

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

PREOMICS GMBH AND BIOGNOSYS AG.,
Petitioner,

v.

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

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

**PATENT OWNER'S PRELIMINARY RESPONSE
PURSUANT TO 37 C.F.R. § 42.107**

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I. INTRODUCTION

Scientists at the Harvard Medical School-affiliated Brigham & Women's teaching hospital ("BWH"), led by Professor Omid Farokhzad, were awarded the '360 Patent because they solved an important technical problem plaguing proteomics. Researchers had tried using mass spectrometry ("mass spec") to analyze the identity and concentration of proteins that formed a coating (called a corona) on the outside of a nanoparticle when it was incubated in a biological sample (e.g., blood serum), but the complexity of the protein mixture made the readings unreliable and uninformative beyond a very narrow dynamic range. A handful of proteins were present in such high concentrations that the concentration of the low-abundant proteins of interest could not be detected reliably. In many cases, proteins of interest might not be detected at all. Accordingly, it was common for practitioners to use a biological sample to individually screen a library of nanoparticles to identify an individual particle that was best suited to quantify target protein(s) of interest (those enriched to the greatest extent in the corona of that particular particle), effectively *narrowing* the dynamic range.

It turns out that narrowing the dynamic range works well only if one knows ahead of time which protein(s) matter. When the protein(s) that matter are known, targeted enrichment could be useful (e.g., for targeted delivery of therapeutics or toxins). When it was unknown which protein(s) matter (e.g., when assaying a

biological sample), proteomics researchers were left to essentially search one-by-one until they found a particle that provided a useful assay with a narrow dynamic range.

This was like using a magnet to find a rare metallic needle in a haystack—if the hay itself is made out of metal. By virtue of its sheer abundance, the metallic hay obscures the rare metal needle. But assaying the proteins in a biological sample involves not just identifying the proteins, but also reliably identifying their concentrations. The task here is thus not just to find one needle among the pile of metal hay, but to reliably detect the number of rare needles in the pile so that their concentration can be reliably assayed. As the '360 Patent explains, existing proteomics approaches were wholly inadequate to the task.

As evidenced by the literature overcome during prosecution, which is very similar to the references asserted by Petitioner herein, the challenged claims distinguish the prior art by assaying ***a subset of proteins*** produced from the ***different biomolecule coronas*** formed after at least two different particle types contact a biological sample. The challenged 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.” EX1001, 19:61-20:10. The patent further explains that it is “***the combination*** of protein-nanoparticle and protein-protein interactions that is uniquely derived using” multi-nanoparticles with different physicochemical properties that allows consideration of the “different concentrations” at which “each type of protein” is present “on the surface of different nanoparticles” to provide “additional proteomic information.” EX1001, 20:10-14.¹ In particular, assaying a subset of proteins from the coronas from the different particle types enables detection, in the subset, of the proteins in the biological sample at concentrations across a dynamic range comprising at least 6 orders of magnitude, as required by all challenged claims. All Petition grounds fail to contend with the claimed invention, and thus fail for the same reason the claims were allowed in the first place.

Rather than grappling with the patent’s innovation, Petitioner ignores it completely. Petitioner’s asserted references follow the prior art approach of using a sample to screen different particles to find the particle best suited to reduce dynamic range for the proteins of interest, the opposite of the claimed invention. Just like the art asserted during prosecution, this one-at-a-time approach can be useful for finding a particle suited for payload delivery, but it does not teach or

¹ All bold-italic emphases added.

suggest assaying ***the subset*** of proteins produced from the different biomolecule coronas from the different particle types. Nor does it suggest expanding the dynamic range.

Accordingly, institution of *inter partes* review of claims 1-4, 6-7, 17, and 22-25 of U.S. Patent No. 11,435,360 (the '360 patent) should be denied.

II. BACKGROUND

A. State of the Art

In the art, it was known that a protein corona could be formed around certain particles exposed to a biological sample (e.g., a person's blood plasma). E.g., EX1006 (Walkey), 2439-2440; EX1010 (Hajipour), 1210-1211. The protein composition (identity and concentration) of the corona depended both on the composition of the biological sample and on the physicochemical properties of the particle. *See, e.g.*, EX1010, 1212-1215; EX1006, 2439; EX2001 (Hadjidemetriou), 8142. Researchers sought to exploit these insights in one of two ways.

Some researchers, including Walkey (EX1006), Hadjidemetriou (EX2001), Hu (EX1008) and Cai (EX1005), were interested in identifying the proteins highly abundant in an individual protein corona that were “‘seen’ by the components of the biological system and influence[] the downstream biological behavior” such as cellular association and uptake for drug delivery. EX1006, 2440, 2446-2447; EX2001, Abstract, 8151. Because the goal was targeted payload delivery, this

literature analyzed each corona individually, to identify its effect for narrowing the dynamic range.

Other researchers, like Hajipour (EX1010) and Zaccaria (EX1007), were concerned with identifying specific biomarkers associated with disease states. EX1010, 1210-1211; EX1007, 1869-1870. They likewise analyzed each corona individually, suiting their interest in identifying a nanoparticle that was tuned to selectively isolate biomarkers of interest to provide disease diagnosis and prognosis. EX1010, 1213-1214, 1216-1217. Similar to protein depletion and protein separation approaches, nanoparticle-targeted protein enrichment attempted to improve detection sensitivity by limiting the dynamic range of the sample being assayed.

For each purpose, the literature taught the importance of focusing on an individual protein corona to narrow the dynamic range. For example, Walkey et al. individually examined coronas formed on 105 different nanoparticles by assaying them one at a time. EX1006, 2441 (“A total of 105 formulations were prepared by grafting 3 quasi-spherical gold nanoparticle cores...with one of 67 surface ligands” and “[a]fter synthesis, each formulation was incubated with undiluted human serum” to form coronas), *id.* (“The composition of the protein corona around each formulation was characterized qualitatively using [PAGE] and semi-quantitatively using [LC-MS/MS].”). Consistent with standard practice at the time, Walkey

focused on the results of individual corona in a one-at-a-time approach. *E.g.*, EX1006, Fig. 3 (“the serum protein fingerprint formed around each nanoparticle formulation” separately assayed to “identif[y] serum proteins”); *id.*, 2441 (“each formulation was incubated” and “purified”); *id.* (“the protein corona around each formulation was characterized”).

Similarly, Hajipour focused on the individual corona that formed on either polystyrene or silica nanoparticles using a one-at-a-time approach. “After incubation with the various plasma samples, the hard corona-coated NPs were analyzed under the same conditions” and “the protein patterns of the various hard coronas” were each individually assayed. EX1010, 1212; *see also id.*, Fig. 2 (“SDS-PAGE gel...of human plasma proteins obtained from (a and b) polystyrene NP-protein complexes and (c and d) from silica NP-protein complexes”), Figs. 3-4 (same), Figs. 5-6 (histogram differences based on particle type).

Likewise, Hadjidemetriou et al. focused on the corona individually. EX2001, 8143 (evaluating bare, PEGylated, and targeted particles, “[t]he protein coronas formed after intravenous administration of the three liposome nanoscale systems...were characterized by recovering the liposomes from blood circulation”), 8151 (“Identification of the corona proteins enabled us to correlate the liposome type with the protein molecules absorbed both *in vitro* and *in vivo*.”); 8152 (there is a “unique character and morphology of the protein corona for each

nanomaterial” leading to different bioactivities); *see also id.*, Fig. 2 (separate analysis of protein corona formation on each particle type), Fig. 4a-c (separate assays for each type of particle, both *in vivo* and *in vitro*), Fig. 5 (most abundant proteins by particle type), Fig. 6 (cellular uptake by particle type), 8143 (prior art concerned with “the specific NPs used” in incubations). As discussed below in Section II.E, the Grounds references asserted in the Petition similarly approach the corona individually for purposes of narrowing dynamic range.

The literature specifically explained that narrowing the dynamic range by focusing on the abundant proteins on an individual corona was important because using mass spec to assay protein concentrations was not reliable across a wide dynamic range. Though mass spec had the theoretical ability to detect protein concentrations that differed from one another by up to 4-6 orders of magnitude, a problem arose when applying mass spec to a complex mixture of low- and highly-abundant proteins in a biological sample such as blood plasma. Hajipour explained the problem as follows:

Human plasma contains more than 10 000 proteins of which a very small fraction of proteins are highly abundant...and the rest of proteins are very low abundant. This small fraction of highly abundant proteins constitutes approximately more than 90% of plasma protein concentrations, ***which hampers the identification of low abundant proteins in plasma by mass spectrometry analysis.***

EX1010, 1218; *see also id.* (“[T]he amount of stained proteins observed in the SDS-band is not enough for mass spectrometry analysis (in this regard, highly abundant proteins are exceptions).”). Walkey similarly emphasized the problem of applying mass spec to a biological sample, stating succinctly: “Using spectral counting [LC-MS data] to estimate the relative abundance of ***low-abundance proteins within complex mixtures is inaccurate.***” EX1006, 2451; *see also* EX1015, 786 (“The technical challenges in the analysis of plasma/serum proteome is that their proteins are present in unequal concentrations as a few are so dominant (e.g., albumin and globulin present at almost 90% of total proteins by weight) that ***they mask the detection of other proteins, especially low abundant ones,*** which are considered to be of clinical importance.”), 786 (“Without fractionation 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, 1869-70 (despite constant advances in mass spec and sample fractionation methods, “the limited dynamic range of current mass spectrometers hinders the entire proteome coverage of biological fluids” rendering “the low-abundant protein compartment where disease-associated biomarkers are thought to reside [as] still ***unattainable***”); *id.* (where highly-abundant proteins mask the “biologically interesting low-abundant proteins, it is difficult to investigate” the full proteome).

