

**UNITED STATES PATENT AND TRADEMARK OFFICE**

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**BEFORE THE PATENT TRIAL AND APPEAL BOARD**

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SAREPTA THERAPEUTICS, INC.,

Petitioner,

v.

Genzyme Corporation,

Patent Owner

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CASE: IPR2026-00270

U.S. PATENT NO. 12,298,313

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**PATENT OWNER'S PRELIMINARY RESPONSE**

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**PATENT OWNER’S UPDATED TABLE OF EXHIBITS**

| <b>Exhibit No.</b> | <b>Description</b>                                                                                                                                                                                                                                   |
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| 2001               | Declaration of Katherine A. Helm                                                                                                                                                                                                                     |
| 2002               | <i>Genzyme Corporation v. Sarepta Therapeutics Inc.</i> (1:24-cv-00882) D.I. 78 – Joint Stipulation to Amend Scheduling Order                                                                                                                        |
| 2003               | <i>Genzyme Corporation v. Sarepta Therapeutics Inc.</i> (1:24-cv-00882) D.I. 59 – Notice of Service of Plaintiff’s Initial Claim Charts on Infringement                                                                                              |
| 2004               | <i>Genzyme Corporation v. Sarepta Therapeutics Inc.</i> (1:24-cv-00882) D.I. 68 – Notice of Service of Defendant’s Initial Invalidity Contentions                                                                                                    |
| 2005               | <i>Genzyme Corporation v. Sarepta Therapeutics Inc.</i> (1:24-cv-00882) D.I. 28 - Notice of Service of Plaintiff’s First Set of Requests for Production of Documents and Things                                                                      |
| 2006               | <i>Genzyme Corporation v. Sarepta Therapeutics Inc.</i> (1:24-cv-00882) D.I. 35 - Notice of Service of Defendant’s First Set of Requests of Production of Documents and Things to Plaintiff and First Set of Interrogatories and Initial Disclosures |
| 2007               | <i>Genzyme Corporation v. Sarepta Therapeutics Inc.</i> (1:24-cv-00882) D.I. 37 - Notice of Service of Plaintiff’s Second Set of Interrogatories and Second Set of Requests for Production of Documents and Things                                   |
| 2008               | <i>Genzyme Corporation v. Sarepta Therapeutics Inc.</i> (1:24-cv-00882) D.I. 45 – Notice of Service of Plaintiff’s First Set of Interrogatories and Third Set of Requests for Production of Documents and Things                                     |
| 2009               | <i>Genzyme Corporation v. Sarepta Therapeutics Inc.</i> (1:24-cv-00882) D.I. 67 - Notice of Service of Defendant’s Second Set of Requests of Production of Documents and Things to Plaintiff and First Set of Interrogatories                        |
| 2010               | <i>Genzyme Corporation v. Sarepta Therapeutics Inc.</i> (1:24-cv-00882) D.I. 83 - Notice of Service of Defendant’s Amended Second Set of Requests of Production of Documents and Things to Plaintiff and First Set of Interrogatories                |
| 2011               | <i>Genzyme Corporation v. Sarepta Therapeutics Inc.</i> (1:24-cv-00882) D.I. 87 - Notice of Service of Defendant’s Third Set of Requests of Production of Documents and Things to Plaintiff                                                          |
| 2012               | <i>Genzyme Corporation v. Sarepta Therapeutics Inc.</i> (1:24-cv-                                                                                                                                                                                    |

| Exhibit No. | Description                                                                                                                                                                                                                 |
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|             | 00882) D.I. 91 – Notice of Service of Plaintiff’s Responses to Defendants Third Set of Requests for Production of Documents and Things                                                                                      |
| 2013        | <i>Genzyme Corporation v. Sarepta Therapeutics Inc.</i> (1:24-cv-00882) D.I. 92 - Notice of Service of Plaintiff’s Fourth Set of Requests for Documents and Things                                                          |
| 2014        | <i>Genzyme Corporation v. Sarepta Therapeutics Inc.</i> (1:24-cv-00882) D.I. 99 – Notice of Service of Plaintiff’s Fifth Set of Requests for Documents and Things                                                           |
| 2015        | <i>Genzyme Corporation v. Sarepta Therapeutics Inc.</i> (1:24-cv-00882) D.I. 102 – Notice of Service of Defendant’s Fourth Set of Requests of Production of Documents and Things to Plaintiff                               |
| 2016        | <i>Genzyme Corporation v. Sarepta Therapeutics Inc.</i> (1:24-cv-00882) D.I. 110 – Notice of Service of Plaintiff’s Sixth Set of Requests for Production of Documents and Things                                            |
| 2017        | <i>Genzyme Corporation v. Sarepta Therapeutics Inc.</i> (1:24-cv-00882) D.I. 76 – Second Amended Complaint                                                                                                                  |
| 2018        | <i>Genzyme Corporation v. Sarepta Therapeutics Inc.</i> (1:24-cv-00882) - Defendant’s Supplemental Initial Invalidity Contentions                                                                                           |
| 2019        | U.S. Patent No. 11,698,377                                                                                                                                                                                                  |
| 2020        | Prosecution History Excerpts for U.S. Patent No. 11,698,377                                                                                                                                                                 |
| 2021        | Van Vilet, Kim et al., Adeno-Associated Virus Capsid Serotype Identification: Analytical Methods Development and Application, 159 <i>Journal of Virological Methods</i> 167–177 (2009)                                      |
| 2022        | Bark, Steven et al., High-Temperature Protein Mass Mapping Using A Thermophilic Protease, 123 <i>J. Am. Chem. Soc.</i> 1774-76 (2001)                                                                                       |
| 2023        | <i>Genzyme Corporation v. Sarepta Therapeutics Inc.</i> (1:24-cv-00882) D.I. 108 – Joint Claim Construction Chart                                                                                                           |
| 2024        | Expert Declaration of John A. McLean, Ph.D. (“McLean Decl.”)                                                                                                                                                                |
| 2025        | Curriculum Vitae of John A. McLean, Ph.D.                                                                                                                                                                                   |
| 2026        | Wörner and Heck et al., “Adeno-associated virus capsid assembly is divergent and stochastic,” <i>Nature Communications</i> , 12:1642 (2021): 1-9 (“Wörner”)                                                                 |
| 2027        | Kontogiannis et al., “Characterization of AAV vectors: A review of analytical techniques and critical quality attributes,” <i>Mol. Ther. Methods and Clinical Development</i> , Vol. 32 (Sept. 2024): 1-17 (“Kontogiannis”) |

| <b>Exhibit No.</b> | <b>Description</b>                                                                                                                                                                                                                                                                        |
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| 2028               | Salganik et al., “Evidence for pH-Dependent Protease Activity in the Adeno-Associated Virus Capsid,” <i>Journal of Virology</i> , 86:21 (Nov. 2012):11877-11885 (“Salganik”)                                                                                                              |
| 2029               | Supplemental Materials to Ansong et al., “Top-down proteomics reveals a unique protein S-thiolation switch in <i>Salmonella Typhimurium</i> in response to infection-like conditions,” <i>Proceedings of the National Academy of Sciences</i> 110:25 (2013): 10153-10158 (“Ansong Supp.”) |
| 2030               | Lancaster and Ha et al., “Characterization of N-glycosylation profiles from mammalian and insect derived chikungunya VLP,” <i>Journal of Chromatography B</i> , 1032 (2016): 218-223 (“Lancaster”)                                                                                        |
| 2031               | ICH Harmonised Tripartite Guideline: Validation of Analytical Procedures: Text and Methodology Q2(R1) (Nov. 2005) (“ICH Guideline”)                                                                                                                                                       |
| 2032               | Boys et al., “Protein Oxidative Modifications During Electrospray Ionization: Solution Phase Electrochemistry or Corona Discharge-Induced Radical Attack?,” <i>Analytical Chemistry</i> 81 (2009): 4027-4034 (“Boys”)                                                                     |
| 2033               | Knolhoff, Callahan, and Croley, “Mass Accuracy and Isotopic Abundance Measurements for HR-MS Instrumentation: Capabilities for Non-Targeted Analyses,” <i>J. Am. Soc. Mass Spectrom.</i> 25 (2014): 1285-1294 (“Knolhoff”)                                                                |
| 2034               | Valeja et al., “Three Dimensional Liquid Chromatography Coupling IEC/HIC/RPC for Effective Protein Separation in Top-Down Proteomics,” <i>Analytical Chemistry</i> 87(10) (2015): 5363-5371 (“Valeja”)                                                                                    |
| 2035               | Han, Aslanian, & Yates, “Mass spectrometry for proteomics,” <i>Curr. Op. in Chem. Biol.</i> 12 (2008): 483-490 (“Han and Yates”)                                                                                                                                                          |
| 2036               | May & McLean, “Advanced Multidimensional Separations in Mass Spectrometry: Navigating the Big Data Deluge,” <i>Annu. Rev. Anal. Chem.</i> 9 (2016):387-409 (“May and McLean”)                                                                                                             |
| 2037               | Wright & Wright, “Advances in LC: Bioanalytical Method Transfer,” <i>Bioanalysis</i> 8(17) (2016):1837-1844 (“Wright”)                                                                                                                                                                    |

## I. INTRODUCTION

This is Petitioner’s third petition challenging three related patents from the same patent family. The Director has already denied institution of *inter partes* review petitions challenging two related patents sharing the same specification in view of the same art and arguments presented by Petitioner here. *See* IPR2026-00167, Paper No. 13 (May 15, 2026); IPR2026-00168, Paper No. 13 (May 15, 2026). As explained in this Preliminary Response, denial of institution is also merited here.

The patent challenged here, U.S. Patent No. 12,298,313 (EX1001, the “’313 patent”), is directed to methods of detecting post-translational modifications and heterogeneity in preparations of adeno-associated virus (“AAV”) particles by performing liquid chromatography-mass spectrometry (“LC-MS”) on intact proteins in the absence of a gel separation step, as well as methods of preparing pharmaceutical compositions of such particles. These methods represent a significant advancement over prior-art approaches that relied on enzymatic digestion followed by peptide tandem mass spectrometry (“MS/MS”) analysis—a fundamentally different analytical paradigm that destroys information about intact protein masses, post-translational modifications, and capsid protein stoichiometry.

