UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

SAREPTA THERAPEUTICS, INC.,

Petitioner

v.

Genzyme Corporation,

Patent Owner

U.S. Patent No. 12,123,880

"Methods for Detecting AAV"

IPR2025-00168

PETITION FOR *INTER PARTES* REVIEW

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LIST OF CHALLENGED CLAIMS

Claim	Element
1 [pre]	A method of analyzing a preparation of AAV particles, the method comprising
1[a]	a) denaturing the AAV particles,
1[b]	b) subjecting the denatured AAV particles to liquid chromatography/mass spectroscopy (LC/MS) intact protein analysis, and
1[c]	c) determining the masses of one or more viral proteins (VPs) of the particles in the preparation,
1[d]	wherein the method is performed in the absence of a gel separation step.
2	The method of claim 1, wherein the VPs comprise VP1, VP2, and VP3 capsid proteins and one more variants of VP1, VP2, or VP3 capsid proteins.
3	The method of claim 1, wherein the AAV particles are denatured with acetic acid, guanidine hydrochloride and/or an organic solvent.
4	The method of claim 1, wherein the liquid chromatography is reverse phase liquid chromatography, size exclusion chromatography, hydrophilic interaction liquid chromatography, or cation exchange chromatography.
5	The method of claim 1, wherein the liquid chromatography is reverse phase liquid chromatography.
6	The method of claim 5, wherein the reverse phase chromatography is performed with a C4 column.

Claim	Element
7	The method of claim 1, wherein the liquid chromatography is ultra-performance liquid chromatography (UPLC).
8	The method of claim 1, wherein the mass spectrometry comprises assisted calibration.
9	The method of claim 8, wherein sodium iodide is used as a calibrant.
10[pre]	A method of determining post-translational modifications of viral proteins (VPs) in a preparation of viral particles, the method comprising
10[a]	a) denaturing the viral particles,
10[b]	b) subjecting the denatured viral particles to liquid chromatography/mass spectroscopy (LC/MS) intact protein analysis, and
10[c]	c) determining the masses of one or more VPs of the viral particles
10[d]	wherein a deviation of one or more of the masses of the one or more VPs from the theoretical masses of VPs that have not undergone post-translational modifications is indicative of post-translational modifications of the VPs,
10[e]	and wherein the method is performed in the absence of a gel separation step.
11	The method of claim 10, wherein the liquid chromatography is reverse phase liquid chromatography, size exclusion chromatography, hydrophilic interaction liquid chromatography, or cation exchange chromatography.
12	The method of claim 10, wherein the viral particles comprise a viral vector encoding a heterologous transgene.

Claim	Element
13	The method of claim 10, wherein the liquid chromatography is reverse phase chromatography.
14	The method of claim 13, wherein the reverse phase chromatography is a C4 or C8 reverse chromatography.
15	The method of claim 10, wherein the liquid chromatography is ultra-performance liquid chromatography (UPLC).
16	The method of claim 10, wherein the mass spectrometry comprises assisted calibration.
17	The method of claim 16, wherein sodium iodide is used as a calibrant.
18[pre]	A method of determining the extent of deamidation of viral proteins (VPs) in a preparation of viral particles, the method comprising
18[a]	a) denaturing the viral particles,
18[b]	b) subjecting the denatured viral particles to liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) intact protein analysis, and
18[c]	c) determining the percentage of deamidated VPs in the preparation, wherein the method is performed in the absence of a gel separation step.
19	The method of claim 18, wherein the liquid chromatography is reverse phase chromatography, wherein the reverse phase chromatography is performed with a C4 column.
20	The method of claim 18, wherein the liquid chromatography is reverse phase chromatography, wherein the reverse phase chromatography is performed with a C8 column.

Claim	Element
21[pre]	The method of claim 2, wherein the one [or] more variants of VP1, VP2, or VP3 capsid proteins comprise one or more post-translational modifications, wherein the method comprises
21[a]	determining the masses of the VP1, VP2, and VP3 capsid proteins and the one more variants of VP1 VP2 or VP3 capsid proteins,
21[b]	and wherein a deviation of one or more of the masses of the variants of VP1, VP2, or VP3 capsid proteins from the theoretical masses of VP1, VP2, and VP3 capsid proteins that have not undergone post-translational modifications is indicative of post-translational modifications of the variants of VP1, VP2, or VP3 capsid proteins.

Sarepta Therapeutics, Inc. ("Sarepta" or "Petitioner") respectfully requests *inter partes* review of claims 1-21 (the "challenged claims") of U.S. Patent No. 12,123,880 ("the '880 patent") (EX1001). The '880 patent is assigned to Genzyme Corporation.

I. INTRODUCTION

Adeno-associated virus (AAV) has been studied for decades as a useful tool to deliver therapeutic genes to patients to treat diseases such as Duchenne Muscular Dystrophy, cystic fibrosis, Parkinson's disease, hemophilia B, and Canavan disease. For both preclinical and clinical process development, researchers have sought to develop efficient and accurate analytical methods to monitor the purity and stability of AAV compositions.

For years before the earliest priority date for the '880 patent, researchers were using methods such as reversed-phase high pressure liquid chromatography (RP-HPLC), ultra-performance liquid chromatography (UPLC), and liquid chromatography-mass spectrometry (LC-MS) of both enzymatically digested and intact proteins, including liquid chromatography-tandem MS (LC-MS/MS), to monitor the purity and stability of preparations of viral particles and other proteins. It was also well known in the art that widely available software could be used to calibrate a mass spectrometer, and also to deconvolute and interpret mass

spectrometry (MS) data. Similarly, the choice of calibrant for a spectrometer would have required nothing more than routine experimentation.

The challenged claims are directed to methods for the analysis of preparations of AAV particles, and viral particles more generally. In particular, the challenged claims are directed to methods using LC-MS of intact proteins to analyze and characterize viral particles, including their serotype and the presence of post-translational modifications (PTMs). The challenged claims recite straightforward and well known aspects of LC-MS methods, such as choosing a C4 or C8 column, using RP-HPLC and UPLC, and calibrating the mass spectrometer using software and particular calibrants. All of these aspects of the claimed methods were known in the prior art for years before the earliest possible priority date for the ’880 patent.

Challenged claims 1-17 and 21 are obvious over the combination of two prior art references, Satkunanathan and Shytuhina. Challenged claims 7 and 15 are also obvious over the additional combination of three prior art references, Satkunanathan, Shytuhina, and Ansong. Challenged claims 8, 9, 16, and 17 are also obvious over the additional combination of three prior art references, Satkunanathan, Shytuhina, and Byeon. Claims 18-20 are obvious over the combination of two prior art references, Shytuhina and Zabrouskov. Claim 20 is

also obvious over the additional combination of three prior art references, Shytuhina, Zabrouskov, and Yuan.

Satkunanathan and Shytuhina. Satkunanathan studied three different AAV serotypes (AAV2, AAV5, and AAV8), focusing in particular on analyzing the protein composition present in purified AAV vectors of each serotype. 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. Satkunanathan states that their work is directed towards improving the production of AAV vectors for gene therapy. Among the unique proteins identified by LC-MS/MS in each purified preparation, Satkunanathan identified AAV2, AAV5, and AAV8 capsid proteins.

A POSA would have understood, however, that, given the structure of the AAV capsid proteins, vp1, vp2, and vp3, it would have been difficult, perhaps impossible, using Satkunanathan's method, to determine whether a fragment towards the C terminus of a given capsid protein originated from vp1, vp2, or vp3. A POSA would further have understood that enzymatic digestion is a laborious, time-consuming process, which can introduce artificial modifications, such as cyclization of N-terminal glutamine, and deamidation.

A POSA at the time would have been motivated to improve methods for characterizing AAV proteins for process development. A POSA would have

understood that more precise, accurate characterization to improve process development would involve the capability to distinguish AAV serotypes, identify PTMs, and monitor degradation products.

Shytuhina discloses the development and application of an RP-HPLC-MS method for analysis of intact viral structural proteins for process development for a vaccine. In particular, Shytuhina disclosed using their HPLC-MS method to identify PTMs of intact viral structural proteins.

Shytuhina is directed towards development of a vaccine against Chikungunya virus, a mosquito-borne virus that causes acute illness including fever, rash, and severe arthralgia. The Chikungunya virus-like particle (VLP) that Shytuhina studied has three structural proteins – E1 (envelope protein 1), E2 (envelope protein 2), and a capsid protein. The capsid and envelope are organized as follows: an outer surface composed of 240 copies of glycoproteins E1 and E2 embedded in a lipid bilayer surrounding a nucleocapsid made of 240 copies of capsid protein.

Shytuhina states that their goal was to develop an RP-HPLC assay that would separate E1, E2, and capsid proteins of Chikungunya particles. This assay would evaluate and quantitate the mass and purity of the vaccine product, and would be a tool to assess both protein degradation and PTMs for formulation and process development. Shytuhina used LC-MS intact protein analysis to validate their RP-HPLC method, and to identify specific PTMs on the E1 and E2 proteins. Shytuhina

discusses monitoring PTMs as a key element of process development.

Shytuhina identified E1 and E2 PTMs, including N-glycosylation and acylation, by matching the observed mass to the theoretical molecular weight. Shytuhina states that they likely also observed deamidation of E2. Shytuhina notes that Chikungunya particles produced in different cell lines exhibit different PTMs. Shytuhina states that it is therefore important to monitor PTMs to ensure product lot-to-lot consistency throughout the vaccine development cycle.

A POSA would have understood 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. A POSA would also have understood at the relevant time that the different capsid protein sequences would be reflected in different masses identified through LC-MS and that therefore the determined masses of the capsid proteins would be indicative of AAV serotypes such as those Satkunanathan studied. A POSA would therefore have been motivated to combine Satkunanathan and Shytuhina.

Challenged claims 1-17, and 21 are therefore obvious over the combination of Satkunanathan and Shytuhina.

Satkunanathan, Shytuhina, and Ansong. For the reasons discussed above, a POSA would have been motivated to combine Satkunanathan and Shytuhina.

Ansong discloses the use of intact LC-MS with a UPLC column to study the proteome of *Salmonella typhimurium*. Using intact LC-MS with UPLC, Ansong identified 563 unique proteins including 1665 proteoforms generated by PTMs. They found 12 proteins with an N-terminal acetylation modification, among other PTMs. A POSA would have been aware that UPLC could offer certain benefits as compared with HPLC, including increased resolution, sensitivity and speed of the chromatographic analysis, which are desirable for a chromatographic method to analyze proteins for in-process controlA POSA would therefore have likely tested Ansong's UPLC approach, in applying Shytuhina's intact LC-MS method to the separation and identification of AAV capsid proteins.

Challenged claims 7 and 15 are therefore also obvious over the combination of Satkunanathan, Shytuhina, and Ansong.

Satkunanathan, Shytuhina, and Byeon. For the reasons discussed above, a POSA would have been motivated to combine Satkunanathan and Shytuhina. Byeon discloses the use of LC-MS analysis to identify the molecular structure of a variant peak detected by RP-HPLC of rHu-EPO (Human Recombinant Erythropoietin). Byeon discloses that the mass spectrometer was calibrated with NaI before acquisition. Byeon also discloses that the mass spectra obtained in experiments were processed with a Maximum Entropy program (MaxEnt) to deconvolute multiply charged ESI data.

A POSA would have understood that LC-MS systems require calibration. A POSA would have been motivated to use Byeon's method of assisted calibration to calibrate the mass spectrometer because such assisted calibration was an efficient and straightforward method of calibration. In particular, a POSA would have been motivated to use Byeon's method to avoid complications resulting from adding a calibrant to the sample being analyzed, which would have required ensuring compatibility between the sample and the internal calibrant. In addition, a POSA would have understood NaI to be a desirable and commonly available calibrant, including for assisted calibration using software.

Challenged claims 8, 9, 16, 17 are therefore also obvious over the combination of Satkunanathan, Shytuhina, and Byeon.

