

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

**PATENT OWNER'S RESPONSE
PURSUANT TO 37 C.F.R. § 42.120**

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I. INTRODUCTION

All petition challenges (based on Cai, Hu, or Zaccaria, respectively) rely on mass spectrometry (MS) signals recorded from what both the scientific literature and Petitioners' expert calls the "shotgun" approach to MS proteomics. In a shotgun proteomic approach, a protein source is extensively fragmented, the fragments are analyzed by MS, and the mass spectral data is analyzed algorithmically to make "predictions" of the "theoretical peptides possible." EX1003, ¶39.

But the scientific literature reports that prior approaches presented only limited sensitivity/specificity as well as poor reproducibility, and significantly limited the dynamic range of proteins that could be reliably detected. Shotgun approaches were recognized as "noisy" with multiple sources of error and interference complicating accurate and reliable detection, particularly for low abundance components. The literature described a limited dynamic range in MS proteomics studies as "typically 3 orders of magnitude." Alternate methods that attempted improvement—methods not employed in *any* of the cited grounds references—were reported as increasing the dynamic range of detection to about 5 orders of magnitude. In contrast, each of the challenged claims requires, *inter alia*, assaying proteins present in the biological sample at concentrations across a dynamic range comprising at least 6 orders of magnitude. See e.g., Claim 1.

The present petition fails to demonstrate that any of the relied upon Cai, Hu or Zaccaria references defied the limited range of detection reported in the scientific literature. Indeed, none of the cited references satisfies the dynamic range of detection required by the challenged claims. For alleged unpatentability over the Cai reference (Grounds 2 and 3), the petition cites uncritically to a comment in Cai regarding generating “emPAI” values spread over eight orders of magnitude. The petition overlooks that Cai itself describes its emPAI values as a “semi-quantitative” indication of **amount** of protein on particles, not concentration at all—certainly not concentration in a “biological sample” as recited in the claims.

The petition’s obviousness arguments for Hu (Ground 1) and Zaccaria (Ground 4) fare no better. For each of these grounds, Petitioners combed through the raw data tables in the supplemental materials in search of a “low abundance” protein. With no critical analysis of these cherry-picked data entries, and admission of no disclosure in Hu or Zaccaria regarding protein concentrations, the petition advances an inherency theory based on what it argues would be “normally present.” As explained by Dr. Cima, a professor at MIT with expertise in proteomics (EX2023, ¶¶1-8), the petition fails to establish that the references detected the identified proteins in the first place, and certainly not at the identified concentrations. And, of course, the exacting standard for inherency is not met by what might “normally” be present (i.e., mere probability); instead, the inherent

disclosure must *necessarily* be present—a showing Petitioners never satisfied. As such, each ground at least fails to demonstrate prior art disclosure of assaying at the 6 orders of magnitude dynamic range as specifically recited in the challenged claims. See Claim 1(c).

As discussed in detail herein, the challenges fail for these and additional reasons. EX2023, ¶¶9-14. Once one plays archeologist with the record, as the petition burdens Patent Owner and the Board to do, the deficiencies of the present challenge become apparent. Accordingly, the present challenge should be rejected and the claims confirmed as not unpatentable.

II. TECHNOLOGICAL BACKGROUND

All petition challenges rely on MS signals recorded from what Petitioners' expert, Dr. Vargas, calls the “bottom-up or shotgun” approach to MS proteomics on which she focused her analysis. EX1003, ¶32.

In a shotgun proteomic approach, a protein source is obtained and then subjected to extensive fragmentation into smaller peptides for subsequent mass spectrometry analysis. EX2017, 1; *see also* EX1003, ¶¶32-39, EX2022, 99:16-20, EX2023, ¶¶25-26; *see generally* EX2023, ¶¶15-24. Using the MS instrument, the smaller fragments are ionized and their mass spectra recorded. The MS instrument itself does not detect or identify proteins from the original source, rather protein prediction is accomplished bioinformatically by analyzing the mass spectral data

using software (e.g., algorithmic analysis) and database resources. EX2023, ¶¶25-26, 32, EX1030, 3-4. As Dr. Vargas puts it, search algorithms and database information is used to analyze the raw mass spectral data and make “predictions” of the “theoretical peptides possible.” EX1003, ¶39 (describing “fragmentation predictions” for the possibility of a protein being present).

While shotgun approaches have been a valuable tool, researchers recognized prior approaches presented only limited sensitivity/specificity as well as poor reproducibility, significantly limiting the dynamic range of protein detection. As Dr. Cima explains, such shortcomings with shotgun approaches were widely reported in the scientific literature at the time. EX2023, ¶¶27-28, EX2017, 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, 2 (“many studies also have reported difficulties with reproducibility and accuracy when analyzing small quantities of analytes in complex samples such as biological fluids”).

While highly abundant proteins could be more reliably detected using shotgun approaches, these approaches lacked sensitivity, specificity and were prone to experimental error and/or artifacts for low abundance components. EX2017, 12 (“in shotgun proteomics, only the more highly abundant part of the proteome is reproducibly identified”), EX2019, 1820 (“low abundance components

are masked by the (bio)chemical background”), EX2020, 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.”).

Shotgun approaches were recognized as “noisy” with multiple sources of error and interference complicating accurate and reliable detection, particularly for low abundance components. EX2023, ¶¶27-28. One such source comes from the sample itself, which typically presents a highly complex mixture of various proteins present in vastly differing amounts. EX2020, 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.”). Such complex samples can further exacerbate ionic interference or suppression effects in the instrumentation, a known cause of inaccuracy on MS instrumentation. EX2023, ¶¶29-30; EX2018, 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, 13 (“ion suppression may also impair analyte detection”), EX2019, 1820 (“The nominal dynamic range of mass spectrometers can be further constrained by ion suppression effects”). Sample contamination is another source of experimental error, particularly when working with complex biological samples. EX2023, ¶31; EX2018, 3 (“Disregarding sample cleanup, especially when complex matrices are involved, will lead to poor performance.”), *id.*, 3 (Describing as a source of experimental error “exogenous substances, molecules not present in the original sample but from contamination during sample preparation”).

Bioinformatic analysis is yet another source of error, as the accuracy of protein predictions are limited by the quality and quantity of the mass spectral data, the software, and database resources. EX2023, ¶32; EX2021, 3575. High abundance proteins produce a large quantity of peptide fragments whereas low abundance proteins do not. EX2023, ¶32. Moreover, short peptide fragments commonly map back to multiple different proteins bioinformatically, thereby confounding prediction of the source protein, and databases can be limited in protein sequence information, data of different isoforms, and the like. EX2021, 3580.

As reflected in the literature, researchers were aware of limitations in shotgun approaches and several measures were employed to navigate issues of

limited sensitivity and accuracy. For one, testing that did not replicate the same result was an indication of likely experimental error or artifact rather than a detection event. EX2023, ¶¶33-34; EX2022, 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”); *see also* EX1007, 1874 (Zaccaria emphasizing the need for qualitative and quantitative reproducibility).

Additionally, recognizing noise limited reliability in a dataset, researchers restricted their analysis to what they viewed as a reasonably reliable subset of the proteomics data generated. EX2017, 12 (“in shotgun proteomics, only the more highly abundant part of the proteome is reproducibly identified”), EX2019, 1820 (describing a limited dynamic range in MS proteomics studies as “typically 3 orders of magnitude”). This typically consisted of a subset of the most highly abundant proteins predicted. EX1008, 359 & Table 2 (analysis of the “top 20 most abundant plasma proteins”; threshold of “plasma proteins with an average MWSpC ≥ 1 ”), EX1007, 1874 (“we compared the 20 most abundant identified proteins (ie, the 20 proteins with the higher MS/MS queries)”).

Additionally, as discussed in the literature, different methods were required to expand the dynamic range of detection. For example, Dr. Vargas discusses a “wide dynamic range” and “[t]argeted approaches like multiple reaction monitoring (MRM).” EX1003, ¶58. Dr. Vargas includes a block quote from the

Nanjappa reference (EX1014) discussing MRM assays. First, as explained by Dr. Cima, none of the Hu, Cai nor Zaccaria references relied upon in the petition materials employ such MRM assays. EX2023, ¶35. Dr. Vargas admits as much. EX1003, ¶32. Dr. Vargas also admitted during deposition she had not even looked at the references cited in the portion of Nanjappa she block quotes at paragraph 58 of her declaration. See e.g., EX2017 (“Lange”) cited as reference #39 and EX2019 (“Stahl-Zeng”) cited as reference #40 in Nanjappa. Stahl-Zeng describes protein detection in shotgun proteomics approaches as limited to “typically 3 orders of magnitude.” EX2019, 1810. Lange describes MRM approaches (also referred to as “selected reaction monitoring”) as improving detection to “a linear response over a wide dynamic range of up to five orders of magnitude.” EX2017, 1. Both references discuss the “limited sensitivity” and “poor reproducibility” of prior art shotgun proteomics assays. EX2017, 1; EX2019, 1810.

III. BRIEF SUMMARY OF GROUND REFERENCES

A. Hu (EX1008)

Hu evaluated protein corona formation of differently-sized Fe₃O₄ nanoparticles exposed to pooled blood plasma to “facilitate the exploration of the bio-distributions and biological fates” of the particles “in biological systems.” EX1008, Abstract, 354. For its evaluation, Hu pooled blood from 20 different

donors, which were “mixed together in order to eliminate individual differences.”

EX1008, 355.

Hu individually incubated—in replicate—a portion of its mixed plasma with nanoparticles of 30-, 200-, or 400-nm diameter. EX1008, 355; EX2022, 92:2-97:18 (Dr. Vargas confirming). Hu explicitly focused on “the top 20 most abundant plasma proteins” that formed the coronas (EX1008, Table 2, Fig. 6), as those represented plasma proteins that are most relevant to understanding the biological fate of the nanoparticles. *Id.*, 359-360 & Table 2; EX2023, ¶¶87-88. Hu discarded from further consideration plasma proteins that lacked “an average MWSpC ≥ 1 .” *Id.*, 358-360. Neither Hu nor the supplementary information provide concentrations for any proteins.

B. Cai (EX1005)

Cai evaluated the biochemical properties of protein corona formed on multi-walled carbon nanotubes (MWCNTs) and carbon black (CB). EX1005, Abstract. For its evaluation, Cai used different populations of immortalized HeLa cell lines passaged in various conditions, including populations grown in SILAC encoding media. *Id.*, 584-585.

In a first experiment, Cai individually incubated a HeLa cell lysate with either CNTs or CB. EX1005, 584, Fig. 1; EX2022, 104:10-107:8 (Dr. Vargas confirming). Isolated corona proteins were segregated and subjected to SDS-

PAGE. EX1005, Figure 1; EX2022, 125:14-127:22 (Dr. Vargas confirming no mass spectrometry).

In a separate experiment, Cai metabolically labeled what it describes as three separate populations of HeLa cells, each grown in its own isotopically-labeled media. EX1005, 586-587. Each population was contacted with only a single particle type or none at all. One labeled population was incubated with CNT particles (no CB); a different population was incubated with CB (no CNT particles); the third population was not incubated with particles at all. *Id.* A mixture of proteins was then subjected to shotgun MS. EX1005, Fig. 3, 587.

Cai calculated emPAI values for proteins in its post-pulldown SILAC mixture, but made no effort to assess the **concentrations** of proteins in any one of the starting HeLa cell lysates. EX1005, 587. The calculated emPAI values are described as “semi-quantitative” indications of abundance (not concentration) of proteins in the post-pulldown SILAC mixture. *Id.*; EX2023, ¶¶64-65.