Petitioner challenges claims 1-27 of the ’313 patent (the “challenged claims”) on three grounds of obviousness, asserting that the claimed methods

require “nothing more than routine experimentation.” Pet. at 60. Institution should be denied. Petitioner’s case rests on mischaracterization of the prior art, hindsight reconstruction, and unsupported expert opinions that ignore critical technical obstacles in the references Petitioner cites. A person of ordinary skill in the art (“POSA”) would recognize that these references are incompatible in the combinations Petitioner posits. Petitioner’s failure to identify a clear motivation to combine the asserted references and to establish a reasonable expectation of success result in the Petition’s irremediable failure to establish unpatentability. *In re Magnum Oil Tools Int’l Ltd.*, 829 F.3d 1364, 1380-81 (Fed. Cir. 2016).

Ground I of the Petition alleges claims 1-27 are obvious over Satkunanathan (EX1005) combined with Shytuhina (EX1006), asserting that both references concern “efficient and precise methods for analysis of viral particle composition for the purpose of process development” and that “[a] POSA would have been motivated to combine” them. Pet. at 27. Grounds II and III rely on the same combination of primary references as Ground I, adding tertiary references for dependent claims. Petitioner does not support the motivation and reasonable expectation of success critical to its obviousness assertions, and the cited art is riddled with errors, inaccuracies, and omissions that would preclude motivation to combine and reasonable expectation of success.

***First***, there is no motivation to combine Shytuhina and Satkunanathan.

Satkunanathan studied AAV serotypes using “bottom-up” liquid chromatography followed by tandem mass spectrometry (“LC-MS/MS”), an analytical approach requiring enzymatic digestion of proteins to identify proteins present in preparations of purified AAV vectors. Tandem mass spectrometry analysis is an analytical technique distinct from traditional mass spectrometry. McLean Decl., ¶128; EX1005-Satkunanathan, 930-31. The explicit purpose of Satkunanathan was to identify host-cell proteins that co-purify with AAV vectors in order to understand cellular contribution to AAV assembly—a discovery-proteomics objective requiring identification of many unknown proteins. McLean Decl., ¶132; EX1005-Satkunanathan, 930.

Shytuhina, on the other hand, discloses a bespoke reversed-phase high-performance liquid chromatography-mass spectrometry (“RP-HPLC-MS”) method, attempting to analyze intact Chikungunya virus structural proteins specifically for vaccine process development. McLean Decl., ¶157; EX1006-Shytuhina, 192. Shytuhina’s stated purpose was to quantitate known Chikungunya virus-like particle structural proteins and validate a Chikungunya-specific assay to replace SDS-PAGE (*i.e.*, gel separation) and colorimetric methods for vaccine quality control. McLean Decl., ¶134; EX1006-Shytuhina, 192. There is no motivation to combine Satkunanathan and Shytuhina because a POSA would view these references as analytically distinct; they are designed and used for very

different purposes in the art. McLean Decl., ¶¶136-141.

**Second**, Shytuhina’s method is not “efficient and precise” as Petitioner repeatedly claims—rather, its data quality and mass accuracy are poor and unreliable, and its methodology was abandoned by the lab that originally published it. The mass-accuracy error of Shytuhina is over 2000 ppm, which is more than two orders of magnitude higher than what a POSA would have considered to be acceptable accuracy for calculating protein masses at the time, including for determining post-translational modifications and heterogeneity. Shytuhina’s method was difficult to develop, inefficient, specific to Chikungunya virus, and would not be viewed as broadly applicable. McLean Decl., ¶¶84-101.

**Third**, and relatedly, Petitioner has failed to provide more than hindsight reconstruction for combining Shytuhina and Satkunanathan to develop an intact LC-MS method for AAV viral particles. While Petitioner asserts that “the intact LC-MS method of Shytuhina would have allowed efficient, reliable, accurate analysis of individual AAV capsid proteins, including distinguishing vp1, vp2, and vp3 from one another and from those of other serotypes, and assessing PTMs and other variations and truncations” (Pet. at 4), this assertion is based on unsupported expert opinion and is belied by the art. AAV has specific challenges for intact analysis that distinguish it from Chikungunya virus, including high particle stability, a variety of post-translational modifications, and numerous co-purifying

host proteins absent in Shytuhina. McLean Decl., ¶¶102-110; *see also TriVascular, Inc. v. Samuels*, 812 F.3d 1056, 1066 (Fed. Cir. 2016) (noting the Board must “be careful not to allow hindsight reconstruction of references . . . without any explanation as to how or why the references would be combined to produce the claimed invention”). Petitioner cites no prior-art teaching that post-translational modification analysis for AAV by intact capsid protein mass measurement was known, feasible, or would provide sufficient mass resolution given the variety of post-translational modifications and processing variants known in the art, and the mass accuracy limitations found in Shytuhina. A POSA would simply not be motivated to use Shytuhina’s inaccurate intact LC-MS method to analyze viral particles. McLean Decl., ¶¶118-122.

**Fourth**, Petitioner fails to establish a reasonable expectation of success. Petitioner asserts that “the techniques required to make the claimed combinations . . . were well known . . . and would have required nothing more than routine experimentation” and that “[i]ntact LC-MS analysis of AAV capsid proteins would have been even more straightforward” than Shytuhina’s glycoprotein analysis because “[g]lycoproteins are more variable and therefore more difficult to analyze.” Pet. at 59-60. These assertions are contradicted by Shytuhina’s disclosure. Shytuhina reports that the Chikungunya virus-like particle system was technically challenging because of the hydrophobic nature of the viral

glycoproteins and the presence of lipids. McLean Decl., ¶103; EX1006-Shytuhina, 194. As described in detail below, Shytuhina's disclosures establish that intact virus-like particle protein analysis required method development specific to Chikungunya glycoproteins. Petitioner provides no evidence that the Chikungunya-specific methods disclosed by Shytuhina would apply to AAV or other viral capsids (which have entirely different structures, assembly properties, and protein composition), no teaching on how to adapt them, and no basis for concluding that similar or different challenges would not require comparable system-specific development work for AAV or other viral particles. McLean Decl., ¶¶123-130. This is especially true because Shytuhina's method did not even work in Shytuhina—a POSA would understand its data were too inaccurate to be applicable to analyzing populations of any viral particles, including AAV.

***Fifth***, a POSA would understand that Shytuhina and Satkunanathan are technically incompatible, such that a POSA would have to choose one method or the other, not a combination of the two. Satkunanathan's method involves enzymatically digesting proteins before subjecting them to LC-MS/MS. McLean Decl., ¶153; EX1005-Satkunanathan, 930-31. Conversely, Shytuhina's method involves carrying out MS on intact Chikungunya proteins that have not been enzymatically digested. McLean Decl., ¶152; EX1006-Shytuhina, 192. Their workflows operate on different analyte forms (peptides vs. intact proteins), use

different separation strategies (nano-LC peptide gradients vs. high-temperature intact protein RP-HPLC), employ different MS acquisition modes (data-dependent MS/MS vs. intact mass profiling), and require different data analysis software (peptide database searching vs. intact mass deconvolution). McLean Decl., ¶¶142-158. A POSA would recognize these as mutually exclusive analytical approaches, not techniques amenable to combination. McLean Decl., ¶159.

Petitioner's case relies heavily on the declaration of Dr. Joshua J. Coon, EX1003 ("Coon Decl."). As demonstrated in the rebuttal provided by Dr. John McLean (EX2024, "McLean Decl."), Dr. Coon's opinions are contradicted by the specific technical disclosures in the references he cites. His unsupported assertions of "routine experimentation," "straightforward" combination, and "reasonable expectation of success" ignore the clear challenges in the art (including Shytuhina's extensive method development challenges and poor mass accuracy), mischaracterize the analytical objectives of both references, and rest on conclusory statements unsupported by reference-based teachings.

Dr. McLean explains the deficiencies in Dr. Coon's opinions, including in Dr. Coon's lengthy background technical section. For example, Dr. Coon fails to distinguish between art that uses top-down, intact LC-MS from art that uses bottom-up MS/MS; a POSA would understand these are distinct analytical techniques. McLean Decl., ¶29. Dr. Coon disparages methods employing

enzymatic digestion as “laborious” and “time-consuming” while ignoring the disadvantages of intact methods like Shytuhina’s. McLean Decl., ¶30. And Dr. Coon opines that a POSA would expect intact analysis to work for large proteins (*e.g.*, 100 to 200 kDa and larger) without considering the shortcomings of intact LC-MS methods like Shytuhina’s. McLean Decl., ¶31.

Dr. Coon’s opinion 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” is particularly problematic. Coon Decl., ¶713. This reasoning misidentifies Shytuhina’s challenges (which were primarily hydrophobicity and virus-like particle disassembly, not glycan variability), provides no evidence of whether AAV capsids would present similar or different challenges, and offers no experimental data or prior-art teaching to support the assertion. *Qiagen N. Am. Holdings, Inc. v. HandyLab, Inc.*, No. 2020-2249, 2021 WL 5024387, at \*5 (Fed. Cir. Oct. 29, 2021) (“host of specific technical difficulties” can defeat proposed modifications and combinations). Indeed, Petitioner ignores that AAV capsids present their own structural obstacles and difficulties: highly stable icosahedral particles requiring disassembly, potential carry-over issues, sample complexity from co-purifying host proteins, pH-dependent cleavage, and unknown ionization efficiency if analyzed under Shytuhina’s conditions. McLean Decl., ¶¶100-101; EX2026-Wörner; EX2027-

Kontogiannis; EX2028-Salganik.

Ground II's addition of Yuan for dependent claims 6, 19, and 27 for column selection does nothing to remedy the deficiencies of the base combination. In Ground III, Petitioner attempts to further combine the problematic Shytuhina-Satkunanathan combination with Zabrouskov, a reference describing the use of an MS/MS method to analyze deamidation in a single well-studied, well-behaved protein. Zabrouskov does not teach or suggest how to discern post-translational modifications in large, intact viral capsid proteins, how to analyze mixed populations of particles, or how to handle challenges specific to AAV viral particles.

