

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

Petition for *Inter Partes* Review

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LIST OF EXHIBITS

Exhibit No.	Description
1001	U.S. Patent No. 11,435,360
1002	File History of U.S. Patent No. 11,435,360 (<i>excerpts</i>)
1003	Declaration of Dr. Jessica Vargas
1004	<i>Curriculum Vitae</i> of Dr. Jessica Vargas
1005	Cai <i>et al.</i> , “Characterization of Carbon Nanotube Protein Corona by Using Quantitative Proteomics,” <i>Nanomedicine: Nanotechnology, Biology and Medicine</i> 9 (2013) 583-593, with its supplementary data (Table S2) (“Cai”)
1006	Walkey <i>et al.</i> , “Protein Corona Fingerprinting Predicts the Cellular Interaction of Gold and Silver Nanoparticles,” <i>ACS Nano</i> 8 (2014) 2439-2455 (“Walkey”)
1007	Zaccaria <i>et al.</i> , “Accessing to the Minor Proteome of Red Blood Cells Through the Influence of the Nanoparticle Surface Properties on the Corona Composition,” <i>International Journal of Nanomedicine</i> 10 (2015) 1869-1883, with its Supplemental Materials (“Zaccaria”)
1008	Hu <i>et al.</i> , “Nanoparticle Size Matters in the Formation of Plasma Protein Coronas on Fe ₃ O ₄ Nanoparticles,” <i>Colloids and Surfaces B: Biointerfaces</i> 121 (2014) 354-361, including Appendix A (Table S1) (“Hu”)
1009	Bryk <i>et al.</i> , “Quantitative Analysis of Human Red Blood Cell Proteome,” <i>Journal of Proteome Research</i> 16 (2017) 2752-2761, including supporting information (“Bryk”)

Exhibit No.	Description
1010	Hajipour <i>et al.</i> , “Personalized Protein Coronas: a “Key” Factor at the Nanobiointerface,” <i>Biomaterials Science</i> 2 (2014) 1210-1221 (“Hajipour”)
1011	U.S. Patent No. 10,866,242 (“Farokhzad”)
1012	Declaration of Nancy Adams
1013	<i>Curriculum Vitae</i> of Nancy Adams
1014	Nanjappa <i>et al.</i> , “Plasma Proteome Database as a Resource for Proteomics Research: 2014 Update,” <i>Nucleic Acids Research</i> 42 (2014) D959-D965 (“Nanjappa”)
1015	Ahmed, “Sample Preparation and Fractionation for Proteome Analysis and Cancer Biomarker Discovery by Mass Spectrometry,” <i>Journal of Separation Science</i> 32 (2009) 771-798 (“Ahmed”)
1016	Cao <i>et al.</i> , “Latent Transforming Growth Factor-Beta Binding Protein-1 in Circulating Plasma as a Novel Biomarker for Early Detection of Hepatocellular Carcinoma,” <i>International Journal of Clinical and Experimental Pathology</i> 8(12) (2015) 16046-16054 (“Cao”)
1017	U.S. Provisional Patent Application No. 62/435,409 (“the ’409 Provisional”)
1018	Ishihama <i>et al.</i> , “Exponentially Modified Protein Abundance Index (emPAI) for Estimation of Absolute Protein Amount in Proteomics by the Number of Sequenced Peptides per Protein,” <i>Molecular & Cellular Proteomics</i> 4.9 (2005) 1265-1272 (“Ishihama”)
1019	U.S. Patent No. 8,796,184 (“Chilkoti”)

Exhibit No.	Description
1020	Archival website for http://www.plasmaproteomedatabase.org/faq.html as of Feb. 19, 2015, <i>available at</i> https://web.archive.org/web/20150219211334/http://www.plasmaproteomedatabase.org/faq.html
1021	Dean, L. “Blood Groups and Red Cell Antigens,” Chapter 1: Blood and the cells it contains, Bethesda (MD): National Center for Biotechnology Information (US) (2005), <i>available at</i> https://www.ncbi.nlm.nih.gov/books/NBK2263/ . (“Dean”)
1022	Norouzi, N. <i>et al.</i> , “Sorting cells by their density,” PLoS ONE, 12(7):e0180520 (2017) (“Norouzi”)
1023	Archival website for https://www.ncbi.nlm.nih.gov/books/NBK2261/ as of Mar. 20, 2014, <i>available at</i> https://web.archive.org/web/20140320071602/https://www.ncbi.nlm.nih.gov/books/NBK2261/
1024	Graves <i>et al.</i> , “Molecular Biologist's Guide to Proteomics,” <i>Microbiology and Molecular Biology Reviews</i> 66(1), (2002) 39-63 (“Graves”)
1025	Berg <i>et al.</i> , <i>Biochemistry, Sixth Edition</i> . W.H. Freeman and Company (2007) (<i>excerpts</i>) (“Berg”)
1026	McDonald <i>et al.</i> , “Shotgun Proteomics and Biomarker Discovery,” <i>Disease Markers</i> 18(2), (2002) 99-105 (“McDonald”)
1027	Aebersold <i>et al.</i> , “Mass Spectrometry-Based Proteomics,” <i>Nature</i> 422(6928), (2003) 198-207 (“Aebersold”)

Exhibit No.	Description
1028	Yates <i>et al.</i> , “Proteomics by Mass Spectrometry: Approaches, Advances, and Applications,” <i>Annual Review of Biomedical Engineering</i> 11(1) (2009) 49-79 (“Yates”)
1029	Feist <i>et al.</i> , “Proteomic Challenges: Sample Preparation Techniques for Microgram-Quantity Protein Analysis from Biological Samples,” <i>International Journal of Molecular Sciences</i> 16(2) (2015) 3537-3563 (“Feist”)
1030	Szabo <i>et al.</i> , “Challenges and Developments in Protein Identification Using Mass Spectrometry,” <i>Trends in Analytical Chemistry</i> 69 (2015) 76-87 (“Szabo”)
1031	Bantscheff <i>et al.</i> , “Quantitative Mass Spectrometry in Proteomics: A Critical Review,” <i>Analytical and Bioanalytical Chemistry</i> , 389 (2007) 1017-1031 (“Bantscheff”)
1032	Harris, D. <i>Quantitative Chemical Analysis, Seventh Edition</i> . W.H. Freeman and Company (2007) (<i>excerpts</i>)

LISTING OF CLAIMS

Element	Claim Language
Claim 1	
1[pre]	A method for assaying a biological sample, comprising:
1[a][1]	(a) contacting the biological sample with a plurality of particles comprising different particle types
1[a][2]	to permit biomolecules of the biological sample to bind to the plurality of particles and form coronas around the plurality of particles,
1[a][3]	wherein the coronas corresponding to the different particle types (i) differ based on particle type, and (ii) comprise overlapping and distinct proteins;
1[b]	(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;
1[c]	(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.
Claim 2	
2	The method of claim 1, wherein the detecting of proteins in the biological sample comprises detecting a protein present in the sample at a concentration greater than 1 mg/ml and a protein present in the sample at a concentration of less than 10 ng/ml.
Claim 3	
3	The method of claim 1, wherein the plurality of particles comprise iron oxide.
Claim 4	
4	The method of claim 1, wherein the assaying is performed using gel-electrophoresis, liquid chromatography, mass spectrometry, nuclear magnetic resonance spectroscopy, Fourier transform infrared spectroscopy, circular dichroism, Raman spectroscopy, or any combination thereof.
Claim 6	
6	The method of claim 1, wherein the dynamic range of the proteins detected spans 6 or more orders of magnitude.

Element	Claim Language
Claim 7	
7	The method of claim 1, wherein the detected proteins comprise proteins present in concentrations of 100 ng/ml or less in the biological sample.
Claim 17	
17	The method of claim 1, wherein the assaying further comprises digesting the proteins.
Claim 22	
22	The method of claim 1, wherein the biological sample is selected from the group consisting of: blood, plasma, serum, lung lavage, menstrual blood, urine, processed tissue samples, amniotic fluid, cerebrospinal fluid, tears, saliva, sperm, chopped tissue, homogenized tissue, or any lysates or combinations thereof.
Claim 23	
23	The method of claim 1, wherein the coronas that form around the plurality of particles further comprise a species of biomolecules selected from the group consisting of polysaccharides, sugars, lipids, lipoproteins, metabolites, oligonucleotides, or any combination thereof.
Claim 24	
24	The method of claim 1, wherein at least one particle of the plurality of particles enriches for peptides other than albumin such that a ratio of an amount of albumin to a protein X that binds to the at least one particle is less than a ratio of an amount of albumin to the protein X in the biological sample.
Claim 25	
25	The method of claim 1, wherein the different particle types differ from each other by one or more physicochemical properties, wherein the one or more physicochemical properties are selected from the group consisting of: composition, size, surface charge, hydrophobicity, hydrophilicity, surface functionality, surface topography, surface curvature, shape, and any combination thereof.

PreOmics GmbH and Biognosys AG (together, “Petitioner”) request *inter partes* review of claims 1-4, 6-7, 17, and 22-25 of U.S. Patent No. 11,435,360 (EX1001, the “’360 Patent”), assigned to The Brigham and Women’s Hospital, Inc. (“Patent Owner”).

I. Introduction

The ’360 Patent discloses and claims simple concepts that were already well known by the time of the patent. The single independent claim is directed to a method for assaying a biological sample using particles to analyze the proteins that form coronas around the particles. Using particles, such as nanoparticles, to form coronas to analyze various proteins found in a biological sample was well known in the field before the ’360 Patent. As was the alleged novelty: detecting proteins “at concentrations across a dynamic range comprising at least 6 orders of magnitude.” The prior art in this Petition discloses the same method claimed in the ’360 Patent, including the claimed “dynamic range.”

As explained below and in the declaration of Dr. Vargas, there is nothing inventive about the challenged claims.

II. Mandatory Notices (37 C.F.R. §42.8)

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

PreOmics GmbH and Biognosys AG are the sole real parties in interest.

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

Petitioner is not aware of any other matter involving the ’360 Patent.

C. Counsel and Service Information (37 C.F.R. §§42.8(b)(3)-(4))

Petitioner designates the following lead and back-up counsel:

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III. Requirements for *Inter Partes* Review (37 C.F.R. §42.104)

A. Grounds for Standing (37 C.F.R. §42.104(a))

Petitioner certifies the '360 Patent is available for IPR, and Petitioner is not barred or estopped from requesting IPR challenging the claims on the identified grounds.

B. Identification of Challenge (37 C.F.R. §42.104(b)(1)-(2)) and Relief Requested (37 C.F.R. §42.22(a)(1))

Petitioner requests the Board institute IPR because the challenged claims are anticipated and/or obvious under AIA 35 U.S.C. §102 and/or §103 based on the following:

Ground	Prior Art	Basis	Claims
1	Nanoparticle size matters in the formation of plasma protein coronas on Fe ₃ O ₄ nanoparticles, <i>Colloids and Surfaces B: Biointerfaces</i> (2014) (“Hu”; EX1008)	§103	1-4, 6-7, 17, 22-25
2	Characterization of carbon nanotube protein corona by using quantitative proteomics, <i>Nanomedicine: Nanotechnology, Biology and Medicine</i> (2013) (“Cai”; EX1005)	§102	1, 4, 6, 17, 22, 25
3	Cai	§103	1, 4, 6, 17, 22, 25
4	Accessing to the minor proteome of red blood cells through the influence of the nanoparticle surface properties on the corona composition, <i>International Journal of Nanomedicine</i> (2015) (“Zaccaria”; EX1007)	§103	1-2, 4, 6-7, 17, 22-23, 25

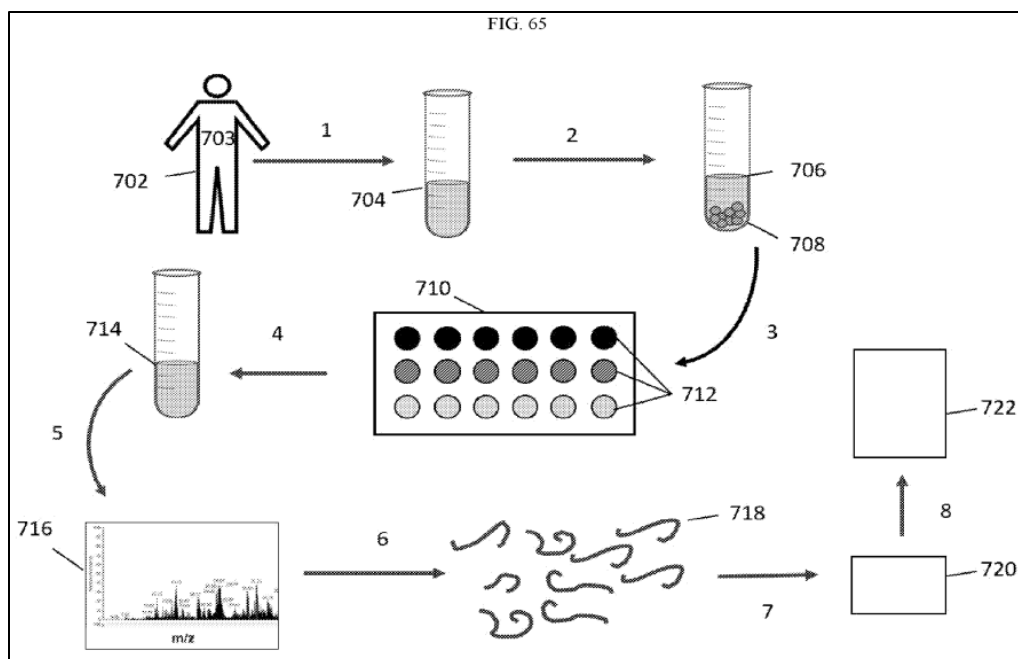
IV. Relevant Information Concerning the Contested Patent

A. '360 Patent Overview

The '360 Patent claims a method for assaying a biological sample. It claims priority, through a series of applications, to a provisional filed December 16, 2016. EX1001, Cover Page, 1:6-13.

The method in claim 1 (the sole independent claim) involves contacting a biological sample with a plurality of particles with different particle types, allowing biomolecules from the sample to bind and form coronas around them, where the coronas differ based on particle type and comprise overlapping and distinct proteins. EX1001, 86:52-87:3. The method further includes removing a subset of the particles comprising the coronas. *Id.* The remaining protein subset is assayed with an instrument to detect proteins at concentrations across a dynamic range of at least 6 orders of magnitude, which, per the claim, results in assaying the biological sample. *Id.*

Figure 65, below, “is a [s]chematic diagram of a method of distinguishing states of a biological sample.” EX1001, 14:25-26, 21:14-15 (“FIG. 65 shows an exemplary scheme of the... disclosed array system.”).



EX1001, FIG. 65.

In Figure 65, a biological sample 704 (*e.g.*, blood, plasma, cell lysates, etc.) is collected from a subject 702 expressing a biological state 703 (“*e.g.*, a disease state” or “healthy people (non-diseased state)”), where, “[i]n some embodiments, plasma 706 can be separated from blood cells 708 of subjects expressing a biological state 703.” EX1001, 21:14-29. The plasma 706 is incubated with sensor array 710 comprising a plurality of particles 712 with different physicochemical properties to allow biomolecules (*e.g.*, proteins) in the plasma to bind to the particles. EX1001, 21:30-36. The biomolecules bound to the particles can be isolated in a protein solution 714 for further analysis. EX1001, 21:37-39. The solution can be characterized by liquid chromatography-tandem mass spectrometry (LC-MS/MS) 716 and analyzed to determine biomolecule fingerprint 718. EX1001, 21:43-50. A

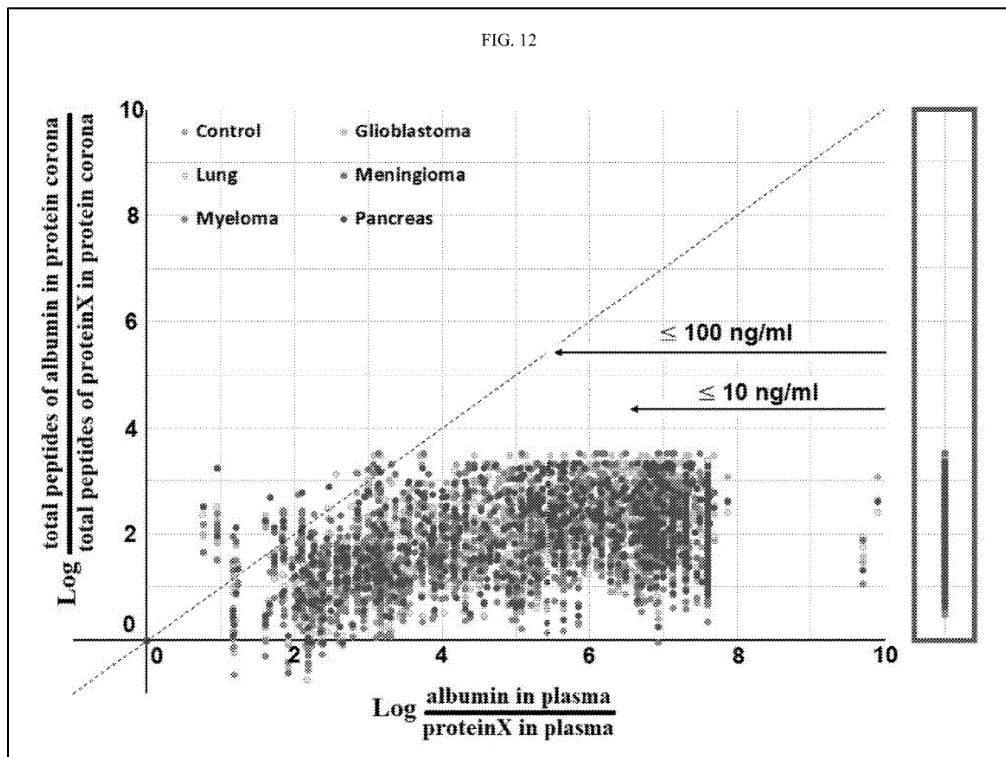
computer 720 can then be used to generate an association 722 between the biological state 703 and biomolecule fingerprint 718. EX1001, 21:51-54.

