumentation. It is also my opinion that a POSA would have expected such conditions to have been successful when applied to rAAV preparations as of January 20, 2015.

### B. The Claims of the '288 Patent

50. There are eight independent claims in the '288 patent (claims 1-2, 4-8, and 12) all of which claim methods that involve using SV-AUC to characterize the presence, absence, or relative amounts of different AAV particle species in a sample. Independent claim 1 recites:

> A method of characterizing a preparation of recombinant adeno-associated viral (AAV) particles comprising the steps of
>
> c) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant AAV particles is monitored at time intervals, (the "subjecting limitation")
>
> b) plotting the differential sedimentation coefficient distribution value (c(s)) versus the sedimentation coefficient in Svedberg units (S), (the "plotting limitation" and
>
> c) integrating the area under each peak in the c(s) distribution to determine the relative concentration of each peak, wherein each peak represents a species of recombinant AAV particle (the "integrating limitation").

51. Two of the steps recited in claim 1—the "subjecting" and "plotting" limitations recited in steps a and b—are present in all claims of the '288 patent. The third limitation—the "integrating" limitation—also appears in many, but not all, of the claims.

### C. The Prosecution History

52. I understand that when seeking the claims in the '288 patent, the applicant represented that:

> one of skill in the art would not have predicted with a reasonable expectation of success that methods described for a virus such as adenovirus could be applied to recombinant AAV particles. Adenoviruses and AAV are distinctly different viruses in a number of ways, but they especially differ in terms of size as adenoviruses are about 500-1000% larger than AAV in terms of both genome size and actual particle size. . . . One of skill in the art would not have assumed that the use of AUC would have allowed for the characterization of variant recombinant viral genomes or empty viral capsid particles in a preparation of recombinant AAV particles, much less with the sensitivity, precision, and accuracy discussed below.

Ex.1002, 541. I do not agree. As explained above, before the priority date of the '288 patent, the prior art had already recognized that AUC "a powerful tool for quantitative characterization of structural heterogeneity of rAAV preparations, allowing precise and selective observation of viral capsid sedimentation in real time." Ex.1008, 6. AUC was also known to be applicable to a wide range of

molecular weights, both larger and smaller than rAAVs. Ex.1005, 145 ("The range of molecular weights suitable for AUC exceeds that of any other solution technique from a few hundred Daltons (e.g., peptides, dyes, oligosaccharides) to several hundred-million Daltons (e.g., viruses, organelles)."). A POSA would have understood that AUC would allow characterization of empty rAAV particles with sensitivity, precision, and accuracy. In fact, Le Bec—which was published before the '288 patent's priority date—proved that to be the case. Ex.1003.

53. Further, that adenovirus is much larger than AAV particles would actually lead a POSA to more confidently predict AUC would work with rAAV preparations. The relatively larger size of adenovirus requires that the ultracentrifuge be operated at much lower rotor speeds than is needed for rAAVs, pushing the lower limits of the capability of the ultracentrifuge. So while a POSA may or may not have been able to predict the applicability of AUC to particles larger than adenovirus, a POSA would have predicted AUC to work on particles smaller than adenovirus, like rAAVs.

54. Further, I understand that the patent applicant argued that the application was based on "the inventors' surprising finding that AUC could be applied to preparations of recombinant AAV particles to identify variant recombinant viral genomes or empty viral capsid particles impurities with incredible sensitivity, precision, and accuracy." As explained above, a POSA

would not find the application of AUC of rAAV particles surprising, especially in view of the prior art.

55. Finally, I understand that the patent examiner relied on the applicant's statements when deciding to allow the claims in the '288 patent. Specifically, the examiner stated:

> Analytical ultracentrifugation (AUC) was well-described in the art as a means to separate biological molecules, including viruses. While it would be obvious to apply AUC to separation of AAV particles and routine to optimize said parameters, AUC was not noted as having been applied to AAV separation routinely, and the general methods of separating AAV in the art were not very accurate or specific, especially when it came to variation within the AAV genome or the AAV subtype, and separation of empty AAV particles, which were known to cause unwanted effects when AAV preparations were used *in vivo*.

Ex.1002, 561. I agree that AUC was well-described in the art for separating biological molecules, including viruses. I also agree that it would be obvious to apply AUC parameters to the separation of AAV particles and routine to optimize the parameters for doing so. This is confirmed by many publications, including those cited in this declaration. While I agree that with the development of gene therapy drug products there was a need for an accurate characterization method

with regard to the separation and quantification of empty AVV particles, the use of AUC was already recognized and described as such a method in the art.

56. The patent examiner continued:

> Applicants have surprisingly found parameters for AUC that allow for high levels of separation of AAV particles, including accurate separation of AAV subtypes and empty particles. The closest prior art is that of record, which teaches general methods of AUC, but the argument that the results were surprising and/or unexpected (see p. 4 of "Remarks" starting at ¶2) was determined to be convincing after a thorough search of the art.

Ex.1002, 561. As explained above, I disagree that what the applicants claimed as their invention was surprising. The applicability of SV-AUC to AAV particles in Le Bec is unsurprising. Indeed, the inventors in Le Bec did not even suggest the approach was in any way novel and they did not seek to claim invention. Rather, SV-AUC is an established method that has long been used for the very purpose for which it has been applied in the '288 patent to separate both particles larger than AAVs (e.g., adenovirus), smaller than AAVs (peptides and oligosaccharides), and to search for the presence of other intermediates of the complete assembled capsid protein shell that were very similar in size to the empty and full AAV particles. The Examiner appears to have not been aware of or considered the Le Bec reference in deciding to allow the '288 patent claims.

## VI. LEVEL OF ORDINARY SKILL IN THE ART

57. From counsel, I understand that patentability must be analyzed from the perspective of a "person of ordinary skill in the art" ("POSA") in the same field of the challenged patent as of the "effective filing date" of the claims. I understand the effective filing date of the '288 patent to be January 20, 2015. I further understand that a POSA is a hypothetical individual presumed to know the relevant art as of the effective filing date and who has the same level of skill as the ordinary practitioner of the art at issue.

58. In my opinion, a POSA working in the field of the '288 patent on January 20, 2015, would have possessed at least a B.S. in biology, chemistry, chemical engineering, biochemistry, biophysics, pharmaceutical science, or a related discipline, with more than two years of industry, laboratory, and/or clinical experience in analyzing or characterizing biomolecules, including viruses or viral vectors. Such a person may be familiar with, or consult with someone familiar with, the development, formulation, and/or administration of viral vectors for gene therapy and quality standards required to market such products.

59. For this declaration, I have applied this definition of a POSA, and my opinions are offered from the perspective of a POSA as that hypothetical person would have understood matters on January 20, 2015.

## VII. ANTICIPATION ANALYSIS

### A. Legal Standard

60. I have been informed by counsel and understand that a prior art reference "anticipates" an asserted claim if it places the invention in the possession of the public. That is, I understand the question is whether one skilled in the art would reasonably understand or infer from the prior art reference's teaching that every claim element was disclosed in that single reference. I understand that the reference does not need to disclose the elements in the exact same terms as used in the patent.

61. I also understand from counsel that a reference can anticipate a claim even if it does not expressly spell out all the limitations arranged or combined as in the claim, if a person of skill in the art, reading the reference, would at once envisage the claimed arrangement or combination.

62. I further understand that even if a prior-art reference does not expressly disclose every limitation of a claim, the reference may still anticipate the element if the missing descriptive element is "inherent" in the disclosure. A claim element is inherent if the missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill.

63. Finally, I understand that anticipation does not require actual performance of the suggestions in the disclosure, only that a person having ordinary skill in the art could practice the invention without undue experimentation.

### B. PCT Publication of International Application Number PCT/EP2014/052978, entitled "Methods for the Production of Double-Stranded AAV Viral Particles" ("Le Bec")

64. The reference referred to as "Le Bec" is a publication of International Application Number PCT/EP2014/052978, entitled "Methods for the Production of Double-Stranded AAV Viral Particles." Ex.1003, (21). I understand that Le Bec was published on August 21, 2014 that it qualifies as prior art to the '288 patent.

65. Le Bec discloses methods to produce double stranded/"self-complementary" adeno-associated virus particles ("scAAV") compositions for gene therapy applications using two different methods, one involving insect derived sf9 cells, and another employing a human embryonic kidney cell line called HEK293. These scAAV particles are recombinant AAV particles like those claimed in the '288 patent. Le Bec reports characterization data for its AAV preparations, including "[a]nalysis of empty and full AAV viral particles" using analytical ultracentrifugation.

66. Le Bec is from the same field of endeavor as the '288 patent (methods for characterizing recombinant viral vectors using analytical

ultracentrifugation) and is reasonably pertinent to the problem the ’288 patent sought to solve (the need for a generic assay to characterize recombinant viral preparations). The ’288 patent describes its field of invention as relating to methods to characterize recombinant viral vectors, including rAAV particles using analytical ultracentrifugation. Ex.1001, 1:17-22.

67. Le Bec explained that “[t]he sedimentation coefficient of the various AAV viral particles (empty, full, aggregate) and other present populations (subparticles, contaminant proteins, aggregate) in the purified products was determined by real-time centrifugation.” Ex.1003, 15:19-23. Further, “[c]entrifugation of the samples was carried out at a speed of 16,000 rpm using 100 µl or 400 µl of undiluted pure vectors, sedimentation was followed by absorbance at the wavelength of 276 nm, and the sedimentation coefficient of the various populations was obtained using the software SEDFIT.” Ex.1003, 15:23-28. Finally, Le Bec noted that the densities of empty and full AAV viral particles had been reported in the literature, and that “[k]nowing that the difference in density is sufficiently significant to distinguish them by centrifugation, we have implemented a method today for analytically separating by ultracentrifugation and quantifying the different species present in an AAV viral preparation.” Ex.1003, 18:1-6.

68. Figures 2 and 3 of Le Bec depict c(s) v. S plots:

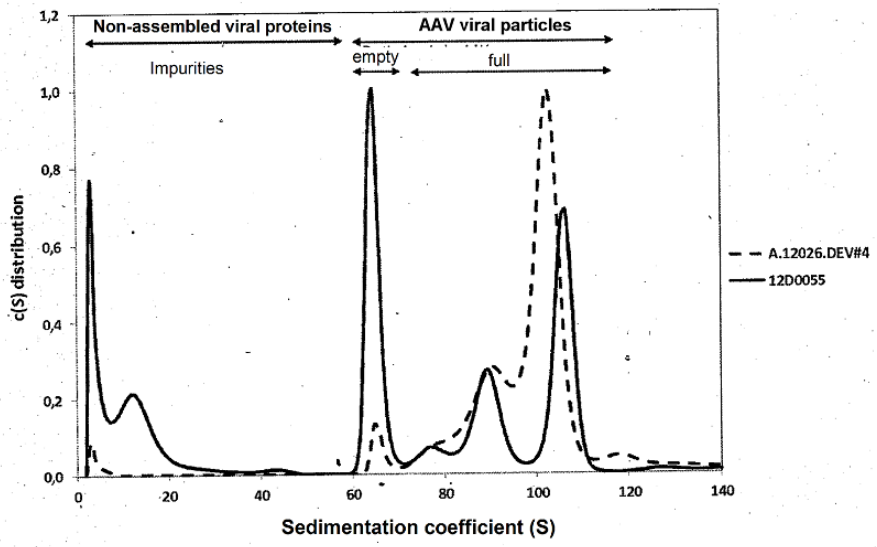



Figure 2

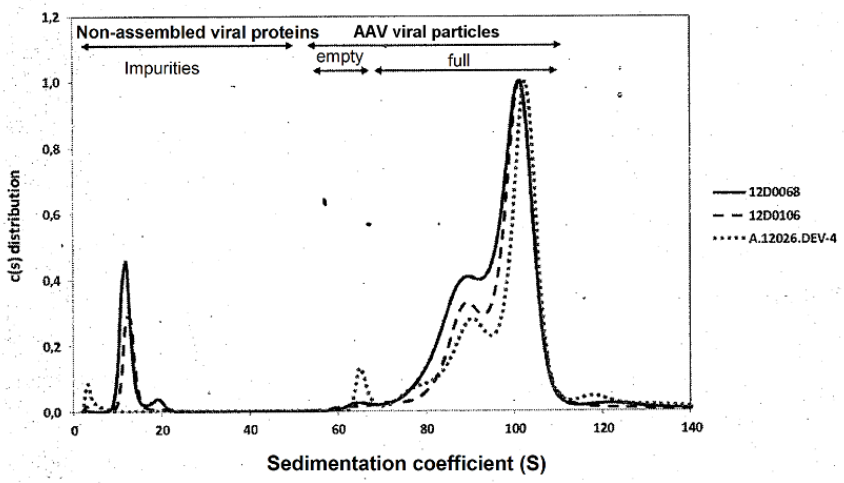



Figure 3

69. Figure 2 compares the AAV particles produced in the sf9 system (dashed line) with those prepared in the HEK293 system (solid line). Le Bec explained that the distribution profile showed the different populations: (1) < 60S represents non-assembled viral proteins or contaminants, (2) empty AAV particles are shown by the peak at around 65S, and (3) a peak at 90S corresponds to

particles with the viral genome in single-stranded form, and (4) a peak at 105S corresponds to particles with the viral genome in double-stranded form. Ex.1003, 18:14-25.

70. Further, Le Bec indicates the results in Figure 2 showed that the viral preparation made with the HEK293 system cells has less than 25% full particles (only 10% of which containing the double stranded genome). Ex.1003, 19:1-5. Empty AAV particles are a substantial portion of this composition, as shown by the large peak at 65S in Figure 2.

71. The data show that viral particles made with the sf9 system contained very few empty particles and were enriched in particles containing the double stranded genome relative to those produced using HEK293. Ex.1003, 19:7-15. As shown in Figure 3 (a c(s) v. S plot of three lots of sf9-produced AAV particles), the AUC analysis showed consistent distribution profiles between lots, with few empty viral particles.