Patent Owner respectfully requests that the Board deny institution of *inter partes* review.

## **II. BACKGROUND**

### **A. The '313 patent**

The '313 patent, titled "Methods for Detecting AAV," issued on May 13, 2025, and is assigned to Patent Owner Genzyme Corporation. The specification discusses using intact protein LC-MS as an analytical tool to evaluate viral preparations and to discern their characteristics, including the presence of any post-translational modifications or heterogeneity on the viral capsid proteins. The specification describes methods of denaturing rAAV samples before LC-MS

analysis and details the LC-MS intact protein analysis that is the basis of the invention.

The earliest priority date for the '313 patent claims is August 15, 2016. Petitioner applies this priority date. Pet. at 14.

The 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, 1:40-44, 51:63-52:26; McLean Decl., ¶33. The inventors of the '313 patent disclosed methods for improving this process, and particularly for serotype identification and the detection of any post-translational modifications and heterogeneity on the viral capsid proteins. *See, e.g.*, EX1001, 20:19-23.

The capsid of an AAV particle is made up of three distinct proteins, VP1, VP2, and VP3, and the masses of each viral capsid protein ("VP") is unique for each serotype. *Id.*, 57:20-24, 20:16-19, Table 3. Thus, following mass spectrometry, the '313 patent teaches that AAV serotype can be accurately determined by comparing the measured masses of each of the VPs within a sample against their theoretical masses. *Id.* Additionally, the '313 patent teaches that post-translational modifications can be determined by identifying peaks on the mass spectrometer data that are similar to, but slightly different than, the respective peaks for each of the respective VPs. *See id.*, 26:7-9, 54:53-55:7, Figs. 2A and 2B.

Different serotypes can be difficult to differentiate, especially serotypes having high sequence homology and overlapping epitopes, which result in similar masses. McLean Decl., ¶35. Indeed, the '313 patent discloses the small differences in mass between the VPs of different AAV serotypes. EX1001, Tables 4-6. Post-translational modifications and heterogeneity are similarly difficult to track given the high resolution needed to accurately separate and measure VPs from their modified forms. McLean Decl., ¶35.

The prior art included purported methods to discern post-translational modifications, heterogeneity, consistency, and identity, 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, 1:55-66. Gel-LC-MS/MS was another potential method, but it entailed multiple steps performed over multiple days. EX1001, 1:62-67. These steps included SDS-PAGE, in-gel digestion using a protease, and LC-MS/MS, which, despite taking significant amounts of time, ultimately “provid[ed] limited sequence coverage” as the inventors of the '313 patent explained. *Id.*, 1:55-66; *see also id.*, 52:42-48. Thus, the inventors explained that “it would be useful to have improved methods of characteriz[ing] viral particles,” including quicker and more accurate ways to characterize VPs in a given sample. *Id.*, 2:2-3.

The '313 patent inventors achieved improved speed and accuracy for characterizing VPs 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 steps. *Id.*, 2:16-20, 52:27-59, 53:35-51, 54:35-55:7. This method included denaturation of the intact protein, which “allowed for clean mass spectrum” prior to LC. *Id.*, 53:35-38, 54:30-32. The inventors overcame the issue of VP1 and VP2 peak suppression through changes of various chromatographic column parameters. *Id.*, 54:36-52.

The inventors compared the theoretical masses for VP1, VP2, and VP3 for several AAV serotypes against the masses determined experimentally via their intact protein analysis method. *Id.*, Table 2. The results showed that intact protein analysis via the inventors’ disclosed method could accurately differentiate 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.*, 54:53-55:4. They also checked for glycosylation on the VPs. *Id.*, 55:4-7. The inventors compared these results against LC-MS/MS peptide mapping and validated the accuracy of their post-translational modification results. *Id.*, 53:65-54:22, 55:8-50, 56:60-67.

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.*, 59:17-28. *See also id.*, 64:20-30.

**B. Prosecution History of the ’313 patent and Related Family**

During the prosecution of the ’313 patent, Genzyme filed a terminal disclaimer related to U.S. Patent No. 11,698,377, which is at issue in IPR2026-00167 (the “-167 IPR”), which the Director declined to institute. The relevant substance of the prosecution of this patent family occurred during prosecution of the ’377 patent; the claims of the ’313 patent issued without rejection following the terminal disclaimer. *See* EX1002, 231, 267-269. That prosecution history confirms the novelty of the inventors’ disclosed methods. Claim 1 of the ’377 patent originally 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.” -167 IPR, EX1002, 522. The prosecution centered on two prior art references, Vliet and Bark. In an obviousness rejection, the Examiner conceded that Vliet did not teach directly subjecting the denatured AAV particle to LC-MS, but argued that Bark supplied that teaching. *Id.*, 1799.

In response, Genzyme amended claim 1 of the '377 patent 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. 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. In fact, Vliet taught away from performing the disclosed method in the absence of a gel separation step, as it 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 without the use of a protein digestion step. *Id.*, 1832-33. Instead, 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.

In a final rejection, the Examiner argued that Bark supplied 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.* Genzyme therefore 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 (emphasis added). 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” and that the limitation 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.

The Examiner then allowed claim 1 of the ’377 patent 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. Those claims issued as the ’377 patent. The claims of the ’313 patent issued as filed without substantive rejection. *See generally* EX1002.

### **C. The Challenged Claims**

The challenged claims at issue here in the ’313 patent are directed to methods for detecting post-translational modifications of viral proteins and determining the heterogeneity of viral particles in preparations of AAV particles, and of preparing pharmaceutical compositions of AAV particles, using intact protein LC-MS analysis without gel separation. Claims 1, 11, and 20 are

independent claims, and recite the following:

1. A method of detecting post-translational modifications of one or more viral proteins (VPs) in a preparation of adeno-associated virus (AAV) particles, the method comprising
  - a) denaturing the AAV particles,
  - b) subjecting the denatured AAV particle to liquid chromatography/mass spectrometry (LC/MS) **intact protein analysis**;
  - c) determining the masses of one or more VPs; and
  - d) determining any deviation of the determined masses of the one or more VPs from the theoretical masses of corresponding VPs that have not undergone post-translational modifications to detect a deviation in the compared masses,wherein the VPs comprise VP1, VP2 and VP3 capsid proteins, and wherein the method is performed **in the absence of a gel separation step**.
11. A method of determining the heterogeneity of viral particles in a preparation of adeno-associated virus (AAV) particles comprising VP1, VP2 and VP3 capsid proteins, the method comprising
  - a) denaturing the AAV particles;
  - b) subjecting the denatured AAV particles to liquid chromatography/mass spectrometry (LC/MS) **intact protein analysis**, thereby separating the peaks of the VP1, VP2 and VP3 capsid proteins;
  - c) deconvoluting the peaks of the VP1, VP2 and VP3 capsid proteins; and
  - d) determining the masses of one or more of the VP1, VP2 and VP3 capsid proteins and additional capsid proteins within one or more of the deconvoluted peaks,wherein the method is performed **in the absence of a gel separation step**.
20. A method of preparing a pharmaceutical composition of adeno-associated virus (AAV) particles, the method comprising:
  - monitoring AAV particles for consistency and/or identity;wherein the AAV particles comprise viral proteins (VPs) comprising VP1, VP2 and VP3 capsid proteins of an AAV particle capsid,

wherein the AAV particle is monitored for consistency and/or identity by:

- a) extracting an aliquot of an AAV particle preparation;
- b) denaturing the AAV particles;
- c) subjecting the denatured AAV particles to liquid chromatography/mass spectrometry (LC/MS) ***intact protein analysis***;
- d) determining the masses of one or more VPs of the AAV particles; and
- e) comparing the determined masses of the one or more VPs to theoretical masses of corresponding VPs, wherein the theoretical masses of corresponding VPs are those VPs of known AAV serotypes and/or those that have not undergone undesired post-translational modifications; and
- f) determining if there is any deviation of the determined masses of the one or more VPs from the theoretical masses of the corresponding VPs;

wherein the determination of any deviation of the determined masses of the one or more VPs from the theoretical masses of corresponding VPs thereby monitors the AAV particles for consistency and/or identity;

wherein the monitoring for consistency and/or identity is performed ***in the absence of a gel separation step***; and

wherein if less than an undesirable amount of deviation is determined during the monitoring for consistency and/or identity, the AAV particles are combined with one or more pharmaceutically acceptable excipients to form the pharmaceutical composition.

Claims 2-10, 12-19, and 21-27 depend therefrom and specify particular post-translational modifications, chromatography columns, and denaturation methods.

#### **D. Level of Ordinary Skill in the Art**

Petitioner asserts that a POSA 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, or alternatively at least a Master's or Bachelor's Degree with a corresponding number of additional years of experience. Pet. at 15.

For purposes of this Preliminary Response, Patent Owner agrees with this general framework. Patent Owner further emphasizes that a POSA should have specialized experience in viral vector characterization, bottom-up and top-down LC-MS method development, and experience troubleshooting complex analytical challenges.

### **III. CLAIM CONSTRUCTION**

Petitioner states that “the challenged claims have generally been analyzed according to their plain and ordinary meaning.” Pet. at 26. Patent Owner generally agrees that claim terms should be given their plain and ordinary meaning as understood by a POSA in light of the specification. Patent Owner reserves the right to address specific claim construction issues should the Board institute review.

### **IV. OVERVIEW OF THE PRIOR ART**

#### **A. Satkunanathan (EX1005)**

Petitioner states that “Satkunanathan used LC-MS/MS, involving enzymatic digestion of proteins before MS analysis, to identify the proteins present in preparations of these purified AAV vectors.” Pet. at 16. As Petitioner recognizes, Satkunanathan used bottom-up proteomics, not intact protein analysis. Indeed, the

methods described in Satkunanathan require a complete tryptic digestion and peptide-based identification workflow, very different from intact protein mass analysis. McLean Decl., ¶53; EX1005-Satkunanathan, 930-31. Satkunanathan's digestion method necessarily destroys any information about intact mass, capsid protein stoichiometry, or post-translational modification patterns on intact proteins. McLean Decl., ¶53-54. And like the Bark reference cited during prosecution of the '377 patent, Satkunanathan digests the protein prior to analysis. EX1005-Satkunanathan, 930-31; EX2022-Bark. Genzyme amended or cancelled all claims reciting digestion during prosecution of patents in this family. -167 IPR, EX1002, 2251 (Response to Office Action).

