

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

PREOMICS GMBH AND BIOGNOSYS AG.,
Petitioners,

v.

THE BRIGHAM AND WOMEN'S HOSPITAL, INC.,
Patent Owner.

Case No. IPR2024-01473
Patent No. 11,435,360

DECLARATION OF DR. MICHAEL J. CIMA, PH.D.

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I, Dr. Michael J. Cima, Ph.D., declare as follows:

I. QUALIFICATIONS

1. I am over the age of eighteen (18) and otherwise competent to make this Declaration.

2. I have been retained through counsel for Patent Owner in the above-captioned *inter partes* review (IPR). I am being compensated for my time in connection with this IPR at my standard consulting rate, which is \$800.00 per hour. My compensation does not depend in any way on the outcome of the IPR. I have been asked to evaluate and provide my opinions regarding the testimony of Dr. Jessica Vargas (EX1003). In so doing I have considered the state of the art before December 16, 2016, for methods of assaying biological samples in the field of proteomics.

3. I am the David H. Koch Professor of Engineering in the Department of Materials Science and Engineering at Massachusetts Institute of Technology with expertise in proteomic studies and protein analysis. Additionally, I serve as an Investigator at the Koch Institute for Integrative Cancer Research at MIT, the Director of the Convergence Products Research Laboratory at MIT, and the Faculty Director of the Lemelson-MIT Program. I served as Associate Dean for Innovation at the School of Engineering at MIT and Co-Director of the MIT Innovation Initiative for five years.

4. I obtained a Ph.D. in Chemical Engineering from the University of California at Berkeley in 1986. Thereafter, I joined the Massachusetts Institute of Technology (“MIT”) faculty in 1986 as an Assistant Professor. I was promoted to a full Professor in 1995 and was elected to the National Academy of Engineering in 2011 and to the National Academy of Inventors in 2016.

5. I have published extensively in the scientific field, having authored or co-authored over 300 peer reviewed scientific publications, including on protein enrichment and analysis. I am also listed as an inventor on at least fifty U.S. patents. I am a recognized expert in the field of medical devices and materials processing and am involved in materials and engineered systems for the improvement in human health. My research includes advanced forming technology for macro- and micro-devices, colloid science, MEMs and other micro components for medical devices that are used for drug delivery and diagnostics, and high-throughput development methods for formulations of materials and pharmaceutical formulations.

6. I have used proteomic studies and protein analysis since well before December 16, 2016, which I understand is the priority date of the patent. I am, for example, the lead investigator on a platform to micro-invasively sample interstitial fluid (ISF) in the brain. These very small samples are then processed through a LC/MS/MS system to reveal the proteomic profile from different parts of the brain.

In the early 2000s I was a principal scientist at a company called Transform Pharmaceuticals. Many of our bioanalytical methods for drug development were based on LC/MS/MS. I therefore consider myself an expert on the use of proteomic analysis.

7. Throughout the remainder of this Declaration, I will refer to the field of proteomics, and specifically assaying biological samples across a wide dynamic range, as the relevant field or the relevant art. In formulating my opinions, I have relied upon my training, knowledge, and experience in the relevant art. A copy of my current curriculum vitae is attached to this declaration as EX2024, and it provides a description of my academic and employment history.

8. By 2016, I had an advanced degree (Ph.D. in Chemical Engineering), experience in the field of proteomics, experience with methods for detecting proteins in biological samples (including with mass spectrometry assays), and knowledge of the analytical methods employed to separate, detect, and analyze proteins in biological samples. I also had at least three years of experience in the fields of proteomics and protein analysis, including protein analysis with mass spectrometry. I understand that Petitioners in this matter defined the level of skill of the person of ordinary skill in the art (“POSA” or “skilled artisan”) as someone with at least a bachelor’s degree in chemistry, biochemistry, bioengineering, or a related field, and three years’ experience in the field of proteomics, protein

analysis, or mass spectrometry. Petition, 11; EX1003 (Vargas Decl.), ¶¶24-26. I have also been informed that the PTAB Board in this case further stated “a POSA would have an understanding of both proteins and the analytical techniques, e.g., mass spectrometry, that can be used to characterize them.” I also understand the Board stated that while Petitioners’ definition of a POSA only requires a “bachelor’s degree,” the references relied upon in the petition materials and the educational background of the inventors here indicate “a graduate degree is more typical for a POSA.” I agree. As an expert in the field of proteomics and protein analysis, I am qualified to provide an opinion as to what such a POSA would have understood, known, or concluded as of 2016. Indeed, since the 1990’s, I have accumulated significant training and experience in the field of proteomics and protein analysis.

II. MATERIALS REVIEWED AND SUMMARY OF OPINIONS

9. I have reviewed the ’360 patent as well as the petition, the declaration by Dr. Vargas (EX1003) and references cited therein, and references cited and discussed in this declaration. While I considered the materials as a whole, my review and discussion focused on claims 1-4, 6-7, 17 and 22-25 (the challenged claims) of the ’360 patent, which are the claims addressed in the petition.

10. In my opinion, none of the references (Cai, Hu, or Zaccaria) that Dr. Vargas and the petition rely on would be viewed as disclosing or rendering obvious

the '360 patent claims that are challenged here. I provide more detailed discussion below.

11. For example, the petition materials do not address what Dr. Vargas agreed is an express definition of the claimed sample. The Cai reference addresses different lysates obtained from different HeLa cell populations. HeLa cells are immortalized cells obtained commercially and cultured in a laboratory; they bear no resemblance to a sample that would be obtained from an individual. And none of the different HeLa cell populations are contacted by a plurality of different particle types and assayed by mass spectrometry.

12. In addition, none of the cited references disclose detecting proteins found in the sample at concentrations across an “at least 6 orders of magnitude” dynamic range as required by the challenged claims. For the Cai reference (Grounds 2 and 3), the petition relies on a comment in Cai regarding observing “emPAI” values spread over eight orders of magnitude. Cai itself describes its emPAI values as a “semi-quantitative” indication of **amount** of protein on particles, not concentration. And identified emPAI values relate to proteins in a post-enrichment mixture; they certainly do not identify concentration in the original HeLa cell population or any lysate obtained.

13. The obviousness arguments for Hu (Ground 1) and Zaccaria (Ground 4) also fail for similar reasons. For each of these grounds, the petition and Dr.

Vargas rely on a few selected entries from the raw data tables in supplemental materials from Hu and Zaccaria, arguing these are a mix what they call “low and high abundance” proteins. Dr. Vargas and the petition concede that neither Hu nor Zaccaria provide protein concentrations but argue what they contend would be “normally present” for the identified proteins. There are many problems with the argument. There is no real discussion or analysis of the identified entries, and Dr. Vargas and the petition fail to establish the references detected identified proteins in the first place. Also, protein concentrations vary considerably among individual patients and it is improper to cite to different papers for speculation about what concentrations may or may not exist in different studies like Hu and Zaccaria. It is my opinion that each of Hu, Zaccaria, and Cai at least fails to demonstrate prior art disclosure of assaying at the claimed 6 orders of magnitude dynamic range. *See* EX1001 (’360 Patent), Claim 1(c).

14. As I detail further below, each of the petition and Dr. Vargas’ challenges fail for a multitude of additional reasons.

III. TECHNOLOGICAL BACKGROUND

A. Primer on Peptides

15. Proteins in the human body are polymers (polypeptides) composed of amino acids. The body uses twenty natural amino acids, shown in the table below. Each one has a common name, a three letter code, and a single letter code, as

shown below. Each amino acid has an amine group that is alpha (one carbon removed) from a carboxylic acid. The amino acids in a protein are linked through an amide bond (called a peptide bond).

Symbol	Name	Mol. form.	M_r	$T_m / ^\circ\text{C}$	$\text{p}K_a$	$\text{p}K_b$	$\text{p}K_s$	pI	S
Ala	Alanine	$\text{C}_3\text{H}_7\text{NO}_2$	89.09	297	2.34	9.69		6.00	167
Arg	Arginine	$\text{C}_6\text{H}_{14}\text{N}_4\text{O}_2$	174.20	238	2.17	9.04	12.48	10.76	181
Asn	Asparagine	$\text{C}_4\text{H}_7\text{N}_2\text{O}_3$	132.12	236	2.02	8.80		5.41	25
Asp	Aspartic acid	$\text{C}_4\text{H}_7\text{NO}_4$	133.10	270	1.88	9.60	3.65	2.77	5
Cys	Cysteine	$\text{C}_3\text{H}_7\text{NO}_2\text{S}$	121.15	178	1.96	10.28	8.18	5.07	
Gln	Glutamine	$\text{C}_5\text{H}_{10}\text{N}_2\text{O}_3$	146.15	185	2.17	9.13		5.65	42
Glu	Glutamic acid	$\text{C}_5\text{H}_9\text{NO}_4$	147.13	249	2.19	9.67	4.25	3.22	
Gly	Glycine	$\text{C}_2\text{H}_5\text{O}_2$	75.07	290	2.34	9.60		5.97	251
His	Histidine	$\text{C}_6\text{H}_9\text{N}_3\text{O}_2$	155.16	277	1.82	9.17	6.00	7.59	43
Ile	Isoleucine	$\text{C}_6\text{H}_{13}\text{NO}_2$	131.17	284	2.36	9.60		6.02	34
Leu	Leucine	$\text{C}_6\text{H}_{13}\text{NO}_2$	131.17	337	2.36	9.60		5.98	23
Lys	Lysine	$\text{C}_6\text{H}_{14}\text{N}_2\text{O}_2$	146.19	224—225	2.18	8.95	10.53	9.74	6
Met	Methionine	$\text{C}_5\text{H}_{11}\text{NO}_2\text{S}$	149.21	283	2.28	9.21		5.74	56
Phe	Phenylalanine	$\text{C}_9\text{H}_9\text{NO}_2$	165.19	284	1.83	9.13		5.48	29
Pro	Proline	$\text{C}_5\text{H}_9\text{NO}_2$	115.13	222	1.99	10.60		6.30	1622
Ser	Serine	$\text{C}_3\text{H}_7\text{NO}_3$	105.09	228	2.21	9.15		5.68	422
Thr	Threonine	$\text{C}_4\text{H}_9\text{NO}_3$	119.12	253	2.09	9.10		5.60	97
Trp	Tryptophan	$\text{C}_{11}\text{H}_{12}\text{N}_2\text{O}_2$	204.23	282	2.83	9.39		5.89	12
Tyr	Tyrosine	$\text{C}_9\text{H}_{11}\text{NO}_3$	181.19	344	2.20	9.11	10.07	5.66	0.5
Val	Valine	$\text{C}_5\text{H}_{11}\text{NO}_2$	117.15	292-295	2.32	9.62		5.96	58

EX2029, 7-1. As I will discuss in detail below, amino acids can be difficult to differentiate by weight alone, as many of them have nearly identical weights (e.g., leucine and isoleucine both have molecular weights of 131.17).

16. The human genome encodes roughly 20,000 canonical human protein sequences. Each such sequence is subject to post translational modification (PTM) so the number of all proteins found in the human body is thought to be on the order of 100,000 to 1,500,000 (considering all types of PTM).

B. Primer on Proteomics

17. Proteomics attempts to determine what proteins are present in biological samples. One approach is based on mass spectrometry (MS). As the

name suggests, mass spectrometers determine the mass of the various chemical constituents. But mass by itself is a rather crude characterization of proteins. Imagine trying to identify laptop models by weight; some laptops might have extra memory or a lighter screen, and as a consequence two laptops with the same weight are not necessarily the same model. One approach to identify such a laptop is to weigh the various parts of each laptop: each laptop is broken into individual subsystems; each subsystem is weighed. The ability to distinguish one model from another is improved, though still very imperfect (i.e., the weight of a subsystem often will not unambiguously differentiate from other subsystems). A similar approach is taken in mass spectrometry.

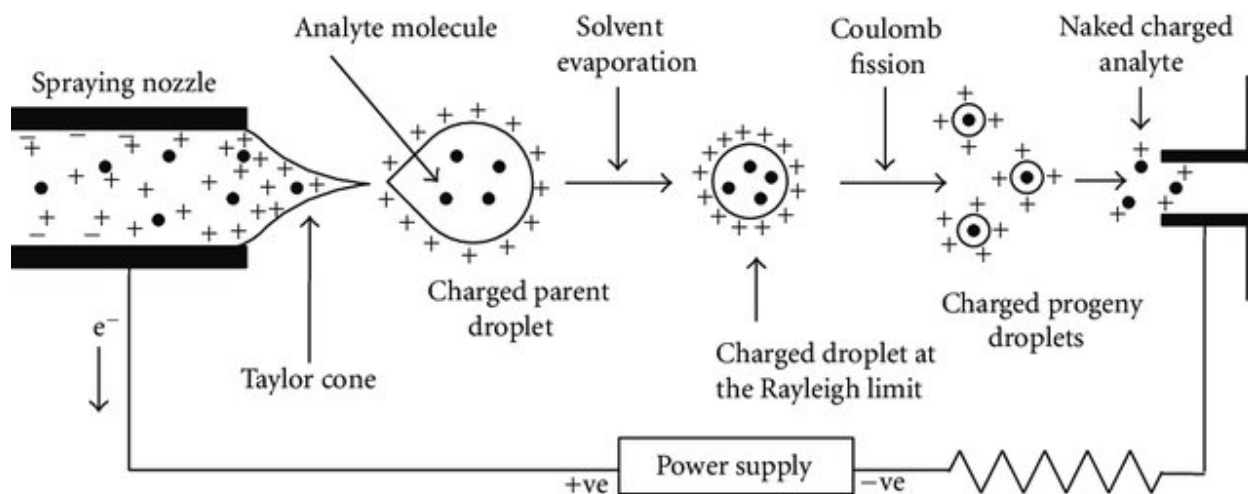
18. Mass spectrometry separates molecules on the basis of mass to charge ratio (m/z). Large molecules not only have larger mass but also adopt much higher and variable charge in the ionization process discussed below. A single large protein molecule therefore appears as many peaks in the mass spectrum and is often outside the sensitive range of the detector. That variability is superimposed on the isotope variability that naturally occurs in the MS measurement, also discussed below. The result is a mass spectrum that is very difficult to interpret. The identification of a digested peptide in an aliquot is thus a useful but imperfect way to try to identify the presence of a protein.