Because the complexity of the mixture rendered the mass spec signals of low-abundant proteins unreliable noise, protein identifications with low-abundant signals were commonly disregarded as the literature instead assayed the most abundant proteins for an individual corona. *See, e.g.*, EX1010, 1218 (“[The hard corona mainly consists of albumin, fibrinogen, immunoglobulin, complement factors and apolipoproteins. These are the highly abundant proteins of plasma...[B]ut the amount of stained proteins observed in the SDS-band is not enough for mass spectrometry analysis (in this regard, highly abundant proteins are exceptions).”), Fig. 2; EX1006, 2451 (Walkey “prun[ed]” over 83% of the identified proteins from mass spec to “eliminate[...]low-abundance proteins” from consideration); *id.* (“The pruning procedure eliminated 656 low-abundance proteins. Together, the 129 remaining proteins defined the serum protein fingerprint.”); *id.* (“Using spectral counting to estimate the relative abundance of low-abundance proteins within complex mixtures **is inaccurate**”); EX2001, 8148 (evaluating the “[m]ost-abundant proteins (top-20) identified” for each particle); *id.*, Fig. 5 (“Most-abundant proteins (top-20)”); EX1007, 1874 (“[W]e compared the 20 most abundant identified proteins”); *id.* (reproducibility “for these 20 most abundant proteins”). The state of the art thus employed mass spec to analyze the subset of proteins from an individual corona of a given particle type across only a limited dynamic range. It did not assay a biological sample by assaying the subset

of proteins from the coronas of different particle types at concentrations across a dynamic range comprising at least 6 orders of magnitude.

B. Summary of the Invention

The '360 Patent expressly recognizes the above-described prior art problems and provides its sensor array approach as 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 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) in their concentrations. EX1001, 19:19-27. The specification thus explains that “existing technologies” required a “trade-off” between coverage depth and processing throughput, which led practitioners to, e.g., deplete highly abundant proteins (i.e., narrow dynamic range). EX1001, 19:27-37. “Despite such efforts,” the patent explains, “mass-spectrometry approaches to plasma proteomics have not met with robust success” and “no prior study, proteomic or otherwise, has reported accurate prediction and classification” for a number of different diseases. EX1001, 19:37-46. The specification thus identified that the complexity and differential concentrations of proteins in the biological sample imposed severe limits

precluding one from approaching the theoretical dynamic range that was potentially available through mass spec proteomics.

In contrast to existing literature, the '360 Patent provided an innovative solution to this technical obstacle. The challenged 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. The patent further explains that “***the combination*** of protein-nanoparticle and protein-protein interactions that is uniquely derived” by assaying the proteins using multi-nanoparticles with different physicochemical properties provides “additional proteomic information.” EX1001, 20:10-14. The '360 Patent thus takes an opposite and counterintuitive approach as compared to the prior art.

In the challenged patent, the noise problem that plagued the prior art is resolved by ***increasing input*** into the assay (i.e., different coronas from multiple particle types) rather than using the “disease-specific protein corona” of a single particle type to target a particular disease state. EX1001, 19:47-20:10. Instead of weeding out the noise by being selective about which “needles” to attract, the '360 Patent embraces the noise and uses multiple particle types together, enriching a

mass of results. The patent thus rests on a very counterintuitive insight: using a targeted approach worsens the noise problem, but expanding the inputs (e.g., analogous to adding more hay to the stack) resolves it.

In particular, as recited in claim 1, the '360 Patent assays the biological sample by detecting, in the subset of proteins from the coronas of the different particle types, proteins at concentrations across a dynamic range comprising at least 6 orders of magnitude. The method proceeds when a plurality of particles comprising different particle types contact (e.g., are incubated in) the biological sample to permit biomolecules of the sample to bind to the plurality of particles and form coronas around the plurality of particles. The coronas corresponding to the different particle types differ based on the particle type and therefore comprise distinct proteins, but they also comprise overlapping proteins. At least a subset of the plurality of particles comprising the coronas is removed from the biological sample, thereby producing a subset of proteins from the biological sample. It is this subset of proteins that the claims require to be assayed. Because the subset of proteins in the assay derive from different particle types, an instrument (e.g., mass spec) can be used 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 text of claim 1, from which all other claims depends, is reproduced here with color and emphases added.

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.**

C. Practical Implications of this Very Promising Invention

The '360 Patent explains that the practical implications of assaying a biological sample to detect the concentrations of the subset of proteins from multiple particle types were significant. Whereas mass spec had a potential dynamic range of 4-6 orders of magnitude (EX1001, 20:20-32), this potential remained unrealized when applied to the complexity of biological samples. See Discussion in Section II.A above. Assaying the subset of proteins from the

multiple particle types not only enabled mass spec's untapped potential for assaying a biological sample, it far exceeded it by achieving a dynamic range of at least 6 orders of magnitude, including as high as ten. For example, the '360 Patent explains:

The present sensor array has a sensitivity and dynamic range of ten (10) orders of magnitude in terms of protein detection using mass-spectroscopy approaches. The present assay is able to detect proteins that are found in the sub-ng range within a sample. This assay or approach has a much greater dynamic range than current assays for measuring proteins within a sample. For example, mass spectrometry only has a dynamic range 4-6 order[s] of magnitude. This novel sensor array has the ability to sample a greater dynamic range than has previously been achievable. The present sensor array allows for detection and determination of low abundant **and** rare proteins that we[re] not previously able to be detected.

EX1001, 20:20-32. In other words, mass spec previously could be used to either detect abundant protein concentrations **or** to detect rare protein concentrations six orders of magnitude lower, but the claimed invention detects and determines **both** low-abundant and rare protein concentrations (i.e., assays the subset of proteins from the two coronas at concentrations **across** a dynamic range of at least 6 orders of magnitude).

Professor Farokhzad founded Seer, Inc. to develop this groundbreaking technology and bring to market his insight that using multiple particle types

enables assaying throughout much of the dynamic range of the proteome itself. He went beyond the prior art approach of screening different nanoparticles, each narrowing the dynamic range, and instead selected a combination of multiple nanoparticles (e.g., a panel), enabling the detection of more proteins across a wider dynamic range. EX2002 (Blume), Abstract. He illustrated that using “multi-nanoparticle protein corona” enables “[r]apid, deep and precise profiling of the plasma proteome.” *Id.*, Title. This innovation has attracted over 100 million dollars of investments. EX2003 (Bonislawski). It has changed the face of proteomics.

D. During Prosecution, the Examiner Recognized the Invention Was Only Found in the Inventors’ Own Work

The Petition mischaracterizes the prosecution history by presenting the allowance as a failure of the Examiner to recognize that mass spectrometry theoretically could be used to detect proteins at concentrations differing by 6 orders of magnitude. Pet., 7-11. But the prosecution history repeatedly reflects Examiner recognition of prior art application of mass spectrometry to proteomics analysis, and Examiner recognition that mass spectrometry theoretically could detect protein concentrations differing by 6 orders of magnitude. EX1002, 1642-1644 (Examiner: “the measurement of the biomolecule fingerprint by mass spectrometry (a method taught by the disclosure to measure a broad dynamic range of at least 6 magnitude...)”), 1734-1736 (similar); EX1001, 20:20-32. Allowance

was never based on uncertainty over whether mass spectrometry theoretically could measure protein concentrations differing by 6 orders of magnitude.

What the Examiner confronted, but the Petition ignores, is that the literature teaches mass spec fails its potential to accurately detect protein concentrations separated by 6 orders of magnitude when applied to the complexity of a biological sample. Indeed, like Petitioner, the Examiner initially asserted the claims were obvious over references using mass spectrometry to individually analyze corona proteins of different particle types. In the first substantive office action, the Examiner asserted the challenged claims were obvious over a combination of Hajipour (EX1010), Walkey (EX1006), and Hadjidemetriou (EX2001). EX1002, 1636-1648. Each of the three references individually analyzed a distinct protein corona for multiple particle types to identify which protein(s) were most enriched in each corona (i.e., to narrow the dynamic range). EX1010, 1212-1215 (Hajipour separately analyzed the corona of polystyrene particles and the corona of silica particles using SDS-PAGE); EX1006, 2441, 2451 (Walkey separately analyzed individual coronas from 105 different gold nanoparticles using LC-MS); EX2001, 8147-8148 (Hadjidemetriou separately analyzed corona of bare, PEGylated, or targeted liposomes using both SDS-PAGE and LC-MS); *see also* Section I.A, above. In a prosecution interview subsequent to the rejection over Hajipour, Walkey, and Hadjidemetriou, the Applicant explained that assaying the subset of

proteins from multiple different particle types (e.g., using a sensor array), unlike the prior art, allowed the identification of biomarkers associated with disease state across a range of at least 6 orders of magnitude. EX1002, 1712, 1720-1730; *see also* EX1001, 15:37-42.

As the Examiner recognized in the next rejection, the prior art did not assay the subset of proteins from the coronas of the multiple particle types to assay the biological sample. EX1002, 1731-1743. The only place the Examiner could find this claimed innovation was in the inventors' own disclosure. The Examiner recognized the inventors' sensor array "has the ability to sample a greater dynamic range than has previously been achievable" by the prior art. EX1002, 1736-1737. But Applicant explained to the Examiner that the inventor's own disclosure was unavailable as prior art. EX1002, 1751, 1755, 1758. Unable to rely on Applicant's invention as prior art, the Examiner allowed the claims. EX1002, 1800-1803. The Examiner recognized that the closest reference was Applicant's own sensor array, and that the other references (analyzing individual corona, one-by-one, even using mass spec) did not disclose "measuring a subset of particles comprising coronas from a biological sample wherein the proteins are detected with an instrument that measures concentration across a dynamic range of at least 6 orders of magnitude." EX1002, 1800-1803.

E. The Ground References Fail to Distinguish the Prosecution Art

Just like the literature asserted during prosecution, the Petition's ground references also analyze protein concentration of each corona individually. For example, Zaccaria's (Ground 4) mass spectrometry analysis was done separately for a subset of peptides, which were derived from the subset of proteins derived from each of the distinct particles. EX1007, 1873-1877, *see also id.*, 1871 ("each duplicated eluate...were separately diluted" and processed by SDS-PAGE, then individual bands were cut and analyzed by LC-MS/MS); *id.*, 1873 ("a deeper characterization of **each NP corona** was performed" by LC-MS/MS); *id.*, Abstract (study is "[t]aking advantage of the NP potential to harvest different subsets of proteins" and "the capture of specific proteins on the three different surfaces"); *id.*, 1870 (study comprised "analysis of the different NP-treated samples" and "highlighting subsets of proteins specific to each kind of NPs"); *id.*, 1871 ("each kind of NPs were individually incubated in two replicates"); *id.*, 1872 (particles "were separately incubated in duplicates"); *id.* ("a protein profile of each condition" was obtained). This is further evidenced by Zaccaria's detailed discussion of relative protein abundance based on particle type (EX1007, 1874-1878, Table 2, Fig. 3) and supplemental information distinguishing identified proteins based on association with a given particle, as opposed to identification of proteins from the combined assays (*id.*, 17-120 (supplemental information)).

