UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

SAREPTA THERAPEUTICS, INC.,
Petitioner

v.

GENZYME CORPORATION,
Patent Owner.

Case: IPR2026-00167
Patent No. 11,698,377

DECLARATION OF JOHN A. MCLEAN IN SUPPORT OF PATENT OWNER'S PRELIMINARY RESPONSE

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I. INTRODUCTION

1. I submit this declaration in connection with Patent Owner's Preliminary Response in the *Inter Partes* Review IPR2026-00167 (the "IPR") of claims 1-20 ("challenged claims") of U.S. Patent No. 11,698,377 (the "'377 patent"; Ex. 1001). I have been retained by Dechert LLP, counsel for Patent Owner Genzyme Corporation in this matter. I am being compensated for my time at my standard rate of $700 per hour plus actual expenses. The opinions in this declaration are my own, and my compensation is not dependent in any way upon the outcome of the IPR of the '377 patent.

II. PROFESSIONAL QUALIFICATIONS

1. I provide the below summary of my professional and educational qualifications. For a more complete account of my professional and educational experience, I have attached as EX2025 my curriculum vitae, which provides significant detail on my background, credentials, and includes a list of my publications and invited presentations between 1998 and 2025.

2. I received my Bachelor of Science degree in Chemistry from the University of Michigan in 1995. I received my M.Phil and Ph.D. in Chemistry from The George Washington University in 1998 and 2001, respectively.

3. I was a postdoctoral fellow at The George Washington University in the Department of Chemistry in 2001 under Professor Akbar Montaser, and a Postdoctoral Research Associate at Texas A&M University from 2001 to 2006

under Professor David H. Russell.

4. As a PhD student I invented new technologies for the direct sample introduction of limited, expensive, and toxic samples into inductively coupled plasma mass spectrometers (ICPMS) for ultratrace elemental analysis.

5. In my postdoctoral research at Texas A&M University, I designed, developed, and constructed ion mobility and ion mobility-mass spectrometry instrumentation for characterization of complex biological samples.

6. In my independent scientific career as a faculty member at Vanderbilt University, I have continued the design, development, and construction of ion mobility, mass spectrometry, and coupled ion mobility-mass spectrometry instrumentation (IM-MS). This is primarily in the areas of complex biological samples in health and medicine and in systems, synthetic, and chemical biology. I am a noted pioneer in such instrumentation, such as imaging IM-MS, IM-MS/MS, LC-IM-MS, LC-IM-MS/MS, MS, LC-MS, integrated omics generally, and proteomics, lipidomics, glycomics, metabolomics, and exposomics specifically. We have also led numerous research projects in drug discovery and new strategies for drug characterization using MS and IM-MS techniques in clinical, targeted, and untargeted analyses from complex sample biological samples.

7. Since 2006, I have served in a number of different capacities on the faculty of Vanderbilt University, including as Chair of the Vanderbilt University

Faculty Senate, Chair of the Department of Chemistry, and Associate Provost for Graduate Education.

8. Since 2014 I have served as the founding director of the Center for Innovative Technology, which is a shared core resource that provides broadscale analyses of complex biological samples using MS, LC-MS, LC-MS/MS, IM-MS, LC-IM-MS, and LC-IM-MS/MS with collaborative efforts nationally and internationally.

9. Currently, I am the Stevenson Professor of Chemistry and Dean of Research and Graduate Education for the College of Arts & Science at Vanderbilt. I am an elected Fellow of the National Academy of Inventors, an elected Fellow of the American Association for the Advancement of Science, a Fellow of the Royal Society of Chemistry, and Inaugural Fellow of the American Society for Mass Spectrometry. I have received numerous awards in my career related to mass spectrometry, including the Charles H. Herty Medal from the American Chemical Society (2024), the Eastern Analytical Symposium Award for Outstanding Achievements in Mass Spectrometry (2023), the Chancellor's Award for Research (2022), the American Society for Mass Spectrometry Research Award (2008), the Bunsen- Kirchhoff Prize from the Society of German Chemists (GDCh - 2001), an R&D-100 Award (1999), and numerous teaching awards.

10. Since 2014 my laboratories have been recognized as an Agilent

Thought Leader Award laboratory. Agilent Technologies is a world leader in mass spectrometry instrumentation pertinent to this matter. According to Agilent, the Thought Leader Program, selects a few very influential thought leaders in well-defined focus areas. My laboratory is the first Agilent Thought Leader program in mass spectrometry and more generally in integrated biology.

11. Since 2014 my laboratories have been designated by Waters Corporation as a Waters Center of Innovation. Waters Corporation is a world leader in chromatography and mass spectrometry instrumentation pertinent to this matter. According to Waters, the Waters Centers of Innovation Program recognizes analytical scientists facilitating breakthroughs in health and life science research, food safety, environmental protection, sports medicine and many other areas.

12. I have published over 200 peer-reviewed articles in my career, primarily focusing on mass spectrometry, ion mobility-mass spectrometry, and including numerous publications in the fields of LC-MS and LC-MS/MS. Many of my papers focus on LC-MS and LC-MS/MS of proteins, including biological materials, bacteria, and toxins.

13. I am named on 30 patents and patent applications, including many related to mass analysis, mass spectrometry, IM-MS, and characterization of chemical and biological samples by mass spectrometry.

14. I have presented invited lectures at over 200 meetings and symposia

in my career and presented and contributed to many hundreds more, with the focus of these presentations being mass spectrometry, LC-MS, LC-MS/MS, and IM-MS including of proteins.

15. I have conferred degrees on over two-dozen students, who have gone on to take leading roles in analytical laboratories in industry (including at AbbVie, Janssen, AstraZeneca, Sanofi, Waters Corporation, and Agilent Technologies) and academia (including Vanderbilt University, University of Toronto, University of Georgia, and University of North Carolina).

III. MATERIALS CONSIDERED

16. My analysis is based on my years of education, research, experience, and background, as well as my investigation and study of relevant materials for this declaration, including the Petition and Dr. Coon's declaration (EX1003, "Coon Decl."). In addition to the Petition and Coon Declaration, I reviewed and considered the materials identified in Dr. Coon's declaration and additional literature cited throughout my declaration and as set forth in the list below.

Exhibit	Description
EX1001	U.S. Patent No. 11,698,377 ("the '377 patent")
EX1002	Prosecution history of U.S. Patent No. 11,698,377 ("the '377 prosecution history")
EX1003	Expert Declaration of Joshua J. Coon, Ph.D.
EX1004	*Curriculum vitae* of Joshua J. Coon, Ph.D.

Exhibit	Description
EX1005	Satkunanathan *et al*., "Establishment of a Novel Cell Line for the Enhanced Production of Recombinant Adeno-Associated Virus Vectors for Gene Therapy," *Human Gene Therapy* 25.11 (2014): 929-941 ("Satkunanathan")
EX1006	Shytuhina *et al*., "Development and application of a reversed-phase high-performance liquid chromatographic method for quantitation and characterization of a Chikungunya virus-like particle vaccine," *Journal of Chromatography A* 1364 (2014): 192-197 ("Shytuhina")
EX1007	Alqahtani, "Analysis of purified wild type and mutant adenovirus particles by SILAC based quantitative proteomics," *Journal of General Virology* 95.11 (2014): 2504-2511 ("Alqahtani")
EX1008	Anacleto and Boyd, "Calibration of ion spray mass spectra using cluster ions," *Organic Mass Spectrometry* 27.6 (1992): 660-666 ("Anacleto")
EX1009	Ansong *et al*., "Top-down proteomics reveals a unique protein S-thiolation switch in *Salmonella Typhimurium* in response to infection-like conditions," *Proceedings of the National Academy of Sciences* 110.25 (2013): 10153-10158 ("Ansong")
EX1010	Ayuso *et al*., "Manufacturing and Characterization of a Recombinant Adeno-Associated Virus Type 8 Reference Standard Material," *Human Gene Therapy* 25.11 (2014): 977-987 ("Ayuso")
EX1011	Becerra *et al*., "Direct mapping of adeno-associated virus capsid proteins B and C: a possible ACG initiation codon," *Proceedings of the National Academy of Sciences* 82.23 (1985): 7919-7923 ("Becerra")
EX1012	Bondarenko *et al*., "Mass Measurement and Top-Down HPLC-MS Analysis of Intact Monoclonal Antibodies on a Hybrid Linear Quadrupole Ion Trap-Orbitrap Mass Spectrometer," *Journal of the American Society for Mass Spectrometry* 20.8 (2009): 1415-1424 ("Bondarenko")
EX1013	Burova and Ioffe, "Chromatographic purification of recombinant adenoviral and adeno-associated viral vectors: methods and implications," *Gene Therapy* 12.1 (2005): S5-S17 ("Burova")
EX1014	Byeon *et al*., "Structural Identification of a Non-Glycosylated Variant at Ser126 for O-Glycosylation Site from EPO BRP, Human Recombinant Erythropoietin by LC-MS Analysis," *Molecules and Cells* 38.6 (2015): 496-505 ("Byeon")

Exhibit	Description
EX1015	Cecchini *et al*., "Toward exascale production of recombinant adeno-associated virus for gene transfer applications," *Gene Therapy* 15.11 (2008): 823-830 ("Cecchini")
EX1016	Chelius *et al*., "Analysis of the adenovirus type 5 proteome by liquid chromatography and tandem mass spectrometry methods," *Journal of Proteome Research* 1.6 (2002): 501513 ("Chelius")
EX1017	Chen *et al*., "Molecular characterization of adeno-associated viruses infecting children," *Journal of Virology* 79.23 (2005): 14781-14792 ("Chen")
EX1018	Coon *et al*., "Tandem mass spectrometry for peptide and protein sequence analysis." *Biotechniques* 38.4 (2005): 519523 ("Coon 2005")
EX1019	Coon, "Collisions or electrons? Protein Sequence Analysis in the 21st Century," *Anal. Chem*. (2009): 3208-3215 ("Coon 2009")
EX1020	Cueto-Rojas, "Interferon-α 2b quantification in inclusion bodies using Reversed Phase-Ultra Performance Liquid Chromatography (RP-UPLC)," *Journal of Chromatography B* 878.13-14 (2010): 1019-1023 ("Cueto-Rojas")
EX1021	Davis *et al*., "Rational Design and Engineering of a Modified Adeno-Associated Virus (AAV1)-Based Vector System for Enhanced Retrograde Gene Delivery," *Neurosurgery* 76.2 (2015): 216-225 ("Davis")
EX1022	Dong *et al*., "Proteomics analysis of co-purifying cellular proteins associated with rAAV vectors," *PLoS One* 9.2 (2014): e86453 ("Dong")
EX1023	Glish and Vachet, "The Basics of Mass Spectrometry in the Twenty-First Century," *Nature Reviews Drug Discovery* 2.2 (2003): 140-150 ("Glish")
EX1024	Good and Coon, "Advancing proteomics with ion/ion chemistry," Mass Spectrometry For Proteomics Analysis Review, *Biotechniques* 40.6 (2006): 783-789 ("Good")
EX1025	Grimm and Kay, "From Virus Evolution to Vector Revolution: Use of Naturally Occurring Serotypes of Adeno-Associated Virus (AAV) as Novel Vectors for Human Gene Therapy," *Current Gene Therapy* 3.4 (2003): 281-304 ("Grimm and Kay")