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; 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 by intact mass analysis. McLean Decl., ¶55. Even if it were, such serotype analysis would require digestion and a bottom-up LC-MS/MS approach. McLean Decl., ¶55.

**B. Shytuhina (EX1006)**

According to Petitioner's expert, Shytuhina discloses an LC-MS method to characterize Chikungunya virus-like particles and to "evaluate and quantitate the mass and purity of the [Chikungunya] vaccine product." Coon Decl., ¶267.

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." EX1006-Shytuhina, 192-193. Notably, Shytuhina does not reference AAV. *Id.*

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, 194. 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." EX1006-Shytuhina, 194. The authors also required elevated temperature (60°C) and a detergent pretreatment protocol

because of “carry-over of E1 and E2 between HPLC runs.” EX1006-Shytuhina, 194.

These disclosures teach a POSA that Shytuhina’s method was difficult to develop and was built specifically to try to analyze Chikungunya glycoproteins embedded in lipid bilayers. McLean Decl., ¶62. 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. McLean Decl., ¶63; EX1006-Shytuhina, 193-94. This modifier incompatibility demonstrates that even Shytuhina’s attempt to optimize RP-HPLC conditions were not directly applicable to intact LC-MS without significant adaptation. McLean Decl., ¶63.

Petitioner provides no basis for its assumption that these Chikungunya-specific conditions would transfer to AAV capsids, let alone be “more straightforward” for AAV capsids (*e.g.*, Pet. at 60), 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). McLean Decl., ¶64.

Finally, Shytuhina’s intact LC-MS analysis required “10 consecutive

injections of 25  $\mu$ L” of virus-like particle sample with signal averaging to achieve sufficient sensitivity and intensity to draw any conclusions. 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. As explained below, Shytuhina exhibited 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. McLean Decl., ¶¶65-66; EX2026-Wörner, 3-4. A POSA would have serious concerns about Shytuhina’s accuracy and efficiency and would not view Shytuhina’s method as a viable way to identify AAV serotypes, post-translational modifications, or heterogeneity in AAV. McLean Decl., ¶65.

**C. Zabrouskov (EX1038)**

Petitioner combines Zabrouskov with Shytuhina and Satkunanathan to address dependent claims 7-10, related to particular post-translational modifications. Zabrouskov studied deamidation in ribonuclease A (RNase A), a small, 13.7 kDa monomeric pancreatic enzyme. McLean Decl., ¶68. Zabrouskov uses electrospray ionization (ESI) on RNase A to introduce gaseous ions into a Fourier-transform MS. EX1038-Zabrouskov, 987. It does not employ a viral denaturing step and does not teach or suggest an intact LC-MS analysis of an intact viral particle. McLean Decl., ¶68.

RNase A is a well-studied lab reagent, not a virus. McLean Decl., ¶69. It does not form a viral capsid, does not have distinct subunit proteins corresponding to VP1, VP2, and VP3, and does not have a serotype. Zabrouskov's method does not examine a viral particle with distinct capsid protein subunits. McLean Decl., ¶69.

Further, Zabrouskov performs only top-down tandem-MS on a pre-prepared protein sample. 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. McLean Decl., ¶70; Coon Decl., ¶360 (citing EX1038-Zabrouskov, 989).

**D. Yuan (EX1037)**

Petitioner combines Yuan as a tertiary reference with Satkunanathan and Shytuhina to address dependent claims 6, 19, and 27, related to column selection. Yuan simply discloses an LC method using gel separation (SDS-PAGE) for human papillomaviruses. EX1037-Yuan, 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. McLean Decl., ¶80.

**V. GROUND I: SATKUNANATHAN AND SHYTUHINA FAIL TO RENDER THE CHALLENGED CLAIMS OBVIOUS**

Ground I alleges that the challenged claims are obvious over the

combination of Satkunanathan and Shytuhina based on the assertion that both references are directed toward methods for analysis of viral particle composition for process development, and that a POSA would have been motivated to combine them with a reasonable expectation of success because the required techniques were well-known and would require nothing more than routine experimentation.

This ground fails for multiple independent reasons.

**A. A POSA Would Not Be Motivated to Combine Satkunanathan and Shytuhina**

“[C]ombinations that change the basic principles under which the prior art was designed to operate, or that render the prior art inoperable for its intended purpose, may fail to support a conclusion of obviousness.” *Plas-Pak Indus., Inc. v. Sulzer Mixpac AG*, 600 F. App’x 755, 758 (Fed. Cir. 2015) (citing *In re Ratti*, 270 F.2d 810, 813 (C.C.P.A. 1959); *In re Gordon*, 733 F.2d 900, 902 (Fed. Cir. 1984)); *Uniroyal, Inc. v. Rudkin-Wiley Corp.*, 837 F.2d 1044, 1052 (Fed. Cir. 1988) (reversing obviousness finding “[i]n view of the antithetical principles of operation and absence of any teaching or suggestion to combine these prior art devices”).

Satkunanathan and Shytuhina take very different approaches to solve very different problems such that a POSA would not be motivated to combine them. Satkunanathan’s stated purpose is 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. The analytical strategy in Satkunanathan is built around bottom-up discovery proteomics: identifying many unknown host cell proteins that co-purify with three identified AAV serotypes after digestion, validating identifications by Western blotting, and performing functional studies via shRNA knockdown to determine which proteins affect AAV production. McLean Decl., ¶132; EX1005-Satkunanathan, 929-930. Satkunanathan's method depends on in-solution tryptic digestion and protein identification using specific software and score thresholds. McLean Decl., ¶133; EX1005-Satkunanathan, 931.

By contrast, Shytuhina's stated goal is to develop "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, Abstract. The authors sought to replace or augment existing, analytical methods such as SDS-PAGE and colorimetric assays. *Id.* Shytuhina attempted to characterize three known Chikungunya virus-like particle structural proteins (capsid, E1, E2) for lot-to-lot consistency monitoring and used intact LC-MS specifically to identify post-translational modifications in the Chikungunya virus-like particle proteins. McLean Decl., ¶135; EX1006-Shytuhina, 193-94.

These are distinct analytical paradigms designed for very different purposes. McLean Decl., ¶136. Satkunanathan's bottom-up discovery proteomics method

requires comprehensive coverage of an unknown proteome across three known AAV serotypes, peptide-level identification with confidence scoring, and database searching across thousands of potential proteins. McLean Decl., ¶137. Shytuhina’s targeted intact-protein assay discloses, conversely, separation of three known proteins, validation for vaccine release testing, and a failed attempt at post-translational modification characterization by intact mass deconvolution. McLean Decl., ¶138.

Petitioner asserts that “both [references] are directed towards efficient and precise methods for analysis of viral particle composition for the purpose of process development” and “[b]oth use liquid chromatography-mass spectrometry techniques to characterize purified viral preparations, identifying properties such as differences among different viral serotypes, or post-translational modifications.” Pet. at 27. This abstract characterization ignores what each reference was trying to accomplish and why.

Further, even assuming a POSA could have combined these references, “[o]bviousness concerns whether a skilled artisan not only *could have made* but *would have been motivated to make* the combinations or modifications of prior art to arrive at the claimed invention.” See *Belden Inc. v. Berk-Tek LLC*, 805 F.3d 1064, 1073 (Fed. Cir. 2015) (emphasis added). “Without any explanation as to how or why the references would be combined to arrive at the claimed invention, we are

left with only hindsight bias that *KSR* warns against.” *Metalcraft of Mayville, Inc.*

*v. The Toro Co.*, 848 F.3d 1358, 1367 (Fed. Cir. 2017).

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. McLean Decl., ¶139; EX2027-Kontogiannis, 6-7. A POSA reading Shytuhina, on the other hand, would understand that it teaches a questionable attempt at a quantitative 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. McLean Decl., ¶140; EX1006-Shytuhina, 194. Nothing in either reference suggests abandoning its approach to pursue the other’s objective using the other’s methodology for a completely different viral system. McLean Decl., ¶140. Indeed, 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. McLean Decl., ¶141; EX2034-Valeja, 7.