19. Comparison of the identified digested peptide with databases for proteins can be a necessary condition for the presence of a protein but it is not a sufficient condition because different proteins often share sections of identical linear peptide sequences. An additional challenge this approach presents is how researchers may compare the mass spectrum of their digested peptides to a given database. Some criteria can be established to determine the probability that a mass spectrum is actually that of a digested peptide in the database. But no matter how high the bar is set on accepting a match, when applied to thousands of proteins in an assay, it becomes likely that some assignments will be wrong. This is why replication of experiments is so important in proteomics and is particularly important for proteins at low concentrations.

20. Another difficulty arises from how the sample is manipulated prior to MS analysis. The volume introduced into the machine consists of a mixture of digested peptides. This is true even if liquid chromatography is used because separation by liquid chromatography is never perfect. Each volume of peptides coming off the column thus will be a mixture despite being enriched or depleted in certain peptides.

21. Even when a single digested peptide is analyzed, the MS result will vary even for a given digested peptide. MS separates molecules from one another

based on the charge-to-mass ratio of ions in the gas phase. Shown below is a schematic of the electrospray ionization (ESI) process frequently used.

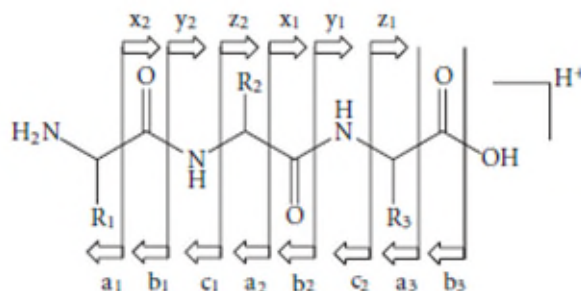


EX2016 (Banerjee), 7. The charged analyte ions vary in the charge that they carry because of the stochastic nature of the charge on the initial drops. The variation in charge means that the charge-to-mass ratio is expected to vary even for a given digested peptide.

22. The MS system usually consists of two mass spectrometers in tandem. The first is used to sequentially select among the charge-to-mass ratios of tryptic peptides present at any moment. From here on, we mostly refer to the difference between ions as a mass-to-charge ratio. For any given mass-to-charge ratio, there is not enough information to determine the exact peptide. Let's say the peptide has 50 amino acids. There are many possible peptides with those same amino acids in a different order. All of them have the same mass. Here is where the strategy discussed above for the laptops is used. The MS-MS system introduces a

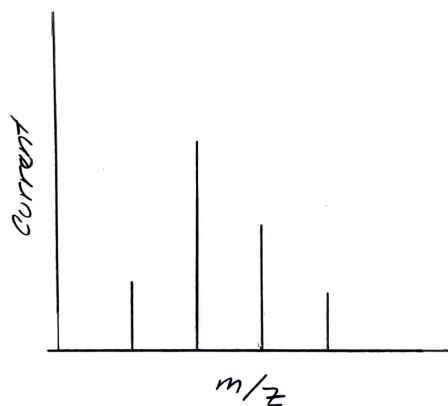
“fragmentation” step between the MS units. Rather than just measuring the mass of the tryptic peptide coming off the column, the instrument measures the mass of individual parts of the peptide.

23. Fragmentation is usually accomplished by Collision-Induced Dissociation (CID). A peptide ion is accelerated and injected into a cell with nitrogen (or other inert gas). Collisions between the ion and the neutral gas molecules have sufficient energy to break bonds in the peptide. The acceleration potential in the CID cell is selected to primarily break the peptide bonds. A very small peptide is shown below.



EX2016 (Banerjee), 20 & Fig. 17. The bonds most likely to break are those designated as b or y in the figure above (representing the amide bonds). The number of fragments can be quite large as each amide bond has the possibility of dissociating. Not all amide bonds have the same strength so the numbers of each fragment will vary. It is important to realize that many molecules of the same peptide are being introduced to the CID cell. Thus, a large variety of fragments are being produced each from a different dissociation event.

24. The stream of fragments is then separated by a second MS device according to the mass over charge ratio of each fragment. The numbers of each fragment ion per unit time (current) is recorded by a detector. When plotted as a function of m/z , the result is a mass spectrum with many peaks. In the case of very clean mass spectrum, it is theoretically possible in some cases to deduce the peptide from the mass spectrum. That is not typical because the spectrum is complicated by many issues. Consider for example, the case of a single fragment with a known charge. The detector will actually find several peaks for that one ion due to isotopic composition (see figure drawn below).



Carbon-13 represents about 1% of carbon atoms found terrestrially. The balance is carbon 12. The lightest form of the fragment will have only C12 atoms. If there a large number of carbon atoms in the fragment the chances of finding a fragment composed with only C12 is very small. The strongest peak in the spectrum actually has some C13 simply because of probability. The situation may be such that the

lightest peak is below the sensitivity of the detector. That means, not all expected peaks may be present in the mass spectrum. The same problem exists for each of the fragments generated. Another issue is that mass is not necessarily unique for all amino acids. Leucine and isoleucine have, for example, exactly the same mass. Thus, uncertainty exists in interpretation of the mass spectrum.

C. Challenges in Shotgun Proteomics

25. Each of the petition and Dr. Vargas' challenges to the '360 Patent—based on Cai, Hu, or Zaccaria—rely on mass spectrometry (MS) signals recorded from the “bottom-up or shotgun” approach of MS proteomics. EX1003 (Vargas Decl.), ¶32. Dr. Vargas' analysis focused on the shotgun approach. EX1003 (Vargas Decl.), ¶32. Her declaration provides a description of this approach. EX1003 (Vargas Decl.), ¶¶33-39. Additional discussion is provided below, which provides some background information a POSA would consider.

26. In shotgun proteomics, a protein source is first obtained and then subjected to extensive fragmentation—i.e., into smaller peptides—for subsequent mass spectrometry analysis. EX2017 (Lange), 1 (“Proteomic studies are commonly performed using a shotgun approach, in which the sample proteins are enzymatically degraded to peptides, which are then analyzed by mass spectrometry (MS).”). Using MS instrument, these smaller peptide fragments are ionized, and then the spectrometer acquires mass spectra of the ionized peptides, identifying the

mass-to-charge ratio (m/z). I note that the MS instrument itself does not detect or identify proteins from the original source; instead, peptide and protein prediction is accomplished bioinformatically by analyzing the mass spectral data using software (e.g., algorithmic analysis) and database resources. EX1030 (Szabo), 3-4. As Dr. Vargas explains, search algorithms and database information was used to analyze the raw mass spectral data and make “predictions” of the “theoretical peptides possible.” EX1003 (Vargas Decl.), ¶39 (describing “fragmentation predictions” for the possibility of a protein being present).

27. Though shotgun proteomic approaches were understood to be a valuable tool in proteomics studies, the scientific literature reported these prior approaches yielded only limited sensitivity and specificity, as well as poor reproducibility. These prior art approaches of shotgun proteomics also significantly limited the dynamic range of proteins that could be reliably detected. These shortcomings with shotgun proteomic approaches were widely described in the scientific literature at the time. EX2017 (Lange), 1 (“consistency [has] not been achieved by the current shotgun proteomic approaches. Besides their limited sensitivity, a main shortcoming of these methods is poor reproducibility...”), EX2018 (Volmer), 2 (“many studies also have reported difficulties with reproducibility and accuracy when analyzing small quantities of analytes in complex samples such as biological fluids”).

28. Highly abundant proteins could be relatively reliably detected using shotgun approaches, but the literature describes these approaches as lacking the sensitivity and specificity required for characterization of low abundance proteins. Further, the shotgun proteomic approach was prone to experimental error and/or artifacts, especially for those low abundance components. EX2017 (Lange), 12 (“in shotgun proteomics, only the more highly abundant part of the proteome is reproducibly identified”), EX2019 (Stahl-Zeng), 1820 (“low abundance components are masked by the (bio)chemical background”), EX2020 (Keshishian), 16 (“using single fragment transitions to monitor for peptides in complex proteomes can be highly misleading and result in erroneous detection of the presence and/or the level of a peptide because what is actually being detected is an interference that cannot be distinguished from the analyte of interest.”).

29. Matching via shotgun proteomics was known to have multiple sources of error and interferences which complicated any accurate and reliable detection, particularly for low abundance proteins. This is described throughout the literature. For example, the sample itself was a known complication in the biological systems evaluated in proteomics. Biological samples typically present a highly complex mixture of various proteins that are present in vastly differing amounts. EX2020 (Keshishian), 16 (“Sensitivity for detection and quantitation by MS is largely limited by the sample background”); *id.*, 13 (“Plasma or serum represent ‘worst

case’ scenarios with respect to complexity given the enormous number of proteins present”), 16 (“Given the complexity of the plasma matrix and the low resolving power of triple quadrupole instruments, it is anticipated that many interferences from co-eluting peptides will often reside within the mass selection windows of the mass analyzers.”).

30. Such complex biological samples are known to further exacerbate ionic interference or suppression effects in the instrumentation, which is a known cause of inaccuracy on MS instrumentation. EX2018 (Volmer), Title (“Ion Suppression: A Major Concern in Mass Spectrometry”), 1 (“Ion suppression negatively affects several analytical figures of merit, such as detection capability, precision, and accuracy.”); EX2020 (Keshishian), 13 (“Noninstrumental factors such as analyte-on-analyte ion suppression may also impair analyte detection”), EX2019 (Stahl-Zeng), 1820 (“The nominal dynamic range of mass spectrometers can be further constrained by ion suppression effects”).

31. Another known source of experimental error is sample contamination, particularly when researchers are working with complex biological samples. EX2018 (Volmer), 3 (“Disregarding sample cleanup, especially when complex matrices are involved, will lead to poor performance.”), *id.*, 3 (a source of experimental error are “exogenous substances, molecules not present in the original sample but from contamination during sample preparation”).

32. Another source of error in shotgun proteomics are the bioinformatic analyses, since the accuracy of protein predictions are limited by the quality and quantity of the mass spectral data as well as software and database resources. EX2021 (Lubec), 3575. High abundance proteins yield a large quantity of peptide fragments, while low abundance proteins yield a relatively small quantity of peptide fragments. Additionally, short peptide fragments are known to frequently bioinformatically map back to multiple different proteins, thus further complicating any prediction of the source protein, and databases may be limited in protein sequence information, the data of different isoforms and the like. EX2021 (Lubec), 3573, 3580; EX2012 (Nesvizhskii), 2111-2112. Digestion fragments of abundant proteins could also be falsely identified as peptides from low-abundant proteins, as an example of so-called “interferences.” EX2020 (Keshishian), 7.

33. False positive identifications were known to result from the application of shotgun proteomics. It was known, for example, that “[i]n the absence of robust statistical and computational methods, proteomic datasets contain significant numbers of false positives, and statements referring to computational analysis of MS/MS data as e.g. ‘the Achilles heels of proteomics’ are common in the literature.” EX2012 (Nesvizhskii), 2093. Large databases further exacerbated false positive detections. *Id.*, 2098 (“Searching large databases also reduces the sensitivity of peptide identification by introducing more false

identifications (the likelihood of obtaining a high scoring random match increases with increasing database size).”). The use of automated processing, which was frequently applied with such large datasets, “produce a match for almost every input MS/MS spectrum, [though] only a fraction of those peptide to spectrum matches (PSMs) are true” and “[i]n certain datasets...incorrect PSMs are the majority.” *Id.*, 2100. The use of decoy databases does not capture all significant sources of false identification. *Id.*, 2104. Overall, “the field is essentially lacking a robust and statistically powerful model with a practical software implementation for the analysis of very large proteomic datasets.” *Id.*, 2115.

34. The scientific literature describes such limitations for shotgun proteomics approaches, necessitating several measures to navigate issues of limited sensitivity and accuracy. As one example, testing that did not replicate the same result was indicative of likely experimental error or artifact as opposed to a detection event. I understand Dr. Vargas agreed with this at her deposition. EX2022 (Vargas Depo. Tr.), 43:20-44:7 (“Typically you conduct tests in replicates to ensure reproducibility.... So there’s no -- there should be no differences between the duplicates”). Additionally, researchers restricted their analysis to what they viewed as a reasonably reliable subset of the proteomics data generated—typically what was identified as a more abundant subset of proteins, since complications with the probability-based matching limited reliability in a given dataset. EX2017

(Lange), 12 (“in shotgun proteomics, only the more highly abundant part of the proteome is reproducibly identified”); EX2019 (Stahl-Zeng), 1820 (describing a limited dynamic range in MS proteomics studies as “typically 3 orders of magnitude”); EX1008 (Hu), 359 & Table 2 (analysis of the “top 20 most abundant plasma proteins”; threshold of “plasma proteins with an average MWSpC ≥ 1 ”); EX1007 (Zaccaria), 1874 (“we compared the 20 most abundant identified proteins (ie, the 20 proteins with the higher MS/MS queries)”).

35. Further, different methods were needed to expand the dynamic range of detection, as was discussed in the literature. For instance, Dr. Vargas discusses a “wide dynamic range” and application of “[t]argeted approaches like multiple reaction monitoring (MRM).” EX1003 (Vargas Decl.), ¶58. Her declaration cites to Nanjappa (EX1014) which in turn discusses MRM (“Multiple Reaction Monitoring”) assays. None of the Hu, Cai, or Zaccaria references employ such MRM assays. Nanjappa cites to several references. *See, e.g.*, EX2017 (Lange) cited as reference #39 and EX2019 (Stahl-Zeng) cited as reference #40 in Nanjappa. Stahl-Zeng explains that protein detection in shotgun proteomics was limited to “typically 3 orders of magnitude.” EX2019 (Stahl-Zeng), 1810. Meanwhile, Lange describes MRM approaches as improving detection to “a linear response over a wide dynamic range of up to five orders of magnitude.” EX2017 (Lange), 1. Each of these references also discuss the “limited sensitivity” and “poor reproducibility”

of prior art shotgun proteomics assays. EX2017 (Lange), 1; EX2019 (Stahl-Zeng), 1810.