Exhibit	Description
EX1026	Han, *et al*. "Extending top-down mass spectrometry to proteins with masses greater than 200 kilodaltons," *Science* 314.5796 (2006): 109-112 ("Han")
EX1027	Huang *et al*., "In Vivo Deamidation Characterization of Monoclonal Antibody by LC-MS/MS," *Analytical Chemistry* 77.5 (2005): 1432-1439 ("Huang")
EX1028	Loo *et al*., "Tandem Mass Spectrometry of Very Large Molecules: Serum Albumin Sequence Information from Multiply Charged Ions Formed by Electrospray Ionization," *Analytical Chemistry* 63.21 (1991): 2488-2499 ("Loo")
EX1029	Mahoney and Hermodson, "Separation of Large Denatured Peptides by Reverse Phase High Performance Liquid Chromatography; Trifluoroacetic Acid as a Peptide Solvent," *Journal of Biological Chemistry* 255.23 (1980): 1119911203 ("Mahoney")
EX1030	Merten and Al-Rubeai, *Viral Vectors for Gene Therapy*, Vol. 737, Totowa, NJ: Humana Press, 2011 ("Merten")
EX1031	Rayaprolu *et al*. "Comparative analysis of adeno-associated virus capsid stability and dynamics," *Journal of Virology* 87.24 (2013): 13150-13160 ("Rayaprolu")
EX1032	Rhoads *et al*., "Neutron-Encoded Mass Signatures for Quantitative Top-Down Proteomics," *Analytical Chemistry* 86.5 (2014): 2314-2319 ("Rhoads")
EX1033	Richards *et al*., "Proteome sequencing goes deep," *Current Opinion in Chemical Biology* 24 (2015): 11-17 ("Richards")
EX1034	Riley *et al*., "Activated Ion Electron Transfer Dissociation for Improved Fragmentation of Intact Proteins," *Analytical Chemistry* 87.14 (2015): 7109-7116 ("Riley")
EX1035	Rouse *et al*., "Top-down characterization of protein pharmaceuticals by liquid chromatography/mass spectrometry: Application to recombinant factor IX comparability – A case study," *Therapeutic proteins: Methods and Protocols*, Totowa, NJ: Humana Press, 2005, 435-460 ("Rouse")
EX1036	Siuzdak, "Probing Viruses with Mass Spectrometry," *Journal of Mass Spectrometry* 33.3 (1998): 203-211 ("Siuzdak")
EX1037	Yuan *et al*., "Reversed-phase high-performance liquid chromatography of

Exhibit	Description
	virus-like particles," *Journal of Chromatography A* 816.1 (1998): 21-28 ("Yuan")
EX1038	Zabrouskov *et al*., "Stepwise deamidation of ribonuclease A at five sites determined by top-down mass spectrometry," *Biochemistry* 45.3 (2006): 987-992 ("Zabrouskov")
EX1039	Zubarev and Makarov, "Orbitrap Mass Spectrometry," *Analytical Chemistry*, (2013): 5288-5296 ("Zubarev")
EX1040	*Genzyme Corp. v. Sarepta Therapeutics, Inc. and Sarepta Therapeutics Three, LLC*, Second Amended Complaint, C.A. No. 24-cv-00882-RGA (D. Del.)
EX2021	Van Vliet *et al.*, "Adeno-associated virus capsid serotype identification: Analytical methods development and application," *J. Virol. Methods* 159 (2009): 167-177 ("Vliet")
EX2022	Bark *et al.*, "High-Temperature Protein Mass Mapping Using a Thermophilic Protease," *J. Am. Chem. Soc.* 123 (2001): 1774-1775 ("Bark")
EX2024	Expert Declaration of John A. McLean, Ph.D.
EX2025	*Curriculum Vitae* of John A. McLean, Ph.D.
EX2026	Wörner and Heck *et al.*, "Adeno-associated virus capsid assembly is divergent and stochastic," *Nature Communications*, 12:1642 (2021): 1-9 ("Wörner")
EX2027	Kontogiannis *et al.*, "Characterization of AAV vectors: A review of analytical techniques and critical quality attributes," *Mol. Ther. Methods and Clinical Development*, Vol. 32 (Sept. 2024): 1-17 ("Kontogiannis")
EX2028	Salganik *et al.*, "Evidence for pH-Dependent Protease Activity in the Adeno-Associated Virus Capsid," *Journal of Virology*, 86:21 (Nov. 2012):11877-11885 ("Salganik")
EX2029	Supplemental Materials to Ansong *et al*., "Top-down proteomics reveals a unique protein S-thiolation switch in *Salmonella Typhimurium* in response to infection-like conditions," *Proceedings of the National Academy of Sciences* 110:25 (2013): 10153-10158 ("Ansong Supp.")

Exhibit	Description
EX2030	Lancaster and Ha *et al.*, "Characterization of *N*-glycosylation profiles from mammalian and insect derived chikungunya VLP," *Journal of Chromatography B*, 1032 (2016): 218-223 ("Lancaster")
EX2031	ICH Harmonised Tripartite Guideline: Validation of Analytical Procedures: Text and Methodology Q2(R1) (Nov. 2005) ("ICH Guideline")
EX2032	Boys *et al.*, "Protein Oxidative Modifications During Electrospray Ionization: Solution Phase Electrochemistry or Corona Discharge-Induced Radical Attack?," *Analytical Chemistry* 81 (2009): 4027-4034 ("Boys")
EX2033	Knolhoff, Callahan, and Croley, "Mass Accuracy and Isotopic Abundance Measurements for HR-MS Instrumentation: Capabilities for Non-Targeted Analyses," *J. Am. Soc. Mass Spectrom.* 25 (2014): 1285-1294 ("Knolhoff")
EX2034	Valeja *et al.*, "Three Dimensional Liquid Chromatography Coupling IEC/HIC/RPC for Effective Protein Separation in Top-Down Proteomics," *Analytical Chemistry* 87(10) (2015): 5363-5371 ("Valeja")
EX2035	Han, Aslanian, & Yates, "Mass spectrometry for proteomics," *Curr. Op. in Chem. Biol.* 12 (2008): 483-490 ("Han and Yates")
EX2036	May & McLean, "Advanced Multidimensional Separations in Mass Spectrometry: Navigating the Big Data Deluge," *Annu. Rev. Anal. Chem.* 9 (2016):387-409 ("May and McLean")
EX2037	Wright & Wright, "Advances in LC: Bioanalytical Method Transfer," *Bioanalysis* 8(17) (2016):1837-1844 ("Wright")

IV. LEGAL STANDARDS

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

A. Obviousness

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

- the scope and content of the prior art;
- the differences between the prior art and the claims at issue;
- the knowledge of a person of ordinary skill in the pertinent art; and
- if present, objective factors indicative of non-obviousness, sometimes referred to as "secondary considerations."

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

- combining prior-art elements according to known methods to yield predictable results;
- simple substitution of one known element for another to obtain predictable results;
- use of a known technique to improve similar devices in the same way;
- applying a known technique to a known device ready for improvement to yield predictable results;
- applying a technique or approach that would have been "obvious to try," i.e., choosing from a finite number of identified, predictable solutions, with a reasonable expectation of success;
- known work in one field of endeavor may prompt variations of it for use in either the same field or a different one based on design incentives or other market forces if the variations would have been predictable to one of ordinary skill in the art;
- some teaching, suggestion, or motivation in the prior art that would have led one of ordinary skill to modify the prior-art reference or to combine prior-art reference teachings to arrive at the claimed invention.

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

21. I have been informed that for a combination of references to render

the claimed invention obvious, the POSA must have been able to arrive at the claimed invention by altering or combining the applied references and must have had a reasonable expectation of success in doing so. I understand that the claim is considered as a whole, such that the obviousness analysis requires consideration of whether the claimed combination of elements was rendered obvious by the cited prior art.

22. I also have been informed that a claim can be obvious if it is the product of routine experimentation or optimization.

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

V. THE LEVEL OF ORDINARY SKILL IN THE ART

24. I have been informed that obviousness is analyzed from the perspective of the POSA in the relevant field at the time of the effective filing date of the '377 patent, which I understand was August 15, 2016. I understand that the Petition and Coon Declaration applied the following definition for the POSA in their analyses:

> A POSA working in the technical field of the ’377 patent would have had at least a Ph.D. in biochemistry, chemistry, pharmaceutical sciences, or a related field, and between one and four years of post-doctoral experience in the field of chemistry or pharmaceutical sciences, including analytical techniques such as chromatography and mass spectrometry. Alternatively, a POSA would have had at least a Master’s or Bachelor’s Degree in biochemistry, chemistry, pharmaceutical sciences, or a related field, with a corresponding number of additional years of experience in the field of chemistry or pharmaceutical sciences.

Coon Decl., ¶ 231.

25. For this Declaration I will apply the same POSA definition as applied by Petitioners and Dr. Coon. I reserve the right to amend or supplement the definition of a POSA as additional information becomes available.

VI. SCIENTIFIC AND FACTUAL BACKGROUND

A. Background Scientific Concepts Relevant to the ’377 Patent

26. Dr. Coon provides a lengthy overview of the technical background of this art field and the prior art. *See* Coon Decl., ¶¶62-204. I do not intend to address and rebut each paragraph of Dr. Coon’s declaration; instead, I will address the points most relevant to my opinions.

27. I agree with Dr. Coon’s general definitions for techniques for LC-MS, LC-MS/MS, “top-down” and “bottom-up” proteomics, and for scientific concepts like AAV capsids and serotypes.

28. For example, Dr. Coon is correct in differentiating between "bottom-up" proteomics (Coon Decl., ¶130), where a protein is digested, separated, and interrogated with tandem-MS, from "top-down" proteomics (Coon Decl., ¶¶166-169), where an intact protein is analyzed.

29. I disagree, however, with many of Dr. Coon's characterizations. Dr. Coon does not differentiate between references that use "top-down, intact LC-MS" (*see* Coon Decl., ¶176) from those that use top-down MS/MS (*see* Coon Decl., ¶183). Despite both techniques being referred to as "top-down" in the literature, a POSA would consider these analytical methods distinct. For top-down MS/MS, while intact proteins are introduced into the mass spectrometer, this only effectuates the MS/MS part of the experiment, which couples two (or more) mass analyzers in sequence with an intermediate fragmentation step. *See* EX1038-Zabrouskov, 987-88.

30. Dr. Coon also opines that "[i]t was known in the art, however, that enzymatic digestion is a laborious, time-consuming process, which can introduce artificial modifications, such as cyclization of N-terminal glutamine, and deamidation." Coon Decl., ¶158 (citing EX1012-Bondarenko). But every analytical method has trade-offs and limitations. It was known in the art that instrumental factors can also introduce artificial modifications like protein oxidation, even under standard operating conditions. EX2032-Boys, 4027-28. And

as discussed in detail below, Shytuhina's method required bespoke and optimized preparation and analytical conditions, and even then showed significant carry-over, proteins sticking to the column, inaccurate mass measurements for minor peaks, and more generally inaccurate mass measurements for virtually all intact peaks as discussed below. EX1006-Shytuhina, 194.

31. Further, Dr. Coon's characterization that a "POSA would have understood that analysis of larger proteins (*e.g.*, 100 to 200 kDa and larger) using top down LC-MS, including LC-MS/MS, could require adjusting certain conditions of the MS analysis to ensure sufficient cleavage of the protein" is not supported by the prior art through the lens of Satkunanathan and Shytuhina. Coon Decl., ¶184 (citing EX1026-Han). As discussed below, a POSA in 2015 or 2016 with Satkunanathan and Shytuhina in hand would not have sought to apply Shytuhina's intact LC-MS method to AAV or other viral proteins because of issues with resolution, minor peak analysis, and difficulty in sample preparation, particularly given the shortcomings with the method used and data produced by Shytuhina. *See, e.g.*, EX1006-Shytuhina, 194.

B. The '377 Patent

32. The '377 patent (EX1001) is titled "Methods for Detecting AAV." The specification discusses using intact protein LC-MS as an analytical tool to evaluate viral preparations and to discern characteristics including the presence of

post-translational modifications and the identity of the capsid serotype. The specification describes methods of denaturing rAAV samples before LC-MS analysis and discusses LC-MS intact protein analysis.