The '360 Patent relies upon admittedly known instruments and methods to perform the assaying (*e.g.*, mass spectrometry such as a liquid chromatography-tandem mass spectrometry (LC-MS/MS) apparatus; gel electrophoresis; etc.). EX1001, 21:43-50, 41:29-38, 75:20-77:23.

The '360 Patent also admits that mass spectrometry “has a dynamic range [of] 4-6 order[s] of magnitude” and asserts that its invention enables a broader range, namely, a “dynamic range of ten (10) orders of magnitude in terms of protein detection using mass spectrometry approaches.” EX1001, 20:20-37, 72:1-25. Importantly, however, as explained further below, the challenged claims *only* require the admittedly known 6-orders range.

The '360 Patent also relies on an admittedly known database (“the plasma proteome database (see website at plasma-proteome-database.org)”) to obtain the plasma protein concentrations for purposes of determining orders of magnitude of the dynamic range, rather than measuring the concentrations directly. EX1001, 66:63-67:67 (discussing Table 5), 69:30-67 (explaining manual lookup of detected protein concentrations in known database to determine orders of magnitude of the dynamic range), FIG. 12 (below, depicting results of lookup showing orders of magnitude); *see also* EX1014, D959-D962 (explaining same known plasma

proteome database and noting “wide dynamic range of protein abundance, greater than 10 orders of magnitude....” and “MRM [multiple reaction monitoring] assays” “multi-step process provides superior specificity and allows for accurate quantification of protein levels over a wide dynamic range of abundance”); EX1012 §II.E & ¶¶43-48 (explaining publication of Nanjappa (EX1014) in December 2013).



EX1001, FIG. 12.

B. Prosecution History

Originally filed application claims 1 and 2 recited:

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 broad dynamic range, thereby assaying the biological sample.

2. The method of claim 1, wherein the broad dynamic range comprises at least 6 orders of magnitude.

EX1002, 136.

The Examiner issued an Office Action rejecting original application claims 1-5, 10-13, 19, 23-27, and 30. EX1002, 1637. The Examiner found the term “broad dynamic range” indefinite. EX1002, 1641.

The Examiner also found various claims (including claims 1 and 2) unpatentable under §103 over Hajipour (EX1010) in view of Walkey (EX1006). EX1002, 1642. The Examiner found that Hajipour “do[es] not specifically teach 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 – see

section 00176).” EX1002, 1642. “[S]ection 00176” (*i.e.*, Paragraph 00176) of the ’360 Patent application stated:

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 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[sic] not previously able to be detected.

EX1002, 1511-1512.

The Examiner further stated that “Walkey teaches that protein corona fingerprinting of nanoparticles can be characterized by mass spectrometry (p. 2441-42) and Fig. 1.” EX1002, 1642.

An interview was conducted, after which Applicant amended application claim 1 by cancelling claim 2 and adding its limitation to claim 1 such that limitation (c) of claim 1 read: “(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.” EX1002, 1720. Applicant stated: “as discussed during the

interview... Walkey does not teach or suggest [a] ‘dynamic range comprising at least 6 orders of magnitude.’” EX1002, 1726. Neither the Examiner’s nor Applicant’s Interview Summaries provided any reasoning regarding Walkey not disclosing this limitation. EX1002, 1712, 1730

The Examiner issued another office action, this time rejecting the claims under §103 as being unpatentable over Hajipour in view of Walkey and Farokhzad (EX1011). EX1002, 1734-1737. The ’360 Patent is a divisional of application No. 17/099,331, which is a continuation-in-part of application No. 15/880,627 (which issued as Farokhzad). EX1001, Cover Page.

The Examiner stated that, with respect to limitation (c): “Although Hajipour... in view of Walkey... teach mass spectrometry procedures they do not specifically recite the utility of an instrument to measure concentrations across a dynamic range of at least 6 orders of magnitude.” EX1002, 1736.

However, per the Examiner, Farokhzad discloses that limitation, citing to portions of Farokhzad’s specification also in the ’360 Patent (including “Section 00176” quoted above). *Compare* EX1002, 1736-1737 (Examiner citing Farokhzad (EX1011), 20:20-45, 39:33-60) *with* EX1001, 20:20-45, 39:46-40:6.

In response, Applicant stated that Farokhzad is not eligible as prior art. EX1002, 1755. The Examiner then allowed the claims, stating the following reason: “The instant method requires measuring a subset of particles comprising coronas

from a biological sample wherein the proteins are detected with an instrument that measures concentrations across a dynamic range of at least 6 orders of magnitude.”

EX1002, 1801.

However, as explained above in Section IV.A and below in Sections V.B.1, V.D, and V.E.1 (regarding limitation 1[c]), although the Examiner allowed the claims because of the particular “dynamic range,” the ’360 Patent admits that such range was already known, which knowledge is further confirmed by the prior art herein.

C. Person of Ordinary Skill in the Art

Several factors may be considered in determining the qualifications of a person of ordinary skill in the art (“POSA”). M.P.E.P. §2141.03. Petitioner submits that a POSA would have had at least a bachelor’s degree in chemistry, biochemistry, bioengineering, or a related field, and three years’ experience in the field of proteomics, protein analysis, or mass spectrometry. EX1003 ¶¶24-27. This description is approximate—a higher education or skill level might make up for less experience, and vice-versa. EX1003 ¶26. Petitioner’s expert, Dr. Vargas, was at least a POSA as of the ’360 Patent’s earliest possible priority date, December 16, 2016. EX1003 ¶¶5-10, 27; EX1004, 1-3.

D. The '360 Patent's Priority Date

Patent Owner bears the burden of showing each challenged claim is entitled to the priority date of its provisional. *See Tech. Licensing Corp. v. Videotek, Inc.*, 545 F.3d 1316, 1324, 1326-27 (Fed. Cir. 2008); *PowerOasis, Inc. v. T-Mobile USA, Inc.*, 522 F.3d 1299, 1304-05 (Fed. Cir. 2008); *see also Dynamic Drinkware, LLC v. Nat'l Graphics, Inc.*, 800 F.3d 1375, 1381 (Fed. Cir. 2015).¹ This requires Patent Owner to show the provisional contains adequate written description for the later-claimed invention, *PowerOasis*, 522 F.3d at 1306-07, including that the provisional's disclosure "describe[s] the claimed invention with ***all*** its limitations," *Tronzo v. Biomet, Inc.*, 156 F.3d 1154, 1158 (Fed. Cir. 1998).² *See also Novozymes A/S v. DuPont Nutrition Biosciences APS*, 723 F.3d 1336, 1344-46 (Fed. Cir. 2013) (claims not entitled to provisional date where provisional failed to provide written description support). The inquiry requires an analysis of the actual disclosure in the application ***itself***; that an earlier application may render obvious the later-claimed invention "is not sufficient to meet the written description requirement." *Tronzo*, 156

¹ Petitioner has established a *prima facie* invalidity case herein.

² Unless noted, all emphasis has been added.

F.3d at 1158; *Lockwood v. Am. Airlines, Inc.*, 107 F.3d 1565, 1571-72 (Fed. Cir. 1997).

Here, the '360 Patent's earliest priority claim is to Prov. App. 62/435,409 ("the '409 Provisional"; EX1017), filed December 16, 2016. EX1001, Cover Page.

Because the prior art herein pre-dates the '409 Provisional with the sole exception being Bryk, a state-of-the-art background reference used in Ground 4 only (published July 8, 2017, EX1009), Petitioner focuses on the '409 Provisional.³

No challenged claim is entitled to claim priority to the '409 Provisional because the provisional does not provide written description support for at least the following limitations found in all challenged claims (directly or by dependency): 1[a][3] ("wherein the coronas corresponding to the different particle types (i) differ based on particle type, and (ii) comprise overlapping and distinct proteins"); and 1[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"). EX1003 ¶¶75-90.

³ Petitioner submits that even if Bryk is not considered, Zaccaria still renders obvious the claims challenged in Ground 4. *See* §V.E.

The '409 Provisional includes a basic description of its proposed method of assaying proteins in a biological sample and various applications of the same, *see generally* EX1017, but it lacks certain disclosure, figures, and tables added to the '360 Patent related to, *e.g.*, measuring protein concentration and relative abundance, including disclosure regarding the dynamic range, orders of magnitude of the protein subset, protein concentrations, reference to the Plasma Proteome Database to lookup concentrations, or the coronas differing based on particle type and comprising overlapping and distinct proteins. *See, e.g.*, EX1001, 8:80-23, 8:48-59, 20:20-32, 39:46-65, 66:63-72:60, Table 5, FIGs. 10B, 12; EX1003 ¶¶79-86. The closest the '409 Provisional comes is its generic statement that “[b]lood plasma contains several thousands of different proteins with twelve orders of magnitude differences in concentrations of these proteins,” EX1017, [00112], which says nothing about the specific claimed requirements in limitation 1[c] (including assaying with an instrument the claimed protein subset to detect, in the subset, proteins at the claimed concentrations across the claimed dynamic range). EX1003 ¶¶87-90.

E. Claim Construction (37 C.F.R. §42.104(b)(3))

This Petition construes terms consistent with the understanding a POSA would have had at the time of the invention. *See Phillips v. AWH Corp.*, 415 F.3d 1303, 1313-14 (Fed. Cir. 2005). Only terms subject to a legitimate dispute relevant to the invalidity issues in this IPR need to be construed here. *See Nidec Motor Corp.*

v. Zhongshan Broad Ocean Motor Co., 868 F.3d 1013, 1017 (Fed. Cir. 2017). For purposes of this proceeding only, Petitioner submits that no express construction of terms is necessary.

V. The Specific Grounds of Unpatentability

As evidenced by the prior art herein and Dr. Vargas's Declaration (EX1003), there was nothing new or novel about the challenged claims. As Dr. Vargas explains and the prior art shows, it was well known prior to the '360 Patent to use different types of particles to assay and detect a large range of concentrations of proteins in a biological sample (including six or more orders of magnitude). *See* §V.B-E. The prior art teaches the claimed method, and a POSA would have found the challenged claims anticipated and/or obvious. Authentication and public availability evidence for selected prior art references is included in the Declaration of Nancy Adams (EX1012; EX1013 (CV)) and further discussed herein.

A. Exemplary Prior Art and State of the Art

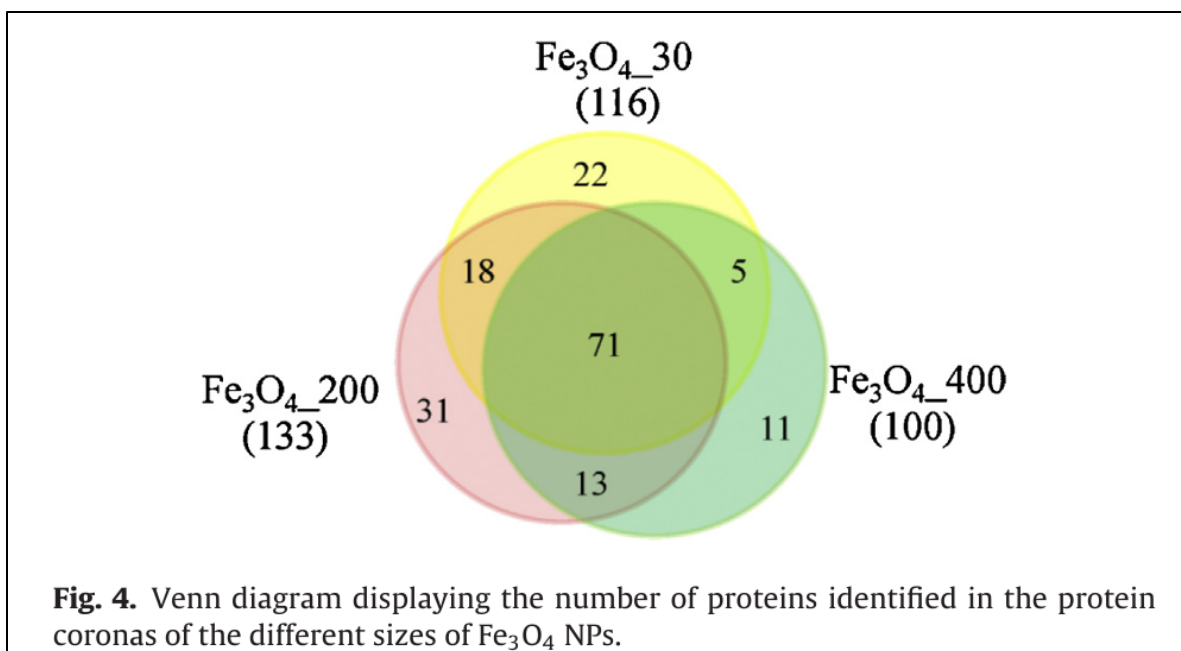
1. Hu

Hu published more than a year before the '360 Patent's earliest possible priority. Hu published by at least September 2014 in *Colloids and Surfaces B: Biointerfaces*, along with its supplementary data (Table S1 in its Appendix). EX1008, 354, 357, 361; EX1012 §II.B & ¶¶16-25 (explaining publication of Hu and its supplementary data); EX1003 ¶¶98, 92. Hu was made available online in June 2014. EX1008, 354; EX1012 ¶¶17-21. Hu was not disclosed during prosecution.

Hu discloses the formation and analysis of protein corona composition on three different sizes of Fe₃O₄ (iron oxide) nanoparticles NPs (30nm, 200 nm, 400 nm). EX1008, 355-360, Table 2 (below), Figure 4 (below). Hu discloses that the size of Fe₃O₄ NPs influences the composition and abundance of plasma proteins in the corona, which is crucial for understanding their bio-distribution and biological fate in applications like cell and protein separation. EX1008, 359.

Rank	Fe ₃ O ₄ -30	Fe ₃ O ₄ -200	Fe ₃ O ₄ -400
1	Platelet factor 4	Apolipoprotein A-I	Apolipoprotein E
2	Apolipoprotein E	Serum albumin	Vitronectin
3	Vitronectin	Apolipoprotein C-III variant 1	Histidine-rich glycoprotein
4	Platelet basic protein	Apolipoprotein C-I	Platelet basic protein
5	IGK@ IGK@ protein	Apolipoprotein E	Platelet factor 4
6	IGKC Anti-RhD monoclonal T125 kappa light chain	Isoform LMW of Kininogen-1	Complement C1q subcomponent subunit C
7	Putative uncharacterized protein	Vitronectin	Complement C1q subcomponent subunit A
8	Complement C1q subcomponent subunit C	Platelet factor 4	Isoform 1 of Gelsolin
9	IGHG1	Histidine-rich glycoprotein	APOC1 Apolipoprotein C-I
10	Apolipoprotein C-III variant 1	Platelet basic protein	Complement C3 (Fragment)
11	IGHG1	Isoform HMW of Kininogen-1	Prothrombin (Fragment)
12	IGHG1	Isoform 1 of Gelsolin	Serum albumin
13	Prothrombin (Fragment)	Apolipoprotein A-II	C1QB Uncharacterized protein
14	Complement C1q subcomponent subunit A	Complement C3 (Fragment)	IGHG1
15	CLEC3B Putative uncharacterized protein DKFZp686H17246	Isoform 1 of Inter-alpha-trypsin inhibitor heavy chain H4	IGHG1
16	Apolipoprotein A-I	clusterin isoform 3	IGHG1
17	Isoform LMW of Kininogen-1	ITIH4 protein	APOH Beta-2-glycoprotein 1
18	Complement C3 (Fragment)	inter-alpha (globulin) inhibitor H4 isoform 2 precursor	IGKC Putative uncharacterized protein
19	Beta-2-glycoprotein 1	Prothrombin (Fragment)	Complement component 4B
20	IGHM Putative uncharacterized protein DKFZp686I15212	Apolipoprotein C-II	IGKC Anti-RhD monoclonal T125 kappa light chain

EX1008, Table 2.



EX1008, FIG. 4.