IV. UNDERSTANDING OF RELEVANT LAW

36. I have not been asked to provide, nor will I provide, in this declaration, any opinions on the law. Counsel for Patent Owner has informed me of some relevant legal principles that apply in this proceeding.

37. I am informed that the burden of proving every proposition of unpatentability in this proceeding rests on the Petitioner and must be proven by a preponderance of the evidence. This burden of proof applies to each factual matter in each Ground raised in the Petition, including specific arguments raised by the Petitioners regarding anticipation, and obviousness.

38. I understand claim terms are generally given their plain and ordinary meaning as understood by one of ordinary skill in the art in light of the specification. I also understand that claim terms are generally not limited by the embodiments described in the specification.

39. I am informed that a claim is unpatentable as anticipated if each and every element of the claim is described, either expressly or inherently, in a single prior art reference in the same configuration in which those elements are claimed. To establish anticipation by inherency, the inherent teaching must necessarily result from what is described in the prior art reference. The mere probability or

possibility that a certain thing may result from a given set of circumstances is not sufficient to demonstrate inherency.

40. A claimed invention is unpatentable for obviousness if the differences between the invention and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to the person of ordinary skill in the art, in view of the prior art. I understand that the U.S. Supreme Court set out the framework for the obviousness inquiry as follows:

The scope and content of the prior art are to be determined; differences between the prior art and the claims at issue are to be ascertained; and the level of ordinary skill in the pertinent art resolved. Against this background, the obviousness or nonobviousness of the subject matter is determined. Such secondary considerations as commercial success, long felt but unsolved needs, failure of others, etc., might be utilized to give light to the circumstances surrounding the origin of the subject matter sought to be patented.

41. Secondary considerations of non-obviousness are sometimes referred to as objective indicia of non-obviousness, and these must be considered in every case in which they are present.

42. I understand that the steps of a method claim may be performed in any order unless a particular order is expressed or implied by the claim, or is determined by physical or logical constraints..

V. CLAIM CONSTRUCTION

D. “a biological sample”

43. I understand that Dr. Vargas identified in the specification of the '360 Patent an express definition for the term, “a biological sample.” EX2022 (Vargas Depo. Tr.), 33:3-36:9 (Dr. Vargas confirming the definition). Specifically, the specification describes that “[t]he term ‘sample’ refers to a biological sample or a complex biological sample obtained from ***a subject***.”¹ EX1001 ('360 Patent), 20:33-45. The specification further explains: “The terms ‘individual,’ ‘subject,’ and ‘patient’ are used interchangeably.” EX2022 (Vargas Depo. Tr.), 36:10-37:8; EX1001 ('360 Patent), 50:26-36. I agree with Dr. Vargas that the specification defines the term “sample” in this manner.

44. As discussed further below, the express definition of the '360 Patent for “a biological sample” would exclude, e.g., a lysate taken from an immortalized cell line that is engineered and grown in a laboratory (such as Cai’s HeLa cell populations) rather than obtained from a subject. Likewise, the '360 Patent definition excludes a mixture of materials from different donors for the purpose of eliminating individual differences.

E. “a subset of the plurality of particles”

¹ Bold-italic emphases throughout are added by me unless indicated otherwise.

45. Independent claim 1 (step 1(b)) requires “separating at least a subset of the plurality of particles comprising the coronas from the biological sample,” thereby producing a subset of proteins from the biological sample that is thereafter assayed in step 1(c). Further, the term “the coronas” is introduced and defined in step 1(a), which requires that “the coronas” correspond “to different particle types” and “differ based on particle type” and “comprise overlapping and distinct proteins.” EX1001 (’360 Patent), Claim 1, 86:52-87:3; *see also* EX2022 (Vargas Depo. Tr.), 30:21-31:13, 48:1-49:25 (describing “at least”).

46. As the language of claim 1 indicates, step 1(b) is not satisfied where the corona on the identified subset of particles does not differ based on particle type (i.e., where the subset of particles consists of only one particle type) or does not comprise overlapping and distinct proteins. E.g., EX2022 (Vargas Depo. Tr.), 27:21-28:3, 28:22-29:7. As discussed further below, for each of Hu, Cai, and Zaccaria, the petition and Dr. Vargas identify a subset of particles for step 1(b) that is never shown to include “the coronas” comprising overlapping and distinct proteins. A corona on a single particle type does not comprise “overlapping and distinct” proteins as required by the claims. *See, e.g.*, EX2022 (Vargas Depo. Tr.) 24:19-24.

VI. CAI’S STUDY DOES NOT DISCLOSE ALL ELEMENTS

47. The petition and Dr. Vargas opine that Cai anticipates claims 1, 4, 6, 17, 22, and 25 of the '360 Patent. Pet., 3, 46-61; EX1003 (Vargas Decl.), ¶192. They also contend that Cai renders obvious claims 1, 4, 6, 17, 22, and 25 of the '360 Patent. Pet. 62-64; EX1003 (Vargas Decl.), ¶236. I disagree with both of these contentions. A POSA would not have read Cai (EX1005) as disclosing or making obvious any of the challenged claims.

48. First, while the petition and Dr. Vargas assert that Cai discloses a method of assaying “a biological sample,” they point to a lysate of cells from an immortalized HeLa cell line. But a lysate taken from an immortalized cell line engineered and cultured in a laboratory (e.g., Cai’s HeLa cell populations) is not “a biological sample” as that claim term is defined in the challenged patent.

49. Second, the petition and Dr. Vargas mix and match different experiments of Cai without explanation. Dr. Vargas admits (and I agree) that Cai’s Figures 1 and 3 illustrate different experiments that use different HeLa cell populations (the alleged “samples”) in distinct protocols and analyses. For example, Cai’s Figure 1 experiment does not conduct the mass spectrometry analysis (relied upon by Dr. Vargas and the petition for the claimed assaying (step 1(c))). See EX2022 (Vargas Depo. Tr.), 107:9-20, 123:21-124:3, 128:3-17, 129:7-12. Cai’s SILAC-encoded MS experiment (illustrated in Cai’s Figure 3) individually contacted **two different lysates** (each from a different populations of

HeLa cells), each with only ***a single particle type***. None of those individual SILAC populations (light, medium, or heavy) were contacted with a ***plurality*** of different particles as required in element 1(a).

50. Regarding limitation 1(c), the emPAI values relied upon by the petition and Dr. Vargas for assaying across “at least six orders of magnitude” is described by Cai as a “semi-quantitative” index of ***abundance***, not the claimed ***concentration***. Further, emPAI is described by Cai as reflecting abundance ***on the particles***, not the abundance in any HeLa cell population or a lysate thereof.

A. Cai’s HeLa Cell Lysate Is Not “A Biological Sample” As Claimed.

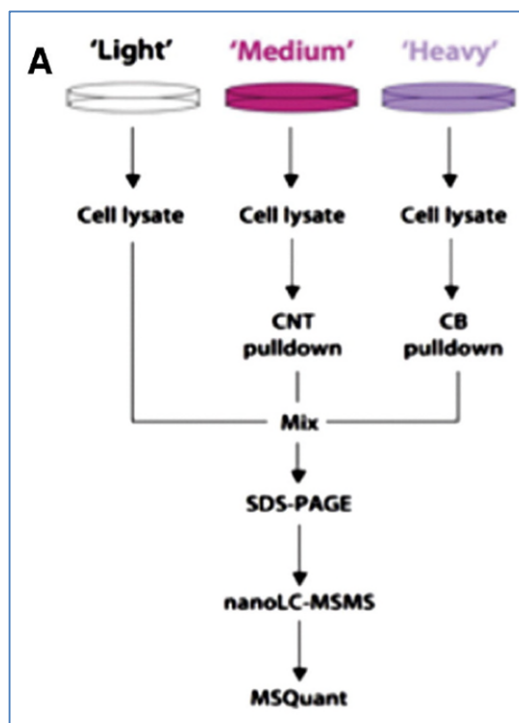
51. Neither Dr. Vargas nor the petition demonstrate that Cai discloses the claimed “method for assaying a biological sample,” including applying the method steps to the biological sample as specifically required by the claims, since the petition and Dr. Vargas rely exclusively on lysate of HeLa cells as the identified biological sample. *See, e.g.*, EX2022 (Vargas Depo. Tr.) 134:3-16; EX1003 (Vargas Decl.), ¶¶194-197.

52. HeLa cells are not obtained from an individual subject. I understand Dr. Vargas confirmed at deposition that HeLa cells are vendor-purchased immortalized cells from cell lines cultured in labs. EX2022 (Vargas Depo. Tr.), 117:11-21, 124:4-16, 136:19-137:18. I agree. In fact, HeLa cells differ quite significantly compared to cells that would be obtained from any subject. EX2026

(Heng) (“it might even be argued that the HeLa genome is no longer a human genome.”).

53. As discussed above, Dr. Vargas identified a definition in the specification that states: “The term ‘sample’ refers to a biological sample or a complex biological sample obtained from **a subject**.” EX1001 (’360 Patent), 20:33-45. The specification also describes: “The terms ‘individual,’ ‘subject,’ and ‘patient’ are used interchangeably.” EX1001 (’360 Patent), 50:26-36. A HeLa cell lysate is derived from cultured, immortalized HeLa cells, rather than from a biological sample as required by the claims.

54. Regarding Cai’s SILAC experiment (depicted in Cai Figure 3), Cai describes each of its HeLa cell lysates as **distinct populations** of cells. EX1005 (Cai), 586 (In SILAC study, “[c]ell lysates were prepared from **three populations** of HeLa cells that had been metabolically encoded using three different SILAC media”). See EX2022 (Vargas Depo. Tr.), 133:25-135:2, 106:22-107:1, 135:3-136:22. As discussed and illustrated in Cai, in the SILAC



experiment depicted in Fig. 3, above, each different population was contacted by at

most one single particle type—not by the multiple particle types required by the claims. Cai’s SILAC study thus does not satisfy claim step 1(a), as required, because it involves experiments with three different (plural) populations and no (singular) population is ever contacted by a plurality of particle types. *See* EX2022 (Vargas Depo. Tr.), 131:8-132:5.

55. While the petition cites to different experiments (Fig. 1 and Fig. 3) for different aspects of the challenged claims, neither of Cai’s experiments disclose a biological sample or the method claimed. Each of Cai’s different experiments operate on different HeLa cell populations, none of which qualify as a “biological sample” as defined in the ’360 patent. The petition and Dr. Vargas rely on the MS analysis in Cai’s Figure 3 experiment to satisfy the assaying across six orders of magnitude requirement in step (c). Pet., 60; EX1003 (Vargas Decl.), ¶¶228-229. But, as indicated above, the Figure 3 experiment does not contact any HeLa cell population with a plurality of different particle types. Regarding, Cai’s Figure 1 study, that is not subject to MS analysis and Cai does not assay the material across at least six orders of magnitude, as required by the claims.

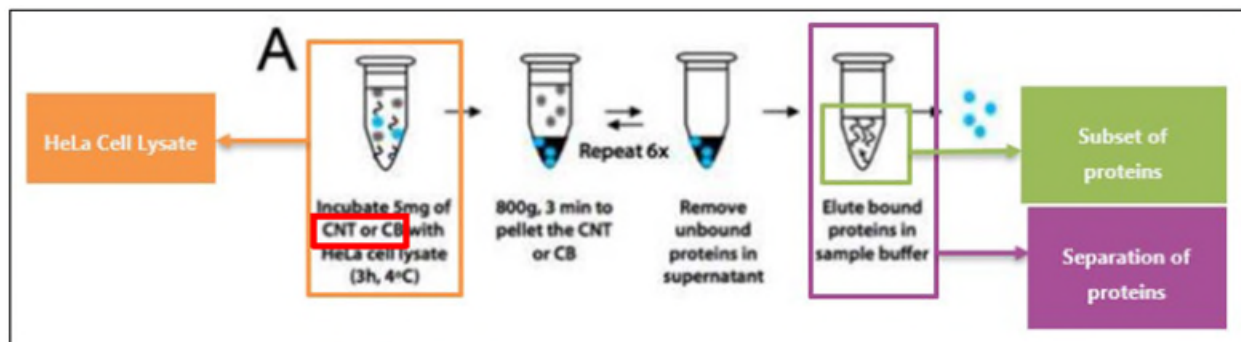
56. As such, each of the experiments reflected in Cai Figure 1 and 3 are different experiments on different populations of HeLa cells. None of those HeLa cell populations (or lysates) are obtainable from an individual subject. And each of those different experiments is missing several aspects of the claimed method.

**B. Cai Fails to Disclose Pulling Down a Subset of Particles
Comprising the Coronas (Element 1(b)).**

57. As discussed above (Section V.B), the plain and ordinary meaning of the claims require separating from the biological sample at least “a subset of the plurality of particles comprising the coronas.” The claims also require the separated particles to have both (1) different particle types and (2) coronas corresponding to the different particle types comprising overlapping and distinct proteins. *See* EX2022 (Vargas Depo. Tr.), 27:21-28:3. Neither Dr. Vargas nor the petition demonstrate that Cai discloses this claim element.