33. The inventors of the ’377 patent teach that characterization and monitoring of viral capsid proteins during manufacturing is important to gene therapy product development, as it affects potency and infectivity, and in turn safety and efficacy, of rAAV therapeutics. EX1001, 1:34-38, 51:59-52:22. The inventors of the ’377 patent disclose methods for improving this process, and particularly for serotype identification and the tracking of any post-translational modifications on the viral capsid proteins. *See, e.g.*, *id.*, 20:14-18.

34. The inventors of the ’377 patent teach that the capsid of an AAV particle is made up of the three distinct proteins, VP1, VP2, and VP3, and the masses of each viral capsid protein (“VP”) is unique for each serotype. *Id.*, 57:19-23, 20:11-14, Table 3. Following mass spectrometry, the serotype of AAV can be accurately determined by comparing measured VP masses within a sample against their theoretical masses. *Id.* Additionally, post-translational modifications can be determined by identifying mass spectral peaks that are similar to, but characteristically different from, the respective peaks for each of the respective VPs owing to the specific post-translational modification. *See id.*, 26:1-3, 54:49-55:4, Figs. 2A and 2B.

35. Different serotypes can be difficult to differentiate, especially serotypes having high sequence homology and overlapping epitopes, which result in similar masses. The ’377 patent discloses these small differences in mass between the VPs of different AAV serotypes. EX1001, Tables 4-6. And post-translational modifications are similarly difficult to differentiate, given the high resolution needed to accurately separate and measure VPs from their modified forms.

36. The prior art included purported methods to identify different AAV serotypes, but many of them, including SDS-PAGE gel separation, antibody-based ELISA, and Western blot assays, had “banding patterns and antibodies [that were] not specific enough to differentiate between different AAV serotypes.” EX1001, 1:52-56. Gel-LC/MS/MS was another potential method, entailing multiple steps performed over multiple days. EX1001, 1:56-60. These steps included SDS-PAGE, in-gel digestion using a protease, and LC/MS/MS, which were lengthy and laborious but ultimately “provid[ed] limited sequence coverage.” EX1001, 1:52-56; *see also id.*, 52:38-44. Thus, the inventors explained that “it would be useful to have improved methods of characterizing viral particles,” including faster and more accurate ways to characterize VPs in a given sample. *Id.*, 1:63-64.

37. The ’377 patent inventors disclose that they achieved faster and more accurate characterization through a novel method for direct LC/MS intact protein

analysis to identify AAV serotype and post-translational modifications without the use of digestion/gel separation. *Id.*, 2:10-13; 52:23-55, 53:30-47, 54:31-55:4. This method included denaturation of the intact protein, which allowed for better separation reproducibility in LC and higher spectral quality in MS. *Id.*, 53:31-34; 54:26-28. Additionally, the inventors overcame the issue of VP1 and VP2 peak suppression through changes of various chromatographic column parameters. *Id.*, 54:31-51.

38. The inventors compared the theoretical masses for VP1, VP2, and VP3 for several AAV serotypes against their masses determined experimentally via the disclosed intact protein analysis method. *Id.*, Table 2. The results showed that intact protein analysis via the inventors' disclosed method accurately differentiated between the serotypes tested. *Id.* Further, to check for post-translational modifications, the inventors monitored samples for the presence of acetylation on VP1 and VP3. *Id.*, 54:49-55-1. They also checked for glycosylation on the VPs. *Id.*, 55:1-4. The inventors compared these results against digested proteins analyzed by bottom-up LC-MS/MS and validated the accuracy of their post-translational modification results. *Id.*, 53:61-54:18, 55:5-48, 56:61-67.

39. From the LC-MS experimental results, the inventors concluded that "intact protein measurement of VP1, VP2 and VP3 is highly specific as [a serotype] identity test" and that "intact protein analysis and LC/MS/MS can be

used to profile VPs to monitor . . . posttranslational modifications . . . to ensure product consistency during VLP production." *Id.*, 59:11-21, 64:2-2-9.

40. The challenged claims are directed to methods for determining the serotype of AAV and other viral particles using intact protein LC-MS analysis without gel separation. Claims 1 and 2 are independent claims, with claims 3-20 depending from either claim 1 or claim 2 and specifying particular denaturing methods, chromatography types, and calibration methods.

C. Prosecution History

41. As originally submitted to the Patent Office, claim 1 recited "[a] method to determine the serotype of an adeno-associated virus (AAV) particle comprising (a) denaturing the AAV particle; (b) subjecting the denatured AAV particle to liquid chromatography/mass spectrometry (LC/MS); and (c) determining the masses of VP1, VP2 and VP3 of the AAV particle; wherein the specific combination of masses of VP1, VP2 and VP3 are indicative of the AAV serotype." EX1002 at 522.

42. The Patent Office's rejections during prosecution centered on two prior art references, Vliet (EX2021) and Bark (EX2022). The Examiner conceded that Vliet did not teach directly subjecting the denatured AAV particle to LC-MS, but the Examiner argued that a second reference, Bark supplied that teaching. *Id.*, 1799.

43. I understand that Genzyme amended claim 1 to include an additional limitation, making clear that the claimed method does not include a gel separation step: "wherein the method is performed in the absence of a gel separation step." *Id.*, 1821, 1831.

44. Genzyme explained that while Vliet disclosed a method for identifying the serotype of an AAV particle, the disclosed method included loading the AAV particles on a 10% polyacrylamide gel and performing SDS-PAGE prior to LC-MS/MS. *Id.*, 1832. Genzyme argued that Vliet instructed a POSA to not perform the disclosed method in the absence of a gel separation step, as Vliet taught that SDS-PAGE was necessary to separate the three overlapping VPs and any impurities in a given sample. *Id.* Genzyme also explained that Bark did not cure the missing teachings of Vliet by subjecting intact, denatured proteins to LC/MS, because Bark included the use of a protein digestion step. *Id.*, 1832-33. Specifically, Bark taught the use of a thermotolerant protease that solved several issues with proteolysis, including the long run times associated with the process. *Id.*, 1833-34.

45. In a final rejection, the Examiner argued that Bark cured the missing teaching of Vliet because it taught performing its claimed method in the absence of a gel separation step to save the time associated with gel separation. *Id.*, 2120. Additionally, the Examiner noted that the claims did not recite performing the

method without the use of a protease. *Id.*

46. Genzyme then amended claim element 1(b) to recite "*directly* subjecting the denatured AAV particle to liquid chromatography/mass spectrometry (LC/MS) *intact protein analysis*." *Id.*, 2146, 2155. Genzyme also rebutted the Examiner's characterization of Bark by pointing to Bark's teachings that "[t]he primary techniques for proteomic analysis consist of 2-D gel electrophoreses followed by proteolytic digestion." *Id.*, 2155-56. The limitation of the technique that Bark sought to solve was not the time associated with running a gel separation step, but "the time required for adequate proteolysis and the resistance of some proteins to digestion." *Id.*, 2155-56. Thus, Bark did not relate to intact protein analysis.

47. The Examiner allowed claim 1 and other claims, stating in the reasons for allowance that "neither Vliet nor Bark teaches or fairly suggest directly subjecting a denatured AAV or viral particle to liquid chromatography/mass spectrometry (LC/MS) intact protein analysis, i.e., without using a protease." *Id.*, 2186.

D. Challenged Claims

48. Claims 1 and 2 of the '377 patent are independent claims. Therefore, all claims share the elements recited by these claims. Claims 1 and 2 recite, in part

1. A method to ***determine the serotype*** of an adeno-associated virus (AAV) particle comprising

(a) denaturing the AAV particle,
(b) directly subjecting the denatured AAV particle to liquid chromatography/mass spectrometry (LC/MS) ***intact protein analysis***, and
(c) determining the masses of VP1, VP2 and VP3 of the AAV particle;
wherein the specific combination of masses of VP1, VP2 and VP3 are indicative of the AAV serotype,
and wherein the method is performed ***in the absence of a gel separation step***.

2. A method to ***determine the serotype*** of a viral particle comprising
(a) denaturing the viral particle,
(b) directly subjecting the denatured viral particle to liquid chromatography/mass spectrometry (LC/MS) ***intact protein analysis***, and
(c) determining the masses of one or more capsid proteins of the viral particle;
wherein the specific combination of masses of one or more capsid proteins are indicative of the virus serotype,
and wherein the method is performed ***in the absence of a gel separation step***.

Ex. 1001, 109:18-43. Elements 1(b) and 2(b) therefore require performing LC-MS on intact proteins, an advance over prior-art approaches that relied on enzymatic digestion followed by peptide tandem mass spectrometry ("MS/MS") analysis.

VII. CLAIM CONSTRUCTION

49. I have been informed by counsel to interpret the language of the claims according to their plain and ordinary meaning as understood by a person of ordinary skill in the art, which was applied by Dr. Coon. Coon Decl., ¶337.

VIII. OVERVIEW OF THE PRIOR ART

50. Dr. Coon provides a lengthy overview of the prior art references in his declaration. As noted, I do not intend to provide a paragraph-by-paragraph rebuttal to these scientific background sections; rather, I will point out where I specifically disagree with Dr. Coon's characterizations or conclusions. My lack of reference to a particular point does not signal any agreement with that point in Dr. Coon's declaration.

A. Satkunanathan (EX1005)

51. Satkunanathan is a research article published in the journal Human Gene Therapy in November 2014. It is entitled "Establishment of a Novel Cell Line for the Enhanced Production of Recombinant Adeno-Associated Virus Vectors for Gene Therapy."

52. Dr. Coon provides an overview of Satkunanathan at ¶¶237-265 of his declaration. I further emphasize that Satkunanathan used bottom-up proteomics, not intact protein analysis. EX1005-Satkunanathan, 930-31.

53. The methods described in Satkunanathan are very different from those described in the '377 patent; they represent a complete tryptic digestion and peptide-based identification workflow. EX1005-Satkunanathan, 930-31. Satkunanathan's method is similar to the method used in the Bark reference cited during prosecution, in that it relies on peptide digestion followed by MS/MS

analysis for peptide analysis. Satkunanathan, therefore, fails to teach intact protein analysis.

54. Because digestion breaks the protein apart, Satkunanathan's digestion method necessarily destroys information about intact mass, capsid protein stoichiometry, or post-translational modification patterns on intact proteins. *See id.*

55. Further, Satkunanathan's purpose was to "analyz[e] host cellular proteins co-produced and copurified with AAV vectors" to "identify differences in protein composition among the three AAV serotypes AAV2, AAV5, and AAV8; better understand the role of cellular proteins in AAV assembly; and ultimately improve AAV vector production." EX1005-Satkunanathan, 930. The AAV serotypes were known *ab initio* in Satkunanathan (*id.*); as such, a POSA reading it would understand it does not teach or suggest that its method would be applicable or even helpful to determining AAV serotype or post-translational modifications by intact mass analysis.

B. Shytuhina (EX1006)

56. Shytuhina is an article published in the Journal of Chromatography A in 2014 entitled "Development and application of a reversed-phase high-performance liquid chromatographic method for quantitation and characterization of a Chikungunya virus-like particle vaccine."

57. Dr. Coon provides an overview of Shytuhina at ¶¶266-313 of his

declaration. Shytuhina discloses a reverse-phase HPLC and LC-MS method intended to specifically characterize Chikungunya virus-like particles to "evaluate and quantitate the mass and purity of the [Chikungunya] vaccine product." Coon Decl., ¶277.

58. Shytuhina's Chikungunya virus-like particle method required extensive development, expressly teaching that the disclosed method was tailored to address Chikungunya-specific technical challenges. EX1006-Shytuhina, 194. Shytuhina discloses a number of viruses for which "HPLC methods have been applied for the identification and quantitation of virus proteins and VLPs including influenza virus, lentivirus, Sendai virus, poliovirus, human papillomavirus VLP, adenovirus types 3 and 5, and Hepatitis B VLP." *Id.*, 192-193. Shytuhina does not reference AAV. *Id.*

59. Dr. Coon fails to provide any opinion that Shytuhina teaches that its methods could be applied to viral particles besides Chikungunya. Rather, he opines without citations that working with AAV capsid proteins would have been "more straightforward" than Shytuhina's glycoproteins because glycoproteins "are more variable and therefore more difficult to analyze than AV capsid proteins by intact LC-MS." *See* Coon Decl., ¶525.