### C. Claim 1 anticipated by Le Bec

#### 1. "A method of characterizing a preparation of recombinant adeno-associated viral (AAV) particles comprising the steps of…"

72. Le Bec teaches a method for characterizing a viral preparation of rAAV particles that can analytically separate, detect, and quantify the presence of empty capsids, full capsids, and aggregates based on sedimentation coefficients.

Ex.1003, 16:25-29 ("We thus obtained scAAV9-SMN viral preparations whose titer in viral genome, the purity, the identity and the quantification of empty and full viral particles were determined."); *see also id.*, 15:17-23 ("The sedimentation coefficient of the various AAV viral particles (empty, full, aggregate) and other present populations (subparticles, contaminant proteins, aggregate) in the purified products was determined by real-time centrifugation."); 18:1-6 ("Knowing that the difference in density is sufficiently significant to distinguish them by centrifugation, we have implemented a method today for analytically separating by ultracentrifugation and quantifying the different species present in an AAV viral preparation."). The scAAV preparations of Le Bec contain rAAV particles.

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

73. Le Bec teaches applying analytical ultracentrifugation to an rAAV preparation to determine sedimentation coefficients of the particles contained in the viral preparation. Ex.1003, 15:23-28 ("Centrifugation of the samples was carried out at a speed of 16,000 rpm using 100 µl or 400 µl of undiluted pure vectors, sedimentation was followed by absorbance at the wavelength of 276 nm, and the sedimentation coefficient of the various populations was obtained using the software SEDFIT."). In my opinion, these conditions teach boundary sedimentation velocity conditions.

74. As explained above, when the ’288 patent application was filed in 2015, there were primarily two different types of analytical ultracentrifugation experiments, including SV-AUC and SE-AUC. Le Bec explained that sedimentation coefficients of the various populations were determined, confirming that the method Le Bec sought to use required the measurement of the velocity at which different boundaries were sedimenting. Those skilled in the art would use SV-AUC if a sedimentation coefficient or sedimentation coefficient distribution profile was the information sought from a sample.

75. As described above, boundary sedimentation results when a sufficient centrifugal force is applied to a sample such that particles migrate in a velocity cell at characteristic velocities. Under these conditions, species can be separated, identified, and quantified based on their particular velocity. One achieves this force by setting an appropriate rotor speed based on the known or expected sedimentation coefficients of the particles of interest, generally using the fastest rotor speed available that will permit enough scans/cell to be acquired. The ’288 patent comments that “[i]t is within the purview of the skilled artisan to optimize the parameters of an AUC for different types of viral particles. Ex.1001, 26:4-6. I agree.

76. Le Bec discloses that rAAV particles of interest will range from approximately 60S to 110S. A POSA may predict an appropriate rotor speed for

particles with those anticipated sedimentation coefficients based on the knowledge in the art, as pointed out by publications like as Cole, which generally teaches SV-AUC and discloses maximum rotor speeds for analyzing species at 90S and 270S, respectively:

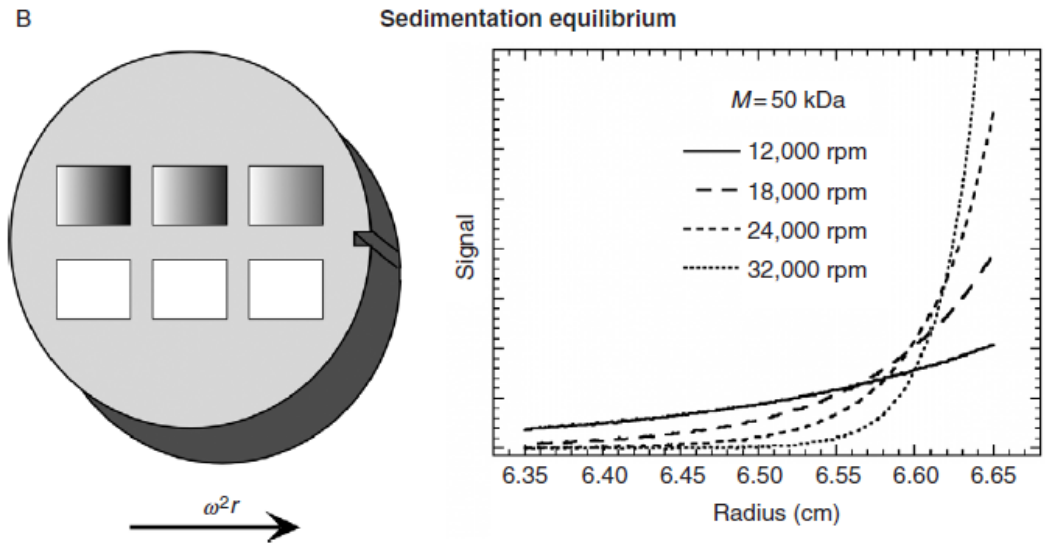
**Table III**
**Maximum Rotor Speeds for Sedimentation Velocity Experiments**

| S[a] | $M_{app}$[b] | rpm[c] |
|---|---|---|
| 10 | 200,000 | 55,000 |
| 15 | 400,000 | 50,000 |
| 30 | 1,000,000 | 30,000 |
| 90 | 5,000,000 | 20,000 |
| 270 | 25,000,000 | 10,000 |

Ex.1005, 164 (Table 3). Knowing that 20,000 rpm for species at 90S and 10,000 rpm for 270S, a POSA would understand a rotor speed of 16,000 rpm sufficient to create sedimentation boundary conditions for the characterization of rAAV particles with sedimentation coefficients of 60S and 110S as described in Le Bec.

77. Additionally, Le Bec discloses a pair of c(s) v. S plots it generated from its AUC data. Ex.1003, Figs. 2 and 3. Creating such plots requires data generated from samples sedimenting under boundary sedimentation velocity conditions. That is how one would acquire data about the unique sedimentation velocities of species in a composition necessary to construct such a plot. And typically, such plots are generated by a software program, like the SEDFIT software identified in Le Bec.

78. The data presented in Figures 2 and 3 are not the type of data that would result from an SE-AUC experiment. SE-AUC experiments are primarily concerned with the static profile (independent of time) of concentration of all species as a function of radial positions within the cell after equilibrium is reached. These experiments require lower rotor speeds. As further reflected by Cole, a POSA would understand the results of SE-AUC experiments to generally be plotted as concentration or detector signal readout vs. radial position (and thus consist of only one scan for a given rotor speed):




*See* Ex.1005, 162, Fig. 1.

79. Finally, Le Bec also discloses that "sedimentation was followed by absorbance at the wavelength of 276 nm" in "real-time." Ex.1003, 15:19-28. A POSA would understand in "real-time" to indicate that multiple scans are taken at time intervals as the sedimentation experiment is occurring. For an absorbance

experiment as that disclosed by Le Bec, as evidenced by the prior art (such as Cole), a POSA would understand that at least 30 scans are typically necessary. Ex.1005, 164. Further, use of the SEDFIT software also inherently requires that sedimentation was monitored at time intervals. A velocity cannot be calculated from a single time point; it requires the tracking of the dynamic movement of material dissolved or suspended in the sample solution being held within the AUC cell over time.

### 3. "b) plotting the differential sedimentation coefficient distribution values (C(s)) versus the sedimentation coefficient in Svedberg units (S)"

80. Figures 2 and 3 of Le Bec are plots of "the differential sedimentation coefficient distribution values (c(s)) versus the sedimentation coefficient in Svedberg units (S)."

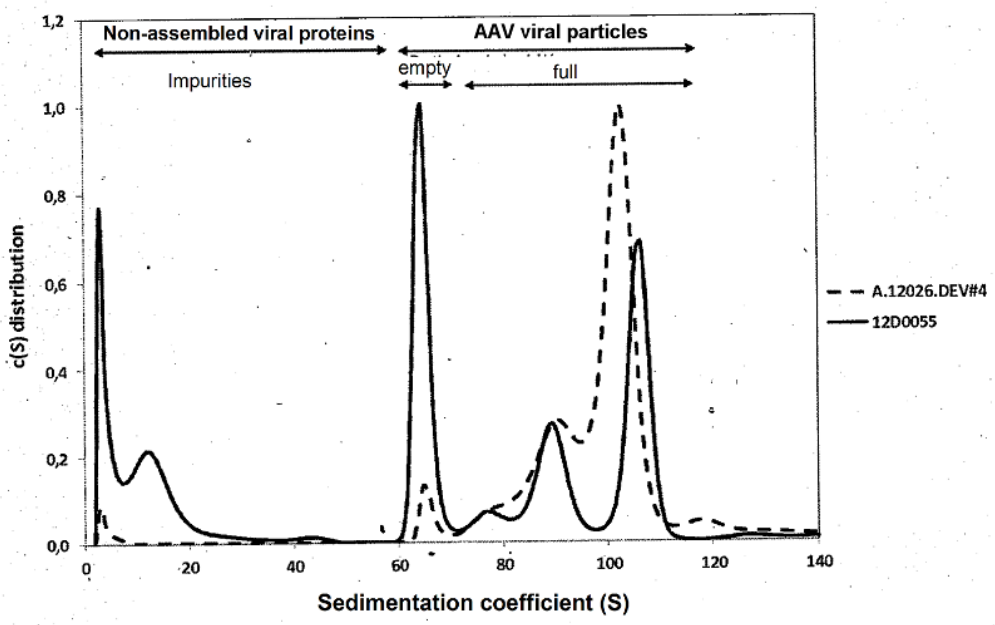



Figure 2

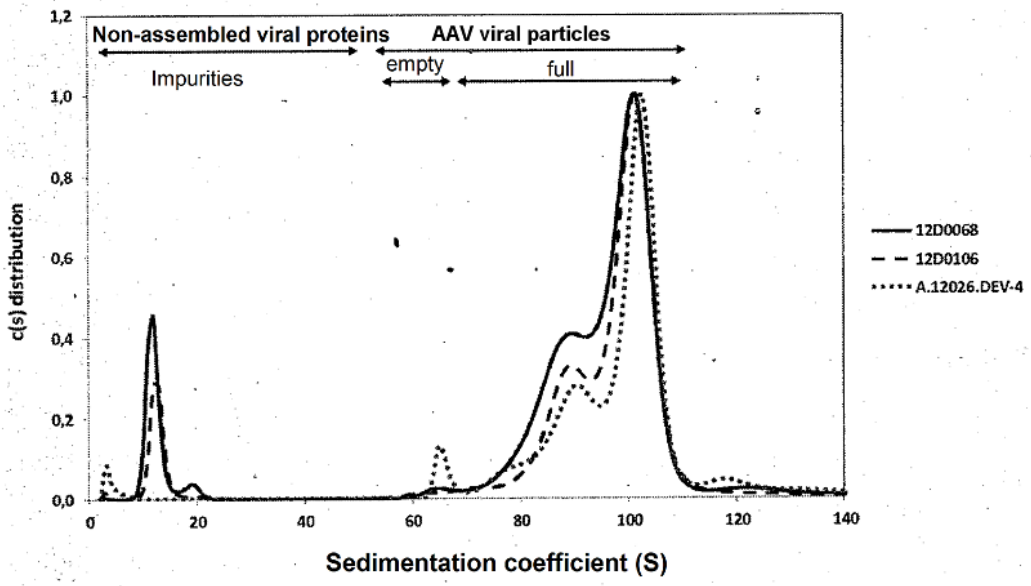



**Figure 3**

81. I have generated these types of plots from SV-AUC experiments on viral particles using the SEDFIT software (as was done in Le Bec). *See* Ex.1004, 27, Figs. 6C, 6D, 8C. It is common and routine to plot the differential sedimentation coefficient (c(s)) on one axis and the sedimentation coefficient (S) on the other axis, which facilitates additional analyses, including the determination of relative concentrations. *See* Ex.1005, 25 ("This c(s) distribution is similar to a chromatogram: each species produces a peak at its characteristic sedimentation coefficient, and the area under each peak gives the concentration of that species (in signal units of OD or fringes).").

#### 4. "c) integrating the area under each peak in the C(s) distribution to determine the relative concentration of each peak, wherein each peak represents a species of recombinant AAV particle"

82. Applying its SV-AUC data, Le Bec reports values of the relative amount of rAAV particles. *See, e.g.*, Ex.1003, 19:11-15 ("These three batches all show a similar distribution profile and confirm that the sf9 system makes it possible to produce AAV viral particles essentially composed of full AAV particles (> 80%), with a very high percentage of viral genome in double-stranded form (> 50%)."). A POSA would understand that quantitation of species in an SV-AUC experiment like that disclosed in Le Bec would have been determined by integration of the area under the peak of a c(s) v. S plot due to Le Bec's disclosure of such plots and that it used SEDFIT to determine the sedimentation coefficients of the different species in its rAAV preparations. Ex.1003, 15:19-28; Figs. 2 and 3. Moreover, because Le Bec reported percentages, the data is a relative concentration.

83. As explained above in paragraphs 27-40, the SEDFIT software referenced in Le Bec and available prior to January 2015 calculated relative concentrations by integrating the area under the peaks of a sedimentation coefficient distribution profile.

84. Because Le Bec disclosed relative amounts rAAV species in its preparation and analyzed its data using a c(s) model in SEDFIT, Le Bec teaches

"integrating the area under each peak in the c(s) distribution to determine the relative concentration of each peak." At a minimum, a POSA would have known how to integrate SV-AUC data of rAAV particles from the teaching of Le Bec using the standard functionality built into the SEDFIT software.

85. Thus, in my opinion, the method described in claim 1 of the '288 patent is anticipated by Le Bec.

### D. Claim 4 is anticipated by Le Bec

#### 1. "A method to determine the presence of empty capsids or capsid particles comprising variant sized recombinant AAV genomes in a preparation of recombinant AAV particles comprising the steps of"

86. Le Bec teaches a method for characterizing a viral preparation of rAAV particles that can analytically separate, detect, and quantify the presence of empty capsids, full capsids, and aggregates based on sedimentation coefficients as well as the capable of distinguishing AAV particles filled with different genome sizes. Ex.1003, 16:25-29 ("We thus obtained scAAV9-SMN viral preparations whose titer in viral genome, the purity, the identity and the quantification of empty and full viral particles were determined."); *see also id.*, 15:17-23 ("The sedimentation coefficient of the various AAV viral particles (empty, full, aggregate) and other present populations (subparticles, contaminant proteins, aggregate) in the purified products was determined by real-time centrifugation."); 18:1-6 ("Knowing that the difference in density is sufficiently significant to

distinguish them by centrifugation, we have implemented a method today for analytically separating by ultracentrifugation and quantifying the different species present in an AAV viral preparation.”).