2. Cai

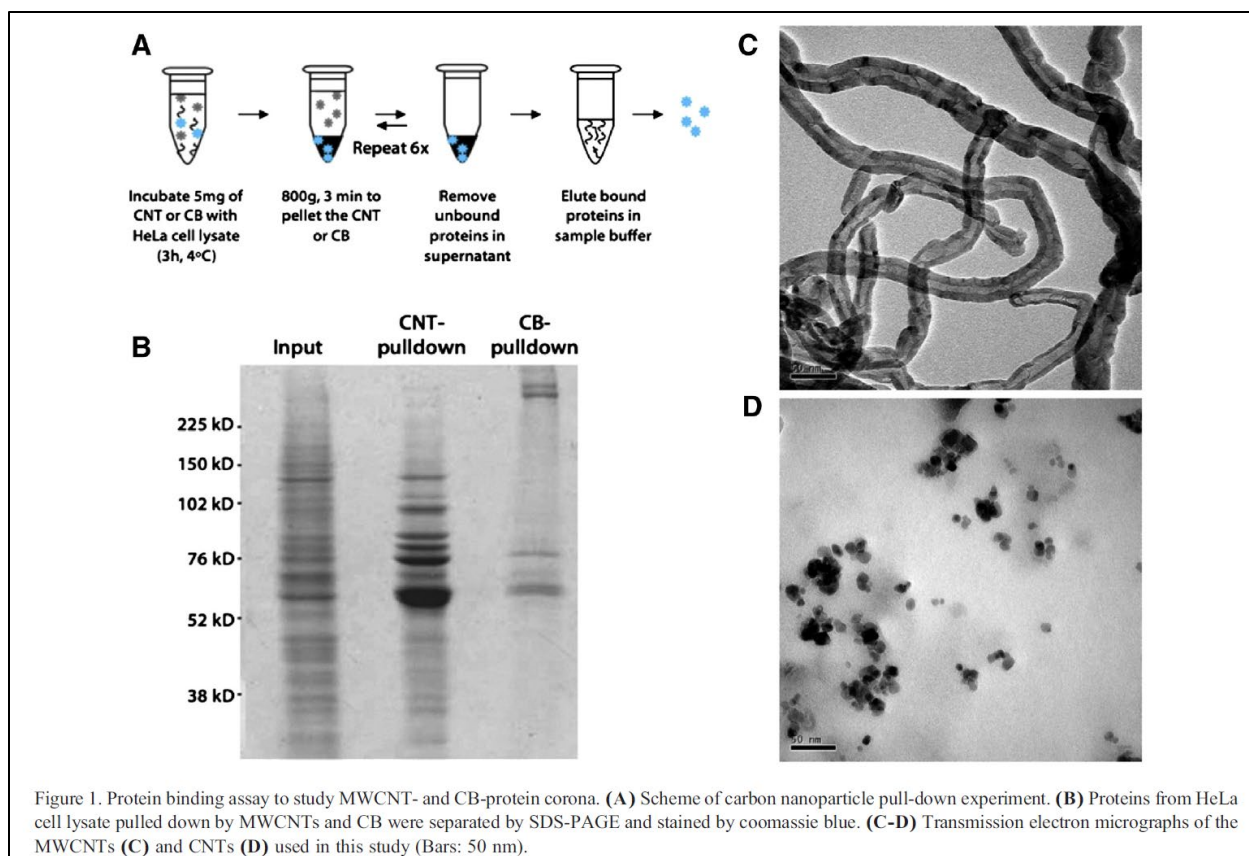
Cai published more than a year before the '360 Patent's earliest possible priority. Cai published by at least July 2013 in *Nanomedicine: Nanotechnology, Biology and Medicine*, along with its supplementary data (Table 2 in its Appendix A, also referred to as "Supplementary Table 2: Data reported in Figure 3"). EX1005, 583, 588; EX1012 §II.C & ¶¶26-33 (explaining publication of Cai and its supplementary data); EX1003 ¶¶92-93. Cai was not disclosed during prosecution.

Cai discloses the formation and analysis of protein corona composition on carbon nanotubes ("CNTs") and carbon black ("CB") nanoparticles. EX1005, 583. Analysis was performed on these protein coronas using mass spectrometry-based proteomics. EX1005, 587. The analysis revealed that CNTs and CB bind a

significant amount of proteins during the formation of their protein coronas and this binding is affected by the diameter and length of the nanoparticles. EX1005, 585. Larger diameter and longer CNTs exhibit higher protein binding efficiency. EX1005, 586. Thus, Cai discloses that different nanoparticle types having different physicochemical properties are forming differing coronas. EX1005, 586-587.

Cai also discloses that peptides from the protein coronas were eluted, and mass spectrometry was performed on them. EX1005, 585. The spectra were searched against the UniProt database using the Mascot search engine, which is a computerized analysis method. EX1005, 585. The relative abundance of Multi-Walled CNT-binding proteins was spread over 8 orders of magnitude. EX1005, 587. Cai's proteomics analysis identified 778 proteins selectively enriched by Multi-wall CNTs (MWCNTs) as compared to CB, indicating that the two nanoparticles have distinct protein binding specificities, with only a small number of CB-binding proteins also binding to MWCNTs. EX1005, 587.

Figure 1, below, depicts the protein binding assay to study MWCNT- and CB-protein corona. EX1005, 586.

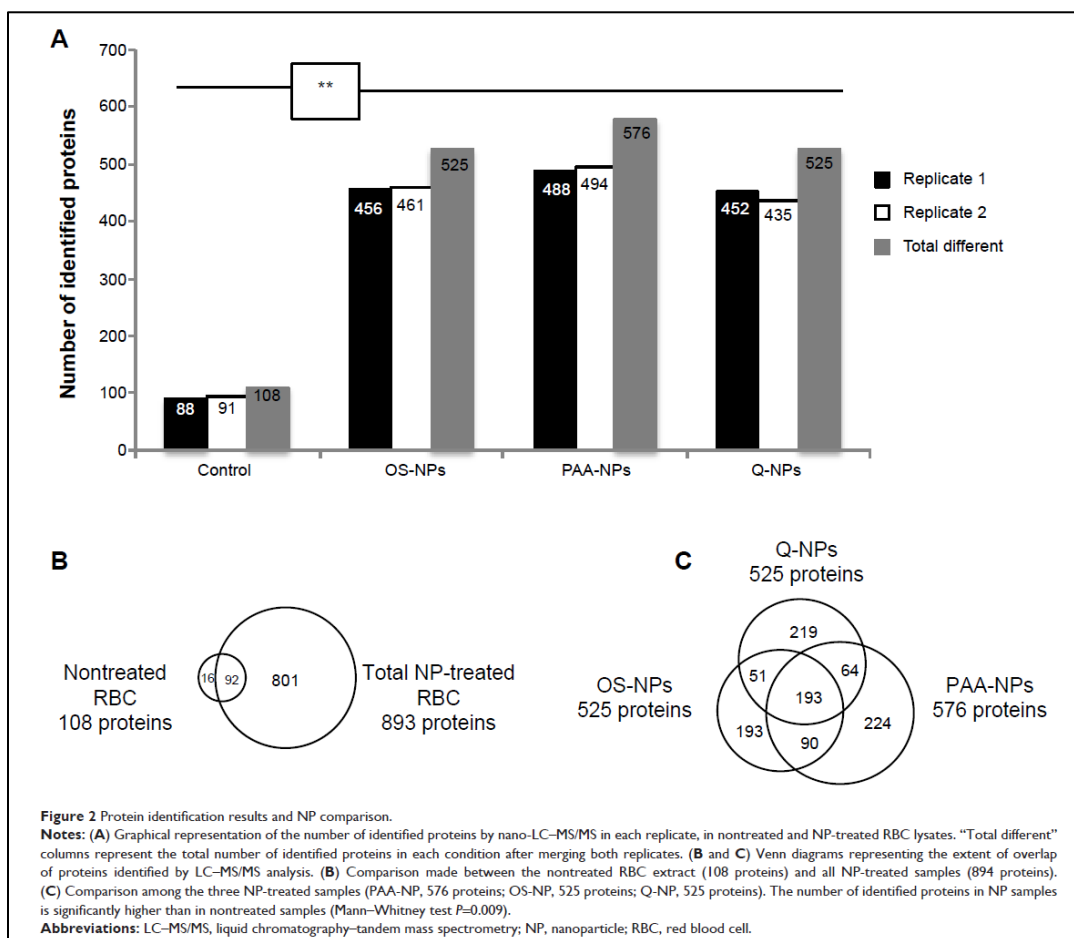


EX1005, FIG. 1.

3. Zaccaria

Zaccaria published more than a year before the '360 Patent's earliest possible priority. Zaccaria published by at least March 2015 in the *International Journal of Medicine*, along with its supplementary materials. EX1007, 1868-69, 1871, 1873-74, 1878, 1880, 1883; EX1012 §II.A & ¶¶12-15 (explaining publication of Zaccaria); EX1003 ¶¶109-113 (explaining corresponding publication of Zaccaria's supplementary materials); *see also* EX1003 ¶92. Zaccaria is included as one of many references in the '360 Patent's "References Cited," but it was not discussed or relied upon by the Examiner during prosecution.

Zaccaria discloses incubating three types of superparamagnetic nanoparticles (“NPs”) for protein capture in red blood cell (“RBC”) lysates and identifying and quantifying their respective eluted coronas using nano-LC-MS/MS. EX1007, 1870. Figure 2, below, shows the “total different” proteins identified from the coronas of each type of nanoparticle and the overlap of proteins between them. EX1007, 1873.



EX1007, FIG. 2.

Zaccaria further discloses that “low-abundant proteins identified in nontreated sample were present in higher abundance on NPs,” because “the higher affinity of several low-abundant proteins for the different kinds of chemical functionalization

allowed their accumulation on the NP surfaces.” EX1007, 1881. Accordingly, with the decrease of high-abundance proteins and increase of low-abundance proteins, “NP treatment greatly reduces the range of concentration in the sample, making detectable more peptides by the MS instruments.” EX1007, 1881.

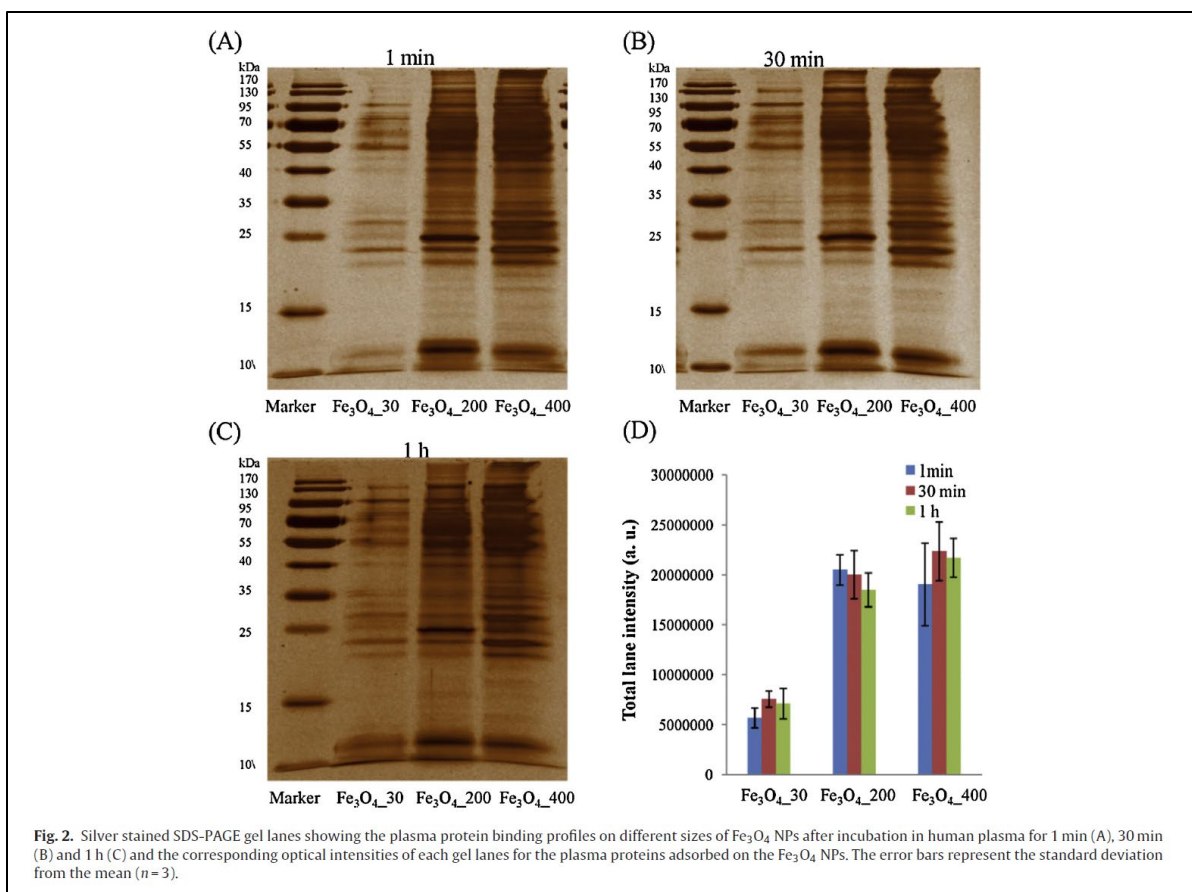
B. Hu Renders Obvious Claims 1-4, 6-7, 17, and 22-25

1. Claim 1

1[pre] “A method for assaying a biological sample, comprising:”

Hu discloses 1[pre], regardless of whether it is limiting. EX1003 ¶¶127-131; *see also* limitations 1[a]-1[c].

Hu discloses a method to “quantif[y] the [human blood] plasma protein coronas bound on three different sizes of pristine Fe₃O₄ NPs (~30 nm, 200 nm and 400 nm) by means of label-free quantitative proteomic strategy.” EX1008, 355; EX1003 ¶128. Hu discloses that “[i]n this study, the plasma protein coronas formed on pristine Fe₃O₄ NPs [nanoparticles] of three different particle sizes were investigated for the first time.” EX1008, 360; 356-57 (“The protein coronas bound on the Fe₃O₄ NPs after 1 h of incubation with human plasma were used to investigate the size effects on the formation of protein coronas on the Fe₃O₄ NPs.”); 357 and FIG. 2 (below); EX1003 ¶128.



EX1008, FIG. 2.

For quantification, Hu relies on spectral counts of proteins searched against a known plasma proteome database, the same type of counting method employed by the '360 Patent. *Compare* EX1008, 357 (“based on the spectral counting (SpC) method, we applied the label-free quantitative proteomic strategy to quantitatively analyze the plasma proteins bound”), 356 *with* EX1001, 66:65-66 (“The concentrations are values obtained using spectral counting from the plasma proteome database”), 66:63-67:67, 69:30-67, FIG. 12, Table 5; EX1003 ¶¶129, 55-59, 100; *see also* §IV.A (discussing known plasma protein database); EX1014,

D959-D962 (explaining same); EX1020, 1.

A POSA would have understood that Hu's method of analyzing human blood plasma proteins using Fe₃O₄ NPs discloses the claimed "method for assaying a biological sample...." EX1003 ¶130; *see also* EX1001, 20:33-45 ('360 Patent describing that "biological samples include, but are not limited to, systemic blood, plasma,... cell lysates,... processed tissue samples,... and the like"), 28:11-18.

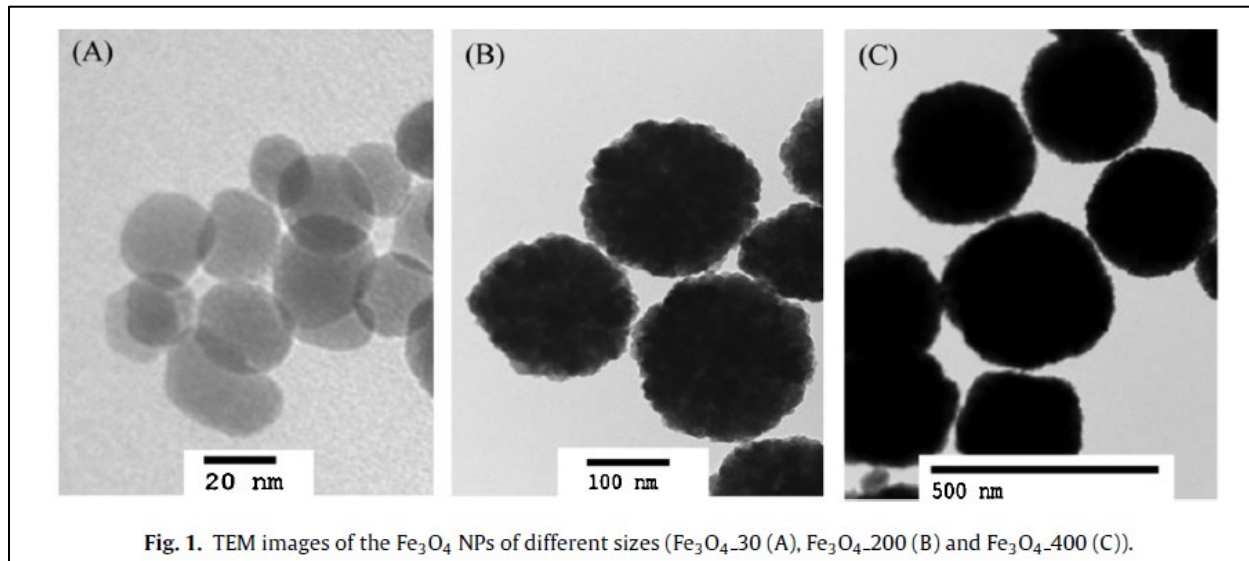
1[a][1] "(a) contacting the biological sample with a plurality of particles comprising different particle types"

Hu discloses limitation 1[a][1], *e.g.*, contacting the biological sample (*e.g.*, human blood plasma in Hu) with various particles comprising different particle types (*e.g.*, Fe₃O₄ (iron oxide nanoparticles) of various sizes and surface curvatures). EX1003 ¶¶132-137. Notably, the '360 Patent provides the same examples. EX1001, 20:2-7 (biological sample may be human blood plasma), 75:24-52 ("iron oxide nanoparticles"); *see also* 20:33-45, 21:14-33, cls. 3, 22; EX1003 ¶133.