60. To address Chikungunya-specific technical challenges, Shytuhina screened and modified various mobile phase compositions and found that "E1

[protein] tended to stick to the column," requiring a "strong organic solvent such as 2-propanol to elute it." EX1006-Shytuhina, 194.

61. Shytuhina further reported that a "lack of TFA in the mobile phases led to the elution of VLPs in the void volume of the column" and that "TFA appeared to help disassemble the VLPs and facilitate the binding of proteins to the stationary phase." *Id.* The authors also required elevated temperature (60°C) and a detergent pretreatment protocol because of "carry-over of E1 and E2 between HPLC runs." *Id.*

62. These disclosures in Shytuhina teach a POSA that Shytuhina's method was difficult to develop and was optimized specifically for Chikungunya glycoproteins embedded in lipid bilayers, requiring substantial empirical screening to identify conditions that could disassemble virus-like particles, elute hydrophobic proteins, and manage carry-over. *Id.*, 192-94.

63. Moreover, Shytuhina had to create a bespoke protocol because, while "TFA was necessary in the mobile phase to promote VLP interaction with the stationary phase," it also "caused ion suppression"; thus, "formic acid was used as the mobile phase modifier" instead of TFA for the intact LC-MS analysis. *Id.*, 193-94. This modifier incompatibility demonstrates that even Shytuhina's optimized RP-HPLC conditions were not directly applicable to intact LC-MS without significant adaptation. *Id.*

64. Dr. Coon does not provide any opinion that the Chikungunya-specific conditions from Shytuhina would transfer to AAV capsids, which have entirely different structural organization (non-enveloped icosahedral protein shells versus lipid-enveloped glycoprotein virus-like particles) and protein composition (non-glycosylated capsid proteins VP1/VP2/VP3 versus heavily glycosylated envelope proteins E1/E2). *Id.*, 192-93. Shytuhina does not provide any detail about the specific challenges faced when analyzing AAV or other viral particles by intact LC-MS analysis.

65. Further, as Dr. Coon points out, Shytuhina's intact LC-MS analysis required "10 consecutive injections of 25 μL" of virus-like particle sample with signal averaging to achieve sufficient sensitivity and intensity. Coon Decl., ¶288 (citing EX1006-Shytuhina, 193). Even then, "[d]ue to the mass heterogeneity of both E1 and E2, the deconvoluted masses of the minor peaks were less accurate," leading to many peaks not being assigned. EX1006-Shytuhina, 194. This indicates significant limitations in mass accuracy and sensitivity that would be exacerbated in more complex samples containing multiple capsid protein variants and host cell proteins like AAV. EX2026-Wörner, 3-4.

66. Finally, as set forth below, there are serious concerns about the accuracy and efficiency of the analytical method used in Shytuhina. These issues were known before the priority date of the '377 patent, such that a POSA would

have been aware of them and would not have been motivated to use Shytuhina's intact LC-MS method in other contexts or in combination with other references, like Satkunanathan or Zabrouskov.

C. Zabrouskov (EX1038)

67. Zabrouskov is a 2006 Biochemistry paper entitled "Stepwise Deamidation of Ribonuclease A at Five Sites Determined by Top Down Mass Spectrometry." Dr. Coon addresses Zabrouskov only for background in his declaration related to the '377 patent. *See, e.g.*, Coon Decl., ¶¶179-183.

68. Zabrouskov is directed to the study of deamidation in ribonuclease A (RNase A), a small, 13.7 kDa monomeric pancreatic enzyme. Zabrouskov uses electrospray ionization (ESI) on RNase A to introduce gaseous ions into a Fourier-transform MS. Zabrouskov does not employ a viral denaturing step and does not teach or suggest an intact LC-MS analysis of an intact viral particle.

69. RNase A is a standard protein that can be found in many mass spectrometry laboratories because it is an inexpensive, well-studied, well-understood mass spectrometry standard. Zabrouskov states "…For the extensively studied case of reduced bovine ribonuclease A…" EX1038-Zabrouskov, 987. RNase A is not a virus, does not form a viral capsid, does not have distinct subunit proteins corresponding to VP1, VP2, and VP3, and does not have a serotype.

Unlike the claimed method in the ’880 patent, Zabrouskov’s method does not examine a viral particle with distinct capsid protein subunits.

70. Further, Zabrouskov does not involve an LC step coupled to the mass spectrometer. Instead, Zabrouskov performs only top-down tandem-MS/MS on a pre-prepared protein sample. As. Dr. Coon points out, Zabrouskov teaches that the precision of the values is inversely proportional to the mass of the sample, such that larger samples will have lower mass precision. Coon Decl., ¶359 (citing EX1038-Zabrouskov, 989).

D. Ansong (EX1009)

71. Ansong is a 2013 article from the Proceedings of the National Academy of Sciences entitled “Top-down proteomics reveals a unique protein S-thiolation switch in *Salmonella Typhimurium* in response to infection-like conditions. Dr. Coon provides an overview of Ansong at ¶¶314-323 of his declaration.

72. Dr. Coon uses Ansong in combination with Satkunanathan and Shytuhina to address dependent claims relevant to UPLC (’377 patent claims 8 and 17). Dr. Coon does not provide any opinion that Ansong discloses an intact LC-MS method for analyzing viral proteins in the absence of a gel separation step, or would provide any motivation or limitation relevant to any independent claim.

73. Ansong studied *Salmonella typhimurium* bacterial proteins to identify post-translational modifications (specifically S-thiolation) in response to infection-like conditions. EX1009, 10153. Ansong provides no teaching regarding viral particle analysis, virus-like particle disassembly, capsid protein separation, or serotype identification.

74. The analytical challenges and sample preparation requirements for bacterial lysates are entirely different from those for intact viral particles. EX1006-Shytuhina, 194; EX1005-Satkunanathan, 930-31; EX1009-Ansong, 10154.

E. Byeon (EX1014)

75. Byeon is a 2015 Molecules and Cells paper entitled "Structural Identification of a Non-Glycosylated Variant at Ser126 for O-Glycosylation Site from EPO BRP, Human Recombinant Erythropoietin by LC-MS Analysis." Dr. Coon provides an overview of Byeon at ¶¶324-333 of his declaration.

76. Dr. Coon uses Byeon in combination with Satkunanathan and Shytuhina to address dependent claims relevant to calibration ('377 patent claims 9, 10, 18, and 19). Dr. Coon does not provide any opinion that Byeon discloses an intact LC-MS method for analyzing viral proteins in the absence of a gel separation step or would provide any motivation or limitation relevant to any independent claim.

77. Byeon provides no teaching regarding viral particle analysis, virus-like particle disassembly, capsid protein separation, or serotype identification. Byeon analyzed recombinant human erythropoietin (rHu-EPO), a glycoprotein therapeutic, to identify a non-glycosylated variant at a specific glycosylation site. EX1014, 501-02. This is post-translational modification characterization of a single known protein, not serotype or post-translational modification identification of viral capsid proteins in complex samples. *See id.*

F. Yuan (EX1037)

78. Yuan is a 1998 Journal of Chromatography article entitled "Reversed-phase high-performance liquid chromatography of virus-like particles." Dr. Coon addresses Yuan only for background in his declaration related to the '377 patent. *See, e.g.*, Coon Decl., ¶¶443-444.

79. Dr. Coon does not provide any opinion that Yuan's method would be relevant to any independent claim of the '880 patent. Rather, he combines Yuan with Shytuhina and Zabrouskov for dependent claim 20, related to column selection.

80. Yuan simply discloses an LC method using gel separation (SDS-PAGE) for human papillomaviruses. EX1037, 21. Yuan analyzes a single protein of such viruses, L1, using LC, and teaches using a C4 or C8 column for the

analysis. *Id.*, 21-22. Yuan does not teach or suggest an intact LC-MS/MS method for analyzing viral particles in the absence of a gel separation step.

IX. GROUND I: A POSA WOULD NOT BE MOTIVATED TO COMBINE SATKUNANATHAN AND SHYTUHINA AND WOULD NOT HAVE HAD A REASONABLE EXPECTATION OF SUCCESS

81. I understand that the Petitioner bears the burden of proving that there is a reasonable likelihood that the challenged claims are unpatentable as obvious based on the art and arguments set forth in its Petition. The inadequacies in the Petition's proofs identified herein are so significant that, in my opinion, no reasonable reviewer would come to that conclusion. Dr. Coon's opinions fail to establish that it would have been obvious for a POSA to combine Satkunanathan and Shytuhina to arrive at the claimed inventions in the '377 patent.

A. Independent Claims 1 and 2

1. Dr. Coon Fails to Provide Evidence That a POSA Would Consider Shytuhina's LC-MS Method to Be Efficient and Precise

82. Dr. Coon's opinion that a POSA would be motivated to combine Shytuhina with Satkunanathan is premised on the assumption that "a POSA would have been motivated to apply Shytuhina's efficient and precise intact LC-MS method to improve Satkunanathan's use of LC-MS/MS." *See, e.g.*, Coon Decl., ¶351. This is critical to Dr. Coon's opinion and to Petitioner's argument: Dr. Coon makes this assertion no fewer than ***thirty-eight times*** in his declaration. *See* Coon Decl., ¶¶ 339, 351, 360, 365, 369, 374, 381, 384, 394, 399, 403, 408, 409, 416,

419, 425, 432, 435, 438, 448, 455, 460, 466, 472, 475, 481, 484, 487, 497, 504, 509, 515, 535, 543, 562, 568, 575, 581.

83. In other words, Dr. Coon bases his motivation to combine opinion on the assumption that, because Shytuhina's method is apparently "efficient and precise," a POSA would have sought to combine it with Satkunanathan to arrive at the claimed invention. In my opinion, Dr. Coon's assumption is unsupported by the disclosure in Shytuhina and is driven by hindsight in view of the efficiency and precision of Genzyme's claimed method. Shytuhina's method for intact LC-MS analysis is neither efficient nor precise, defeating any motivation to combine. Indeed, given the problems with Shytuhina's results and conclusions and the difficulty the authors faced in preparing and analyzing the Chikungunya viral particles of interest—which would be readily apparent to a POSA—a POSA would not have sought to apply those teachings elsewhere.

Any Reference to Precision in Shytuhina Is Not Attributable to Its Intact LC-MS Method

84. As explained above, Dr. Coon believes that Shytuhina's method is "efficient and precise," such that a POSA at the time would have adopted its methodology and combined it with Satkunanathan. Yet Shytuhina reports no precision or accuracy data for its intact LC-MS analysis. The only precision data in Shytuhina—Table 1, which Dr. Coon presumably relies upon—is ascribable to UV absorption at 280 nm during RP-HPLC separation, not to mass spectrometry.

EX1006-Shytuhina, Table 1, 195. UV absorption is unrelated to the claimed invention and is a fundamentally different detection method than mass spectrometry. It provides no information about the performance of Shytuhina's intact protein mass analysis. Dr. Coon's repeated assertion that Shytuhina's method is "efficient and precise" therefore lacks any evidentiary support in the reference.

85. While Shytuhina does not explicitly state the analytical methodology used to generate the precision data shown in Table 1, there are several indications that would cause a POSA to ascribe the precision and accuracy data to UV absorption data rather than intact protein LC-MS.

86. Shytuhina's studies of quantitative measurement to demonstrate linearity, accuracy, and precision are performed in the context of an International Conference on Harmonization (ICH) document. ICH guidance documents are prepared by the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use and are used by the US Food and Drug Administration as a guidance document in the pharmaceutical industry:

> *3.4. RP-HPLC assay performance assessment*
>
> The International Conference on Harmonisation (ICH) outlines expectations on pharmaceutical product development and registration. ICH Q2(R1) requires that an analytical assay that is intended for quantitative measurement of the active moiety of a drug substance or drug product needs to demonstrate linearity, accuracy and precision [29].