**2. “a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant AAV particles is monitored at time intervals”**

87. See analysis for claim 1, ¶¶73-79.

**3. “b) plotting the differential sedimentation coefficient distribution value (C(s)) versus the sedimentation coefficient in Svedberg units (S),”**

88. See analysis for claim 1, ¶¶80-81.

**4. “wherein the presence of one or more peaks other than the peak for full capsid particles comprising intact recombinant AAV genomes indicates that presence of capsid particles comprising variant sized genomes and/or empty capsids”**

89. Le Bec describes characterizing the viral preparation of rAAV particles by determining relative concentrations from sedimentation coefficient data. When such data is plotted with the differential sedimentation coefficient distribution values, c(s), on one axis and the sedimentation coefficient values, S on the other axis, the peaks that appear at different sedimentation coefficients indicate the presence of a quantity of particles sedimenting at that velocity. *See, e.g.*, Ex.1004, 25. A POSA would inherently understand that peaks other than the peak for full rAAV particles would indicate the presence of other species and be able to quantify them.

90. Thus, in my opinion, the method described in claim 4 of the ’288 patent is anticipated by Le Bec.

### E. Claim 5 is anticipated by Le Bec

#### 1. “A method of measuring the relative amount empty capsids in a preparation of recombinant AAV particles comprising the steps of”

91. Le Bec teaches a method for characterizing a viral preparation of rAAV particles that can analytically separate, detect, and quantify the presence of empty capsids, full capsids, and aggregates based on sedimentation coefficients. Ex.1003, 16:25-29 (“We thus obtained scAAV9-SMN viral preparations whose titer in viral genome, the purity, the identity and the quantification of empty and full viral particles were determined.”); *see also id.*, 15:17-23 (“The sedimentation coefficient of the various AAV viral particles (empty, full, aggregate) and other present populations (subparticles, contaminant proteins, aggregate) in the purified products was determined by real-time centrifugation.”); 18:1-6 (“Knowing that the difference in density is sufficiently significant to distinguish them by centrifugation, we have implemented a method today for analytically separating by ultracentrifugation and quantifying the different species present in an AAV viral preparation.”).

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

92. See analysis for claim 1, ¶¶73-79.

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

93. See analysis for claim 1, ¶¶80-81.

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

94. See analysis for claim 1, ¶¶82-85.

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

95. Le Bec reports the results from its AUC method in terms of the relative amounts of species observed and derives those values from its c(s) v. S plots. Le Bec further discloses that the 90S and 105S peaks on its c(s) v. S plots correspond to rAAV particles with single- and double-stranded genomes, respectively, while "empty AAV viral particles" correspond to a peak at "65S." Ex.1003, 18:21-25; *see also* Figures 2 and 3. Further, Le Bec comments that "we detected very few empty AAV viral particles with the sf9 system compared to the HEK293 system." Ex.1003, 18:27-29. From this statement, a POSA would

understand Le Bec to have used the peak size at 65S to quantify the relative amount of empty rAAV particles to reach this conclusion. *See, e.g.*, Ex.1004, 25.

96. Furthermore, a POSA would understand that the amount of empty rAAV particles could be compared to the amounts of other components of the viral preparation, whether to the full rAAV particles or to the total amount of rAAV particles in the sample, including through use of the SEDFIT software. *See* paragraphs 27-40. In my 2007 article, I described the variety of comparisons that could be made from SV-AUC data. Ex.1004, 17. These comparisons are routine and a POSA would understand how to implement any of them.

97. Thus, in my opinion, the method described in claim 5 of the '288 patent is anticipated by Le Bec.

### F. Claim 6 is anticipated by Le Bec

#### 1. "A method of measuring the relative amount of capsid particles comprising variant recombinant AAV genomes or empty AAV capsid particles in a preparation of recombinant AAV particles comprising the steps of"

98. Le Bec teaches a method for characterizing a viral preparation of rAAV particles that can analytically separate, detect, and quantify the presence of empty capsids, full capsids, and aggregates based on sedimentation coefficients. Ex.1003, 16:25-29 ("We thus obtained scAAV9-SMN viral preparations whose titer in viral genome, the purity, the identity and the quantification of empty and full viral particles were determined."); *see also id.*, 15:17-23 ("The sedimentation

coefficient of the various AAV viral particles (empty, full, aggregate) and other present populations (subparticles, contaminant proteins, aggregate) in the purified products was determined by real-time centrifugation."); 18:1-6 ("Knowing that the difference in density is sufficiently significant to distinguish them by centrifugation, we have implemented a method today for analytically separating by ultracentrifugation and quantifying the different species present in an AAV viral preparation.").

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

99. See analysis for claim 1, ¶¶73-79.

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

100. See analysis for claim 1, ¶¶80-81.

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

101. See analysis for claim 1, ¶¶82-85.

**5. "d) comparing the amount of recombinant AAV particles having an S value[] that do[es] not correspond to recombinant AAV particles comprising intact AAV genomes to the amount of recombinant AAV particles having an S value that corresponds to recombinant AAV particles comprising intact AAV genomes or to the total amount of recombinant AAV particles in the preparation."**

102. See analysis for claim 5, step (d). ¶¶95-96. A POSA would understand that the amount of rAAV particles not corresponding to the peak for full particles could be compared to the amounts of other components of the viral preparation, whether to the full rAAV particles or to the total amount of rAAV particles in the sample. For example, in my 2007 article, I described the variety of comparisons that could be made from SV-AUC data. Ex.1004, 17, 25. These comparisons are routine and a POSA would understand how to implement any of them.

103. Thus, in my opinion, the method described in claim 6 of the '288 patent is anticipated by Le Bec.

**G. Claim 7 is anticipated by Le Bec**

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

104. Le Bec teaches a method for characterizing a viral preparation of rAAV particles that can analytically separate, detect, and quantify the presence of empty capsids, full capsids, and aggregates based on sedimentation coefficients.

Ex.1003, 16:25-29 ("We thus obtained scAAV9-SMN viral preparations whose titer in viral genome, the purity, the identity and the quantification of empty and full viral particles were determined."); *see also id.*, 15:17-23 ("The sedimentation coefficient of the various AAV viral particles (empty, full, aggregate) and other present populations (subparticles, contaminant proteins, aggregate) in the purified products was determined by real-time centrifugation."); 18:1-6 ("Knowing that the difference in density is sufficiently significant to distinguish them by centrifugation, we have implemented a method today for analytically separating by ultracentrifugation and quantifying the different species present in an AAV viral preparation.").

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

105. See analysis for claim 1, ¶¶73-79.

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

106. See analysis for claim 1, ¶¶80-81.

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

107. See analysis for claim 1, ¶¶82-85.

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

108. See analysis for claim 5, step (d), ¶¶95-96. A POSA would understand that the amount of rAAV particles not corresponding to the peak for full particles or empty particles could be compared to the amounts of other components of the viral preparation, whether to the full rAAV particles, to the empty particles, or to the total amount of rAAV particles in the sample. For example, in my 2007 article, I described the variety of comparisons that could be made from SV-AUC data. Ex.1004, 17. Figure 2 of Le Bec depicts a peak at approximately 77S that does not correspond to the peak for empty particle (65S) or the peaks for single- and double-stranded particles (90S and 105S, respectively). These comparisons are routine and a POSA would understand how to implement any of them.

109. Further, Le Bec uses a self-complementary AAV vector that is designed to be packaged with a double-strand-forming genome. Le Bec describes these "double stranded" genome containing AAV species (particles) as "full" virus particles that sediment at around 105S. The AAV species sedimenting at 90S, Le Bec describes as "singled-stranded" are AAV particles having less DNA (smaller genome) incorporated into their protein capsid (or shell) (*see* Ex.1017) and as a

result have a proportionally lower sedimentation coefficient. A POSA would thus understand from Le Bec that SV-AUC can identify AAV species with different amounts of DNA and quantify them relative to other AAV species and/or other material present in the sample.

110. Thus, in my opinion, the method described in claim 7 of the ’288 patent is anticipated by Le Bec.

### H. Claim 8 is anticipated by Le Bec

#### 1. “A method of measuring the relative amount of recombinant AAV particles comprising intact AAV genomes in a preparation of recombinant AAV particles comprising the steps of”

111. Le Bec teaches a method for characterizing a viral preparation of rAAV particles that can analytically separate, detect, and quantify the presence of empty capsids, full capsids, and aggregates based on sedimentation coefficients. Ex.1003, 16:25-29 (“We thus obtained scAAV9-SMN viral preparations whose titer in viral genome, the purity, the identity and the quantification of empty and full viral particles were determined.”); *see also id.*, 15:17-23 (“The sedimentation coefficient of the various AAV viral particles (empty, full, aggregate) and other present populations (subparticles, contaminant proteins, aggregate) in the purified products was determined by real-time centrifugation.”); 18:1-6 (“Knowing that the difference in density is sufficiently significant to distinguish them by centrifugation, we have implemented a method today for analytically separating by

ultracentrifugation and quantifying the different species present in an AAV viral preparation.”).

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

112. See analysis for claim 1, ¶¶73-79.

**3. “b) plotting the differential sedimentation coefficient distribution value (C(s)) versus the sedimentation coefficient in Svedberg units (S),”**

113. See analysis for claim 1, ¶¶80-81.

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

114. See analysis for claim 1, ¶¶82-85.

**5. “d) comparing the amount of recombinant AAV particles having an S value[] corresponding to recombinant AAV particles comprising intact AAV genomes to the amount of recombinant AAV particles having an S value corresponding to empty capsid particles, to capsid particles comprising variant recombinant AAV genomes, and/or to the total amount of recombinant AAV particles in the preparation”**

115. See analysis for claim 5, step (d), ¶¶95-96. A POSA would understand that the amount of rAAV particles corresponding to the peak for full particles could be compared to the amounts of other components of the viral preparation, whether to the empty rAAV particles, to the particles having variant genomes, or to the total amount of rAAV particles in the sample. In my 2007

article, I described the variety of comparisons that could be made from SV-AUC data. Ex.1004, 17, 25. These comparisons are routine and a POSA would understand how to implement any of them.

116. Thus, in my opinion, the method described in claim 8 of the ’288 patent is anticipated by Le Bec.

**I. Claim 10 is anticipated by Le Bec**

117. Claim 10 depends from claim 4, and further requires that “wherein the presence of a peak that corresponds to the S value of empty capsid particles indicates the presence of empty capsid particles; or the presence of one or more peaks other than the peak for full capsid particles comprising intact recombinant AAV genomes or empty capsid particles indicates that presence of capsid particles comprising variant sized genomes.”

118. As discussed above, a POSA would understand from Le Bec that the presence of a peak on a c(s) v. S plot indicates the presence of a species corresponding to that sedimentation coefficient in the tested composition. See analysis for claim 4, ¶89.

119. Thus, in my opinion, the method described in claim 10 of the ’288 patent is anticipated by Le Bec.

**J. Claim 11 is anticipated by Le Bec**

120. Claim 11 depends from claim 10, and further requires that

### 1. "wherein the presence of one or more peaks other than the peak for full capsid particles comprising intact recombinant AAV genomes or empty capsid particles indicates [the] presence of capsid particles comprising variant sized genomes, and "wherein the capsid particles comprising variant sized genomes comprises truncated genomes, aggregates, recombinants and/or DNA impurities compared to the intact recombinant AAV genome"

121. In my opinion, a POSA would understand the presence of a peak on a c(s) v. S plot to indicate the presence of a species corresponding to that S-value in the tested composition. See analysis for claim 4, ¶89.

122. Le Bec discloses that "[t]he sedimentation coefficient of the various AAV viral particles (empty, full, aggregate) and other present populations (subparticles, contaminant proteins, aggregate) in the purified products was determined by real-time centrifugation." Ex.1003, 15:19:23. At a minimum, a POSA would understand from Le Bec that SV-AUC could be performed on rAAV particles without undue experimentation and would understand how to interpret the results of an SV-AUC experiment because Le Bec included experimental data and described the results. Further, a POSA would understand Le Bec to teach the use of SV-AUC to distinguish the presence of rAAV aggregates (or other particles having variant genomes) from empty capsids or full particles because of the peak differences on its c(s) v. S plots owing to their different sedimentation coefficients. *See* ¶109 above. Indeed, Figure 2 of Le Bec depicts a peak at approximately 77S

that does not correspond to the peak for empty particle (65S) or the peaks for single-stranded and double-strand-forming particles (90S and 105S, respectively).

123. Thus, in my opinion, the method described in claim 11 of the ’288 patent is anticipated by Le Bec.

### K. Claim 12 is anticipated by Le Bec

#### 1. “A method of determining the heterogeneity of recombinant AAV particles in a preparation of recombinant AAV particles comprising the steps of…”

124. Le Bec teaches a method for characterizing a viral preparation of rAAV particles that can analytically separate, detect, and quantify the presence of empty capsids, full capsids, and aggregates based on sedimentation coefficients. Ex.1003, 16:25-29 (“We thus obtained scAAV9-SMN viral preparations whose titer in viral genome, the purity, the identity and the quantification of empty and full viral particles were determined.”); *see also id.*, 15:17-23 (“The sedimentation coefficient of the various AAV viral particles (empty, full, aggregate) and other present populations (subparticles, contaminant proteins, aggregate) in the purified products was determined by real-time centrifugation.”); 18:1-6 (“Knowing that the difference in density is sufficiently significant to distinguish them by centrifugation, we have implemented a method today for analytically separating by ultracentrifugation and quantifying the different species present in an AAV viral preparation.”).

125. Additionally, a POSA would be familiar with the terms homogeneity (one species in the sample) and heterogeneity (more than one species in the sample), and would understand Le Bec's reference to the determination of the "different species in an AAV viral preparation" to be a direct measure of the heterogeneity of the sample. *See, e.g.*, Ex.1004, 17, 31.