**B. Shytuhina’s LC-MS Method Is Inefficient, and Its Data Are Imprecise and Inaccurate**

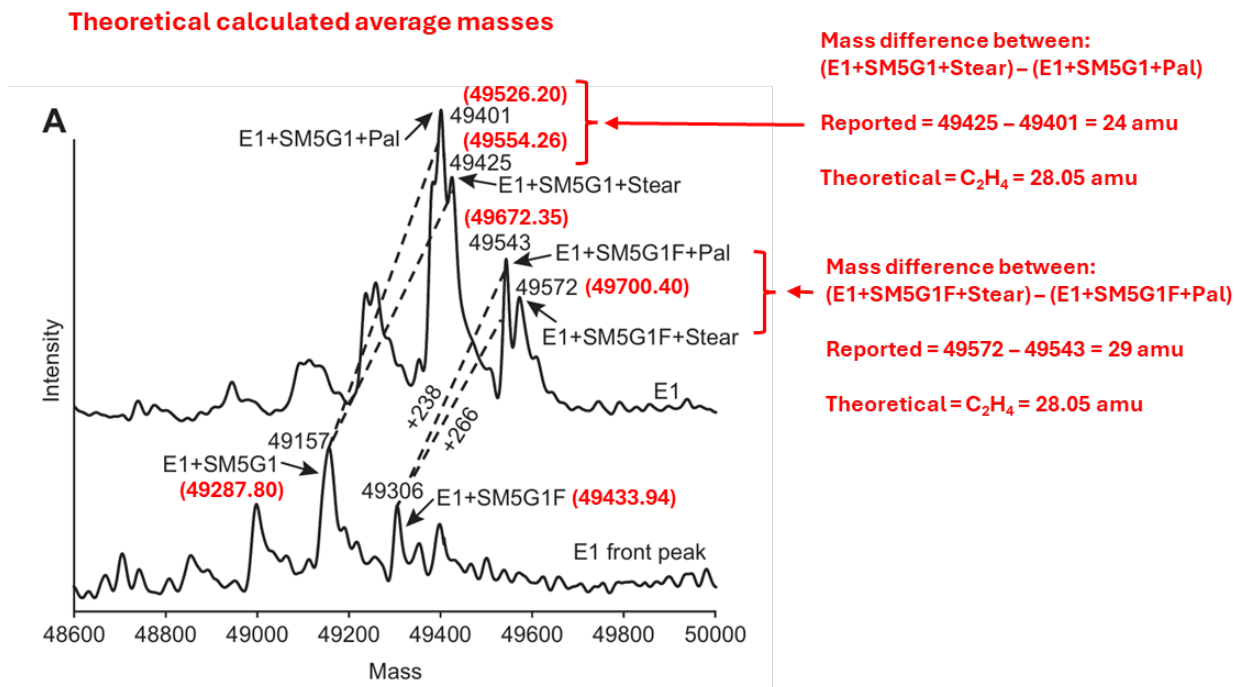
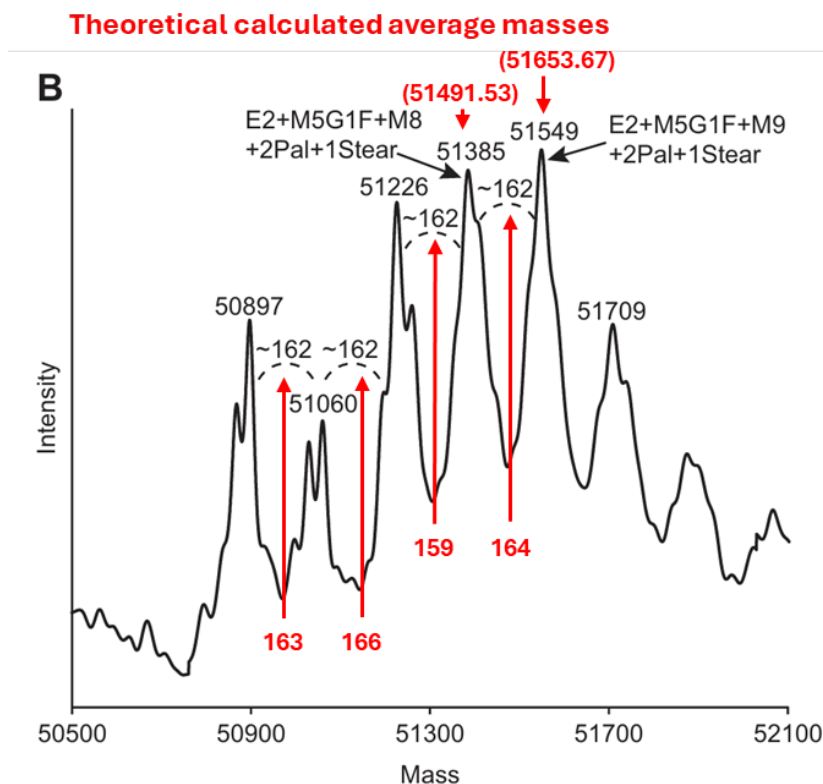
The Petition’s motivation to combine argument is premised on the assumption that both Satkunanathan and Shytuhina “are directed toward efficient and precise methods for analysis of viral particle composition for the purpose of process development.” Pet. at 27. Dr. Coon states that Shytuhina’s method is “efficient and precise” fifty-seven times in his declaration. McLean Decl., ¶82. As set forth below, Shytuhina’s method is neither efficient nor precise, defeating Petitioner’s proposed motivation to combine. Given the problems with Shytuhina’s results and conclusions, a POSA would not have sought to apply Shytuhina’s teachings elsewhere, including to Satkunanathan. McLean Decl., ¶83; *see Raytheon Techs. Corp. v. Gen. Elec. Co.*, 993 F.3d 1374, 1381-82 (Fed. Cir. 2021) (reversing Board determination of obviousness; noting that “the evidence of record must still establish that a skilled artisan could have made the claimed invention”).

As an initial matter, the assay precision reported in Shytuhina (*see* EX1006-Shytuhina, Table 1) is not for intact protein LC-MS analysis. Rather, it is ascribable to UV absorption at 280 nm, which is not relevant to the claimed invention. McLean Decl., ¶84. 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...” McLean Decl., ¶87; EX1006-Shytuhina, 195.

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. McLean Decl., ¶88. Shytuhina does not report any precision values for its intact mass analysis by LC-MS. McLean Decl., ¶88. Thus, there is no precision data for Shytuhina’s intact mass method. Further, the “assay accuracy” reported by Shytuhina is merely a measure of the mass of the sample, not the accuracy of the mass spectra data. McLean Decl., ¶89; EX1006-Shytuhina, 195-96.

Shytuhina’s mass spectra data are highly and troublingly inaccurate as well. Dr. McLean calculated the theoretical values of the assigned peaks in Shytuhina, which were not reported in that reference. McLean Decl., ¶¶89-90. Dr. McLean determined that the reported masses were off by over 100 Da from theoretical, and the difference between peaks was off by up to 4 Da:



McLean Decl., ¶90. Dr. McLean then applied the mass accuracy equation from the literature to the values from Shytuhina, and calculated mass accuracy errors of over

2000 ppm for each peak. McLean Decl., ¶91. A POSA would consider any mass accuracy value greater than 20 ppm to be questionable, and anything over 100 ppm to be highly inaccurate, unreliable, and not suitable for intact protein characterization. McLean Decl., ¶91. A POSA faced with the very poor data in Shytuhina would not choose to apply its method more broadly and would in fact be cautioned from following its approach. McLean Decl., ¶91. Because the combination of Shytuhina and Satkunanathan does not “establish that a skilled artisan could have made the claimed invention,” Ground I fails. *Raytheon Techs.*, 993 F.3d at 1381.

**C. The Laboratory that Developed Shytuhina Abandoned Its Intact LC-MS Method**

A later paper from the same laboratory as Shytuhina further underscores that the intact protein LC-MS experiment in Shytuhina would not have been considered precise or efficient for protein analysis by a POSA. The paper was published in *Journal of Chromatography B* on April 21, 2016 (before the August 2016 priority date of the '313 patent), with lead author Catherine Lancaster and the same corresponding author at Merck. McLean Decl., ¶¶92-94; EX2030-Lancaster, 218.

The Lancaster authors jettisoned the intact protein LC-MS methodology highlighted by Petitioner 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. McLean Decl., ¶95; EX2030-Lancaster, 219-21. The peaks were then further identified by LC-MS analysis of the released and derivatized carbohydrates. McLean Decl., ¶95; EX2030-Lancaster, 220-21. All of the observed molecular weights in Lancaster were within 0.02 Da of the calculated molecular weights, exhibiting high precision and accuracy. McLean Decl., ¶95; EX2030-Lancaster, Table 1. In contrast, Shytuhina's observed molecular weights varied by up to 140 Da from theoretical, and the theoretical mass values were not even provided in Shytuhina. McLean Decl., ¶95; EX1006-Shytuhina, 195-96, Fig. 2.

A POSA would read Shytuhina and Lancaster together as abrogating the utility of Shytuhina's method, and a POSA would not infer that Shytuhina's method was efficient or precise. McLean Decl., ¶96. 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. McLean Decl., ¶96; EX2030-Lancaster, Table 1.

Because Lancaster was published in April 2016—four months before the priority date—a POSA would have been aware that Shytuhina's laboratory had moved away from intact LC-MS, providing real-time evidence that the approach

was inadequate and eliminating any reasonable motivation to combine Shytuhina's method with Satkunanathan's for AAV or other viral analysis. Rather, a POSA would infer that direct, intact protein LC-MS had such significant limitations based on Shytuhina that it simply did not work, and that the approach to characterize modifications in the absence of the protein from Lancaster was preferable. McLean Decl., ¶96. As a result, a POSA would not be motivated to use Shytuhina or combine it with any reference, including with Satkunanathan's digestion LC-MS/MS method.

**D. Shytuhina's Intact LC-MS Method Is Inefficient and Only Applicable, at Best, to Chikungunya Viral Particles**

Shytuhina's method development was driven by Chikungunya-specific constraints and challenges. One of the Chikungunya viral proteins, E1, stuck to the column during method development, requiring modification of the procedure to include a strong organic solvent system. McLean Decl., ¶98; 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. McLean Decl., ¶98; EX1006-Shytuhina, 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. McLean Decl., ¶98; EX1006-Shytuhina, 193-94. 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. McLean Decl., ¶98; EX1006-Shytuhina, 193-94.

Even if Shytuhina's method worked (which a review of its data calls into serious question), a POSA would view these system-specific challenges and substantial method development as roadblocks to developing a method for other viral particles like AAV because Shytuhina's method is bespoke and fit for its specific purpose. McLean Decl., ¶99. Petitioner ignores AAV-specific constraints, including that AAV capsids are highly stable, non-enveloped icosahedral protein shells, have non-glycosylated capsid proteins, and have different particle stability and disassembly requirements. McLean Decl., ¶100; EX2026-Wörner, 3-5; EX2027-Kontogiannis, 6-9; EX1006-Shytuhina, 194.

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. McLean Decl., ¶101. And a POSA would have no reasonable expectation that Chikungunya-focused conditions would be successful for AAV or other viral systems besides Chikungunya, or that the development effort would be worthwhile for Satkunanathan's host protein identification objectives. McLean Decl., ¶101;

EX1006-Shytuhina, 193-94. This is especially true considering the poor quality and unreliable results from Shytuhina.