EX1006-Shytuhina, 195. Reference 29 in Shytuhina above, cited for the proposition that quantitative measurement "needs to demonstrate linearity, accuracy, and precision" is provided as EX2031.

87. In the paragraph preceding the citation to EX2031 with respect to quantitative measurement, Shytuhina describes that UV absorption was used to measure percent recovery: "We evaluated the recoveries of E1 and E2 from RP-HPLC in comparison to capsid protein using UV absorbance…Although the fluorescent signal detected in the RP-HPLC assay provides high sensitivity, the fluorescence is sensitive to the local environment of the protein. Because of this, the signal is not proportional to the number of tryptophan residues in each protein…" EX1006-Shytuhina, 195.

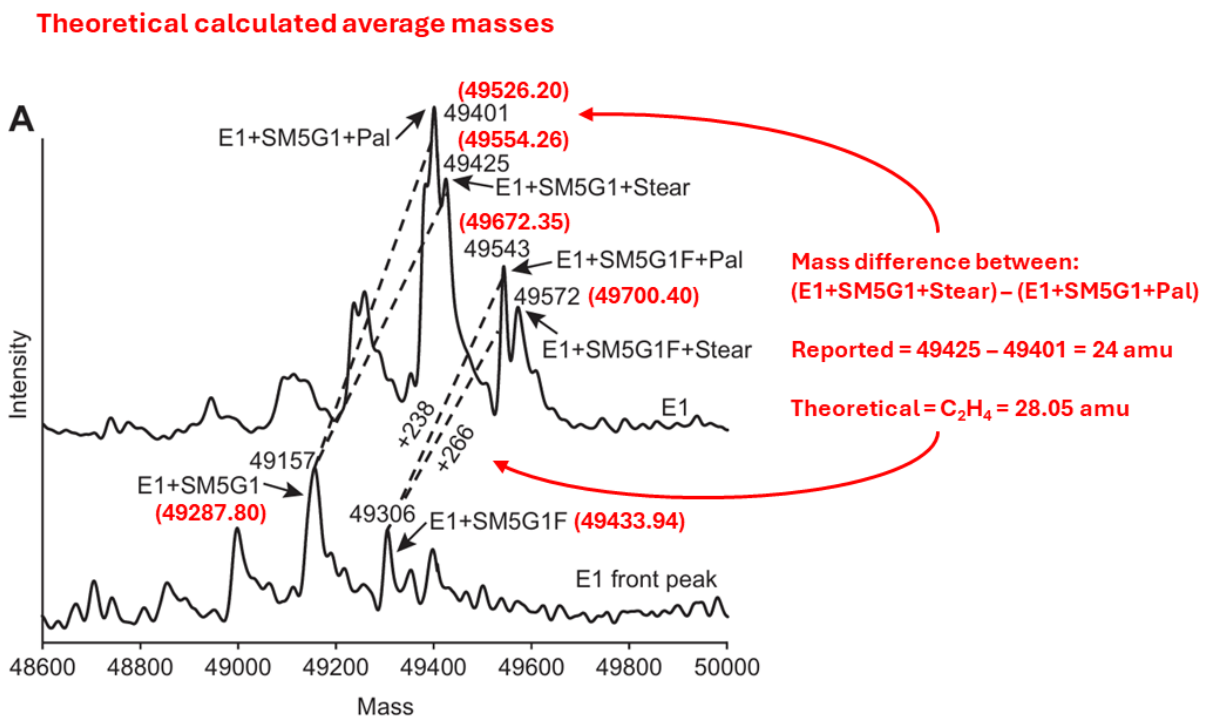
88. Shytuhina states that in its precision determination, "[t]he retention time did not drift during the analysis." EX1006-Shytuhina, 195. A POSA would understand that retention time is referring to the spectroscopic measurement during RP-HPLC, not during mass spectrometry analysis. Shytuhina does not report any precision values for its intact mass analysis by LC-MS.

89. Shytuhina does not report any mass accuracy data, because it does not include any theoretical mass information. The mass accuracy equation is:

$$mass\ \ accuracy\ \ error(ppm) = \frac{m/z_{theor} - m/z_{obs}}{m/z_{theor}} \times 10^6$$

Where "m/z_{theor}" is the theoretical mass/charge ratio and "m/z_{obs}" is the observed mass/charge ratio. EX2033-Knolhoff, 1287. Without theoretical mass data, Shytuhina cannot report on the accuracy of its method. The "assay accuracy" reported in Table 2 of Shytuhina is simply the "percentage of measured mass compared to the theoretical mass" of the total amount of sample in solution, which provides no information about the accuracy of the mass spectral data. EX1006-Shytuhina, 195-96.

90. Figure 2 of Shytuhina reports a number of peaks with assigned mass values. Because Shytuhina does not provide mass accuracy data, I have annotated Figure 2A below in red with the theoretical calculated average masses, that are assigned by Shytuhina, for the observed peaks:



Both the positions of the peaks and the spacing of the peaks show extremely high error. For example, the theoretical calculated average mass for the E1+SM5G1+Pal peak is 49526.20 Da, but it is observed at 49401 Da in Shytuhina, a difference of 125.20 Da. The E1+SM5G1+Stear peak has a theoretical calculated average mass of 49554.26, but is observed at 49425, a difference of 129.426 Da. The theoretical difference between these peaks is 28.05 Da, but is reported as 24 Da. In my opinion, a POSA examining Shytuhina's data would consider it highly inaccurate and not a suitable method for intact protein characterization. Figure 2B shows similarly high but somewhat different error, including errors even in the reported peak differences, as annotated below:

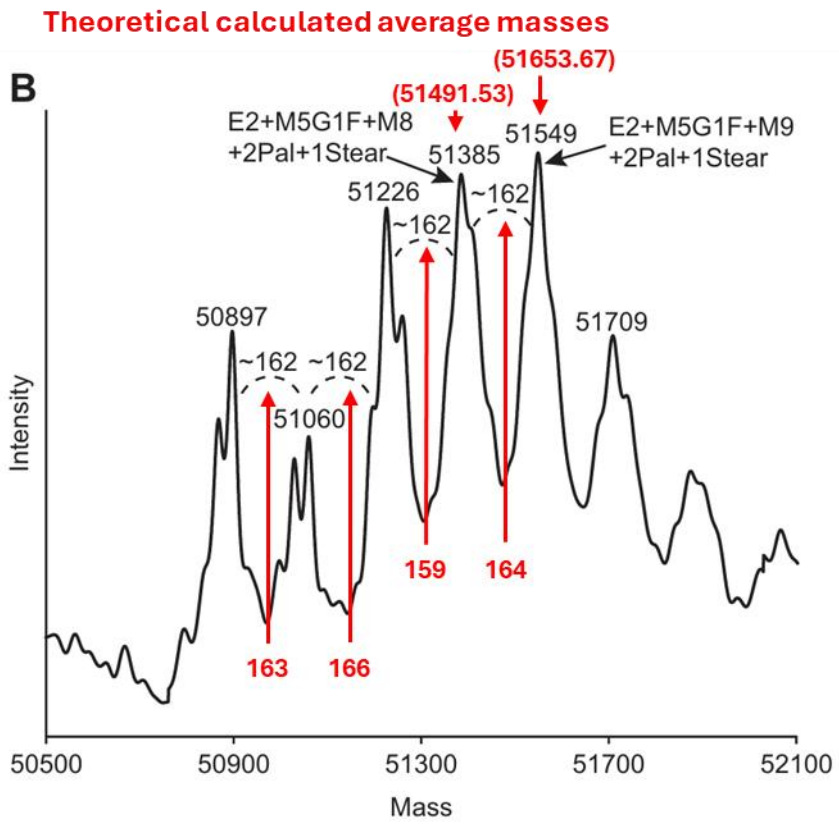


Figure 2B only has two peaks assigned, which differ from the theoretical values by 106.53 Da and 104.67 Da respectively. And even the reported peak differences are labeled incorrectly, or in the best light as approximately 162, further demonstrating very poor mass accuracy measurements. There are four peak spacings that are labeled as "~162," likely representing mannose units, but the actual reported differences are 163, 166, 159, and 164, a difference of ± 4 Da.

91. Applying the mass accuracy equation from Knolhoff to the values in Figure 2, I calculated mass accuracies for Shytuhina's peaks to range from 2026 ppm to 2653 ppm. For the MS instrumentation used, a POSA would expect a mass accuracy of 10-20 ppm, or better, depending on the specific method of calibration.

A POSA would consider any mass accuracy over 20 ppm to be questionable, and anything over 100 ppm to be highly inaccurate and not useful. EX2035-Han and Yates, Table 1. A POSA would not trust mass spectra with a mass accuracy of 2000 ppm or higher, as is the case with Shytuhina. Rather, the very poor mass accuracy and poor data quality in Figure 2 of Shytuhina would caution a POSA not to apply Shytuhina's method in any other contexts, for AAV or otherwise. EX1006-Shytuhina, 195-96.

A Later Paper from the Same Laboratory as Shytuhina Provided Significantly Better Accuracy, Precision, and Efficiency with a Different Method

92. A later paper further underscores that the intact protein LC-MS experiment in Shytuhina was not considered precise or efficient for protein analysis by a POSA. This information comes from the authors themselves in a paper written by the same group that wrote Shytuhina.

93. It is understood in the community and would be understood by a POSA that the corresponding author of Shytuhina, Sha Ha (a Merck scientist), served as the key individual for supervising and directing the work that Shytuhina performed in the manuscript. EX1006-Shytuhina, 192.

94. Sha Ha, again as corresponding author while a Merck scientist, published the follow up manuscript in Journal of Chromatography B on April 21,

2016 (before the August 2016 priority date of the ’377 patent), with lead author Catherine Lancaster. EX2030-Lancaster, 218.

95. In Lancaster, the follow-up manuscript from the group at Merck, the authors did not use the intact protein methodology highlighted by Coon as “efficient and precise.” In Lancaster, which also examined Chikungunya virus, the authors characterized the glycosylation post-translational modifications on the viral proteins E1 and E2 by enzymatically releasing the glycans from the protein using PNGase-F followed by labelling the carbohydrates with a fluorescent label for detection using LC with fluorescence detection. EX2030-Lancaster, 219-21. The peaks were then further identified by LC-MS analysis of the released and derivatized carbohydrates. EX2030-Lancaster, 220-21. All of the observed molecular weights in Lancaster were within 0.02 Da of the calculated molecular weights, exhibiting high mass accuracy. EX2030-Lancaster, Table 1. In contrast, Shytuhina does not report a mass accuracy measurement range, because theoretical mass values are not provided. One can, however, infer poor mass measurement accuracy, because the mass differences expected to be 162 Da were actually 163, 166, 159, and 164, a difference of $\pm$ 4 Da, or about 3% from theoretical mass. EX1006-Shytuhina, 195-96, Fig. 2. Shytuhina does report assay accuracy 96-106% for the total amount of material quantified in their analyses using different analytical techniques. EX1006-Shytuhina, Table 2.

96. A POSA would read Shytuhina and Lancaster together as questioning the utility of Shytuhina's method and would not infer that Shytuhina's method was efficient or precise. The authors chose not to use the Shytuhina methodology of intact protein LC-MS for PTM characterization in Lancaster, instead developing a different, highly precise, highly accurate method, which does not involve intact protein LC-MS. EX2030-Lancaster, Table 1. The mass accuracies I calculated in Lancaster based-on the data in Table 1 result in values of -7.4 to 12.8 ppm. This would be considered an acceptable range of mass accuracy (within $\pm$ 20 ppm) by a POSA in light of Han and Yates and is dramatically improved by over two orders of magnitude over that in Shytuhina (>2000 ppm). A POSA would infer from Shytuhina and Lancaster that direct, intact protein LC-MS may have such significant limitations that the approach to characterize modifications in the absence of the protein was preferable, particularly in light of the many limitations of the Shytuhina method discussed throughout my report.