C. Zaccaria (EX1007)

Zaccaria individually evaluated protein corona formation of differently-coated magnetic nanoparticles exposed to pooled red blood cell lysate. EX1007, Abstract, 1870. Similar to Hu, Zaccaria used pooled material from different donors. EX1007, 1870-1871.

Zaccaria individually incubated—in replicate—a portion of its pooled hemolysate with PAA-, OS- and Q-coated nanoparticles. EX1007, 1870-1871; EX2022, 45:10-46:9 (Dr. Vargas confirming). Each individual incubation was digested (extensively fragmented) and subjected to shotgun MS. EX1007, 1871; EX2022, 46:10-50:12 (Dr. Vargas explaining washing and desorption); EX2023, ¶114.

Zaccaria emphasized the importance of qualitatively and quantitatively reproducible data, characterizing proteins as reproducibly identified if the protein appeared in each sample replicate and revealed a PAI ratio close to the expected value of 1 between replicates. EX1007, 1874. Zaccaria provides its raw data in supplementary materials (EX1007, 1874), which Petitioners append to the article. EX1007, 17-120. Neither the Zaccaria article nor the supplementary material provide concentrations for any proteins.

IV. SUMMARY OF THE INVENTION

The '360 Patent recognizes the above-described prior art problems and provides a solution for achieving a wider dynamic range. The patent explains that the dynamic range available for proteomic assay using mass spec was limited by error and confounding noise. The specification explains, for example, that “the sensitivity and specificity of mass spectrometry approaches” to proteomic analyses before the challenged patent “have not been adequate...due to the high noise

created by the 10000 of proteins comprising the human proteome” and the wide disparity (e.g., 10-orders of magnitude difference, 35 mg/ml vs. 1 pg/ml) of protein concentrations expected in blood. EX1001, 19:19-27. The ’360 Patent explains, as “[c]ompared with previous approaches limited to the surface of a single nanoparticle,” sampling a complex biological sample “using multi-nanoparticles with different physicochemical properties...effectively reduces the noise in the vast proteomic information available, yielding more-accurate early differentiation of the proteomic signature that is characteristic of a disease” without protein depletion. EX1001, 19:61-20:10.

Claim 1 recites the following:

1. A method for assaying a biological sample, comprising:

(a) contacting the biological sample with a plurality of particles comprising different particle types to permit biomolecules of the biological sample to bind to the plurality of particles and form coronas around the plurality of particles, wherein the coronas corresponding to the different particle types (i) differ based on particle type, and (ii) comprise overlapping and distinct proteins;

(b) separating at least a subset of the plurality of particles comprising the coronas from the biological sample by removing the subset of the plurality of particles thereby producing a subset of proteins from the biological sample;

(c) assaying the subset of proteins of (b) with an instrument 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.

V. CLAIM CONSTRUCTION

In an *inter partes* review, claim terms “shall be construed using the same claim construction standard that would be used to construe the claim in a civil action under 35 U.S.C [§] 282(b). 37 C.F.R. §42.100(b); *Phillips v. AWH Corp.*, 415 F.3d 1303, 1312 (Fed. Cir. 2005) (en banc). Petitioners offer no constructions. Pet. 14-15. However, Petitioners’ challenges fail to give effect to the claim terms “a biological sample” and “a subset of the plurality of particles comprising the coronas.”

A. “a biological sample”

As an initial matter, the petition fails to give effect to what both parties’ experts agree is an express definition in the specification for the claim term “a biological sample.” Claims will be understood according to an express definition provided in the specification of the patent. *Southwall Techs., Inc. v. Cardinal IG Co.*, 54 F.3d 1570, 1578 (Fed. Cir. 1995) (“[T]he definition in the patent documents controls the claim interpretation.”); *Phillips*, 415 F.3d at 1321 (“the specification ‘acts as a dictionary when it expressly defines terms used in the claims or when it defines terms by implication.’”); *Volkswagen Group of Am., Inc.*

v. *Mich. Motor Techs., LLC*, IPR2020-00229 Paper 13, 9-12 (Jul. 6, 2020) (petition deficient for failure to apply express definition provided in the specification).

Both parties' experts agree that the specification of the '360 Patent provides an express definition for this term. EX2023, ¶¶43-44; EX2022, 33:3-36:9 (Dr. Vargas confirming the definition), in particular at 35:25-36:9 (lines 32-33 of column 20 in specification "set up the definition and after that [lines 34 et seq.] there are specific examples"). The definition in the specification states: "The term 'sample' refers to a biological sample or a complex biological sample obtained from **a subject**." EX1001, 20:33-45. The specification further states: "The terms 'individual,' 'subject,' and 'patient' are used interchangeably." *Id.*, 50:26-36; EX2022, 36:10-37:8 (Dr. Vargas confirming); EX2023, ¶¶43-44. Thus, the claimed "a biological sample" is limited by definition to what is obtained from an **individual** subject or patient. The definition would exclude a lysate taken from an immortalized cell line that is engineered and grown in a laboratory (e.g., Cai's HeLa cell populations) rather than obtained from a subject. Additionally, the definition excludes a mixture of materials from different donors for the purpose of eliminating individual differences, thereby precluding the ability to assay a biological sample from any given subject (e.g., pooled blood plasma or RBCs in Hu and Zaccaria, respectively). The petition never proposed nor substantiated any contrary construction.

B. “a subset of the plurality of particles comprising the coronas”

All petition grounds fail because what the petition identified as the claimed “a subset...” in each of the asserted references does not satisfy that limitation according to the plain language of the claim. Claim construction begins with the plain language of the claims. *Phillips*, 415 F.3d at 1312.

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 must be assayed in step 1(c). The plain and ordinary language of the claims thus requires **a** subset (singular) **of the plurality of particles** comprising **the coronas** (plural). The definition for the term “the coronas” is found unambiguously 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, 86:52-87:3. Accordingly, 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). Here, where the claim recites separating at least a subset (singular) of the plurality of particles comprising “the coronas” (plural), the plain language of the claim requires a single subset of particles that comprises “the coronas,” as recited in the claims. *See, e.g., Harari v. Lee*, 656 F.3d 1331, 1341 (Fed. Cir. 2011) (“The plain language of the claim

clearly indicates that only *a single bit line* is used...” (emphasis original); *see also Insituform Techs., Inc. v. Cat Contracting, Inc.*, 99 F.3d 1098, 1105 (Fed. Cir. 1996) (where, for example, a claim “refers to ‘a cup’ and ‘the cup’ repeatedly,” it “suggest[s] that only one cup is involved.”).

The petition never proposed nor substantiated any contrary construction. Indeed, both parties’ experts agree that “the coronas” must correspond “to different particle types,” “differ based on particle type,” and “comprise overlapping and distinct proteins.” EX2023, ¶¶45-46; EX2022, 26:6-27:2 (“Q. You agree that those are both two requirements for what the claim calls ‘the coronas,’ correct? A. Yes.”). What the petition identifies in Hu, Cai, and Zaccaria as the claimed “subset” of particles does not satisfy this express claim requirement. *See* Sections VI.B, VIII.B, IX.B, below. In each of Hu, Cai, and Zaccaria, the petition identifies a subset of particles for step 1(b) that the petition fails to show comprises “the coronas” comprising overlapping and distinct proteins. EX2023, ¶46. By definition, the corona on a single particle type does not comprise “overlapping and distinct” proteins as required. *Id.*

While the petition offers no construction, the Institution Decision offered preliminary commentary questioning whether the “at least a subset” of “limitation 1(b) could be met by separating particles from only one of the different particle types” in limitation 1(a). Paper 11, 21-22. First, the Board stated “we discern

nothing in the language of claim 1 that requires that the contacting of the biological sample with ‘different particle types’ recited in limitation 1(a) occur in the same reaction vessel or at precisely the same time.” *Id.*; *see also* Paper 11, 25. Of course, there is no pre-requisite that limitation 1(a) (contacting) duplicate the requirements expressly recited in limitation 1(b) (separating), which requires “a” (singular) subset comprising “the coronas” (plural). It is immaterial whether said subset is obtained after (1) contacting a single reaction vessel with a plurality of different particles; or (2) contacting multiple reaction vessels and then combining contents together. Even if limitation 1(a) is agnostic in this regard, limitation 1(b) still requires separating a subset (singular) comprising “the coronas” (plural), which “differ based on particle type” and comprise “overlapping and distinct proteins.” EX1001, Claim 1.

Second, emphasizing the term “at least a subset,” the Board reasoned “limitation 1(b) does not require that all of the protein coronas formed in limitation 1(a) be separated.” Paper 11, 22; *see also id.*, 25. Patent Owner agrees that limitation 1(b) does not require separating literally every single one of the particles with all of the coronas formed in limitation 1(a). But it does require separating a subset of particles and it requires that subset comprise coronas (plural) defined in the claims as “differ[ing] based on particle type” and comprising “overlapping and distinct proteins.” EX1001, Claim 1.

Indeed, Petitioners' expert recognized limitation 1(b)'s "at least a subset" language reflects a practical consideration that 100% of the particles might not be separated from the biological sample. EX2022, 27:21-28:3 ("Q. Is it correct then when you were performing your analysis for this case, do you believe that the coronas in element 1B did not have to satisfy the requirements of the coronas in step 1A? A. I think they have to satisfy 1A, but it could still be a subset of the total number of the plurality of particles that were contacted in part A."), 28:22-29:7 (Q. "So you're saying there, there needs to be at least two particle types; is that correct? A. The plurality of particles, operationally, how you do this is that it would be [at least two] types."), 30:21-31:3; *see also* EX2022, 47:10-48:23. But this common-sense language provides no justification for eliminating from limitation 1(b) the express claim requirement that the subset of particles that are separated from the biological sample are particles comprising "the coronas," not merely a corona of an individual particle type.

Third, the Board concluded that claim 1 is not limited to a "sensor array" or state that the different particle types must be **contacted** with the biological sample at the same time. Paper 11, 25. Patent Owner does not argue that claim 1 is limited to detecting on a sensor array. *See, e.g.*, Claim 14. And step (b) is directed to separating from the biological sample, whereas contacting (step (a)) and assaying (step (c)) are separately recited steps. *See, e.g.*, Claim 13 (further addressing "the

assaying”). As discussed above, even if limitation 1(a) is agnostic regarding how the biological sample is contacted, that would not dispense with the language recited in limitation 1(b).

Petitioners bear the burden of addressing how the claims are to be construed (37 C.F.R. §42.104(b)(3))—and it is not Patent Owner’s burden to disprove a construction never made. Yet, there is strong reason to reject a construction (which Petitioners never proposed nor substantiated) that limitation 1(b) could be met by separating only one type of particle from the biological sample, as such a construction would impermissibly render the words “comprising the coronas” superfluous. “A claim construction that gives meaning to all the terms of the claim is preferred over one that does not do so.” *Merck & Co. v. Teva Pharms. USA, Inc.*, 395 F.3d 1364, 1372 (Fed. Cir. 2005); *see also Stumbo v. Eastman Outdoors, Inc.*, 508 F.3d 1358, 1362 (Fed. Cir. 2007) (construction that would render a phrase “superfluous” is “a methodology of claim construction that this court has denounced.”); *Bicon, Inc. v. Straumann Co.*, 441 F.3d 945, 950–51 (Fed. Cir. 2006) (refusing to construe claim terms in a way that made other claim limitations meaningless); *Elekta Instrument S.A. v. O.U.R. Sci. Int’l, Inc.*, 214 F.3d 1302, 1307 (Fed. Cir. 2000) (construing claim to avoid rendering a claim limitation superfluous); *Gen. Am. Transp. Corp. v. Cryo-Trans, Inc.*, 93 F.3d 766, 770 (Fed.

Cir. 1996) (similar). Such construction would impermissibly render the words “comprising the coronas” superfluous.