Hu discloses that Fe₃O₄ nanoparticles were "incubated in a constant amount of human plasma at 37°C with shaking at 1000 rpm for 1 h." EX1008, 355; EX1003 ¶134. Hu discloses that "[f]urthermore, to investigate the plasma protein adsorption dynamics of the Fe₃O₄ NPs, Fe₃O₄ NPs of different sizes and with a total surface area of 0.005 m² were also incubated with the same amount of human plasma at 37°C with shaking at 1000 rpm for 1 min and 30 min." EX1008, 355; EX1003 ¶134. Hu also discloses that "Fe₃O₄_30 (~30 nm), dithiothreitol (DTT), iodoacetamide

(IAA),TPCK-treated trypsin, bovine serum albumin (BSA), formic acid(FA), tetraethyl orthosilicate (TEOS) and (3-aminopropyl) tri-ethoxysilane (APTES) were obtained from Sigma–Aldrich (St. Louis, MO, USA).... Fe₃O₄ NPs with sizes of ~200 and ~400 nm were synthesized according to previous literature.” EX1008, 355; EX1003 ¶134.

Figure 1, below, depicts TEM imaging of the different sized Fe₃O₄ NPs. EX1008, 356; EX1003 ¶135.



EX1008, FIG 1.

A POSA would thus have understood that Hu discloses that a biological sample (*e.g.*, human blood plasma) is contacted with, per Hu “Fe₃O₄ NPs of different sizes” including 30, 200, and 400 nm, as well as different surface curvature, which constitute particles consisting of different types, per the ’360 Patent. EX1003 ¶¶136-137; *see also* EX1008, 355, 360; EX1001, cl. 25 (“different particle types” may

differ by “size” and “surface curvature,” among other things), 33:27-67, 86:10-22, FIG. 13. A POSA would further have understood that Hu’s disclosed incubation encompasses the claimed contacting (also like the ’360 Patent). EX1003 ¶137; EX1001, 9:3-5, 21:30-42, 76:22-35, 83:1-63, cls. 18-19.

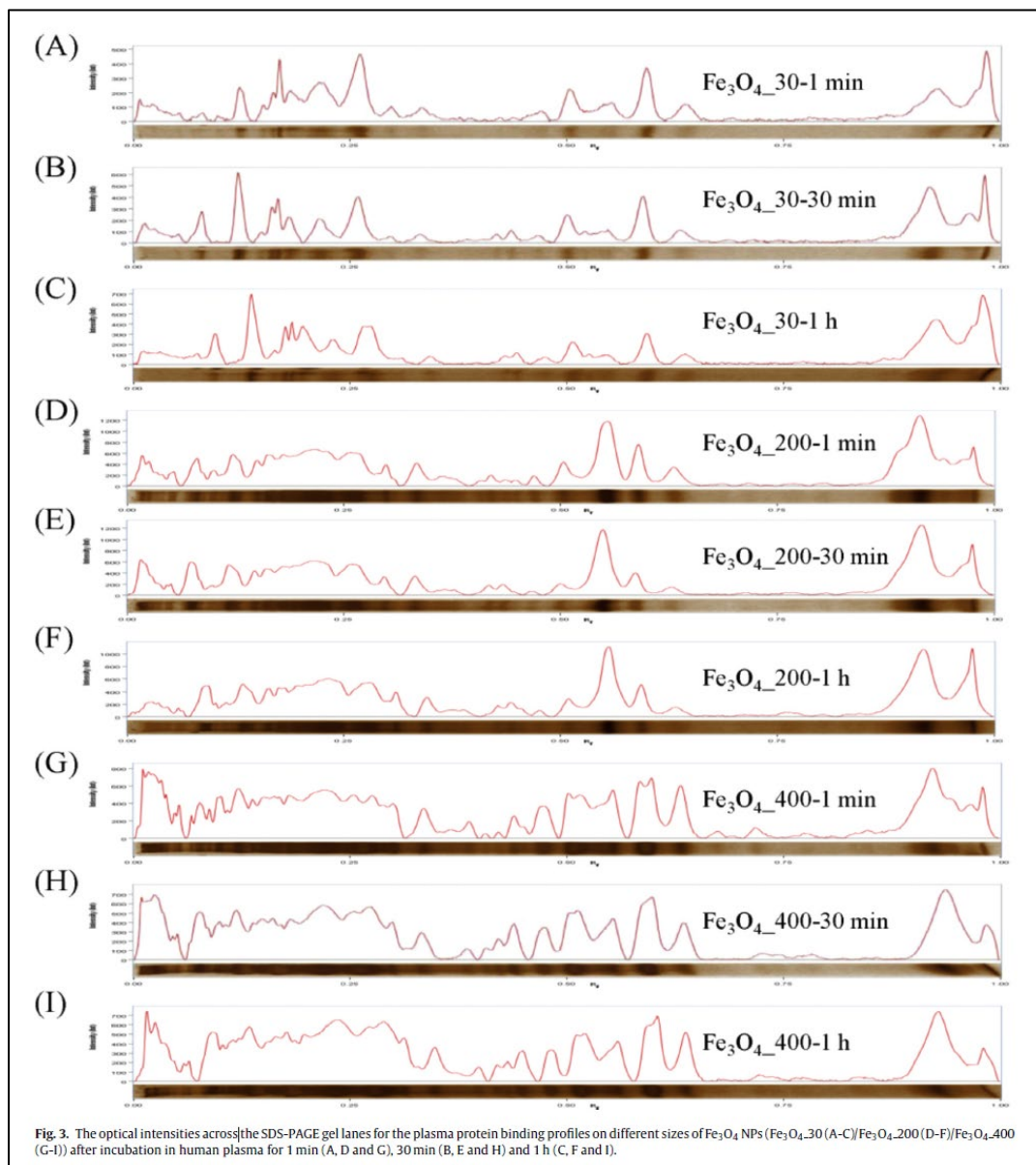
1[a][2] “to permit biomolecules of the biological sample to bind to the plurality of particles and form coronas around the plurality of particles,”

Hu discloses limitation 1[a][2], *e.g.*, the contacting explained regarding limitation 1[a][1] above permits biomolecules (*e.g.*, proteins in Hu) of the sample to bind to the Fe₃O₄ nanoparticles and form coronas around them. EX1003 ¶¶138-143; EX1001, 15:7-11, 17:29-39, 18:26-38 (biomolecules can be proteins and proteins form coronas around nanoparticles).

As described in limitation 1[a][1], Hu discloses incubation of human blood plasma with different Fe₃O₄ nanoparticles. *See also, e.g.*, EX1008, 355-56; limitation 1[a][1]; EX1003 ¶¶138-139, 134, 137. For example, Hu discloses that 30, 200, and 400 nanometer Fe₃O₄ NPs were incubated with human blood plasma for various intervals (1 minute; 30 minutes; 1 hour). EX1008, 355-56; EX1003 ¶139.

Hu further discloses analysis of the protein coronas that formed around the Fe₃O₄ NPs during that incubation period resulting in the NPs being bound to the human blood plasma proteins. EX1008, 355; EX1003 ¶¶140-142, 41-42; *see also* EX1007, 1869. In Figure 3, below, Hu depicts “[t]he optical intensities across the

SDS-PAGE gel lanes for the plasma protein binding profiles on different sizes of Fe_3O_4 NPs ($\text{Fe}_3\text{O}_4_{30}$ (A-C)/ $\text{Fe}_3\text{O}_4_{200}$ (D-F)/ $\text{Fe}_3\text{O}_4_{400}$ (G-I)) after incubation in human plasma for 1 min (A, D and G), 30 min (B, E and H) and 1 h (C, F and I).”
EX1008, 358; EX1003 ¶140.



EX1008, FIG. 3.

Notably, Hu's disclosure is consistent with the '360 Patent, which describes that "'biomolecule corona' encompasses 'protein corona' which is a term used in the art to refer to the proteins, lipids and other plasma components that bind nanoparticles when they come in contact with biological samples...." and "encompasses both the soft and hard protein referred to in the art." EX1001, 17:29-59; *see also* 18:26-39 ("biomolecule" can be, *e.g.*, "proteins"); EX1003 ¶143.

A POSA would have understood that Hu's incubation step permits proteins (the claimed "*biomolecules*") in the human blood plasma (the claimed "*biological sample*") to bind to the Fe₃O₄ particles to form protein coronas around the particles. EX1003 ¶143.

1[a][3] "wherein the coronas corresponding to the different particle types (i) differ based on particle type, and (ii) comprise overlapping and distinct proteins;"

Hu discloses limitation 1[a][3], *e.g.*, Hu discloses that its protein coronas differ based on particle type (*e.g.*, size and composition) and comprise overlapping and distinct plasma proteins. EX1003 ¶¶144-150.

Hu discloses that the size and composition of the protein coronas formed on the Fe₃O₄ nanoparticles is affected by the diameter of the nanoparticle. EX1008, 358; EX1003 ¶¶144, 42. Hu discloses that "[f]or instance, serum albumin was observed in the protein corona of the Fe₃O₄_200 NPs with an average MWSpC [Molecular Weighted Spectra Count, a measure of abundance] of 9.01, while the average

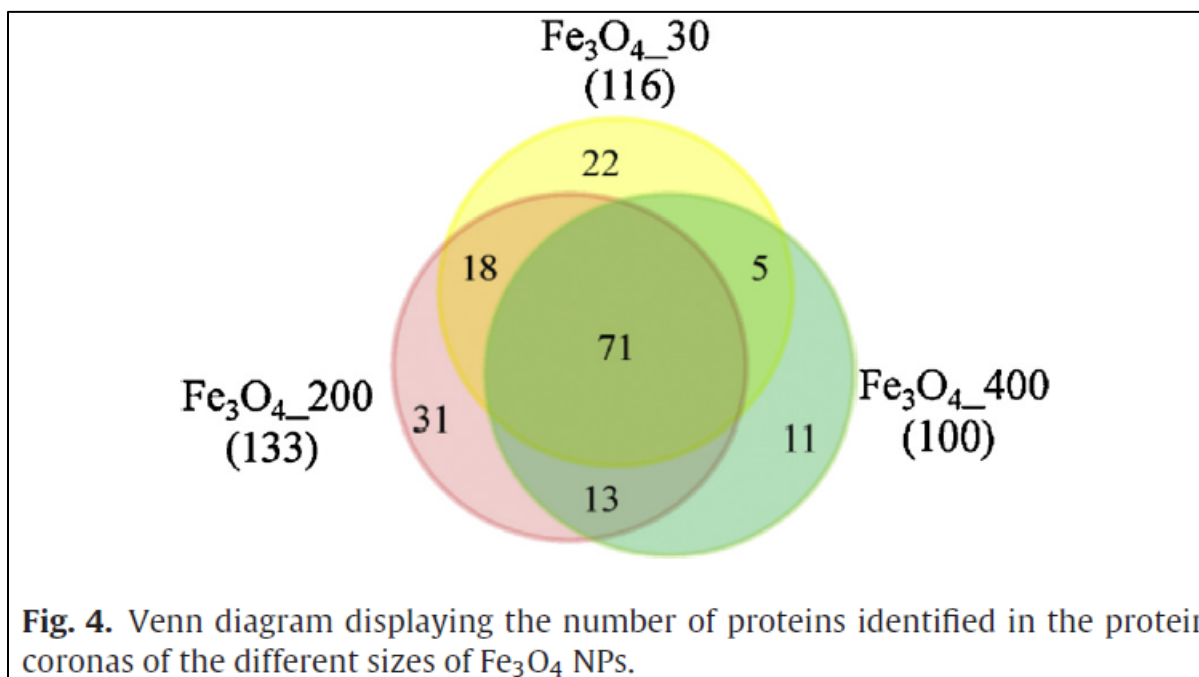
MWSpC for serum albumin in the protein coronas of the Fe₃O₄_30 and Fe₃O₄_200 NPs were 1.27 and 2.50, respectively, indicating the selective binding of plasma proteins on different sizes of Fe₃O₄ NPs.” EX1008, 358; EX1003 ¶144. In Table 2, below, Hu compares the top twenty most abundant plasma proteins bound to the 30 nm, 200 nm, and 400 nm Fe₃O₄ nanoparticles. EX1008, 359; EX1003 ¶145.

Rank	Fe ₃ O ₄ _30	Fe ₃ O ₄ _200	Fe ₃ O ₄ _400
1	Platelet factor 4	Apolipoprotein A-I	Apolipoprotein E
2	Apolipoprotein E	Serum albumin	Vitronectin
3	Vitronectin	Apolipoprotein C-III variant 1	Histidine-rich glycoprotein
4	Platelet basic protein	Apolipoprotein C-I	Platelet basic protein
5	IGK@ IGK@ protein	Apolipoprotein E	Platelet factor 4
6	IGKC Anti-RhD monoclonal T125 kappa light chain	Isoform LMW of Kininogen-1	Complement C1q subcomponent subunit C
7	Putative uncharacterized protein	Vitronectin	Complement C1q subcomponent subunit A
8	Complement C1q subcomponent subunit C	Platelet factor 4	Isoform 1 of Gelsolin
9	IGHG1	Histidine-rich glycoprotein	APOC1 Apolipoprotein C-I
10	Apolipoprotein C-III variant 1	Platelet basic protein	Complement C3 (Fragment)
11	IGHG1	Isoform HMW of Kininogen-1	Prothrombin (Fragment)
12	IGHG1	Isoform 1 of Gelsolin	Serum albumin
13	Prothrombin (Fragment)	Apolipoprotein A-II	C1QB Uncharacterized protein
14	Complement C1q subcomponent subunit A	Complement C3 (Fragment)	IGHG1
15	CLEC3B Putative uncharacterized protein DKFZp686H17246	Isoform 1 of Inter-alpha-trypsin inhibitor heavy chain H4	IGHG1
16	Apolipoprotein A-I	clusterin isoform 3	IGHG1
17	Isoform LMW of Kininogen-1	ITIH4 protein	APOH Beta-2-glycoprotein 1
18	Complement C3 (Fragment)	inter-alpha (globulin) inhibitor H4 isoform 2 precursor	IGKC Putative uncharacterized protein
19	Beta-2-glycoprotein 1	Prothrombin (Fragment)	Complement component 4B
20	IGHM Putative uncharacterized protein DKFZp686I15212	Apolipoprotein C-II	IGKC Anti-RhD monoclonal T125 kappa light chain

EX1008, Table 2.