Likewise, Hu (Ground 1) analyzed particle types individually, one-by-one. EX1008, 355, Table 2; *see also id.*, 359 (“[T]he distinctive properties of the plasma protein coronas formed on the different sizes of Fe₃O₄ NPs.”); *id.*, 355 (“Here, we quantified the plasma protein **coronas** bound on three different sizes of pristine Fe₃O₄ NPs.”), *id.*, 356 (“each sample was analyzed in at least three replicates”), Table S1 (showing 3 replicates of MWSpC for each sample corresponding with each particle size), Fig. 2(c) (samples run on a particle-by-particle basis), Fig. 5(b) (showing proteins identified and uniquely bound to different particles).

Similarly, Cai (Grounds 2 and 3) teaches its “[c]ell lysates were incubated with 5 mg of CNTs **or** CB [carbon black],” washed and thereafter analyzed. EX1005, 584; *see also id.*, Fig. 2(b); *id.*, Fig. 1(a), 585 (“the same protein mixture” was tested “[i]n parallel” with CB); *id.*, 586 (separately, a “CNT pull-down was performed on the ‘medium’ lysate, and a CB pull-down was performed on the ‘heavy’ lysate”); *id.*, 587 (“Our MS analysis confirmed that the number of proteins enriched by the MWCNT-binding is much higher than that enriched by CB.”). Each of Petitioner’s ground references—like the art asserted during prosecution—applied the individual approach that was routinely used in the field to narrow the dynamic range.

Like the art asserted during prosecution, the Ground references also identify the most abundant proteins in a given corona, and discard low-abundant signals.

See, e.g., EX1008 (Hu), Table 2 (“The top 20 most abundant plasma proteins bound on the Fe₃O₄ NPs of different sizes”), *id.*, 360 (“we list the top 20 most abundant plasma proteins”), 358-359 (limiting evaluation to “plasma proteins with an average MWSpC [percentage normalized spectral count for a given protein] ≥ 1 in the protein corona on the Fe₃O₄ NPs”), 359-360 (“proteins with extremely low relative abundances are not expected to be critical factors that determine the bio-distributions” studied); EX1007 (Zaccaria), 1874 (comparing “the 20 most abundant identified proteins (i.e., the 20 proteins with the higher MS/MS queries”), *id.* (“results confirmed the reproducibility of capture of each kind of NPs for these 20 most abundant proteins”), *id.*, 1870 (“the low abundant protein compartment where disease-associated biomarkers are thought to reside is still unattainable”), *id.* (“the presence of hemoglobin...masks the 2% of biologically interesting low-abundant proteins [so] it is difficult to investigate the deep RBC proteome”); EX1005 (Cai), 592 (directing future development with focus on the “proteins with high affinity to CNT” identified in the study). None of the Ground references discloses step (c) as required by all challenged claims.

1. Brief Summary of Hu (EX1008)

Hu is directed to evaluating nanoparticle uptake into cells and “the bio-distributions and biological fates” of particles in biological systems. EX1008, Abstract. Hu separately incubated 30-nm, 200-nm, or 400-nm Fe₃O₄ particles in a

plasma mixture from 20 different individuals. EX1008, 355. Following corona formation, captured proteins from the corona of each individual particle type were digested and individually analyzed via shotgun proteomics using mass spectrometry. *Id.*

Noting that protein coronas “can critically impact the biological identity” of the particle, Hu individually identified the 20 proteins that were most highly abundant on each particle size because those most-abundant proteins drove the biological fate of the particles. EX1008, 359-360 & Table 2, Abstract. Hu pruned out all results with spectral counts below 1. EX1008, Fig. 6 Caption (“Relative protein abundance of proteins (normalized to molecular weight, MWSpC) satisfying the criterion that $\text{MWSpC} \geq 1$ in the coronas of the different sizes of Fe_3O_4 NPs.”). Even the most abundant spectral results in Hu were just over 10. *See, e.g.*, EX1008, 11 (entry IPI00021841.1 Apolipoprotein A-I middle particle type: $\text{MWSpC} = 10.11$). Accordingly, Hu narrowed its theoretical dynamic range for an individual particle type from less than 4 to just about 1 order of magnitude.

2. Brief Summary of Cai (EX1005)

Cai also focused on corona composition of the individual particle types, one-by-one. Like Hu, Cai also wanted to explore the impact of the individual corona on cell uptake and biological fate of the individual particle type in the biological system. Specifically, Cai asserts that its studies provide “the biochemical basis for

the assessment of the suitability of CNTs [carbon nanotubes] as biomedical tools, and as an emerging pollutant.” EX1005, Abstract.

Cai teaches there is an “apparent link between CNT [carbon-nanotube] protein binding and toxicity,” that proteins with high affinity to bind with CNTs are “potential targets” for disruption to cellular structure and functioning after CNTs enter the cell, and that the binding of these proteins to CNTs “could complicate the efficacy of CNTs as biosensing probes or delivery tools for therapeutic cargos.” EX1005, 592. Cai demonstrated that CNTs are able to selectively enrich some specific proteins from protein mixtures and taught that CNTs should be modified and blocked to “minimize” non-specific protein interactions (i.e., narrowing their dynamic range). *Id.* In investigating the capacity of CNTs for protein binding, Cai discloses two separate studies.

In the first study, Cai individually incubated multi-wall carbon nanotubes (“MWCNTs”), **or** carbon black (“CB”) particles, in separate HeLa cell lysates. EX1005, 585-587. Proteins that bound stably to the given carbon matrix were separated from one another using SDS-PAGE. EX1005, 585 & Fig. 1A-B (reproduced, right). Coomassie-blue

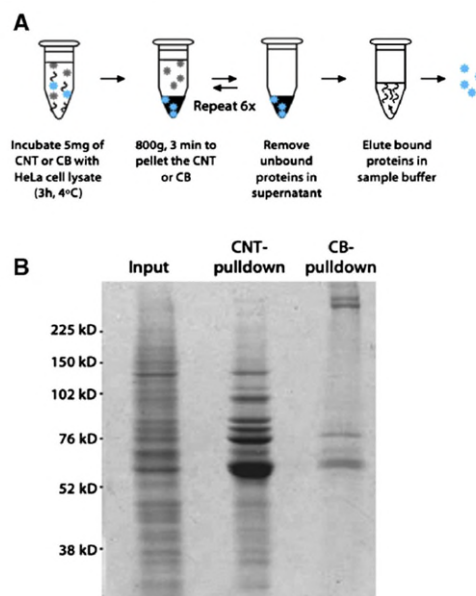
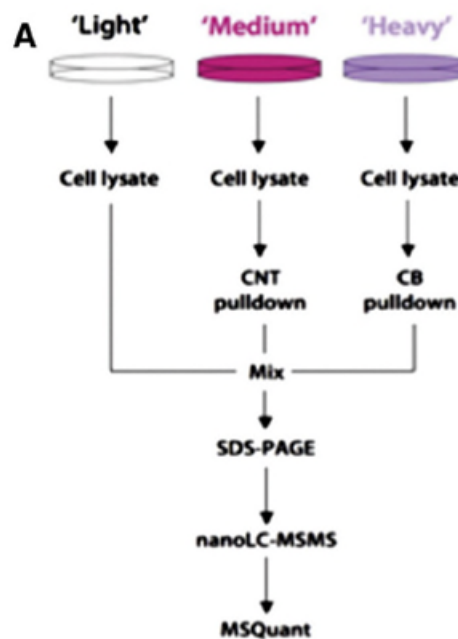


Figure 1. Protein binding assay to study MWCNT- and CB-protein corona. (A) Scheme of

staining intensity was used to estimate gross protein loading after 3 hours of incubation. EX1005, 585. Cai reported that MWCNTs with diameters wider than 20 nm did not further increase protein binding capacity, as the amount of protein pulled down by CNTs with diameters from 20-100 nm is essentially the same, but that MWCNTs with diameters less than 10 nm “bound to virtually no proteins.” EX1005, 586 & Fig. 2. Cai similarly reported that MWCNT length had no effect on the amount of protein bound. EX1005, 586 & Fig. 2.

Cai’s second study individually incubated CBs or MWCNTs having a diameter of 20-40 nm in separate HeLa cell lysates. EX1005, 4 & Fig. 3

(reproduced, right). The MWCNTs were incubated in “medium”-SILAC-media-metabolically-encoded HeLa cell lysate. EX1005, 586 (CNT pull-down). Separately, CBs were incubated in “heavy”-SILAC-media-metabolically-encoded HeLa cell lysate. *Id.* (CB pull-down). Equal volumes of these differentially-labeled proteins were then spiked



with an equal amount of unfractionated “light”-SILAC-media-metabolically-encoded HeLa cell lysate. *Id.* The proteins ***in this mixture*** were separated from one another using SDS-PAGE and proteins extracted from some individual bands

of the mixture proteins were then analyzed with “the ‘gel-LC-MS/MS’ approach.”
EX1005, 586-587.

Cai reports some protein relative abundance that it says spreads over 8 orders of magnitude, and explains that this abundance arises from a semi-quantitative indication of the overall abundance of protein *in the mixture*:

An examination of the SILAC ratio of each quantified protein and its empirical peptide abundance index (emPAI), a semi-quantitative indication of the overall abundance of protein *in the mixture*, reveals no strong correlation between the MWCNT- and CB-binding efficiencies of a protein and its abundance in the lysate. The relative abundance of the MWCNT-binding proteins spread over 8 orders of magnitude.

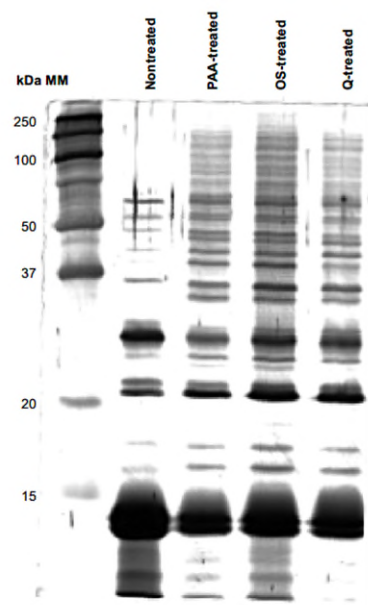
EX1005, 587. Cai does not report that the relative abundance of a protein *in the mixture* corresponds to the relative abundance of the protein *in a lysate*. Nor does Cai’s measurement *in the mixture* map to the subset of proteins comprising the coronas produced from two different particle types exposed to a single biological sample.