Shytuhina's Method Is Bespoke, Inefficient, and Riddled with Difficulties

97. Shytuhina states that developing an LC method for the Chikungunya virus-like particle system was technically challenging due to the presence of "lipophilic and hydrophobic glycoproteins." EX1006-Shytuhina, 194. Shytuhina's method development was driven by Chikungunya-specific constraints.

98. One of the Chikungunya viral proteins, E1, stuck to the column during method development; therefore, the procedure was modified to include a strong organic solvent system. EX1006-Shytuhina, 193-94. Detergent pre-treatment was required to reduce carry-over, and the authors had to balance the use of TFA for LC and formic acid for MS. *Id.*, 193-94. TFA was needed to "promote VLP interaction with the stationary phase" for chromatographic separation but caused "ion suppression" in the mass spectrometer, requiring a switch to formic acid. *Id.* A POSA would recognize this switch as a non-trivial trade-off requiring empirical testing, not a routine and globally applicable use of LC-MS. *See id.*

99. These challenges demonstrate to a POSA that intact virus-like particle protein LC-MS is system-specific and requires substantial method development. As a result, a POSA would view Shytuhina's method as bespoke and fit for its specific purpose, not as a broadly applicable methodology ready for combination with other methods in the art.

100. Indeed, nothing in Shytuhina teaches or suggests that its Chikungunya-specific solutions would transfer to AAV capsids, which have fundamentally different structure and challenges: non-enveloped icosahedral protein shells (AAV) versus lipid-enveloped glycoproteins (Chikungunya); non-glycosylated capsid proteins (AAV VP1/VP2/VP3) versus heavily N- and O-glycosylated envelope proteins (Chikungunya E1/E2); and different particle

stability and disassembly requirements. EX2026-Wörner, 3-5; EX2027-Kontogiannis, 6-9; EX1006-Shytuhina, 194.

101. A POSA would recognize that attempting to apply Shytuhina's method to AAV or other viral particles would require redeveloping solutions to substantial challenges—capsid disassembly conditions, protein adsorption management, carry-over reduction, MS-compatible mobile phases—for a completely different viral system, with no expectation that Chikungunya-focused conditions would work for AAV ('377 patent claim 1) or other viral systems besides Chikungunya ('377 patent claim 2), or that the development effort would be worthwhile for Satkunanathan's host protein identification objectives. EX1006-Shytuhina, 193-94.

2. Dr. Coon Fails to Provide Evidence that Shytuhina and Satkunanathan Could Be Used to Develop an Intact Method for AAV or Other Viral Particles

102. Dr. Coon asserts that "intact LC-MS analysis of AAV capsid proteins would have been even more straightforward for a POSA than working with the glycoproteins Shytuhina analyzed" because "glycoproteins are more variable and therefore more difficult to analyze than AAV capsid proteins by intact LC-MS" and that "[a]pplying intact LC-MS to AAV capsid proteins at the relevant date would have been considerably more routine and straightforward than applying the technique to monoclonal antibodies." Coon Decl., ¶¶525, 530. This assertion

mischaracterizes Shytuhina's recited technical challenges and relies on hindsight from the teachings of Genzyme's patents. Further, it misidentifies the source of Shytuhina's technical challenges and ignores the challenges that AAV capsids present.

103. Shytuhina expressly states that its development challenges were driven by "the hydrophobic nature of the viral glycoproteins and the presence of lipids," not primarily by glycan variability. EX1006-Shytuhina, 193. The primary obstacles included structural and physicochemical issues, such as viral proteins sticking to the column requiring specific strong organic solvents to elute, a bespoke system using different acids for virus-like particle disassembly and mass spectral analysis, the need for detergent to reduce carry-over, and ion suppression from the use of TFA. *Id.*, 193-94. Even after optimization, Shytuhina's carry-over remained significant, at 4%. *Id.*, 194.

104. AAV capsids present their own structural challenges that Dr. Coon does not address. For example, AAV capsids are among the most stable viral particles known, with icosahedral symmetry and extensive inter-subunit contacts. EX1031-Rayaprolu, 13153. The art cited by Dr. Coon fails to teach whether AAV capsids would disassemble under Shytuhina's Chikungunya-optimized conditions.

105. While AAV capsid proteins are not glycosylated, they may exhibit a variety of post-translational modifications across serotypes (acetylation,

phosphorylation, deamidation, isomerization, ubiquitination, SUMOylation, etc.) (EX2027-Kontogiannis, 8) and N-terminal processing variants that complicate intact mass assignment. *See, e.g.*, ’377 Patent, Example 4. And as noted above, Satkunanathan’s AAV samples contain numerous co-purifying host proteins that would increase analytical complexity for intact mass analysis compared to Shytuhina’s purified three-protein virus-like particle samples. EX1005-Satkunanathan, 930. Shytuhina does not teach or suggest how its method could be used to analyze AAV samples given these complications.

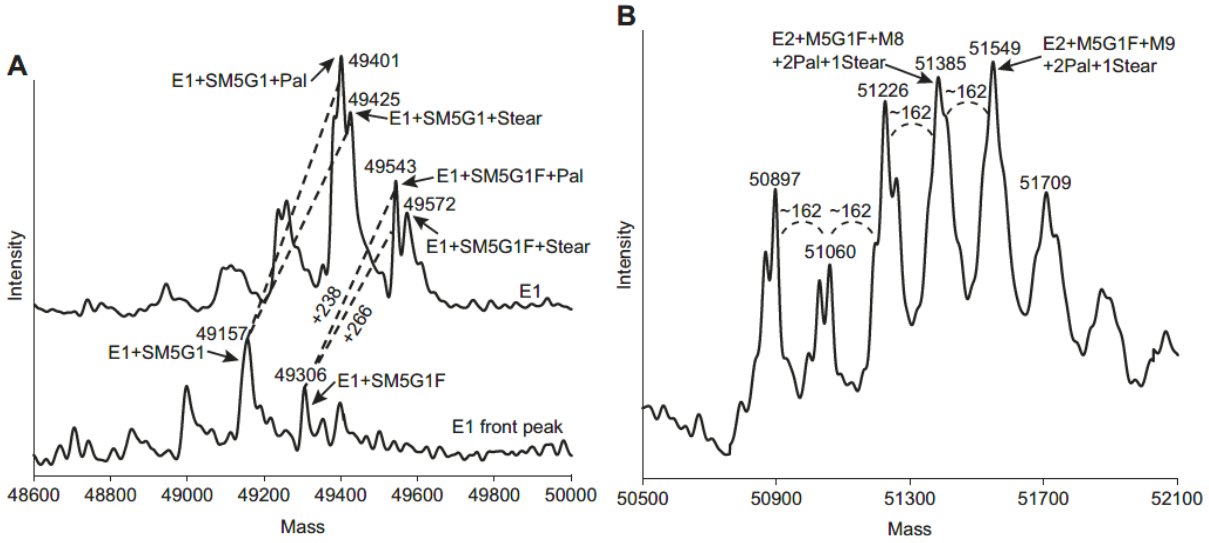
106. Relatedly, AAV viral particles and other viral particles present different analytical challenges requiring mass resolution sufficient to distinguish serotype-specific sequence variants from post-translational modifications and from other processing variants, which is not taught or suggested by Shytuhina or Satkunanathan. Indeed, as explained above, Shytuhina’s intact mass analysis demonstrated poor resolution and mass accuracy incapable of distinguishing minor differences in unknown samples. EX1006-Shytuhina, 195, Figs. 2A & 2B.

107. Further complicating matters, just one AAV serotype, AAV9, can have 1,891 possible capsid masses, and each mass can have 37 charge states, resulting in up to 69,967 different ion species. EX2026-Wörner, 5. This does not even take into account mass variations arising from post-translational modifications or co-purifying host proteins discussed above. A POSA would

understand that AAV is more complex than Shytuhina's method could plausibly analyze.

108. In addition, Dr. Coon provides no evidence from the prior art that AAV capsids would disassemble under Chikungunya-optimized conditions or that a POSA could identify appropriate conditions from the teachings of Shytuhina. Dr. Coon further fails to explain whether or not AAV capsid proteins would exhibit adsorption or different carry-over issues from Shytuhina or Satkunanathan, or whether Shytuhina's Chikungunya-optimized column chemistry, temperature, mobile phase composition and modifier system would work for AAV.

109. Finally, even setting aside the misidentification of Shytuhina's challenges, the unaddressed AAV-specific obstacles, and unacceptable mass accuracy, Shytuhina's method, even after significant attempts at optimization, still produced very noisy mass spectra, with broad peaks unsuitable for quantitation. *See* EX1006-Shytuhina, Figs. 2A & 2B:



A POSA would not look to a method with such severe limitations as a starting point for AAV analysis and would not conclude that adapting it to a structurally distinct viral system would be "more straightforward" as Dr. Coon suggests.

110. Given these difficulties, Dr. Coon's failure to explain why the development effort required for AAV or other viral protein systems would have been routine, rather than comparable to or more extensive than Shytuhina's, renders his opinion unpersuasive. In my opinion, Dr. Coon's assertion that AAV analysis would be "more straightforward" (EX1003, ¶525) is hindsight speculation unsupported by experimental data, prior art teaching, or analysis of the specific structural and analytical differences between Chikungunya virus-like particles and AAV or other viral capsids available to a POSA at the relevant time.

3. The Prior Art Teaches That Intact Protein LC-MS Would Be Unsuitable for AAV Serotype Identification and Detection of Post-Translational Modifications

111. Satkunanathan analyzed previously identified AAV2, AAV5, and

AAV8 digested capsid proteins (not intact proteins) using peptide MS/MS database searching. EX1005-Satkunanathan, 930. Shytuhina, on the other hand, used intact LC-MS to try to identify post-translational modifications in Chikungunya by "matching the observed mass to the theoretical molecular weight" based on "known glycoprotein acylation and expected N-linked glycans," characterizing multiple glycoforms of E1 and E2 proteins from a single Chikungunya isolate—not distinguishing between different viral serotypes. EX1006-Shytuhina, 194.

112. Satkunanathan successfully identified AAV capsid proteins for three serotypes (AAV2, AAV5, AAV8) along with numerous co-purifying host cell proteins using a bottom-up LC-MS/MS approach. EX1005-Satkunanathan, 939. Digestion followed by peptide tandem MS/MS method worked. It provided comprehensive protein identification, statistical confidence through spectral matching, and enabled downstream functional validation. *Id.*

113. Satkunanathan's Supplementary Table S1 identifies "Capsid protein VP1 OS=Adeno-associated virus 2," "Capsid protein OS=Adeno-associated virus-5," and "Capsid protein OS=Adeno-associated virus-8" as unique proteins for each respective serotype, demonstrating that the peptide MS/MS approach successfully distinguished capsid proteins among different AAV serotypes. *Id.*, p. 15.

114. Satkunanathan specifically teaches that peptide MS/MS can provide information about AAV serotype and capsid proteins, *id.*, 932, and a POSA would

not view a bottom-up method like Satkunanathan's to be helpful to developing an intact method for determining serotypes or the presence of post-translational modifications.

115. Dr. Coon cites no prior-art reference teaching that viral serotypes or viral post-translational modifications can be reliably distinguished by intact VP mass measurement. Shytuhina tried and failed to do so for Chikungunya. And Dr. Coon does not point to any prior art teaching that the mass differences between AAV serotype capsid proteins or any viral serotype capsid proteins are large enough for differential identification given potential unknown post-translational modifications, processing variants, and mass measurement accuracy limitations. EX2028-Salganik, 11879-80; EX2027-Kontogiannis, 7-8.