Zabrouskov and Shytuhina. Zabrouskov disclosed the use of intact MS/MS to identify multiple deamidation sites, at asparagine and glutamine, on Ribonuclease A (RNase A). Shytuhina discloses that they may have observed deamidation of E2 as a separate peak in carrying out HPLC. A POSA would have understood that the intact LC-MS method of Shytuhina might not have provided sufficient resolution to identify all deamidations in a given protein. A POSA would therefore have been motivated to combine Shytuhina's intact LC-MS method with Zabrouskov's intact MS/MS method, which had sufficient resolution to identify multiple different sites of deamidation on RNase A.

Challenged claims 18-20 are therefore obvious over the combination of Zabrouskov and Shytuhina.

Zabrouskov, Shytuhina, and Yuan. A POSA would have understood the need for routine optimization of column chromatography conditions, such as the column, column length, solvent, and solvent gradient for example, in carrying out liquid chromatography for LC-MS methods, such as those disclosed in Shytuhina. Yuan discloses a RP-HPLC method to detect a capsid protein of HPV, the L1 protein. Yuan tested both C4 and C8 columns, finding that either column could be used with equal efficiency to purify the L1 capsid protein. A POSA would therefore have likely tested both C4 and C8 columns, in applying Shytuhina's intact LC-MS method to the characterization of deamidation PTMs in viral proteins.

Challenged claim 20 is therefore also obvious over the combination of Zabrouskov, Shytuhina, and Yuan.

Petitioner respectfully submits that the challenged claims (1-21) are unpatentable. This conclusion is supported by the prior art of record and the declaration of Dr. Joshua J. Coon (EX1004), an expert in the area of biological mass spectrometry (EX1005). EX1003, ¶¶2-48.

II. MANDATORY NOTICES

A. Real Parties-in-Interest (37 C.F.R. §42.8(b)(1))

Petitioner identifies Sarepta Therapeutics, Inc. and Sarepta Therapeutics Three, LLC as real parties-in-interest.

B. Related Matters (37 C.F.R. §42.8(b)(2))

Petitioner identifies the following related matters. The '880 patent is being asserted in currently-pending litigation: *Genzyme Corp. v. Sarepta Therapeutics, Inc.*, C.A. No. 24-cv-00882-RGA (D. Del.), D.I. 81. EX1040.

Petitioner is unaware of any other lawsuits involving the '880 patent.

C. Related Patent Office Proceedings

This is the first petition challenging a claim of the '880 patent.

Petitioner is concurrently filing petitions requesting *inter partes* review of the claims of two related patents, U.S. Patent No. 11,698,377 ("the '377 patent") and U.S. Patent No. 12,298,313 ("the '313 patent"). The '880 patent issued from U.S. Application No. 18/321,542, which is a division of Application No. 16/325,653, filed as PCT/US2017/046814, which issued as the '377 patent. The '313 patent issued from U.S. Application No. 19/013,863, which is a continuation of U.S. Application 18/801,293, which is a division of U.S. Application No. 18,321,542, which issued as the '880 patent.

D. Lead and Back-up Counsel and Service Information

Petitioner provides the following counsel and service information. Pursuant to 37 C.F.R. §42.10(b), a Power of Attorney accompanies this Petition.

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III. REQUIREMENTS FOR IPR

A. Payment of Fees

The undersigned authorizes the Office to charge the fee required for this Petition for *inter partes* review to Deposit Account No. 50-5708.

B. Grounds for Standing

Petitioner certifies that the ’880 patent is available for IPR and Petitioner is not barred or estopped from requesting IPR on the grounds identified herein. Petitioner further certifies that the prohibitions of 35 U.S.C. §§315 (a)-(b) are inapplicable.

C. Statement of Relief Requested

Petitioner respectfully requests review and cancellation of claims 1-21 of the '880 patent. The challenged claims should be found unpatentable on the following grounds:

Prior Art References
Satkunanathan (EX1005); published in 2014; prior art under AIA §102(a)(1).
Shytuhina (EX1006), published in 2014; prior art under AIA §102(a)(1).
Ansong (EX1009); published in 2013; prior art under AIA §102(a)(1).
Byeon (EX1014); published in May 2015; prior art under AIA §102(a)(1).
Zabrouskov (EX1038); published in 2006; prior art under AIA §102(a)(1).
Yuan (EX1037); published in 1998; prior art under AIA §102(a)(1).

Ground	**Claims**	**Description**
1	1-17, 21	Obvious in view of Satkunanathan and Shytuhina
2	7, 15	Obvious in view of Satkunanathan, Shytuhina, and Ansong
3	8, 9, 16, 17	Obvious in view of Satkunanathan, Shytuhina, and Byeon
4	18-20	Obvious in view of Shytuhina and Zabrouskov
5	20	Obvious in view of Shytuhina, Zabrouskov, and Yuan

Satkunanathan, Shytuhina, Ansong, Byeon, Zabrouskov, and Yuan were not cited to or considered by the Patent Office during prosecution. EX1001; EX1002.

IV. BACKGROUND OF THE TECHNOLOGY

Well over a decade before the earliest possible priority date for the '880 patent, researchers were using liquid chromatography, RP-HPLC in particular, to detect and quantify viral capsid proteins for purification process development. EX1037, Abstract; EX1003, ¶¶190, 88-99, *see also* ¶¶64-65. MS had also been used to explore the properties of viruses, including PTMs, for more than a decade before the earliest priority date for the '880 patent. EX1036, Abstract, 205-206; EX1003, ¶¶191, 100-206. A 1998 review, "Probing Viruses with Mass Spectrometry," stated, "[m]ass measuring viral proteins is now routine and since viruses are typically well characterized, in that the capsid proteins and DNA (or RNA) sequences are known, identifying a virus based on the mass of the protein and enzymatic digestion fragments is relatively straightforward." EX1036, Abstract, 205; EX1033, 11; EX1003, ¶191.

At least as early as 2002, LC-MS had been used to analyze viral composition. EX1016; EX1003, ¶¶195, 130-167, 190-206. Chelius disclosed the use of LC-MS/MS to analyze the composition of adenovirus particles. EX1016, Abstract; EX1003, ¶195. LC-MS/MS was also used before the priority date for process development for rAAV, including identifying the capsid proteins of different serotypes and identifying PTMs. EX1022, Abstract, 2; EX1005, Abstract, Supplementary Table S1; EX1003, ¶196-203. Satkunanathan used LC-MS/MS to explore cellular components associated with three different serotypes of rAAV,

specifically, AAV2, AAV5, and AAV8. EX1005, Abstract; EX1003, ¶197. Before the priority date, researchers had also used MS techniques to study mutant viral proteins in proteomics studies. EX1007, Abstract, 2509; EX1003, ¶¶204-05.

Years before the earliest possible priority date, researchers had also used LC-MS to analyze intact monoclonal antibodies, after reduction and separation using RP-HPLC. EX1012, 1416; EX1003, ¶178; *see also id.*, ¶¶168-89. Bondarenko stated that "it is very attractive to separate the intact protein isoforms by liquid chromatography and then perform on-line mass and top-down [intact] analyses to determine the sites of modifications and their abundances in one short assay." EX1012, 1416; EX1003, ¶¶175, 180.

Also before the priority date, researchers were using LC-MS to analyze intact viral structural proteins for process development. EX1006, Abstract; EX1003, ¶¶206, 168-189. Shytuhina applied LC-MS to analyze intact, undigested viral structural proteins to evaluate PTMs. EX1006, Abstract, 193-94; EX1003, ¶¶206, 259-271.

V. THE '880 PATENT

The '880 patent is titled "Methods for Detecting AAV." EX1001; EX1003, ¶207. The patent names Xiaoying Jin, Catherine O'Riordan, Lin Liu, and Kate Zhang as inventors. EX1001; EX1003, ¶207. The '880 patent issued on October 22, 2024. EX1001; EX1003, ¶207.

The ’880 patent is assigned to Genzyme Corporation. EX1001; EX1003, ¶208.

A. The Claims

The challenged claims are reproduced in the list above. Claims 1, 10, and 18 are independent claims. EX1001, 83:11-20, 41-56, 84:26-36; EX1003, ¶211. Claims 2-8 depend from claim 1, and claim 9 depends from dependent claim 8. EX1001, 83:21-40. Claim 21 depends from dependent claim 2. EX1001, 84:45-55. Claims 11-13, 15, and 16 depend from claim 10. *Id.*, 84:9-16, 19-23. Claim 14 depends from dependent claim 13, and claim 17 depends from dependent claim 16. EX1001, 84:17-18, 24-25. Claims 19 and 20 depend from claim 18. *Id.*, 84:45-55; EX1003, ¶211.

The challenged claims are directed to the use of LC-MS and LC-MS/MS intact protein analysis to characterize preparations of AAV and other viral particles. EX1003, ¶209. The claims require denaturing the viral particles and then subjecting them to intact LC-MS or LC-MS/MS. *Id.* Dependent claims specify particular denaturing methods, particular types of chromatography, and particular methods of calibration and particular calibrants for the mass spectrometer. Certain claims also involve characterization of viral proteins having PTMs, including variants of AAV capsid proteins (vp1, vp2, and vp3), and viral proteins that have been subject to deamidation.

B. The Specification

The specification of the ’880 patent discusses using LC-MS as an analytical tool to evaluate viral preparations. EX1001, 2:7-21; EX1003, ¶¶212-216. The specification discusses using LC-MS to discern characteristics of the viral preparation including the identity of the capsid serotype. EX1001, 2:12-17; EX1003, ¶212. The specification states that the method can be used as an AAV serotype identity test or to monitor viral capsid protein heterogeneity in rAAV gene therapy development. EX1001, 2:14-17; EX1003, ¶213. The specification discusses methods of denaturing rAAV samples before LC-MS analysis. EX1001, 54:22-32; EX1003, ¶214.

The specification discusses LC-MS intact protein analysis. EX1001, 53:32-49; 54:33-55:5; EX1003, ¶215. The specification also discusses LC-MS/MS peptide mapping. EX1001, 53:63-54:20; 55:6-48; 62:58-63:10; EX1003, ¶216. The specification does not disclose LC-MS/MS analysis of intact proteins. EX1003, ¶216.

C. The Prosecution History

The applicants filed a preliminary amendment, and a terminal disclaimer. EX1002, 217-52, 276-78; EX1003, ¶217. The applicants also filed an Amendment after Allowance, which was entered by the Examiner. EX1002, 317-23, 329-31;

EX1003, ¶217. The applicants also filed two Certificates of Correction, which were entered by the Examiner. EX1002, 339-44, 349-57; EX1003, ¶218.

D. Priority Date

The ’880 patent claims priority to U.S. Provisional Application No. 62/375,314, filed Aug. 15, 2016 (“’314 provisional”). EX1003, ¶219. The ’880 patent issued from U.S. Patent Application No. 18/321,542, filed May 22, 2023, which is a division of U.S. Patent Application No. 16/325,653, filed as application No. PCT/US2017/046814 on August 14, 2017, which issued as U.S. Patent No. 11,698,377. EX1003, ¶220. The earliest possible priority date for the ’880 patent claims is the filing date of the ’314 provisional, August 15, 2016. EX1003, ¶221.

VI. LEVEL OF ORDINARY SKILL IN THE ART

A POSA in the technical field of the ’880 patent would have had at least a Ph.D. in biochemistry, chemistry, pharmaceutical sciences, or a related field, and between one and four years of post-doctoral experience in the field of chemistry or pharmaceutical sciences, including analytical techniques such as chromatography and mass spectrometry. EX1003, ¶¶222-225. Alternatively, a POSA would have had at least a Master’s or Bachelor’s Degree in biochemistry, chemistry, pharmaceutical sciences, or a related field, with a corresponding number of additional years of experience in the field of chemistry or pharmaceutical sciences. *Id.*

VII. OVERVIEW OF THE PRIOR ART

A. Satkunanathan

Satkunanathan, titled "Establishment of a Novel Cell Line for the Enhanced Production of Recombinant Adeno-Associated Virus Vectors for Gene Therapy," was published in November 2014, more than one year before the earliest possible filing date for the '880 patent. EX1005. Therefore, Satkunanathan is AIA §102(a)(1) prior art. EX1005; EX1003, ¶231.