3. Brief Summary of Zaccaria

Zaccaria sought to use an individual particle type, one-by-one, to narrow the dynamic range of a MS assay to try to detect a subset of low-abundant proteins of interest. EX1007, 1881 (“through the respective *decrease of high-abundant proteins* and increase of low-abundant proteins, NP treatment *greatly reduces the*

range of concentrations in the sample, making detectable more peptides by the MS instruments”). Zaccaria explained that the individual nanoparticle approach (narrowing the dynamic range) was a necessity because assaying a biological sample using mass spec was otherwise **unattainable**. EX1007, 1869-70 (despite constant advances in MS and sample fractionation methods, “the limited dynamic range of current mass spectrometers hinders the entire proteome coverage of biological fluids” rendering “the low-abundant protein compartment where disease-associated biomarkers are thought to reside still **unattainable**”). Zaccaria explains that hemoglobin represents 98% of the total red blood cell (“RBC”) soluble protein content, masking the remaining 2% of proteins. *Id.*, 1870. Zaccaria thus focused its analysis on “the 20 most abundant identified proteins” for an individual corona. *Id.*, 1873-1874, 1880-1881, Table 2 Caption.

Zaccaria separately incubated non-treated, PAA-treated, OS-treated, or Q-treated particles in red blood cell lysate mixtures created from 5 different individuals. EX1007, 1870, 1872-1873. Following corona formation and separation, a subset of results from an individual particle type were separated using SDS-PAGE to detect a subset of peptides. EX1007, 1871, Fig. 1 (reproduced right); *see also id.*, 20 (“The stacking band was cut and



washed in 100 mM ammonium bicarbonate, reduction and alkylation of cysteines were performed by mixing the gel pieces in 10 mM DTT for 35 min.... Additional cycles of washes was then performed and proteins were digested by incubating the band gel with 0.6 µg of modified sequencing grade trypsin.... The resulting peptides were extracted from the gel by three steps....”). The subset of peptides extracted from an individual band gel was then individually analyzed via mass spectrometry. EX1007, 1871. Zaccaria, like Hu and Cai, calculates abundance as captured on an individual particle type. EX1007, 1872.

III. CLAIM CONSTRUCTION

In an *inter partes* review, a claim is given its ordinary and customary meaning in light of the specification. Petitioner offers no constructions. Pet. 14-15. No construction is necessary to deny institution.

IV. PERSON OF ORDINARY SKILL IN THE ART

A. Definition of a Person of Ordinary Skill in the Art

Patent Owner affirmatively disputes Petitioner’s definition of a POSA as insufficient. The Petition relies on an expert with no proteomics experience, who received her advanced degree in Chemistry in 2017 (after the critical date).

EX1004, 1. The Petition then curates its definition of a person of ordinary skill in the art (POSA) to try to encompass its witness’s lack of relevant experience. Pet., 11 (anyone with a bachelor’s degree in chemistry and three years’ experience in

the field of proteomics, protein analysis, **or** mass spectrometry). But the field of the invention relates to using “sensor arrays for the detection and diagnosis of different disease states” and, particularly, “to diagnose or prognose diseases or disorders.” EX1001, 1:28-31. In particular, the claimed invention is directed to assaying a subset of proteins separated from a biological sample (e.g., blood serum) using the coronas formed around a plurality of particles of different particle types. The level of skill is thus not satisfied by anyone with a bachelor’s degree in chemistry and three years of experience using mass spectrometry, as Petitioner proposes.

Indeed, Petitioner’s definition is deficient because it excuses Petitioner’s expert from having **any** expertise in proteomics assays, protein coronas, or applying mass spec to a biological sample (e.g., blood plasma). A POSA in the field of the ’360 Patent in December 2016 or December 2017 would have had a degree of expertise, either through education, years of work experience, or a combination thereof, specifically in the field of proteomics and protein analysis.

In addition to failing to account for the field of the invention, the Petition’s definition of the level of skill is additionally deficient for failing to consider the qualifications of workers in the field and what the prior art reflects about the state of the field. For example, the inventors’ educational background and experience is relevant to the definition of a POSA. *Daiichi Sankyo Co., Ltd. v. Apotex, Inc.*, 501 F.3d 1254, 1256 (Fed. Cir. 2007). Inventor Dr. Omid Farokhzad was a Professor at

the Harvard Medical School, and directed the Center for Nanomedicine at Brigham and Women's Hospital. His educational experience included an M.D. and a post-doctoral fellowship focusing on nanomedicine and biomaterials. EX2004. Inventor Dr. Morteza Mahmoudi had a Ph.D. in nanoscience and nanotechnology and received additional education specifically focusing on protein corona. EX2005. Finally, inventor Dr. Claudia Corbo had a Ph.D. that included the use of differential proteomics and bioinformatic analyses of protein datasets, and also had postdoctoral research experience directed to proteomics and the use of nanoparticles. EX2006. Thus, a POSA would have had at least three years of experience working with protein coronas and proteomics assays.

B. Petitioner's Expert is Not a Person of Ordinary Skill in the Art

Petitioner's expert—Dr. Vargas—is not a POSA. She lacks the relevant educational background and lacks sufficient experience working with protein coronas and proteomics assays. Instead, Dr. Vargas “is a *chemist* who specializes in compositional analysis of complex materials in support of product development, manufacturing, and root cause analyses.” EX1004, 1. Dr. Vargas does not hold herself to be an expert in proteomics or to have experience with protein coronas. EX1003, ¶¶5-10. Dr. Vargas's expertise is directed toward analytical chemistry and synthesis of polymeric materials. EX1003, ¶¶5-10; EX1004. She does not purport to have performed mass spectrometry techniques specifically for protein

analysis, much less to have applied mass spectrometry for a proteomics assay of a protein corona. Lacking the basic qualifications of a POSA for the '360 Patent, Dr. Vargas's testimony is "neither relevant nor reliable." *Kyocera Senco Indus. Tools v. Int'l Trade Comm'n*, 22 F.4th 1369 (Fed. Cir. 2022).

Because she lacks basic qualification in the field of the challenged patent, Dr. Vargas failed to recognize the teachings in the prior art that the wide dynamic range in protein concentrations in biological samples "hampers the identification of low abundant proteins" by mass spectrometry analysis (EX1010, 1218), that using spectral counting "to estimate the relative abundance of low-abundance proteins within complex mixtures is inaccurate" (EX1006, 2451), 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 (EX1015, 786), and that, despite constant advances in mass spec and sample fractionation methods, "the limited dynamic range of current mass spectrometers hinders the entire proteome coverage of biological fluids" rendering "the low-abundant protein compartment where disease-associated biomarkers are thought to reside [as] still **unattainable**" (EX1007, 1869-1870). Dr. Vargas's oversimplified approach to the challenged patent and prior art confirm she is not qualified to testify.

Petitioner's erroneous POSA definition and unqualified expert incurably taint the Petition case. Petitioner bears the burden of proof, yet the Petition materials lack the perspective or testimony of a person of ordinary skill in the art. Institution can be denied on this basis alone.

V. GROUND 1 (HU) FAILS

Petitioner fails to show that Hu discloses each and every element of the challenged independent claim. Because the Petition fails to show all elements of the claims in the prior art, Petitioner fails to meet its burden of establishing the claims are *prima facie* obvious.

A. Missing Element: Assaying the Subset of Proteins of (b)

As a threshold matter, Ground 1 fails to demonstrate Hu discloses claim step (c), “assaying the subset of proteins of (b),” as required by all challenged claims. In step (b), at least a subset of the plurality of particles comprising “the coronas” is removed from the biological sample, thereby producing a subset of proteins from the biological sample. “The coronas” were formed in step (a) and differ based on particle type. EX1001, claim 1. Step (c) is satisfied only by assaying “the subset of proteins of (b)” to “detect, in the subset, proteins in the biological sample at concentrations across a dynamic range comprising six orders of magnitude.” But the Petition fails to demonstrate that Hu discloses assaying step (c).

To satisfy assaying step (c), the Petition points exclusively to Hu's use of RPLC-MS/MS "carried out on a 1-D nano LC-MS/MS system with 5 μ L of the resuspended peptides injected each time." Pet. 33 (quoting EX1008, 355-356). Rather than assay "the subset of proteins of (b)" produced when the particles "comprising the coronas" were separated from the biological sample, Hu individually assayed proteins produced when a given particle type was separated from the biological sample, one-at-a-time. Rather than assaying the subset of proteins *from the coronas*, as required, Hu discloses that "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 μ L of the resuspended peptides injected each time." EX1008, 355-356. Hu then reported results individually for a given particle type. 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 three different columns); Pet., 33-34 (citing Hu Table 2 and the row corresponding to IPI00973177.1 for a result found individually on only one particle type). Ground 1 thus fails for the same reason the Examiner allowed the claims over Hajipour, Walkey, and Hadjidemetriou—Hu never went beyond the prior art approach of screening different nanoparticles individually, each narrowing the dynamic range.

B. Missing Element: Detect Proteins At Concentrations Across a Dynamic Range Comprising At Least 6 Orders of Magnitude

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. Instead, the Petition admits that Hu does not expressly disclose this limitation. Pet., 35 (“Hu does not specifically state the dynamic range in numerical form”).

The Petition ignores both the data reported by Hu and what Hu teaches about that data. The Petition argues that “serum albumin” was one of the top 20 most abundant proteins in the corona, and that Hu’s “supplementary data” provides “relative protein abundance” results for both serum albumin and LTBP-1. Pet., 33-35. Rather than detecting proteins in the subset across a dynamic range comprising at least 6 orders of magnitude, Hu instead focused on a narrow dynamic range. Indeed, building on protein depletion approaches, Hu discarded all protein identifications with spectral counts below 1 and instead assayed “the top twenty most abundant proteins found bound” to each individual nanoparticle. Pet., 33 (“Hu discloses the top twenty most abundant proteins”). EX1008, 359-360 (“proteins with extremely low relative abundances are not expected to be critical factors that determine the bio-distributions and biological fates of NPs”), Table 2, Fig. 6 Caption (“Relative protein abundance of proteins (normalized to molecular

weight, MWSpC, satisfying the criterion that $MWSpC \geq 1$)). The values reported in Hu's supplementary data are 9.01 and 0.01 for serum albumin and LTBP1 Isoform 4, respectively, which values differ by less than 4 orders of magnitude. EX1008, 10 (IPI00745872.2), 18 (IPI00973177.1). The Petition does not even assert that the values actually reported in Hu satisfy the 6 orders of magnitude limitation of the claims.