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

126. See analysis for claim 1, ¶¶73-79.

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

127. See analysis for claim 1, ¶¶80-81.

**4. "wherein the presence of peaks in addition to the peak representing capsids comprising an intact AAV genome indicates heterogeneity of recombinant particles in the preparation."**

128. A POSA would understand the heterogeneity of a viral composition to be the degree to which it comprises species other than "intact AAV genome[s]." *See* Ex.1004, 31 (differentiating between "EC material and other heterogeneous viral material" from "the intact … monomer."). Le Bec identifies two populations of species, the "non-assembled viral proteins and/or contaminants at < 60S, and rAAV particles between 60S and 100S, with subpopulations representing species of empty rAAV particles and full rAAV particles. Ex.1003, 18:8-32. A POSA

would further understand from Le Bec's distribution profiles that there were peaks other than those identified as "full," which indicate heterogeneity in the composition. *See id.* (describing "material other than the major peak corresponding to the intact virus monomer" as "[t]ypes and sources of heterogeneity").

129. Thus, in my opinion, the method described in claim 12 of the '288 patent is anticipated by Le Bec.

**L. Claim 13 is anticipated by Le Bec**

130. Claim 13 depends from claim 12, and further requires that "the presence of additional peaks indicates the presence of empty capsid particles and/or recombinant AAV particles comprising variant genomes." Le Bec expressly identifies the peak corresponding to empty rAAV particles at 65S, which is an "additional peak." Figure 2 of Le Bec also depicts a small peak appearing at approximately 77S, which is lower than the peak identified for single-stranded DNA and which likely represents a particle comprising a variant genome.

131. Thus, in my opinion, the method described in claim 13 of the '288 patent is anticipated by Le Bec.

**M. Claim 14 is anticipated by Le Bec**

132. Claim 14 depends from claim 13, and further requires that "the variant genomes are truncated AAV genomes, aggregates, recombinants and/or DNA impurities compared to the intact recombinant AAV genome." Le Bec

expressly identifies the peak corresponding to empty rAAV particles, which is an "additional peak," and appears at 65S. The "single-stranded" particles at 90S do not contain the full double-strand-forming or full self-complementary viral genome. *See* ¶109, Ex.1017. And Figure 2 of Le Bec also depicts a small peak appearing at approximately 77S, which is lower than the peak identified for single-stranded DNA and which may comprise a particle comprising a truncated AAV genome. In any event, a POSA would immediately appreciate the applicability of the method to identifying variant genomes from the Le Bec disclosure showing the clear applicability of SV-AUC to rAAV particles, because Le Bec shows the rAAV particles with "single-stranded" DNA (which have less DNA incorporated into the viral particle) have a lower sedimentation coefficient than the rAAV particles identified as having "double-stranded" DNA. A POSA would have been able to apply this routine and well-known method to rAAV particles, including those species having variant genomes.

133. Thus, in my opinion, the method described in claim 14 of the '288 patent is anticipated by Le Bec.

**N. Claim 15 is anticipated by Le Bec**

134. Claim 15 depends from claim 12, and further requires "integrating the area under each peak in the c(s) distribution to determine the relative

concentration of each species of recombinant AAV particles." See analysis for claim 1, ¶¶82-85.

**O. Claim 17 is anticipated by Le Bec**

135. Claim 17 depends from claim 1, and further requires "wherein the sedimentation of recombinant AAV particles is monitored by absorbance or interference." Le Bec discloses that its rAAV particle sedimentation is monitored by optical absorbance. Ex.1003, 15:25-26 ("[T]he sedimentation was followed by absorbance at the wavelength of 276 nm").

136. Thus, in my opinion, the method described in claim 17 of the '288 patent is anticipated by Le Bec.

**P. Claim 18 is anticipated by Le Bec.**

137. Claim 18 depends from claim 1, and further requires "wherein the preparation is an aqueous solution." Le Bec discloses that the preparations it is analyzing "are concentrated and formulated in PBS buffer…." Ex.1003, 14:15-16. A POSA would understand "PBS buffer" to mean phosphate buffered saline, a common buffer used in biological or chemical laboratories to maintain a biologically relevant pH. The solvent used in PBS is water, so a POSA would understand PBS to be an aqueous solution.

138. Thus, in my opinion, the method described in claim 18 of the '288 patent is anticipated by Le Bec.

### Q. Claim 20 is anticipated by Le Bec

139. Claim 20 depends from claim 1, and further requires "wherein the c(s) values are determined by an algorithm that comprises Lamm equation solutions." The Lamm equation has been long known as the equation that describes the sedimentation of a component or molecular species under ultracentrifugation in traditional sector-shaped cells. I understand it is named for Ole Lamm, who derived it during his Ph.D studies in 1929. Furthermore, the SEDFIT software is known to use solutions to the Lamm equation to generation a sedimentation coefficient distribution profile. Le Bec expressly stated use of the SEDFIT software. Ex.1003, 15: 23-28.

140. Thus, in my opinion, the method described in claim 20 of the '288 patent is anticipated by Le Bec.

### R. Claim 21 is anticipated by Le Bec

141. Claim 21 depends from claim 20, and further requires "wherein the algorithm is the SEDFIT algorithm." As discussed above, Le Bec discloses that "the sedimentation coefficient of the different populations was obtained using the software SEDFIT." Ex.1003, 15:27-28.

142. Thus, in my opinion, the method described in claim 21 of the '288 patent is anticipated by Le Bec.

### S. Claim 23 is anticipated by Le Bec

143. Claim 23 depends from claim 1 and further comprises "wherein the ultracentrifugation utilizes an ultracentrifuge comprising an ultracentrifuge velocity cell." SV-AUC experiments require a special sample cell sometimes referred to as a "velocity cell." Ex.1005, 144. A POSA would understand that the cell has a special sector shape necessary for an SV-AUC experiment, otherwise the data can be compromised. Specifically, without use of a sector shaped cell, the particles can interact with the cell walls and cause convection, distorting the sedimentation of the particles. As explained in the 2015 book chapter I published with John Philo:

> This sector shape is actually required, and is a critical element of any successful SV-AUC experiment. This need for a sector shape cell arises due to the radial nature of the centrifugal field and molecular flow. It is essential that the flow remains parallel to the walls of the sector. Any deviation from the appropriate sector shape and its appropriate alignment within the centrifugal field could lead to the flow hitting the walls, producing a local concentration increase that could violate the necessary stability criteria of a nonnegative density gradient across the entire sample solution running from the meniscus to the base of the solution compartment. If a negative density gradient is encountered anywhere along this path, solution inversion (mixing) will occur as a result of the density difference, leading to convective

> transport of the protein molecules causing erroneous sedimentation data to be generated.

Ex.1011, 220. A POSA would know about the equipment requirements (especially in view of detailed disclosures in the prior art such as Cole), and would have understood Le Bec to have involved the use of a velocity cell. And a POSA would have known how to implement the use of a velocity cell along with the teaching of Le Bec.

144. Thus, in my opinion, the method described in claim 23 of the ’288 patent is anticipated by Le Bec.

**T. Claim 32 is anticipated by Le Bec**

145. Claim 32 depends from claim 1 and further requires that “the boundary sedimentation velocity is performed at about 3,000 rpm to about 20,000 rpm and/or at about 4°C. to about 20° C.” Le Bec teaches that “centrifugation of the samples was carried out at a speed of 16,000 rpm.” Ex.1003, 15:23-25. This rotor speed falls within the range of claim 32.

146. Thus, in my opinion, the method described in claim 32 of the ’288 patent is anticipated by Le Bec.

## U. Claim 33 is anticipated by Le Bec

### 1. "A method of evaluating a process for the production of recombinant AAV particles comprising"

147. Le Bec teaches a method for characterizing a viral preparation of rAAV particles that can analytically separate, detect, and quantify the presence of empty capsids, full capsids, and aggregates based on sedimentation coefficients. Ex.1003, 16:25-29 ("We thus obtained scAAV9-SMN viral preparations whose titer in viral genome, the purity, the identity and the quantification of empty and full viral particles were determined."); *see also id.*, 15:17-23 ("The sedimentation coefficient of the various AAV viral particles (empty, full, aggregate) and other present populations (subparticles, contaminant proteins, aggregate) in the purified products was determined by real-time centrifugation."); 18:1-6 ("Knowing that the difference in density is sufficiently significant to distinguish them by centrifugation, we have implemented a method today for analytically separating by ultracentrifugation and quantifying the different species present in an AAV viral preparation."). The results of Le Bec's SV-AUC characterization method were relied on to evaluate the effectiveness of the two processes for generating rAAV particles and conclude which process was superior.

### 2. "the method of claim 1,"

148. See analysis for claim 1, ¶¶72-85.

**3. "wherein an increase in the relative amount of recombinant AAV particles comprising intact AAV genomes compared to the relative amount of empty capsid particles and/or recombinant AAV capsid particles with variant recombinant AAV genomes compared to a reference preparation of recombinant AAV particles indicates an improvement in the production of recombinant AAV particles."**

149. In my opinion, a POSA would recognize that Le Bec discloses that because the sf9-based preparation has more intact rAAV particles and fewer empty capsid particles relative to the HEK293-based preparation, the sf9-based method constitutes "an improvement in the ratio of particles containing viral DNA to empty particles with an increased percentage of particles containing a double-stranded genome." Ex.1003, 3:26-29; *see also* 3:4-22 (describing benefits of double stranded particles and undesirability of empty particles). Thus, Le Bec teaches use of SV-AUC to assess an increase in the relative amount of full AAV particles, which in turn reflects an improved method of production.

150. Thus, in my opinion, the method described in claim 33 of the '288 patent is anticipated by Le Bec.

## VIII. OBVIOUSNESS ANALYSIS

### A. Legal Standard

151. I have been informed by counsel and I understand that an obviousness analysis concerns whether the differences between the prior art and the subject matter claimed in the patent are such that, at the time the invention was

made, the subject matter as a whole would have been obvious to a POSA of which the subject matter pertains.

152. I have been informed by counsel and I understand that obviousness is a factual inquiry, and that the factors guiding the analysis include: (1) the scope and content of the prior art; (2) the differences between the prior art and the claims at issue; (3) the level of ordinary skill in the art; (4) and any so-called objective secondary factors of non-obviousness (for example, commercial success, long-felt but unmet need, unexpected results, and copying).

153. I have been informed by counsel and I understand that obviousness may be shown by a single prior art reference or by a combination of prior art references. I understand that it is important to consider whether there was a reason (e.g., a teaching, suggestion, or motivation) to combine elements found in the prior art, although an explicit teaching, suggestion, or motivation to combine prior art references is not required. Rather, the following factors may be considered in evaluating the existence of a motivation to combine: (1) the inter-related teachings of multiple references; (2) the effects of demands known to those of ordinary skill in the field or present in the marketplace; and (3) the background knowledge possessed by a POSA.

154. I have been informed by counsel and I understand that, to evaluate the obviousness of the ’288 patent claims over a given combination of prior art

references, I should consider whether the prior art references disclose every limitation of the challenged claims either explicitly or inherently, as those references are read by the POSA at the time of the invention. Then I am to determine whether that combination makes the claimed invention, as a whole, more likely than not obvious to the POSA.

155. I have been informed by counsel and I understand that a patent claiming a combination of elements from the prior art is obvious if the alleged improvement is no more than the predictable use of prior art elements according to their established functions. When there is a design need or market pressure to solve a problem and the prior art suggests a finite number of identified, predictable solutions, a skilled artisan has good reason to pursue the known options within their technical grasp. If this leads to the anticipated success, it is likely that the product is not one of innovation but rather one of ordinary skill and common sense.

156. I have been informed by counsel and I understand that under an obviousness analysis, all that is required is a reasonable expectation of success in combining the prior art references to arrive at the claimed embodiments. A guarantee or absolute certainty of success based on the prior art is not required.

## B. Summary of Prior Art References

### 1. PCT Publication of International Application Number PCT/EP2014/052978, entitled "Methods for the Production of Double-Stranded AAV Viral Particles" ("Le Bec")

157. A summary of Le Bec is provided above. *See* ¶¶64-71.

### 2. Berkowitz, S.A. & Philo, J.S. "Monitoring the homogeneity of adenovirus preparations (a gene therapy delivery system) using analytical ultracentrifugation" 362 ANAL BIOCHEM. 16-37 (2007) ("Berkowitz")

158. The reference referred to as Berkowitz is a scientific article entitled "Monitoring the homogeneity of adenovirus preparations (a gene therapy delivery system) using analytical ultracentrifugation." I co-authored this article with John Philo and our objective was to make those in biotechnology, especially the gene therapy area, aware of the existing and unique capabilities of an old biophysical technique called analytical ultracentrifugation (which at the time of our writing was making a return to mainstream science) to characterize large complex biopharmaceutical such as viruses (viral vectors). It was published in 2007 (which complements an earlier publication in 2006 (S.A. Berkowitz, Role of analytical ultracentrifugation in assessing the aggregation of protein biopharmaceuticals, AAPS, 8(3) (2006) E590-605). I also understand that Berkowitz qualifies as prior art and is cited in the '288 patent. Ex.1001, 19:4-6.

159. Berkowitz is from the same field of endeavor as the '288 patent (methods for characterizing recombinant viral vectors and assessing homogeneity

via SV-AUC) and is reasonably pertinent to the problem the ’288 patent sought to solve (the need for an assay to characterize recombinant viral preparations).

160. Berkowitz teaches the use of AUC to characterize adenovirus preparations developed for gene therapy applications (a much larger and even more complex virus particle than AAV). Ex.1004, 16. Berkowitz explains that for all biologic drug products requiring marketing approval, “drug product quality (with regard to homogeneity and purity) and consistency of manufacturing are of key importance,” but notes that at the time the article was written (in 2007) there was not an agreed-upon approach for characterizing adenoviral homogeneity. *Id.* Berkowitz notes that an adenoviral preparation might include empty capsids, incomplete or aberrant particles, sub particles, and/or aggregates that need to be identified and quantified. Ex.1004, 16-17.