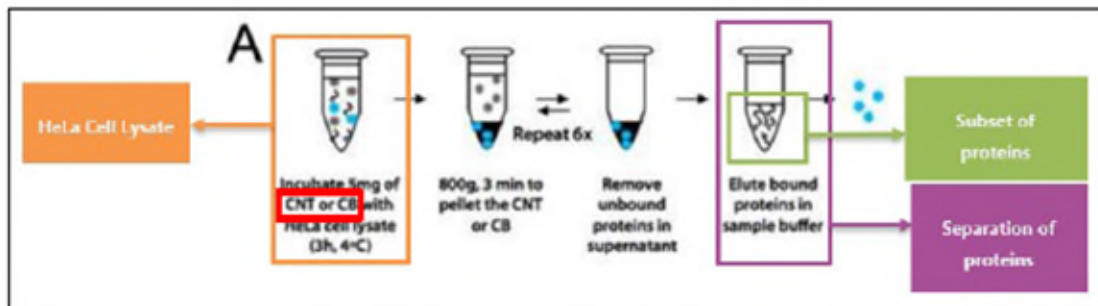
58. When discussing the separating step of claim element (b), both the petition and Dr. Vargas rely on Cai’s Figure 1A—and **Figure 1A alone**—which depicts single particle types in a single HeLa cell lysate. Pet., 57-58 (citing EX1005 (Cai), Fig. 1A); EX1005 (Cai), 585 (specifically relating to Fig. 1A); EX2022 (Vargas Depo. Tr.), 155:19-156:10; EX1003 (Vargas Decl.), ¶¶223-224. Dr. Vargas’s annotations of Cai’s Figure 1A are clear that there is never disclosed separating a subset (singular) of particles comprising coronas (plural) from more than one particle type. Instead, Cai’s Figure 1 samples were incubated **individually** in parallel (EX1005 (Cai), Fig. 1A “CNT **or** CB” which Dr. Vargas annotated in red (reproduced below)); EX1003 (Vargas Decl.), ¶202 (Dr. Vargas admitting Cai’s samples are incubated in parallel)); EX2022 (Vargas Depo. Tr.), 102:20-

104:8, 104:10-17, 106:22-107:1, 119:19-120:9. Further, the proteins are desorbed prior to assay. EX2022 (Vargas Depo. Tr.), 133:5-11, 132:6-133:2.



59. I understand that Dr. Vargas acknowledged in deposition that the different particle types of Cai were always *individually* incubated in its pull-down experiments, and that every separation step created a subset of particles comprising a corona from only one particle type. I agree. Cai does not disclose separating a subset of particle comprising “the coronas” corresponding to multiple particle types, as required by the claims.

60. In fact, the discussion in the petition and Dr. Vargas’ declaration fail to identify the claimed subset of particles. Though the petition identifies the “subset of proteins of (b)” from the CNT *or* the CB pulldown with green annotation—it never maps to the claimed subset of *particles* comprising “the coronas.” Pet., 57-58 (Petitioners’ annotated Fig. 1A, reproduced below).



61. The petition and Dr. Vargas provide limited argument alleging that MWCNTs of diameters 20-40 nm constitute a different particle type from MWCNTs of diameters less than 10 nm. This fails to meaningfully address the challenged claims and certainly fails to show anticipation by Cai. First, the experiment relied upon by Dr. Vargas and the petition to generate the alleged “subset of proteins” (Cai’s Figure 1) never employed MWCNTs with diameters less than 10 nm. Second, Cai describes that MWCNTs having diameters less than 10 nm “bound to virtually no proteins.” EX1005 (Cai), 586. In contrast to MWCNTs of diameters less than 10 nm, Cai teaches that “the amount of proteins pulled down by CNTs of diameters from 20-100 nm is essentially the same.” EX1005 (Cai), 586. Cai also teaches that MWCNTs length likewise did not impact protein binding amount. EX1005 (Cai), 586. Cai’s disclosure of 20-40 nm and 10 nm CNTs thus did not describe separating a subset of particles comprising “the coronas” of different particle types, and this disclosure instead merely indicates the absence of a corona from a different particle type in that experiment. Accordingly, this argument fails to establish that Cai anticipates the claims.

C. Cai Fails to Disclose Element 1(c).

62. Claim 1 step (c) requires assaying the subset of proteins to “detect, in the subset, proteins in the biological sample at concentrations across a dynamic range comprising at least 6 orders of magnitude, thereby assaying the biological sample.” In my opinion, Cai does not disclose this limitation as argued in the petition materials.

63. For this claim element, both the petition and Dr. Vargas rely exclusively on emPAI values differing by at least six orders of magnitude. Pet., 60; EX1003 (Vargas Decl.), ¶229. Specifically, the petition materials cite to a comment in Cai that emPAI values for the MWCNT-binding proteins “spread over 8 orders of magnitude.” EX1005, 587 (Figure 3F). There is no meaningful discussion of this aspect of Cai, which the petition materials clearly misunderstand.

64. First, the petition materials confuse abundance and concentration. Cai itself confirms that emPAI is not a measure of **concentration**. It is instead described as a semi-quantitative indication of abundance. EX1005 (Cai), 587 (“empirical peptide abundance index (emPAI), a **semi-quantitative** indication of the **overall abundance** of protein **in the mixture**”). Abundance is not the same as concentration. Rather, abundance refers to the signal intensity of ions, which is proportional to the number of ions detected. See, e.g., EX1031 (Bantscheff), 1018 (“quantification is achieved by comparing their respective signal intensities”).

Such relative intensity does not represent a direct measure of concentration because ionization efficiency varies between compounds, thus a higher intensity (abundance) does not always mean a higher concentration. *See* Section III, above. In contrast, concentration refers to the actual amount of a substance in a volume. emPAI is ***semi-quantitative*** because it assumes that there is a correlation between the number of peptides sequenced per protein and the amount of protein present in the mixture, but this fails to account for, e.g., the differences in ionization efficiency and multiple charge states. *See* EX2021 (Lubec), 3573 (“Spectral Quality” includes basic components of “charge state differentiation,” “total signal intensity” and “signal-to-noise estimates”).

65. Second, the relied-upon emPAI does not demonstrate abundance, much less concentration, in the lysate identified by the petition and Dr. Vargas as the “biological sample.” Instead, Cai explains that emPAI is a semi-quantitative measure of abundance of proteins ***in the mixture of proteins*** created during the SILAC experiment. I understand that Dr. Vargas explained during her deposition that this refers to previously-bound proteins placed into what Cai Fig. 3 labels the “Mix,” but that mixture is not the HeLa cell population contacted with particles or the lysate. I agree. As I understand, Dr. Vargas also confirmed the Mix comprises a mixture of proteins from the ***pulled-down corona*** from two of the three lysates created from the three populations of HeLa cells. EX1005, Fig. 3. Thus, emPAI is

clearly a measure of abundance of “bound proteins” (i.e., present in the pull-down) rather than a measure of concentration in the HeLa cells or their lysate. EX1003 (Vargas Decl.), ¶229 (“the relative abundance of **bound proteins** ‘spread over 8 orders of magnitude’ shows that Cai’s MS analysis detected protein concentrations in the subset across a dynamic range comprising the claimed ‘*at least 6 orders of magnitude.*’” (citing EX1005, 587)). Thus, not only does Dr. Vargas confuse abundance with concentration, she confuses the Mix with what she separately identifies as a sample.

66. Third, even if emPAI is considered, the emPAI values in Cai that are relied upon by the petition and Dr. Vargas are at the extreme ends of high and low abundance, and are not found within the narrow range that had been validated for the emPAI procedure. Cai relies on Ishihama (EX1018) as representing the extent to which emPAI methodology has been validated. EX1005 (Cai), 587 (ref. 30 is Ishihama)). But Ishihama only validated emPAI over a narrow range. EX1018 (Ishihama), 1268 (number of peptides having linear relationship “from 3 to 500 fmol”); *id.* (Ishihama’s study testing quantification in the range of from 30 fmol to 1.8 pmol/μL). While Cai observed a wide spread of emPAI values, it cites Ishihama, which confirms a substantially narrower range of validated emPAI values. *Compare* EX1018 (Ishihama), Table 1 (Ishihama’s emPAI ranging from 0.10 to 15.68) *with* EX1003 (Vargas Decl.), ¶229 (relying on Cai’s emPAI values

of 0.02 to 2,254,683). Cai reports that a wide spread of emPAI values is an observation that some proteins were selectively enriched; but Cai is not reporting absolute protein amounts in contradiction to underlying validation work reported in the scientific literature.

67. Thus, for at least the reasons above, Cai has not been shown to anticipate the challenged claims.

D. Cai Obviousness Fails

68. The petition and Dr. Vargas argue claims 1, 4, 6, 17, 22 and 25 would alternatively have been obvious. Specifically, they argue that to the extent Cai fails to disclose limitation 1[c] it would have been obvious to convert Cai's emPAI values "into more traditional concentration numbers." Pet. 62-64; EX1003 (Vargas Decl.), ¶¶236-241. This argument otherwise relies on its Cai anticipation argument from Ground 2 and proposes only converting Cai's emPAI values to mol % based on a formula from the Ishihama reference.

69. As a threshold matter, the petition and Dr. Vargas still fail to distinguish between protein content and concentration. Molar percent (mol %) of protein content is indicative of relative abundance; it is not a measure of concentration. This can be seen from the Ishihama formula presented in the petition, which is a simple calculation for the fraction of a given emPAI value

relative to the sum of all emPAI values (multiplying the fraction by 100 gives percentage).

$$\text{Protein content (mol \%)} = \frac{\text{emPAI}}{\sum (\text{emPAI})} \times 100 \quad (\text{Eq. 3})$$

EX1018 (Ishihama), 1267 & Equation 3. This is still, at its core, the same emPAI number that is “roughly proportional to protein abundance” with all the caveats and issues I described above. I also note that Ishihama only tested the linearity of its emPAI and related calculations with concentrations of proteins having a relatively high concentration—from 30 fmol to 1.8 pmol/μL (EX1018 (Ishihama))—and observed error as high as three-fold the detected values. Ishihama’s abundance-based calculations are not a substitute for concentration. Thus, even if a POSA was inclined to make such a calculation, it still reflects relative emPAI (“semi-quantitative indication” of abundance in the mixture) fraction rather than concentration.

70. Furthermore, the obviousness argument does not change any of my criticisms discussed above in the anticipation challenge. For example, “mol %” of the protein mixture, even if determined, still would not correspond to the features the petition identifies as the biological sample (Section VI.A-B). Additionally, Cai’s abundance index, regardless of units of expression, at best reflects an amount of protein that bound to particles in the mixture extract rather than the amount in

the lysate (identified in the petition as the claimed “biological sample”).

Furthermore, this proposed modification does nothing to change the fact that the identified HeLa cell lysate are not a sample from an individual, or that Cai’s SILAC experiment does not contact any population with a plurality of different particle types to satisfy claim element 1(a), or Cai’s failure to disclose pulling down a subset of particles comprising the coronas, as required by claim element 1(b).

E. Dependent Claims

71. The petition and Dr. Vargas argue that dependent claims 4, 6, 17, 22 and 25 are anticipated by Cai for the “same reasons” set forth for independent claim 1. Pet. 60-61; EX1003 (Vargas Decl.), ¶¶230-235. As discussed above, the petition fails to establish Cai anticipates claim 1.

72. For at least the reasons above, Petitioners fail to show Cai renders the challenged claims anticipated or obvious.

VII. HU’S STUDY DOES NOT DISCLOSE ALL ELEMENTS

73. The Vargas Declaration and petition opine that Hu (EX1008) renders obvious claims 1-4, 6-7, 17, and 22-25 of the ’360 Patent. EX1003 (Vargas Decl.), ¶126. I disagree. A POSA would not have read Hu (EX1008) as disclosing or making obvious any of the challenged claims.

74. First, Hu (like Cai) fails to disclose assaying a biological sample as claimed. The petition and Dr. Vargas identify Hu's mixture of blood from 20 different individuals as the purported biological sample, but the petition and Dr. Vargas fail to explain how such a mixture satisfies the patent's explicit definition of a biological sample.

75. Second, this ground also fails because Hu does not disclose separating a subset of particles comprising the coronas, as required by step 1(b). Hu instead individually separated subsets of particles each comprising the corona of only an individual particle type.

76. Finally, the Hu ground fails regarding the six orders of magnitude limitation of step (c) for several reasons. Hu does not expressly disclose concentrations or any dynamic range; turning to alleged inherent disclosure, the petition and Dr. Vargas latch onto one particular entry (mentioning LTBP-1) found among the many entries in the raw data table from Hu's supplement. Pet., 35-36. The petition and Dr. Vargas, however, fail to establish Hu actually detected LTBP-1 as opposed to simply reporting a non-reproducible signal indistinguishable from background, as is commonly found in raw data from shotgun proteomics assays. Indeed, Hu's table lists only a weak signal for the "LTBP-1" entry that was not confirmed in Hu's replicates.

77. Even assuming LTBP-1 detection occurred, the petition and Dr. Vargas fail to establish the identified low concentration is necessarily present in Hu's sample. They argue serum albumin and LTBP-1 are "normally present" at certain concentrations identified in the petition. Pet. 36. I disagree with their representation of "normally present," and the citation to different references does not demonstrate protein concentrations in Hu's sample.

A. Hu Fails to Disclose Assaying "A Biological Sample" As Claimed.

78. The petition and Dr. Vargas fail to demonstrate that Hu discloses the claimed "method for assaying a biological sample." Pet. 21-25; EX1003 (Vargas Decl.), ¶¶127-131. Hu's study combines blood taken from 20 different individuals that are mixed prior to assay. EX1008 (Hu), 355 ("Human blood was *taken from 20 different healthy donors*" and processed, then the resultant plasma was "*mixed together* in order to *eliminate individual differences*."). As indicated above, Dr. Vargas identified a definition in the challenged patent, but does not explain how Hu's mixture is "a biological sample" as defined by the '360 Patent.