1. The Petition's Inherency Argument Fails

Under the guise of conclusory language about what a POSA "would have understood, or at least found it obvious," Petitioner pivots away from Hu's reported values in favor of different values it manufactures through alleged inherency. Pet., 35-36. Specifically, the Petition argues that serum albumin and LTBP-1 "existed" at "concentrations in the biological sample being assayed" in Hu that differed by six orders of magnitude. Pet. 35-37. This is fundamentally an inherency argument, as admitted by Petitioner's expert. EX1003, ¶161 ("Although Hu does not explicitly disclose 'assaying the subset of ... proteins in the biological sample at concentrations across a dynamic range comprising at least 6 orders of magnitude,' this is inherently disclosed by Hu"). But the Petition's inherency argument fails for at least two reasons.

First, the Petition fails to tie together the sample in Hu to the extrinsic references it relies upon for its inherency argument. The Petition points to Hu's

discarded spectral count ($0.01 \leq 1$) for LTBP1 Isoform 4. Pet., 34, 36; EX1008, 18 (Table S1, row corresponding to “IPI00973177.1”). Petitioner then pivots to two separate references (Cao, EX1016 and Ahmed, EX1015) to argue that LTBP1 was present in **Cao’s** blood serum sample at a concentration at least 6 orders of magnitude lower than serum albumin protein was present in **Ahmed’s** blood serum sample. Pet., 36. The Petition thus attempts to incorporate into Hu two **different values** reported in two **different references** discussing results from two **different experiments** involving two **different samples**. Pet. 36 (discussing EX1015-EX1016). But the Petition fails to demonstrate necessary correspondence between Cao or Ahmed and the sample tested in Hu.

The Petition entirely fails to justify its assumption that either Cao or Ahmed report protein concentrations **for Hu’s samples**. Hu does not cite Cao or Ahmed. Nor do they cite Hu. Rather than establish that protein concentrations are inherently identical in different samples, the Petition’s extrinsic evidence instead declares the opposite: protein concentrations vary significantly between samples. *See, e.g.*, EX1015, 786 (“Standardizing sample preparation procedures for plasma/serum profiling..., as well as fractionation techniques employed either to generate several fractions or to selectively obtain a particular subset of peptides/protein, although time-consuming and error prone is critical for obtaining reliable biomarkers and building a biomarker pattern, since **slight changes in a**

given sample preparation could lead to very different protein profiles.”), 786 (protein half-lives range from a few days to just a few hours). The Petition never justifies its baseless assumption that values reported in Cao or Ahmed necessarily are the concentrations for the sample in Hu.

Second, the Petition’s inherency argument points to the wrong protein as the reference point. The (discarded) LTBP1 spectral count in Hu is for “Isoform 4.” EX1008, 18 (Table S1, row IPI00973177.1: “Isoform 4”). Cao teaches “[t]here are four isoforms of LTBP1 (LTBP-1, -2, -3, and -4)” and reported concentration values from Cao’s sample *for isoform 1*. EX1016, 16051 (LTBP-1). The Petition fails to demonstrate that the LTBP-1 concentration relied upon in Cao is for the same protein as in Hu.

2. Petitioner’s Obviousness Theory Fails

After admitting Hu lacks express disclosure of the dynamic range limitation of the claims, the Petition makes a conclusory and entirely unsubstantiated argument that “a POSA would have...found it obvious.” Pet., 35 (“Hu does not specifically state the dynamic range in numerical form”).

Petitioner has the burden of showing a POSA would have been motivated to modify a prior art reference to arrive at the claimed invention. It is not sufficient to show that a modification *could* be made; Petitioner must establish the POSA would have been motivated to do so. *PersonalWeb Techs., LLC v. Apple, Inc.*, 848

F.3d 987 (Fed. Cir. 2017); *see also Polaris Indus., Inc. v. Arctic Cat, Inc.*, 882 F.3d 1056, 1068 (Fed. Cir. 2018) (error to focus “on what a skilled artisan would have been able to do, rather than what a skilled artisan would have been motivated to do at the time of the invention.”); *Belden Inc. v. Berk-Tek LLC*, 805 F.3d 1064, 1073 (Fed. Cir. 2015) (“[O]bviousness concerns whether a skilled artisan not only *could have made* but *would have been motivated to make* the combinations or modifications of prior art to arrive at the claimed invention.”) (emphases original).

To support its contention, Petitioner first explicitly relies on improper hindsight. Pet., 37 (arguing “the ’360 Patent discloses looking up known protein concentrations in a known database to determine the dynamic range” and thus a POSA would do the same). Petitioner may not use the ’360 Patent as a guide. To do so is impermissible hindsight reasoning. *Apple Inc. v. Voip-pal.com*, IPR2016-01201, Paper 53 (Nov. 20, 2017).

The Petition’s conclusory argument that a “POSA was clearly motivated and understood the need, desirability and routine nature of an analysis resulting in broader dynamic ranges including 6 orders of magnitude and beyond” (Pet. 37-38) improperly conflates knowledge that there was a wide disparity between the concentrations of various proteins found in the blood, into a disclosure to use mass spectrometry from multiple particle types to assay the biological sample across that

entire dynamic range. Petitioner's conclusory and unsubstantiated argument is contradicted by Petitioner's own evidence.

Petitioner submitted multiple references that, like the references asserted during prosecution, belie the Petition's conclusory and unsubstantiated obviousness assertions. These references discuss the difficulties and inaccuracies of mass spec analysis of protein concentrations across a broad dynamic range. *See, e.g.*, EX1010, 1218 (the "small fraction of highly abundant proteins...***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***" and one should apply a "pruning procedure" to eliminate "low-abundance proteins"); 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 (despite constant advances in mass spec and sample fractionation methods, "the limited dynamic range of current mass spectrometers hinders the entire proteome coverage of biological fluids" rendering "the low-abundant protein compartment where disease-associated biomarkers are thought to reside [as] still ***unattainable***"). These obstacles explain why Hu expressly limits its analysis to the 20 most abundant proteins and prunes from its evaluation any proteins with spectral counts below 1. EX1008, 360

(“Here, we list the top 20 most abundant plasma proteins comprising the protein coronas formed on the Fe₃O₄ NPs”), Table 2 (same), Fig. 6 Caption (discarding MWSpC < 1). As explained above in Section II.E.1, Hu’s pruning procedure **narrows** the dynamic range to no more than two orders of magnitude (MWSpC range of 1-10) rather than expanding it to 6. The Petition’s conclusory and uncorroborated obviousness argument is thus belied by its own evidence.

Instead of confronting its own evidence detailing how the state of the art contradicts its obviousness assertions, the Petition pretends this is an overlapping range case. Pet., 38-39 (citing *Iron Grip*, *Peterson*, and *Pfizer*). But Petitioner cannot invoke overlapping range case-law without presenting evidence 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 the prior art did not actually disclose the claimed range. *ModernaTX, Inc. v. Arbutus Biopharma Corp*, 18 F.4th 1364 (Fed. Cir. 2021). Instead, before a presumption of obviousness can be applied, the patent challenger “would have to first show that, despite the lack of an express disclosure in the references, a person of ordinary skill would have nevertheless understood” that a prior art range overlaps with the claimed range. When there is no prior art range, Petitioner cannot flip the burden onto Patent Owner to address the nonexistent prior art ranges.

Here, Hu does not disclose assaying step (c) across a dynamic range comprising 6 orders of magnitude, for all the reasons discussed above. There is thus no overlap between the claimed range (at least 6 orders of magnitude) and a prior art range. 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) explained that currently-available mass spec technologies were “inaccurate” and “unattainable” for assaying across a wide dynamic range. That is the opposite of an overlapping prior art range. This point was vetted during prosecution. See Section II.D above. The Petition presents no good reason to revisit it again.

For each of the reasons discussed above, Ground 1 fails to demonstrate a reasonable likelihood of success and Petitioner fails to meet its burden to establish that Hu renders obvious the challenged claims.

VI. GROUND 2 (CAI ANTICIPATION) FAILS

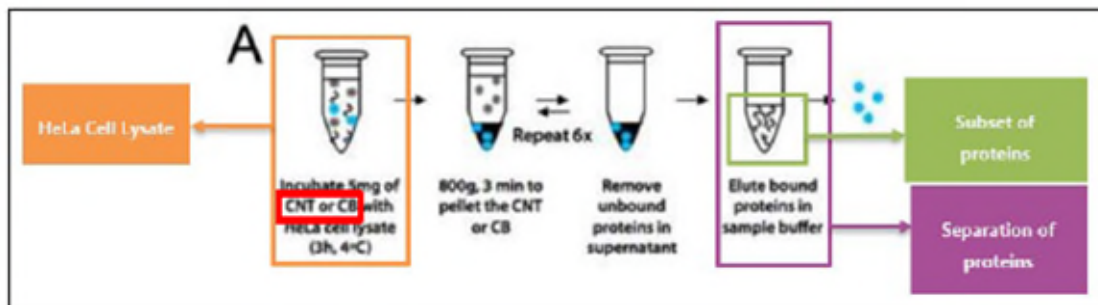
Petitioner’s back-up grounds fare no better than its primary ground. Because Hu (Ground 1) does not disclose all claim steps, Petitioner then asserts in a back-up ground (Ground 2) that Cai anticipates the independent claim. But there is a critical disconnect in Petitioner’s claim mapping, which fails to satisfy the standards for anticipation. The Petition’s inability to map Cai to show actual

anticipation of claim 1 likely explains Petitioner's decision to assert this reference only as a back-up Ground after its (also meritless) inherency argument in Ground 1.