161. Berkowitz encouraged the use of AUC to assess the homogeneity of adenovirus preparations, noting that it had been used on other viral preparations in the past (in fact AUC has a long past history in being applied to study of a wide range of virus preparations in terms of their physical properties, structure and purity). Ex.1004, 17.

162. Berkowitz further teaches that “simple peak area integration, taking into account radial dilution effects . . . allows the percentage of [empty capsid] material present in a virus preparation to be readily calculated.” Ex.1004, 21.

Berkowitz provides examples of these calculations for empty capsids and aggregates in Table 2. Ex.1004, 29 (Table 2).

163. Thus, Berkowitz demonstrates the use of SV-AUC to determine the homogeneity of viral preparations, including the voluminous information that can be extracted from SV-AUC data and the ability to calculate relative concentrations of viral particles using "simple peak integration". Ex.1004.

### 3. Cole, J.L. et al., "Analytical Ultracentrifugation: Sedimentation Velocity and Sedimentation Equilibrium", *in* METHODS IN CELL BIOLOGY, VOL. 84 (2008) ("Cole")

164. The reference referred to as Cole is a chapter in METHODS IN CELL BIOLOGY, VOL. 84 entitled "Analytical Ultracentrifugation: Sedimentation Velocity and Sedimentation Equilibrium." It was published in 2008, and I understand that it qualifies as prior art with regards to the '288 patent. Ex.1005. Cole was cited in the '288 patent. Ex.1001, 19:61-64.

165. Cole provides a general overview of various analytical ultracentrifugation techniques—including SV-AUC—and explained the various parameters and analyses that could be adjusted depending on the experimental system and samples being tested. Cole is from the same field of endeavor as the '288 patent (analytical methods for characterizing biomolecules, including viruses) and is reasonably pertinent to the problem the '288 patent sought to solve

(providing an assay that could be applied to characterize recombinant viral preparations).

166. Cole explains that a common application of SV-AUC is to determine the distribution of sedimentation coefficients for different macromolecules, *e.g.*, by their c(s) values, explaining that "often a simple relationship between s [sedimentation coefficient] and M [effective particle mass] may be used to identify particular peaks as belonging to certain oligomers (e.g., dimer, trimer, etc.) or certain fragments of the monomer." Ex.1005, 149. In particular, "[s]edimentation coefficient distributions are used widely in the pharmaceutical industry to assess the stability of protein formulations and to characterize preparations of inherently heterogeneous samples (e.g., vaccines based on bacterial cell wall preparations)." Ex.1005, 149.

167. Cole also describes the instrumentation routinely used in SV-AUC analyses. Analytical ultracentrifuges are distinguished from other high-speed centrifuges by "the specialized rotors, sample holders and optical systems that permit the observation of samples during sedimentation." Ex.1005, 150. Samples are loaded into cells that permit the passage of light allowing for optical assessments during centrifugation. These "fundamental measurements" are known as "scans" and "are acquired at intervals ranging from minutes (for velocity sedimentation) to hours (for equilibrium sedimentation)." Ex.1005, 151. Cole

instructs in detail as to how to determine appropriate parameters to use for an SV-AUC experiment and how to perform the data analysis steps necessary to interpret the data. Ex.1005, 161-168.

#### 4. Schuck, P., "Size-Distribution Analysis of Macromolecules by Sedimentation Velocity Ultracentrifugation and Lamm Equation Modeling" 78 BIOPHYS. J. 1606-1619 (2000)

168. The reference referred to as Schuck is a scientific article entitled "Size-Distribution Analysis of Macromolecules by Sedimentation Velocity Ultracentrifugation and Lamm Equation Modeling." It was published in 2000, and I understand that it is prior art with regards to the '288 patent. Ex.1007. Schuck was cited in the '288 patent specification. Ex.1001, 17:56-59; 20:42-44; 20:65-21:1; 21:5-10; 24:28-30; 44:18-20; 45:44-46; 54:11-13.

169. Schuck is from the same field of endeavor as the '288 patent (concerning data analyses useful for determining sedimentation coefficients from SV-AUC data) and is reasonably pertinent to the problem the '288 patent sought to solve (providing useful data analysis tools for determining sedimentation coefficients from SV-AUC).

170. Schuck describes methods for analyzing analytical ultracentrifugation data using the SEDFIT software program (referenced in the '288 patent and prior art references), which Peter Schuck created and freely distributed to the public. Schuck discusses using SV-AUC to assess polymer size

distributions, and calls it "one of the principal problems in the study of biological macromolecules. . . ." In fact, SV-AUC is considered the gold standard in assessing macromolecular purity due to its solid theoretical first principles that it operates under, coupled with its unique ability to characterize a macromolecular sample in its native environment without any sample preparation (where it could be altered, absorbed into to high surface area materials, or filtered out of solution) other than possibly sample dilution. Ex.1007, 1606.

171. Schuck explains that the Lamm equation can be used to model boundaries of sedimenting particles, using a number of different models, the most common being that the sample consists of one or more independent ideal behaving discrete species (commonly referred to as the "continuous c(s) model." Ex.1007, 1607. Schuck "describes an extension of the Lamm equation analysis for the characterization of continuous size distributions of macromolecules." Ex.1007, 1607. In particular, "a method for direct boundary modeling for the size-distribution analysis in sedimentation velocity analytical ultracentrifugation" is described. Ex.1007, 1615. Schuck also describes important considerations scientists must consider when analyzing data from an SV-AUC experiment, including with respect to the regularization of data.

172. Finally, Schuck describes that "[a]ll computational methods were implemented into the Windows-based ultracentrifugal analysis program SEDFIT . . . ." Ex.1007, 1610.

### 5. Zhao, H. et al., "Overview of Current Methods in Sedimentation Velocity and Sedimentation Equilibrium Analytical Ultracentrifugation" 71 Curr. Protocols in Protein Sci. 20.12.1-20.12.49 (2013) ("Zhao")

173. The reference referred to as Zhao is a scientific review entitled "Overview of Current Methods in Sedimentation Velocity and Sedimentation Equilibrium Analytical Ultracentrifugation." Peter Schuck, the creator of the SEDFIT software used for SV-AUC analysis, is a co-author. It was published in 2013, and I understand it is prior art with regards to the '288 patent. Ex.1016.

174. Zhao is from the same field of endeavor as the '288 patent (concerning data analyses useful for determining sedimentation coefficients from SV-AUC data) and is reasonably pertinent to the problem the '288 patent sought to solve (providing useful data analysis tools for determining sedimentation coefficients from SV-AUC).

175. Zhao reviews basic principles of AUC analysis discussed above, but also discusses the need to regularize data obtained using AUC methods, because the observed "spikes" in the data are "highly unreliable and do not reflect the true information content of the data." Ex.1016, 10. Consequently, Zhao teaches that one must regularize the data to find the distributions that avoid "misleading details of

the peak structure" and minimize "over-interpretation." Ex.1016, 10. To achieve a fit that balances these concerns, "the error surface of the fit is skewed by a regularization penalty term and minimized, while the magnitude of the penalty term is adjusted such that the difference in the quality of fit to the raw data of the penalized solution does not diminish relative to the un-penalized overall best-fit by more than a permissible factor governed by F-statistics on a given, user-determined confidence level (e.g., P = 0.683)." Ex.1016, 10. Basically the fitting process is controlled so the fitting process doesn't start fitting the inherent noise in the data.

176. Zhao characterizes regularization as "an important and well-studied problem in data analysis in many fields of physical science" for which "[t]here is a large body of literature." Ex.1016, 11. Specifically, Zhao identifies "Tikhonov" and "maximum-entropy regularization" as regularization methods implemented in SEDFIT. Ex.1016, 11. Zhao explains that Tikhonov regularization is appropriate for "macromolecules with a broad intrinsic size distribution…or heavily glycosylated proteins…" Ex.1016, 11. On the other hand, maximum-entropy regularization "is particularly attractive for systems that likely have a few intrinsically discrete species, such as purified protein samples." Ex.1016, 11. Furthermore, maximum-entropy "is the default choice in SEDFIT," but "for typical SV data . . . both Tikhonov and maximum entropy regularization will result in very similar distributions." Ex.1016, 11. It should be noted that both forms of

regularization can be applied to the data analysis at the same time so experimenters can decide which one might be best for their particular situation.

177. Zhao also teaches about SV-AUC data analysis generally, indicating that the "continuous c(s) or just c(s)" method or model is the most common. Ex.1016, 30. In particular, "[u]seful parameters…include a resolution of 50-100, an s-range spanning the expected sedimentation coefficients, a starting frictional ratio of 1.2-1.4 (this parameter is set to be refined) . . . and a regularization P-value of 0.68." Ex.1016, 30. Zhao indicates that that P-value of 0.68 "controls the regularization level used . . . and a value of 0.68 strikes an excellent balance between the resolution of the distribution and the accuracy of its information content." Ex.1016, 30.

### C. Claims 1, 4-8, 10-15, 17-18, 20-21, 23 and 32-33 are Obvious in View of Le Bec

178. As discussed above, it is my opinion that Le Bec anticipates the method described in claims 1, 4-8, 10-15, 17-18, 20-21, 23, and 32-33 of the '288 patent. These claims would also have been obvious to a POSA based on the Le Bec reference alone.

#### 1. Claim 1

179. As discussed above, it is my opinion that Le Bec anticipates the method described in claim 1 of the '288 patent. *See* ¶65-77.

180. Additionally, claim 1 would have been obvious from the teaching of Le Bec. At least as of January 2015, and indeed well before then, the POSA was aware of the need to monitor viral preparations for gene therapy such as rAAV preparations for product homogeneity and purity because of requirements set by the clinic and regulatory agencies such as the Food and Drug Administration to market such a product. Ex.1004, 16; Ex.1008, 1, 6. This need motivated me in developing and publishing the 2007 article on SV-AUC techniques for adenoviral preparations (which had been a dominant viral vector of choice in the gene therapy area) to remind and educate those in the biotechnology area of this old analytical technology, which was making a comeback into main stream science with improved analytical capability that could fill important gaps in our ability to characterize gene therapy drug products (e.g., viral vectors and nanoparticles). Ex.1004, 16.

181. A POSA would understand boundary SV-AUC to be the top choice of the AUC-based approaches because of its ability to easily monitor the different particle sizes involved in virus characterization due to its enormous dynamic size range, high resolution, the absence of a high surface area that could absorb or interact with the sample, the ability to separate material not only on the basis of size and mass, but also density, the ability to test samples in their native state, and the additional information available regarding product homogeneity as compared

to SE-AUC. Ex.1003, 17:29-19:15; Ex.1005 145, 161-168. Indeed, these attributes of SV-AUC have led to AUC being referred to as the "Gold Standard" in the field of biophysical characterization of biological macromolecules and macromolecular complexes (Ex.1018, 22), which would include complexes such as AAV.

182. Further, integrating the area under the peaks revealed in an c(s) vs S (distribution of sedimentation coefficient) plot generated in an SV-AUC experiment is a standard technique used to determine the relative concentrations of components in the analyzed sample (where relative refers to either the total area of the distribution plot or to some defined range of its x-axis, sedimentation coefficient expressed in unit of S). Ex.1004, 25. As discussed above, peak integration was available in the SEDFIT software, and a POSA would have found it obvious to utilize the peak integration function to quantify peak size, as this lends necessary precision to the assessment of the viral preparation. Le Bec's own use of the SEDFIT software and reporting of relative concentrations of AAV particles would have indicated to a POSA that AAV particles could be effectively separated by SV-AUC and that relative concentrations could be determined by integrating the areas under the peaks using the SEDFIT software functionality, the same way it has been applied in other SV-AUC experiments.

183. Claim 1 would have been obvious to a POSA in view of Le Bec.

### 2. Claims 4-8 and 10-15

184. As discussed above, Le Bec anticipates the method described in claim 1 of the ’288 patent, and also claim 1 would have been obvious in view of Le Bec. Claims 4-8 are independent claims that have some limitations in common with claim 1, adding only additional limitations concerning comparisons that could be made between the relative amounts of species identified and quantified in an SV-AUC experiment. Claim 12 adds only that the presence of a peak in addition to the peak for full rAAV particles indicated heterogeneity.

185. For the reasons set forth above, a POSA would have been motivated to assess homogeneity of rAAV preparations in pursuit of a drug product of high quality (with regard to homogeneity and purity). Le Bec noted that empty AAV capsids were undesirable (Ex.1003, 3:16-22), which was generally recognized in the field. *See* Ex.1008, 1, 6. A POSA would have been motivated to use SV-AUC to compare the relative concentrations of rAAV particles present in a viral preparation, including the concentration of empty rAAV particles, full rAAV particles, and other species of rAAV particles (truncated genomes, aggregates, or other viral degradation products), to each other and/or to the total number of rAAV particles in the preparation. By doing these comparisons, a POSA would be seeking to quantify heterogeneity and thus assess sample purity. These

determinations would require only simple mathematical comparisons routinely performed by a POSA.

186. Claims 10 and 11 are claims that depend from claim 4, but further include comparison limitations like those included in claims 5-8. Claims 10 and 11 would have been obvious in view of Le Bec for the same reasons as claims 5-8.

187. Claim 13 depends from claim 12 and additionally recites that "the presence of additional peaks indicates the presence of empty capsid particles and/or recombinant AAV particles comprising variant genomes." Claim 14 depends from claim 13 and merely adds additional limitations concerning what falls within the understanding of "recombinant AAV particles comprising variant genomes." A POSA would understand that particles with different sedimentation coefficients and appearing at different peaks (different s values) represent, among other things, empty rAAV particles and/or AAV particles comprising variant genomes because that is how SV-AUC experiments work, as taught by the prior art. *See* Ex.1003, 18:8-23; Ex.1004, 25. Claims 13 and 14 would have been obvious in view of Le Bec.

188. Claim 15 depends from claim 12 and adds a limitation concerning integrating the area under each peak to determine the relative concentration. This limitation would have been obvious for the reasons set forth above with regard to the integration limitation of claim 1. *See* ¶¶82-85, 179-183.