Satkunanathan studied three different AAV serotypes (AAV2, AAV5, and AAV8), focusing in particular on analyzing the protein composition present in purified AAV vectors of each serotype. EX1005, Abstract; EX1003, ¶232. 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. EX1005, Abstract, 930-31; EX1003, ¶¶233-34. Satkunanathan states that their work is directed towards improving the production of AAV vectors for gene therapy. EX1005, Abstract, 930; EX1003, ¶235. Satkunanathan discusses the problem of pre-existing immune responses in patients, requiring administration of higher titers and, presumably, the design of vectors based on different AAV serotypes. EX1005, Abstract; EX1003, ¶235.

Satkunanathan digested purified and concentrated vector samples with trypsin before LC-MS/MS. EX1005, 930-31; EX1003, ¶¶236-41. Equal amounts of total

proteins from three different types of purified AAV vector samples, that is, AAV2-GFP, AAV5-GFP, and AAV8-GFP were subjected to LC-MS/MS analysis. EX1005, 932; EX1003, ¶¶242-58. To minimize data variation, three batches of samples were prepared for each type of vector, with each batch pooled from 40 tissue culture plates (150 mm diameter). EX1005, 932; EX1003, ¶243. Three MS runs were performed for each batch of samples. EX1005, 932; EX1003, ¶243.

For data analysis, including mass spectra processing and database searching, up to two missed tryptic cleavages were considered, and methionine oxidation was set as a dynamic modification. EX1005, 931; EX1003, ¶239. Notably, Dong had identified oxidation of methionine as a PTM found in the AAV2 capsid proteins. EX1022, Abstract, 2; EX1003, ¶239.

Out of eight proteins found to be by two serotypes, five were shared by AAV2 and AAV5, indicating a relative similarity between AAV2 and AAV5 vectors. EX1005, 932, Supplementary Table S1; EX1003, ¶246. Twenty-six were unique to individual serotypes of vectors. EX1005, 932, Supplementary Table S1; EX1003, ¶246. As shown in the excerpt from Supplementary Table S1 below, among the unique proteins for each serotype, Satkunanathan identified AAV2, AAV5, and AAV8 capsid proteins:

SUPPLEMENTARY TABLE S1. (CONTINUED)

Protein ID	*AAV2*	*AAV5*	*AAV8*
Actin, gamma 1 OS=Homo sapiens GN=ACTG1 PE=3 SV=1 - [F5H0N0_HUMAN]		+	
Annexin A2 (Fragment) OS=Homo sapiens GN=ANXA2 PE=4 SV=1 - [H0YKZ7_HUMAN]		+	
ATP synthase subunit alpha OS=Homo sapiens PE=2 SV=1 - [B4DY56_HUMAN]		+	
ATP synthase-coupling factor 6, mitochondrial OS=Homo sapiens GN=ATP5J PE=1 SV=1 - [ATP5J_HUMAN]		+	
Capsid protein VP1 OS=Adeno-associated virus 2 (isolate Srivastava/1982) PE=1 SV=2 - [CAPSD_AAV2S]	+		
Capsid protein OS=Adeno-associated virus - 5 GN=cap PE=1 SV=1 - [Q9YIJ1_9VIRU]		+	
Capsid protein OS=Adeno-associated virus - 8 PE=1 SV=1 - [Q8JQF8_9VIRU]			+

EX1005, Supplementary Table S1 (excerpt) (yellow highlights added); EX1003, ¶¶247-48.

Satkunanathan also found a serotype-specific role for an AAV-associated cellular protein, YB1. EX1005, Abstract, 938); EX1003, ¶249. Satkunanathan found that knockdown of YB1 improved AAV2 and AAV8 production by 45- and 9-fold, respectively, but had no significant effect on AAV5 production. EX1005, 938; EX1003, ¶249.

Satkunanathan therefore teaches the importance of identifying and characterizing different AAV serotypes accurately for rAAV vector purification and production. EX1003, ¶252. Satkunanathan's discussion of the problem of pre-existing immunity among patients to various AAV serotypes further underscores the need to ensure the serotypic purity of any preparation of rAAV for possible clinical use. EX1005, 929; EX1003, ¶252.

Satkunanathan also teaches that as of 2014, it was routine to identify capsid proteins of different AAV serotypes using LC-MS/MS. EX1005, Supplementary Table S1; EX1003, ¶253.

B. Shytuhina

Shytuhina, titled, "Development and application of a reversed-phase high-performance liquid chromatographic method for quantitation and characterization of a Chikungunya virus-like particle vaccine," was published in 2014, more than a year before the earliest possible priority date for the '880 patent (August 15, 2016), and is therefore AIA §102(a)(1) prior art. EX1006; EX1003, ¶259.

Shytuhina discloses the development and application of an RP-HPLC-MS method for analysis of intact viral structural proteins for process development for a vaccine. EX1006, Abstract; EX1003, ¶¶260-306. In particular, Shytuhina disclosed using their HPLC-MS method to identify PTMs on intact viral structural proteins. EX1006, Abstract; EX1003, ¶260, *see also* ¶¶226-30.

Shytuhina is directed towards development of a vaccine against Chikungunya virus, a mosquito-borne virus that causes acute illness including fever, rash, and severe arthralgia. EX1006, Abstract, 192; EX1003, ¶261. Shytuhina states that to support process and formulation development effectively, it is highly desirable to have a sensitive and robust method available that can be automated to measure both vaccine purity and antigen specific vaccine mass. EX1006, 192; EX1003, ¶266.

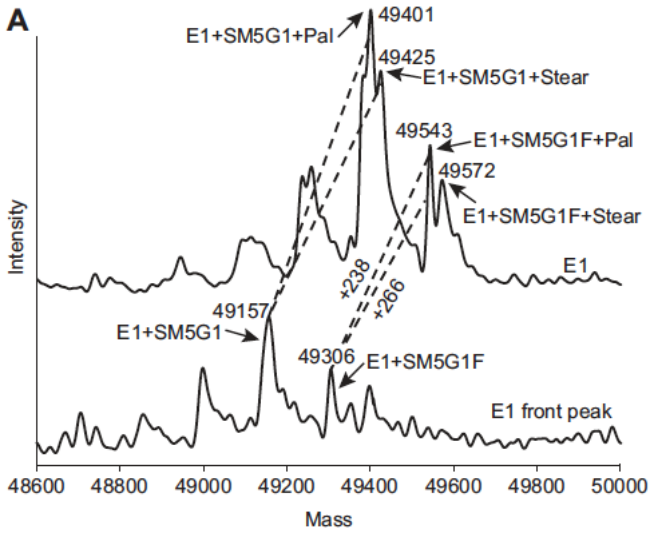
Shytuhina discloses that HPLC was an attractive analytical tool, in light of its high sensitivity and reproducibility. EX1006, 192; EX1003, ¶267. Shytuhina notes that HPLC had been applied for the identification and quantitation of viral proteins and VLPs from a variety of other viruses, including serotypes of adenovirus (types 3 and 5), influenza, lentivirus, Sendai virus, poliovirus, human papillomavirus VLP, and Hepatitis B VLP. EX1006, 192-93; EX1003, ¶267.

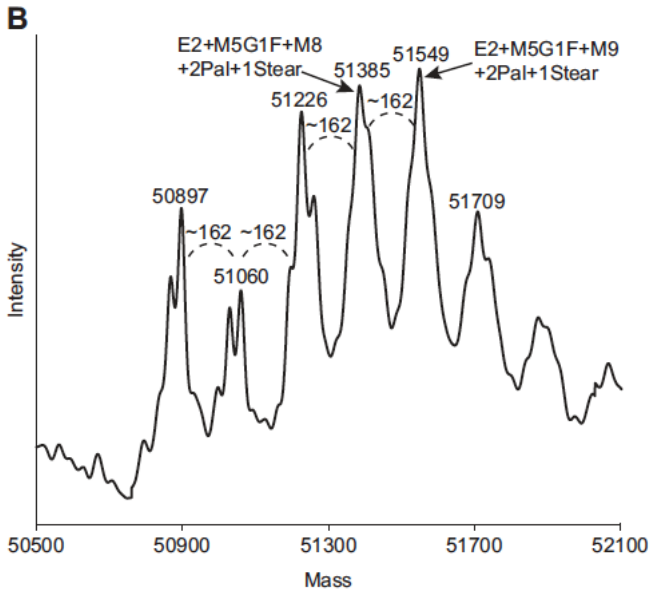
The Chikungunya VLP has three structural proteins – E1 (envelope protein 1), E2 (envelope protein 2), and a capsid protein. EX1006, 193; EX1003, ¶269. The capsid and envelope are organized as follows: an outer surface composed of 240 copies of glycoproteins E1 and E2 embedded in a lipid bilayer surrounding a nucleocapsid made of 240 copies of capsid protein. EX1006, 193; EX1003, ¶269.

Shytuhina states that their goal was to develop a RP-HPLC assay that would separate E1, E2, and capsid proteins of Chikungunya VLPs. EX1006, 193; EX1003, ¶270. This assay would evaluate and quantitate the mass and purity of the vaccine product. EX1006, 193; EX1003, ¶270. This method would be a tool to assess both protein degradation and PTMs for formulation and process development. EX1006, 193; EX1003, ¶270.

Shytuhina used LC-MS intact protein analysis to validate their RP-HPLC method, and to identify specific PTMs on the E1 and E2 proteins. EX1006, 193-96; EX1003, ¶271. Shytuhina discusses monitoring PTMs as a key element of process

development. EX1006, 196-97; EX1003, ¶271. PTMs were identified by comparing observed masses to theoretical masses of expected N-glycoforms with and without acylation. EX1006, 194; EX1003, ¶285. Shytuhina first carried out RP-HPLC, and then carried out RP-HPLC-MS, where the chromatography was online with the mass spectrometer. EX1006, 193-94; EX1006, 194; EX1003, ¶¶286-303. Figure. 2, reproduced below, shows the deconvoluted spectra for E1 (Fig. 2A) and E2 (Fig. 2B).





EX1006, 194-95, Fig. 2; EX1003, ¶298.

As shown in the figures above, multiple masses were observed with each representing different glycosylation and acylation modifications. EX1006, 194-95, Fig. 2; EX1003, ¶299. Based on known glycoprotein acylation and expected N-linked glycans, Shytuhina identified glycoprotein modifications in several major peaks by matching the observed mass to the theoretical molecular weight. EX1006, 194-95, Fig. 2; EX1003, ¶300.

Shytuhina detected one N-glycosylation and one acylation – either palmitoylation (Pal) or stearoylation (Stear) – for the majority of E1 (Figure. 2A, top trace). EX1006, 194-95, Figure. 2; EX1003, ¶301. They detected a small amount of glycosylated but deacylated E1. EX1006, 194-95, Figure 2A, bottom trace; EX1003, ¶302. Shytuhina explains that this result was not surprising, given that

protein acylation is a reversible process, and enzymatic depalmitoylation of viral glycoprotein had been demonstrated. EX1006, 194-95, Figure 2; EX1003, ¶302.

Fig. 2B shows that E2 contained two N-glycosylations and three acylations. EX1006, 194-95, Figure 2; EX1003, ¶303.

Shytuhina states that they likely observed deamidation of one of the viral structural proteins they studied, the E2 protein of chikungunya virus. EX1006, 196; EX1003, ¶304. Specifically, in carrying out RP-HPLC, they observed an E2 degradant peak that eluted slightly earlier than the regular E2, suggesting that the degradant was more hydrophilic. EX1006, 194, 196, Figure 1A; EX1003, ¶304.

Shytuhina explains that Chikungunya VLPs produced in different cell lines exhibit different PTMs. EX1006, 196-97; EX1003, ¶306. Shytuhina states that it is therefore important to monitor PTMs to ensure product lot-to-lot consistency throughout the vaccine development cycle. EX1006, 197; EX1003, ¶306.