A. The Petition Inconsistently and Incoherently Maps Cai to Claim 1

The Petition argues that “the relative abundance of bound proteins ‘spread over 8 orders of magnitude’ shows that Cai’s MS analysis detected protein concentrations in the subset across a dynamic range comprising the claimed ‘*at least 6 orders of magnitude*,’ which is further confirmed by the concentrations detected in Cai.” Pet., 60 (citing EX1005, 587; EX1003, ¶¶229, 97). Petitioner’s expert specifies that the “8 orders of magnitude” in Cai refers to the range of emPAI values reported in Table S2 of Cai for Cai’s second experiment. EX1003, ¶97 (emPAI ranging from 0.02-2,254,683). But the claims do not merely require assaying across a dynamic range comprising at least 6 orders of magnitude ***in some abstract sense***. They require “assaying, in the subset, proteins in the biological sample at concentrations across a dynamic range comprising at least 6 orders of magnitude[.]” EX1001, claim 1. The Petition merely assumes, without demonstrating, that the “8 orders of magnitude” difference in emPAI values discloses detecting, in the subset of (b), proteins in the biological sample at concentrations across a dynamic range comprising at least 6 orders of magnitude.

This unsupported assumption misinterprets Cai and is inconsistent with their own mapping of the claims to the Cai reference. First, there is a disconnect between what the Petition maps as the “subset of proteins of (b)” and what the Petition maps as being assayed for step (c). Though step (c) requires assaying “the subset of proteins of (b),” Petitioner inconsistently points to different subject matter from different experiments as “the subset” without explanation. The Petition mapped the claimed “subset of (b)” to the individual subset of proteins eluted in sample buffer in Cai’s first experiment. Pet., 57-58 (see annotated EX1005, Fig. 1A, reproduced below).



But when they arrived at step (c), which also refers to the subset of (b), the Petition changed gears entirely and without explanation. While step (c) refers to “the subset” of step (b), the Petition jumps to a different experiment and different content of Cai for the “at least 6 orders of magnitude” limitation—Petitioner’s expert specifies that the “8 orders of magnitude” in Cai refers to the range of emPAI values reported in Table S2 of Cai for Cai’s second experiment. EX1003, ¶197 (emPAI ranging from 0.02-2,254,683).

Moreover, there is also a disconnect between what Petitioner identifies as “the biological sample,” “the subset,” and the different subject matter Petitioner identifies as producing a dynamic range of “8 orders of magnitude.” Cai does not attribute the “8 orders of magnitude” to the concentrations in either the “subset of (b)” or “the biological sample” mapped by the Petition. Pet., 46-47 (mapping “biological sample” to “complete protein lysate of HeLa cells” from Cai’s first (protein loading) experiment). When discussing “8 orders of magnitude,” Cai is referring to emPAI values from the second experiment. Cai explains that emPAI is a semi-quantitative indication of the overall abundance of protein *in the mixture*. EX1005, 587 (“empirical peptide abundance index (emPAI), a semi-quantitative indication of the overall abundance of protein in the mixture”). This particular mixture in Cai was manufactured by spiking “[e]qual volumes of proteins eluted from these pull-down experiments” with “an equal amount of unfractionated ‘light’ lysate.” EX1005, 586. The Petition fails to demonstrate that the concentration of proteins in *this mixture* is either the concentration of proteins from the pulldown or from the lysate. The Petition’s alleged “anticipation” mapping, which inconsistently spans *different components of different experiments* that the Petition haphazardly combines, fails even to connect the six orders of magnitude mapping to what the Petition alleges constitutes the claimed biological sample or the claimed subset of proteins.

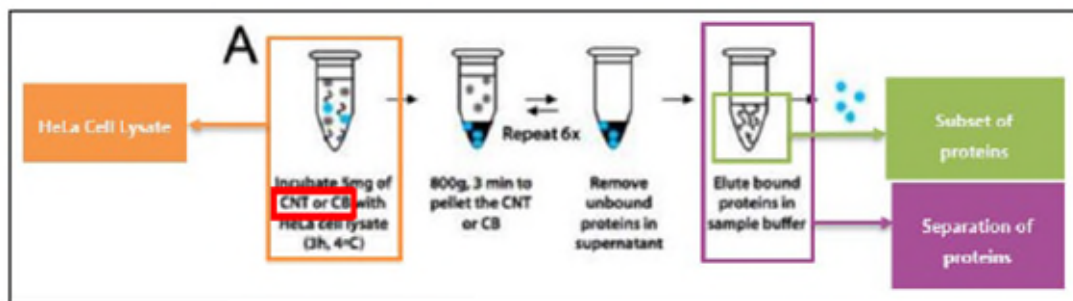
Petitioner's Ground 2 mapping is also inconsistent with its Ground 1 challenge, where the Petition argued the dynamic range reflected the concentration of proteins *in the sample*. Pet., 35-37. By Petitioner's expert's own admission, it is the protein concentrations *in the mixture* that are at issue in its Ground 2 Cai challenge. EX1003, ¶¶228-229.

The Petition's meandering conglomeration of disparate embodiments does not satisfy the legal standard for anticipation. "In order to anticipate the claimed invention, a prior art reference must 'disclose all elements of the claim within the four corners of the document,' and it 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)). Petitioner fails to establish its mapping of Cai shows prior art disclosure of all claim elements as arranged in the claims. Accordingly, institution of Ground 2 should be denied.

B. Missing Element: Forming (a) and Separating (b) "the coronas"

As illustrated by the graphic from page 58 of the Petition (discussed above), the Petition mapping also fails to satisfy the requirements of claim step (b). Claim step (b) requires separating a "plurality of particles comprising the coronas" from the biological sample by removing that plurality of particles "thereby producing a subset of proteins." EX1001, claim 1. The "coronas" were formed around the

plurality of particles in step (a) and, as recited in step (a), correspond “to the different particle types.” *Id.* The Petition highlights in red Cai’s individual incubation in experiment 1 of CNTs **or** CBs with HeLa cell lysate. Pet., 57-58. The Petition then points to the proteins eluted from the individual particle type as “the Subset of proteins” (highlighted in green). Just as with Hu, the subset of proteins of an individual particle type mapped in Cai does not satisfy the claim requirement of step (b) because the Petition fails to demonstrate that the mapped “subset of proteins” is produced by separating a plurality of particle comprising “the coronas” from the two different particle types. Ground 2 fails for this additional reason.



The Petition argument 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 at least two 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. Second, Cai teaches that MWCNTs of diameters less than 10 nm “bound to virtually no proteins.” EX1005, 586. In contrast to MWCNTs of diameters less than 10 nm, Cai teaches that “the

amount of proteins pulled down by CNTs of diameters from 20-100 nm is essentially the same.” EX1005, 586. Cai also teaches that MWCNTs length similarly had no impact on protein binding amount. EX1005, 586. Rather than coronas from two different particle types, the Petition’s back-up argument merely identifies a corona from one particle type and recognizes the absence of a second corona. The Petition’s backup argument for different coronas is thus flatly contradicted by the express teachings of Cai.

Thus, for at least the reasons above, Petitioner fails to show Cai anticipates the challenged claims.

VII. GROUND 3 (CAI OBVIOUSNESS) FAILS

Failing to establish that Cai renders the challenged claims anticipated, Petitioner pivots to an argument that Cai by itself renders the claims obvious. Pet., 62-64. But Petitioner’s obviousness challenge fails to address or cure the deficiencies of its anticipation challenge. *Id.*; *see supra*, Section VI.

Petitioner’s obviousness challenge fails to establish that a POSA would have combined (or have had a reasonable expectation of success in combining) the distinct scientific studies of Cai. Pet., 62-64. Instead, Petitioner simply haphazardly combines various aspect of different studies without rationale, justification, or demonstration of reasonable expectation of success. Pet., 62. The Supreme Court made clear in *KSR Int’l Co. v. Teleflex Inc.*, 550 U.S. 398, 418 (2007) that there

must be “a reason” for a POSA “to combine the elements in the way the claimed new invention does” with a reasonable expectation of success. When a petitioner challenges a patent “combining multiple embodiments from a single reference...***there must be a motivation to make the combination and a reasonable expectation that such a combination would be successful***, otherwise a skilled artisan would not arrive at the claimed combination.” *In re Stepan Co.*, 868 F.3d 1242 (Fed. Cir. 2017); *see also Abiomed, Inc. v. Maquet Cardiovascular, LLC*, IPR2017-01204, Paper 9 (Oct. 23, 2017) (petitions were “deficient because of the failure to explain sufficiently and support the challenges” where, as here, “Petitioner [] ***fails to provide a sufficient rationale to combine the teachings of [a reference]’s different embodiments***. Petitioner simply fails to explain sufficiently *why* one skilled in the art would have found it obvious to combine those teachings.”); *Activision Blizzard, Inc. v. Acceleration Bay, LLC*, IPR2016-00726, Paper 11 (Sept. 9, 2016) (Petitioner failed to show obviousness where, as here, Petitioner “***does not explain why those disjunctive descriptions may be treated as related to each other as if one embodiment***, or if not one embodiment why a person having ordinary skill in the art would have combined the different embodiments”); *Peddinghaus Corp. v. Ficep Corp.*, IPR2020-01707, Paper 15 (May 11, 2021). Petitioner provides no reason to modify or combine Cai’s studies as mapped in the Petition, nor does it provide any indication of an expectation of

success in so combining the disparate studies. Petitioner's Ground 3 challenge fails for at least this reason.

Insofar as the Petition argues it would have been obvious to convert Cai's emPAI values "into more traditional concentration numbers" (Pet. 62-63), this argument does not remedy the deficient mapping in the Petition. More "traditional concentration numbers" of the spiked mixture still would not correspond to the features the Petition maps as the biological sample or the subset of proteins.

Petitioner's conclusory overlapping ranges argument (Pet., 63-64) fails for at least the same reasons as detailed in Ground 1. *Supra*, Section V.B.

Thus, for at least the reasons above, Petitioner fails to show Cai renders the challenged claims obvious.