Moreover, Petitioners’ own expert identified that the use of coronas from at least two particle types is integral to the invention. EX2022, 24:19-24; *see also* EX1001, 19:65-20:2 (distinguishing the invention “[c]ompared with previous approaches limited to the surface of a single nanoparticle”). Construing limitation 1(b) to a corona from the surface of a single nanoparticle would render the use of a plurality of particle types in step (a) superfluous to the claimed method. *UltimatePointer, L.L.C. v. Nintendo Co., Ltd.* 816 F.3d 816, 823 (Fed. Cir. 2016) (construction that reads out the stated invention is not correct); *Eon Corp. IP Holdings v. Silver Spring Networks, Inc.*, 815 F.3d 1314, 1321 (Fed. Cir. 2016) (specification’s description of the invention is “context [that] must be considered in determining the ordinary meaning, as the ‘construction that stays true to the claim language and most naturally aligns with the patent’s description of the invention will be, in the end, the correct construction.’”) (quoting *Renishaw PLC v. Marposs Societa’ per Azioni*, 158 F.3d 1243, 1250 (Fed. Cir. 1998)).

Accordingly, there is no reason to depart from the plain language of the claim, and certainly the petition offers no reason to do so. The petition offers no argument at all regarding how the claims are to be construed.

VI. GROUND 2 (CAI ANTICIPATION) FAILS

Petitioners assert in Ground 2 that Cai anticipates claims 1, 4, 6, 17, 22 and 25. Pet. 3, 46-61. This Ground and independent claim 1 was the focus of the Board's analysis in the Institution Decision. Paper 11, 16-23. But the petition fails to satisfy the standards for anticipation for all challenged claims.

First, when asserting that Cai discloses a method of assaying "a biological sample," Petitioners point to a lysate of cells from an immortalized HeLa cell line. Pet., 46-50; EX1006, Fig. 1. But a lysate taken from an immortalized cell line that is 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.

Second, the petition's mixing and matching of different experiments of Cai without explanation does not support a legally valid basis for anticipation. *Net MoneyIN, Inc. v. VeriSign, Inc.*, 545 F.3d 1359, 1371 (Fed. Cir. 2008); *see also In re Arkley*, 455 F.2d 586, 587 (CCPA 1972). Both experts agree, Cai's Figs. 1 and 3 reflect different experiments, using different HeLa cell populations (alleged "samples") for distinct protocols and analysis. Cai's Fig. 1 experiment does not conduct the MS analysis upon which the petition relies for the claimed assaying (step 1(c)). When asked at deposition, Petitioners' expert attempted to pivot to the SILAC-encoded MS experiment depicted in Cai Fig. 3 (Pet., 58-60), but that only further underscores the deficiency of the anticipation allegation. The SILAC

experiment individually contacted two different lysates (each from a different populations of HeLa cells) each with only a single particle type.¹ Thus, even if a HeLa cell lysate is viewed as “a sample” as claimed (it is not), none of those individual populations (light, medium, or heavy) were contacted with a plurality of different particles as recited in element 1(a). Neither experiment discloses the subject matter as arranged in the challenged claim, and amalgamating different aspects of different experiments without explanation does not, and cannot, support an anticipation challenge.

Third, Petitioners’ anticipation challenge also fails for limitation 1(c). For limitation 1(c), the emPAI values relied upon by Petitioners for the assaying across at least six orders of magnitude limitation is described by Cai as a “semi-quantitative” index of abundance, not concentration as claimed. And emPAI is described by Cai as reflecting abundance in the “mixture” produced by mixing untreated HeLa lysate with (i) proteins eluted from CNTs, and (ii) proteins eluted from CB. EX1006, 584. Such mixture is multiple steps removed from a HeLa cell

¹ As shown in Cai’s Fig. 3, the “light” HeLa lysate was contacted with no particles; the “medium” HeLa lysate was contacted with CNT only; the “heavy” HeLa lysate was contacted with CB only.

lysate, which itself is not the identified biological sample as claimed. For each of these reasons, Petitioners' anticipation challenge fails. EX2023, ¶¶47-50.

A. Cai's HeLa Cell Lysate Is Not "A Biological Sample" As Claimed.

As a threshold matter, Ground 2 fails to demonstrate that Cai discloses the claimed "method for assaying a biological sample," including applying the method steps to the claimed biological sample as specifically required, because Petitioners exclusively rely on lysate of HeLa cells as the identified biological sample.

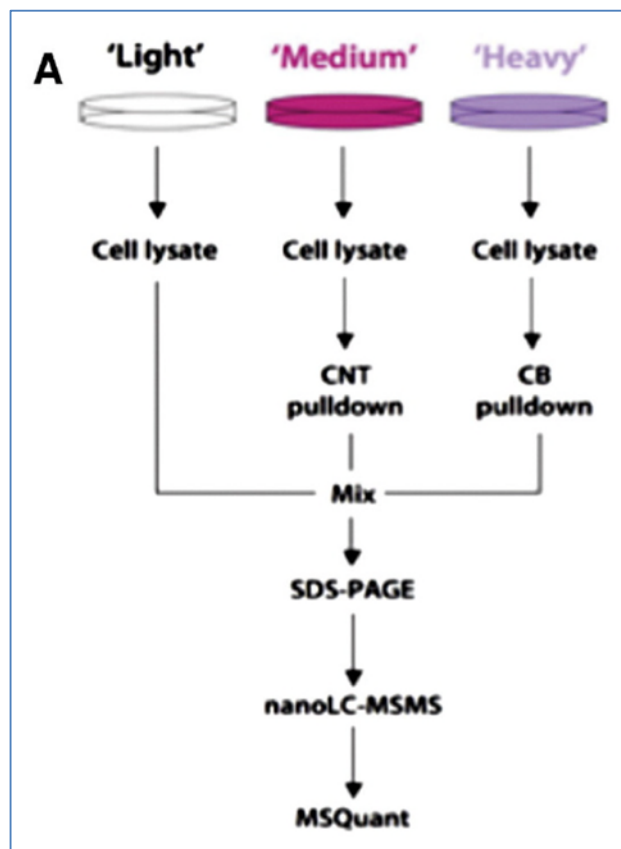
When asserting that Cai discloses a method of assaying "a biological sample" and the step of "contacting" the "biological sample," Petitioners point exclusively to a lysate of cells from an immortalized HeLa cell line. Pet., 46-50; EX1006, Fig. 1. But the petition fails to explain how the HeLa cells in Cai would satisfy the requirements of the claimed biological sample. HeLa cells are not obtained from an individual subject but are instead vendor-purchased immortalized cells from a cell line cultured in a lab. EX2022, 117:11-21 (immortalized cell line), 123:23-124:16 ("commercially available"); EX2023, ¶¶51-52. Both parties' experts agree that the specification of the '360 Patent provides an express definition for the biological sample. EX2023, ¶53; EX2022, 33:3-36:9, in particular at 35:25-36:9 (lines 32-33 of specification column 20 "set up the definition and after that [lines 34 et seq.] there are specific examples"). Specifically, the specification definition states: "The term 'sample' refers to a

biological sample or a complex biological sample obtained from *a subject*.”

EX1001, 20:33-45; EX2022, 34:15-36:9 (Dr. Vargas confirming the specification “set up” this “definition”). The specification further states: “The terms ‘individual,’ ‘subject,’ and ‘patient’ are used interchangeably.” EX1001, 50:26-36; EX2022, 36:10-37:8 (Dr. Vargas confirming the same; no recollection of citing this).

To the extent Petitioners rely on the SILAC experiment depicted in Fig. 3, this does not remedy the deficiency. Indeed, during deposition, Dr. Vargas tried to pivot to Fig. 3 for the contacting step as a whole based on a reference to Fig. 3 for the element of corona formation. EX2022, 130:25-131:5 (making an inference), 149:25-150:25, 163:8-164:15; *see also* Pet. 52. But the lysates in Fig. 3 are all still derived from cultured, immortalized HeLa cells, rather than from a biological sample, as claimed. The SILAC experiment thus fails to remedy the deficiencies in the petition’s “biological sample” identification.

If Petitioners are to pivot to Fig. 3 for anticipation, it only further



underscores deficiencies to its challenge. Cai describes each of its cell lysates as

distinct populations of cells. EX1005, 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”). Dr. Vargas confirmed during deposition that each distinct lysate (represented by the separate Petri dishes in the SILAC experiment depicted in Fig. 3) was contacted by at most one particle type, not by the multiple particle types. EX2022, 131:8-132:5 (confirming individual contacting and separation of individual corona types). Cai’s SILAC study thus does not satisfy claim step 1(a), as required, because it experiments with three different populations and no (singular) population is ever contacted by a plurality of particle types. EX2023, ¶54.

The piecemeal mapping of different aspects of the claim to different experiments in Cai should not camouflage the failure of either experiment to disclose the elements as arranged in the claims. For example, the petition relies on the MS analysis in the Fig. 3 experiment to satisfy the assaying across six orders of magnitude requirement in step (c). Pet., 60. The petition does not allege, much less demonstrate, that Cai’s Fig. 1 study assayed the material across at least six orders of magnitude, as required. Pet., 58-60. Instead, Dr. Vargas admitted during her deposition that Fig. 1 identifies gross protein bands using Coomassie blue staining and evaluates CNT and CB structure using TEM—it does not identify individual proteins and never mentions mass spec. EX2022, 107:9-20 (Fig. 1 showing TEM),

125:21-126:2 (no discussion of mass spec), 128:3-17. Dr. Vargas also admitted during deposition that Fig. 1 contains no disclosure of SILAC encoding. EX2022, 129:7-12 (confirming “no disclosure of SILAC encoding in that experiment”). Moreover, the different experiments operate on different HeLa cell populations, none of which qualify as a “biological sample” as defined in the ’360 patent specification and claimed. EX2023, ¶55.

Of course, anticipation is not satisfied by mixing and matching embodiments. “In order to anticipate the claimed invention, a prior art reference...must ‘disclose those elements arranged as in the claim.’” *Microsoft Corp. v. Biscotti, Inc.*, 878 F.3d 1052, 1068 (Fed. Cir. 2017) (quoting *Net MoneyIN, Inc. v. VeriSign, Inc.*, 545 F.3d 1359, 1369 (Fed. Cir. 2008)). At institution, the Board raised an “at once envisage argument” that is not found in the petition. *Compare* Paper 11, 20 (citing cases) *with* Petition (not citing cases or invoking such a theory). The Board raised that even if Fig. 1 and Fig. 3 are different experiments (which, of course they are), a skilled artisan might at once envisage that the pull-down conducted in the Fig. 3 experiment would involve similar pull-down steps (e.g., pellet by low-speed centrifugation) as in the Fig. 1

experiment.² Though the petition never advanced an “at once envisage” theory of anticipation in this respect or any other, such an “envisage” theory does not remedy the petition’s deficiencies.

Even if true that a skilled artisan would at once envisage that the pull-down referenced in Cai Fig. 3 employs the same low-speed centrifugation pulldown procedure as in Fig. 1, that does not solve any of the deficiencies discussed above. HeLa cells, including any of the different populations from the Fig. 1 or Fig. 3 experiments, still do not satisfy the definition for “biological sample.” If Petitioners are relying on Fig. 1 to satisfy the biological sample, that material is never assayed across at least six orders of magnitude, as required by the challenged claims. Petitioners rely on the Fig. 3 experiment for the “at least six orders of magnitude” assaying step, but this experiment used only a single particle type (or no particle) in each different HeLa cell population. As discussed above, this fails to

² While such a theory of anticipation would still be deficient for reasons discussed herein, the Federal Circuit has cautioned about improper extension of *Kennametal*. *Nidec Motor Corp. v. Zhongshan Broad Ocean Motor Co. Ltd*, 851 F.3d 1270 (Fed. Cir. 2017) 1274-75 (“*Kennametal* does not permit the Board to fill in missing limitations simply because a skilled artisan would immediately envision them.”).

satisfy the claimed contacting “a biological sample” (singular) with “a plurality of different particles comprising different particle types” as claimed (step 1(a)).