1. A POSA Would Understand that Shytuhina and Satkunanathan Do Not Address AAV-Specific Challenges for Intact LC-MS

Petitioner asserts that “[i]ntact LC-MS analysis of AAV capsid proteins would have been even more straightforward for a POSA than working with the glycoproteins Shytuhina analyzed” because “[g]lycoproteins 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.” Pet. at 61. This assertion fails for two independent reasons: it misidentifies Shytuhina’s recited technical challenges and ignores the distinct and unaddressed challenges that AAV capsids would present.

Dr. Coon’s comparative reasoning rests on a mischaracterization of Shytuhina. Shytuhina’s method development challenges were not primarily driven by glycan variability. McLean Decl., ¶103. Rather, as Shytuhina expressly states, the method was technically challenging due to “the hydrophobic nature of the viral glycoproteins and the presence of lipids.” EX1006-Shytuhina, 193. The primary obstacles were structural and physicochemical: viral proteins sticking to the column, requiring strong organic solvents to elute; the need for TFA to promote virus-like particle disassembly and stationary phase interaction; ion suppression

caused by that same TFA, requiring a switch to formic acid for MS analysis; and significant carry-over requiring detergent pretreatment and elevated temperature. McLean Decl., ¶103; EX1006-Shytuhina, 193-94. Because Petitioner’s “more straightforward” argument is premised on glycan variability being the dominant challenge in Shytuhina, and it was not, its comparative reasoning fails.

Critically, Dr. Coon does not provide any reference-based reason why AAV would be easier or more straightforward to analyze by intact LC-MS. The art indicates that AAV would present different, equally difficult challenges that Petitioner and Dr. Coon do not address, including:

- Capsid stability: AAV capsids are among the most stable viral particles known, with icosahedral symmetry and extensive inter-subunit contacts. McLean Decl., ¶104; EX1031-Rayaprolu, 13153. The art cited by Petitioner fails to teach whether AAV capsids would disassemble under Shytuhina’s Chikungunya-specific conditions. McLean Decl., ¶¶104, 108.
- Protein properties: 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.*, ’313 patent, Example 4; McLean Decl., ¶105.

- Sample complexity: 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. McLean Decl., ¶145; EX1005-Satkunanathan, 930.
- Identification requirements: Unlike Shytuhina's post-translational modification characterization of known proteins, the claimed invention requires using intact mass to identify post-translational modifications—a different analytical challenge 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 the cited art. McLean Decl., ¶106. As one example, 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. McLean Decl., ¶107; EX2026-Wörner, 5. This number does not even take into account post-translational modifications or co-purifying host proteins. *Id.*

Even setting aside Petitioner's misidentification of Shytuhina's stated challenges, lack of explanation of any AAV-specific obstacles, and unacceptable mass accuracy, Shytuhina's data is very noisy, with broad peaks unsuitable for quantitation, even after significant and lengthy attempts at optimization. McLean Decl., ¶109; see EX1006, Figs. 2A & 2B. A POSA would not look to a method

with severe limitations and results like Shytuhina's for analysis of complex viral systems like AAV. McLean Decl., ¶109.

Petitioner's assertion that AAV intact LC-MS analysis would be "more straightforward" than Shytuhina (Pet. at 60) is simply speculation unsupported by any 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. The art cited by Petitioner suggests that intact protein LC-MS of AAV or of viral particles generally would be unlikely to be successful given the limitations above. McLean Decl., ¶110. Petitioner relies on nothing but hindsight and the success of the inventions in the '313 patent to argue that those inventions would be straightforward, which is impermissible and insufficient for obviousness. *See W.L. Gore. & Assoc. v. Garlock, Inc.*, 721 F.2d 1540, 1553 (Fed. Cir. 1983) (using an invention's teachings to inform a POSA "is to fall victim to the insidious effect of a hindsight syndrome wherein that which only the inventor taught is used against its teacher").

2. Petitioner Fails to Demonstrate that Satkunanathan and Shytuhina Together Could Be Used for Detection of Post-Translational Modifications or Heterogeneity

The combination of Satkunanathan and Shytuhina does not teach or suggest using intact LC-MS protein analysis for detection of post-translational modifications or heterogeneity in AAV. Satkunanathan analyzed previously

identified AAV2, AAV5, and AAV8 digested capsid proteins (not intact proteins) using peptide MS/MS database searching. EX1005-Satkunanathan, 930. Shytuhina used intact LC-MS to attempt 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. EX1006-Shytuhina, 194.

In other words, 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. McLean Decl., ¶112; EX1005-Satkunanathan, 939. Digestion followed by peptide tandem MS/MS method worked. McLean Decl., ¶112. It provided comprehensive protein identification, statistical confidence through spectral matching, and enabled downstream functional validation. McLean Decl., ¶112. Satkunanathan specifically teaches that peptide MS/MS can provide information about AAV serotype and capsid proteins and a POSA would not view a bottom-up method like Satkunanathan’s to be helpful to developing an intact method for determining the presence of post-translational modifications. McLean Decl., ¶¶113-114.

Neither Petitioner nor Dr. Coon cites any prior-art reference teaching that viral post-translational modifications can be reliably distinguished by intact VP

mass measurement. And Petitioner does not point to any prior art teaching that the mass differences between post-translationally modified AAV capsid proteins or any viral capsid proteins are large enough for differential identification given potential unknown post-translational modifications, processing variants, and mass measurement accuracy limitations. McLean Decl., ¶115; EX2028-Salganik, 11879-80; EX2027-Kontogiannis, 7-8.

Petitioner's citation to Huang further shows the success of digestion methods followed by LC-MS/MS. Huang's method, like Satkunanathan, followed a bottom-up approach using peptide digestion. McLean Decl., ¶116; EX1027-Huang. Huang's method was used to detect deamidation in antibodies—a very small change in the mass of a protein (approximately 1 Da). McLean Decl., ¶116; EX1038-Zabrouskov, 987. Petitioner 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 employing bottom-up proteomics rather than intact LC-MS methods to detect small changes in mass arising from different viral protein serotypes, post-translational modifications, or capsid heterogeneity. McLean Decl., ¶116. While such methods can be “laborious” and “time-consuming,” they are accurate and precise, unlike Shytuhina's highly inaccurate and messy intact LC-MS method. McLean Decl., ¶117.

All techniques have advantages and disadvantages. Indeed, techniques like

electrospray ionization mass spectrometry can introduce artificial modifications like protein oxidation. McLean Decl., ¶117; EX2032-Boys, 4027-28. But a POSA would not have been motivated to abandon a working, accurate method (Satkunanathan's peptide MS/MS proteomics) to pursue an untested, inaccurate approach (adapting Shytuhina's bespoke, Chikungunya-specific intact virus-like particle method to AAV), particularly when the references themselves provide no teaching or suggestion that such adaptation would work, how to accomplish it, or that it would address either reference's objectives. McLean Decl., ¶118; EX1005-Satkunanathan, 930-31; EX1006-Shytuhina, 193-95. A POSA would not be so motivated, particularly because the group that worked on the Shytuhina paper subsequently abandoned its intact LC-MS approach in favor of a more accurate and precise methodology. McLean Decl., ¶118; EX2030-Lancaster, 218-221.

Further, Petitioner provides no opinion or evidence that the prior art taught a POSA that intact mass measurement would provide advantages over established identification methods (*e.g.*, peptide MS/MS, immunological assays, sequencing), that intact mass measurements would even work, or that a POSA could validate that observed intact masses correspond to specific post-translational modifications rather than other modifications or degradation variants. McLean Decl., ¶119; EX2028-Salganik, 11879-80. 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 attempt at optimizing a Chikungunya system, mass accuracy was insufficient for complete characterization. McLean Decl., ¶120; EX1006-Shytuhina, 194.

As explained above, a POSA would understand that Shytuhina’s intact mass method had severe limitations in mass accuracy 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 and calculated *ex ante* by a POSA. McLean Decl., ¶121. Shytuhina’s method is simply unsuitable for detecting small changes in mass as found between VPs of different serotypes, post-translational modifications, or capsid heterogeneity. McLean Decl., ¶122.

**E. The Petition Does Not Set Forth a Reasonable Expectation of Success for the Combination of Satkunanathan and Shytuhina**

Petitioner asserts 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.” Pet. at 59-60. This is insufficient. Even if all the techniques raised by Petitioner were available to a POSA, the combination of those techniques must have a reasonable expectation of success. *See, e.g., Juniper Networks, Inc. v. Correct Transmission, LLC*, No. 2023-1046,

2024 WL 3517862, at \*5 (Fed. Cir. July 24, 2024). For the same reasons stated above for motivation to combine, the combination of Shytuhina and Satkunanathan would not have had a reasonable expectation of success.

In addition, Petitioner relies on Dr. Coon’s testimony 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. Pet. at 61 (citing Coon Decl., ¶717). Petitioner uses this testimony to conclude that application of these techniques to AAV “would have been considerably more routine and straightforward.” *Id.* Petitioner’s conclusion does not follow from the evidence, and Dr. Coon’s statements fail to even cite evidence. Even conceding that researchers had been using intact LC-MS on proteins for years before the priority date, why had no one successfully applied intact LC-MS to AAV?

Petitioner and Dr. Coon do not say. McLean Decl., ¶126. Petitioner’s assertion that intact LC-MS had been used in other contexts for years before Genzyme successfully applied such methods to AAV refutes Petitioner’s argument that doing so would have been obvious, and supports at least the objective indicia of long-felt need in the art, failure of others, and unexpected results to achieve the claimed invention. McLean Decl., ¶127; *see also Leo Pharm. Prods., Ltd. v. Rea*, 726 F.3d 1346, 1354 (Fed. Cir. 2013).

***Long-felt but unmet need.*** The need for improved methods for identifying viral serotypes and detecting post-translational modifications in viral capsid proteins was well-recognized in the art long before the priority date. EX1022-Dong, 1; EX1036-Siuzdak, 205. Indeed, Dr. Coon acknowledges that he has been working on such improvements since 2005. Coon Decl., ¶11. Dr. Coon further acknowledges that prior art methods like SDS-PAGE, ELISA, Western blot, and Gel-LC-MS/MS were “laborious,” “time-consuming,” and could “introduce artificial modifications.” Coon Decl., ¶158 (citing EX1012-Bondarenko, 1415). Despite the recognized need for improved methods, no prior-art reference taught or suggested using intact protein LC-MS to analyze AAV particles or detect post-translational modifications without a gel separation step. McLean Decl., ¶¶126-127. The long-felt, unmet need for such a method supports the non-obviousness of the claimed invention.