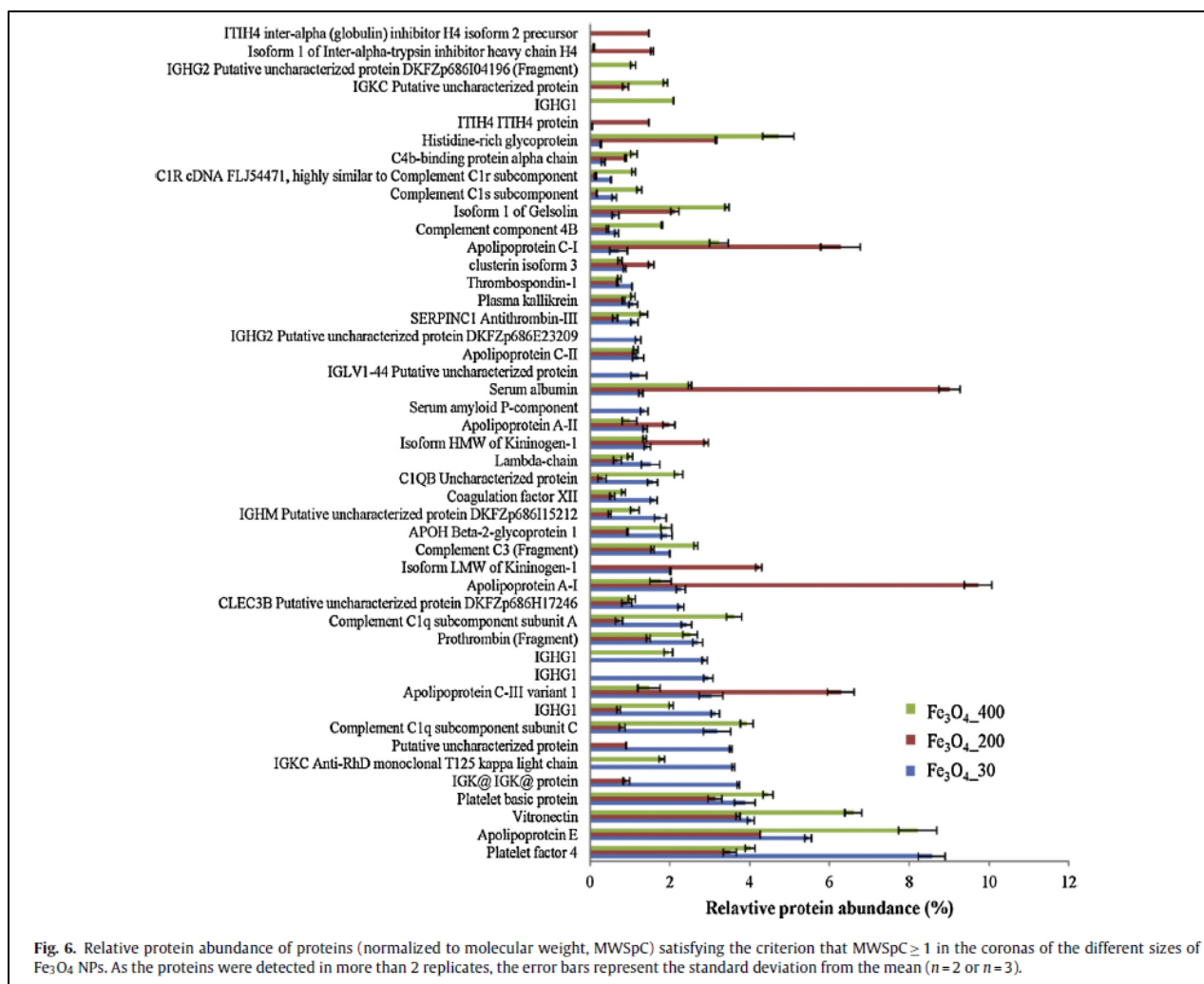
Figure 4, below, shows a Venn diagram comparing the number of distinct and overlapping proteins found in the coronas of each nanoparticle. EX1008, 357, 359; EX1003 ¶146. “In total, there were 117, 133, and 100 plasma proteins identified in the protein coronas of the Fe₃O₄_30, Fe₃O₄_200 and Fe₃O₄_400 NPs, respectively.... A total of 71 plasma proteins were detected in the protein coronas of Fe₃O₄ NPs of the three different sizes, with 22, 31 and 11 distinct proteins for the Fe₃O₄_30, Fe₃O₄_200 and Fe₃O₄_400 NPs, respectively (shown in Fig. 4).” EX1008, 357;

EX1003 ¶147.



EX1008, FIG. 4.

A POSA would have understood that the differences in the types and amounts of proteins bound between the various diameters of Fe_3O_4 nanoparticles shows that the corresponding coronas of the different types of particles differ. EX1003 ¶148. Hu further illustrates the distinct and overlapping proteins bound to the different nanoparticle coronas in Figure 6, below, which shows relative protein abundance for a subset of proteins from the sample. EX1008, 360; EX1003 ¶149.



EX1008, FIG. 6.

In addition, to the above-described disclosure in Hu—as shown and depicted in Table 2 and Figures 4 and 6—Hu further explains that its coronas corresponding to the different particle types both (i) differ based on particle type (*e.g.*, size and composition), and (ii) comprise overlapping and distinct proteins:

It was found that although there were 71 common plasma proteins bound on the different sizes of Fe₃O₄ NPs, the relative protein abundances for these proteins differed. For instance, serum albumin

was observed in the protein corona of the Fe₃O₄_200 NPs with an average MWSpC of 9.01, while the average MWSpC for serum albumin in the protein coronas of the Fe₃O₄_30 and Fe₃O₄_200 NPs were 1.27 and 2.50, respectively, indicating the selective binding of plasma proteins on different sizes of Fe₃O₄_NPs. Additionally, platelet factor 4 was observed in the corona of Fe₃O₄_30 with an average MWSpC of 8.57, which accounted for 3.51% and 4.01% in the protein coronas of the Fe₃O₄_200 and Fe₃O₄_400 NPs, respectively. Furthermore, the plasma proteins with an average MWSpC ≥ 1 in the protein corona on the Fe₃O₄ NPs are illustrated in Fig. 6, which also reveals the distinctive properties of the plasma protein coronas formed on the different sizes of Fe₃O₄ NPs.

EX1008, 358-59; EX1003 ¶150.

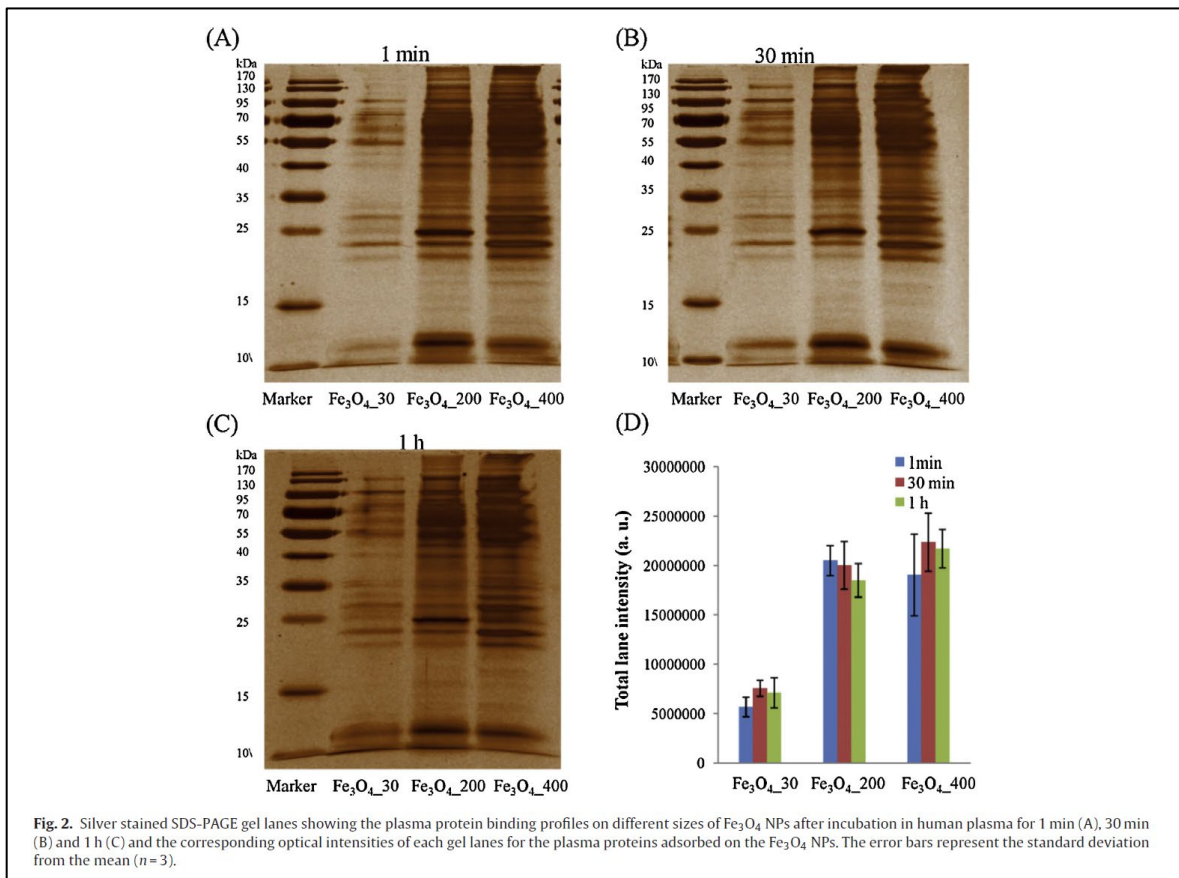
1[b] “(b) separating at least a subset of the plurality of particles comprising the coronas from the biological sample by removing the subset of the plurality of particles thereby producing a subset of proteins from the biological sample;”

Hu discloses limitation 1[b]. EX1003 ¶¶151-153.

Hu discloses separating out the nanoparticles from the biological sample by magnetic means, followed by washing “to remove the loosely bound and unbound proteins,” isolating the particles and their coronas, and thereby producing a subset of proteins from the sample (namely, those that are bound and not removed during washing). EX1008, 355; *see also* 356; EX1003 ¶151.

Figure 2, below, depicts “the adsorption dynamics of the human plasma

protein coronas on the Fe_3O_4 NPs” (the bound subset). EX1008, 356-57; EX1003 ¶152. As noted in the Figure 2 caption, Figure 2 shows “plasma protein binding proteins on different sizes of Fe_3O_4 NPs after incubation in human plasma” for certain times. EX1008, FIG. 2; EX1003 ¶152.



EX1008, FIG. 2.

A POSA would further have understood that based on the binding affinities for Fe_3O_4 nanoparticles disclosed in Hu, separating out the particles and their coronas from the biological sample would produce a subset of proteins from the sample (as described and depicted in Hu, including Figure 2). EX1003 ¶153.

1[c] “(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.”

It would have been obvious to include as part of Hu, limitation 1[c]. EX1003 ¶¶154-173, 126.

Hu discloses assaying the subset of proteins using “RPLC” (*i.e.*, Reverse Phase Liquid Chromatography) in conjunction with tandem mass spectrometry (RPLC-MS/MS). EX1008, 355-56; EX1003 ¶¶155, 99-103. Hu also discloses that “[t]he analysis was carried out on a 1-D nano LC-MS/MS system with 5 µL of the resuspended peptides injected each time.” EX1008, 355-56; EX1003 ¶155. Hu further discloses using known “Protein Pilot software (AB SCIEX, USA)” to analyze and search the known International Protein Index for MS/MS spectral data protein matches. EX1008, 356; EX1003 ¶155.

In Table 2, below, Hu discloses the top twenty most abundant proteins found bound to the three different types of Fe₃O₄ nanoparticles, which includes, among others, “serum albumin.” EX1008, 359-60; EX1003 ¶156.

Rank	Fe ₃ O ₄ -30	Fe ₃ O ₄ -200	Fe ₃ O ₄ -400
1	Platelet factor 4	Apolipoprotein A-I	Apolipoprotein E
2	Apolipoprotein E	Serum albumin	Vitronectin
3	Vitronectin	Apolipoprotein C-III variant 1	Histidine-rich glycoprotein
4	Platelet basic protein	Apolipoprotein C-I	Platelet basic protein
5	IGK@ IGK@ protein	Apolipoprotein E	Platelet factor 4
6	IGKC Anti-RhD monoclonal T125 kappa light chain	Isoform LMW of Kininogen-1	Complement C1q subcomponent subunit C
7	Putative uncharacterized protein	Vitronectin	Complement C1q subcomponent subunit A
8	Complement C1q subcomponent subunit C	Platelet factor 4	Isoform 1 of Gelsolin
9	IGHG1	Histidine-rich glycoprotein	APOC1 Apolipoprotein C-I
10	Apolipoprotein C-III variant 1	Platelet basic protein	Complement C3 (Fragment)
11	IGHG1	Isoform HMW of Kininogen-1	Prothrombin (Fragment)
12	IGHG1	Isoform 1 of Gelsolin	Serum albumin
13	Prothrombin (Fragment)	Apolipoprotein A-II	C1QB Uncharacterized protein
14	Complement C1q subcomponent subunit A	Complement C3 (Fragment)	IGHG1
15	CLEC3B Putative uncharacterized protein DKFZp686H17246	Isoform 1 of Inter-alpha-trypsin inhibitor heavy chain H4	IGHG1
16	Apolipoprotein A-I	clusterin isoform 3	IGHG1
17	Isoform LMW of Kininogen-1	ITIH4 protein	APOH Beta-2-glycoprotein 1
18	Complement C3 (Fragment)	inter-alpha (globulin) inhibitor H4 isoform 2 precursor	IGKC Putative uncharacterized protein
19	Beta-2-glycoprotein 1	Prothrombin (Fragment)	Complement component 4B
20	IGHM Putative uncharacterized protein DKFZp686I15212	Apolipoprotein C-II	IGKC Anti-RhD monoclonal T125 kappa light chain

EX1008, Table 2.

Hu further discloses additional data in its Appendix A, which it refers to as “supplementary data” and “Table S1”, which data provides “detailed identification results” for the plasma proteins identified in the various-sized protein coronas, such as “Latent Transforming Growth Factor-Beta Binding Protein-1” (LTBP-1) among others. EX1008, 361, 357-358; *see also* EX1008, 18 (Table S1, row corresponding to “IPI00973177.1”);⁴ EX1003 ¶¶157-158. Hu explains, with respect to Table S1:

⁴ Pagination for the supplementary data is indicated by the exhibit page numbers in the lower righthand corner of EX1008. Pagination for the remainder is to the article page numbers on the upper righthand/left-hand corners.

In total, there were 117, 133 and 100 plasma proteins identified in the protein coronas of the Fe₃O₄_30, Fe₃O₄_200 and Fe₃O₄_400 NPs, respectively (the detailed identification results were listed in Table S1). A total of 71 human plasma proteins were detected in the protein coronas of Fe₃O₄ NPs of the three different sizes, with 22, 31 and 11 distinct proteins for the Fe₃O₄_30, Fe₃O₄_200 and Fe₃O₄_400 NPs, respectively (shown in Fig. 4). For example, serum albumin, apolipoprotein A-I and Platelet factor, among others, were observed to bind on the surfaces of all three types of Fe₃O₄ NPs, while ceruloplasmin and serum amyloid P-component, among others, were found to be uniquely bound on the surface of only the Fe₃O₄_30 NPs. Fibronectin, serotransferrin and serum paraoxonase/arylesterase 1 were observed to only be adsorbed on the Fe₃O₄_200 NPs, and coagulation factor XIII A chain and inter-alpha (Globulin) inhibitor H2 were only bound on the Fe₃O₄_400 NPs (Table S1).

EX1008, 357; EX1003 ¶157. Hu further explains its equation for MWSpC and that “[b]y using [the] equation, the relative protein abundances for the plasma proteins bound on the Fe₃O₄ NPs were calculated and listed in Table S1.” EX1008, 357-358; EX1003 ¶¶159-160.

Hu does not specifically state the dynamic range in numerical form, but a POSA would have understood, or at least found it obvious, from Hu’s disclosure and the knowledge of a POSA—including the disclosed bound proteins such as in Tables S1 and 2 and as further discussed above in this section for limitation 1[c] and in the sections above for limitations 1[a][3] and 1[b]—that proteins in the subset are

detected with the instrument at concentrations across a dynamic range comprising six or more orders of magnitude. EX1003 ¶¶161-166, 126. For example, a POSA would have understood that if proteins are found to be bound to the Fe₃O₄ nanoparticles, then those proteins (such as serum albumin and LTBP-1) existed in certain concentrations in the biological sample being assayed. EX1003 ¶162. A POSA would have understood that serum albumin is normally present in healthy human plasma at concentrations ranging from 35 to 52 mg/mL and LTBP-1 in concentrations ranging from 1.7 to 6.0 ng/mL including because both proteins are well-known components of human plasma. EX1003 ¶¶163-165, 126, 102-108; EX1015, 786-87, Table 2 (disclosing known reference values for the concentration of serum albumin in healthy human blood); EX1012 §II.F & ¶¶49-54 (explaining publication of Ahmed (EX1015) in March 2009); EX1016, 16046-48, Table 1 (disclosing known reference values for the concentration of LTBP-1 in healthy human blood); EX1012 §II.G & ¶¶55-59 (explaining publication of Cao (EX1016) on December 1, 2015).

Accordingly, the method of Hu detects the bound proteins in the biological sample at concentrations greater than 10 mg/mL (10^{-2}) (*i.e.*, serum albumin) and less than 10 ng/mL (10^{-9}) (*i.e.*, LTBP-1), comprising a dynamic range of at least six orders of magnitude. EX1003 ¶¶166, 103-104.

As further confirmation of the obviousness of inclusion of the claimed dynamic range in Hu's method, as explained in Section IV.A, the '360 Patent discloses looking up known protein concentrations in a known database to determine the dynamic range. *See* §IV.A; EX1001, 66:63-69:67, 82:16-21, FIG. 12; EX1014, D959-D962; EX1003 ¶¶167-170, 56-59. Indeed, 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. EX1003 ¶170; EX1014, D959-D962 (explaining the known Plasma Proteome Database relied upon in the '360 Patent and desirability of analyzing lower abundance proteins; noting “wide dynamic range of protein abundance[s], greater than 10 orders of magnitude....” and “MRM [multiple reaction monitoring] assays...” “multi-step process provides superior specificity and allows for accurate quantification of protein levels over a wide dynamic range of abundance[s]”); EX1020, 1 (Plasma Proteome Database FAQs noting, *e.g.*, 9000 proteins in plasma and serum reported and use of mass spectrometry); EX1005, 585-87; EX1007, 1869-70, 1872-1873, 1881.