EX2023, ¶56. Performing the pull-down in Fig. 3 using the same low-speed centrifugation procedure illustrated in Fig. 1 does not remedy any of these deficiencies.

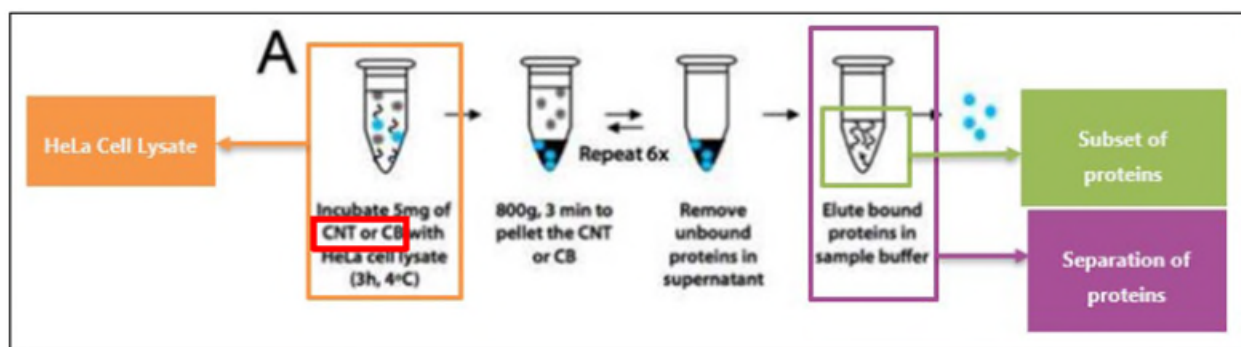
Petitioners are at best inconsistent in the claim mapping of which lysate is considered to be the biological sample. *Compare* Pet., 48-49 (the “biological sample” is “HeLa cell lysate” contacted with CNTs “of various diameters and lengths” illustrated by Fig. 1A) *and* Pet., 58 (similar) *with* Pet., 58-59 (“the biological sample” is any one of multiple SILAC metabolically-encoded HeLa cell lysates). Cai describes each of its cell lysates as distinct and—in addition to the legally impermissible mixing of different experiments—none of these different populations constitute a biological “sample” as defined in the ’360 patent specification. To the extent Cai’s SILAC study is considered vis-à-vis claim 1 as a whole, it at least fails to satisfy claim step 1(a) and thus cannot anticipate the challenged claims. EX2023, ¶56.

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

As discussed above (Section V.B), all claims require separating from the biological sample at least “a subset of the plurality of particles comprising the coronas.” The claims 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. Section V.B, above. Even putting aside the improper mix-and-match claim mapping in the petition, Petitioners still fail to show that Cai discloses this claim element.

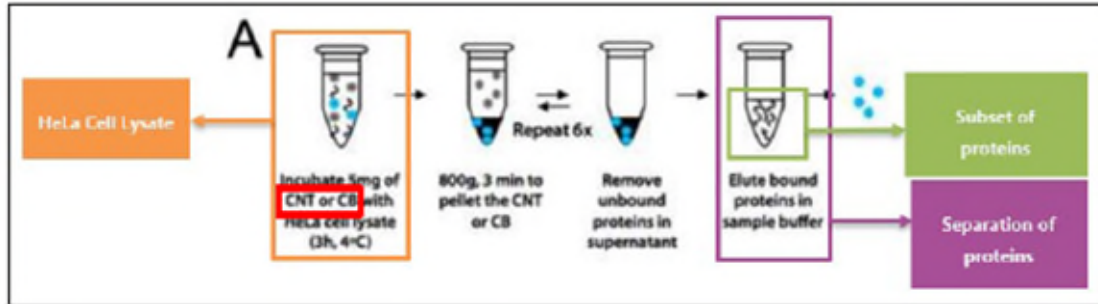
In discussing the separating step of claim element (b), Petitioners rely on Cai's Figure 1A—and **Figure 1A alone**—which depicts single particle types in a single HeLa cell lysate. Pet., 57-58 (citing EX1005, Fig. 1A; EX1005, 585 (specifically relating to Fig. 1A); and EX2022, 155:19-156:10 (“I only specifically referenced figure 1 to address this particular claim” element and confirming she “did not cite figure 3”). But Petitioners do not even allege that the subset of separated particles depicted in Cai's Fig. 1A comprises “the coronas” (or are assayed by MS). Nor could they. Petitioners' and Dr. Vargas's annotations make clear that in Cai's Figure 1A, there is never disclosed separating a subset of particles comprising coronas from more than one particle type. Instead, samples are incubated individually in parallel (EX1005, Fig. 1A “CNT **or** CB” annotated in red in the petition (reproduced below); EX1003, ¶202 (Dr. Vargas admitting Cai's samples are incubated in parallel)).



During her deposition, Dr. Vargas confirmed that the different particle types were always *individually* incubated in Cai’s pull-downs, and that every separation step created a subset of particles comprising a corona from only one particle type. EX2022, 104:1-17 (CNT vial has no CB; CB vial has no CNT), 106:22-107:1 (confirming “what you’re left with is, in one vial you have the CNTs and whatever proteins bound to the CNTs and in the other vial you have CBs, and whatever proteins bound to the CBs”), 118:17-20 (“looking at either CB or CNT being separated from lysates of the HeLa cells”), 119:19-120:9 (confirming only the CB corona on particles in one vial and only the CNT corona on particles in the other vial). Cai thus does not disclose separating a subset of particles comprising “the coronas” corresponding to multiple particle types, as required by the claims. EX2023, ¶¶57-59.

This deficiency in Petitioners’ anticipation ground is further illustrated by the Petitioners’ failure to even identify the claimed subset of particles. EX2023, ¶60. Though the petition maps 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).



The petition’s argument (Pet., 50) that MWCNTs of diameters 20-40 nm constitute a different particle type from MWCNTs of diameters less than 10 nm (Pet., 56) cannot save Ground 2 for multiple reasons. First, the experiment used to generate the alleged “subset of proteins” relied upon in the mapping did not employ MWCNTs with diameters less than 10 nm. EX2023, ¶61. Second, Cai teaches that MWCNTs of diameters less than 10 nm “bound to virtually no proteins.” EX1005, 586. EX2023, ¶61. 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, 586. Cai also teaches that MWCNTs length similarly had no impact on protein binding amount. EX1005, 586. Rather than disclose separating a subset of particles comprising “the coronas” of different particle types, Cai’s disclosure cited in the petition’s CNT-only argument merely indicates the absence of a corona from a different particle type in

that experiment. EX2023, ¶61. Accordingly, this argument likewise fails to establish anticipation.

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

Step (c) further 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.” The petition fails to demonstrate Cai discloses this limitation. EX2023, ¶62.

The petition relies exclusively on emPAI values differing by at least six orders of magnitude to satisfy this claim element. Pet., 60. But this argument fails for numerous reasons. As a threshold matter, Cai itself confirms that emPAI is not a measure of concentration. It is described as a semi-quantitative indication of abundance. EX1005, 587 (“empirical peptide abundance index (emPAI), a *semi-quantitative* indication of the *overall abundance* of protein *in the mixture*”); EX2022, 144:24-147:15 (Dr. Vargas confirming this provides the context for her reliance on Cai, though she omitted it from her quotation of Cai). As Dr. Cima confirms, abundance is not the same as concentration. EX2023, ¶¶63-64.

Second, the relied-upon emPAI does not demonstrate abundance, much less concentration, in the lysate identified by Petitioners as the “biological sample.” EX2023, ¶65. Instead, Cai explains that emPAI is a semi-quantitative measure of abundance of proteins in the *mixture* of proteins created during the SILAC

experiment. Dr. Vargas confirmed 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 alleged biological sample. EX2022, 131:8-133:2 (desorbed proteins), 133:25-134:7 (“The mix is the three different lysates” but “I would identify the lysate as the biological sample”), 148:7-22 (Dr. Vargas’ understanding that emPAI is from the mixture). She also confirmed that the Mix comprises a mixture of proteins from the **pulled-down corona** from each of two of the three lysates created from the three populations of HeLa cells. *Id.*, 134:21-136:18 (“particle-free mixture of proteins from three populations of HeLa cells” is subsequently analyzed). Even the petition admits that emPAI is a measure of abundance of “bound proteins” (i.e., present in the pull-down) rather than a measure of concentration in the alleged biological sample. Pet., 60 (“the relative abundance of **bound proteins** ‘spread over 8 orders of magnitude’” (citing EX1005, 587)). Petitioners thus failed to demonstrate the eight orders of magnitude variation in emPAI values measures **concentrations** of the proteins **in the sample**, as Petitioners purported to do in Grounds 2 and 3. Pet., 35-37.

Third, even if emPAI is considered, Petitioners seize on an observation in Cai regarding emPAI values at the extremes without critical consideration of this disclosure. Importantly, the emPAI values in Cai relied upon by Petitioners are not found within the narrow range that had been validated for this procedure. Despite

reporting a wide range of emPAI values, Cai (EX1005, 587 (ref. 30)) relies on Ishihama (EX1018) as representing the extent to which emPAI methodology has been validated, but Ishihama only validated emPAI over a narrow range that does not support Petitioners argument. EX1018, 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). EX2023, ¶66. The petition overlooks this distinction in uncritically seizing on, and misinterpreting, this statement in Cai. *Compare* EX1018, Table 1 (Ishihama 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). As Dr. Cima explains, Cai cites the wide spread of emPAI values to support its observation that some proteins were selectively enriched, but Cai is not conflating emPAI spread with sample concentrations—contrary to the scientific literature—as Petitioners attempt to do. EX2023, ¶¶66-67.

Thus, for at least the reasons above, Petitioners fail to show Cai anticipates the challenged claims.

D. Dependent Claims

The petition argues 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. As discussed above, the petition fails to establish Cai anticipates claim 1. EX2023, ¶¶71-72. Its anticipation challenge to the dependent claim thus similarly fails.

VII. GROUND 3 (CAI OBVIOUSNESS) FAILS

Failing to establish that Cai renders the challenged claims anticipated, the petition argues claims 1, 4, 6, 17, 22 and 25 alternatively would have been obvious. Specifically, the petition argues 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. The argument otherwise relies on its anticipation argument in Ground 2 and proposes only converting Cai's emPAI values to mol % based on a formula from the Ishihama reference. EX2023, ¶68.

As a threshold matter, the petition still fails to distinguish between protein content and concentration. As Dr. Cima explains, molar percent (mol %) of protein content is indicative of relative abundance, it is not a measure of concentration. EX2023, ¶69. This is readily apparent 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). 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 rather than concentration.

Furthermore, Petitioners' argument does not remedy the deficiencies discussed above. Section VI. For example, "mol %" of the protein mixture, even if determined, still would not correspond to the features the petition maps as the

biological sample (Section VI.A). Also as discussed above, Cai's abundance index at best reflects an amount of protein recovered from the particles and added to the "mixture" rather than the amount in the lysate (identified in the petition as the claimed "biological sample"). Converting bound-then-recovered protein abundance into bound-then-recovered protein concentration, even if determined (which the petition never did), still does not satisfy the claim under Petitioners' own claim mapping, and therefore fails to remedy the deficient anticipation case.