C. Ansong

Ansong, titled, "Top-down proteomics reveals a unique protein S-thiolation switch in *Salmonella Typhimurium* in response to infection-like conditions," was published on June 18, 2013, more than a year before the earliest possible priority date for the '880 patent (August 15, 2016), and is therefore AIA §102(a)(1) prior art. EX1009; EX1003, ¶307.

Ansong used intact LC-MS with a UPLC column to study the proteome of *Salmonella typhimurium*. EX1009, Abstract, 10153-54; EX1003, ¶¶307-16. Ansong states that a number of factors contributed to the improved proteome coverage using their platform compared with prior studies. EX1009, Abstract, 10154; EX1003, ¶312. Ansong states that these factors included the use of a UPLC system with long columns (80 cm) and long gradients (250min), and particular software tool for intact protein identification based on spectral alignment. EX1009, Abstract, 10154; EX1003, ¶¶313-14.

Using intact LC-MS with UPLC, Ansong identified 563 unique proteins including 1665 proteoforms generated by PTMs. EX1009, Abstract; EX1003, ¶315. They found 12 proteins with an N-terminal acetylation modification, among other PTMs. EX1009, Abstract, 10154-55; EX1003, ¶316.

D. Byeon

Byeon, titled, "Structural Identification of a Non-Glycosylated Variant at Ser126 for O-Glycosylation Site from EPO BRP, Human Recombinant Erythropoietin by LC/MS Analysis," was published online on May 27, 2015, more than a year before the earliest possible priority date for the '880 patent (August 15, 2016), and is therefore AIA §102(a)(1) prior art. EX1014; EX1003, ¶317.

Byeon used LC-MS analysis to identify the molecular structure of a variant peak detected by RP-HPLC of rHu-EPO (Human Recombinant Erythropoietin).

EX1014, Abstract; EX1003, ¶¶318-26. Byeon used UPLC-MS for the analysis, and calibrated the mass spectrometer, which was calibrated with NaI before acquisition. EX1014, 497; EX1003, ¶¶318-20. Byeon also discloses that the mass spectra obtained in experiments were processed with a Maximum Entropy program (MaxEnt) to deconvolute multiply charged ESI data. EX1014, 497; EX1003, ¶321. Byeon concluded that their method could be used to monitor rHu-Epo-related degradation on the carbohydrate structure and therefore could be applied as a purity test method. EX1014, 504; EX1003, ¶326.

E. Zabrouskov

Zabrouskov, "Stepwise Deamidation of Ribonuclease A at Five Sites Determined by Top Down Mass Spectrometry," was published in 2006, more than a year before the earliest possible priority date for the '880 patent (August 15, 2016), and is therefore AIA §102(a)(1) prior art. EX1038; EX1003, ¶327.

Zabrouskov intact MS/MS to identify multiple deamidation sites, at asparagine and glutamine, on Ribonuclease A (RNase A). EX1038, Abstract; EX1003, ¶¶328-329; *see also id.*, ¶¶330-61. Zabrouskov also carried out site-specific quantitation of deamidation at the various sites in RNase A. EX1038, Abstract; EX1003, ¶¶328-331.

Zabrouskov notes, nonetheless, that it was known in the art that deamidation poses a special challenge, in that the covalent $–NH_2 \rightarrow –OH$ modification produces

only a 0.984 Da mass increase, closely matching the 1.002 Da spacing of the molecular ion isotope peaks. EX1038, 987; EX1003, ¶332. As a result, it is difficult to distinguish deamidated forms from ^{13}C isotopic forms. EX1003, ¶332.

Despite this known difficulty, Zabrouskov successfully used top-down MS/MS to distinguish five stepwise deamidation sites in RNase A. EX1038, 987; EX1003, ¶333.

According to Zabrouskov, rates of deamidation of asparagine and glutamine residues depend on protein primary sequence, three-dimensional structure, and solution parameters – increased pH and temperature and enhanced denaturation accelerate deamidation. EX1038, 987; EX1003, ¶334.

Deamidation of Asn is generally favored over that of Gln, in part through operation of a cyclic imide reaction mechanism that also favors the Asn67-Gly68 sequence found in RNase A, while other neighboring residues also show an influence statistically. EX1038, 987; EX1003, ¶335. However, some Asn and Gln residues are extremely resistant to *in vivo* deamidation. EX1038, 987; EX1003, ¶335.

Zabrouskov states that in "bottom-up" MS proteomics, which uses enzymatic digestion of proteins before MS analysis, initial digestion of the protein gives peptides with mass spectra that often provide a fast, reliable identification of a

protein, but are much less useful in characterizing PTMs. EX1038, 987; EX1003, ¶336.

Zabrouskov calculated percentages of deamidated products in the various reactions. EX1038, 990; EX1003, ¶¶341-361. For example, Zabrouskov reported that, for the monodeamidated fraction, Asn67 was the first residue to deamidate and the deamidation of this reside was nearly complete in 1 hour (0:1:2 deamidations at a 5:43:52 ratio), with <10% monodeamidation at other sites. EX1038, 990; EX1003, ¶361.

F. Yuan

Yuan, titled, "Reversed-phase high-performance liquid chromatography of virus-like particles," was published in 1998, more than a year before the earliest possible priority date for the '880 patent (August 15, 2016), and is therefore AIA §102(a)(1) prior art. EX1037; EX1003, ¶362.

Yuan discloses an RP-HPLC method for analysis of virus-like particles (VLPs) of human papillomavirus (HPV) for process development. EX1037, Abstract; EX1003, ¶363; *see also id.*, ¶¶364-85. In particular, Yuan's method is directed towards identifying the L1 capsid protein that comprises 90-95% of HPV capsids. EX1037, 21; EX1003, ¶364. Yuan explains that the HPV capsid is typically composed of 72 pentameric capsomers of L1 arranged on a skewed icosahedral lattice. EX1037, 21; EX1003, ¶364. A second structural protein, L2, represents the

remaining 5-10% of the capsid. EX1037, 21; EX1003, ¶364. Yuan states that their method is quantitative and can be used to facilitate HPV purification process development. EX1037, 21; EX1003, ¶365.

Yuan discusses the importance of the first step of dissociating the VLPs before placing them on the HPLC column. EX1037, 22; EX1003, ¶¶366-371. Yuan explains that the VLPs they used in the analysis were approximately 55 nm in diameter and contained a single capsid protein, L1, with a molecular mass of about 55,000. EX1037, 22; EX1003, ¶371. Yuan explains that to quantitate the L1 capsid protein, it was necessary to dissociate the VLPs prior to RP-HPLC analysis. EX1037, 22; EX1003, ¶¶371-376.

Yuan tested both C4 and C8 columns, noting that "either the C4 or C8 column can be used with equal efficiency of separation providing calibration is carried out with purified reference standard material." EX1037, 23; EX1003, ¶¶377-385.

VIII. CLAIM CONSTRUCTION

In this petition, the challenged claims have generally been analyzed according to their plain and ordinary meaning. For the term "variants/variant," the petition analyzes the challenged claims according to the construction that Genzyme has proposed in the litigation for this term – to mean "AAV mutant capsid protein[s]." EX1003, ¶365.

IX. DETAILED EXPLANATION OF GROUNDS

A. Ground 1: Claims 1-17, 21 Are Obvious Over Satkunanathan and Shytuhina

A POSA would have been motivated to combine Satkunanathan and Shytuhina because both are directed towards efficient and precise methods for analysis of viral particle composition for the purpose of process development. EX1003, ¶¶390-391; *see also id.*, ¶¶392-426. Both use liquid chromatography-mass spectrometry techniques to characterize purified viral preparations, identifying properties such as differences among different viral serotypes, or post-translational modifications. *Id.*, ¶¶390-391. Satkunanathan's method, however, involves enzymatically digesting proteins before subjecting them to LC-MS, while Shytuhina's method involves carrying out MS on intact proteins that have not been enzymatically digested. *Id.*

Satkunanathan studied proteins that co-purify along with each of three different AAV serotypes, AAV2, AAV5, and AAV8. *Id.*, ¶392. As part of this analysis, Satukunanathan also identified capsid proteins for each of the three serotypes studied. *Id.*, ¶¶393-396. In addition, Satkunanathan's settings included the PTM methionine oxidation as a dynamic modification. *Id.*, ¶397. Given that Dong had determined that AAV capsid proteins undergo this PTM, Satkunanathan's analysis likely included the identification of some capsid proteins containing this PTM. *Id*.

A POSA would have understood that, given the structure of the AAV genome, LC-MS analysis involving enzymatic digestion before MS analysis would not have enabled reliable and accurate separation and characterization of the AAV viral capsid proteins (vp1, vp2, and vp3) from one another, and from the capsid proteins of different serotypes. *Id.*, ¶401, *see also* ¶¶66-87.

A POSA at the time would have been motivated to improve methods for characterizing AAV proteins for process development, as Satkunanathan teaches, including identifying and characterizing different serotypes, identifying and characterizing PTMs, and identifying and characterizing individual AAV capsid proteins (vp1, vp2, and vp3). *Id*., ¶¶398-400. A POSA would also have been aware that it had been known since the 1980s that AAV capsid proteins undergo N-terminal truncation and acetylation *in vivo*. EX1011, 7920; EX1003, ¶399.

In particular, a POSA would have understood 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. EX1003, ¶¶401-403. Shytuhina discloses the use of intact LC-MS of viral structural proteins to identify and monitor PTMs to improve process development. *Id.*, 402.

1. Claim 1: "A method of analyzing a preparation of AAV particles, the method comprising"

Satkunanathan uses LC-MS/MS to analyze preparations of AAV2, AAV5, and AAV8 particles, including identifying the capsid proteins for each serotype. EX1005, Abstract, Supplementary Table S1; EX1003, ¶405.

Shytuhina uses intact LC-MS to identify PTMs of viral structural proteins. EX1006, 194; EX1003, ¶406.

Satkunanathan, in combination with Shytuhina, therefore meets this limitation. EX1003, ¶¶404-408.

(a) "a) denaturing the AAV particles,"

Shytuhina teaches carrying out two denaturing steps – treatment with Zwittergent and RP-HPLC – before subjecting the denatured viral particles to LC-MS intact protein analysis. EX1006, 193-94; EX1003, ¶410.

For the first step, Shytuhina teaches incubating the samples with 5% Zwittergent 3-12 detergent. EX1006, 193-94; EX1003, ¶411. Shytuhina states that pre-treatment with Zwittergent improved recovery, and that "Zwittergent 3-12 detergent is believed to solubilize and stabilize the glycoproteins [viral structural proteins] and prevent non-specific binding during chromatography." EX1006, 194; EX1003, ¶411. A POSA at the time would have understood that "solubilizing" glycoproteins involves denaturing them. EX1003, ¶411.

For the second step, Shytuhina carried out an RP-HPLC separation, before subjecting the denatured samples to intact LC-MS, which then involved another RP-

HPLC column coupled to the mass spectrometer. EX1006, 193; EX1003, ¶412. Shytuhina states that for the initial RP-HPLC separation (before LC-MS), they used TFA in the mobile phase on the column, and that "TFA appeared to help disassemble the VLPs and facilitate the binding of proteins to the stationary phase." EX1006, 194; EX1003, ¶412.

Satkunanathan, in combination with Shytuhina, therefore meets this limitation. EX1003, ¶¶409-413.

(b) "b) subjecting the denatured AAV particles to liquid chromatography/mass spectrometry (LC-MS) intact protein analysis, and"

See claim 1(a). EX1003, ¶415. Shytuhina discloses that after the initial RP-HPLC, which denatures the viral particles, the denatured viral particles are then separated and analyzed by intact LC-MS using a Waters Acquity LC system coupled to a Synapt G2 mass spectrometer. EX1003, ¶416. The denatured viral particles are therefore subjected to LC-MS intact protein analysis. EX1006, 193-94; EX1003, ¶416.

Satkunanathan, in combination with Shytuhina, therefore meets this limitation. EX1003, ¶¶414-417.

(c) "c) determining the masses of one or more viral proteins (VPs) of the particles in the preparation,"

Using LC-MS/MS, Satkunanathan determined the masses of capsid proteins for each of three different AAV serotypes, AAV2, AAV5, and AAV8. EX1005, Supplementary Table S1; EX1003, ¶419.