VIII. GROUND 4 (ZACCARAI) FAILS

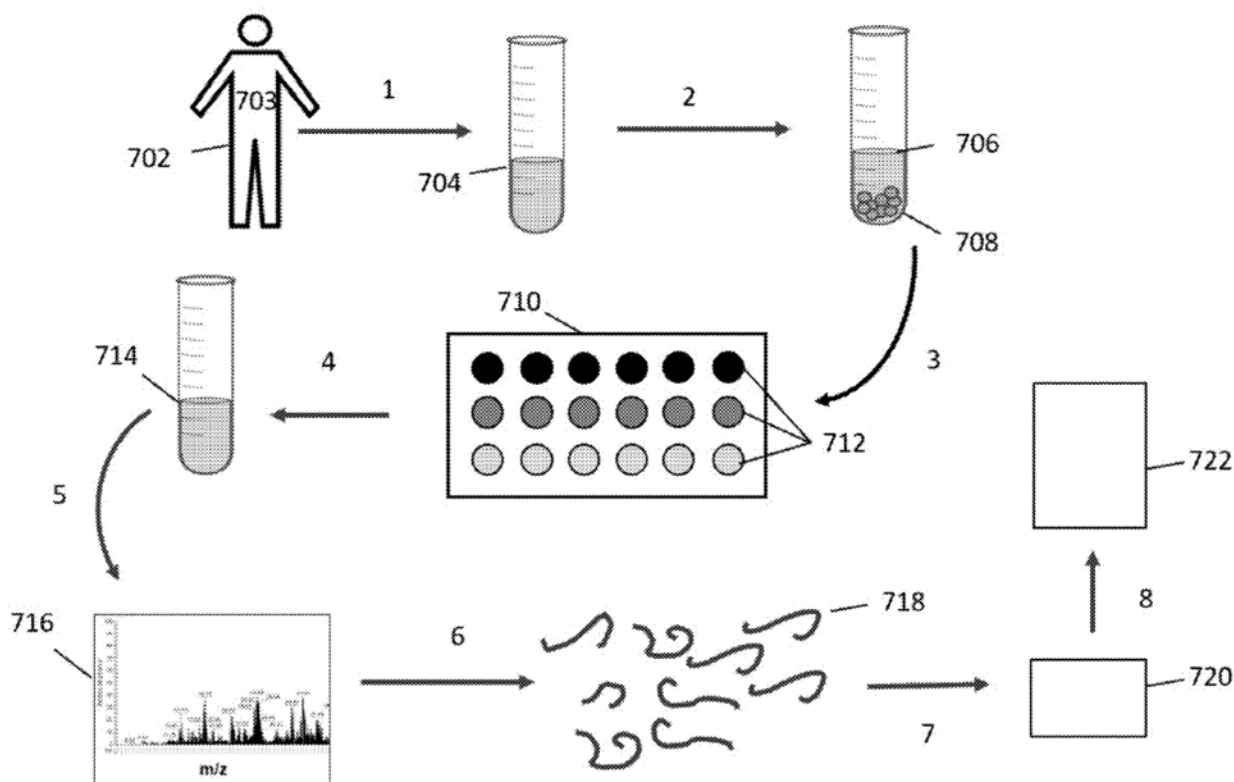
Ground 4 does not remedy the deficiencies of Grounds 1-3. Like the failed Petition grounds that preceded it, Ground 4 fails to provide claim mapping tying Zaccaria's disclosure to all elements of the claim as arranged in the claim, or any rationale for modifying Zaccaria with a reasonable expectation of success.

A. Missing Element: Forming (a) and Separating (b) "the coronas"

As discussed above, step (a) of claim 1 requires contacting the biological sample with a plurality of particles comprising different types and forming different **coronas** around the different particle types. Step (b) of claim 1 requires

separating a subset of particles “comprising *the coronas*” from the biological sample. That subset (“the subset”) must be assayed in step (c). *See also* Pet., 4-5 (citing EX1001, Fig. 65).

FIG. 65



The Petition fails to demonstrate this approach is reflected in Zaccaria.

As discussed above, Zaccaria used an individual particle type, one-by-one, to individually form and then separate a resulting corona, one at a time. Zaccaria explains that different hemolysates were individually and separately contacted with one nanoparticle type. *See* Section II.E.3, above; EX1007, 1871 (“Five hundred

micrograms of each kind of NPs were individually incubated in two replicates for 45 minutes with 200 μ L of hemolysate containing 1 mg of total protein.”). Zaccaria then says the nanoparticles are likewise separated from the individual hemolysate one at a time. *Id.*, 1871 (“Then, NPs were attracted to one side of the vial by an external magnet, and supernatant was removed. NPs were washed three times in 500 μ L PBS.”). The Petition even confirms this one at a time approach. Pet., 65 (“500 μ g of each kind of NPs...were ***separately incubated*** in duplicates..., followed by extensive washing to remove all unbound proteins.” (quoting EX1007, 1871-1872)). Indeed, Zaccaria subsequently discusses its individual “characterization of each nanoparticle corona.” EX1007, 1872. While Zaccaria’s one-at-a-time approach may form and separate a corona for a particle type, that approach lacks “the subset” having “the coronas” formed from a plurality of different particle types. The Petition fails to demonstrate otherwise. The Petition merely points to the one-at-a-time contacting and analyzing of individual corona without providing any explanation for why this disclosure satisfies the claim. Pet., 71.

B. Missing Element: Assaying the Subset of (b)

There is a disconnect between what the Petition argues is “the subset of step (b)” and what the Petition says is assayed “with an instrument” according to step (c). As explained before, assaying step (c) requires assaying the subset of proteins

of step (b). The Petition, however, disregards the disclosure in Zaccaria it relies upon for the claimed subset of proteins of (b) and pivots to a different disclosure for assaying step (c). Claim step (b) requires producing “a subset of proteins from the biological sample” by removing “at least a subset of the plurality of particles” from the biological sample. EX1001, claim 1. The Petition thus points to “isolating the particles with a magnet and removing the supernatant” as allegedly “separat[ing] the particles with coronas from the rest of the biological sample,” leaving “only the subset of particles” that are “bound to the nanoparticles” for further protein analysis after the “NP-associated proteins were desorbed.” Pet., 71. For the assaying step (c), the Petition relies upon Zaccaria’s identification of “the twenty most abundant proteins identified from NPs corona” using “nano-LC-MS/MS protein identification.” Pet., 72-73 (citing EX1007, 1873, Table 2). Those two things are different.

What the Petition points to as the subset of step (b) was not put through LC-MS/MS. Instead, following additional isolation and processing steps, a different combination is subjected to LC-MS/MS. Specifically, Zaccaria discloses that individually incubating 500 µg of a given nanoparticle in 200 µL of hemolysate containing 1 mg of total protein, magnetically pulling down the particles, and disposing of the supernatant, resulted in 19 µg of protein. EX1007, 1871-1872. Again, this is what the Petition identifies as the separated subset of step (b). But

Zaccaria makes clear there is subsequent isolation and processing of the 19 µg of protein such that the MS analysis is not ultimately performed on the subset of (b). Instead, Zaccaria takes a 10 µg portion of the 19 µg subset and applies that portion of the subset to a gel. EX1007, 1871. Once bands are separated on the gel, certain bands are cut out for further processing. An individual band is subjected to trypsin digestion and extraction procedures, and the resulting extracted peptide from a digested band is then subject to LC-MS/MS. *Id.*; see also EX1007, 20 (supplemental materials). The Petition omits all of this and, without explanation, points to a different portion for assaying than the subset it identifies as the subset of (b).

In other words, Zaccaria makes clear that “the subset” separated from the sample is not what is assayed for SBF1 and HBA1, the assaying step cited in the Petition for step (c). Specifically, the Petition argues that Zaccaria Table 2 discloses that hemoglobin subunit alpha (HBA1) was among the 20-most abundant proteins on the particles, and that the supplementary materials identify SBF1 as a “lower abundant” protein on particle Q. Pet., 74 (citing EX1007, 58; EX1003, ¶¶270, 119). But Zaccaria indicates that SBF1 and HBA1 are assessed in two separate instrument detection steps due to the large difference in their molecular weight. Zaccaria reports the molecular weights of the peptides (SBF1 and HBA1) found on the particles, which Zaccaria Fig. 1 indicates would have been far apart in

Zaccaria's different assays of different subsets.

EX1007, 25 (Hemoglobin subunit alpha: 49 rows from the bottom, three columns from the left: MW of 15304.95 daltons) 28 (SBF1: 23 rows from the top, three columns from the left: MW of 210,294.08 daltons), 1872 Fig. 2 (reproduced right, red annotations added) & Caption ("bands corresponding to alpha and beta hemoglobin subunits (around 14 kDa)").

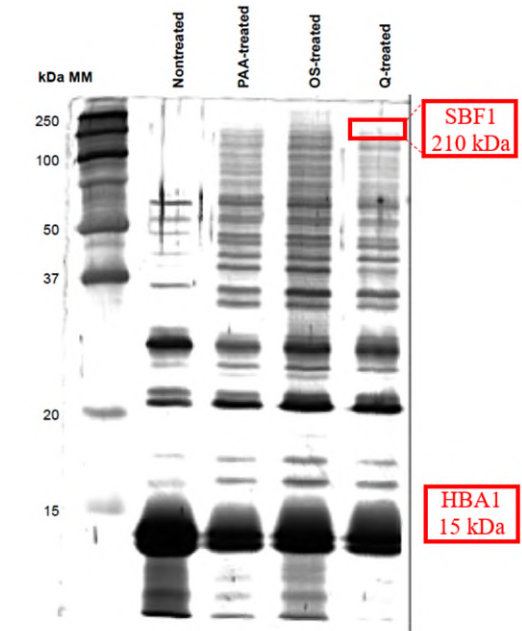


Figure 1 SDS-PAGE of nontreated and NP-treated RBC lysates.
Notes: The same quantity of proteins was loaded on gel for the different tracks. We can see the diminution of hemoglobin proportion in NP-treated samples for which we can distinct two bands corresponding to alpha and beta hemoglobin subunits (around 14 kDa).
Abbreviations: SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; NP, nanoparticle; RBC, red blood cell; MM, molecular markers.

The Petition thus fails to demonstrate that Zaccaria's mass spec experiment assayed the subset of (b), rather than analyzing only a subset of peptides extracted from a gel segment, representing merely a subset of a subset of proteins derived from an individual particle type.

C. Missing Element: Detect Proteins At Concentrations Across a Dynamic Range Comprising At Least 6 Orders of Magnitude

As with Hu in Ground 1, the Petition concedes that Zaccaria in Ground 4 fails to expressly disclose the 6 orders of magnitude limitation of the claims. Pet., 74 ("Zaccaria does not specifically state the dynamic range in numerical form"). For the two proteins relied upon by the Petition for the 6 orders of magnitude element—hemoglobin subunits alpha (HBA1) and SBF1 (Pet., 74 (citing EX1007, 58; EX1003, ¶¶270, 119))—Dr. Vargas testified that the MS/MS queries for the

former were in the range of 244-1563, whereas the MS/MS queries for the latter were “reported to be 10.” EX1003, ¶270.² The Petition does not assert that this difference in abundance of no more than 3 orders of magnitude satisfies claim step (c).