Moreover, the '360 Patent does not ascribe any particular significance to the particular claimed “at least 6 orders of magnitude.” *See generally* EX1001; EX1003 ¶¶171-173. To the contrary, the '360 Patent concedes there was none: it admits that known mass spectrometry can detect proteins at a dynamic range of 4-6 order of

magnitude using known techniques such as LC-MS/MS and that the patent's purported invention enables a "dynamic range of ten (10) orders of magnitude in terms of protein detection...." EX1001, 20:20-37, 72:1-25; EX1003 ¶¶171-173, 53-54. That known mass spectrometry was allegedly limited to "4-6 order[s] of magnitude" is of no moment, as claim 1 only requires "at least 6 orders of magnitude" (not the 10 orders allegedly enabled by the '360 Patent). EX1003 ¶¶171-173, 53-54. And, in fact, broad dynamic ranges for low and high abundance proteins (including those encompassing the claimed range) were well known and understood to be typically and advantageously detected when assaying proteins of various abundance in biological samples using known instruments, such as LC-MS/MS. EX1003 ¶¶170-173, 33-34, 36-38, 99-104, 155; EX1014, D959-D962; EX1020, 1; EX1005, 585-87; EX1007, 1869-70, 1872-1873, 1881; EX1019, 12:4-59; EX1007, 354-55; EX1001, 72:1-25.

In other words, there is nothing inventive in claim 1 by claiming a dynamic range that was already well known and understood (and calculable from Hu's data and detectable using known techniques). *See, e.g., Iron Grip Barbell Co. v. USA Sports, Inc.*, 392 F.3d 1317, 1322 (Fed. Cir. 2004) ("Where the difference between the claimed invention and the prior art is some range or other variable within the claims, the patentee must show that the particular range is critical, generally by showing that the claimed range achieves unexpected results."); *In re Peterson*, 315

F.3d 1325, 1329-31 (Fed. Cir. 2003) (patentee failed to show claimed range is “critical” and “unexpected results [were] commensurate in scope with the claimed range” where patent specification failed to provide examples of unexpected results “for the entire claimed range”); *see also, e.g., Pfizer Inc. v. Sanofi Pasteur Inc.*, 94 F.4th 1341, 1347-48 (Fed. Cir. 2024).

2. Claim 2

“The method of claim 1, wherein the detecting of proteins in the biological sample comprises detecting a protein present in the sample at a concentration greater than 1 mg/ml and a protein present in the sample at a concentration of less than 10 ng/ml.”

Hu renders obvious claim 1. §V.B.1. Hu discloses claim 2. EX1003 ¶¶174-176.

Hu discloses using samples of human blood from healthy donors to form protein coronas on different types of Fe₃O₄ nanoparticles. EX1008, 355-56; EX1003 ¶175. As explained regarding claim 1 above (and similar to Cai’s method discussed below in §V.C.1), Hu discloses that after separating out the proteins using SDS-PAGE, the proteins were analyzed using liquid chromatography and tandem mass spectrometry (RPLC-MS/MS). EX1008, 355-56; EX1003 ¶175.

As explained regarding claim 1 above, Hu discloses various proteins in different abundances found bound to the three different types of Fe₃O₄ nanoparticles, which include, *e.g.*, serum albumin and LTBP-1. EX1008, 355, 357, 359, Table 2;

see also 18 (Table S1 including LTBP-1); EX1003 ¶176. And as explained regarding limitation 1[c] above, a POSA would have understood that if proteins are found to be bound to the Fe₃O₄ nanoparticles, then those proteins (*i.e.*, serum albumin and LTBP-1) existed in certain concentrations in the biological sample being assayed, and that serum albumin is normally present in healthy human plasma at concentrations ranging from 35 to 52 mg/mL and LTBP-1 in concentrations ranging from 1.7 to 6.0 ng/mL including because both proteins are well-known components of human plasma. EX1015, 786-87, Table 2; EX1016, 16046-48, Table 1; EX1003 ¶¶176, 162-165, 103-108. Accordingly, the detecting includes detecting a protein in the sample at a concentration greater than 1 mg/mL (serum albumin) and a protein at less than 10 ng/mL (LTBP-1). EX1003 ¶176.

3. Claim 3

“The method of claim 1, wherein the plurality of particles comprise iron oxide.”

Hu renders obvious claim 1. §V.B.1. Hu discloses claim 3 for the same reasons as 1[a][1], *e.g.*, Hu discloses using Fe₃O₄ (iron oxide) nanoparticles of various sizes. EX1003 ¶177; EX1008, 355-57, Table 1, FIG. 2.

4. Claim 4

“The method of claim 1, wherein the assaying is performed using gel-electrophoresis, liquid chromatography, mass spectrometry, nuclear magnetic resonance spectroscopy, Fourier transform infrared spectroscopy, circular dichroism, Raman spectroscopy, or any combination thereof.”

Hu renders obvious claim 1. §V.B.1. Hu discloses claim 4. EX1003 ¶¶178-180.

Hu discloses using gel-electrophoresis (SDS-PAGE), liquid chromatography (LC), and mass spectrometry (MS) to characterize and assay the protein coronas of the Fe₃O₄ nanoparticles. EX1008, 357 (Fig. 2(A)-(C) “Silver stained SDS-PAGE gel lanes showing the plasma protein binding profiles on different sizes of Fe₃O₄ nanoparticles”); EX1008, 355 (“The digested peptides from the human plasma protein corona were dissolved in 100 µL of 0.1% FA solution. The analysis was carried out on a 1-D nano LC-MS/MS system with 5 µL of the resuspended peptides injected each time.”); EX1003 ¶¶179-180; §§VI.B.1-2.

5. Claim 6

“The method of claim 1, wherein the dynamic range of proteins detected spans 6 or more orders of magnitude.”

Hu renders obvious claim 1. §V.B.1. Hu discloses, or it would have been obvious to include as part of Hu, the dynamic range of claim 6, for the same reasons as 1[c]. EX1003 ¶181.

6. Claim 7

“The method of claim 1, wherein the detected proteins comprise proteins present in concentrations of 100 ng/ml or less in the biological sample.”

Hu renders obvious claim 1. §V.B.1. Hu discloses or it would have been obvious to include as part of Hu, claim 7 for the same reasons as discussed in §§V.B.1-2 (namely, LTBP-1 is present in healthy human plasma at concentrations between 1.7 to 6.0 ng/mL). EX1003 ¶182.

7. Claim 17

“The method of claim 1, wherein the assaying further comprises digesting the proteins.”

Hu renders obvious claim 1. §V.B.1. Hu discloses claim 17. EX1003 ¶¶183-184.

Hu discloses digesting the proteins from the nanoparticle protein corona with the enzyme trypsin as part of the assaying method, just like in the '360 Patent. EX1008, 355 (“...[T]he solution was diluted with 100 mM NH₄HCO₃ to 1 M urea, and the proteins were digested with trypsin at 37°C overnight with shaking (the amount of trypsin applied to digest the plasma proteins was set to an enzyme:protein ration of 1:40 according to the amount of adsorbed proteins”); EX1001, 59:20-21 (“...digested by adding 2 micrograms of trypsin”), 59:58-59 (“For the database search, trypsin was specified as the proteolytic enzyme...”); EX1003 ¶184.

8. Claim 22

“The method of claim 1, wherein the biological sample is selected from the group consisting of: blood, plasma, serum, lung lavage, menstrual blood, urine, processed tissue samples, amniotic fluid, cerebrospinal fluid, tears, saliva, sperm, chopped tissue, homogenized tissue, or any lysates or combinations thereof.”

Hu renders obvious claim 1. §V.B.1. Hu discloses claim 22 for the same reasons as it discloses 1[pre] and 1[a] (human blood plasma). EX1003 ¶185; §V.B.1.

9. Claim 23

“The method of claim 1, wherein the coronas that form around the plurality of particles further comprise a species of biomolecules selected from the group consisting of polysaccharides, sugars, lipids, lipoproteins, metabolites, oligonucleotides, or any combination thereof.”

Hu renders obvious claim 1. §V.B.1. Hu discloses claim 23. EX1003 ¶¶186-187.

Hu discloses biomolecule coronas formed around nanoparticles, wherein the coronas comprise lipoproteins, namely Apolipoproteins. EX1008, 359 (Table 2, below, “Apolipoprotein A-1,” “Apolipoprotein C-1,” etc.); EX1003 ¶187.

Rank	Fe ₃ O ₄ _30	Fe ₃ O ₄ _200	Fe ₃ O ₄ _400
1	Platelet factor 4	Apolipoprotein A-I	Apolipoprotein E
2	Apolipoprotein E	Serum albumin	Vitronectin
3	Vitronectin	Apolipoprotein C-III variant 1	Histidine-rich glycoprotein
4	Platelet basic protein	Apolipoprotein C-I	Platelet basic protein
5	IGK@ IGK@ protein	Apolipoprotein E	Platelet factor 4
6	IGKC Anti-RhD monoclonal T125 kappa light chain	Isoform LMW of Kininogen-1	Complement C1q subcomponent subunit C
7	Putative uncharacterized protein	Vitronectin	Complement C1q subcomponent subunit A
8	Complement C1q subcomponent subunit C	Platelet factor 4	Isoform 1 of Gelsolin
9	IGHG1	Histidine-rich glycoprotein	APOC1 Apolipoprotein C-I
10	Apolipoprotein C-III variant 1	Platelet basic protein	Complement C3 (Fragment)
11	IGHG1	Isoform HMW of Kininogen-1	Prothrombin (Fragment)
12	IGHG1	Isoform 1 of Gelsolin	Serum albumin
13	Prothrombin (Fragment)	Apolipoprotein A-II	C1QB Uncharacterized protein
14	Complement C1q subcomponent subunit A	Complement C3 (Fragment)	IGHG1
15	CLEC3B Putative uncharacterized protein DKFZp686H17246	Isoform 1 of Inter-alpha-trypsin inhibitor heavy chain H4	IGHG1
16	Apolipoprotein A-I	clusterin isoform 3	IGHG1
17	Isoform LMW of Kininogen-1	ITIH4 protein	APOH Beta-2-glycoprotein 1
18	Complement C3 (Fragment)	inter-alpha (globulin) inhibitor H4 isoform 2 precursor	IGKC Putative uncharacterized protein
19	Beta-2-glycoprotein 1	Prothrombin (Fragment)	Complement component 4B
20	IGHM Putative uncharacterized protein DKFZp686I15212	Apolipoprotein C-II	IGKC Anti-RhD monoclonal T125 kappa light chain

EX1008, Table 2.

10. Claim 24

“The method of claim 1, wherein at least one particle of the plurality of particles enriches for peptides other than albumin such that a ratio of an amount of albumin to a protein X that binds to the at least one particle is less than a ratio of an amount of albumin to the protein X in the biological sample.”

Hu renders obvious claim 1. §V.B.1. Hu discloses claim 24. EX1003 ¶¶188-190.

As explained above regarding claim 1, Hu discloses using three different sizes of Fe₃O₄ nanoparticles to detect proteins in human plasma. EX1008, 355-56; EX1003 ¶189; §V.B.1. Hu further discloses that “[i]t was found that as the most abundant protein in human plasma, serum albumin was the 2nd most abundant protein in the protein corona around the Fe₃O₄_200 NPs, while it was the 12th most abundant protein in the protein corona on the Fe₃O₄_400 NPs and was not among the

top 20 most abundant proteins for the Fe₃O₄_30 NPs (27th).” EX1008, 360; Table 2 (below); EX1003 ¶189. Hu further discloses in Table 2 that all three sizes of nanoparticles enrich for Apolipoproteins over and above serum albumin. EX1008, 356-57, 359; EX1003 ¶189.

Rank	Fe ₃ O ₄ _30	Fe ₃ O ₄ _200	Fe ₃ O ₄ _400
1	Platelet factor 4	Apolipoprotein A-I	Apolipoprotein E
2	Apolipoprotein E	Serum albumin	Vitronectin
3	Vitronectin	Apolipoprotein C-III variant 1	Histidine-rich glycoprotein
4	Platelet basic protein	Apolipoprotein C-I	Platelet basic protein
5	IGK@ IGK@ protein	Apolipoprotein E	Platelet factor 4
6	IGKC Anti-RhD monoclonal T125 kappa light chain	Isoform LMW of Kininogen-1	Complement C1q subcomponent subunit C
7	Putative uncharacterized protein	Vitronectin	Complement C1q subcomponent subunit A
8	Complement C1q subcomponent subunit C	Platelet factor 4	Isoform 1 of Gelsolin
9	IGHG1	Histidine-rich glycoprotein	APOC1 Apolipoprotein C-I
10	Apolipoprotein C-III variant 1	Platelet basic protein	Complement C3 (Fragment)
11	IGHG1	Isoform HMW of Kininogen-1	Prothrombin (Fragment)
12	IGHG1	Isoform 1 of Gelsolin	Serum albumin
13	Prothrombin (Fragment)	Apolipoprotein A-II	C1QB Uncharacterized protein
14	Complement C1q subcomponent subunit A	Complement C3 (Fragment)	IGHG1
15	CLEC3B Putative uncharacterized protein DKFZp686H17246	Isoform 1 of Inter-alpha-trypsin inhibitor heavy chain H4	IGHG1
16	Apolipoprotein A-I	clusterin isoform 3	IGHG1
17	Isoform LMW of Kininogen-1	ITIH4 protein	APOH Beta-2-glycoprotein 1
18	Complement C3 (Fragment)	inter-alpha (globulin) inhibitor H4 isoform 2 precursor	IGKC Putative uncharacterized protein
19	Beta-2-glycoprotein 1	Prothrombin (Fragment)	Complement component 4B
20	IGHM Putative uncharacterized protein DKFZp686I15212	Apolipoprotein C-II	IGKC Anti-RhD monoclonal T125 kappa light chain

EX1008, Table 2.

Accordingly, a POSA would have understood that serum albumin is the most abundant protein in human plasma and that the disclosed methods are capable of enriching for peptides other than albumin such that a ratio of an amount of albumin to a certain Apolipoprotein that binds to the nanoparticles disclosed in Hu is less than a ratio of an amount of albumin to Apolipoprotein in the biological sample. EX1003 ¶190.

11. Claim 25

“The method of claim 1, wherein the different particle types differ from each other by one or more physicochemical properties, wherein the one or more physicochemical properties are selected from the group consisting of: composition, size, surface charge, hydrophobicity, hydrophilicity, surface functionality, surface topography, surface curvature, shape, and any combination thereof.”

Hu renders obvious claim 1. §V.B.1. Hu discloses claim 25 for the same reasons as claim 1 (e.g., size, surface curvature). EX1003 ¶191.

C. Cai Anticipates Claims 1, 4, 6, 17, 22, and 25

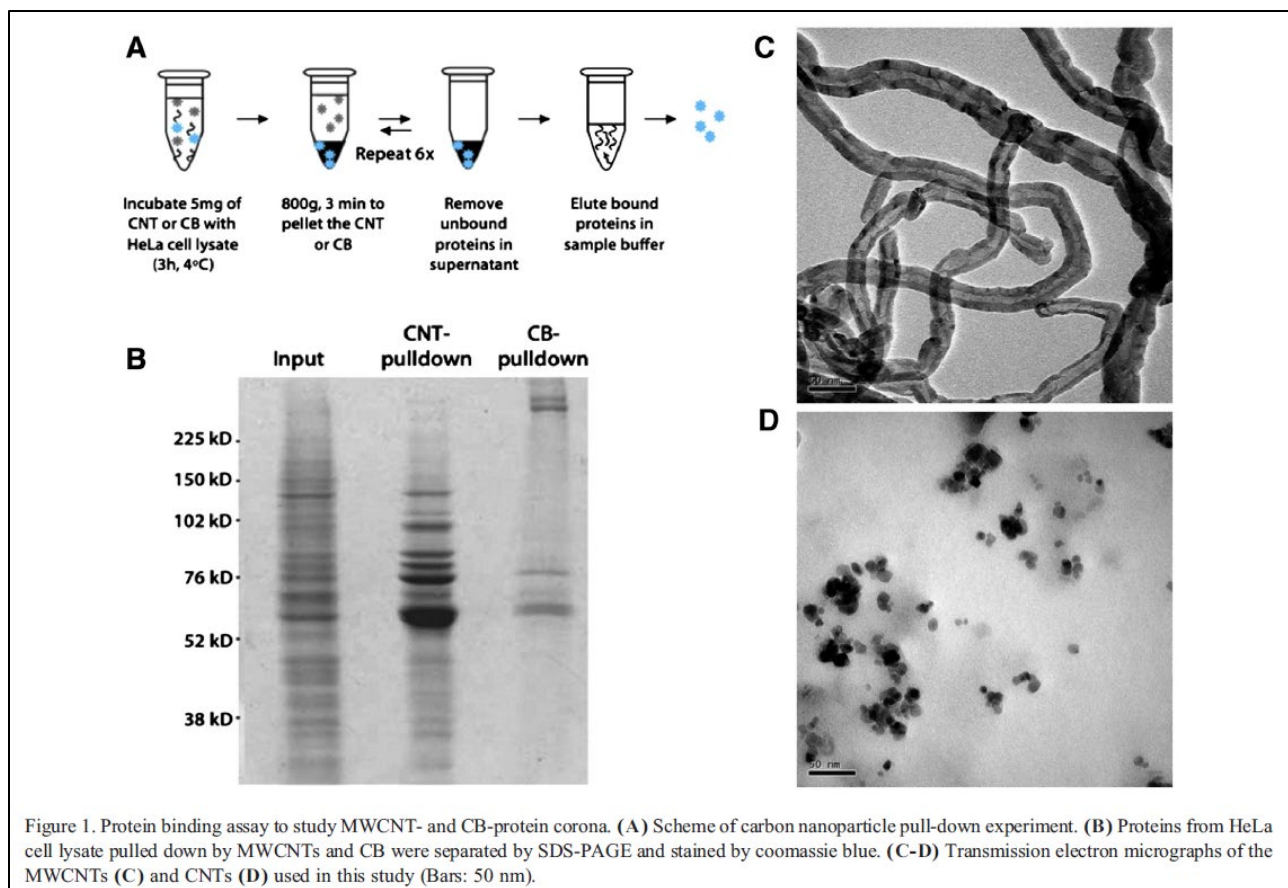
1. Claim 1

1[pre] “A method for assaying a biological sample, comprising:”

Cai discloses 1[pre], regardless of whether it is limiting. EX1003 ¶¶193-198; *see also* limitations 1[a]-1[c].