***Failure of others.*** As noted above, Dr. Coon concedes that researchers had been using intact LC-MS on proteins (including reduced monoclonal antibodies) “for years before the relevant date.” Coon Decl., ¶717. Yet despite years of availability of the underlying techniques, no researcher successfully applied intact LC-MS in the absence of a gel separation step to AAV serotype identification or post-translational modification detection before the ’313 patent inventors. McLean Decl., ¶¶126-127. Petitioner and Dr. Coon offer no explanation for this

conspicuous gap. The failure of others to achieve the claimed invention despite the supposed availability of the component techniques is strong evidence that the combination was not obvious. *In re Cyclobenzaprine HCl Extended-Release Capsule Pat. Litig.*, 676 F.3d 1063, 1082 (Fed. Cir. 2012) (evidence that “skilled artisans struggled to attain” the claimed invention “is classic evidence of nonobviousness”).

**Unexpected results.** The ’313 patent inventors demonstrated that intact protein LC-MS could accurately differentiate between AAV serotypes and detect post-translational modifications and heterogeneity with high specificity. The prior art suggested these results were unlikely to be achievable. McLean Decl., ¶115; EX2028-Salganik, 11879-80; EX2027-Kontogiannis, 7-8. As Dr. McLean explains, the prior art as of August 2016 taught that “accurate mass measurement of intact proteins alone does not allow for unequivocal protein identification,” requiring additional techniques like tandem MS/MS for a complete picture. McLean Decl., ¶141; EX2034-Valeja, 7. The inventors’ success in applying intact protein LC-MS alone, without digestion or gel separation, for post-translational modification identification, was unexpected in light of the prior art. *See Leo Pharm. Prods.*, 726 F.3d at 1354.

Petitioner 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 even as good as) established LC-MS/MS identification methods for AAV, or that a POSA could validate intact mass-based serotype assignments. McLean Decl., ¶128. Further, the prior art cited by Petitioner does not establish that a POSA would have a reasonable expectation of success in characterizing post-translational modifications with any level of confidence or accuracy based on Satkunanathan and Shytuhina. McLean Decl., ¶128.

Shytuhina acknowledges that “[d]ue to mass heterogeneity of both E1 and E2, the deconvoluted masses of the minor peaks were less accurate” and therefore were not assigned. EX1006-Shytuhina, 194. This demonstrates limitations in intact mass measurement accuracy even for a purportedly optimized Chikungunya system like Shytuhina’s. McLean Decl., ¶129. A POSA would have no reasonable basis to expect that intact mass measurement could reliably identify AAV serotypes, post-translational modifications, or capsid heterogeneity without substantial development and validation work not taught in the prior art. McLean Decl., ¶129.

Shytuhina does not even provide the theoretical masses used for comparison, or quantitative values for mass accuracy, leaving a POSA questioning the mass accuracy of its identifications. McLean Decl., ¶130. A POSA digging into the

numbers reported by Shytuhina would expose the inaccuracy of that data and would expect Shytuhina's method to fail. McLean Decl., ¶90. This further refutes Petitioner's assertion that a POSA would have a reasonable expectation of success in combining Shytuhina's method with Satkunanathan's.

**F. Satkunanathan's and Shytuhina's Methods Are Incompatible**

A POSA further would not be motivated to combine Satkunanathan and Shytuhina because they disclose incompatible sample preparation and analytical methods. For example, 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. McLean Decl., ¶151; EX1005-Satkunanathan, 930-31. The output of this preparation is a complex peptide mixture suitable for nano-LC separation and MS/MS fragmentation. McLean Decl., ¶151; EX1005-Satkunanathan, 931.

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. McLean Decl., ¶152; 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. McLean Decl., ¶152; EX1006-Shytuhina, 195.

A POSA would consider these sample preparation protocols to be mutually exclusive. Satkunanathan's trypsin digestion destroys intact protein mass information by cleaving proteins into peptides. McLean Decl., ¶153. Shytuhina's intact protein approach cannot be combined with a method requiring enzymatic digestion without defeating the express purpose of intact mass analysis. McLean Decl., ¶153. Importantly, Patent Owner's argument is not that Satkunanathan cannot be "bodily incorporated" into Shytuhina, or vice versa. *Allied Erecting & Dismantling Co. v. Genesis Attachments, LLC*, 825 F.3d 1373, 1381 (Fed. Cir. 2016). Rather, given the references' incompatibility on the fundamental point of intact protein analysis vs. sample digestion, a POSA attempting to combine these references would recognize the need to choose one approach or the other and would not be motivated to combine them. McLean Decl., ¶¶154-155.

Petitioner asserts that "Shytuhina teaches carrying out two denaturing steps—treatment with Zwittergent and RP-HPLC" and that Satkunanathan in combination with Shytuhina meets the "denaturing the AAV particles" limitation. Pet. at 30. But Petitioner fails to explain why a POSA would assume that Shytuhina's Chikungunya-specific denaturation protocol would apply to AAV. 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. McLean Decl., ¶155; EX1006-Shytuhina, 194. AAV capsids are non-enveloped protein shells with no lipid bilayer. McLean Decl., ¶154. Shytuhina provides no teaching that Zwittergent treatment would be appropriate for, or effective at, disassembling non-enveloped AAV capsids or other non-enveloped capsids. McLean Decl., ¶154.

Similarly, Shytuhina’s finding that “TFA appeared to help disassemble the VLPs” applies only to Chikungunya’s virus-like particle VLP structure—an outer lipid envelope with embedded glycoproteins surrounding a nucleocapsid. McLean Decl., ¶155; EX1006, 194. Whether TFA would disassemble AAV’s highly stable icosahedral protein capsid is unknown and not taught by Shytuhina. Shytuhina notes that “without TFA, the VLPs eluted in the void volume,” demonstrating that virus-like particle disassembly was critical for chromatographic separation. EX1006-Shytuhina, 194. But, again, this Chikungunya-specific finding does not translate to AAV without experimental validation which is absent in Petitioner’s cited art. McLean Decl., ¶155; EX1006-Shytuhina, 194.

1. The data acquisition and data analysis methods of Satkunanathan and Shytuhina are incompatible

Similarly, the references’ data acquisition and analysis methods are incompatible. 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).” McLean Decl., ¶156; 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. McLean Decl., ¶156; EX1005-Satkunanathan, 931.

Shytuhina used “a Synapt G2 mass spectrometer” operated in “Sensitivity mode and spectra were acquired in an *m/z* range of 1000-2500,” tailored to intact Chikungunya protein detection. McLean Decl., ¶157; 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.” EX1006-Shytuhina, 193. Observed masses were matched to “theoretical molecular weight[s]” of known Chikungunya proteins with expected post-translational modifications. McLean Decl., ¶157; EX1006-Shytuhina, 194.

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. McLean Decl., ¶158. Shytuhina's intact mass profiling acquires spectra of intact multiply-charged Chikungunya proteins, deconvolutes charge states to determine neutral masses, and attempts to match observed masses to undisclosed theoretical masses of known target proteins with predicted post-translational modification states. McLean Decl., ¶158.

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. McLean Decl., ¶159.

2. Satkunanathan and Shytuhina disclose different sample complexity and different analytical challenges

Satkunanathan and Shytuhina have disparate analytical challenges and different complexity such that a POSA would not be motivated to combine them. 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. McLean Decl., ¶142; 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. McLean Decl., ¶142; EX1005-Satkunanathan, pp. 14-15. The

art teaches that this level of sample complexity is ideal for discovery proteomics using peptide MS/MS with comprehensive database searching. McLean Decl., ¶142.

On the other hand, 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. McLean Decl., ¶143. In addition to the issues raised above, a POSA would recognize that attempting to apply Shytuhina's intact protein approach to Satkunanathan's complex AAV samples would introduce significant analytical challenges not present in Shytuhina's three-protein system. For example,

- Dynamic range: 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. McLean Decl., ¶145; EX2026-Wörner, 6.
- Peak capacity: separating intact AAV capsid proteins (VP1 ~87 kDa, VP2 ~73 kDa, VP3 ~62 kDa) ('313 patent, 52:27-32) plus numerous host proteins on a

single RP-HPLC column may not provide adequate resolution, especially if proteins exhibit similar retention behavior. McLean Decl., ¶146.

- Mass accuracy: 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 as described above, Shytuhina’s mass accuracy, at over 2000 ppm, is and was unacceptable. Adding sample complexity and size would exacerbate this problem. McLean Decl., ¶147.
- pH-dependent cleavage: 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.” McLean Decl., ¶148; EX2028-Salganik, 11879-80. Petitioner’s references do not teach a POSA how to use a 0.1% TFA solution (pH  $\approx$  2.1) at high temperature for separation of intact AAV VPs without inducing cleavage. McLean Decl., ¶148.
- Method validation: 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.

McLean Decl., ¶149.

Petitioner provides no explanation for how these analytical challenges would be addressed or why a POSA would combine Shytuhina's three-protein Chikungunya vaccine assay with Satkunanathan's complex AAV preparations for an entirely different analytical purpose. As such, Petitioner's proposed combination fails to render any claim obvious.

#### **G. Additional Independent Claim Limitations**

The arguments above regarding motivation to combine and reasonable expectation of success of the claimed combinations of prior art apply generally, regardless of whether the challenged claims relate to post-translational modifications, heterogeneity, preparation of pharmaceutical compositions, particle identity, or particle consistency. As stated above, Shytuhina describes a bespoke intact LC-MS method for analysis of known Chikungunya proteins. Its method development was laborious and even after optimization had poor data quality, precision, and accuracy, as well as significant drawbacks. McLean Decl., ¶162. In contrast, Satkunanathan discloses a working, accurate, bottom-up LC-MS method that uses digestion to study differences in protein composition between three AAV serotypes. McLean Decl., ¶162. 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. McLean Decl., ¶118.