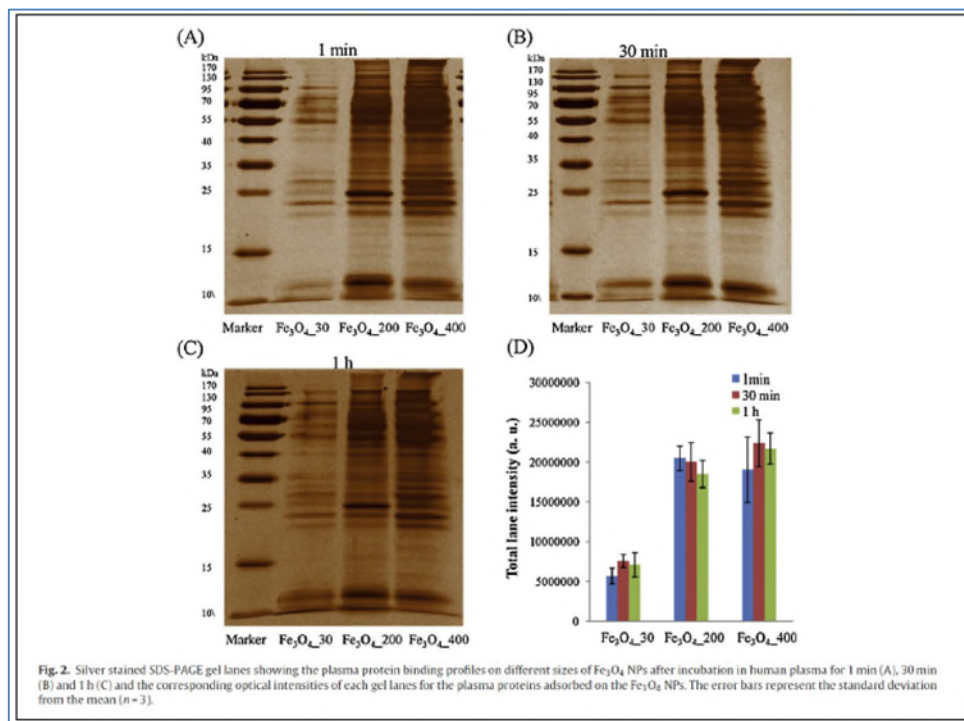
B. Hu Fails to Disclose Separating a Subset of the Plurality of Particles Comprising the Coronas As Required By 1(b).

79. The petition and Dr. Vargas also fail to demonstrate that Hu discloses separating the claimed "subset of the plurality of particles comprising the coronas" as required by all challenged claims. The claims require the separated subset of

particles to have both (1) different particle types and (2) coronas corresponding to the different particle types comprising overlapping and distinct proteins. Section V.B, above.

80. The petition and Dr. Vargas inconsistently identify what they consider to be “the subset.” *Compare* Pet., 31-32 (“the bound subset” depicted by Fig. 2, showing SDS-PAGE gel lanes) and EX1003 (Vargas Decl.), ¶¶151-153 (similar) *with* Pet., 33-36 (“the subset” referring to proteins derived from singular corona types detected by mass spectrometry) and EX1003 (Vargas Decl.), ¶¶154-173 (similar). But their identified “subset” of particles fails in each scenario. As I explain below, if Hu’s SDS-PAGE gel is relied on for disclosure of “the subset of the plurality of particles comprising coronas” it fails because (1) the SDS-PAGE gel is not shown to contain **any** particles comprising coronas (much less a plurality of particles containing the coronas required by the claims), and (2) it is never assayed to detect proteins across a dynamic range comprising at least 6 orders of magnitude. Alternatively, if Hu’s mass spectrometry assay is relied on for disclosure of “the subset of the plurality of particles comprising coronas,” then it also fails because each of Hu’s mass spectrometry samples comprise only one corona—not the required **coronas** having overlapping and distinct proteins. Section V.B, above.

81. Discussing claim element 1(b), the petition and Dr. Vargas assert Hu’s Figure 2 (reproduced below) depicts “the bound subset.” Pet., 31-32; EX1003 (Vargas Decl.), ¶¶151-153. But Hu’s Figure 2 does not disclose the claimed “subset of the plurality of particles comprising the coronas.” Rather, a POSA would have understood Figure 2 at best illustrates proteins that were *previously* bound to nanoparticles as part of a protein corona.



82. Hu explains that, prior to running its SDS-PAGE, its “nanoparticle-protein complexes” were “pelleted, resuspended in SDS-PAGE loading buffer, then boiled at 95 °C for 5 min and finally separated with 1-D SDS-PAGE.” EX1008 (Hu), 355. Such boiling denatures the protein corona, removing it from the nanoparticle. EX2025 (Voet), 159. Reliance on Hu’s Fig. 2 for the “subset of

the plurality of particles comprising the coronas” is insufficient because it does not disclose **any** particles comprising coronas, much less a subset of the particles comprising **coronas** comprising overlapping and distinct proteins. Instead, in Hu’s Figure 2 it is the **detached** proteins that are “separated with 1-D SDS-PAGE to illustrate the binding patterns of the plasma proteins on the” nanoparticles. EX1008 (Hu), 355. Neither Hu’s Figure 2 nor the preparation of its SDS-PAGE indicate separating a subset of the plurality of particles comprising the multiple coronas as required by claim 1. I understand that Dr. Vargas confirmed during her deposition that there are no particles when the material is loaded onto the SDS-PAGE gels. EX2022 (Vargas Depo. Tr.), 99:3-15 (confirming her “understanding it’s just the proteins” loaded rather than the particles). I agree.

83. Even if the petition or Dr. Vargas were to attempt to pivot to Hu’s particles that are digested and analyzed by mass spectrometry via shotgun MS as “the subset,” this also fails. EX1008 (Hu), 355; Pet., 33-36. I understand that Dr. Vargas confirmed at her deposition that the separation step in Hu generates a subset of particles comprising only the corona of **a single particle type**. EX2022 (Vargas Depo. Tr.), 92:2-97:18 (each of 30-, 200-, and 400-nm samples incubated individually). I agree with Dr. Vargas; Hu never describes separating a subset of particles comprising coronas from multiple particle types. Consistent with its preparation, Hu later describes LC-MS/MS analysis on a corona **individually**. See,

e.g., EX1008 (Hu), 355-356 (“digested peptides from **the** human plasma protein **corona** were dissolved” and then the “analysis was carried out on a 1-D nano LC-MS/MS system with 5 µL of the resuspended peptides injected each time.”), EX1008 (Hu), 359 (Table 2 providing three separate lists of the “top 20 most abundant plasma proteins bound of the Fe₃O₄ NPs of different sizes”), 10 (Table S1 reporting results individually under three different columns). The subset of particles separated during Hu’s pull-down thus comprised only the corona from a **single** particle type, not multiple **coronas** with overlapping and distinct proteins as required. Section V.B. Accordingly, Hu fails to describe separating a “subset of the plurality of particles comprising **the coronas**,” which have overlapping and distinct proteins, as required by all challenged claims.

C. Hu Fails to Disclose Detecting Proteins At Concentrations Across a Dynamic Range Comprising At Least 6 Orders of Magnitude As Claimed.

84. Step (c) further requires that the assay must “detect, in the subset, proteins in the biological sample at concentrations across a dynamic range comprising at least 6 orders of magnitude, thereby assaying the biological sample.” The petition and Dr. Vargas fail to demonstrate Hu discloses this limitation. Pet. 33-39; EX1003 (Vargas Decl.), ¶¶154-173.

85. The petition and Dr. Vargas’ argument rely specifically on two proteins (serum albumin and LTBP-1) written in Hu’s supplementary data table.

Pet. 35-36. Acknowledging no concentrations or dynamic range is expressly found in Hu, they argue inherent disclosure in Hu because the proteins are “normally present” at concentrations that would span 6 orders of magnitude. As discussed, the petition and Dr. Vargas fail to demonstrate the identified concentrations of these proteins are necessarily present in Hu.

i. Failure To Demonstrate Hu Actually Detected LTBP1 at a concentration of 1.7-6.0 ng/mL.

86. To allege inherent disclosure, the petition and Dr. Vargas rely on a single entry found among the many entries in the raw data table from Hu’s supplement. Pet., 35-36; EX1003 (Vargas Decl.), ¶¶166 (explicitly alleging serum albumin and LTBP-1 in Hu meets “the claim limitation of ‘at least 6 orders of magnitude.’”), ¶162 (“Two of those proteins detected from Hu are serum albumin and LTBP-1.”). But the authors of Hu never meaningfully discussed LTBP-1. Likewise, the petition and Dr. Vargas’ declaration lack meaningful discussion of this entry and fail to establish that Hu actually detected LTBP-1 as assumed, let alone at the identified concentration.

87. As discussed above (Section III), shotgun proteomics approaches like that applied in Hu extensively degrade proteins into small peptide fragments, acquire spectral data, and analyze the raw mass spectral data to make “predictions” of the “theoretical peptides possible.” EX1003 (Vargas Decl.), ¶39 (“fragmentation predictions”). It was known that prior art shotgun proteomics were prone to

experimental error—especially for low abundance components. See e.g., EX2020 (Keshishian), 16 (discussing “erroneous detection of the presence and/or the level of a peptide because what is actually being detected is an interference that cannot be distinguished from the analyte of interest.”).

88. Accounting for the uncertainty and complications associated with shotgun mass spectrometry proteomics such as errors in predictions of matches (Section III, above)—particularly with low-abundant proteins—POSAs commonly tested samples in multiple replicates and viewed non-replicable results as indication of likely experimental error or artifact rather than a detection event. Researchers—including Hu—also restricted their analysis to what they viewed as a reasonably reliable subset of the proteomics data generated. EX1008 (Hu), 359 & Table 2 (analysis of the “top 20 most abundant plasma proteins”; threshold of “plasma proteins with an average MWSpC ≥ 1 ”); *see also* EX1007 (Zaccaria), 1874 (“we compared the 20 most abundant identified proteins (ie, the 20 proteins with the higher MS/MS queries)”).

89. Dr. Vargas and the petition identify LTBP-1 based on an entry in Hu’s raw data table found in its “supplementary data,” but they offer little discussion. EX1003 (Vargas Decl.), ¶¶161-162. But a POSA would be highly skeptical that Hu had actually detected LTBP-1. As explained in further detail below, a POSA

would more reasonably concluded Hu is simply reporting a non-reproducible match to a normalized spectral count that did not meet the threshold of detection.

90. The below image shows the column headings from page 10 and the entry from page 18 of EX1008 (Hu), providing a summary of what was actually reported regarding LTBP-1 in Hu's supplemental information. See also, EX2013 (accurately summarizing this information). Annotations indicate the particle type employed and the number of replicates attempted. As shown in the image from EX2013 below, of nine attempts on three different particles, seven of those attempts failed to even postulate the presence of LTBP-1 (isoform 4) at all.

IPI	Names	pI	MW (kD)	Fe ₃ O ₄ _30					Fe ₃ O ₄ _400					Fe ₃ O ₄ _400				
				MWSpC ₋₁	MWSpC ₋₂	MWSpC ₋₃	Average	SD	MWSpC ₋₁	MWSpC ₋₂	MWSpC ₋₃	Average	SD	MWSpC ₋₁	MWSpC ₋₂	MWSpC ₋₃	Average	SD
IPI00973177.1	LTBP1 Isoform 4 of Latent-transforming growth factor beta-binding protein 1	5.63	186.74						0.01	0.01	0.01	0.00						

Fe ₃ O ₄ _30 (30 nm)		
Replicate 1	Replicate 2	Replicate 3
Fe ₃ O ₄ _400 [sic] (200 nm)		
Replicate 1	Replicate 2	Replicate 3
	0.01	0.01
Fe ₃ O ₄ _400 (400 nm)		
Replicate 1	Replicate 2	Replicate 3

(blank spaces indicate that the corresponding proteins were not identified in the analysis).

91. I observe that in no instance does Hu's table report signal for all replicates of any tested particle. Where Hu's table does report a MWSpC (percentage normalized spectral count) value, it is extremely small (0.01) and well-below the ≥ 1 threshold Hu discusses elsewhere as being instituted for its analysis.

EX1008 (Hu), 359. Indeed, the MWSpC value of 0.01 is the lowest non-zero value reported in any entry in Hu’s supplementary data table. LTBP-1 is not discussed by the authors in the Hu article itself, and is certainly not identified among the list of any proteins Hu claimed it reliably detected. EX1008, Table 2.

92. Given the weakness of the MWSpC (percent normalized spectral count) and lack of reproducibility, a POSA would more reasonably conclude that any non-replicable signal was more likely experimental artifact rather than an actual detection event. The petition and Dr. Vargas’ argument ignores the known sensitivity limitations and experimental error common in shotgun proteomics assays like Hu. Section III, above. They do not meaningfully address the raw data reported in Hu itself—they merely cite an entry without addressing it critically as a POSA would do.

ii. The “Normally Present” Argument Does Not Make Sense.

93. Since Hu does not disclose any dynamic range or concentrations of any proteins in its plasma mixture, the petition and Dr. Vargas argue inherent disclosure. EX1003 (Vargas Decl.), ¶161 (“this is inherently disclosed by Hu”). Specifically, they identify two proteins—serum albumin and LTBP-1—which they argue are “normally present” at identified concentrations differing by six orders of magnitude (though citing to references other than Hu). Pet. 35-37; EX1003 (Vargas Decl.), ¶¶163, 165-166. This argument fails for at least several reasons.

94. First, the petition and Dr. Vargas cite to Ahmed and Cao but these references do not establish concentrations for serum albumin and LTBP-1 in Hu. EX1003 (Vargas Decl.), ¶165. Concentrations of different proteins vary considerably among different individuals. EX1001, 56:35-38. These references do not address Hu's plasma testing or even discuss Hu. Moreover, Ahmed does not address Cao's samples, and Cao does not address Ahmed. In fact, the spectral count in Hu mapped to LTBP1 is for "Isoform 4." EX1008, 18 (Table S1, row IPI00973177.1); EX2013 (excerpt reproduced below)). But Cao teaches "[t]here are four isoforms of LTBP1s (LTBP-1, -2, -3, and -4)" and discusses concentration values from Cao's sample for Isoform 1. EX1016, 16051 (LTBP-1). Dr. Vargas does not appear to recognize that Hu and Cao are addressing different proteins.