Cai discloses a method to “utili[ze] stable isotope labeling by amino acids in cell culture (SILAC)-based quantitative proteomics to characterize the binding of human cellular proteins to two forms of carbon nanoparticles: namely multi-walled carbon nanotubes (MWCNTs) and carbon black (CB).” EX1005, Abstract, 583; EX1003 ¶194. Cai further discloses: “[i]n the present study we exposed CNTs [carbon nanotubes] to a complex protein mixture, and report the large-scale identification of the resulting corona proteins. A complete protein lysate of HeLa cells was used as an archetypal human proteome, which provided the representative

inventory of proteins for the binding assay.” EX1005, 591; *see also* 585 (“To test the binding of complex mixtures of proteins, we incubated multi-wall CNTs (MCWNTs) with a mean diameter of 20-40 nm with a cell lysate prepared from the human cell line HeLa. Proteins from HeLa cells were chosen because they provide a representative source of the human proteome.”); 584 (“Cell lysates were incubated....”); 586 & FIG. 1 (below, depicting “Protein binding assay to study MWCNT- and CB-protein corona”); EX1003 ¶¶194-195.



EX1005, FIG. 1.

A POSA would have understood that Cai's method of analyzing the protein lysate from HeLa cells using various CNTs alone or with CB discloses the claimed "method for assaying a biological sample...." EX1003 ¶¶196-197; *see also* EX1001, 20:32-45, 28:11-18.

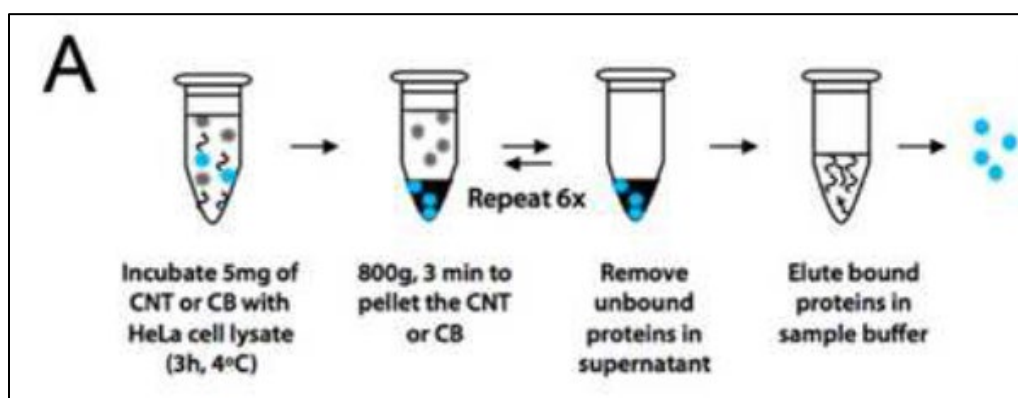
1[a][1] "(a) contacting the biological sample with a plurality of particles comprising different particle types"

Cai discloses limitation 1[a][1], *e.g.*, contacting the biological sample (*e.g.*, HeLa cell lysate) with various particles comprising different particle types (*e.g.*, CNTs with different particle types such as sizes or CNTs and CB). EX1003 ¶¶199-206.

Cai discloses that (i) "[c]ell lysates were incubated with 5 mg of CNTs or CB for 3 hrs at 4 °C", EX1005, 584; EX1003 ¶200; (ii) HeLa cells were harvested by trypsinization and then the lysates were collected for further proteomics analysis, EX1005, 584; EX1003 ¶200; (iii) "[t]o test the binding of a complex mixture of proteins to CNTs, we incubated pristine multiwall CNTs (MWCNTs) with a mean diameter of 20-40 nm with a cell lysate prepared from the human cell line HeLa", EX1005, 585; EX1003 ¶200; and (iv) "[i]n some experiments, 5 mg of 7 CNTs of different sizes were incubated with the same amount of FBS (4 mg) or cell lysate proteins (5 mg)." EX1005, 584; EX1003 ¶200. Cai also explains that "[d]ifferent types of CNTs with various diameters and lengths made by a chemical vaporization deposition (CVD) method were purchased from Nanotech Port Co. Ltd.," EX1005,

590, which a POSA would have understood are the CNTs utilized in Cai’s method. EX1003 ¶201; EX1005, 584 (“Properties of the CNTs used in this study....”).

Additionally, Cai discloses that “[i]n parallel, CB, a pure elemental carbon in the form of colloidal nanoparticles, was also tested for the binding capacity for the same protein mixture.” EX1005, 585; EX1003 ¶202. Figure 1(A), below, is an exemplary schematic of the disclosed method in Cai:



EX1005, FIG. 1(A); *see also* 586; EX1003 ¶202.

A POSA would have understood that Cai discloses that a biological sample (*e.g.*, HeLa cell lysate) is contacted with, per Cai, “[d]ifferent types of CNTs” of various diameters and lengths, which constitute particles consisting of different types (like the ’360 Patent). EX1003 ¶¶203-204; *see also* EX1005, 585 (“We further characterized the possible influence of CNT length and dimension on its protein binding capacity. Fetal bovine serum (FBS) proteins were incubated with CNTs of various types, diameters, and lengths....”); EX1001, cl. 25, 33:27-67, 86:10-22, FIG. 13. *see also* §V.B.1, limitation 1[a][1]. And, as explained in Section V.B.1

(regarding Hu), a POSA would further have understood that incubation encompasses the claimed contacting, as in the '360 Patent. EX1003 ¶205; EX1001, 9:3-5, 21:30-42, 76:22-35, 83:1-63, cls. 18-19; §V.B.1, limitation 1[a][1].

Additionally and in the alternative to the “[d]ifferent types of CNTs” alone constituting the claimed “*plurality of particles comprising different particle types*,” a POSA would also have understood that CB is a different type of nanoparticle from CNTs with different compositions and sizes. EX1005, Abstract (MWCNTs and CBs are “two forms of carbon nanoparticles”), 583-84 (describing properties of CNTs), 585 (describing MWCNTs and CBs), 585 (“[t]he diameters of CBs appear significantly smaller than MWCNTs,” and that “[t]his suggests the dimensions of carbon nanomaterials may affect the formation of protein corona”); EX1003 ¶206. Accordingly, a POSA would have understood that the biological sample (*e.g.*, HeLa cell lysate) is in contact with different particle types (*e.g.*, CNTs and CB) under this alternative as well. EX1003 ¶206.

1[a][2] “to permit biomolecules of the biological sample to bind to the plurality of particles and form coronas around the plurality of particles,”

Cai discloses limitation 1[a][2], *e.g.*, the contacting explained regarding limitation 1[a][1] above permits biomolecules (*e.g.*, proteins) of the sample to bind to the CNTs and CB and form coronas around the particles. EX1003 ¶¶207-215.

As described in limitation 1[a][1], above, Cai discloses incubation of cell lysate with different CNTs and CB particles. *See also, e.g.*, EX1005, 585-586; EX1003 ¶208; limitation 1[a][1]. For example, Cai discloses that 20-40 nm length MWCNTs were incubated with cell lysate prepared from HeLa cells, and the same samples were incubated with CB. EX1005, 585; EX1003 ¶208; *see also* limitation 1[a][1].

As further explained below, Cai also discloses analysis of the protein coronas that formed around the CNTs and CB during the incubation period resulting in the CNTs and CB being bound to the HeLa cell lysate. EX1005, 583-589 (sections entitled “Protein corona of carbon nanoparticles,” “Systematic characterization of carbon nanoparticle-protein corona,” “Functional classification of the carbon nanotube-protein corona,” “Physiochemical properties of CNT-binding proteins”); EX1003 ¶209.

For example, Cai discloses that “[a] comparison of the gel electrophoretic profiles of total HeLa cell lysate and the proteins pulled down by MWCNTs indicated that some proteins were selectively enriched into the CNT protein corona.” EX1005, 586; EX1003 ¶210.

Cai also discloses:

To test the binding of a complex mixture of proteins to CNTs, we incubated pristine multi-wall CNTs (MWCNTs) with a mean diameter of 20-40 nm with a cell lysate prepared from the human cell

line HeLa. Proteins from HeLa cells were chosen because they provide a representative source of the human proteome. In parallel, CB, a pure elemental carbon in the form of colloidal nanoparticles, was also tested for the binding capacity for the same protein mixture.... Both MWCNT and CB formed loose agglomerates that could be collected by low-speed centrifuge after protein binding.... Remarkably, MWCNT bound to a significant amount of HeLa proteins....

EX1005, 585; *see also* 584 (“MWCNT (diameter 20-40 nm)-binding proteins”; “CB-binding proteins”), 583 (“protein corona on CNT”), 584 (“The binding of cellular proteins to CNTs....”), 584 (“protein corona of CNTs and carbon black (CB)”); EX1003 ¶¶211-212.

Cai also discloses “the formation of protein corona” as a result of the protein binding. EX1005, 585; *see also* 584, 586, 589, FIGs. 1B, 3; EX1003 ¶213. Cai further discloses that CNTs—such as those used in its process—“are known to absorb a mixture of proteins to form a ‘protein corona’.” EX1005, 583; EX1003 ¶213.

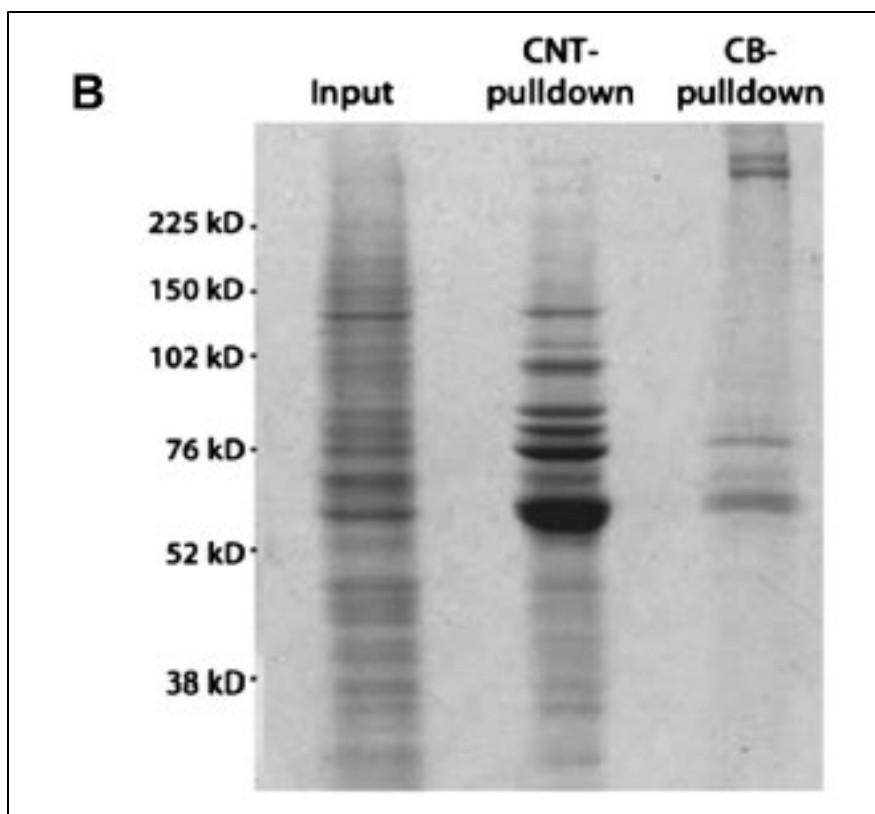
Cai’s disclosure is consistent with the ’360 Patent, which discloses the admittedly known “biomolecule coronas” such as “protein corona[s]” that “bind nanoparticles when they come into contact with biological samples or biological systems.” EX1001, 17:29-59, 18:26-39; EX1003 ¶214.

A POSA would have understood that Cai's incubation step permits proteins (the claimed "*biomolecules*") in the cell lysate (the claimed "*biological sample*") to bind to the CNTs and CB particles to form protein coronas around the particles. EX1003 ¶215.

1[a][3] "wherein the coronas corresponding to the different particle types (i) differ based on particle type, and (ii) comprise overlapping and distinct proteins;"

Cai discloses limitation 1[a][3]. EX1003 ¶¶216-222.

Cai discloses that its "result confirms the roles of the size of carbon nanomaterials in protein binding, and is consistent with previous studies where protein binding was more efficient with larger CNTs or nanoparticles than smaller ones." EX1005, 586; EX1003 ¶217. For example, Figure 1(B), below, shows the difference in HeLa cell proteins bound to MWCNTs and CBs. EX1005, 585 ("MWCNTs bound to a significant amount of HeLa cell proteins, whereas much less proteins were pulled down by the same amount of CB (Figure 1, B)."; "dimensions of carbon nanomaterials may affect the formation of protein corona"); EX1003 ¶¶217-218.



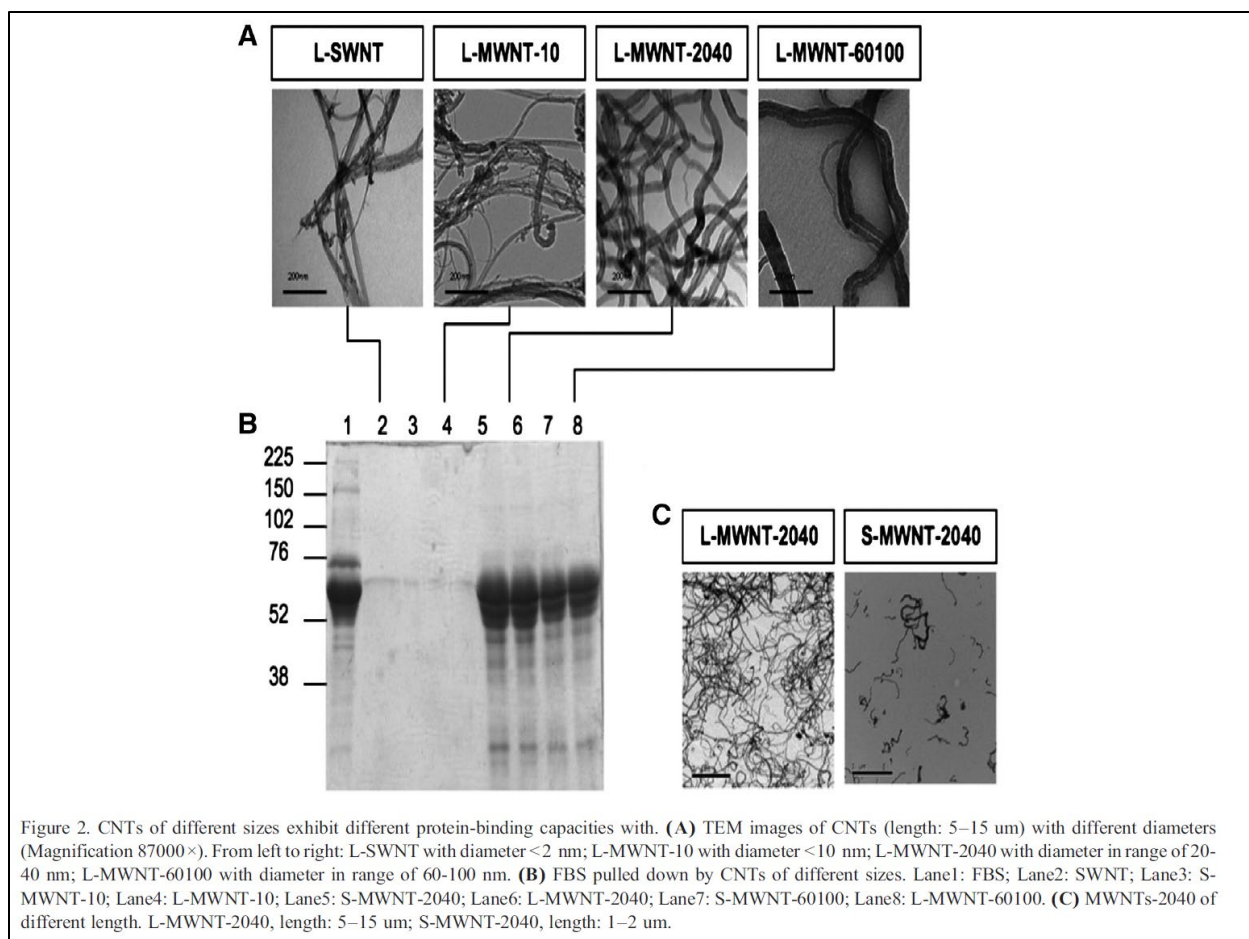
EX1005, FIG. 1(B).