Additionally, Petitioners' proposed modification does nothing to remedy the failure of the identified HeLa cell lysate to qualify as the claimed biological sample, the failure of Cai's SILAC experiment 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). Petitioners' conclusory obviousness argument thus fails for at least the same reasons as detailed in Ground 2. *Supra*, Section VI; EX2023, ¶70.

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

VIII. GROUND 1 (HU) FAILS

Petitioners fail to show that Hu discloses or render obvious claims 1-4, 6-7, 17 or 22-25. EX2023, 73-77. First, Hu (like Cai) fails to disclose assaying a biological sample as claimed. The petition identifies Hu's mixture of blood plasma

from 20 different individuals, but the petition fails to explain how such a mixture satisfies the patent's definition of a biological sample.

Second, Ground 1 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.

Finally, Ground 1 fails regarding the six orders of magnitude limitation of step (c) for several reasons. Petitioners acknowledge that Hu does not expressly disclose concentrations or any dynamic range. Pet. 35. For alleged inherent disclosure, Petitioners 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, however, fails to establish Hu actually detected LTBP-1 as opposed to simply reporting a non-reproducible signal indistinguishable from background noise, as is commonly found in raw data from shotgun proteomics assays.

Even assuming LTBP-1 detection occurred, the petition fails to establish the identified low concentration is inherently present in Hu's sample. Petitioners' inherency case is self-defeating as based on the (unsubstantiated) argument that serum albumin and LTBP-1 are "normally present" at concentrations identified in the petition. Pet. 36. The exacting standard for inherency requires disclosure that is

“necessarily present,” not the mere probability or possibility of what is “normally” observed. As discussed in further detail below, Ground 1 fails.

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

Ground 1 fails to demonstrate that Hu discloses the claimed “method for assaying a biological sample.” Pet. 21-25. Hu’s study combines blood plasma taken from 20 different individuals that was mixed prior to assay. The petition fails to establish that such a mixture is “a biological sample” as its own expert agrees is defined by the ’360 Patent. Section V.A, above; EX2023, ¶78; EX2022, 36:4-37:8 (Dr. Vargas confirming the definition of “sample”).

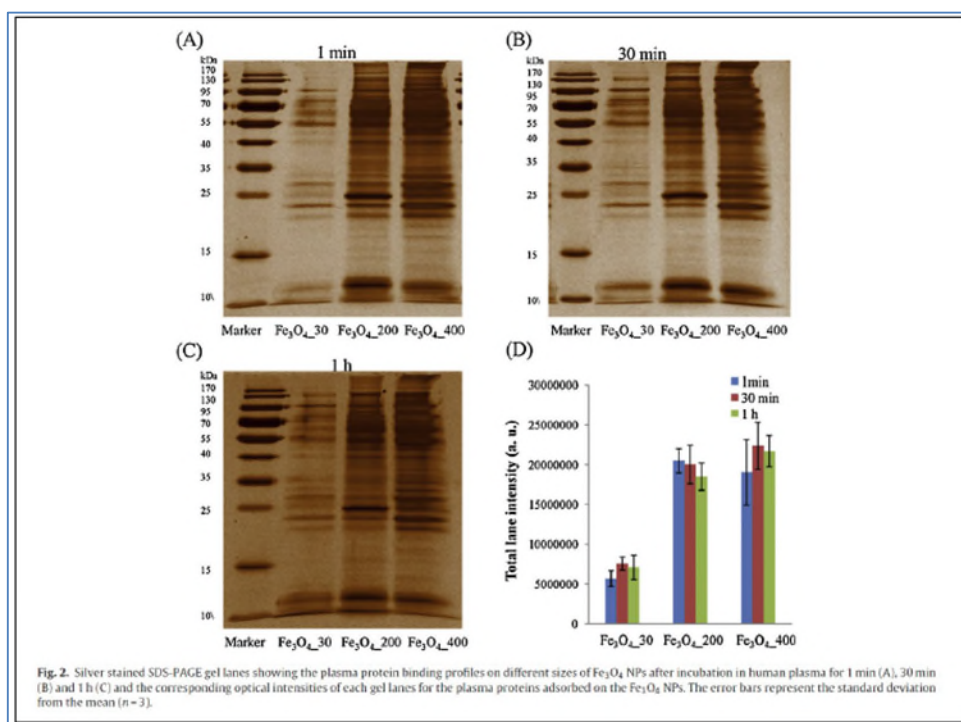
B. Hu Fails to Disclose Separating a Subset of the Plurality of Particles Comprising the Coronas As Required By 1(b).

Ground 1 also fails to demonstrate Hu discloses separating the claimed “subset of the plurality of particles comprising the coronas” as required by all challenged claims. EX2023, ¶79. 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.

Petitioners inconsistently map what they consider to be “the subset” in the petition. *Compare* Pet., 31-32 (“the bound subset” depicted by Fig. 2, showing SDS-PAGE gel lanes) *with* Pet., 33-36 (“the subset” referring to proteins derived from singular corona types detected by mass spectrometry). But Petitioners’ identified “subset” of particles fails in each scenario. EX2023, ¶80. As detailed

further below, if Petitioners rely on Hu’s SDS-PAGE gel for disclosure of “the subset of the plurality of particles comprising coronas,” this fails because (1) the SDS-PAGE gel is not shown to contain **any** particles comprising coronas (much less a plurality of particles comprising 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 Petitioners rely on the particles from which proteins ultimately were subjected to Hu’s shotgun mass spectrometry for disclosure of “the subset” of the plurality of particles, then it also fails because each of Hu’s subsets of particles comprises only one corona—not the required **coronas** having overlapping and distinct proteins. Section V.B, above.

When discussing claim element 1(b), the petition asserts Hu’s Figure 2 (reproduced below) depicts “the bound subset.” Pet., 31-32. But Hu’s Figure 2 does not disclose the claimed “subset of the plurality of particles comprising the coronas.” A POSA would have understood Figure 2 to at best illustrate proteins that were **previously** bound to nanoparticles as part of a protein corona. EX2023, ¶ 81.



Hu explains that, prior to running its SDS-PAGE, its “nanoparticle-protein complexes” were individually “pelleted, resuspended in SDS-PAGE loading buffer, then boiled at 95 °C for 5 min and finally separated with 1-D SDS-PAGE.” EX1008, 355. Such boiling denatures the protein corona, removing it from the nanoparticle. EX2023, ¶82; EX2025, 159. Thus, Petitioners’ reliance on Hu’s Fig. 2 for the “subset of the plurality of particles comprising the coronas” is insufficient because it discloses neither *particles* comprising the coronas nor a subset of the particles comprising the *coronas* having overlapping and distinct proteins. EX2023, ¶82. Indeed, 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. EX2023, ¶82. Dr. Vargas confirmed during her

deposition that there are no particles when the material is loaded onto the SDS-PAGE gels. EX2022, 99:3-15 (Dr. Vargas confirming her “understanding it’s just the proteins” loaded rather than the particles).

Even if Petitioners attempt to pivot to Hu’s particles that are digested and the proteins analyzed by shotgun mass spectrometry as “the subset,” this also fails. EX1008, 355; Pet., 33-36. Dr. Vargas confirmed during her deposition that the separation step in Hu generates a subset of particles comprising only the corona of a single particle type. EX2022, 92:2-97:18 (each of 30-, 200-, and 400-nm samples incubated individually). Hu never describes separating a subset of particles comprising coronas from multiple particle types. EX2023, ¶83. Indeed, Hu later describes LC-MS/MS analysis on a corona **individually**. *See, e.g.*, EX1008, 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, 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 different columns); EX2022, 98:6-99:8 (individual samples desorbed then analyzed). 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. EX2023, ¶83.

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.

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 fails to demonstrate Hu discloses this limitation. Pet. 33-39; EX2023, ¶84.

The petition relies on two proteins (serum albumin and LTBP-1) mentioned in Hu’s supplementary data table. Pet. 35-36. Acknowledging that no concentrations or dynamic range is expressly found in Hu, the petition argues inherent disclosure in Hu because the proteins are “normally present” as concentrations that would span 6 orders of magnitude. But Petitioners’ uncritical review of Hu’s raw data tables fails to establish Hu actually detected the identified proteins in the first place, and certainly fails to demonstrate the identified concentrations of these proteins are inherently and necessarily present in Hu’s tested material. EX2023, ¶85.

1. Petitioners Fail To Demonstrate Hu Actually Detected LTBP1 at a concentration of 1.7-6.0 ng/mL.

For alleged inherent disclosure, Petitioners latch onto one entry among the many entries in the raw data table from Hu's supplement. Pet., 35-36. The petition, however, fails to establish Hu actually detected LTBP-1 as Petitioners assumed, let alone at the identified concentration. EX2023, ¶86.

As discussed above (Section II), shotgun proteomics approaches like Hu extensively degrade obtained proteins into small peptide fragments. EX1008, 355 (“digested with trypsin”; “digested proteins”). Following MS analysis of the degraded fragments, protein prediction is accomplished bioinformatically (e.g., using algorithms and databases) by analyzing the raw mass spectral data to make “predictions” of the “theoretical peptides possible.” EX1003, ¶39 (“fragmentation predictions”). Prior art shotgun proteomics approaches were known to lack sensitivity and specificity, and were prone to experimental error for low abundance components. See e.g., EX2020, 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.”). EX2023, ¶87.

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. EX2023, ¶88; EX2022, 43:20-44:7 (“Typically you conduct tests

in replicates to ensure reproducibility...there should be no differences between the duplicates”). Additionally, researchers (including Hu) restricted their analysis to what they viewed as a reasonably reliable subset of the proteomics data generated. EX1008, 359 & Table 2 (analysis of the “top 20”; threshold of “plasma proteins with an average MWSpC ≥ 1 ”), EX1007, 1874 (“we compared the 20 most abundant identified proteins (ie, the 20 proteins with the higher MS/MS queries)”).

The petition identifies LTBP-1 in an entry buried in Hu’s voluminous raw data table found in the “supplementary data,” but offer little discussion and no critical review. As explained by Dr. Cima, a POSA would be highly skeptical that Hu actually detected LTBP-1 as Petitioners assume, as opposed to simply reporting a non-reproducible signal that did not meet the threshold of detection. EX2023, ¶89.

While Petitioners failed to cite the actual pages of the supplemental data on which they found the LTBP1 entry, the below images show the column headings from page 10 and the entry from page 18 of EX1008. See also, EX2013.

Annotations indicate the particle type employed and the number of replicates attempted. As shown in the image from EX2013 shown below, LTBP1 (isoform 4) was not detected in any of the replicates performed with the 30nm and 400nm particles, while only two of three attempts with the 200nm particles allegedly detected LTBP1 (with MWSpC values of only 0.01).

IPI	Names	pI	MW (kD)	Fe ₃ O ₄ _30					Fe ₃ O ₄ _400					Fe ₃ O ₄ _400				
				MWSpC _3	MWSpC _2	MWSpC _3	Average	SD	MWSpC _3	MWSpC _2	MWSpC _3	Average	SD	MWSpC _3	MWSpC _2	MWSpC _3	Average	SD
IP100973177.1	LTBP1 Isoform 4 of Latent-transforming growth factor beta-binding protein 1	5.63	186.74						0.01	0.01	0.01	0.09						

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

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

In no instance does Hu's table report signal for all replicates of any tested particle. EX2023, ¶¶90-91. 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 imposed. EX1008, 359. In fact, 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 in the Hu article itself, and certainly not identified among the list of any proteins Hu claimed it reliably detected. EX1008, Table 2 & Fig. 6; EX2023, ¶¶91-92.