95. It was also known in the field of proteomics that protein concentrations vary significantly between samples and that even variations in preparation method impact testing results. See, e.g., EX1015 (Ahmed), 786 ("Standardizing sample preparation procedures for plasma/serum profiling..., as well as fractionation techniques employed either to generate several fractions or to selectively obtain a particular subset of peptides/protein, although time-consuming and error prone is critical for obtaining reliable biomarkers and building a biomarker pattern, since slight changes in a given sample preparation could lead to very different protein profiles."), 786 (protein half-lives range from a few days to

just a few hours). For example, the preparation and analysis methods of Hu and Cao differed in various respects. Hu and Cao examined different cohorts with a different composition of age and gender. EX1014 (Nanjappa), D960 (explaining that differences in age and gender matter for protein composition). Additionally, Hu and Cao differed in sample processing and handling (e.g., centrifugation, storage) as well as assaying method (e.g., shotgun proteomics and MS in Hu versus ELISA kit in Cao). I summarize some differences between Hu and Cao below:

Hu (EX1008)	Cao (EX1016)
Samples from 20 healthy individuals (no further details provided)	Samples from healthy individuals: 39 females, 65 males, mean age 47.6 years
Centrifuged 5 min. at 1000 g (temperature not provided)	Centrifuged 10 min. at 1000 g and 4 °C
Following storage and upon thawing, plasma centrifuged 2 min. at 16,000 g “to further reduce the presence of red and white blood cells”	Not described in Cao
Used immediately upon thawing, never refrozen	Not described in Cao

96. Second, the petition and Dr. Vargas mischaracterize the cited Ahmed and Cao references as reporting “normally present” concentrations of identified proteins. EX1003 (Vargas Decl.), ¶165. Even if Cao and Ahmed observed particular concentrations in those particular samples tested, they are not broadly reporting what is “normally present” in a more general manner. See e.g., EX1016 (Cao), 16047-16048 (observing concentrations employing ELISA).

97. In fact, it is unclear why Ahmed and Cao specifically are relied upon for alleged concentrations of serum albumin and LTBP-1, respectively. For example, the petition materials cite specifically and only to Cao in asserting that LTBP-1 is “normally present” at concentrations ranging from “1.7 to 6.0 ng/mL.” I see no explanation why only Cao is cited or why no other sources are provided. Other resources available at the time identify significantly different LTBP-1 concentrations than what the petition and Dr. Vargas present.

98. For example, the Peptide Atlas database is an on-line resource known to researchers in proteomics and a database I’ve used personally. The Peptide Atlas is available at the following hyperlink: <https://peptideatlas.org/>. Searching Peptide Atlas database for LTBP-1 in the most recent human plasma dataset identifies LTBP-1 at an estimated concentration of **220.0 ng/mL**. See EX2027 (below). The identified “10x uncertainty” indicates expected concentrations can be as high as 2200.0 ng/mL. This is far greater (by approximately 2-3 orders of magnitude) than the concentration presented in the petition and by Dr. Vargas. EX1003 (Vargas Decl.), ¶¶165-166.

PeptideAtlas	
Search	All Builds
Current Build	Queries
SRMAtlas	Submission
SWATH/DIA	
Peptide	Protein
PeptideAtlas Build: Human Plasma 2023-04	
Protein Name: Q14766-4	
Blood Plasma	
Experiment Categories: ?	QUERY
Show/hide extra fields	
Q14766-4 Status: indistinguishable representative ?	
Q14766-4	
Protein Name	Q14766-4
Gene Symbol	
PEFF PName	Latent-transforming growth factor beta-binding protein 1 isoform iso 4
PEFF GName	LTBP1
PEFF NcbiTaxId	9606
PEFF TaxName	Homo Sapiens
PEFF Length	1722
PEFF SV	4
PEFF EV	207
PEFF PE	1
PEFF ModResPsi	(191 MOD:00798 half cysteine)(201 MOD:00798 half cysteine)(195 MOD:00798 half cysteine)(207 MOD:00798 half cysteine)(209 MOD:00798 half cysteine)(218 MOD:00798 half cysteine)(321 MOD:00046 O-phospho-L-serine)(403 MOD:00798 half cysteine)(413 MOD:00798 half cysteine)(407 MOD:00798 half cysteine)(419 MOD:00798 half cysteine)(421 MOD:00798 half cysteine)(430 MOD:00798 half cysteine)(559 MOD:00798 half cysteine)(581 MOD:00798 half cysteine)(568 MOD:00798 half cysteine)(594 MOD:00798 half cysteine)(582 MOD:00798 half cysteine)(597 MOD:00798 half cysteine)(630 MOD:00798 half cysteine)(641 MOD:00798 half cysteine)(636 MOD:00798 half cysteine)(650 MOD:00798 half cysteine)(652 MOD:00798 half cysteine)(665 MOD:00798 half cysteine)(679 MOD:00798 half cysteine)(702 MOD:00798 half cysteine)(689 MOD:00798 half cysteine)(714 MOD:00798 half cysteine)(703 MOD:00798 half cysteine)(717 MOD:00798 half cysteine)(704 MOD:00798 half cysteine)(729 MOD:00798 half cysteine)(787 MOD:00046 O-phospho-L-serine)(788 MOD:00798 half cysteine)(890 MOD:00798 half cysteine)(885 MOD:00798 half cysteine)(899 MOD:00798 half cysteine)(901 MOD:00798 half cysteine)(914 MOD:00798 half cysteine)(920 MOD:00798 half cysteine)(932 MOD:00798 half cysteine)(927 MOD:00798 half cysteine)(941 MOD:00798 half cysteine)(943 MOD:00798 half cysteine)(956 MOD:00798 half cysteine)(962 MOD:00798 half cysteine)(973 MOD:00798 half cysteine)(968 MOD:00798 half cysteine)(982 MOD:00798 half cysteine)(975 MOD:00035 (2S,3R)-3-hydroxyasparagine)(985 MOD:00798 half cysteine)...
PEFF ModRes	(167 N-linked (GlcNAc...) asparagine)(296 O-linked (GalNAc...) serine)(304 O-linked (GalNAc...) threonine)(326 O-linked (GalNAc...) threonine)(347 N-linked (GlcNAc...) asparagine)(357 O-linked (GlcNAc...) threonine)(378 N-linked (GlcNAc...) asparagine)(424 N-linked (GlcNAc...) asparagine)(438 O-linked (GlcNAc...) serine)(460 O-linked (GlcNAc...) serine)(461 O-linked (GlcNAc...) threonine)(463 O-linked (GlcNAc...) threonine)(467 O-linked (GlcNAc...) threonine)(469 O-linked (GlcNAc...) threonine)(470 O-linked (GlcNAc...) serine)(501 O-linked (GlcNAc...) serine)(533 O-linked (GlcNAc...) threonine)(536 O-linked (GlcNAc...) serine)(537 O-linked (GlcNAc...) threonine)(535 O-linked (GlcNAc...) serine)(520 N-linked (GlcNAc...) asparagine)(647 O-linked (GlcNAc...) serine)(768 O-linked (GlcNAc...) serine)(769 O-linked (GlcNAc...) threonine)(785 O-linked (GlcNAc...) threonine)(801 O-linked (GlcNAc...) threonine)(810 O-linked (GlcNAc...) serine)(938 O-linked (GlcNAc...) serine)(1020 O-linked (GlcNAc...) serine)(1060 O-linked (GlcNAc...) serine)(1142 O-linked (GlcNAc...) asparagine)(1164 N-linked (GlcNAc...) asparagine)(1198 N-linked (GlcNAc...) asparagine)(1225 O-linked (GlcNAc...) asparagine)(1334 O-linked (GlcNAc...) threonine)(1367 N-linked (GlcNAc...) asparagine)(1367 N-linked (GlcNAc...) (paucimannose) asparagine)(1367 N-linked (GlcNAc...) (complex) asparagine)(1489 O-linked (GlcNAc) serine)(1688 O-linked (GlcNAc) serine)
PEFF VariantSimple	(3 R)(7 M)(7 T)(8 S)(9 W)(10 F)(13 C)(13 S)(14 E)(14 G)(14 V)(18 P)(18 S)(20 L)(22 Y)(23 R)(23 S)(24 G)(27 W)(28 L)(28 V)(30 F)(30 H)(31 L)(31 M)(32 A)(33 Q)(34 Q)(34 R)(35 D)(37 S)(42 T)(42 V)(43 F)(44 S)(45 V)(47 R)(47 W)(49 L)(49 R)(50 H)(50 P)(53 R)(56 I)(58 I)(60 D)(62 C)(63 G)(64 S)(65 N)(67 E)(67 V)(69 P)(71 T)(71 V)(76 Y)(77 A)(78 R)(79 F)(79 G)(81 I)(81 P)(83 K)(84 A)(84 I)(84 S)(85 L)(87 M)(88 N)(90 A)(92 D)(92 S)(94 G)(95 M)(96 H)(98 H)(100 L)(100 Q)(100 S)(100 T)(101 L)(101 S)(102 L)(104 R)(104 S)(105 L)(105 Q)(106 S)(107 D)(107 Q)(108 A)(109 N)(110 L)(112 V)(113 D)(113 H)(117 H)(119 Q)(120 L)(121 D)(121 H)(121 S)(122 A)(122 T)(123 A)(123 D)(123 S)(124 D)(125 Q)(126 L)(129 P)(129 V)(130 Q)(130 R)(131 Y)(133 R)(134 R)(135 R)(136 G)(136 S)(137 E)(138 I)(140 H)(140 S)(141 F)(141 P)(142 E)(142 M)(142 R)(143 M)(144 R)(144 S)(145 E)(145 K)(145 L)(146 D)(146 K)(146 Q)(147 S)(148 H)(148 R)(149 N)(151 V)(153 A)(153 C)(153 P)(154 G)(154 T)(155 M)(157 A)(158 Y)(159 R)(160 R)(163 P)(165 R)(166 I)(166 L)(167 H)(167 S)(168 I)(171 R)(172 P)(172 W)(175 L)(178 G)(178 R)(179 N)(180 G)(180 P)(180 V)(183 F)(183 P)(184 P)(184 R)(185 K)(186 G)(186 R)(190 N)(191 S)(193 L)(193 S)(194 L)(195 I)(195 P)(196 E)(196 L)(197 D)(199 E)(199 R)(203 Q)(203 W)(204 R)(205 R)(206 F)(207 R)(208 L)(210 E)(211 L)(214 R)(215 A)(216 I)(217 D)(217 V)(220 A)(221 K)(221 L)(221 M)(221 V)(225 G)(226 A)(227 I)(227 L)(227 T)(228 L)(229 S)(230 I)(232 I)(234 I)(236 A)(238 T)(239 D)(239 T)(239 V)...
PEFF VariantComplex	(8 W)(9 W)(89 S)(97 I)(96 Q)(100 PP)(104 I)(106 I)(105 I)(106 I)(106 PP)(106 I)(106 PP)(106 I)(106 PP)(124 I)(124 G)(124 H)(124 P)(124 Q)(124 R)(124 S)(124 T)(124 V)(124 W)(124 X)(124 Y)(124 Z)(124 AA)(124 AB)(124 AC)(124 AD)(124 AE)(124 AF)(124 AG)(124 AH)(124 AI)(124 AJ)(124 AK)(124 AL)(124 AM)(124 AN)(124 AO)(124 AP)(124 AQ)(124 AR)(124 AS)(124 AT)(124 AU)(124 AV)(124 AW)(124 AX)(124 AY)(124 AZ)(124 BA)(124 BB)(124 BC)(124 BD)(124 BE)(124 BF)(124 BG)(124 BH)(124 BI)(124 BJ)(124 BK)(124 BL)(124 BM)(124 BN)(124 BO)(124 BP)(124 BQ)(124 BR)(124 BS)(124 BT)(124 BU)(124 BV)(124 BW)(124 BX)(124 BY)(124 BZ)(124 CA)(124 CB)(124 CC)(124 CD)(124 CE)(124 CF)(124 CG)(124 CH)(124 CI)(124 CJ)(124 CK)(124 CL)(124 CM)(124 CN)(124 CO)(124 CP)(124 CQ)(124 CR)(124 CS)(124 CT)(124 CU)(124 CV)(124 CW)(124 CX)(124 CY)(124 CZ)(124 DA)(124 DB)(124 DC)(124 DD)(124 DE)(124 DF)(124 DG)(124 DH)(124 DI)(124 DJ)(124 DK)(124 DL)(124 DM)(124 DN)(124 DO)(124 DP)(124 DQ)(124 DR)(124 DS)(124 DT)(124 DU)(124 DV)(124 DW)(124 DX)(124 DY)(124 DZ)(124 EA)(124 EB)(124 EC)(124 ED)(124 EE)(124 EF)(124 EG)(124 EH)(124 EI)(124 EJ)(124 EK)(124 EL)(124 EM)(124 EN)(124 EO)(124 EP)(124 EQ)(124 ER)(124 ES)(124 ET)(124 EU)(124 EV)(124 EW)(124 EX)(124 FY)(124 FZ)(124 GA)(124 GB)(124 GC)(124 GD)(124 GE)(124 GF)(124 GG)(124 GH)(124 GI)(124 GJ)(124 GK)(124 GL)(124 GM)(124 GN)(124 GO)(124 GP)(124 GQ)(124 GR)(124 GS)(124 GT)(124 GU)(124 GV)(124 GW)(124 GX)(124 GY)(124 GZ)(124 HA)(124 HB)(124 HC)(124 HD)(124 HE)(124 HF)(124 HG)(124 HI)(124 HJ)(124 HK)(124 HL)(124 HM)(124 HN)(124 HO)(124 HP)(124 HQ)(124 HR)(124 HS)(124 HT)(124 HU)(124 HV)(124 HW)(124 HX)(124 HY)(124 HZ)(124 IA)(124 IB)(124 IC)(124 ID)(124 IE)(124 IF)(124 IG)(124 IH)(124 II)(124 IJ)(124 IK)(124 IL)(124 IM)(124 IN)(124 IO)(124 IP)(124 IQ)(124 IR)(124 IS)(124 IT)(124 IU)(124 IV)(124 IW)(124 IX)(124 IY)(124 IZ)(124 JA)(124 JB)(124 JC)(124 JD)(124 JE)(124 JF)(124 JG)(124 JH)(124 JI)(124 JJ)(124 JK)(124 JL)(124 JM)(124 JN)(124 JO)(124 JP)(124 JQ)(124 JR)(124 JS)(124 JT)(124 JU)(124 JV)(124 JW)(124 JX)(124 JY)(124 JZ)(124 KA)(124 KB)(124 KC)(124 KD)(124 KE)(124 KF)(124 KG)(124 KH)(124 KI)(124 KL)(124 KM)(124 KN)(124 KO)(124 KP)(124 KQ)(124 KR)(124 KS)(124 KT)(124 KU)(124 KV)(124 KW)(124 KX)(124 KY)(124 KZ)(124 LA)(124 LB)(124 LC)(124 LD)(124 LE)(124 LF)(124 LG)(124 LH)(124 LI)(124 LJ)(124 LK)(124 LL)(124 LM)(124 LN)(124 LO)(124 LP)(124 LQ)(124 LR)(124 LS)(124 LT)(124 LU)(124 LV)(124 LW)(124 LX)(124 LY)(124 LZ)(124 MA)(124 MB)(124 MC)(124 MD)(124 ME)(124 MF)(124 MG)(124 MH)(124 MI)(124 MJ)(124 MK)(124 ML)(124 MN)(124 MO)(124 MP)(124 MQ)(124 MR)(124 MS)(124 MT)(124 MU)(124 MV)(124 MW)(124 MX)(124 MY)(124 MZ)(124 NA)(124 NB)(124 NC)(124 ND)(124 NE)(124 NF)(124 NG)(124 NH)(124 NI)(124 NJ)(124 NK)(124 NL)(124 NM)(124 NO)(124 NP)(124 NQ)(124 NR)(124 NS)(124 NT)(124 NU)(124 NV)(124 NW)(124 NX)(124 NY)(124 NZ)(124 OA)(124 OB)(124 OC)(124 OD)(124 OE)(124 OF)(124 OG)(124 OH)(124 OI)(124 OJ)(124 OK)(124 OL)(124 OM)(124 ON)(124 OO)(124 OP)(124 OQ)(124 OR)(124 OS)(124 OT)(124 OU)(124 OV)(124 OW)(124 OX)(124 OY)(124 OZ)(124 PA)(124 PB)(124 PC)(124 PD)(124 PE)(124 PF)(124 PG)(124 PH)(124 PI)(124 PJ)(124 PK)(124 PL)(124 PM)(124 PN)(124 PO)(124 PP)(124 PQ)(124 PR)(124 PS)(124 PT)(124 PU)(124 PV)(124 PW)(124 PX)(124 PY)(124 PZ)(124 QA)(124 QB)(124 QC)(124 QD)(124 QE)(124 QF)(124 QG)(124 QH)(124 QI)(124 QJ)(124 QK)(124 QL)(124 QM)(124 QN)(124 QO)(124 QP)(124 QQ)(124 QR)(124 QS)(124 QT)(124 QU)(124 QV)(124 QW)(124 QX)(124 QY)(124 QZ)(124 RA)(124 RB)(124 RC)(124 RD)(124 RE)(124 RF)(124 RG)(124 RH)(124 RI)(124 RJ)(124 RK)(124 RL)(124 RM)(124 RN)(124 RO)(124 RP)(124 RQ)(124 RS)(124 RT)(124 RU)(124 RV)(124 RW)(124 RX)(124 RY)(124 RZ)(124 SA)(124 SB)(124 SC)(124 SD)(124 SE)(124 SF)(124 SG)(124 SH)(124 SI)(124 SJ)(124 SK)(124 SL)(124 SM)(124 SN)(124 SO)(124 SP)(124 SQ)(124 SR)(124 SS)(124 ST)(124 SU)(124 SV)(124 SW)(124 SX)(124 SY)(124 SZ)(124 TA)(124 TB)(124 TC)(124 TD)(124 TE)(124 TF)(124 TG)(124 TH)(124 TI)(124 TJ)(124 TK)(124 TL)(124 TM)(124 TN)(124 TO)(124 TP)(124 TQ)(124 TR)(124 TS)(124 TT)(124 TU)(124 TV)(124 TW)(124 TX)(124 TY)(124 TZ)(124 UA)(124 UB)(124 UC)(124 UD)(124 UE)(124 UF)(124 UG)(124 UH)(124 UI)(124 UJ)(124 UK)(124 UL)(124 UM)(124 UN)(124 UO)(124 UP)(124 UQ)(124 UR)(124 US)(124 UT)(124 UU)(124 UV)(124 UW)(124 UX)(124 UY)(124 UZ)(124 VA)(124 VB)(124 VC)(124 VD)(124 VE)(124 VF)(124 VG)(124 VH)(124 VI)(124 VJ)(124 VK)(124 VL)(124 VM)(124 VN)(124 VO)(124 VP)(124 VQ)(124 VR)(124 VS)(124 VT)(124 VU)(124 VV)(124 VW)(124 VX)(124 VY)(124 VZ)(124 WA)(124 WB)(124 WC)(124 WD)(124 WE)(124 WF)(124 WG)(124 WH)(124 WI)(124 WJ)(124 WK)(124 WL)(124 WM)(124 WN)(124 WO)(124 WP)(124 WQ)(124 WR)(124 WS)(124 WT)(124 WU)(124 WV)(124 WW)(124 WX)(124 WY)(124 WZ)(124 XA)(124 XB)(124 XC)(124 XD)(124 XE)(124 XF)(124 XG)(124 XH)(124 XI)(124 XJ)(124 XK)(124 XL)(124 XM)(124 XN)(124 XO)(124 XP)(124 XQ)(124 XR)(124 XS)(124 XT)(124 XU)(124 XV)(124 XW)(124 XX)(124 XY)(124 XZ)(124 YA)(124 YB)(124 YC)(124 YD)(124 YE)(124 YF)(124 YG)(124 YH)(124 YI)(124 YJ)(124 YK)(124 YL)(124 YM)(124 YN)(124 YO)(124 YP)(124 YQ)(124 YR)(124 YS)(124 YT)(124 YU)(124 YV)(124 YW)(124 YX)(124 YY)(124 YZ)(124 ZA)(124 ZB)(124 ZC)(124 ZD)(124 ZE)(124 ZF)(124 ZG)(124 ZH)(124 ZI)(124 ZJ)(124 ZK)(124 ZL)(124 ZM)(124 ZN)(124 ZO)(124 ZP)(124 ZQ)(124 ZR)(124 ZS)(124 ZT)(124 ZU)(124 ZV)(124 ZW)(124 ZX)(124 ZY)(124 ZZ)
PEFF Processed	(1 23 PEFF:0001021 signal peptide)(24 1722 PEFF:0001020 mature protein)
Identification Status	indistinguishable representative
ProteinProphet Probability	1.00000
Mult Hyp Testing Probability	0.00000
Estimated concentration	220.0 ng/ml (10x uncertainty)
Distinct Peptides	192
Total Observations	16958
Normalized PSMs per 100K	4.695
GO biological_process	GO:0007179:transforming growth factor beta receptor signaling pathway, GO:0008150:biological_process
GO cellular_component	GO:0005578:proteinaceous extracellular matrix, GO:0005576:extracellular region
GO molecular_function	GO:0005024:transforming growth factor beta receptor activity, GO:0019838:growth factor binding, GO:0005509:calcium ion binding, GO:0005488:binding