### 3. Claims 17-18, 20-21, 23, and 32-33

189. As discussed above, Le Bec anticipates the additional limitations recited in claims 17-18, 20-21, 23, and 32-33, which depend from or incorporate claim 1, but these claims also would have been obvious in view of Le Bec alone.

190. Claim 17 recites the use of absorbance or interference to monitor sedimentation. These are two of the three methods that a POSA would have known were available for monitoring sedimentation in an SV-AUC experiment, and in fact it is possible to use both in the same experiment. Le Bec used absorbance.

191. Claim 18 recites that the preparation is an aqueous solution. The preparation in Le Bec was an aqueous solution and it would have been obvious to use an aqueous solution (i.e. a solution where the solvent is water), because aqueous solutions and buffers are commonly used when studying all biological material (including viral preparations) in their native state and is by far the most common solvent used in SV-AUC experiments.

192. Claim 20 recites that the c(s) values are determined by an algorithm that comprises Lamm equation solutions. The Lamm equation has been long known as the equation that describes the sedimentation of a single component or molecular species during ultracentrifugation in traditional sector-shaped cells. I understand it is named for Ole Lamm, who derived it during his Ph.D studies in 1929. The SEDFIT software, discussed above, uses the Lamm equation to simulate

the actual sedimentation boundaries of the resulting experimental SV-AUC data to determine what collection of Lamm equation solutions would best model the actual experimental SV-AUC data. A POSA would have been motivated to determine c(s) values in order to determine purity or homogeneity of the sample and it would have been obvious to a POSA to use computer software programs that use an algorithm that comprises the Lamm equation to determine c(s) values rather than attempt to calculate c(s) values by hand.

193. Claim 21 recites that the algorithm of claim 20 is "the SEDFIT algorithm." The SEDFIT software, was created by Peter Schuck and made publicly available for free in 2000. The SEDFIT software uses the Lamm equation to simulate sedimentation boundaries to model the resulting experimental SV-AUC data. The SEDFIT software was specifically referenced in Le Bec and it would have been obvious to a POSA to use the SEDFIT software with the claimed invention.

194. Claim 23 recites the use of an ultracentrifuge and ultracentrifuge velocity cell. A POSA would know that in an analytical ultracentrifugation experiment, use of an ultracentrifuge and special sector-shaped velocity cell would be necessary. As explained in the 2015 book chapter I published with John Philo:

> This sector shape is actually required, and is a critical element of any successful SV-AUC experiment. This need for a sector shape cell arises due to the radial nature of the centrifugal field and molecular flow. It is essential that the flow remains parallel to the walls of the

> sector. Any deviation from the appropriate sector shape and its appropriate alignment within the centrifugal field could lead to the flow hitting the walls, producing a local concentration increase that could violate the necessary stability criteria of a nonnegative density gradient across the entire sample solution running from the meniscus to the base of the solution compartment. If a negative density gradient is encountered anywhere along this path, solution inversion (mixing) will occur as a result of the density difference, leading to convective transport of the protein molecules causing erroneous sedimentation data to be generated.

Ex.1011, 220. A POSA would appreciate the potential problems with not using a sector-shaped cell, and thus it would have been obvious to use the ultracentrifuge velocity shaped cell.

195. Claim 32 recites a specific range of rotor speeds (3,000 rpm to 20,000 rpm) and/or a specific range of temperatures (about 4°C to about 20°C). Le Bec provides an example of using a rotor speed of 16,000 rpm. A POSA would have the skill to optimize the rotor speed and temperature parameters as needed based on the rAAV samples.

196. Claim 33 recites a method of evaluating a process for the production of rAAV particles that relies on the method of claim 1. Specifically, the SV-AUC method of claim 1 is used and an increase in the relative amount of full rAAV particles relative to empty particles indicates an improvement in the method of preparing the rAAV particles. Le Bec provides an example of using SV-AUC to compare two different methods of producing particles and evaluating them based on the relative percentage of full AAV particles. SV-AUC is a well-known and

understood tool for the separation and quantification of biomolecules, and it would have been obvious to a POSA to use the method to evaluate different methods for producing AAV particles.

### D. Claims 1-26 and 31-33 Are Obvious Over Le Bec In View Of Berkowitz and/or Cole

197. As discussed above, it is my opinion that Le Bec anticipates the methods of claim 1, 4-8, 10-15, 17-18, 20-21, 23, and 32-33 of the ’288 patent and/or renders them obvious. It is further my opinion these claims would have been obvious in view of Le Bec when combined with Berkowitz and/or Cole. Additionally, the additional limitations recited in claims 2-3, 26, and 31-32 would also be obvious in view of Le Bec combined with Berkowitz and/or Cole.

#### 1. Claim 1

198. As discussed above, it is my opinion that Le Bec anticipates and/or renders obvious the method described in claim 1 of the ’288 patent, nevertheless it would have further been obvious in view of Berkowitz.

199. Berkowitz taught the use of SV-AUC to characterize adenovirus preparations for use in gene therapy. The article explained that SV-AUC is a conventional tool with wide applicability to viral preparations and the ability to provide different types of information to assessing homogeneity, including identification and quantification of empty capsids, intact monomers, aggregates, and incompletely formed species. Berkowitz demonstrates that SV-AUC

techniques were widely known, and they had been successfully applied to viral preparations, especially in the early years (prior to 1980's) of biochemistry and molecular biology. For example, Berkowitz instructs as to the "overall capability of boundary sedimentation velocity analysis," refers to it as "conventional," and describes it as "a uniquely useful characterization tool that can assess adenovirus product quality and manufacturing consistency." Ex.1004, 16-18.

200. And while Berkowitz considered adenovirus specifically, a POSA would understand the applicability of the method extends far beyond adenovirus. Indeed, even without the benefits of Le Bec's teaching, a POSA would have expected that application of SV-AUV to AAVs would have been even easier than application to adenovirus, because they have a smaller mass and size, and reduced complexity. Larger particles such as adenovirus, have higher S-values and move more rapidly during centrifugation, which requires running the analytical centrifuge at lower speeds and can complicate sufficient data acquisition (lowest speed of operation is about 3,000 rpm). A POSA would not think AAV were too small, because SV-AUC has been applied to much smaller particles, e.g. monoclonal antibodies, much lower MW proteins, peptides, and even simple organic molecules like sucrose (*see* Ex.1005, 145), and the sedimentation coefficients of empty versus full particles (60S compared to >100S) are sufficiently distinct particles to be readily separated by SV-AUC.

201. A POSA would have been motivated to look to the teachings of Berkowitz to address purity and quality issues implicit in developing viral preparations for gene therapy (Ex.1004, 16; Ex.1008, 1) and understood that they would apply with minimal optimization to rAAV preparations.

202. Further, although integration of areas under the peak was a standard feature of the SEDFIT software, Berkowitz indicated that "quantification" of each particle would be accomplished by integration: "simple peak area integration … allows the percentage of EC [empty capsid] material present in a virus preparation to be readily calculated." Ex.1004, 20. One of the benefits of the c(s) distribution plot described in Berkowitz is its similarity to a chromatogram, where "each species produces a peak at its characteristic sedimentation coefficient, and the area under each peak gives the concentration of that species." Ex.1004, 25. When SEDFIT actually does integration it automatically computes the percentage relative to the total area. A POSA would have been motivated to use the peak integration to determine relative concentrations to assess homogeneity and would have had a reasonable expectation of success because of the disclosures of Le Bec further in view of Berkowitz.

203. Claim 1 would have been obvious in view of Le Bec and Berkowitz.

### 2. Claim 2 is Obvious Over Le Bec In View of Berkowitz, Cole and the Knowledge of a POSA

204. Claim 2 recites a method for evaluating the genome integrity within a monomeric rAAV particle SV-AUC peak of a rAAV samples by subjecting it to boundary sedimentation conditions, to determine its sedimentation coefficient so the size of its genome can be determined when combined with an appropriate standard calibration curve of genome size vs sedimentation coefficient, generated from rAAV particles of known genome sizes. Since all monomeric AAV particles having a completely assembled capsid would have the same frictional coefficient (f) their sedimentation coefficient (s) would be directly related to the molecular weight, buoyant molecular weight ($M_b$) or genome size (where one has assumed that all genomic material packaged inside the AAV capsid is contained within one genome – $s = M_b/f$). *See* ¶21. As discussed above, a POSA would understand Le Bec to disclose the claim limitations directed to performing boundary SV-AUC on rAAV preparations, visualizing the data as c(s) v. S plots, and calculating relative concentrations therefrom.

#### a. "A method to assess vector genome integrity of recombinant AAV particles in a preparation of recombinant AAV particles comprising"

205. Le Bec teaches a method for characterizing a viral preparation of rAAV particles that can analytically separate, detect, and quantify the presence of empty capsids, full capsids, and aggregates based on sedimentation coefficients.

Ex.1003, 16:25-29; 18:1-6. Further, Le Bec explained that "[t]he sedimentation coefficient of the various AAV viral particles (empty, full, aggregate) and other present populations (subparticles, contaminant proteins, aggregate) in the purified products was determined by real-time centrifugation." *Id.*, 15:17-23. A POSA would understand from Le Bec that the SV-AUC method used could be employed to identify and to quantify AAV particles, to characterize the composition and determine the extent to which the AAV particles comprised intact genomes (as demonstrated specifically in the case of Le Bec by the fact that the two full AAV peaks, differing in s value, also differ in their genome size – in this case by a factor of two).

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

206. See analysis for claim 1, ¶¶73-79.

**c. "b) plotting the differential sedimentation coefficient distribution value C(s) versus the sedimentation coefficient in Svedberg units (S), and"**

207. See analysis for claim 1, ¶¶80-81.

**d. "c) identifying species of recombinant AAV particles in the preparation by presence of peaks on the plot corresponding to an S value,"**

Figures 2 and 3 of Le Bec identify "empty" and "full" rAAV particles by peaks on an c(s) v. S plot corresponding to their S values. Ex.1003, Figures 2 and 3.

**e. "wherein the genome size of a particular species of recombinant AAV particles is calculated by comparing the S value of the species to a standard curve generated by S values of recombinant AAV particles comprising encapsidated AAV genomes of known nucleotide sizes."**

208. From Le Bec, a POSA would understand that empty and full rAAV particles have such sufficiently different sedimentation coefficients that the particles can be separated by SV-AUC (and analyzed through computer simulation work that could easily be done using SEDFIT). Further, the textual description of the experimental data reported in Le Bec (with empty capsids traveling at 65S and full capsids moving at between 80S to 110S (Ex.1003, 18:21-25)), mirrors what the inventors in Le Bec reported they expected based on the literature (i.e. reportedly 60S for empty particles and 110S for full particles (Ex.1003, 17:30-35)). Le Bec reported no unexpected deviations from a sedimentation perspective of the AAV capsids.

209. From this, a POSA would have considered the generation of a standard curve with rAAV particles containing genomes of known sizes to have

been obvious. A POSA would have been motivated to utilize a standard curve so as to be able to identify unknown species in the viral preparation to assess purity and homogeneity. Using a known sample with a known characteristic to characterize an unknown sample is a technique that appears in a variety of contexts. It could easily be applied to SV-AUC data on AAV particles with nothing more than routine techniques known to a POSA.

210. SV-AUC experiments are fundamentally biophysical measurements that can be evaluated with resort to mathematical equations. A POSA would have been aware of the definition of a particle's sedimentation coefficient (s) and its relationship to the particle's frictional coefficient and buoyant mass, which is governed by the following equation:

$$s \equiv \frac{v}{\omega^2 r} = \frac{M_b}{f} \qquad (1)$$

211. If you envision a hypothetical system where the rAAV particles are uniform in terms of their size and shape and thus have an identical frictional coefficient, the sedimentation coefficient will change in response to changes in the buoyant mass ($M_b$). That is to say, when the effective mass increases, the sedimentation coefficient will increase, too. In practice, we see this because full rAAV particles, which have a greater effective mass than empty AAV particles, also have a higher sedimentation coefficient (as is also the case with the two full AAV particles reported by Le Bec – one full AAV particle has a genome that is

half the size of the other full AAV particle causing the latter to have a significantly larger s value than the former full AAV particle (*see* ¶109, Ex.1018). A POSA would know that creating a curve by applying SV-AUC to AAV particles having different amounts of viral DNA would allow you to correlate the observed sedimentation coefficient and estimate the amount of DNA in an unknown particle. Indeed, even knowing the few points taught in Le Bec, where an empty AAV particle has a sedimentation coefficient of 65S, a full (single-stranded particle) has a coefficient of 90S, and a full (double-strand-forming particle) has a coefficient of 105S, a POSA could estimate the DNA size of a particle with a sedimentation coefficient between those values (for example the species at ~77S).

212. Further, as discussed above, a particle's buoyant mass is the particle mass, less mass of solvent displaced by the particle, which is a product of the particle's partial specific volume times the solution density. A POSA would understand that solution density is unchanged by the mass of the rAAV particles, and the changes to the partial specific volume would be minor relative to the actual mass changes involved. Ex.1003, 17:30-18:1 (citing density differences of 1.32 $g/cm^3$ for an empty rAAV particle and 1.45 $g/cm^3$ for an rAAV particle with a full, double-stranded particle with a maximum of 4,800 bases). Thus, a POSA would understand the buoyant mass of a particle to track closely with its actual mass for the purposes of calculating an S-value.

213. The other force a POSA would consider for an rAAV particle's expected S-value is the frictional force, *f*. However, for rAAV particles differing only by their genome size, a POSA would expect any changes would be internal and not affect the frictional force of the AAV capsid shell (to a good first approximation). Thus, a POSA would generally expect the S-value of an rAAV particle with a variant genome to vary linearly (to a good first approximation) with its buoyant mass $M_b$ (with the amount of DNA inside the AAV capsid being the major controlling factor influencing the change in a AAV particle's s value). Thus, a POSA would have had a reasonable expectation of success in constructing a standard curve based on rAAV particles of known genomic size using Le Bec's method (given the difference in s value between the "singled-stranded" and "double-stranded" AAVs particle observed by Le Bec), rendering the claim obvious.