It is the Petitioners' burden to prove detection as alleged, Patent Owner does not carry the burden of proving the negative. Yet, given the weakness of the signal and lack of reproducibility, a POSA more reasonably would conclude that any non-replicable signal was more likely experimental artifact rather than an actual detection event. EX2023, ¶88. Petitioners here fail to grapple with the known

sensitivity/specificity limitations and experimental error common in the shotgun MS approach applied in Hu. Nor do Petitioners meaningfully address the raw data reported in Hu itself—they merely invoked an entry without specificity and without addressing the lack of reproducibility and signal that was weak, at best, and non-existent in the vast majority of replicates tested. The petition certainly fails to demonstrate Hu detected LTBP-1 at concentrations of 1.7-6.0 ng/mL, as alleged. EX2023, ¶¶86-92.

2. Petitioners’ Inherency Argument is Unsubstantiated and Self-Defeating.

Hu admittedly does not disclose any dynamic range or concentrations of any proteins in the plasma mixture Hu tested. Instead, Petitioners argue inherent disclosure. EX1003, ¶161 (“this is inherently disclosed by Hu”). Specifically, Petitioners identify two particular proteins—serum albumin and LTBP-1—which it argues (citing to references other than Hu) are “normally present” at identified concentrations differing by six orders of magnitude. Pet. 35-37. EX2023, ¶93. This argument fails for at least several reasons.

Initially, characterizing the identified concentrations as “normally present” tacitly admits that different concentrations (or no concentration at all) are expected in some subjects. The standard for inherency, however, is well-established and is not satisfied by such mere possibilities or probabilities. *Cont’l Can Co. v. Monsanto Co.*, 948 F.2d 1264, 1269 (Fed. Cir. 1991) (“Inherency, however, may

not be established by probabilities or possibilities. The mere fact that a certain thing *may* result from a given set of circumstances is not sufficient.”) (quoting *In re Oelrich*, 666 F.2d 578, 581 (CCPA 1981)) (emphasis original). *See also In re Montgomery*, 677 F.3d 1375, 1380 (Fed. Cir. 2012) (an “inherent result **must inevitably result** from the disclosed steps”); *Bettcher Indus., Inc. v. Bunzl USA, Inc.*, 661 F.3d 629, 639 (Fed. Cir. 2011) (“The mere fact that a certain thing may result from a given set of circumstances is not sufficient.”). Petitioners’ inherency argument is, by definition, improperly based on a mere possibility (“normally present”) and should be rejected for at least this reason.

Additionally, Petitioners’ reliance on two different references (other than Hu) is misplaced for several reasons. First, Petitioners cite to Ahmed and Cao but these references do not establish concentrations for serum albumin and LTBP-1 in the Hu material. These references do not address Hu’s plasma testing or even discuss Hu, which is highly problematic given that concentrations of different proteins vary considerably among different individuals. EX1001, 56:35-38; EX2023, ¶94. Moreover, Ahmed does not address Cao’s samples, and Cao does not address Ahmed. Worse yet, Cao teaches “[t]here are four isoforms of LTBP (LTBP-1, -2, -3, and -4)” and discusses concentration values from Cao’s sample for Isoform 1. EX1016, 16051 (LTBP-1). In contrast, the spectral count in Hu mapped to LTP1 is for “Isoform 4.” EX1008, 18 (Table S1, row IPI00973177.1);

EX2013. But Petitioners fail to recognize that Hu and Cao are not even addressing the same proteins. EX2023, ¶94.

Moreover, Petitioners' own evidence indicates that even variations in preparation method impacts testing results. See, e.g., EX1015 (Ahmed), 786 (“slight changes in a given sample preparation could lead to very different protein profiles.”). Dr. Cima explains various ways in which preparation and analysis methods differed materially between the cited references. EX2023, ¶95. Hu and Cao, for example, examined different cohorts with a different composition of age and gender and differed in sample processing and handling (e.g., centrifugation, storage) as well as assaying method. The petition addresses none of this.

Second, Dr. Cima explains that the petition falsely presents the cited Ahmed and Cao references as reporting “normally present” concentrations of identified proteins when they do no such thing. Neither reference is broadly reporting what is “normally present” in a more general manner. See e.g., EX1016 (Cao), 16047-16048 (observing concentrations employing ELISA). At best, Cao and Ahmed observed particular concentrations in particular materials they tested (different than Hu's). EX2023, ¶96.

In fact, the petition provides no explanation why Ahmed and Cao specifically are relied upon in the first place—presumably Petitioners canvassed the literature to cherry-pick references that fit their litigation narrative. For example,

the petition materials cite specifically and only to Cao in asserting that LTBP-1 is “normally present” at concentrations from “1.7 to 6.0 ng/mL,” but other resources identify different LTPB-1 concentrations. For example, the Peptide Atlas database is an on-line resource known to POSAs at the time. As Dr. Cima reports, the Atlas database estimated LTBP-1 in human plasma at a concentration of 220.0 ng/mL. *See* EX2027 (below); EX2023, ¶98. This is multiple orders of magnitude greater than the concentration presented in the petition. As Dr. Cima explains, even assuming for arguments sake the highest number the petition cites to as what is “normally present” for serum albumin (52 mg/mL), this only differs from LTBP-1 (220ng/mL in the Atlas database) by about five orders of magnitude—not “at least 6 orders of magnitude” as recited in the challenged claims. EX2023, ¶99. There is no explanation why only Cao is cited or why no other sources are provided. EX2023, ¶97.

Accordingly, Petitioners fail to demonstrate that, even if Hu detected serum albumin and LTBP-1, the identified concentrations of those proteins were necessarily present in the plasma tested in Hu. EX2023, ¶100.

3. Petitioners’ Alternate Obviousness Theory Fails.

After admitting Hu lacks express disclosure of the dynamic range limitation of the claims (Pet. 35), the petition makes unsubstantiated alternate argument that “a POSA would have...found it obvious.” Pet., 37-39. The petition improperly

conflates knowledge of a wide disparity between protein concentrations in the blood, with missing disclosure of assaying a biological sample across such a dynamic range using shotgun mass spectrometry. The argument is also misplaced where it discusses mass spectrometry generally rather than Hu’s shotgun approach as relied upon in Ground 1. EX2023, ¶101.

Petitioners’ argument also ignores its own submitted references discussing the difficulties and inaccuracies of prior mass spectrometry analysis of protein concentrations across a broad dynamic range. *See, e.g.*, EX1010, 1218 (discussing complexity that “hampers the identification of low abundant proteins”); EX1006, 2451 (“Using spectral counting to estimate the relative abundance of low-abundance proteins within complex mixtures is ***inaccurate***”); EX1015, 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, 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 II above (discussing EX2017-EX2021); EX2023, ¶102. Moreover, Petitioners’ arguments ignore Hu’s express disinterest in low abundance proteins. EX1008, 360 (“Here, we list the top 20 most abundant plasma proteins comprising the protein coronas formed on the Fe₃O₄ NPs”), Table 2

(same). Hu expressly limits its analysis to no more than two orders of magnitude (MWSpC range of 1-10). EX2023, ¶102; EX1008, 360, Table 2, Fig. 6 Caption (discarding MWSpC < 1).

Petitioners' citation to the '360 patent itself is improper hindsight, as the challenged patent may not be used as a guide. *Apple Inc. v. Voip-pal.com*, IPR2016-01201, Paper 53; EX2023, ¶103. *Otsuka Pharm. Co. v. Sandoz, Inc.*, 678 F.3d 1280, 1296 (Fed. Cir. 2012) ("The inventor's own path itself never leads to a conclusion of obviousness; that is hindsight."); *Sensonics, Inc. v. Aerosonic Corp.*, 81 F.3d 1566, 1570 (Fed. Cir. 1996) ("The invention must be viewed not after the blueprint has been drawn by the inventor...") (citing *Interconnect Planning Corp. v. Feil*, 774 F.2d 1132, 1138 (Fed.Cir.1985)). The petition also mischaracterizes the '360 patent. The cited experimental example discusses machine learning analysis of performed proteomics data and database resources for identification and discrimination of cancer at early stages. EX1001, 62:45-75:20.

Elsewhere, the petition pretends this is an overlapping range case. Pet., 38-39 (citing *Iron Grip*, *Peterson*, and *Pfizer*); EX2023, ¶103. But Petitioners cannot invoke overlapping range case-law when they've never established the existence of an **overlapping prior art range**. Overlapping range case law is inapposite where, as here, the prior art lacks the disclosed range corresponding to what is claimed. In *Moderna v. Arbutus*, the Federal Circuit rejected range case law as inapposite

where a petitioner tries to concoct a range that the prior art did not clearly disclose.

ModernaTX, Inc. v. Arbutus Biopharma Corp, 18 F.4th 1364 (Fed. Cir. 2021).

When there is no prior art range, Petitioners cannot flip the burden onto Patent Owner to address the nonexistent prior art ranges.

As discussed above, Petitioners fail to show Hu discloses assaying step (c) across a dynamic range comprising 6 orders of magnitude as claimed. Prior art knowledge that proteins existed across a wide dynamic range does not provide the range that is claimed (assaying across at least six orders of magnitude). In any event, the literature (e.g., EX1010, 1218; EX1006, 2451; EX1015, 786; EX1007, 1869-1870; Section II, above) explained that prior shotgun proteomics approaches were “inaccurate” and “unattainable” for assaying across such a dynamic range. EX2023, ¶103.

For each of the reasons discussed above, Petitioners fail establish that Hu renders obvious the challenged claims. EX2023, ¶104.

D. Dependent Claims

The petition argues 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. As discussed above, the petition fails to establish Hu renders claim 1 obvious. EX2023, ¶105. Its challenge to the dependent claim thus similarly fails.

In addition, for claim 23, the petition confuses a lipoprotein as recited in the claim with “Apolipoproteins.” Apolipoproteins are proteins, whereas a lipoprotein is a complex of lipids and proteins. EX2028, 631-632; EX2023, ¶106.

IX. GROUND 4 (ZACCARIA) FAILS

Ground 4 fails to demonstrate Zaccaria renders obvious claims 1, 2, 4, 6-7, 17, 22-23 or 25, or any rationale for modifying Zaccaria with a reasonable expectation of success. The obviousness challenge of Ground 4 fails.

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

Rather than disclosing the claimed “method for assaying a biological sample” as the term is defined in the specification, Zaccaria’s study (like Hu) combines materials taken from multiple different individuals that are mixed prior to analysis. EX1007, 1870-1871; EX2022, 51:11-14. The petition fails to explain how such a mixture is “a biological sample” as defined by the ’360 Patent. Pet. 64-66; Section V.A, above; EX2023, ¶¶107-108; EX2022, 36:4-37:8 (Dr. Vargas confirming the definition of “sample”). Thus, Petitioners fail to show that Zaccaria discloses the claimed method for assaying “a biological sample”, which is a feature in each of the challenged claims. See. e.g., Claim 1.

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

Ground 4 also fails 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. 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. EX2023, ¶109.

The Petition asserts 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. Petitioners’ argument is deficient on its face because 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, 39:12-40:1 (“Three separate containers.”), 41:5-17 (separately incubated in duplicates). Indeed, Dr. Vargas confirmed during her deposition that the separation step in Zaccaria generates a subset of particles comprising only the corona of a single particle type. EX2022, 45:10-50:12 (following desorption, “now we have at least a subset of the proteins that came from that individual particle type” for each sample). Zaccaria never describes separating a subset of particles comprising the coronas from multiple particle

types. EX2023, ¶110. 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.* 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

Petitioners argue element 1(c) is satisfied because they claim Zaccaria detected two specific proteins (Hb and SBF1) they claim are found at concentrations across a dynamic range comprising more than six orders of magnitude. Pet. 74, *see generally* 73-77. The petition admits there is no express disclosure of protein concentrations or a dynamic range. Pet., 74 (“Zaccaria does not specifically state the dynamic range in numerical form”); EX2022, 76:19-25 (Zaccaria “does not report the concentrations or the expected concentrations...”). But Petitioners allege inherent disclosure, arguing those proteins are “normally present in RBC lysate” at concentrations spanning 6 orders of magnitude. Pet. 74. Similar to Ground 1, the inherency theory fails for several reasons. EX2023, ¶111.