Shytuhina determined the masses of two viral structural proteins using intact LC-MS. EX1006, 194-95, Fig. 2; EX1003, ¶420.

Satkunanathan, in combination with Shytuhina, thus meets this limitation. EX1003, ¶¶418-421.

(d) "wherein the method is performed in the absence of a gel separation step."

No gel separation step was used as part Shytuhina's intact LC-MS method, or as part of Satkunanathan's LC-MS/MS method. EX1003, ¶423.

Satkunanathan, in combination with Shytuhina, thus meets this limitation. EX1003, ¶¶422-424.

2. Claim 2: "The method of claim 1, wherein the VPs comprise VP1, VP2, and VP3 capsid proteins and one more variants of VP1, VP2, or VP3 capsid proteins."

See claim 1. Satkunanathan identified capsid proteins for each of the three serotypes tested, AAV2, AAV5, and AAV8. EX1005, Supplementary Table S1; EX1003, ¶427. Satkunanathan's settings included the PTM methionine oxidation as a dynamic modification. EX1005, 931; EX1003, ¶425. Given that Dong had determined that AAV capsid proteins undergo this PTM, Satkunanathan's analysis

likely included the identification of some capsid proteins containing this PTM. EX1005, 931; EX1022, Abstract, 2; EX1003, ¶425. A POSA would also have understood that, since the 1980s, it had been known that AAV capsid proteins undergo N-terminal truncation and acetylation *in vivo*. EX1011, 7920; EX1003, ¶427. Before the priority date, researchers had also used MS techniques to study mutant viral proteins in proteomics studies. EX1007, Abstract, 2509; EX1003, ¶¶204-05.

Using intact LC-MS, Shytuhina separated and determined the masses of different post-translationally modified forms of viral structural proteins E1 and E2 from one another and from unmodified forms of the proteins. EX1006, 194-95, Fig. 2; EX1003, ¶¶429-428.

A POSA would further have understood that Shytuhina's intact LC-MS method had sufficiently high resolution to separate capsid protein variants, from capsid proteins that had not been subject to these modifications. EX1003, ¶430. Shytuhina states that "[b]ased on known glycoprotein acylation and expected N-linked glycans, we identified glycoprotein modifications in several major peaks by matching the observed mass to the theoretical molecular weight." EX1006, 194; EX1003, ¶430.

Satkunanathan, in combination with Shytuhina, thus meets this additional limitation of dependent claim 2. EX1003, ¶¶425-431.

3. Claim 3: "The method of claim 1, wherein the AAV particles are denatured with acetic acid, guanidine hydrochloride and/or an organic solvent."

See claim 1. Shytuhina initially separated the viral structural proteins using RP-HPLC, which a POSA would have understood denatures the viral particles. EX1006, 194; EX1003, ¶¶433-434.

For RP-HPLC, mobile phase B contained 30% acetonitrile (ACN), 70% 2-propanol, and 0.1% trifluoroacetic acid (TFA). EX1003, ¶435. A POSA would have understood that both acetonitrile and 2-propanol are organic solvents. EX1029, 11199-200, Table I, Table II; EX1003, ¶435. Shytuhina states, "TFA appeared to help disassemble the VLPs and facilitate the binding of proteins to the stationary phase." EX1006, 193-94; EX1003, ¶436.

A POSA would therefore have understood that the combination of the organic solvents acetonitrile and 2-propanol with TFA in the mobile phase disassembled, *i.e.*, denatured, the VLPs. EX1003, ¶437.

Therefore, the combination of Satkunanathan and Shytuhina meets the additional limitation of dependent claim 3. EX1003, ¶¶432-438.

4. Claim 4: "The method of claim 1, wherein the liquid chromatography is reverse phase liquid chromatography, size exclusion chromatography, hydrophilic interaction liquid chromatography, or cation exchange chromatography."

See claim 1. Shytuhina carried out an initial RP-HPLC separation, followed by RP-HPLC coupled to the mass spectrometer. EX1006, 193; EX1003, ¶440.

Therefore, the combination of Satkunanathan and Shytuhina meets the additional limitation of dependent claim 4. EX1003, ¶¶439-441.

5. Claim 5: "The method of claim 1, wherein the liquid chromatography is reverse phase liquid chromatography."

See claim 1. Shytuhina carried out an initial RP-HPLC separation, followed by RP-HPLC coupled to the mass spectrometer. EX1006, 193; EX1003, ¶443.

Therefore, the combination of Satkunanathan and Shytuhina meets the additional limitation of dependent claim 5. EX1003, ¶¶442-444.

6. Claim 6: "The method of claim 5, wherein the reverse phase chromatography is performed with a C4 column."

See claim 1. Shytuhina discloses that the specific RP-HPLC column they used was a C4 column. EX1006, 193; EX1003, ¶¶446-447. Although Shytuhina does not expressly disclose the column used for the RP-HPLC coupled to the mass spectrometer, a POSA would have understood it was likely that they used the same C4 column that had successfully separated E1 and E2 in the prior RP-HPLC step; EX1003, ¶¶446-447.

In any event, a POSA at the time would have understood that it was generally necessary to test a few different columns, such as C4 and C8 columns, to optimize the desired separation. EX1037, 22-23; EX1003, ¶¶448-451.

Satkunanathan, in combination with Shytuhina, therefore meets the additional limitation of dependent claim 6. EX1003, ¶¶445-452.

7. Claim 7: "The method of claim 1, wherein the liquid chromatography is ultra-performance liquid chromatography (UPLC)."

See claim 1. A POSA would have been aware that researchers were using LC-MS methods such as Shytuhina's, with UPLC columns to study mixtures of proteins and to identify PTMs. EX1009; EX1003, ¶¶454-456. A POSA would have been aware of the advantages UPLC could offer, set out in Ansong, such as the efficient separation and increased sampling of proteins in the mixture and identification of PTMs, using long columns and long gradients. EX1009, 10154; EX1003, ¶455.

A POSA would have been aware that UPLC could offer certain benefits as compared with HPLC, including increased resolution, sensitivity and speed of the chromatographic analysis, which are desirable for a chromatographic method to quantify proteins for in-process control. EX1020, 1019; EX1003, ¶457.

A POSA would therefore have likely tested UPLC, in applying Shytuhina's intact LC-MS method to the separation and identification of AAV capsid proteins. EX1003, ¶458.

Satkunanathan, in combination with Shytuhina, therefore meets the additional limitation of dependent claim 7. *Id.*, ¶¶453-459.

8. Claim 8: "The method of claim 1, wherein the mass spectrometry comprises assisted calibration."

See claim 1. A POSA at the time would have understood that LC-MS systems require calibration, and that calibration could be carried out with an external or internal calibrant. EX1014, 497; EX1008; EX1003, ¶461.

A POSA would further have understood that the term "assisted calibration" in the patent refers to "using software to correlate a peak and/or position of a known standard (e.g., a calibrant) to a specific mass to charge (m/z) ratio." EX1001, 21:47-56; EX1003, ¶462.

A POSA at the relevant time would have been aware that calibration could be carried out using commonly available software, *i.e.*, via assisted calibration. EX1014, 497; EX1003, ¶463.

Satkunanathan, in combination with Shytuhina, therefore meets the additional limitation of dependent claim 8. EX1003, ¶¶460-464.

9. Claim 9: "The method of claim 8, wherein sodium iodide is used as a calibrant."

See claim 8. A POSA would have understood NaI to be a desirable and commonly available calibrant, including for assisted calibration using software. EX1008, 665; EX1014, 497; EX1003, ¶469.

Satkunanathan, in combination with Shytuhina, therefore meets the additional limitation of dependent claim 9. EX1003, ¶¶465-470.

10. Claim 10: "A method of determining post-translational modifications of viral proteins (VPs) in a preparation of viral particles, the method comprising."

Satkunanathan's settings included the PTM methionine oxidation as a dynamic modification. EX1005, 931; EX1003, ¶472. Given that Dong had determined that AAV capsid proteins undergo this PTM, Satkunanathan's analysis likely included the identification of some capsid proteins containing this PTM. EX1005, 931; EX1022, Abstract, 2; EX1003, ¶472.

A POSA would have also understood that it had been known since at least 1985 that AAV capsid proteins are subject to N-terminal truncation and acetylation *in vivo*. EX1011, 7920; EX1003, ¶473.

Shytuhina identified several different glycosylation and acylation modifications on each of two viral structural proteins analyzed by intact LC-MS. EX1006, 194-95, Fig. 2; EX1003, ¶474.

Satkunanathan, in combination with Shytuhina, therefore meets this limitation. EX1003, ¶¶471-476.

(a) "a) "denaturing the viral particles"

See claim 1(a). Satkunanathan, in combination with Shytuhina, therefore meets this limitation. EX1003, ¶¶477-481.

(b) "b) subjecting the denatured viral particles to liquid chromatography/mass spectrometry (LC-MS) intact protein analysis, and"

See claim 1(b). Satkunanathan, in combination with Shytuhina, therefore meets this limitation. EX1003, ¶¶482-485.

(c) "c) determining the masses of one or more VPs of the viral particles"

See claim 1(c). Satkunanathan, in combination with Shytuhina, therefore meets this limitation. EX1003, ¶¶486-489.

(d) "wherein a deviation of one or more of the masses of the one or more VPs from the theoretical masses of VPs that have not undergone post-translational modifications is indicative of post-translational modifications of the VPs,"

Shytuhina states that "[b]ased on known glycoprotein acylation and expected N-linked glycans, we identified glycoprotein modifications in several major peaks by matching the observed mass to the theoretical molecular weight." EX1006, 194; EX1003, ¶493.

Satkunanathan, in combination with Shytuhina, therefore meets this limitation. EX1003, ¶¶490-494.

(e) "and wherein the method is performed in the absence of a gel separation step."

See claim 1(d). Satkunanathan, in combination with Shytuhina, therefore meets this limitation. EX1003, ¶¶495-497.

11. Claim 11: "The method of claim 10, wherein the liquid chromatography is reverse phase liquid chromatography, size exclusion chromatography, hydrophilic interaction liquid chromatography, or cation exchange

chromatography."

See claims 4, 10. Satkunanathan, in combination with Shytuhina, therefore meets this additional limitation of dependent claim 11. EX1003, ¶¶498-501.

12. Claim 12: "The method of claim 10, wherein the viral particles comprise a viral vector encoding a heterologous transgene."

See claim 10. Satkunanathan discloses AAV particles containing a heterologous GFP transgene. EX1005, 930; EX1003, ¶504. A POSA at the time would have understood that rAAV gene therapy vectors were specifically designed to deliver a heterologous transgene to a variety of different target cells. EX1025, Abstract, 289-97; EX1003, ¶503.

Satkunanathan, in combination with Shytuhina, therefore meets this additional limitation of dependent claim 12. EX1003, ¶¶497-505.

13. Claim 13: "The method of claim 10, wherein the liquid chromatography is reverse phase chromatography."

See claims 5, 10. Satkunanathan, in combination with Shytuhina, therefore meets this additional limitation of dependent claim 13. EX1003, ¶¶506-509.

14. Claim 14: "The method of claim 13, wherein the reverse phase chromatography is a C4 or C8 reverse chromatography."

See claims 6, 13. Satkunanathan, in combination with Shytuhina, therefore meets this additional limitation of dependent claim 14. EX1003, ¶¶510-517.

15. Claim 15: "The method of claim 10, wherein the liquid

chromatography is ultra-performance liquid chromatography (UPLC)."

See claims 7, 10. Satkunanathan, in combination with Shytuhina, therefore meets this additional limitation of dependent claim 15. EX1003, ¶¶518-524.

16. Claim 16: "The method of claim 10, wherein the mass spectrometry comprises assisted calibration."

See claims 8, 10. Satkunanathan, in combination with Shytuhina, therefore meets this additional limitation of dependent claim 16. EX1003, ¶¶525-529.