Nor does the Petition dispute that Zaccaria focused its analysis on “the 20 most abundant identified proteins” for a given particle corona, and never identifies fleeting MS/MS counts as low as 10 on the Q-treated particle as providing a reliable concentration across a dynamic range comprising at least 6 orders of magnitude. EX1007, 1873-1874, 1880-1881. Zaccaria teaches that the goal of its use of nanoparticles was to “greatly reduce the range of concentrations in the sample, making detectable more peptides by the MS instruments” by “decreas[ing] high-abundant proteins and increase[ing] low-abundant proteins.” EX1007, 1881. For the Q-treated particle for which the SBF1 count was 10, the lowest MS result incorporated into Zaccaria’s table for that particle was nearly 14-times higher. EX1007, 64 (E3 ubiquitin-protein ligase UBRA4). The dynamic range reported in Zaccaria’s table for the Q particle did not vary by even one order of magnitude. EX1007, 64 (range 136-795). The Petition provides no justification for assuming

² The Q nanoparticle reports a total MS/MS number of 568 for HBA1, and range of 136-795 for 20 most abundant proteins for that particle. EX1007, 64.

that the spectral count of 10 on particle Q represented a reliable detection of the concentration of SBF1, much less across a dynamic range comprising at least 6 orders of magnitude.

Instead of relying on the abundance data reported in Zaccaria, the Petition again pivots to a flawed inherency argument. Pet., 74 (“A POSA would have understood that HBA1 is normally present in RBC lysate in a concentration of 1.47×10^4 pmol/mg and SBF1 in a concentration of 6.62×10^{-6} pmol/mg[.]”). The Petition relies on a post-critical date extrinsic reference (Bryk, EX1009) to establish HBA1 and SBF1 concentrations in a different RBC lysate sample than the one analyzed in Zaccaria. Pet., 74. But just as with its flawed inherency argument based on Hu in Ground 1, Ground 4 of the Petition fails to tie together the sample in Zaccaria to the extrinsic reference it relies upon.

1. The Petition Fails To Prove SBF1 Concentration Across Six Orders of Magnitude Is Inherent to Zaccaria’s Tested Sample

Nothing in Bryk (a post-critical date reference)³ purports to provide the concentration of SBF1 in the sample tested in Zaccaria. Petitioner’s single-

³ Like the Petition’s slipshod approach to the portions of the Grounds addressed herein, its priority challenge also is critically deficient. Because the deficiencies identified herein merit denial of institution regardless of the priority challenge,

reference obviousness challenge is thus predicated on an unfounded presumption that the samples of Zaccaria and Bryk contain the same levels of proteins.

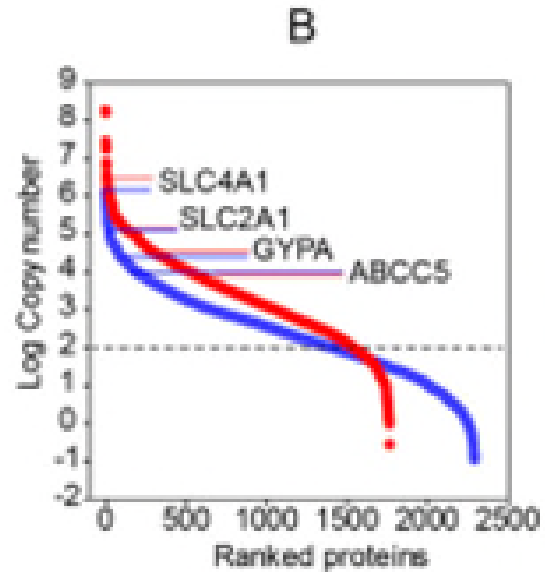
Moreover, the very evidence the Petition cites contradicts the foundation of Ground 4—the Petition’s assertion that SBF1 was a “well-known” component of RBC. Pet., 74. Dr. Vargas cites Bryk for reporting a concentration of 6.62×10^{-6} pmol/mg of SBF1. EX1003, ¶271. Dr. Vargas admits that she plucked out “the lowest non-zero detected concentration” of SBF1 in Bryk’s table. EX1003, ¶271 n.6. By reporting a “zero” detected concentration for SBF1, however, Bryk confirms that the SBF1 concentration relied upon by Dr. Vargas is not inherent to any sample, much less to Zaccaria’s different sample. EX1009, 51 (lowest count for SBF1 in Bryk is “0.00E+00”).

Patent Owner reserves the right to detail deficiencies in the Petition’s priority challenge in a Patent Owner Response if necessary.

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, 2756 &

Fig. 3 Caption (“(B) Distribution of protein copy numbers across all proteins in whole cells (red circles) and WG fractions (blue circles). The dashed line shows **the 100 copy per cell sample purity threshold.**”) (reproduced right). As shown in Figure 3,



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. Accordingly, Bryk does not support Petitioner’s contention that SBF1 was a “well-known” component of Zaccaria’s RBC sample. To the contrary, Bryk undermines any confidence in Petitioner’s assumption that SBF1

concentration in RBC samples were constant or that Zaccaria's nominal result for SBF1 proves that Zaccaria inherently detected, in the subset, proteins in the biological sample at concentrations across a dynamic range comprising at least 6 orders of magnitude.

As with the Petition's failed Hu Ground, its attempt to incorporate into Zaccaria *different values* reported in a *different reference* discussing results from a *different experiment* involving *different samples* fails to demonstrate necessary correspondence with the sample tested in Zaccaria. Accordingly, institution should be denied.

2. The Petition Fails to Prove the Peptide Detected in Zaccaria is the SBF1 Protein Discussed in Bryk

Moreover, Zaccaria admits the SBF1 label may have been misapplied and actually may be an *altogether different protein*. Zaccaria labels SBF1 as a match to a peptide fragment corresponding with AC identifier O95248. EX1007, 28, 39, 58. But Zaccaria admits more than one protein is associated with that peptide fragment. *Id.*, 86 ("Other ACs" include A6PVG8). Zaccaria's "Other ACs" stand for alternative accession numbers in the Uniprot database that matched the detected peptides. *Id.*, 17. Zaccaria explicitly states that for "clarity, *if several proteins match* the same set of peptides, *only one member of the protein group is indicated* in these concise lists." *Id.*; *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.”). Thus, insofar as Zaccaria detected a peptide that might have been associated with SBF1, it is equally likely that the detected peptide fragment was instead from an altogether different protein. Petitioner’s entire assumption for its inherency case is undermined.

D. Petitioner fails to show motivation or reasonable expectation of success

Just as in Ground 1, Ground 4 attempts to manufacture an overlapping range argument based on the wide disparity in protein concentrations in biological samples. Pet., 75-77. But the prior art does not disclose a prior art range overlapping with the claimed range, and this argument fails for the same reason discussed above in Ground 1. *See* Section V.B.2.

The Petition’s conclusory and unsupported argument that it would have been obvious to detect protein concentrations across 6 orders of magnitude because proteins exist across an even wider dynamic range of 10 order of magnitude should be rejected. The Petition asserts incorrectly that Zaccaria is trying to detect “a broad range of protein concentrations” because the ’360 Patent teaches to detect a broad range of protein concentrations. Pet., 76. This kind of hindsight-based analysis is strictly forbidden. *See* Section V.B.2 above.

Indeed, even the portions of Zaccaria cited in this part of the Petition confirm that Zaccaria’s goal was not to expand the dynamic range, but to narrow it. Pet., 75 (“through the respective ***decrease of high-abundant proteins*** and increase

of low-abundant proteins, NP treatment ***greatly reduces the range of concentrations in the sample***, making detectable more peptides by the MS instruments” (quoting EX1007, 1881)). Narrowing the dynamic range was the accepted approach because it was understood that the wide dynamic range of the proteins in the sample rendered mass spec inaccurate, and reliable measurements unattainable. Pet., 76 (despite constant advances in MS and sample fractionation methods, “the limited dynamic range of current mass spectrometers hinders the entire proteome coverage of biological fluids” rendering “the low-abundant protein compartment where disease-associated biomarkers are thought to reside [as] still ***unattainable***” (quoting EX1007, 1869-1870)); *see also* EX1010, 1218 (the “small fraction of highly abundant proteins...***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***” and one should apply a “pruning procedure” to eliminate “low-abundance proteins”); 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 (despite constant advances in mass spec and sample fractionation methods, “the limited dynamic range of current mass spectrometers hinders the entire proteome coverage of biological fluids” rendering “the low-abundant protein

compartment where disease-associated biomarkers are thought to reside [as] still ***unattainable***"). Zaccaria, like the rest of the state of the art, thus directly contradicts the Petition's assertion that mass spec had known "utility" to "measure a dynamic range of ten orders of magnitude to be able to capture the entire proteome of biological fluids." Pet., 77.

Petitioner had the burden of showing a POSA would have been motivated to modify a prior art reference to arrive at the claimed invention with a reasonable expectation of success. See Section V.B.2 above. Neither the Petition's impermissible hindsight, nor its blatant mischaracterization of the references, satisfy that burden.

Therefore, for at least the reasons discussed above, Petitioner fails to meet its burden to establish that Zaccaria renders obvious the challenged claims.

IX. CONCLUSION

Accordingly, for at least the reasons set forth above, institution of *inter partes* review should be denied.

Respectfully submitted,

Date: January 23, 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 12,999 words.

Respectfully submitted,

Date: January 23, 2025

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X. 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	Omid Farokhzad, LINKEDIN, www.linkedin.com/in/omidfarokhzad (last accessed Dec. 19, 2024).
2005	Morteza Mahmoudi, LINKEDIN, www.linkedin.com/in/morteza-mahmoudi-8b76636a (last accessed Dec. 19, 2024).
2006	Claudia Corbo, LINKEDIN, www.linkedin.com/in/claudia-corbo-2a298232 (last accessed Dec. 19, 2024).

CERTIFICATE OF SERVICE

I certify that the foregoing Patent Owner's Preliminary Response Pursuant to 37 C.F.R. § 42.107 and accompanying Exhibits 2001-2006 were served on this 23rd day of January, 2025, on the Petitioner at the following electronic service addresses:

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