Just as in Ground 1, Ground 4 cherry-picks SBF1 (alleged “lower abundant” protein) from the supplemental information and presents it without critical review. But Petitioners fail to demonstrate that Zaccaria actually detected SBF1 in the first

place, rather than reporting non-replicable signal indistinguishable from experimental artifact or noise. Indeed, Zaccaria expressly reports that the identified signal could not be mapped to a single protein, but instead mapped to multiple different accession numbers (different numbers identifying different proteins in a database). The petition overlooks this entirely. The petition certainly fails to demonstrate the identified concentrations of these proteins are inherently and necessarily present in Zaccaria. EX2023, ¶112.

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

The petition argues that Zaccaria's shotgun MS 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. But Petitioners fail to demonstrate that Zaccaria actually detected SBF1, rather than reporting non-replicable signal indistinguishable from experimental artifact or noise. EX2023, ¶113.

As discussed above, shotgun proteomics approaches make "predictions" of the "theoretical peptides possible" based on raw mass spectral data from degraded peptide fragments. As Petitioners' own expert confirms, POSAs viewed non-replicable signal as likely experimental error or artifact, rather than a reliable detection event. EX2022, 43:20-44:7. As Dr. Cima explains, a POSA would more reasonably view Zaccaria's data as indicating the level of detection/specificity was inadequate to detect (and did not in fact detect) this protein. EX2023, ¶114. The

petition fails to demonstrate otherwise and, in fact, fails to provide any critical review or discussion.

Because the petition failed to cite the actual pages of the supplemental data on which they found the SBF1 entry, Patent Owner was forced to play archeologist with the record during the deposition of Dr. Vargas to identify it. EX2022, 54:25-62:3. The image below (see also EX2009) reproduces the column headings from page 25 and the entry from page 28 of EX1007. EX2022, 54:5-69:22 (confirming content of EX2009 corresponding to SBF1 in Zaccaria); EX2023, ¶115.

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																																							
<table> <tr> <th colspan="6">Replicate 1</th><th colspan="5">Q-Treated</th></tr> <tr> <th>Fractions</th><th>Score</th><th>#Peptides</th><th>#Sequences</th><th>Coverage (%)</th><th>#MS/MS</th><th>Fractions</th><th>Score</th><th>#Peptides</th><th>#Sequences</th><th>Coverage (%)</th></tr> <tr> <td>GR_Q1</td><td>223.78</td><td>2</td><td>2</td><td>1</td><td>10</td><td></td><td></td><td></td><td></td><td></td></tr> </table>											Replicate 1						Q-Treated					Fractions	Score	#Peptides	#Sequences	Coverage (%)	#MS/MS	Fractions	Score	#Peptides	#Sequences	Coverage (%)	GR_Q1	223.78	2	2	1	10					
Replicate 1						Q-Treated																																					
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<table> <tr> <th colspan="6">Replicate 1</th><th colspan="5">PAA-Treated</th></tr> <tr> <th>Fractions</th><th>Score</th><th>#Peptides</th><th>#Sequences</th><th>Coverage (%)</th><th>#MS/MS</th><th>Fractions</th><th>Score</th><th>#Peptides</th><th>#Sequences</th><th>Coverage (%)</th></tr> <tr> <td></td><td></td><td></td><td></td><td></td><td></td><td>GR_PAA2</td><td>149.65</td><td>7</td><td>7</td><td>3.4</td></tr> </table>											Replicate 1						PAA-Treated					Fractions	Score	#Peptides	#Sequences	Coverage (%)	#MS/MS	Fractions	Score	#Peptides	#Sequences	Coverage (%)							GR_PAA2	149.65	7	7	3.4
Replicate 1						PAA-Treated																																					
Fractions	Score	#Peptides	#Sequences	Coverage (%)	#MS/MS	Fractions	Score	#Peptides	#Sequences	Coverage (%)																																	
						GR_PAA2	149.65	7	7	3.4																																	

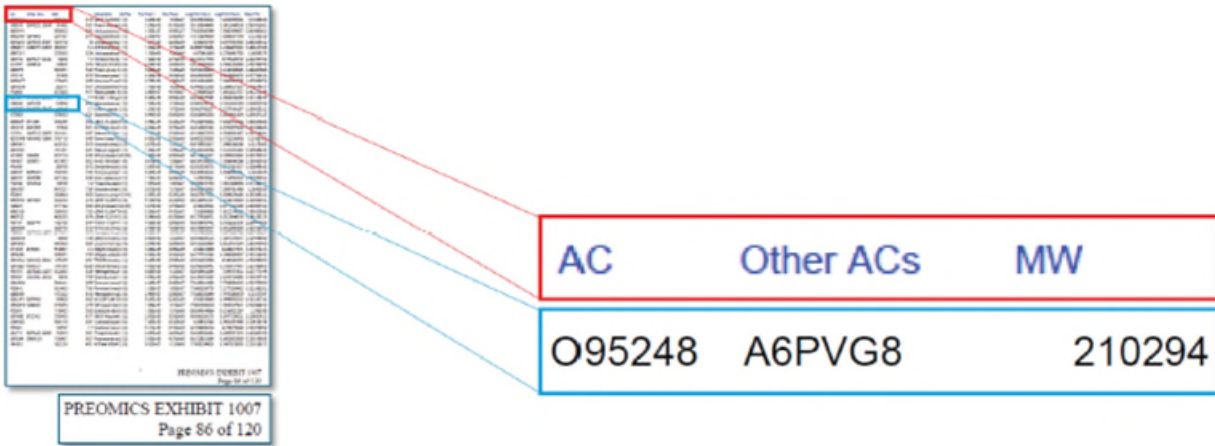
As shown in the image and reported in Zaccaria, in no instance does Zaccaria's table report signal as being replicated for any tested particle. Of six attempts on three different particle types, four of those attempts indicate no signal reported. As Dr. Cima explains, for the few non-replicated instances where signal was reported, it was exceptionally weak. EX2023, ¶116. 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 weak signal gives little confidence of SBF1 detection, as opposed to noise or error, including misassignment of fragments originating from a different protein. EX2023, ¶116.

Indeed, Zaccaria confirms the SBF1 label was arbitrarily applied as just one of several potential accession numbers that might be associated with the signal.³ 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, 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

³ As Dr. Cima explains, different accession numbers are assigned to different proteins in a database. EX2023, ¶117, EX2022, 58:21-24 (Dr. Vargas admitting unfamiliarity with accession numbers.).

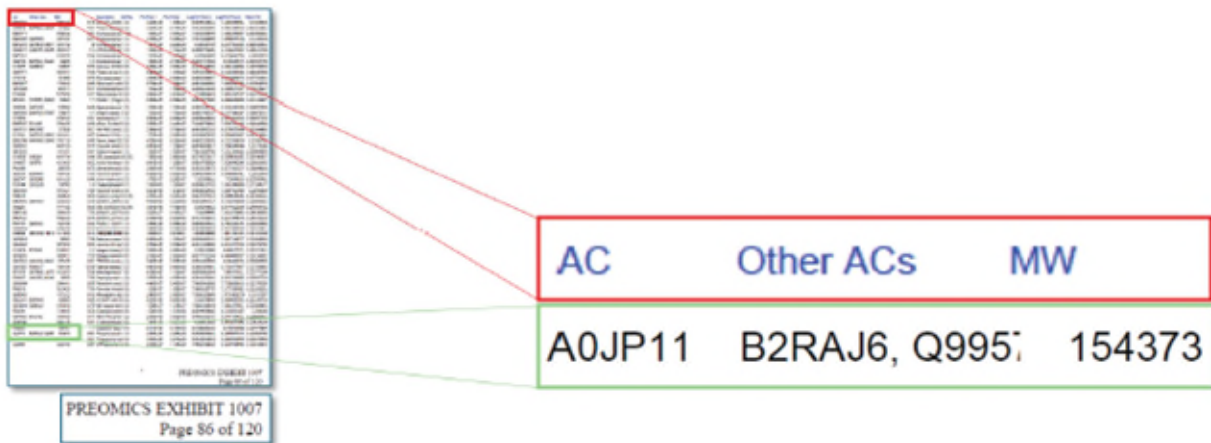
O95248) for the signal Petitioners relied upon, Zaccaria discloses elsewhere that multiple AC identifiers were actually listed for the signal. EX1007, 86 (“Other ACs” include A6PVG8); EX2023, ¶117; EX2014 (below—showing multiple ACs, not just SBF1):



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AC	Other ACs	MW
O95248	A6PVG8	210294

The same issue exists with Zaccaria’s identification of other proteins, like PIK3R4 (cited only against dependent claim 7). EX2015 (below—showing multiple ACs, not just PIK3R4):



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AC	Other ACs	MW
A0JP11	B2RAJ6, Q9957	154373

As Dr. Cima explains, EX2014 and EX2015 (reproduced above) 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. EX2023, ¶117.

This issue was lost on Dr. Vargas, who acknowledged at deposition she could not explain what the “AC” in the column header even stood for. EX2022, 58:21-24 (“Q. Do you know what AC stands for? A. I don’t, offhand.”). As such, Dr. Vargas appears to have adopted uncritically the unsubstantiated attorney argument of Petitioners’ counsel that these signals are indicative of SBF1 detection.

Accordingly, while the petition assumes that Zaccaria detected SBF1 (among other unsubstantiated assertions of protein detection), its uncritical review of the cited entry in a voluminous supplemental data table fails to demonstrate actual detection in the first place. EX2023, ¶118.

2. Petitioners’ Inherency Argument is Self-Defeating

As discussed above, Zaccaria admittedly does not expressly disclose a dynamic range or sample protein concentrations. Pet. 74. Petitioners argue inherency, citing to Bryk (EX1009). Pet. 74-75. The petition specifically identifies two particular proteins—HBA1 and SBF1—which it argues are “normally present” at identified concentrations. Even assuming detection of those proteins in Zaccaria,

which has not been demonstrated (above), the inherency argument still fails.

EX2023, ¶119.

As discussed regarding Ground 1 (Hu) above, Petitioners' argument should be properly credited as an admission by Petitioners that different concentrations (or no concentration at all) of the identified proteins are expected in different subjects. EX2023, ¶120. Thus, Petitioners' inherency argument is, by definition, improperly based on what is presented as a mere possibility ("normally present") and should be rejected accordingly.

Petitioners' own evidence further undermines its inherency argument. Dr. Vargas cites Bryk for reporting 6.62×10^{-6} pmol/mg of SBF1. EX1003, ¶271. But Dr. Vargas admits that she selected out "the lowest non-zero detected concentration" of SBF1 in Bryk's table.