214. Claim 2 would have been obvious in view of Le Bec when combined with Berkowitz and Cole.

**3. Claim 3**

Claim 3 depends from claim 2 and adds the further limitation of integrating the area under each peak to determine the relative concentrations. This limitation would have been anticipated and obvious for the reasons set forth above. *See* ¶73-

79, 179-183. Claim 3 would have been obvious in view of Le Bec when combined with Cole and Berkowitz.

**4. Claims 4-8 and 10-15**

215. As discussed above, it is my opinion that Le Bec anticipates and/renders obvious the methods described in claims 4-8 and 10-15 of the ’288 patent, and they would have further been obvious in view of Berkowitz.

216. Applying SV-AUC to AAV particles would have been obvious in view of Le Bec when combined with Berkowitz for the same reasons outlined for claim 1 above. *See* ¶¶73-79, 179-183.

217. Additionally, Berkowitz explains in greater detail the full scope of available analyses when applying SV-AUC to characterizing viral preparations for potential gene therapy applications, including to identify and quantify empty capsids, intact monomers, aggregates, and incompletely formed species. Specifically, Berkowitz teaches that you can determine:

> (1) amount of intact virus monomer and its heterogeneity, (2) amount of EC [Empty Capsid] material and its heterogeneity, (3) amount of virus aggregation and the distribution of aggregate sizes, (4) detection and quantification of other smaller structural forms of the intact and EC adenovirus particles formed during adenovirus assembly in vivo or from damage that may occur after release from infected cells during all phases of virus processing (which would include cell

> culture production, purification, vialing, or storage), and (5) accurate values for the total concentration of adenovirus material present in a virus sample….

Ex.1004, 17.

218. A POSA would have understood these analyses to be equally applicable to preparations of rAAV particles. A POSA would have been motivated to assess homogeneity of rAAV preparations in pursuit of a drug product of high quality (with regard to homogeneity, purity, and drug product consistency) and determine the percentage of empty rAAV particles (Ex.1008, 1, 6). And a POSA would have expected to apply these analyses with a reasonable expectation of success because they require only simple mathematical comparisons routinely performed by a POSA, and Le Bec itself demonstrated that a heterogenous mixture of AAV particles could be characterized by SV-AUC. Claims 4-8 and 10-15 do nothing more than claim analyses (or assessment of heterogeneity) consistent with the teachings of Berkowitz. That Berkowitz discussed using adenovirus instead of AAVs would not render the teachings of Berkowitz any less applicable. *See* ¶200.

219. Claims 4-8 and 10-15 would have been obvious in view of Le Bec combined with Berkowitz.

**5. Claims 9 and 16**

220. Claim 9 recites a method of monitoring the removal of empty capsids or capsids with variant genomes during purification of an rAAV

preparation by sampling the preparation, analyzing the samples using the method of claim 5, and recognizing that a decrease in empty capsids or capsids with variant genomes reflects removal of those species from the preparation. Claim 16 is similar, but it depends from claim 15 and it focuses on recognition that an increase in capsids containing intact genomes reflects an increase in homogeneity. Claims 5 and 15 are both anticipated and/or obvious, as explained above. Claims 9 and 16 add nothing significant to these claims.

221. The prior art, including Le Bec, teaches that empty capsids are undesirable. Ex.1003, 3:16-22; Ex.1008, 1, 6. Le Bec also taught that AAV particles could be accurately identified and quantified using SV-AUC data, and Gao suggested that AUC should be used for "quantitative characterization of structural heterogeneity of rAAV preparations." Ex.1008, 6.

222. Indeed, Gao stated its results "emphasize the necessity to develop and optimize good manufacturing practice-compatible manufacturing process for efficient removal of empty virus particles from rAAV production/purification process and enhancing therapeutic potency and safety profile of clinical gene therapy vectors." Ex.1008, 6. Thus, a POSA would have been motivated to develop a purification process for removing empty rAAV particles, and for using a characterization assay to confirm removal of such particles.

223. In the 2007 Berkowitz article, we provided an example in which two crude virus preparations were purified by two different purification methods and characterized by looking at analytical ultracentrifugation data. The figures show empty capsid peak(s) for one purification method and an absence of empty capsid peaks for the other purification method. The rAAV preparations in Le Bec were also subjected to purification steps. In the pursuit of a drug product of high quality (with regard to homogeneity and purity), a POSA would be motivated to subject the AAV preparation to a purification processes, and to confirm removal of empty rAAV particles (or increased homogeneity of the viral preparations) using SV-AUC at various steps in the process. In doing so, a smaller peak at the sedimentation coefficient for empty AAV particles would indicate the removal of empty AAV capsids. Similarly, an increase in the relative concentration of full AAV particles would indicate increased homogeneity.

224. A POSA would have had a reasonable expectation of success in monitoring purification of rAAV particles using SV-AUC, because its viability had already been demonstrated in the prior art (Ex.1003, Ex.1008), and because one of the benefits of SV-AUC is its ability to evaluate samples in their native state.

225. Claims 9 and 16 would have been obvious in view of Le Bec combined with Berkowitz.

### 6. Claims 17-25

226. As discussed above, it is my opinion that Le Bec anticipates and/renders obvious the methods described in claim 17-18, 20-21, and 23 of the ’288 patent, and they would have further been obvious in view of Cole and Berkowitz.

227. Claim 17 depends on claim 1 and recites that “the sedimentation of recombinant AAV particles is monitored by absorbance or interference.” Cole teaches that “[t]hree optical systems are available for the analytical ultracentrifuge (absorbance, interference, and fluorescence) that permit precise and selective observation of sedimentation in real time.” Ex.1005, 144. And in the experiments in my 2007 paper, we used interference and UV detectors. A POSA would have been motivated to choose a method of detection that would be effective in detecting sedimentation of rAAV particles to ensure that the SV-AUC method is accurately characterizing the viral preparation. A POSA would have understood that these three detectors were available, and also that while interference and absorbance detection could both be used with rAAV particles (and indeed could have been used at the same time), fluorescence detection might not be a feasible choice, since the pharmaceutical drug product sample may very likely need to be fluorescently labelled, which could alter the virus’s physicochemical properties leading to misleading results. A POSA would not consider fluorescence as an

option for rAAV characterization or testing work. In any event, given the limited number of options, it would have been routine to select the optimal method of detection for rAAV particles.

228. Claim 18 depends on claim 1 and further recites use of an aqueous solution. Le Bec used PBS, which is an aqueous buffer. Cole teaches about buffer requirements and indicates that "[m]ost of the commonly used buffer components are compatible with AUC experiments." Ex.1005, 160. In my 2007 publication, we used "GT buffer," which was an aqueous buffer with relatively high concentration of glycerol, an important stabilizing agent for adenovirus. In my experience, aqueous solutions are commonly used with viral preparations. Because one of the benefits of SV-AUC is testing the sample in its native state, a POSA would have been motivated to test samples in the buffer in which they have been prepared (which are often aqueous buffer), and they would have had a reasonable expectation of success in doing so, at least because the prior art showed SV-AUC can be performed with aqueous buffer.

229. Claim 19 depends from claim 18 and further comprises "wherein the monitoring further comprises comparison to a reference sample, wherein the reference sample comprises the aqueous solution without recombinant AAV particles." Berkowitz teaches that "even for normal boundary sedimentation velocity experiments steps still need to be taken to exactly match the buffer matrix

composition and filling volume between the reference and the sample solutions to eliminate artifacts caused by the sedimentation of buffer components in the case of refractive index detection (interference), or in the case of absorbance detection if the buffer components absorbs light at the wavelength at which the SV-AUC experiment is being monitored." Ex.1004, 21. Cole also teaches use of a reference sample. Ex.1005, 160 ("Samples should be equilibrated into the experimental buffer such that the composition of the reference and sample solutions is identical."), 161 ("The cells are filled with 430 µl of buffer in the reference sector and 420 µl of sample solution in the sample sector.").

230. Using a reference buffer sample in the reference sector channel of an AUC velocity cell in SV-AUC experiments is often employed to ensure that artifacts from the solvent matrix components do not interfere with the sedimentation measuring process on the material of interest. This is especially important when interference detection is used. However, in cases where the solvent matrix components absorb light it could also be important when using absorbance detection. Without an appropriate reference buffer sample in the reference sector channel of an AUC velocity cell, it is possible that the migration of the solvent matrix components during ultracentrifugation in the AAV test sample solution could be detected and disrupt the data analysis on AAV. To ensure no buffer or solvent artifacts are picked up, a POSA would be motivated to use a reference

buffer sample with the same exact solution matrix components as that present in the AAV test sample. This reference buffer solution would lack only the rAAV particles being measured. Doing so would have been accomplished with a reasonable expectation of success because it was known in the art and discussed in articles like Cole.

231. Claims 20 and 21 depend from claim 1 and add limitations concerning use of the Lamm equation and SEDFIT software. These were known and expressly referenced in Le Bec. Further, Cole discusses data analysis in detail, including application of the Lamm equation and use of SEDFIT software for SV-AUC experiments. In order to determine purity and homogeneity of the sample, a POSA would have been motivated to determine c(s) values and would have had a reasonable expectation of success using an algorithm that comprises Lamm equation solutions—like SEDFIT—because of the teachings of Schuck, Le Bec, Cole, and Berkowitz where SEDFIT is discussed and used.

232. Claims 22, 24, and 25 all concern allowing the AAV particles to fully sediment in an SV-AUC experiment. Cole teaches that, in determining rotor speeds for SV-AUC experiments, "[i]t should take a boundary at least 2 h to sediment the full length of the cell (1.5 cm maximum), to ensure sufficient scans will be acquired." Ex.1005, 164. Similarly, Berkowitz reports that for its adenoviral SV-AUC experiments, "scans covering the full range of boundary

movement were analyzed." Ex.1004, 17. Allowing the AAV particles to fully sediment is desirable because it ensures that the most data is collected and enables accurate calculation of sedimentation coefficients. Further, allowing sedimentation of the AAV particles with the smallest sedimentation coefficient to fully sediment ensures that the sedimentation coefficients of all species of interest will be accurately calculated. Thus, a POSA would be motivated to allow the rAAV species to fully sediment so that data could be obtained on the full range of species in the sample. Le Bec obtained data for species with very low sedimentation coefficients (<10S) which would indicate to a POSA that the samples were allowed to run for a long period of time, such that it is likely the rAAV particles with the lowest density and smallest size had cleared an optical window and reached the bottom of a sector of an ultracentrifuge. A POSA would have had a reasonable expectation of success implementing a method in with all rAAV species are allowed to fully sediment as it just requires letting the AUC experiment run for a longer time.

233. Claim 23 depends on claim 1 and further requires use of an ultracentrifuge velocity cell. Cole teaches that sedimentation velocity "experiments are carried out in two-channel cells with sector-shaped compartments (Fig. 1) in order to prevent convection, which would occur if the cell walls were not parallel to radial lines." Ex.1005, 161-162, Fig. 1; *see also* Ex.1011, 220. A POSA would

have been motivated to use a sector-shaped velocity cell to prevent convection, which would distort the SV-AUC data and lead to inaccurate results. Sector shaped cells were well known and described in the art, and a POSA would have been motivated to use a sector shaped velocity cell when running a sedimentation velocity experiment, and would have done so with a reasonable expectation of success.

### 7. Claim 26

234. Claim 26 depends from claim 22 and further comprises "wherein at least 30 scans are used to monitor sedimentation of recombinant AAV particles." Cole explains that when monitoring via optical absorbance as Le Bec did, that "it is necessary to consider the longer scan times and adjust the rotor speed so that at least 30-40 scans are recorded per cell during the movement of the boundary across the cell." Ex.1005, 164. A POSA would have been motivated to collect at least 30 scans/cell per SV-AUC experiment so sufficient data is acquired to ensure accurate and robust results. Setting the number of scans is routine and within the skill of a POSA to optimize and a POSA would have had a reasonable expectation of success modifying the SV-AUC parameters as needed to collect at least 30 scans.

235. Claim 26 would have been obvious in view of Le Bec combined with Cole.

### 8. Claim 31

236. Claim 31 depends from claim 1, and further requires "wherein the sedimentation of recombinant AAV particles is monitored about every 10-60 seconds." Le Bec indicates that it is performing its centrifugation in "real-time," (Ex.1003, 15:23) which indicates collecting scans at certain intervals and would have been necessary to generate the c(s) vs S plots in Figures 2 and 3. Ex.1003, 15:23. Cole teaches scan times of 60-300 seconds for the absorbance-based approach used by Le Bec. Ex.1005,152; Table II. Determining the number of scans to collect is a routine parameter within the purview of a POSA to optimize in any given experiment. In view of Cole, a POSA would have been motivated to take more frequent scans (at the bottom of the Cole range), in order to collect more robust data when using the SV-AUC method disclosed in Le Bec. It should be noted that the time interval or scan rate, which one should use in for collecting data scan when conducting an SV-AUC experiment, is a function of several factors that can be easily modelled in SEDFIT to determine an appropriate scan rate.

237. Claim 31 would have been obvious in view of Le Bec combined with Cole.

### 9. Claim 32

238. Claim 32 depends from claim 1 and further requires "wherein the boundary sedimentation velocity is performed at about 3,000 rpm to about 20,000

rpm and/or at about 4° C to about 20° C." Le Bec discloses that "centrifugation of the samples was carried out at a speed of 16,000 rpm using 100 µl or 400 µl of undiluted pure vectors . . . ." Ex.1003, 15:23-25. Cole teaches that ultracentrifugation should be performed between 4-35º C (which is basically the operating range for Beckman's XL-A/I instrument), but preferably at 20ºC. Ex.1005, 163 ("excessive oil vapor at operating temperatures above 35ºC and difficulty maintaining temperatures below 4ºC limit the useful temperature range."); *see also id.* ("Most experiments are conducted at 20ºC…"). Cole also teaches how to determine what rotor speed to use. Determining rotor speed and optimal temperature is a routine parameter within the purview of a POSA to optimize in any given experiment. In view of Cole, a POSA would have been motivated to and had a reasonable expectation of success in performing ultracentrifugation with rotor speed between 10,000 and 20,000 rpm and between 4ºC to about 20ºC.