99. The concentration of LTBP-1 from Peptide Atlas is much closer to the concentration the petition and Dr. Vargas identify for serum albumin (35 to 52 mg/mL). EX1003 (Vargas Decl.), ¶164. For example, the LTBP-1 value listed in Peptide Atlas (220 ng/mL) converts to 0.00022 mg/mL, or 2.2×10^{-7} g/mL. Even taking the highest number Dr. Vargas cites to as what is “normally present” for serum albumin (52 mg/mL), this converts to 5.2×10^{-2} g/mL. The difference

between 5.2×10^{-2} and 2.2×10^{-7} g/mL is about five orders of magnitude—not “at least 6 orders of magnitude” as recited in the challenged claims.

100. In summary, Hu does not disclose protein concentrations, and the petition materials do not establish concentrations in Hu for the identified proteins (serum albumin and LTBP-1), regardless of whether those proteins were indeed detected. Furthermore, the petition materials mischaracterize the disclosures of Ahmed and Cao, and fail to establish “normally present” concentrations for the identified proteins.

iii. Hu Obviousness Fails.

101. Having admitted that Hu lacks express disclosure of the dynamic range limitation of the claims, the petition and Dr. Vargas make unsubstantiated alternate arguments that “a POSA would have...found it obvious.” Pet., 37-39; *see also* EX1003 (Vargas Decl.), ¶¶167-170. The petition improperly conflates knowledge of a wide disparity between protein concentrations in plasma with missing disclosure of using mass spectrometry to assay the biological sample across such a dynamic range. The argument is also misplaced when it discusses mass spectrometry generally, as opposed to discussion of Hu’s shotgun proteomics approach as relied upon in Ground 1.

102. The petition and Dr. Vargas ignore their own submitted references, which discuss difficulties and inaccuracies of prior mass spectrometry analysis of

protein concentrations across a broad dynamic range. *See, e.g.*, EX1010 (Hajipour), 1218 (discussing complexity that “hampers the identification of low abundant proteins”); EX1006 (Walkey), 2451 (“Using spectral counting to estimate the relative abundance of low-abundance proteins within complex mixtures is ***inaccurate***”); EX1015 (Ahmed), 786 (the technical challenge is that “the complexity of plasma/serum is so overwhelming that important biological information will be in background noise and ***will not be detected by current available MS technologies***”); EX1007 (Zaccaria), 1869-1870 (discussing “the limited dynamic range” of prior methods rendering “the low-abundant protein compartment where disease-associated biomarkers are thought to reside [as] still ***unattainable***”). *See also* Section III, above (discussing EX2017-EX2021). Hu implements a threshold that limits its analysis to no more than two orders of magnitude (MWSpC range of 1-10). EX1008, 360, Table 2 (same), Fig. 6 Caption (discarding MWSpC < 1).

103. The petition and Dr. Vargas then cite to the '360 patent itself as though to use it as a guide. E.g., EX1003 (Vargas Decl.), ¶¶169-173. Elsewhere, the petition argues that there are overlapping ranges at issue in comparing the claims to the prior art, but it is my opinion that the petition never established the existence of an overlapping prior art range. Finally, insofar as the petition and Dr. Vargas rely on prior art shotgun proteomics approaches to disclose this claim

feature, it fails because the literature (e.g., EX1010, 1218; EX1006, 2451; EX1015, 786; EX1007, 1869-1870; Section III, above) explained that prior shotgun proteomics approaches were “inaccurate” and “unattainable” for assaying across such a dynamic range.

104. For each of the reasons discussed above, the petition and Dr. Vargas fail establish that Hu renders obvious the challenged claims.

D. Dependent Claims

105. The petition and Dr. Vargas argue that dependent claims 2-4, 6-7, 17 and 22-25 are obvious for the largely “same reasons” set forth for independent claim 1. Pet. 39-46; EX1003 (Vargas Decl.), ¶¶174-191. As discussed above, the petition fails to establish Hu renders claim 1 obvious.

106. For claim 23, each of the petition and Dr. Vargas confuse a lipoprotein as recited in the claim with “Apolipoproteins.” Apolipoproteins are proteins, whereas a lipoprotein is a complex of lipids and proteins. EX2028 (Voet), 631-632. A lipoprotein is not the same as an apolipoprotein.

VIII. ZACCARIA’S STUDY DOES NOT DISCLOSE ALL ELEMENTS

107. The Vargas Declaration and petition opine that Zaccaria (EX1007) renders obvious claims 1, 2, 4, 6-7, 17, 22, 23, and 25 of the ’360 Patent. EX1003 (Vargas Decl.), ¶242. I disagree. A POSA would not have read Zaccaria (EX1007) as disclosing or making obvious any of the challenged claims.

A. Zaccaria Fails to Disclose Assaying “A Biological Sample” As Claimed.

108. The petition and Dr. Vargas fail to demonstrate that Zaccaria discloses the claimed “method for assaying a biological sample.” Pet. 64-66; EX1003 (Vargas Decl.), ¶¶243-245. Zaccaria’s study (like Hu’s) combines samples taken from multiple different individuals that are mixed prior to assay. EX1007 (Zaccaria), 1870-1871. As indicated above, Dr. Vargas identified a definition in the challenged patent, but does not explain how Zaccaria’s mixture is “a biological sample” as defined by the ’360 Patent.

B. Zaccaria Fails to Disclose Separating a Subset of the Plurality of Particles Comprising the Coronas As Required By 1(b).

109. Both the petition and Dr. Vargas fail to demonstrate Zaccaria discloses separating the claimed “subset of the plurality of particles comprising the coronas” as required by all challenged claims. Pet. 71-72; EX1003 (Vargas Decl.), ¶¶261-266. The claims require the separated subset to have both (1) different particle types and (2) coronas corresponding to the different particle types comprising overlapping and distinct proteins. Section V.B, above.

110. The petition and Dr. Vargas assert that a given particle type in Zaccaria was “separately incubated in duplicates” in RBC lysate, washed extensively “to remove all unbound proteins, leaving only bound proteins,” and the particles in the vial were then “separated” from the biological sample using an

external magnet to hold them in place while the supernatant was removed. Pet., 71; EX1003 (Vargas Decl.), ¶¶261-266. But the identified subset of particles in Zaccaria comprises only one corona—not the required **coronas** having overlapping and distinct proteins. Section V.B, above; EX2022 (Vargas Depo. Tr.), 39:12-40:1 (“Three separate containers.”), 41:5-17 (separately incubated in duplicates). I agree with Dr. Vargas that the separation step in Zaccaria generates a subset of particles comprising only the corona of a single particle type. EX2022 (Vargas Depo. Tr.), 42:16-43:9, 45:10-50:12, 41:8-12. Zaccaria never describes separating a subset of particles comprising coronas from multiple particle types. The subset of particles separated during the pull-down thus comprises only the corona from a single particle type, not multiple **coronas** with overlapping and distinct proteins as required *Id.*, 46:2-50:12, 99:3-15. Accordingly, Zaccaria fails to describe separating a “subset of the plurality of particles comprising **the coronas**,” which have overlapping and distinct proteins, as required by all challenged claims.