17. Claim 17: "The method of claim 16, wherein sodium iodide is used as a calibrant."

See claims 9, 16 above. Satkunanathan, in combination with Shytuhina, therefore meets this additional limitation of dependent claim 17. EX1003, ¶¶530-535.

18. Claim 21

(a) "The method of claim 2, wherein the one more variants of VP1, VP2, or VP3 capsid proteins comprise one or more post-translational modifications, wherein the method comprises:"

See claim 2. EX1003, ¶¶536-538. Using intact LC-MS, Shytuhina separated and determined the masses of different post-translationally modified forms of viral structural proteins E1 and E2 from one another and from unmodified forms of the proteins. EX1006, 194-95, Fig. 2; EX1003, ¶539.

A POSA would have understood that, since the 1980s, it had been known that AAV capsid proteins undergo N-terminal truncation and acetylation *in vivo*. EX1011, 7920; EX1003, ¶538. Before the priority date, researchers had also used MS techniques to study mutant viral proteins in proteomics studies. EX1007, Abstract, 2509; EX1003, ¶¶204-05.

A POSA would further have understood that Shytuhina's intact LC-MS method had sufficiently high resolution to separate capsid protein variants from capsid proteins that had not been subject to these modifications. EX1003, ¶¶540-541. Shytuhina states that "[b]ased on known glycoprotein acylation and expected N-linked glycans, we identified glycoprotein modifications in several major peaks by matching the observed mass to the theoretical molecular weight." EX1006, 194; EX1003, ¶541.

Satkunanathan, in combination with Shytuhina, therefore meets this additional limitation of claim 21. EX1003, ¶¶536-542.

(b) "determining the masses of the VP1, VP2, and VP3 capsid proteins and the one more variants of VP1 VP2 or VP3 capsid proteins, "

See claim 2. EX1003, ¶547. Using intact LC-MS, Shytuhina separated and determined the masses of different post-translationally modified forms of viral structural proteins E1 and E2 from one another and from unmodified forms of the proteins. EX1006, 194-95, Fig. 2; EX1003, ¶547.

A POSA would have understood that, since the 1980s, it had been known that AAV capsid proteins undergo N-terminal truncation and acetylation *in vivo*. EX1011, 7920; EX1003, ¶545. Before the priority date, researchers had also used MS techniques to study mutant viral proteins in proteomics studies. EX1007, Abstract, 2509; EX1003, ¶¶204-05.

A POSA would further have understood that Shytuhina's intact LC-MS method had sufficiently high resolution to separate capsid protein variants, from capsid proteins that had not been subject to these modifications. EX1003, ¶549. Shytuhina states that "[b]ased on known glycoprotein acylation and expected N-linked glycans, we identified glycoprotein modifications in several major peaks by matching the observed mass to the theoretical molecular weight." EX1006, 194; EX1003, ¶548.

Satkunanathan, in combination with Shytuhina, therefore meets this additional limitation of dependent claim 21. EX1003, ¶¶543-550.

(c) "and wherein a deviation of one or more of the masses of the variants of VP1, VP2, or VP3 capsid proteins from the theoretical masses of VP1, VP2, and VP3 capsid proteins that have not undergone post-translational modifications is indicative of post-translational modifications of the variants of VP1, VP2, or VP3 capsid proteins."

See claim 2. Using intact LC-MS, Shytuhina separated and determined the masses of different post-translationally modified forms of viral structural proteins

E1 and E2 from one another and from unmodified forms of the proteins. EX1006, 194-95, Fig. 2; EX1003, ¶554.

A POSA would have understood that, since the 1980s, it had been known that AAV capsid proteins undergo N-terminal truncation and acetylation *in vivo*. EX1011, 7920; EX1003, ¶553. Before the priority date, researchers had also used MS techniques to study mutant viral proteins in proteomics studies. EX1007, Abstract, 2509; EX1003, ¶¶204-05.

A POSA would further have understood that Shytuhina's intact LC-MS method had sufficiently high resolution to separate capsid protein variants, from capsid proteins that had not been subject to these modifications. EX1003, ¶557. Shytuhina states that "[b]ased on known glycoprotein acylation and expected N-linked glycans, we identified glycoprotein modifications in several major peaks by matching the observed mass to the theoretical molecular weight." EX1006, 194; EX1003, ¶556.

Satkunanathan, in combination with Shytuhina, therefore meets this additional limitation of dependent claim 21. EX1003, ¶¶551-558.

19. A POSA Would Have Had a Reasonable Expectation of Success

A POSA would have had a reasonable expectation of success in combining Satkunanathan with Shytuhina. EX1003, ¶559. The techniques required, 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 a POSA at the time and would have required nothing more than routine experimentation. *Id.*, ¶¶559, 564. Similarly, the choice of calibrant for the spectrometer would have required nothing more than routine experimentation. *Id.*, ¶564. A POSA furthermore, using nothing more than routine experimentation, would have been able to select the best column, C4 or C8, for example, to optimize the separation by RP-HPLC, and then the analysis by MS. *Id.*, ¶563.

Using nothing more than routine experimentation, a POSA would have been able to select and optimize a UPLC column for separation followed by analysis using MS, as Ansong showed. *Id.*, ¶561.

Satkunanathan successfully identified the capsid proteins of three different AAV serotypes (AAV2, AAV5, and AAV8). *Id.*, ¶566. Shytuhina successfully identified viral structural proteins using intact LC-MS, including successfully identifying several different PTMs. *Id.*, ¶560.

Intact LC-MS analysis of AAV capsid proteins would have been even more straightforward for a POSA than working with the glycoproteins Shytuhina analyzed. *Id.*, ¶¶560, 562. Glycoproteins are more variable and therefore more difficult to analyze than AAV capsid proteins by intact LC-MS. *Id.*, 562.Moreover, researchers in the field for years before the relevant date, as discussed above, had

been successfully separating reduced monoclonal antibodies and identifying PTMs. *Id.*, ¶567. Applying 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. *Id.*,¶¶565, 567.

20. Secondary Considerations Do Not Change the Conclusion of Obviousness

For evidence of "secondary considerations" to be informative of obviousness, there must be a "nexus" or link between the alleged secondary consideration and the subject matter recited in challenged claims 1-17 and 21 of the '880 patent. EX1003, ¶568. Petitioner is not aware of any secondary considerations of non-obviousness with the required nexus to these claims of the '880 patent. *Id.* For example, Petitioner is not aware of any commercial success attributable to an analytical method of monitoring an AAV preparation using the well known technique of intact LC-MS. *Id.* Similarly, Petitioner is not aware of any licenses directed specifically to the '880 patent or the subject matter recited in challenged claims 1-17, or 21. *Id.*

Finally, Petitioner is not aware of any unexpected results having a nexus to the claimed subject matter. *Id.*, ¶569. The '880 patent does not disclose unexpected properties of the claimed methods. *Id.* Intact LC-MS had been used successfully for years before the relevant date as an analytical technique to monitor proteins including viral structural proteins, for process development. *Id.* Satkunanathan

used LC-MS/MS to identify the capsid proteins of different AAV serotypes, and Shytuhina used intact LC-MS to monitor viral structural proteins, including identifying their PTMs, for process development. *Id.*

To the extent Patent Owner attempts to raise secondary considerations that have only a marginal nexus, if any, to claims 1-17 or 21 of the '880 patent, such evidence of secondary considerations should not outweigh the compelling evidence of obviousness, discussed above. *Id.*, ¶570. Thus, secondary considerations do not affect the conclusion that claims 1-17 and 21 of the '880 patent are obvious over the combination of Satkunanathan and Shytuhina. *Id.*

B. Ground 2: Claims 7 and 15 Are Obvious Over Satkunanathan, Shytuhina, and Ansong

Petitioner incorporates by reference in its entirety the discussion of Ground 1 above. EX1003, ¶571.

Dependent claims 7 and 15 are also obvious over the combination of Satkunanathan, Shytuhina, and Ansong. *Id.*, ¶¶572-586; *see also id.*, ¶¶587-97.

1. Claim 7: "The method of claim 1, wherein the liquid chromatography is ultra-performance liquid chromatography (UPLC)."

See Ground 1, claim 1. As discussed above regarding Ground 1, a POSA would have been motivated to combine Satkunanathan with Shytuhina. EX1003, ¶572. A POSA would have been further motivated to combine Ansong's use of

UPLC with the methods of Satkunanathan and Shytuhina. *Id.*, ¶573. Ansong carried out intact LC-MS using UPLC to analyze the proteome of *Salmonella typhimurium* and identify multiple different PTMs. EX1009, Abstract; EX1003, ¶573.

A POSA would have understood that UPLC is a chromatographic technique that, for example, can use sub-2 μm particles, mobile phases at high linear velocities, and instrumentation that operates at high pressure. EX1020, 1019; EX1003, ¶574. A POSA would have been aware that it was well known in the art that UPLC could offer certain benefits as compared with HPLC, including increased resolution, sensitivity and speed of the chromatographic analysis, which are desirable for a chromatographic method to quantify proteins for in-process control. EX1020, 1019; EX1003, ¶575. A POSA would have been aware of the additional advantages UPLC could offer, for example those set out in Ansong, such as the efficient separation and increased sampling of proteins in a mixture and identification of PTMs, using long columns and long gradients. EX1009, 10154; EX1003, ¶576.

A POSA would therefore have likely tested Ansong's UPLC approach, in applying Shytuhina's intact LC-MS method to the separation and identification of AAV capsid proteins. EX1003, ¶577.

Satkunanathan, in combination with Shytuhina and Ansong, therefore meets the additional limitation of dependent claim 7. EX1003, ¶¶572-578.

2. Claim 15: "The method of claim 10, wherein the liquid

chromatography is ultra-performance liquid chromatography (UPLC)."

See claim 10 of Ground 1, claim 7. Satkunanathan, in combination with Shytuhina and Ansong, therefore meets the additional limitation of dependent claim 15. EX1003, ¶¶579-586.

3. A POSA Would Have Had a Reasonable Expectation of Success

A POSA would have had a reasonable expectation of success in combining Satkunanathan with Shytuhina and Ansong to arrive at the claimed combinations recited in claims 7 and 15. EX1003, ¶¶587-596. 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. *Id.*, ¶588. Similarly, the choice of calibrant for the spectrometer would have required nothing more than routine experimentation. *Id.*

Shytuhina successfully separated glycoproteins by liquid chromatography, specifically RP-HPLC, and analyzed them by intact LC-MS, successfully identifying several different post-translational modifications. *Id.*, ¶589.

Intact LC-MS analysis of AAV capsid proteins would have been even more straightforward for a POSA than working with the glycoproteins Shytuhina

analyzed. *Id.*, ¶590. Glycoproteins are more variable and therefore more difficult to analyze than AAV capsid proteins by intact LC-MS. *Id.*

A POSA furthermore, using nothing more than routine experimentation, would have been able to select the best column, C4 or C8, for example, to optimize the separation by RP-HPLC, and then the analysis by MS. *Id.*, ¶591.

Using nothing more than routine experimentation, a POSA would have been able to select and optimize a UPLC column for separation followed by analysis using MS, as Ansong showed. *Id.*, ¶592.

A POSA would have been able with only routine experimentation to select a calibrant and carry out assisted calibration of a mass spectrometer, as Byeon disclosed. *Id.*, ¶593.

Moreover, a POSA would have had a reasonable chance of success in separating AAV capsid proteins and identifying them, including PTMs, by intact LC-MS. *Id.*, ¶594.

Satkunanathan successfully identified and distinguished three different AAV serotypes, and Shytuhina successfully separated viral structural glycoproteins with multiple different PTMs. *Id.*, ¶595.

Moreover, researchers in the field for years before the relevant date, as discussed above, had been successfully separating reduced monoclonal antibodies and identifying PTMs. *Id.*, ¶596. Applying 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. *Id.*

4. Secondary Considerations Do Not Change the Conclusion of Obviousness

For the reasons discussed above regarding Ground 1, secondary considerations do not alter the conclusion that claims 7 and 15 of the ’880 patent would have been obvious over the combination of Satkunanathan, Shytuhina, and Ansong. EX1003, ¶597.