EX1003, ¶271 n.6; EX2022, 77:1-5

(confirming the same). Other entries in

Bryk report "zero." By reporting a

"zero" result for SBF1 in some donors,

however, Bryk confirms that the SBF1

result relied upon by Dr. Vargas is by no means "necessarily present" in a RBC

lysate. EX1009, 51 (lowest count for SBF1 in Bryk is "0.00E+00"); *see also*,

EX2011 (shown here); EX2023, ¶121. The very same issues arise with Dr. Vargas'

SBF1
EX1009 p. 51

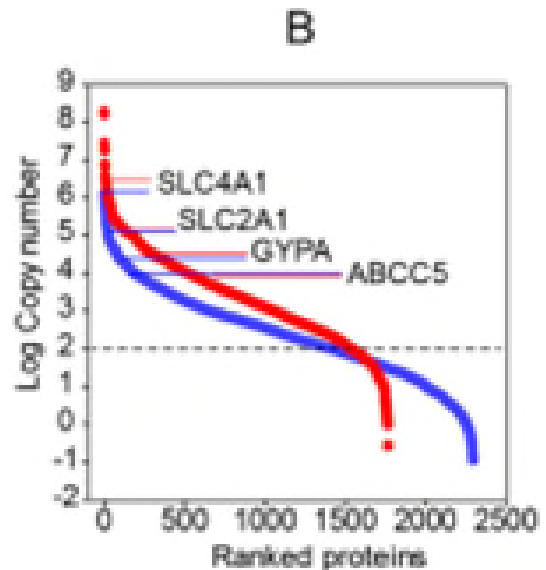
PIK3R4
EX1009 p. 44

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

discussion of PIK3R4 (cited by Petitioners only for dependent claim 7). EX1003, ¶122 (“lowest non-zero” for PIK3R4 is 3.17×10^{-4} pmol/mg); EX1009, 44 (lowest count for PIK3R4 in Bryk is “0.00E+00”); EX2022, 77:1-5 (values in declaration “were the lowest non-zero detected concentrations in Bryk”); EX2011; EX2023, ¶121. Thus, rather than supporting Petitioners’ argument that the asserted concentrations are “normally present,” Bryk confirms the relied upon proteins may not be present at all in a sample—including Zaccaria’s sample.

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)”); EX2023, ¶122. Likewise, Bryk discarded all the PIK3R4 results for the same reason. EX1009, 44 (3.4, 0.0, 0.0, and 0.0 copies); EX2023, ¶122. 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, 2755.

Additionally, Bryk does not establish protein concentrations in Zaccaria's tested material because it is an entirely different reference that employed different methodologies on different samples. Both experts agree. EX2023, ¶123. As Dr. Vargas confirmed during her deposition, numerous differences existed in the preparation protocol employed in Zaccaria as compared to Bryk. EX2022, 51:11-54:4 (confirming details from Zaccaria), 88:21-89:22 (confirming details from Bryk). EX1015, 786 (discussing that even "slight changes in a given sample preparation could lead to very different protein profiles").

Accordingly, Bryk does not support Petitioners' asserted "normally present" concentrations of SBF1, and certainly fails to establish such a concentration in Zaccaria's different RBC sample. To the contrary, Bryk undermines any confidence in Petitioners' assumption that SBF1 (or PIK3R4) was even detected by

Zaccaria in the first place, let alone at concentrations in a sample across a dynamic range comprising at least 6 orders of magnitude as claimed.

Petitioners' remaining argument adds nothing to support obviousness and does not cure the deficiencies identified elsewhere herein. Pet. 75-77; EX2023, ¶124. With improper hindsight, Petitioners cite to the '360 patent itself and illogically argue that Zaccaria is "consistent" with the problem the '360 patent solves. As discussed above, and more pertinent to an obvious challenge, the petition fails to demonstrate the art (including Zaccaria) discloses what is alleged. The petition cites overlapping range caselaw but, as discussed above, failed to substantiate what it argues is "calculable from Zaccaria's data." No range case has been established and the cited cases are inapposite.

Therefore, for at least the reasons discussed above, Petitioners fail to meet their burden to establish that Zaccaria renders obvious the challenged claims. EX2023, ¶125.

D. Dependent Claims

The petition argues 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. As discussed above, the petition fails to establish Zaccaria renders claim 1 obvious. EX2023, ¶126. Its challenge to the dependent claim thus similarly fails.

Additionally regarding claim 2, the unit conversions do not cure the deficiencies discussed regarding claim 1. EX2023, ¶127. For claim 7, the petition relies on the PIK3R4 protein. As discussed above, the petition fails to demonstrate Zaccaria detected this protein, let alone at the concentration identified. Section IX.C; EX2023, ¶128. For claim 23, the petition confuses a lipoprotein as recited in the claim with “Apolipoproteins” as discussed above. EX2023, ¶129.

X. THE PETITION FAILS TO ADDRESS THE CLAIMS FROM THE PERSPECTIVE OF A POSA

In its Institution Decision, the Board seemed to acknowledge the petition’s definition of a POSA as defective. The Board agreed that “a POSA would have an understanding of both proteins and the analytical techniques, e.g., mass spectrometry, that can be used to characterize them.” DI, 9, *see also id.* at 8-9 (acknowledging that both the specification and the cited prior art describe “the analysis of protein coronas”). The Board also acknowledged that while the petition was from the perspective of a person with a “bachelor’s degree,” the art and inventors’ backgrounds indicate “that a graduate degree is more typical for a POSA.” DI, FN5. The Board, however, identified concerns regarding Petitioners’ stated POSA as “go[ing] to the weight we should afford [Dr. Vargas’] testimony at trial.” The identified concerns remain. POPR, 26-30. Patent Owner respectfully agrees that Dr. Vargas’ declaration should be afforded little weight because, while

she may be qualified in mass spectrometry, she lacks any discernable background in proteomics.

While weight accorded is an issue, it is not the only issue. Perhaps more fundamentally, the petition challenge is improperly constructed from the perspective of a hypothetical person who could have the qualifications noted above or could not. Pet. 11. According to the petition, its challenge could, in whole or in part, be from the perspective of a person with a bachelor's degree in chemistry and 3 years' experience in mass spectrometry (e.g., a MS laboratory technician with no understanding of proteomics). It is well-established that obviousness must be assessed from the perspective of a person of ordinary skill. An obviousness challenge constructed from an improper perspective is a case founded in legal error *See Daiichi Sankyo Co., Ltd. v. Apotex, Inc.*, 501 F.3d 1254, 1257 (Fed. Cir. 2007) ("The district court's error in determining the level of ordinary skill in the art of the '741 patent tainted its obviousness analysis.").

XI. CONCLUSION

Accordingly, for at least the reasons set forth above, Petitioners have failed to meet their burden of demonstrating the challenged claims are unpatentable.

Respectfully submitted,

Date: July 24, 2025

/ Michael T. Rosato /
Michael T. Rosato, Lead Counsel
Reg. No. 52,182

CERTIFICATE OF COMPLIANCE

Pursuant to §42.24(d), the undersigned certifies that this paper contains no more than 14,000 words, not including the portions of the paper exempted by §42.24(b). According to the word-processing system used to prepare this paper, the paper contains 13,931 words.

Respectfully submitted,

Date: July 24, 2025

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XII. APPENDIX

EXHIBIT NO.	DESCRIPTION
2001	Marilena Hadjidemetriou et al., <i>In Vivo Biomolecule Corona around Blood-Circulating, Clinically Used and Antibody-Targeted Lipid Bilayer Nanoscale Vesicles</i> , 9(8) ACS NANO, 8142-8156 (2015).
2002	John E. Blume et al., <i>Rapid, deep and precise profiling of the plasma proteome with multi-nanoparticle protein corona</i> , 11 NATURE COMMUNICATIONS, 3662 (2020).
2003	Adam Bonislawski, <i>Seer Closes \$55M Funding Round</i> , GENOMEWEB, Jul. 22, 2020, available at https://web.archive.org/web/20240721111110/https://www.genomeweb.com/proteomics-protein-research/seer-closes-55m-funding-round#.Xxh18p5Kg2w
2004	<i>Omid Farokhzad</i> , LINKEDIN, www.linkedin.com/in/omidfarokhzad (last accessed Dec. 19, 2024).
2005	<i>Morteza Mahmoudi</i> , LINKEDIN, www.linkedin.com/in/morteza-mahmoudi-8b76636a (last accessed Dec. 19, 2024).
2006	<i>Claudia Corbo</i> , LINKEDIN, www.linkedin.com/in/claudia-corbo-2a298232 (last accessed Dec. 19, 2024).
2007	<i>Intentionally left blank</i>
2008	<i>Intentionally left blank</i>
2009	Summary of EX1007 (Zaccaria) SBF1 Entry
2010	Summary of EX1007 (Zaccaria) PIK3R4 Entry
2011	Summary of EX1009 (Bryk) SBF1 and PIK3R4 Entries
2012	Alexey I. Nesvizhskii, <i>A Survey of Computational Methods and Error Rate Estimation Procedures for Peptide and Protein</i>

	<i>Identification in Shotgun Proteomics</i> , 73 J. PROTEOMICS 2092-2123 (2010).
2013	Summary of EX1008 (Hu) LTBP Entry
2014	Summary of EX1007 (Zaccaria) “Other ACs” as alternative to O95248
2015	Summary of EX1007 (Zaccaria) “Other ACs” as alternative to A0JP11
2016	Shidbas Banerjee & Shyamalava Mazumdar, <i>Electrospray Ionization Mass Spectrometry: A Technique to Access the Information Beyond the Molecular Weight of the Analyte</i> , 2012 INT’L J. ANALYTICAL CHEMISTRY, Article ID 282574 1-40 (2012).
2017	Vinzenz Lange et al., <i>Selective reaction monitoring for quantitative proteomics: a tutorial</i> , 4(222) MOLECULAR SYSTEMS BIOLOGY 1-14 (2008).
2018	Dietrich A. Volmer & Lori Lee Jessome, <i>Ion Suppression: A Major Concern in Mass Spectrometry</i> , 24(5) LCGC NORTH AM. 498-510, available at https://www.chromatographyonline.com/view/ion-suppression-major-concern-mass-spectrometry
2019	Jianru Stahl-Zeng et al., <i>High Sensitivity Detection of Plasma Proteins by Multiple Reaction Monitoring of N-Glycosites</i> , 6(10) MOLECULAR & CELLULAR PROTEOMICS 1809-1817 (2007).
2020	Hasmik Keshishian et al., <i>Quantitative, Multiplexed Assays for Low Abundance Proteins in Plasma by Targeted Mass Spectrometry and Stable Isotope Dilution</i> , 6(12) MOLECULAR & CELLULAR PROTEOMICS 2212-2229 (2007).
2021	Gert Lubec & Leila Afjehi-Sadat, <i>Limitations and Pitfalls in Protein Identification by Mass Spectrometry</i> , 107 CHEMICAL REVIEWS 3568-3584 (2007).
2022	Deposition Transcript of Dr. J. Vargas

2023	Declaration of Dr. M. Cima
2024	Curriculum Vitae of Dr. M. Cima
2025	DONALD VOET, JUDITH G. VOET & CHARLOTTE W. PRATT, FUNDAMENTALS OF BIOCHEMISTRY: LIFE AT THE MOLECULAR LEVEL Section 6-4 (2nd ed. 2006)
2026	Henry H. Heng, <i>HeLa Genome Versus Donor's Genome</i> , 501 NATURE 167 (2013).
2027	Q14766-4, PEPTIDEATLAS, https://db.systemsbiology.net/sbeams/cgi/PeptideAtlas/GetProtein?atlas_build_id=559&protein_name=Q14766-4&action=QUERY (last visited September 21, 2025).
2028	DONALD VOET, JUDITH G. VOET & CHARLOTTE W. PRATT, FUNDAMENTALS OF BIOCHEMISTRY: LIFE AT THE MOLECULAR LEVEL Section 19-1 (2nd ed. 2006)
2029	CRC HANDBOOK OF CHEMISTRY AND PHYSICS: A READY- REFERENCE BOOK OF CHEMICAL AND PHYSICAL DATA, 7-1 (David R. Lide ed., 72nd ed. 1991).

CERTIFICATE OF SERVICE

I certify that the foregoing Patent Owner Response Pursuant to 37 C.F.R. § 42.120 and accompanying Exhibits 2009-2029 were served on this 24th day of July, 2025, on the Petitioners at the following electronic service addresses:

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