116. Dr. Coon cites Huang for the proposition that digestion methods followed by LC-MS/MS could be used to detect deamidation in antibodies. Coon Decl., ¶¶156-157 (citing EX1027-Huang). Deamidation is a very small change in the mass of a protein (approximately 1 Da). EX1038-Zabrouskov, 987. Dr. Coon cites no prior art indicating that intact LC-MS could reliably detect a change as small as 1 Da. As such, a POSA would have looked to digestion methods using bottom-up proteomics rather than intact LC-MS methods to detect small changes in mass arising from different viral protein serotypes or from post-translational modifications.

117. Dr. Coon opines that a POSA would want to move away from digestion because it "is a laborious, time-consuming process, which can introduce artificial modifications, such as cyclization of N-terminal glutamine, and deamidation." Coon Decl., ¶158. But every analytical method has limitations, including the method employed in Satkunanathan. Indeed, techniques like electrospray ionization mass spectrometry can introduce artificial modifications like protein oxidation. EX2032-Boys, 4027-28.

118. In my opinion, a POSA would not have been motivated to abandon a working method (Satkunanathan's peptide MS/MS proteomics) to pursue an untested approach (adapting Shytuhina's bespoke, Chikungunya-specific intact virus-like particle method to AAV) when the references themselves provide no teaching that such adaptation would work, how to accomplish it, or that it would address either reference's objectives. EX1005-Satkunanathan, 930-31; EX1006-Shytuhina, 193-95. In my opinion, a POSA would not be so motivated, particularly because the Merck group that worked on the Shytuhina paper subsequently abandoned its intact LC-MS approach in favor of a more accurate and precise methodology. EX2030-Lancaster, 218-221. Rather, a POSA would be motivated to apply an accurate, if cumbersome, method like digestion followed by LC-MS/MS over a highly inaccurate, cumbersome one like Shytuhina's.

119. Dr. Coon further provides no opinion that the prior art taught a POSA

that intact mass measurement would provide advantages over, or similar results to, established serotype identification methods (*e.g.*, peptide MS/MS, immunological assays, sequencing) or that a POSA could validate that observed intact masses correspond to specific serotypes rather than post-translational modifications or degradation variants, or vice versa. EX2028-Salganik, 11879-80.

120. To the contrary, Shytuhina teaches that "[d]ue to mass heterogeneity, the masses of the minor peaks were less accurate and were therefore not assigned," indicating that even in Shytuhina's optimized Chikungunya system, mass accuracy was insufficient for characterization. EX1006-Shytuhina, 194.

121. A POSA would understand that Shytuhina's intact mass method had limitations on characterizing mass accuracy for minor peaks, and thus would not be motivated to use it for other viral particles, AAV or otherwise. Shytuhina does not even tell a POSA which assays are being done for precision and accuracy; these need to be inferred from the ICH guidelines. EX2031.

122. One need only look at Figure 2 to see the poor mass accuracy in Shytuhina, as explained above. A POSA would recognize that this poor mass accuracy makes Shytuhina's method unsuitable for detecting small changes in mass as found between VPs of different serotypes or from post-translational modifications.

4. No Prior Art Teaching Supports Expectation of Success for Serotype Identification or Characterization of Post-Translational Modifications

123. Dr. Coon opines that, because all of the "techniques required to make the claimed combinations, namely RP-HPLC, UPLC, intact LC-MS, and application of software to calibrate a spectrometer, and also to deconvolute and interpret MS data, were well known to people of skill in the art at the time and would have required nothing more than routine experimentation." Coon Decl., ¶522. I disagree.

124. Even if all the techniques were available to a POSA, I understand that the combination of those techniques must have a reasonable expectation of success. For all the reasons stated above, the combination of Shytuhina and Satkunanathan would not.

125. In particular, as set forth in Section IX.A.2, I disagree with Dr. Coon's assertion that intact LC-MS analysis of AAV would be "even more straightforward" than Shytuhina's analysis of glycoproteins. Coon Decl., ¶525.

126. Dr. Coon opines without citation that "researchers in the field for years before the relevant date" had been "separating and characterizing reduced monoclonal antibodies and identifying PTMs, using intact LC-MS." Coon Decl., ¶530. Dr. Coon uses this assertion to state that application of these techniques to AAV "would have been considerably more routine and straightforward." *Id.* Dr.

Coon's conclusion does not follow from the evidence. Although researchers had been using intact LC-MS on proteins for years before the '377 patent, no one successfully conducted intact LC-MS on AAV. Why not? Dr. Coon does not say.

127. I understand that unexpected results, long-felt need in the art, and failure of others to achieve the claimed invention are objective indicia of non-obviousness. In my opinion, Dr. Coon's assertion that intact LC-MS had been used in other contexts for years before Genzyme successfully applied such methods to AAV supports each of these objective indicia.

128. Further, Dr. Coon cites no prior art establishing that intact mass measurements would be expected to provide sufficient mass accuracy and resolution to distinguish AAV serotypes given potential post-translational modifications and processing variants, that intact LC-MS would be superior to (or at least as good as) established LC-MS/MS identification methods for AAV, or that a POSA could validate intact mass-based serotype assignments. Further, the prior art cited by Dr. Coon does not establish that a POSA would have a reasonable expectation of success in characterizing AAV post-translational modifications with any level of confidence or accuracy.

129. Shytuhina's acknowledgment that "[d]ue to mass heterogeneity of both E1 and E2, the deconvoluted masses of the minor peaks were less accurate" and therefore not assigned (EX1006-Shytuhina, 194) demonstrates limitations in

intact mass measurement accuracy even for an optimized Chikungunya system like Shytuhina's. A POSA would have no reasonable basis to expect that intact mass measurement could reliably identify AAV serotypes or post-translational modifications without substantial development and validation work not taught in the prior art.

130. Indeed, Shytuhina does not even provide the theoretical masses that were used for comparison, or quantitative values for mass accuracy, leaving a POSA questioning the mass accuracy of its identifications. This further diminishes a POSA's expectation of success in combining Shytuhina's method with Satkunanathan.

5. Satkunanathan and Shytuhina Address Fundamentally Different Objectives

131. Dr. Coon opines that "[a] POSA would have been motivated to combine Satkunanathan and Shytuhina because both are directed towards efficient and precise methods for analysis of viral particle composition for the purpose of process development." Coon Decl., ¶339. I disagree. Dr. Coon's broad statement that both Satkunanathan and Shytuhina seek to analyze "viral particle composition for the purpose of process development" ignores the stated purpose of each reference.

132. Satkunanathan seeks the "establishment of a novel cell line for the enhanced production of recombinant adeno-associated virus vectors for gene

therapy" and specifically for the identification of host-cell proteins that co-purify with AAV vectors to understand cellular contribution to AAV assembly. EX1005-Satkunanathan, 930. Satkunanathan's strategy is built around bottom-up discovery proteomics: identifying many unknown host cell proteins that co-purify with three identified AAV serotypes, validating identifications by Western blotting, and performing functional studies via shRNA knockdown to determine which proteins affect AAV production. *Id.*, 929-930.

133. Satkunanathan's method depends on in-solution tryptic digestion and protein identification using specific software and score thresholds. *Id.*, 931.

134. By contrast, Shytuhina's stated goal is developing "a sensitive and robust high-performance liquid chromatography (HPLC) method that can quantitate CHIKV VLPs and monitor product purity throughout the manufacturing process." EX1006-Shytuhina, 192. The authors sought to replace or augment existing, disadvantaged analytical methods such as SDS-PAGE and colorimetric assays. *Id.*

135. Shytuhina sought to characterize three known Chikungunya virus-like particle structural proteins (capsid, E1, E2) for lot-to-lot consistency monitoring and used intact LC-MS solely to identify post-translational modifications in the Chikungunya virus-like particle proteins. *Id.*, 193-94. And as discussed above, the same group that worked on the Shytuhina paper subsequently abandoned the intact

LC-MS approach in favor of a more accurate and precise methodology. EX2030-Lancaster, 218-221.

136. In my opinion, Satkunanathan and Shytuhina represent distinct analytical paradigms designed and used for very different purposes in the art.

137. Satkunanathan's discovery proteomics method requires comprehensive coverage of an unknown proteome across three known AAV serotypes, peptide-level identification with statistical confidence scoring, and database searching across thousands of potential proteins. EX1005-Satkunanathan, 931-32.

138. Shytuhina's targeted intact-protein assay requires, conversely, quantitative separation of three known proteins, validation for vaccine release testing, and post-translational modification characterization by intact mass deconvolution. EX1006-Shytuhina, 193, 195-96.

139. A POSA reading Satkunanathan would understand that it teaches identifying co-purifying host cell proteins using in-solution digestion and peptide tandem MS/MS proteomics—a method that was shown to be successful for comprehensive protein identification in complex viral preparations, and is viewed as such even today. EX2027-Kontogiannis, 6-7.

140. A POSA reading Shytuhina, on the other hand, would understand that it teaches an attempt at developing an intact protein assay for three known, specific

Chikungunya virus-like particles—a method requiring extensive system-specific optimization to overcome hydrophobic protein adsorption and disassembly challenges. EX1006-Shytuhina, 194. Nothing in either reference suggests abandoning its proven approach to pursue the other's objective using the other's methodology for a completely different viral system.

141. Indeed, even as of August 2016, the art taught that "accurate mass measurement of intact proteins alone does not allow for unequivocal protein identification," requiring the use of additional techniques, like tandem MS/MS, for a full picture of the protein. EX2034-Valeja, 7.

6. Satkunanathan and Shytuhina Disclose Different Sample Complexities and Different Analytical Challenges

142. Satkunanathan's AAV samples contained viral capsid proteins from three known serotypes along with numerous co-purifying host cell proteins, because Satkunanathan's explicit purpose was to identify and analyze these host proteins. EX1005-Satkunanathan, 930. Satkunanathan's Supplementary Table S1 lists dozens of identified proteins including brain acid soluble proteins, chaperonins, ribosomal proteins, metabolic enzymes, and other cellular components. EX1005-Satkunanathan, pp. 14-15. The art teaches that this level of sample complexity is ideal for discovery proteomics using bottom-up peptide MS/MS with comprehensive database searching. *Id.*, 930-31.

143. In contrast, Shytuhina's Chikungunya virus-like particle samples were purified vaccine preparations designed to contain primarily three structural proteins: capsid, E1, and E2, with defined 1:1:1 stoichiometry in the assembled virus-like particle. EX1006-Shytuhina, 195. This relatively simple protein composition enabled targeted intact protein quantitation with UV absorbance detection and post-translational modification characterization by intact mass measurement. *Id.*, 194-97.

144. A POSA would recognize that applying Shytuhina's intact protein approach to Satkunanathan's complex AAV samples would introduce significant analytical challenges not present in Shytuhina's three-protein system.

145. For example, co-purifying host proteins may be present at varying abundances, potentially suppressing ionization of AAV capsid proteins or creating overlapping peaks that complicate intact mass assignment. EX2026-Wörner, 6.

146. Further, separating intact AAV capsid proteins (VP1 ~87 kDa, VP2 ~73 kDa, VP3 ~62 kDa) ('377 Patent, 52:23-28) plus numerous host proteins on a single RP-HPLC column may not provide adequate resolution, especially if proteins exhibit similar retention behavior. Peak capacity in the MS may also be inadequate if resolution is insufficient to deconvolve the multiplicity of charge-states generated for each protein in samples containing a multiplicity of host cell proteins. *See* EX2036-May and McLean, 394.

147. Shytuhina reports that "[d]ue to the mass heterogeneity of both E1 and E2, the deconvoluted masses of the minor peaks were less accurate," and were therefore not assigned (EX1006-Shytuhina, 194), indicating limitations even in the optimized three-protein Chikungunya system. It is likely that releasing the post-translational modifications from intact proteins for carbohydrate analysis in Lancaster was performed to address this limitation in Shytuhina. EX2030-Lancaster, 219-20. And of course, as I have calculated, the mass accuracy of Shytuhina at over 2000 ppm is unacceptable. Adding sample complexity and size would exacerbate Shytuhina's accuracy problems. *See* EX2036-May and McLean, 394.