C. Ground 3: Claims 8, 9, 16, and 17 Are Obvious Over the Combination of Satkunanathan, Shytuhina, and Byeon

Petitioner incorporates by reference in their entirety the discussions of Grounds 1 and 2 above. EX1003, ¶598.

Dependent claims 8, 9, 16, and 17 are also obvious over the combination of Satkunanathan, Shytuhina, and Byeon. *Id.*, ¶¶599-624; *see also id.*, ¶¶625-34.

1. Claim 8: “The method of claim 1, wherein the mass spectrometry comprises assisted calibration.”

See claim 1 of Ground 1. Satkunanathan and Shytuhina, in combination with Byeon, disclose the additional limitation of dependent claim 8. EX1003, ¶599. As discussed above regarding Ground 1, a POSA would have been motivated to combine Satkunanathan with Shytuhina. *Id.*

As also discussed above, a POSA at the time would have understood that LC-MS systems require calibration. EX1014, 497; EX1008; EX1003, ¶600. A POSA

would further have understood that the term "assisted calibration" in the patent refers to "using software to correlate a peak and/or position of a known standard (e.g., a calibrant) to a specific mass to charge (m/z) ratio." EX1001, 21:47-56; EX1003, ¶601.

A POSA at the relevant time would have been aware that calibration could be carried out using commonly available software, i.e., via assisted calibration. EX1014, 497; EX1003, ¶602.

A POSA would have been motivated to use Byeon's method of assisted calibration to calibrate the mass spectrometer because such assisted calibration was an efficient and straightforward method of calibration. EX1003, ¶603. In particular, a POSA would have been motivated to use Byeon's method of external, assisted calibration to avoid complications resulting from adding a calibrant to the sample being analyzed, which would have required ensuring compatibility between the sample and the internal calibrant. *Id.*

The combination of Satkunanathan and Shytuhina with Byeon therefore discloses the additional limitation of dependent claim 8. *Id.*, ¶¶599-604.

2. Claim 9: "The method of claim 8, wherein sodium iodide is used as a calibrant."

See claim 8. Based on the disclosures of Byeon and the general knowledge in the field, a POSA would have understood NaI to be a desirable and commonly

available calibrant, including for assisted external calibration using software. EX1008, 665; EX1014, 497; EX1003, ¶610.

Satkunanathan, in combination with Shytuhina and Byeon, therefore meets the additional limitation of dependent claim 9. EX1003, ¶¶605-611.

3. Claim 16: "The method of claim 10, wherein the mass spectrometry comprises assisted calibration."

See claim 10 of Ground 1, claim 8. Satkunanathan, in combination with Shytuhina and Byeon, therefore meets the additional limitation of dependent claim 16. EX1003, ¶¶612-617.

4. Claim 17: "The method of claim 16, wherein sodium iodide is used as a calibrant."

See claims 9, 16. Satkunanathan, in combination with Shytuhina and Byeon, therefore meets the additional limitation of dependent claim 17. EX1003, ¶¶618-624.

5. A POSA Would Have Had a Reasonable Expectation of Success

A POSA would have had a reasonable expectation of success in combining Satkunanathan with Shytuhina and Byeon to arrive at the claimed combinations recited in claims 8, 9, 16, and 17. EX1003, ¶625. 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. *Id.* Similarly, the choice of calibrant for the spectrometer would have required nothing more than routine experimentation. *Id.*

Shytuhina successfully separated glycoproteins by liquid chromatography, specifically RP-HPLC, and analyzed them by intact LC-MS, successfully identifying several different post-translational modifications. *Id.*, ¶626.

Using nothing more than routine experimentation, a POSA would have been able to select and optimize a UPLC column for separation followed by analysis using MS, as Ansong showed. *Id.*, ¶627.

Intact LC-MS analysis of AAV capsid proteins would have been even more straightforward for a POSA than working with the glycoproteins Shytuhina analyzed. *Id.*, ¶628. Glycoproteins are more variable and therefore more difficult to analyze than AAV capsid proteins by intact LC-MS. *Id.*

A POSA furthermore, using nothing more than routine experimentation, would have been able to select the best column, C4 or C8, for example, to optimize the separation by RP-HPLC, and then the analysis by MS. *Id.*, ¶629.

A POSA would have been able with only routine experimentation to select a calibrant and carry out assisted calibration of a mass spectrometer, as Byeon disclosed. *Id.*, ¶630.

Moreover, a POSA would have had a reasonable chance of success in separating AAV capsid proteins and identifying them, including PTMs, by intact LC-MS. *Id.*, ¶631.

Satkunanathan successfully identified and distinguished three different AAV serotypes, and Shytuhina successfully separated viral structural glycoproteins with multiple different PTMs. *Id.*, ¶632.

Moreover, researchers in the field for years before the relevant date, as discussed above, had been successfully separating reduced monoclonal antibodies and identifying PTMs. *Id.*, ¶633. Applying 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. *Id.*

6. Secondary Considerations Do Not Change the Conclusion of Obviousness

For the reasons set out regarding Ground 1, secondary considerations do not alter the conclusion that claims 8, 9, 16, and 17 of the ’880 patent are obvious over the combination of Satkunanathan, Shytuhina, and Byeon. EX1003, ¶634.

D. Ground 4: Claims 18-20 Are Obvious Over the Combination of Shytuhina and Zabrouskov

Grounds 1-3 are incorporated herein in their entirety. EX1003, ¶635.

As discussed above, Shytuhina used intact LC-MS to monitor the purity of structural viral proteins for process development. EX1006, Abstract; EX1003, ¶636.

Shytuhina identified various different PTMs, including different N-glycosylations and acylations. EX1006, 194; EX1003, ¶636.

Shytuhina also states that they likely observed deamidation of one of the viral structural proteins they studied, the E2 protein. EX1006, 196; EX1003, ¶637. Specifically, in carrying out RP-HPLC, they observed an E2 degradant peak that eluted slightly earlier than the regular E2, suggesting that the degradant was more hydrophilic. EX1006, 194, Figure 1A, 196; EX1003, ¶637.

Regarding this degradant peak, Shytuhina states: "Proteins are prone to deamidation at high pH and deamidation introduces a net increase in surface charge. EX1003, ¶638. Thus, we speculate at pH 9.0 E2 undergoes deamidation and that this chemical modification affects CHIKV VLP antigenicity." EX1006, 196; EX1003, ¶638.

A POSA would have understood that the intact LC-MS method of Shytuhina might not have provided sufficient resolution to identify all deamidations. EX1003, ¶639. A POSA would therefore have been motivated to combine Shytuhina's intact LC-MS method with Zabrouskov's intact MS/MS method, which had sufficient resolution to identify multiple different sites of deamidation on RNase A. EX1038, Abstract; EX1003, ¶639.

Zabrouskov calculated percentages of deamidated products in the various reactions. EX1038, 990; EX1003, ¶640. For example, Zabrouskov reported that,

for the monodeamidated fraction, Asn67 was the first residue to deamidate and the deamidation of this reside was nearly complete in 1 hour (0:1:2 deamidations at a 5:43:52 ratio), with <10% monodeamidation at other sites. EX1038, 990; EX1003, ¶640.

A POSA would further have understood that the structural proteins of Chikungunya virus studied in Shytuhina are relatively small in molecular weight: the capsid protein is about 33 kDa, while the E1 and E2 proteins are about 55 kDa and 53 kDa, respectively. EX1006, 194, Figure 1B; EX1003, ¶641.

A POSA would have understood that detecting deamidation for proteins of larger molecular weight (the RNase A protein studied in Zabrouskov is about 14 kDa) might require certain adjustments to the intact MS/MS method, which had been well documented and disclosed in the art about a decade before the relevant date. EX1026; EX1003, ¶642.

A POSA would have understood that for the relatively small Chikungunya viral proteins studied by Shytuhina, few if any adjustments might have been necessary. EX1003, ¶643.

To the extent any such modifications were necessary for these relatively small viral proteins, a POSA would have been well aware of such modifications and how to implement them by the relevant date. EX1003, ¶644. A POSA would have understood that certain pre-folding dissociation methods disclosed in Han involving,

for example, additional applied voltages, would have been compatible with Zabrouskov's MS/MS method. EX1026, 110-11; EX1003, ¶645.

A POSA would therefore have been motivated to combine Shytuhina's intact LC-MS methods for monitoring viral protein purity for process development with the MS/MS method of Zabrouskov for detecting deamidation, in the context of well known modifications to that protocol for slightly larger proteins, to the extent any such modifications were necessary. EX1003, ¶645.

1. Claim 18: "A method of determining the extent of deamidation of viral proteins (VPs) in a preparation of viral particles, the method comprising"

Zabrouskov calculated percentages of deamidated products in the various reactions. EX1038, 990. For example, Zabrouskov reported that, for the monodeamidated fraction, Asn67 was the first residue to deamidate and the deamidation of this reside was nearly complete in 1 hour (0:1:2 deamidations at a 5:43:52 ratio), with <10% monodeamidation at other sites. EX1038, 990; EX1003, ¶649.

The combination of Shytuhina and Satkunanathan therefore discloses this limitation. EX1003, ¶¶646-652.

(a) "a) denaturing the viral particles,"

Shytuhina teaches carrying out two denaturing steps – treatment with Zwittergent and RP-HPLC – before subjecting the denatured viral particles to LC-MS intact protein analysis. EX1006, 193-94; EX1003, ¶654.

For the first step, Shytuhina teaches incubating the samples with 5% Zwittergent 3-12 detergent. EX1006, 193-94; EX1003, ¶655. Shytuhina states that pre-treatment with Zwittergent improved recovery, and that "Zwittergent 3-12 detergent is believed to solubilize and stabilize the glycoproteins [viral structural proteins] and prevent non-specific binding during chromatography." EX1006, 194; EX1003, ¶655. A POSA at the time would have understood that "solubilizing" glycoproteins involves denaturing them. EX1003, ¶655.

For the second step, Shytuhina carried out an RP-HPLC separation, before subjecting the denatured samples to intact LC-MS, which then involved another RP-HPLC column coupled to the mass spectrometer. EX1006, 193; EX1003, ¶656. Shytuhina states that for the initial RP-HPLC separation (before LC-MS), they used TFA in the mobile phase on the column, and that "TFA appeared to help disassemble the VLPs and facilitate the binding of proteins to the stationary phase." EX1006, 194; EX1003, ¶656.

A POSA would have further understood that intact MS/MS methods to identify deamidated forms involved denaturing the proteins before carrying out the

intact MS/MS analysis. EX1038, 2-3 (disclosing reduction of protein samples using DTT before intact LC-MS/MS); EX1003, ¶657.

Shytuhina, in combination with Zabrouskov, therefore meets this limitation. EX1003, ¶¶653-658.

(b) "b) subjecting the denatured viral particles to liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) intact protein analysis, and,"

See claim 18(a). EX1003, ¶660. Shytuhina discloses that after the initial RP-HPLC, which denatures the viral particles, the denatured viral particles are then separated and analyzed by intact LC-MS using a Waters Acquity LC system coupled to a Synapt G2 mass spectrometer. *Id.*, ¶661. The denatured viral particles are therefore subjected to LC-MS intact protein analysis. *Id.*, ¶662.

Shytuhina identified various different PTMs, including different N-glycosylations and acylations. EX1006, 194; EX1003, ¶663. Shytuhina also states that they likely observed deamidation of one of the viral structural proteins they studied, the E2 protein. EX1006, 196; EX1003, ¶663.

Zabrouskov used intact MS/MS to identify five stepwise deamidation sites on Ribonuclease A. EX1038, Abstract, 1-2; EX1003, ¶664. As Zabrouskov explains, while bottom-up digestion would not have been suitable to identify deamidated forms of proteins, particularly those in protein complexes such as viral structural

proteins, top-down (intact) MS/MS successfully separated multiple deamidated forms of Ribonuclease A. EX1003, ¶664.

Given Shytuhina's detection of likely deamidation in E2, a POSA would have been motivated to apply Zabrouskov's intact MS/MS method to confirm and identify deamidated forms of the viral proteins. EX1003, ¶665.