As discussed in detail above, Satkunanathan does not teach or suggest anything about post-translational modifications, and Shytuhina's method is inaccurate and unreliable, such that a POSA would never apply it in other contexts. While Dr. Coon opines that Satkunanathan includes methionine oxidation as a dynamic modification (Coon Decl., ¶435), he does not explain how Satkunanathan discloses a "method of determining post-translational modifications of viral proteins" as required by claim 10. McLean Decl., ¶163.

Further, Petitioner relies on a bare, uncited assertion by Dr. Coon that a "POSA would have understood that applying Shytuhina's intact LC-MS method to Satkunanathan's study of variations among different AAV serotypes would have provided a method of detecting post-translational modifications of the AAV capsid proteins in a preparation of AAV particles." Coon Decl., ¶382. In light of all the difficulties described above, a POSA would not have been motivated to combine Shytuhina and Satkunanathan as Dr. Coon has opined, and would not have had a reasonable expectation of success in combining these references. McLean Decl., ¶164.

#### **H. Dependent Claims 2-10, 12-19, and 21-27**

For the same reasons the combination of Satkunanathan and Shytuhina does

not render independent claims 1, 11, and 20 obvious, this combination does not render dependent claims 2-10, 12-19, and 21-27 obvious.

**VI. GROUND II: THE COMBINATION OF SHYTUHINA, SATKUNANATHAN, AND YUAN DOES NOT RENDER CLAIMS 6, 19, OR 27 OBVIOUS**

Ground II adds Yuan to the Satkunanathan-Shytuhina combination to address dependent claims 6, 19, and 27, which requires use of a C8 column for reverse phase chromatography. Pet. at 63.

Yuan does not cure the fundamental deficiencies in Ground I. Petitioner provides no argument that Yuan's method would be relevant to any independent claim. Adding Yuan's column selection to the Satkunanathan-Shytuhina combination does not resolve the fundamental incompatibilities of that combination, which would still face all the problems identified above. Because the combination of Satkunanathan, Shytuhina, and Yuan does not render any independent claim obvious, this combination cannot render dependent claims 6, 19, or 27 obvious.

Petitioner asserts that "[a] POSA would have understood that these columns, C4, C8, and C18 were commonly used columns for separation of viral structural proteins, including AAV capsid proteins," such that "[a] POSA would therefore have likely tested all of them, in applying Shytuhina's intact LC-MS method to the separation and identification of AAV capsid proteins." Pet. at 64. This misses the

point. The question is not whether column selection is routine, but whether the combination of references proposed by Petitioner would motivate a POSA to pursue intact LC-MS analysis of AAV capsids in the first place—which Ground II fails to establish. Choosing a column may be a necessary step if one is performing intact LC-MS, but it does not make intact LC-MS of AAV capsids obvious when the base combination fails to establish motivation or reasonable expectation of success for the analytical approach as claimed. McLean Decl., ¶¶166-168.

Further, Yuan is simply an LC method using gel separation (SDS-PAGE) for human papillomaviruses. EX1037, 21. Yuan analyzes a single protein, L1, using LC, and teaches using a C4 or C8 column for the analysis. EX1037, 21. Yuan does not teach or suggest an intact LC-MS/MS method for analyzing viral particles in the absence of a gel separation step. McLean Decl., ¶167.

**VII. GROUND III: THE COMBINATION OF SHYTUHINA, SATKUNANATHAN, AND ZABROUSKOV DOES NOT RENDER CLAIMS 7-10 OBVIOUS**

Ground III adds Zabrouskov to the problematic Satkunanathan-Shytuhina combination to address dependent claims 7-10, which are directed to determining the sequence of one or more VPs that have undergone particular post-translational modifications. Pet. at 67.

Zabrouskov does not cure the fundamental deficiencies in Ground I. Petitioner provides no argument that Zabrouskov’s method would be relevant to

any independent claim. Adding Zabrouskov's MS/MS method for detecting deamidation to the Satkunanathan-Shytuhina combination does not resolve the fundamental incompatibilities of that combination, which would still face all the problems identified above. Because the combination of Satkunanathan, Shytuhina, and Zabrouskov does not render independent claim 1 obvious, this combination cannot render dependent claims 7-10 obvious.

Further, Zabrouskov does not provide any teaching to render claims 7-10 obvious in combination with Satkunanathan and Shytuhina. As described above, Shytuhina teaches a bespoke, inaccurate, imprecise, inefficient, messy LC-MS method for intact analysis of known Chikungunya proteins. Zabrouskov addresses a different problem than Shytuhina: characterizing stepwise deamidation at five specific sites in reduced bovine ribonuclease A ("RNase A") by MS/MS. McLean Decl., ¶170; EX1038-Zabrouskov, 987. RNase A is a 13.7 kDa single-chain protein. EX1038-Zabrouskov, 987. Zabrouskov focuses on one specific analytical challenge that deamidation presents—the covalent  $\text{NH}_2 \rightarrow \text{OH}$  modification produces only a 0.984 Da mass increase, closely matching the 1.002 Da spacing of molecular ion isotope peaks. *Id.* This makes it difficult to distinguish deamidated RNase A from  $^{13}\text{C}$  isotopes. McLean Decl., ¶170.

A POSA seeking to develop an improved LC-MS method for AAV characterization for gene therapy applications would not be motivated to consult

Zabrouskov's mechanistic study of deamidation kinetics in a single, unrelated model protein for at least the following reasons.

**First**, Zabrouskov teaches that deamidation is a difficult analytical problem with nearly all solutions in the art limited to qualitative identification of a single deamidation site. EX1038-Zabrouskov, 987. And Zabrouskov provides no teaching to solve this difficult problem for AAV viral capsids; rather, Zabrouskov focuses on a single, relatively small standard protein. McLean Decl., ¶171; EX1038-Zabrouskov, 987. The problems with applying Shytuhina's Chikungunya-specific method to AAV are explained in detail above, and Zabrouskov does nothing to remedy these deficiencies or suggest a combination with Shytuhina's intact LC-MS method and Satkunanathan's bottom-up LC-MS/MS method to create an intact LC-MS method for AAV for determining the extent of deamidation or other post-translational modifications. McLean Decl., ¶172.

**Second**, Zabrouskov provides no teaching that its deamidation detection method could be applied to large, intact viral capsid proteins, which are four to six times larger than RNase A. McLean Decl., ¶173. Deamidation is a change of approximately 1 Da, and Zabrouskov's difficulty characterizing this change would be magnified many-fold for AAV. McLean Decl., ¶173. A POSA would not have a reasonable expectation of success in applying Zabrouskov's deconvolution methodology to resolve deamidation in a mixed population of intact 60-87 kDa

viral proteins, particularly given Zabrouskov's teaching that the precision of its method is ***inversely proportional*** to sample mass. McLean Decl., ¶173; EX1038-Zabrouskov, 989.

***Third***, neither Zabrouskov nor Shytuhina nor Satkunanathan explains or remedies the difficulties specific to preparing AAV for intact mass analysis. EX1026-Han and its disclosure of studying post-translational modifications in intact proteins up to 200 kDa is unavailing. Han studied only single-protein systems and did not explain to a POSA how to analyze deamidation for such proteins. McLean Decl., ¶174; EX1026-Han. And Han does not discuss the difficulties in analyzing mixed populations of viral particles. Petitioner's assertion that a POSA would have had a reasonable expectation of success in combining Shytuhina, Satkunanathan, and Zabrouskov (Pet. at 72) is belied by the difficulties analyzing AAV described above.

***Fourth***, unlike the well-characterized, enzymatically reduced, single-chain RNase A studied in Zabrouskov, AAV capsid proteins behave substantially differently. McLean Decl., ¶175. AAV capsid proteins form complex structures in intact capsids, contain a variety of poorly understood modification sites, have unpredictable sequence- and structure-dependent modification patterns, and require denaturation strategies not addressed by Zabrouskov. McLean Decl., ¶175.

***Fifth***, a POSA would expect AAV to have many more overlapping peaks

from different isotopomers across a variety of deamidation sites, which would make Zabrouskov's RNase-specific resolution virtually useless for AAV. McLean Decl., ¶175.

**Sixth**, neither Shytuhina nor Satkunanathan nor Zabrouskov provides any guidance regarding:

- Whether deamidation would occur uniformly across all copies of each VP protein in an assembled capsid;
- How to quantify deamidation or other post-translational modifications when analyzing mixtures of VP1, VP2, and VP3;
- How to distinguish post-translational modifications at specific sites when multiple VP proteins share homologous sequences.
- How to distinguish "N-terminal acetylation" in AAV (cl. 9).

McLean Decl., ¶176.

**Seventh**, for the same reasons stated above for Ground I that a POSA would not combine the intact LC-MS method from Shytuhina with the digestion LC-MS/MS method from Satkunanathan, a POSA would not further combine Shytuhina and Satkunanathan with the MS/MS method from Zabrouskov. Rather, given the references' incompatibility on the fundamental point of intact LC-MS vs. MS/MS, a POSA attempting to combine these references would recognize the need to choose one approach or the other and would not be motivated to combine them. McLean Decl., ¶¶136-141.

As a result, the combination of Shytuhina and Zabrouskov does not render

claims 7-10 obvious.

### **VIII. CONCLUSION**

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

Respectfully submitted,

Dated: June 5, 2026

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**WORD COUNT CERTIFICATION**

Pursuant to 37 C.F.R. § 42.24(d), I certify that this Preliminary Response contains 12,520 words (excluding the title page, table of contents, table of exhibits, claim listing, this certificate, and the certificate of service), as determined by Microsoft Word.

Dated: June 5, 2026

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**CERTIFICATE OF SERVICE**

I certify that today in:

*Sarepta Therapeutics, Inc., v. Genzyme Corp.*, IPR2026-00270  
(U.S. Patent 12,298,313)

I caused to be served a copy of:

PATENT OWNER'S PRELIMINARY RESPONSE  
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