148. AAV undergoes cleavage at pH 5.5 in at least VP1 and VP2, and at pH 5.5 "several new lower-molecular-mass viral bands" appear or increase in intensity, indicating "multiple capsid cleavage products." EX2028-Salganik 2012, 11879-80. Dr. Coon's references do not teach a POSA how to use a 0.1% TFA solution (pH ≈ 2.1) at high temperature for separation of intact AAV VPs without inducing cleavage.

149. Shytuhina validated the RP-HPLC method for quantitation using UV absorbance detection with protein standards. EX1006-Shytuhina, 195. Shytuhina does not teach or suggest validating intact mass measurement for serotype identification in samples containing both viral and host proteins.

150. Dr. Coon provides no explanation for how these analytical challenges would be addressed or why a POSA would expect success applying a three-protein vaccine assay to complex AAV or other viral protein preparations for an entirely different analytical purpose.

The Sample Preparation Methods of Satkunanathan and Shytuhina Are Incompatible

151. Satkunanathan's sample preparation involves digesting samples with trypsin in 50 mM ammonium bicarbonate (pH 8.5) containing 1% Rapigest (an acid-labile surfactant) at 37°C, and terminating the digestion with HCl. EX1005-Satkunanathan, 930-31. The output of this preparation is a complex peptide mixture suitable for nano-LC separation and MS/MS fragmentation. *Id.*, 931.

152. Shytuhina's sample preparation involves incubating virus-like particle samples with 5% Zwittergent 3-12 detergent, followed by RP-HPLC separation on a C4 column at 60°C using mobile phases containing TFA and a strong organic mixture (30% ACN/70% isopropanol) to elute hydrophobic proteins. EX1006-Shytuhina, 194. For MS analysis, the mobile phase was modified to use formic acid instead of TFA to avoid ion suppression. EX1006-Shytuhina, 193. The output of this preparation is intact, denatured Chikungunya virus-like particle proteins suitable for intact mass measurement. *Id.*, 195.

153. These sample preparation protocols are mutually exclusive, and Dr. Coon provides no opinion that these sample preparation protocols could be made

compatible. Satkunanathan's trypsin digestion destroys intact protein mass information by cleaving proteins into peptides. *See* EX1005-Satkunanathan, 930-31. Shytuhina's intact protein approach cannot be combined with a method requiring enzymatic digestion without defeating the express purpose of intact mass analysis. *Id.*; EX1006-Shytuhina, 193-94.

154. Further, Shytuhina's Zwittergent treatment was designed to "solubilize and stabilize the glycoproteins and prevent non-specific binding during chromatography" for Chikungunya enveloped glycoproteins embedded in lipid bilayers. EX1006-Shytuhina, 194. AAV capsids are non-enveloped protein shells with no lipid bilayer. *See* '377 Patent, 51:44-46. Shytuhina provides no teaching that Zwittergent treatment would be appropriate for, or effective at, disassembling non-enveloped AAV capsids or other non-enveloped capsids, and Dr. Coon provides no opinion that it would be.

155. Similarly, Shytuhina's finding that "TFA appeared to help disassemble the VLPs" applies only to Chikungunya's virus-like particle structure—an outer lipid envelope with embedded glycoproteins surrounding a nucleocapsid. EX1006-Shytuhina, 194. Whether TFA would disassemble AAV's highly stable icosahedral protein capsid is not taught or suggested by Shytuhina or Satkunanathan. Shytuhina notes that "without TFA, the VLPs eluted in the void volume," demonstrating that virus-like particle disassembly was critical for

chromatographic separation. *Id.* But, again, this Chikungunya-specific finding does not translate to AAV without experimental validation which is absent in the cited art. *Id.*

The Data Acquisition and Data Analysis Methods of Satkunanathan and Shytuhina Are Incompatible

156. Satkunanathan used an LC-MS/MS system operated in "data-dependent MS/MS mode" where "the five most intense ions were sequentially isolated and passed to the orbi-trap for accurate mass measurement with the resolution of 30,000 parts per million (ppm)." EX1005-Satkunanathan, 931. Data analysis was performed using "Proteome Discoverer 1.2 with built-in Sequest," with protein FASTA databases downloaded from UNIPROT and data included only when Xcorrelation values were greater than 1.5 for +1 charge state, 2.0 for +2, and 2.5 for +3 or higher. *Id.*

157. Shytuhina used "a Synapt G2 mass spectrometer" operated in "Sensitivity mode and spectra were acquired in an m/z range of 1000-2500," optimized for intact Chikungunya protein detection. EX1006-Shytuhina, 193. Data analysis was performed with MassLynx software, with mass spectra deconvoluted using the Maximum Entropy (MaxEnt) algorithm with "output mass range of 40,000 to 60,000." *Id.* Observed masses were matched to "theoretical molecular weight[s]" of known Chikungunya proteins with expected post-translational modifications. *Id.*, 194.

158. These MS workflows operate on entirely different principles. Satkunanathan's data-dependent MS/MS dynamically selects peptide ions based on intensity, fragments them, and matches fragmentation patterns against theoretical peptide sequences in protein databases—enabling identification of unknown proteins. EX1005-Satkunanathan, 931. Shytuhina's intact mass profiling acquires spectra of intact multiply-charged Chikungunya proteins, deconvolutes charge states to determine neutral masses, and matches observed masses to theoretical masses of known target proteins with predicted post-translational modification states—enabling post-translational modification characterization of known Chikungunya proteins. EX1006-Shytuhina, 193-94.

159. A POSA would recognize that the references' approaches to data analysis are mutually exclusive: database searching requires peptide fragmentation spectra; intact mass deconvolution requires intact protein spectra without fragmentation. A POSA cannot perform both analyses on the same sample preparation without fundamentally altering the analytical workflows. *See* EX1005-Satkunanathan, 931; EX1006-Shytuhina, 193-94.

B. Independent Claim 2

160. While claim 1 of the '377 patent is specifically directed to determining the serotype of an AAV particle, I understand that claim 2 of the '377 patent is directed to determining the serotype of a viral particle.

161. My opinions are the same regarding motivation to combine and reasonable expectation of success of the claimed combinations of prior art, regardless of whether the challenged claims relate to AAV or viral particles generally.

162. As stated above, Shytuhina is a bespoke intact LC-MS method for intact analysis of known Chikungunya proteins. Its method development was laborious and even after optimization had poor data quality and significant drawbacks. In contrast, Satkunanathan is a bottom-up LC-MS method that uses digestion to study differences in protein composition between three AAV serotypes and generally worked well. As explained throughout my declaration, there is no motivation to combine these disparate references for viral particle analysis. They would be viewed in the art as incompatible with divergent motivations.

163. For claim 2 of the ’377 patent, neither Shytuhina nor Satkunanathan teaches or suggests using its method to determine the serotype of a viral particle, AAV or otherwise. The AAV serotypes in Satkunanathan were known at the outset, and Shytuhina teaches or suggests nothing about serotypes at all.

164. Further, Dr. Coon makes the bare, uncited assertion that a “POSA would have understood how to use Shytuhina’s intact LC-MS method to determine PTMs in a preparation of viral particles.” Coon Decl., ¶475. In light of all the

difficulties described above, in my opinion a POSA would not have been motivated to combine Shytuhina and Satkunanathan as Dr. Coon has opined.

C. Dependent Claims 3-20

165. Dr. Coon asserts that the combination of Satkunanathan and Shytuhina renders dependent claims 3-20 obvious. Because Dr. Coon's additional opinions regarding these dependent claims do not provide any additional motivation to combine or reasonable expectation of success, it is my opinion that claims 1-20 are not obvious in view of the combination of Satkunanathan and Shytuhina.

X. GROUND II: ANSONG DOES NOT CURE THE FUNDAMENTAL DEFICIENCIES IN GROUND I

166. Dr. Coon does not provide any further opinions regarding a motivation to combine Satkunanathan and Shytuhina with respect to dependent claims 8 and 17. Rather, he applies the Ansong reference to the dependent claims reciting UPLC limitations.

167. Dr. Coon does not provide any opinion that Ansong's method would be relevant to claims 1 or 2 of the '377 patent. Ansong provides no teaching regarding viral particle analysis, virus-like particle disassembly, capsid protein separation, or serotype identification. Ansong studied *Salmonella typhimurium* bacterial proteins to identify post-translational modifications (specifically S-thiolation) in response to infection-like conditions. EX1009-Ansong, 10153. The

analytical challenges and sample preparation requirements for bacterial lysates are entirely different from those for intact viral particles. EX1006-Shytuhina, 194; EX1005-Satkunanathan, 930-31; EX1009-Ansong, 10154.

168. Adding Ansong's UPLC to the Satkunanathan-Shytuhina combination does not resolve the fundamental incompatibilities of that combination, which would still face all the problems identified above.

169. Moreover, Shytuhina's method is optimized for Chikungunya virus-like particles. *See generally* EX1006-Shytuhina. The art does not teach that simply switching from HPLC to UPLC while maintaining Shytuhina's method would work, and it is likely that re-optimizing for UPLC would require additional and substantial method development that Dr. Coon does not consider. EX2037-Wright, 1838-40. Ansong provides no teaching regarding this development for viral particles.

170. Thus, Ansong provides no evidence to cure the deficiencies in Dr. Coon's opinions with respect to independent claims 1 and 2, and does not render obvious the additional limitations of claims 8 and 17.

XI. GROUND III: BYEON DOES NOT CURE THE FUNDAMENTAL DEFICIENCIES IN GROUND I

171. Dr. Coon does not provide any further opinions regarding a motivation to combine Satkunanathan and Shytuhina with respect to dependent

claims 9, 10, 18, and 19. Rather, he applies the Byeon reference to the dependent claims reciting calibration limitations.

172. While Byeon discloses mass spectrometer calibration with NaI, it does not address the substantive challenges of the claimed combination described above. Dr. Coon asserts that "a POSA at the time would have understood that LC-MS systems require calibration" and "would have been motivated to use Byeon's method of assisted calibration . . . because such assisted calibration was an efficient and straightforward method of calibration." Coon Decl., ¶¶569, 572. I disagree.

173. Dr. Coon's reliance on Byeon puts the cart before the horse. Before a POSA would ever consider which calibration method to use for intact LC-MS analysis of AAV capsid proteins, that POSA would first need a reason to pursue intact LC-MS analysis of AAV capsid proteins at all—a motivation that, as demonstrated above, the prior art does not provide. Calibration is a routine prerequisite of any mass spectrometry experiment, not a reason to undertake one. Moreover, multiple calibration approaches were available in the art at the relevant time, and Petitioner provides no explanation for why a POSA would specifically select Byeon's NaI-based assisted calibration over any other available option, or why that selection would be anything other than arbitrary. Byeon's disclosure of a

routine calibration procedure for a single glycoprotein therapeutic therefore adds nothing to cure the fundamental deficiencies in the base combination.

174. Byeon provides no teaching regarding viral particle analysis, virus-like particle disassembly, capsid protein separation, or serotype identification. Byeon analyzed recombinant human erythropoietin (rHu-EPO), a glycoprotein therapeutic, to identify a non-glycosylated variant at a specific glycosylation site. EX1014-Byeon, 501-02. This method involves post-translational modification characterization of a single known protein, not serotype or post-translational modification identification of viral capsid proteins in complex samples. *See id.*

175. Thus, Byeon provides no evidence to cure the deficiencies in Dr. Coon's opinions with respect to independent claims 1 and 2, and does not render obvious the additional limitations of claims 9, 10, 18, and 19.

XII. CONCLUSION

176. The Challenged Claims of the ’377 Patent are not obvious in view of the Grounds set forth by the Petition and by the Coon Declaration.

177. 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 that these statements were made with knowledge that willful false statement 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: March 4, 2026

John A. McLean