Shytuhina, in combination with Zabrouskov, therefore meets this limitation. EX1003, ¶¶659-666.

(c) "c) determining the percentage of deamidated VPs in the preparation,"

Zabrouskov used intact MS/MS to identify five stepwise deamidation sites on Ribonuclease A. EX1038, Abstract, 1-2; EX1003, ¶669. A POSA would have understood that the intact LC-MS method of Shytuhina combined with the intact MS/MS method of Zabrouskov could have been used to identify deamidation of viral proteins in a preparation of viral particles. EX1003, ¶¶669-670. In particular, a POSA would have known that intact LC-MS/MS would have been an effective technique to identify deamidated forms of the AAV capsid proteins. *Id.*, ¶669.

Zabrouskov analyzed the deamidation products after running a deamidation reaction on RNase A for one hour. EX1038, 5; EX1003, ¶¶670-671. They found that a particular asparagine (Asn67) was the first residue to deamidate, and that the deamidation was nearly complete in one hour, with the following ratio of products

observed: 0:1:2 deamidations = 5:43:52, with <10% monodeamidation at other sites. EX1038, 5; EX1003, ¶671.

A POSA would therefore have understood how to apply intact LC-MS/MS to obtain fractions of deamidated viral proteins in a mixture. EX1003, ¶672. A POSA would have been motivated to apply Zabrouskov's intact MS/MS to determine percentages of different deamidation forms and achieve complete characterization of any deamidation degradation products present in the mixture of viral proteins. *Id.*

Shytuhina, in combination with Zabrouskov, therefore meets this limitation. EX1003, ¶¶667-673.

(d) "wherein the method is performed in the absence of a gel separation step."

Both Shytuhina and Zabrouskov carry out their intact LC-MS and intact MS/MS methods in the absence of a gel separation step. EX1003, ¶679.

Shytuhina, in combination with Zabrouskov, therefore meets this limitation. *Id.*, ¶¶674-680.

2. Claim 19: "The method of claim 18, wherein the liquid chromatography is reverse phase chromatography, wherein the reverse phase chromatography is performed with a C4 column."

See claim 18. Shytuhina discloses that the specific RP-HPLC column they used was a C4 column. EX1006, 193; EX1003, ¶683. Although Shytuhina does not expressly disclose the column used for the RP-HPLC coupled to the mass

spectrometer for the intact LC-MS analysis, a POSA would have understood it was likely that they used the same C4 column that had successfully separated E1 and E2 in the previous RP-HPLC step. EX1003, ¶683.

In any event, a POSA at the time would have understood that it was generally necessary to test a few different columns to optimize the desired separation. EX1037, 22-23; EX1003, ¶684.

As discussed above, Yuan discloses a RP-HPLC method to detect a capsid protein of HPV, the L1 protein. EX1037, Abstract; EX1003, ¶685. Yuan tested both C4 and C8 columns, finding that either column could be used with equal efficiency to purify the L1 capsid protein. EX1037, 23; EX1003, ¶685.

Satkunanathan used a reversed phase C18 column for LC-MS/MS. EX1005, 931; EX1003, ¶686. Mahoney used a C8 column for RP-HPLC of large peptides. EX1029, 11199-200, Table I, Table II; EX1003, ¶686.

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. EX1003, ¶687. 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. *Id.*

Shytuhina, in combination with Zabrouskov, therefore meets this limitation. EX1003, ¶¶681-688.

3. Claim 20: "The method of claim 18, wherein the liquid chromatography is reverse phase chromatography, wherein the reverse phase chromatography is performed with a C8 column."

See claims 18, 19. Shytuhina, in combination with Zabrouskov, therefore meets this limitation. EX1003, ¶¶689-696.

4. A POSA Would Have Had a Reasonable Expectation of Success

A POSA would have had a reasonable expectation of success in combining Shytuhina with Zabrouskov. EX1003, ¶697. The techniques required, namely, RP-HPLC, intact LC-MS, and intact MS/MS, to identify and analyze deamidations in proteins, and optimization of liquid chromatography separations were well known to people of skill in the art at the time and would have required nothing more than routine experimentation. *Id.*

Shytuhina successfully separated viral structural glycoproteins by liquid chromatography, specifically RP-HPLC, and analyzed them by intact LC-MS, successfully identifying several different PTMs. *Id.*, ¶698.

A POSA furthermore, using nothing more than routine experimentation, would have been able to select the best column, C4 or C8, for example, to optimize the separation by RP-HPLC, and then the analysis by MS. *Id.*, ¶699.

Zabrouskov successfully identified multiple deamidations in RNase A and quantitated different deamidated products using intact MS/MS. *Id.*, ¶700.

A POSA would have had a reasonable chance of success in applying Zabrouskov's intact MS/MS to analyze deamidation of small viral proteins such as those analyzed in Shytuhina, ranging in molecular mass from about 35 kDa to about 55 kDa. *Id.*, ¶701.

To the extent that a POSA needed to optimize intact MS/MS for viral proteins of this size to identify deamidations, a POSA would have had a reasonable expectation of success in applying the techniques disclosed by Han about a decade before the relevant date and that Han states are suitable even for proteins greater than 200 kDa in size. *Id.*, ¶702.

5. Secondary Considerations Do Not Change the Conclusion of Obviousness

For evidence of "secondary considerations" to be informative of obviousness, there must be a "nexus" or link between the alleged secondary consideration and the subject matter recited in challenged claims 18-20 of the '880 patent. EX1003, ¶703. Petitioner is not aware of any secondary considerations of non-obviousness with the required nexus to these claims of the '880 patent. *Id.* For example, Petitioner is not aware of any commercial success attributable to an analytical method of using intact LC-MS/MS to analyze deamidation of viral proteins. *Id.* Similarly, Petitioner is not aware of any licenses directed specifically to the '880 patent or the subject matter recited in challenged claims 18-20. *Id.*

Finally, Petitioner is not aware of any unexpected results having a nexus to the claimed subject matter. *Id.*, ¶704. The '880 patent does not disclose unexpected properties of the claimed methods. *Id.* Intact LC-MS and intact MS/MS had been used successfully for years before the relevant date as analytical techniques to monitor proteins, including monoclonal antibodies, and viral structural proteins, for process development. *Id.* Combining intact LC-MS with intact MS/MS for the analytical technique LC-MS/MS would not give rise to any unexpected results. *Id.*

Zabrouskov had disclosed the use of intact MS/MS specifically to analyze deamidation of proteins about a decade before the relevant date. *Id.*, ¶705. Around the same time, Han had disclosed optimization of intact MS/MS to analyze deamidation of larger proteins. *Id.*

To the extent Patent Owner attempts to raise secondary considerations that have only a marginal nexus, if any, to claims 18-20 of the '880 patent, such evidence of secondary considerations should not outweigh the compelling evidence of obviousness, discussed above. *Id.*, ¶706. Thus, secondary considerations do not alter the conclusion that claims 18-20 of the '880 patent are obvious over the combination of Shytuhina and Zabrouskov. *Id.*

E. Ground 5: Claim 20 Is Obvious Over the Combination of Shytuhina, Zabrouskov, and Yuan

A POSA would have been motivated to combine Shytuhina's method of using intact LC-MS to monitor viral proteins for PTMs during production of viral-based therapeutics with Zabrouskov's use of intact MS/MS to detect deamidation in proteins to optimize process development for viral vectors. EX1003, ¶707.

1. Claim 20: "The method of claim 18, wherein the liquid chromatography is reverse phase chromatography, wherein the reverse phase chromatography is performed with a C8 column."

See Ground 1, claim 18. A POSA would have understood the need for routine optimization of column chromatography conditions, such as the column, column length, solvent, and solvent gradient for example, in carrying out liquid chromatography for LC-MS methods. EX1003, ¶708.

Shytuhina's method involves separation of the viral proteins by RP-HPLC before analysis by mass spectrometry. EX1006, 193-195. EX1003, ¶709. Shytuhina discloses that the specific RP-HPLC column they used was a C4 column. EX1006, 193; EX1003, ¶710. Although Shytuhina does not expressly disclose the column used for the RP-HPLC coupled to the mass spectrometer for the intact LC-MS analysis, a POSA would have understood it was likely that they used the same C4 column that had successfully separated E1 and E2 in the previous RP-HPLC step. EX1003, ¶710.

In any event, a POSA at the time would have understood that it was generally necessary to test a few different columns to optimize the desired separation and would therefore have been motivated to combine Yuan with Shytuhina and Zabrouskov. EX1037, 22-23; EX1003, ¶711.

Yuan discloses a RP-HPLC method to detect a capsid protein of HPV, the L1 protein. EX1037, Abstract; EX1003, ¶712. Yuan tested both C4 and C8 columns, finding that either column could be used with equal efficiency to purify the L1 capsid protein. EX1037, 23; EX1003, ¶712.

Satkunanathan used a reversed phase C18 column for LC-MS/MS. EX1005, 931; EX1003, ¶713. Mahoney used a C8 column for RP-HPLC of large peptides. EX1029, 11199-200, Table I, Table II; EX1003, ¶713.

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. EX1003, ¶714. 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. EX1003, ¶714.

Shytuhina, in combination with Zabrouskov and Yuan, therefore meets the additional limitation of dependent claim 20. EX1003, ¶707-715.

2. A POSA Would Have Had a Reasonable Expectation of Success

A POSA would have had a reasonable expectation of success in combining Shytuhina with Zabrouskov and Yuan. EX1003, ¶716. The techniques required, namely, RP-HPLC, intact LC-MS, and intact MS/MS, to identify and analyze deamidations in proteins, and optimization of liquid chromatography separations were well known to people of skill in the art at the time and would have required nothing more than routine experimentation. *Id.*

Shytuhina successfully separated viral structural glycoproteins by liquid chromatography, specifically RP-HPLC, and analyzed them by intact LC-MS, successfully identifying several different PTMs. *Id.*, ¶717.

A POSA furthermore, using nothing more than routine experimentation, would have been able to select the best column, C4 or C8, for example, to optimize the separation by RP-HPLC, and then the analysis by MS, as disclosed by Yuan. *Id.*, ¶718

Zabrouskov successfully identified multiple deamidations in RNase A and quantitated different deamidated products using intact MS/MS. *Id.*, ¶719.

A POSA would have had a reasonable chance of success in applying Zabrouskov's intact MS/MS to analyze deamidation of small viral proteins such as those analyzed in Shytuhina, ranging in molecular mass from about 35 kDa to about 55 kDa. *Id.*, ¶720.

To the extent that a POSA needed to optimize intact MS/MS for viral proteins of this size to identify deamidations, a POSA would have had a reasonable expectation of success in applying the techniques disclosed by Han about a decade before the relevant date and that Han states are suitable even for proteins greater than 200 kDa in size. *Id.*, ¶721.

3. Secondary Considerations Do Not Change the Conclusion of Obviousness

Petitioner incorporates by reference in its entirety the discussion of the lack of any secondary considerations from Ground 4. *Id.*, ¶722.

X. CONCLUSION

Sarepta respectfully requests institution of IPR for claims 1-21 of the ’880 patent based on the grounds specified in this Petition.

Respectfully submitted,

December 2, 2025

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WORD COUNT CERTIFICATE OF COMPLIANCE

Pursuant to 37 C.F.R. §42.24(d), Petitioner hereby certifies, in accordance with and reliance on the word count provided by the word-processing system used to prepare this Petition, that the number of words in this paper is 13,580. Pursuant to 37 C.F.R. §42.24(d), this word count excludes the table of contents, table of authorities, mandatory notices under §42.8, certificate of service, certificate of word count, appendix of exhibits, and any claim listing.

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CERTIFICATE OF SERVICE

Pursuant to 37 C.F.R. §42.6 (e) and 37 C.F.R. §42.105, I hereby certify that on December 2, 2025, I caused the foregoing Petition for *Inter Partes* Review, Power of Attorney, and Exhibits 1001–1040 to be served on Patent Owner by depositing them for shipment with Federal Express to the correspondence address of record listed on the Patent Center:

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