239. Claim 32 would have been obvious in view of Le Bec combined with Cole.

**10. Claim 33**

240. Claim 33 recites a method of evaluating a process for the production of rAAV particles that relies on the method of claim 1. Specifically, the SV-AUC method of claim 1 is used as an analytical tool or method to monitor for the

increase in the amount of full rAAV particles relative to the amount of empty particles, which would indicate an improvement in the method of preparing the rAAV particles. Le Bec provides an example of using SV-AUC to compare two different methods of producing particles and evaluating them based on the relative percentage of full AAV particles. Further, Berkowitz teaches the importance of purity (i.e. homogeneity) in pharmaceutical preparations (Ex.1004, 16), and that sedimentation distribution data can be used to compare/evaluate different purification processes (*id.*, 20-21). SV-AUC is a well-known and understood tool for the separation and quantification of biomolecules, and it would have been obvious to a POSA to use the method to evaluate different methods for producing AAV particles. Given the desire for full rAAV particles in gene therapy products (As set forth above), a POSA would have been motivated to monitor the homogeneity rAAV particles using SV-AUC during purification and would have reasonably expected success doing so (as demonstrated by Le Bec).

241. Claim 33 would have been obvious in view of Le Bec combined with Cole.

### E. Claims 27-30 are Obvious Over Le Bec In View Of Berkowitz and Schuck and/or Zhao

#### 1. Claim 27

242. Claim 27 depends from claim 20, and further requires "wherein regularization is applied to a fitting level with a confidence level of F statistic of at

least about 0.68." A POSA would understand that regularization is a data analysis technique used to prevent overfitting data (which would lead the fitting process to fit the inherent noise in the data and needs to be avoided), and F statistics are commonly used to assess significance of a model's fit. Ex.1005, 166; Ex.1016, 10. An F statistic parameter controls the amount of regularization used. Values from 0.5 to 0.999 correspond to probabilities P (confidence levels). A POSA would be motivated to choose a regularization confidence level that smooths the distribution without introducing artifacts into the data. This typically leads many investigators to use a value of 0.68, which corresponds to one standard deviation in the inherent noise level in the collected data.

243. A POSA would have been motivated and expected success from using "a regularization is applied to a fitting level with a confidence level of F statistic of at least about 0.68" because Berkowitz demonstrated regularizing data in SEDFIT resulted in an SV-AUC assay for adenoviral preparations that successfully discriminated between full, empty, and aggregate particles. *See* Ex.1004, 27-30, Figs. 6, 7(A), 7(B), 8(A), 8(B), 8(C). Indeed, a POSA would have considered such practice an essential part of calculating c(s) values from any SV-AUC data in SEDFIT, as Cole and Zhao disclose. Ex.1005, 166 ("The resulting c(s) function is often quite ''spiky,'' and a regularization procedure is performed to produce a smoother distribution function.") Ex.1016, 10 (regularization is

necessary to deal with "misleading details of the peak structure" and minimize "over-interpretation.".) Thus, a POSA would have been motivated to choose confidence level of F statistic of at least about 0.68 to ensure smooth data without artifacts, and would have had a reasonable expectation of success doing so based on express teachings of the prior art.

**2. Claims 28-29**

244. Claims 28 and 29 depend from claim 27 and, in addition to the 0.68 confidence level required by claim 27, require that the regularization is "a second derivative regularization" (claim 28) or "Max entropy regularization" (claim 29) (which are the only two forms of regularization available in SEDFIT). A POSA would have found the use of these regularization methods obvious over Le Bec in view of the combination of Berkowitz, Schuck, and Zhao (in fact both forms of regularization can be applied at the same time to allow the user to determine which one would be best to use).

245. A POSA would consider performing a regularization procedure an essential step in calculating a c(s) distribution and would have been motivated to choose a regularization method for more accurate and robust data analysis. Schuck teaches that during the development of the SEDFIT software, "[a]s regularization methods, maximum entropy regularization and Tikhonov–Phillips regularization with a second derivative operator were studied." Ex.1007, 1617. Schuck further

demonstrates the successful use of maximum entropy regularization: "Shown are results for α = 0 (distributions with spikes, at 10-fold reduced scale) and with maximum entropy regularization and adjusted to a probability of p = 0.68 (smooth curves)." Ex.1007, 1611. (Fig. 1 inset). Moreover, as Zhao confirms, both methods are integrated into SEDFIT and that "for typical SV data…both Tikhonov and maximum entropy regularization will result in very similar distributions." Ex.1016, 11.

246. A POSA regularizing SV-AUC data using SEDFIT as described in Le Bec would have been motivated and have had a reasonable expectation of success in using either "second derivative regularization" or "Max entropy regularization" to analyze SV-AUC data relating to rAAV particles, as these are the available options in the SEDFIT software and described in the prior art.

**3. Claim 30**

247. Claim 30 depends from claim 20, and further requires "wherein the following C(s) parameters are held constant:"

**a. "resolution of about 200 to about 5000,"**

248. In SEDFIT, resolution is the number of S values between the minimum and maximum sedimentation coefficient distribution range that will be considered in the analysis of the SV-AUC data. For example, for a sedimentation coefficient range of 0-50S, a resolution of 100 would result in S-values increments

of every 0.5S. Although in principle resolution values can take any range of values, in my experience, practical ranges typically vary over the range 50 – 300, which can give a useful amount of resolution to a c(s) vs S plot without pushing the amount of computer processing time needed to process the data to excessively long times (because as resolution is increased the amount of computer processing time needed increases exponentially, making data analysis impractical from a time basis). The s value range for the c(s) v. S plots disclosed in Figures 2 and 3 of Le Bec appear to be approximately 1-140S. In my opinion, a POSA would have been motivated to use a resolution value of at least 200 to properly visualize the different rAAV species in the composition over its range of ~1-140S. Moreover, a POSA would be further motivated not to use a resolution greater than about 5000, given the extreme computational effort required to perform the SEDFIT analysis at that level of resolution, which would result in a lengthy amount of computer time to generate results. Indeed, even going to resolution values higher than 500 can require very long computational times. Thus, a POSA would have found it obvious to contain resolution values in the range of about 200 to about 5000 in the method of Le Bec (with values greater than 500 being very unlikely).

**b. "$S_{min}$ is about 1 S to about 100 S",**

249. Le Bec does not disclose the precise $S_{min}$ value it used in its SEDFIT analysis of its SV-AUC data, though its c(s) v. S plots appear to have an axis

which has a minimum s value of ~1S. Ex.1003, Figs. 2 and 3. In any event, a POSA would have been motivated and had a reasonable expectation of success in using an $S_{min}$ value sufficiently less than 60S, but at least about 1S, so that it could properly visualize the empty rAAV particles at 60S along with other AAV degradation material or incompletely assembled AAV material, given Le Bec's recognition that an earlier reference had taught this was the expected S-value for these species. Ex.1003, 17:30-18:1. Choosing the minimum S-value parameter is routine and within a POSA's skill to optimize. A POSA would have found it obvious to use a $S_{min}$ of between an s value of 1S to some s value less than the known S-value for an empty AAV capsid under the experimental conditions being used to do SV-AUC in the method of Le Bec.

**c. "$S_{max}$ is about 100 S to about 5000 S,"**

250. Le Bec does not disclose the precise $S_{max}$ value it used in its SEDFIT analysis of its SV-AUC data, though its c(s) v. S plots appear to show species with up to 140S, which would requcire that the $S_{max}$ value at least 140S. Ex.1003, Figs. 2 and 3. It is clear from the same figures that the $S_{max}$ value is less than "about 5000 S." When performing an analysis on SEDFIT, the program will inform the user if the $S_{max}$ value should be increased. A POSA would understand Le Bec to disclose this limitation.

#### d. "and frictional ratio is about 1.0 or is left to float to a value determined by centrifugation software"

251. The frictional ratio ($f/f_0$) is a number that represents the deviation of the frictional coefficient of the particle (f) from the frictional coefficient of a perfect sphere ($f_0 = 1$). Particles having the same frictional coefficient as a perfect sphere have a frictional ratio of 1.0.

252. Le Bec does not disclose whether it defined a frictional value or left it to float in its SEDFIT analysis of its SV-AUC data, but a POSA would be required to choose one of those options using Le Bec's SV-AUC method and processing the data using SEDFIT. Zhao teaches a useful "starting frictional ratio of 1.2-1.4" but also notes that "this parameter is set to be refined (needs to be optimized via the computer program)" and that "*f/f0* -values between 1.1 – 1.2 are obtained for highly globular particles." Ex.1016, 4, 30. Cole teaches that the "parameter is set to be refined (optimized as noted above)" indicates that "[m]ore complex analysis procedures (model) that do not assume a single value of $f/f_0$ are also implemented in SEDFIT…" Ex.1005 at 167. The prior art thus teaches use of a frictional ratio of 1.0 or allowing the frictional ratio to float.

253. A POSA would have been motivated to and have had a reasonable expectation of success in allowing SEDFIT to determine the frictional ratio because it is known that that approach generally returns more accurate analysis than constraining the $f/f_0$ ratio to a fixed value since the weight-average value of

this parameter for all macromolecular species is being used as the best value for this parameter in the data analysis and this parameter could be influenced by other factors the experimenter might not be aware of causing this parameter to deviate from a value of 1.0. Thus, a POSA would have found the use of Le Bec's method where the "frictional ratio is about 1.0 or is left to float to a value determined by centrifugation software" obvious. And a POSA would have been able to implement either approach with a reasonable expectation of success, as the selection of the frictional ratio is one of the features of the SEDFIT software, as discussed above. *See* ¶¶32-33.

### F. Claim 34 is Obvious Over Colosi In View Of Le Bec

254. Claim 34 recites a method of preparing rAAV particles using host cells and nucleic acids, isolating the rAAV particles, and analyzing them according to the methods of claim 1.

255. I understand that the patent examiner stated the following about Colosi:

> Colosi teaches a method of producing recombinant AAV (rAAV) virus particles comprising:
> (a) introducing an AAV vector into a suitable host cell;
> (b) introducing a AAV helper function vector into the host cell; wherein said vector is comprising:
> an AAV rep coding region;
> an AAV cap coding region; and

> a nucleotide sequence comprising a modified AAV p5 promoter region, such that the modified
> AAV p5 promoter region no longer functions in transcription initiation;
> (c) expressing accessory functions in the host cell; and
> (d) culturing the host cell to produce rAAV virions (reference claims 1-2, 5).
> Colosi teaches that with unlike previously described methods for producing rAAV, the AAV helper function vectors generate 10 high titer stocks of rAAV but do not produce any detectable wild-type AAV (¶[0029]).

Ex.1002, 527-528.

256. I also understand that in response the applicant argued that:

> Colosi described methods of making recombinant AAV particles, but Colosi is silent regarding any method of determining recombinant AAV particle impurities, much less AUC. Given the significant differences between the adenoviruses disclosed in van Tricht as discussed above, one of skill in the art would not have predicted with a reasonable expectation of success that methods described for a virus such as adenovirus could be applied to recombinant AAV particles. One of skill in the art certainly would not have expected the sensitivity, precision, and accuracy discussed above.

Ex.1002, 542. Thus, it is my understanding that the apparent basis for allowance of claim 34 was the alleged absence of the use of AUC with rAAV particles in the prior art under consideration (van Tricht).

257. Le Bec teaches the use of SV-AUC with rAAV particles to characterize the viral preparation and determine impurities. A POSA would have been motivated to use the characterization method of Le Bec to assess the purity and homogeneity of the rAAV particles being made by the method in Colosi. Such a method would allow the preparer of the viral preparations to assess the quality of rAAV particles being produced and assess potential errors in their process. And a POSA would have had a reasonable expectation of success applying SV-AUC because Le Bec taught it was possible to use SV-AUC to separate, identify, and quantify species in an rAAV preparation.

## IX. DECLARATION

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

Date: 06-30-23

Steven Berkowitz, Ph.D

## Exhibit A

In addition to the documents referenced in my declaration, I considered the following materials:

| Exhibit | Description |
|---|---|
| 1001 | U.S. Patent No. 10,429,288 ("the '288 patent") |
| 1002 | Portions of the File History of the '288 patent |
| 1003 | WO2014/125101 A1 and certified English translation ("Le Bec") |
| 1004 | Steven A. Berkowitz & John S. Philo, *Monitoring the homogeneity of adenovirus preparations (a gene therapy delivery system) using analytical ultracentrifugation*, Analytical Biochemistry 362:16-37 (2007) |
| 1005 | James L. Cole et al., *Analytical Ultracentrifugation: Sedimentation Velocity and Sedimentation Equilibrium*, Methods in Cell Biology 84:143-79 (2008) |
| 1007 | Peter Schuck, *Size-Distribution Analysis of Macromolecules by Sedimentation Velocity Ultracentrifugation and Lamm Equation Modeling*, Biophysical J. 78(3):1606-19 (2000) |
| 1008 | Kai Gao et al., *Empty virions in AAV8 vector preparations reduce transduction efficiency and may cause total viral particle dose-limiting side effects*, Molecular Therapy – Methods & Clinical Development 1(9):1-8 (2014) |
| 1011 | Steven A. Berkowitz & John S. Philo, *Characterizing Biopharmaceuticals using Analytical Ultracentrifugation*, *in* Biophysical Characterization of Proteins in Developing Biopharmaceuticals 211-60 (2015) |
| 1015 | U.S. Patent Application Publication No. US 2009/0017542 A1 |
| 1016 | Huaying Zhao et al., *Overview of Current Methods in Sedimentation Velocity and Sedimentation Equilibrium Analytical Ultracentrifugation*, Current Protocols in Protein Science Supplement 71:20.12.1-20.12.49 (2013) |
| 1017 | Douglas M. McCarty, *Self-complementary AAV Vectors; Advances and Applications*, Molecular Therapy 16(10): 1648-56 (2008) |
| 1018 | Timothy R. Dafforn, *So how do you know you have a macromolecular complex?*, Acta Crystallographica D63:17-25 (2007) |