C. Zaccaria Fails to Disclose Detecting Proteins At Concentrations Across a Dynamic Range Comprising At Least 6 Orders of Magnitude As Claimed

111. Step (c) requires that the assay must “detect, in the subset, proteins in the biological sample at concentrations across a dynamic range comprising at least 6 orders of magnitude, thereby assaying the biological sample.” The petition and

Dr. Vargas fail to demonstrate Zaccaria discloses this limitation. Pet. 73-77, EX1003 (Vargas Decl.), ¶¶267, 273.

112. The petition and Dr. Vargas each argue element 1(c) is satisfied by Zaccaria based on two specific proteins (Hb and SBF1) mentioned in Zaccaria's supplementary information. They admit, however, that Zaccaria has no express disclosure of protein concentrations or a dynamic range. Pet., 74, EX1003 (Vargas Decl.), ¶¶270-273, EX2022 (Vargas Depo. Tr.), 76:19-25. Instead, Dr. Vargas and the petition allege inherent disclosure, arguing those proteins are "normally present in RBC lysate" at concentrations spanning at least 6 orders of magnitude. But just as in Ground 1, Ground 4 cherry-picks an allegedly low abundant protein (SBF1) from the supplemental information and presents it without critical review. The petition and Dr. Vargas do not demonstrate that Zaccaria actually detected SBF1, rather than merely reporting non-replicable signal indistinguishable from experimental artifact or background. Zaccaria itself reports that the identified signal could not be mapped to a single protein, and instead mapped to multiple different accession numbers (e.g., different numbers identifying different proteins in a database). The petition overlooks this entirely. As discussed below, the petition and Dr. Vargas fail to demonstrate the identified concentrations of these proteins are necessarily present in Zaccaria.

i. The Petition Fails to Prove SBF1 Was Actually Detected by Zaccaria At The Asserted Concentration

113. The petition and Dr. Vargas argue that Zaccaria's shotgun proteomics assay detected the SBF1 protein, which it claims is a "lower abundant" protein that is "normally present" at a concentration of 6.62×10^{-6} pmol/mg. Pet. 74; EX1003 (Vargas Decl.), ¶271. But they fail to demonstrate that Zaccaria actually detected SBF1, rather than reporting non-replicable #MS/MS indistinguishable from experimental artifact or noise.

114. As discussed above (Section III), shotgun proteomics approaches like that applied in Zaccaria extensively degrade proteins into small peptide fragments, acquire spectral data, and analyze the raw mass spectral data to make "predictions" of the "theoretical peptides possible". EX1003 (Vargas Decl.), ¶39 ("fragmentation predictions"). POSAs viewed non-replicable #MS/MS and corresponding matches to a database as likely experimental error or artifact, rather than a reliable detection event. EX2022 (Vargas Depo. Tr.), 43:20-44:7. A POSA would more reasonably view Zaccaria's mention of SBF1 as indicating a level of detection/specificity that was inadequate to detect (and did not in fact detect) this protein. A POSA would be highly skeptical that Zaccaria had actually detected SBF1. As explained in further detail below, a POSA would more reasonably conclude Zaccaria is simply reporting non-reproducible #MS/MS (and corresponding potential match to a database) that did not meet the threshold of detection.

115. The below image shows the column headings from page 25 and the entry from page 28 of EX1007 (Zaccaria), providing a summary of what was actually reported in Zaccaria’s supplemental information. See also, EX2009 (accurately summarizing this information), EX2022 (Vargas Depo. Tr.), 69:17-22. Annotations indicate the particle type employed and the number of replicates attempted. As shown in the image from EX2009 below, of six attempts on three different particles, four of those attempts failed to even indicate any #MS/MS at all.

AC	ID	MW	pI	Description	Fractions	Score	#Peptides	#Sequences	Coverage (%)	#MS/MS
O95248	MTMR5_HUMAN	210294.08	6.46	Myotubularin-related protein 5 OS=Homo sapiens GN=SBF1 PE=1 SV=3						

Replicate 1						Q-Treated						Replicate 2					
Fractions	Score	#Peptides	#Sequences	Coverage (%)	#MS/MS	Fractions	Score	#Peptides	#Sequences	Coverage (%)	#MS/MS	Fractions	Score	#Peptides	#Sequences	Coverage (%)	#MS/MS
GR_Q1	223.78	2	2	1	10												

Replicate 1						OS-Treated						Replicate 2					
Fractions	Score	#Peptides	#Sequences	Coverage (%)	#MS/MS	Fractions	Score	#Peptides	#Sequences	Coverage (%)	#MS/MS	Fractions	Score	#Peptides	#Sequences	Coverage (%)	#MS/MS

Replicate 1						PAA-Treated						Replicate 2					
Fractions	Score	#Peptides	#Sequences	Coverage (%)	#MS/MS	Fractions	Score	#Peptides	#Sequences	Coverage (%)	#MS/MS	Fractions	Score	#Peptides	#Sequences	Coverage (%)	#MS/MS
												GR_PAA2	149.65	7	7	3.4	13

116. As shown in the image and reported in Zaccaria, in no instance does Zaccaria’s table report #MS/MS matched to SBF1 and replicated in duplicate for any tested particle. For the few non-replicated instances where signal was reported, it was exceptionally weak. Only a few peptide fragments were detected with minute coverage of any ostensibly predicted protein (e.g., Replicate 1 for the “Q-

Treated” reporting 2 peptide fragments and 1% coverage). Furthermore, Zaccaria reports a very low number of MS “hits” registered and used (e.g., software and algorithms) for protein prediction (e.g., Replicate 1 for the “Q-Treated” reporting #MS/MS of 10). By comparison, the proteins discussed in Zaccaria Table 2 report #MS/MS typically at least an order of magnitude larger. Such a small number gives little confidence of accurate assignment to SBF1, as opposed to noise or error, including misassignment of fragments originating from a different protein.

117. Zaccaria explains the SBF1 label was arbitrarily applied as just one of several potential accession numbers that might be associated with the signal. Different accession numbers are assigned to different proteins in a database. Specifically, Zaccaria states that “if several proteins match the same set of peptides, only one member of the protein group is indicated in” the lists Petitioners rely on. EX1007 (Zaccaria), 17; *see also id.*, 1872 (“When several proteins matched exactly the same set of peptides, only one member of the protein group was reported in the final list.”). Though Zaccaria identifies SBF1 as one potential match (AC identifier O95248) for the signal Petitioners relied upon, Zaccaria discloses elsewhere that multiple AC identifiers were actually listed for the signal. EX1007, 86 (“Other ACs” include A6PVG8). The same issue exists with Zaccaria’s identification of other proteins, like PIK3R4 (cited by the petition only against dependent claim 7). *See* EX2022 (Vargas Depo. Tr.), 74:3-75:22, EX2010.

EX2014 and EX2015 (reproduced below) accurately present the information from page 86 of EX1007 where Zaccaria identified the variety of different accession numbers with which the signal potentially could be associated.



AC	Other ACs	MW
O95248	A6PVG8	210294

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EX2014;



AC	Other ACs	MW
A0JP11	B2RAJ6, Q995	154373

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EX2015; *see also* EX2022 (Vargas Depo. Tr.), 58:21-24 (Dr. Vargas not knowing what AC stands for).

118. While the petition assumes that Zaccaria detected SBF1, it fails to demonstrate actual detection.

ii. The “Normally Present” Argument Does Not Make Sense.

119. Since Zaccaria does not expressly disclose a dynamic range or sample protein concentrations, Dr. Vargas and the petition each argue inherency, relying on Bryk (EX1009). Pet. 74-75; EX1003 (Vargas Decl.), ¶¶271-273. The petition and Dr. Vargas specifically identify two particular proteins—HBA1 and SBF1—which they argue are “normally present” at identified concentrations that differ by at least six orders of magnitude. This argument fails for several reasons.

120. First, the petition and Dr. Vargas cite to Bryk, but this reference does not establish concentrations for HBA1 and SBF1 in Zaccaria. Concentrations of different proteins vary considerably among different individuals. EX1001, 56:35-38. Bryk does not address Zaccaria’s hemolysate testing or even discuss Zaccaria. Instead, the petition and Dr. Vargas’ argument admits that different concentrations (or no concentration at all) of the identified SBF1 and PIK3R4 proteins are expected in some subjects.

121. Dr. Vargas and the petition rely on Bryk for reporting 6.62×10^{-6} pmol/mg of SBF1. EX1003, ¶271. But they cherry-picked from Bryk “the lowest non-zero detected concentration” of SBF1 in Bryk’s table. EX1003, ¶271 n.6; EX2022 (Vargas Depo. Tr.), 77:1-77:5 (confirming the same). Other entries in Bryk indicate “zero” detected concentration. Bryk therefore demonstrates that—contrary to Dr. Vargas and the petition’s argument—what is “normally present” in some donors was an apparent absence of the proteins. EX2011 (shown above); *see also* EX2022 (Vargas Depo. Tr.) 83:3-87:15. Rather than supporting the petition and Dr. Vargas’ argument that the identified concentrations are “normally present,” Bryk confirms the relied-upon proteins may not be detectable or present at all in a sample—including Zaccaria’s sample.

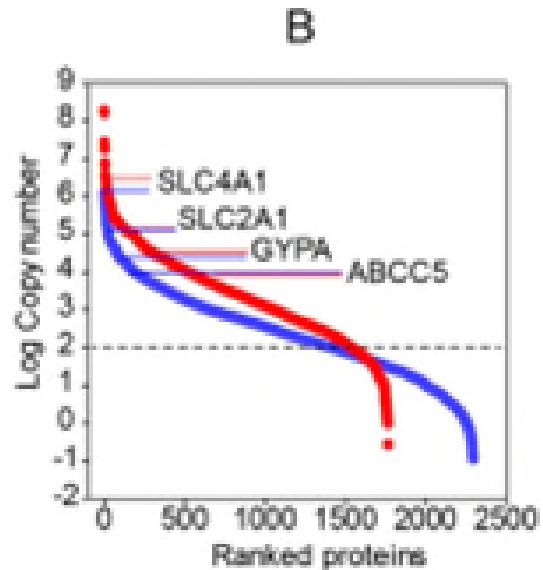
SBF1
EX1009 p. 51

PIK3R4
EX1009 p. 44

Whole erythrocyte			
Concentration (pmol/mg) Donor 1	Concentration (pmol/mg) Donor 2	Concentration (pmol/mg) Donor 3	Concentration (pmol/mg) Donor 4
1.56E-03	0.00E+00	6.62E-06	2.80E-04
3.17E-04	0.00E+00	0.00E+00	0.00E+00

122. Additionally, Bryk discarded **all the SBF1 results** because the number of copies per cell (16.9, 0.0, 0.1, and 3.0) always fell well below its 100-copies “sample purity threshold.” EX1009, 51, 2756

& Fig. 3 Caption (“(B) Distribution of protein copy numbers across all proteins in whole cells (red circles) and WG fractions (blue circles”). Likewise, Bryk discarded all the PIK3R4 results for the same reason. *Id.*, 44 (3.4, 0.0, 0.0, and 0.0 copies). The dashed line of Bryk’s Fig. 3 “shows **the 100 copy**



per cell sample purity threshold.” (reproduced right). As shown in Figure 3, Bryk imposed a horizontal line to depict the threshold below which trace results were discarded. As Bryk explained:

Because a minute contamination of the erythrocyte preparations by other blood cells cannot be avoided **we consider proteins above a threshold of 100 copies as those occurring in erythrocytes**. However, we do not rule out the possibility that some proteins with abundances below this limit may also originate from RBCs. In addition **we cannot exclude contamination** of the RBC and their membrane preparations **by plasma proteins**. For example, the most abundant plasma protein, **albumin, was present at several thousand copies**.

EX1009 (Bryk), 2755.

123. Bryk does not establish protein concentrations in Zaccaria's tested material because it is a different reference that employed different methodologies on different samples. EX1015 (Ahmed), 786 (discussing that even "slight changes in a given sample preparation could lead to very different protein profiles"). Dr. Vargas confirmed at her deposition that numerous differences existed in the preparation protocol employed in Zaccaria as compared to Bryk. EX2022 (Vargas Depo. Tr.), 51:11-54:4. I agree. A POSA could thus not read Bryk's supplemental materials as corresponding to results expected from Zaccaria's hemolysate.

124. The petition and Dr. Vargas' remaining arguments do not support obviousness and do not cure the deficiencies I discussed above. Pet. 75-77; EX1003 (Vargas Decl.), ¶¶274-279. The petition and Dr. Vargas argue that Zaccaria is "consistent" with the problem the '360 patent solves. Pet., 76; EX1003 (Vargas Decl.), ¶277. But, as I explained above, this does not demonstrate the art (including Zaccaria) discloses what is claimed. Additionally, as with the Hu ground above, no prior art range of values has been established.

125. Therefore, for at least the reasons discussed above, the petition and Dr. Vargas fail to establish that Zaccaria renders obvious the challenged claims.

D. Dependent Claims

126. The petition and Dr. Vargas argue that dependent claims 2, 4, 6-7, 17, 22-23 and 25 are obvious for the largely “same reasons” set forth for independent claim 1. Pet. 77-80; EX1003 (Vargas Decl.), ¶¶280-291.

127. As discussed above, the petition fails to establish Zaccaria renders claim 1 obvious. Additionally regarding claim 2, the unit conversions do not cure the deficiencies discussed regarding claim 1.

128. For claim 7, the petition and Dr. Vargas rely on the PIK3R4 protein. As discussed above, the petition fails to demonstrate Zaccaria detected this protein, let alone at the concentration identified. Section VIII.C.

129. For claim 23, the petition confuses a lipoprotein as recited in the claim with “Apolipoproteins” as discussed above. Section VII.D.

IX. CONCLUSIONS

130. In signing this declaration, I recognize that the declaration will be filed as evidence in a contested case before the Patent Trial and Appeal Board of the United States Patent and Trademark Office.

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

Executed July 24, 2025.

/Michael J. Cima/
Michael J. Cima, Ph.D.