Cai further discloses that “[b]y comparing the coomassie-blue staining intensities of the eluted proteins with that of the input, we estimated that about 1.5% of the entire pool of HeLa cell proteins in the incubation are able to bind to the MWCNTs after 3 hrs of incubation, but only less than 0.1% of these proteins are able to bind to the same amount of CB.” EX1005, 585; EX1003 ¶218. A POSA would have understood that the gel image in Figure 1(B) shows that CNT and CB bound different proteins based on the band intensities at different molecular weights. EX1003 ¶219. This is evident, for example by: (i) CNT having bands between 150 kD and above 76 kD that CB does not have (indicating there are no proteins with the

same molecular weights that bound to CB); (ii) the CB sample also having bands above 225 kD that CNT does not have (showing that CNT and CB bind distinct proteins); and (iii) CB and CNT do have bands that overlap in the 52-76 kD range (showing that there are overlapping proteins that bind to both CNT and CB). EX1003 ¶219.

Cai also discloses that (i) its process used “MWCNTs of diameters of 20-40 nm or above [which] could pull-down a significant amount of proteins (Figure 2, *B*, lanes 5-8), whereas those with diameters less than 10 nm bound to virtually no proteins (Figure 2, *B* lanes 3-4)”; and (ii) [t]o test the binding of a complex mixture of CNTs, we incubated pristine multi-wall CNTs (MWCNTs) with a mean diameter of 20-40 nm with a cell lysate prepared from human cell line HeLa”; (iii) “[s]ingle walled CNT, typically with diameters less than 2 nm, also show no significant protein binding (Figure 2, *B*, Lane 2);” and (iv) “[f]or MWCNTs that are wider than 20 nm, an increase in nanotube diameter did not further increase the protein binding capacity, as the amount of proteins pulled down by CNTs with diameters from 20-100 nm is essentially the same (Figure 2, *B*, Lanes 6, 8).” EX1005, 586; EX1003 ¶220.

Figure 2, below, shows the different diameters of multiwalled and single walled carbon nanotubes and their resultant effect on protein binding affinity (as described above). EX1005, 586-87; EX1003 ¶221.



EX1005, FIG. 2.

As evidenced by Cai’s disclosure discussed above, a POSA would have understood that: (i) the difference in the amount of proteins bound between CNTs of diameters 20–40 nm and less than 10 nm shows the corresponding coronas of the different types of particles differ; and (ii) CNTs with diameters 20–100 nm have the same amount of protein and include overlapping corona proteins. EX1003 ¶222. Cai further discloses that “MS analysis confirmed that the number of proteins enriched by MWCNT-binding is much higher than that enriched by CB” and “while a small number of CB-binding proteins also bind to MWCNTs, there is no apparent

correlation between the binding efficiencies of proteins to these two materials.” EX1005, 587; EX1003 ¶222. Cai also discloses that “most of the proteins with high CB-binding efficiencies bind to MWCNTs only marginally or not at all,” and that “[t]his indicates that MWCNTs and CB not only have different protein binding capacities, but also different protein specificities.” EX1005, 587; EX1003 ¶222. A POSA would have understood this to mean that the MWCNTs and CB particles have different coronas based upon these different particle types and also have overlapping and distinct proteins. EX1003 ¶222.

1[b] “(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;”

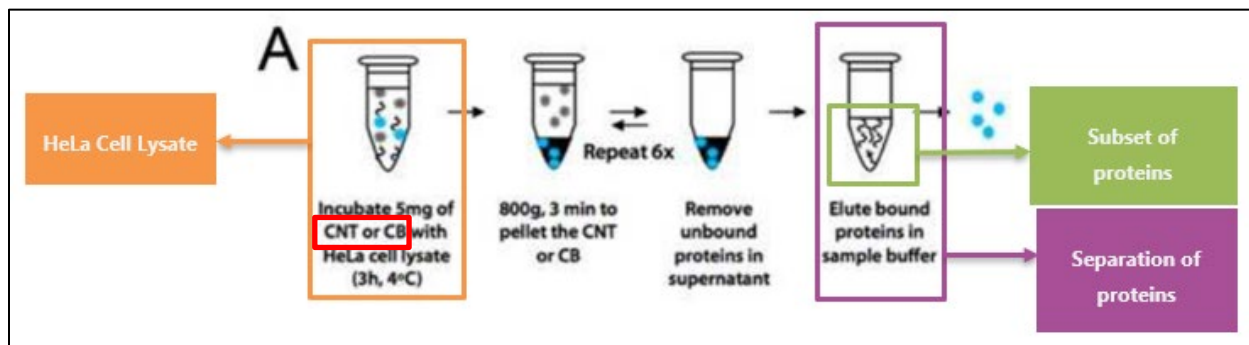
Cai discloses limitation 1[b]. EX1003 ¶¶223-224.

As shown in the annotated version of Figure 1(A) of Cai, below, Cai discloses the **HeLa cell lysate** is incubated with **CNT or CB nanoparticles**, unbound proteins are removed, after which the **bound proteins** are eluted, producing a **subset of proteins**. EX1005, 585; EX1003 ¶223. Per Cai:

Both MWCNT and CB formed loose agglomerates that could be collected by low-speed centrifugations after protein binding. The proteins that bound stably to these carbon matrices were eluted after extensive washing and then analyzed by SDS-PAGE.... By comparing the coomassie-blue staining intensities of the eluted proteins with that

of the input, we estimated that about 1.5% of the entire pool of HeLa cell proteins in the incubation reaction are able to bind to the MWCNTs.

EX1005, 585; EX1003 ¶223.



EX1005, FIG. 1(A) (annotated; blue dots in original).

A POSA would have understood that based on the binding affinities for CNTs and CBs disclosed in Cai, separating these particles and their coronas out from the biological sample would produce a subset of proteins from the biological sample (namely, the subset that were bound as disclosed in Cai). EX1003 ¶224.

1[c] “(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.”

Cai discloses limitation 1[c]. EX1003 ¶¶225-229.

Cai discloses that “[i]t is important to identify the proteins that bind to MWCNTs specifically,” and to do so “we performed proteomics analysis to identify[] the proteins that selectively bind to MWCNT of diameters of 20-40 nm.”

EX1005, 586; EX1003 ¶226. The proteins that selectively bound to MWCNTs of

diameters of 20-40 nm are a subset of proteins that were removed from the biological sample (as described in limitation 1[b]). *See also* EX1003 ¶226.

Cai further discloses that “[t]he mixtures were then separated by SDS-PAGE, and analyzed with the ‘gel-LCMS/MS’ approach.” EX1005, 586-87; EX1003 ¶227. Cai also discloses multiple methods of assaying the isolated protein coronas taken from the bound carbon nanoparticles, including gel electrophoresis, LC-MS/MS with SILAC encoding and Western Blotting. EX1005, 586-87, FIG. 1(B); EX1003 ¶227. A POSA would have understood that the various disclosed methods are performed using devices to detect, assay, and characterize proteins (as in the ’360 Patent). EX1003 ¶227; EX1001, 21:43-50, 41:29-38, 75:20-77:23; *see also* §IV.A. And Cai discloses to perform “[m]ass spectrometry... on an LTQ-orbitrap mass spectrometer system (ThermoElectron, Bremen, Germany) coupled to a Dionex 3000 nano-LC system (Camberley, UK) A 100×0.075 mm Agilent C18 column (3.5 µm particle diameter)....” (also like the ’360 Patent) EX1005, 585; EX1003 ¶227; EX1001, 21:43-50, 41:29-38, 75:20-77:23; *see also* §IV.A.

Cai discloses that “[s]ome of the proteins with the highest binding efficiencies... are low abundance proteins... suggest[ing] that protein binding to MWCNT is unlikely to be non-specific aggregation of the highly abundant proteins.... [i]nstead, the binding of proteins to MWCNT and CB appears to be protein-selective.” EX1005, 587; EX1003 ¶228. In other words, a POSA would have

understood that these results were from assaying the biological sample using MS across a range large enough to detect both low abundant and high abundant proteins in the biological sample. EX1003 ¶¶228, 95-97.

Cai also discloses the results of the “MS analysis confirmed that the number of proteins enriched by MWCNT-binding is much higher than that enriched by CB,” and that “[t]he relative abundance of the MWCNT-binding proteins spread *over 8 orders of magnitude*.” EX1005, 587; EX1003 ¶¶229, 97. A POSA would have understood 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. EX1003 ¶¶229, 97; *see also* §IV.A (explaining the ’360 Patent’s reliance on a known lookup table to determine the concentrations across the dynamic range comprising as least 6 orders of magnitude).

2. Claim 4

Cai discloses claim 1. §V.C.1. Cai also discloses claim 4 for the same reasons it discloses 1[c] (such as mass spectrometry, liquid chromatography, and gel electrophoresis). EX1003 ¶230; §V.C.1.

3. Claim 6

Cai discloses claim 1. §V.C.1. Cai also discloses the dynamic range in claim 6 for the same reasons it discloses 1[c]. EX1003 ¶231; §V.C.1.

4. Claim 17

Cai discloses claim 1. §V.C.1. Cai also discloses claim 17. EX1003 ¶¶232-233. For example, Cai discloses that “[t]he mixed protein samples were electrophoresed, gel-sliced and digested as described previously.” EX1005, 585; *see also* 584; EX1003 ¶232. A POSA would also have understood that Cai discloses digesting proteins with the enzyme trypsin (“trypsin as the enzyme”, EX1005, 585), just as the ’360 Patent. EX1001, 59:20-21 (“digested by adding 2 micrograms of trypsin”), 59:58-59 (“For the database search, trypsin was specified as the proteolytic enzyme....”); EX1003 ¶233.

5. Claim 22

Cai discloses claim 1. §V.C.1. Cai also discloses claim 22 for the same reasons it discloses the biological sample in 1[pre] and limitation 1[a] (namely, HeLa cell lysates). EX1003 ¶234; §V.C.1.

6. Claim 25

Cai discloses claim 1. §V.C.1. Cai discloses claim 25 for the same reasons as claim 1 (*e.g.*, size, shape, surface topography, surface curvature). EX1003 ¶¶235, 206, 96; §V.C.1.

D. Cai Renders Obvious Claims 1, 4, 6, 17, 22, and 25

To the extent that Cai is found not to disclose limitation 1[c] regarding the orders of magnitude (it does, *see* §VI.C.1), Cai would still render obvious claims 1, 4, 6, 17, 22, and 25. EX1003 ¶¶236-241.

A POSA would have understood, or at least found it obvious, from Cai's disclosure and the knowledge of a POSA that proteins in the subset are detected with the instrument at concentrations across a dynamic range comprising six or more orders a magnitude. EX1003 ¶¶237-241.

As explained in Section V.C.1 (limitation 1[c]), Cai discloses binding of both low and high abundance proteins and multiple methods of, and corresponding instruments for, assaying the isolated protein coronas taken from the bound carbon nanoparticles, including gel electrophoresis, LC-MS/MS with SILAC encoding and Western Blotting. *See also* EX1003 ¶238. Cai further discloses that its quantitative analysis of the relative distribution of proteins was done using the "MSQuant programme," where "[i]n total, 778 proteins were accurately quantified (Table S2)." EX1005, 587; EX1003 ¶238. Cai discloses protein concentrations in the form of empirical peptide abundance index data (emPAI). EX1005, 12-17 (Table S2,

“Supplementary Table 2: Data reported in Figure 3”);⁵ EX1003 ¶¶238-239. Cai further refers to Ishihama (EX1018) for the formula for converting emPAI values into more traditional concentration numbers using a formula provided in a citation. EX1007, 587, 593 (n.30); EX1003 ¶239. Ishihama provides:

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

EX1018, 1267; EX1003 ¶239.

A POSA would have understood to apply this formula to the data provided by Cai in Table S2, which results in protein concentrations detected spanning more than 6 orders of magnitude (the largest value was 86.46281858 mol% and the smallest value was 7.66962E-07 mol%, *i.e.*, 8 orders). EX1007, 12-17 (Table S2); EX1003 ¶¶240, 93, 97; *see also* §IV.A; EX1001, 66:63-69:67, 82:16-21, FIG. 12; EX1014, D959-D962; EX1020, 1.

And as explained above in Section V.B.1 (regarding limitation 1[c]), a POSA would have been motivated to search out protein concentrations. There is nothing inventive in claim 1 by claiming a dynamic range that was admittedly already well known and understood (and calculable from Cai’s data and a POSA’s knowledge

⁵ Pagination for Table S2 is to the exhibit page numbers in the lower righthand corner.

including its cited references, and detectable using known techniques). *See* §§V.B.1, V.E; EX1003 ¶241; *see also, e.g., Iron Grip*, 392 F.3d at 1322; *In re Peterson*, 315 F.3d at 1329-31; *Pfizer*, 94 F.4th at 1347-48.

E. Zaccaria Renders Obvious Claims 1, 2, 4, 6-7, 17, 22-23, and 25

1. Claim 1

1[pre] “A method for assaying a biological sample, comprising:”

Zaccaria discloses 1[pre], regardless of whether it is limiting. EX1003 ¶¶243-245; *see* limitations 1[a]-1[c].

Zaccaria discloses a method of assaying blood samples (more particularly, RBC lysates or hemolysates) using superparamagnetic nanoparticles to analyze the erythrocyte minor proteome. EX1007, 1869-70, 1873, 1878; EX1003 ¶¶243-245, 114-117. Zaccaria discloses “the potential capacity of three kinds of NPs [nanoparticles] to capture RBC proteins was determined by qualitative and quantitative proteomics analysis.” EX1007, 1870; EX1003 ¶243. Zaccaria further discloses analyzing the protein corona from the NPs using SDS-PAGE and nano-LC-MS/MS. EX1007, 1872 (“For the three types of NPs, SDS-PAGE analysis of the eluted protein corona revealed a pattern obviously different from nontreated sample.”), 1873 (“To better estimate the protein enrichment offered by the NP treatment, a deeper characterization of each NP corona was performed by nano-LC-MS/MS protein identification.”); EX1003 ¶244.

1[a][1] “(a) contacting the biological sample with a plurality of particles comprising different particle types”

Zaccaria discloses limitation 1[a][1], *e.g.*, contacting the biological sample (*e.g.*, Zaccaria’s red blood cell (RBC) lysate, also referred to as hemolysate) with various particles comprising different particle types (*e.g.*, Zaccaria’s “three kinds” of superparamagnetic nanoparticles (NPs) differing in, *e.g.*, surface chemistry and functionality). EX1003 ¶¶246-249.

For example, Zaccaria discloses using “three kinds of 100nm superparamagnetic NPs that strongly differ in their surface chemistry,” such as “a strong anion exchanger (Q-NP), a strong cation exchanger (PAA-NP), and a hydrophobic (OS-NP) surface to increase the probability to harvest different subsets of proteins.” EX1007, 1878-80; *see also* EX1008, 1870 (“Three different kinds of NPs grafted with different matrices and functional groups... fluidMAG-OS (OS-NP), fluidMAG-PAA (PAA-NP), and fluidMAG-Q (Q-NP).”), Table 1; EX1003 ¶¶247, 114.

Zaccaria discloses that (i) “[f]ive hundred micrograms of each kind of NPs were individually incubated in two replicates for 45 minutes with 200 µL of hemolysate containing 1 mg of total protein”; and (ii) “[a]s particles fulfilled critical quality criteria, 500 µg of each kind of NPs, developing 280 cm² surface area, were separately incubated in duplicates for 45 minutes with 200 µL of RBC lysates ([*i.e.*],

1 mg of proteins), followed by extensive washing to remove all unbound proteins.” EX1007, 1871-72; EX1003 ¶¶247-248.

A POSA would have understood that Zaccaria’s incubation step corresponds to “contacting the biological sample with a plurality of particles,” where the hemolysate or RBC lysate is the biological sample that is being assayed. EX1007, 1880; EX1003 ¶249. And, as explained in Section V.B.1 (regarding Hu), a POSA would further have understood that incubation encompasses the claimed contacting, as in the ’360 Patent. EX1003 ¶249; EX1001, 9:3-5, 21:30-42, 76:22-35, 83:1-63, cls. 18-19; §V.B.1, limitation 1[a][1].

1[a][2] “to permit biomolecules of the biological sample to bind to the plurality of particles and form coronas around the plurality of particles,”

Zaccaria discloses limitation 1[a][2], *e.g.*, the contacting explained regarding limitation 1[a][1] above permits biomolecules (*e.g.*, proteins in Zaccaria) of the sample to bind to the NPs and form coronas that cover the NPs. EX1003 ¶¶250-252; *see also* EX1001, 15:7-11, 17:29-39, 18:26-38 (biomolecules can be proteins and protein coronas bind nanoparticles).

For example, Zaccaria discloses that “[w]hen an NP is introduced into a biological fluid, it is well recognized that proteins immediately cover its surface, resulting in the formation of a protein ‘corona,’ which will rapidly evolve according to the concentration and the affinity of proteins for the NP.” EX1007, 1869; EX1003

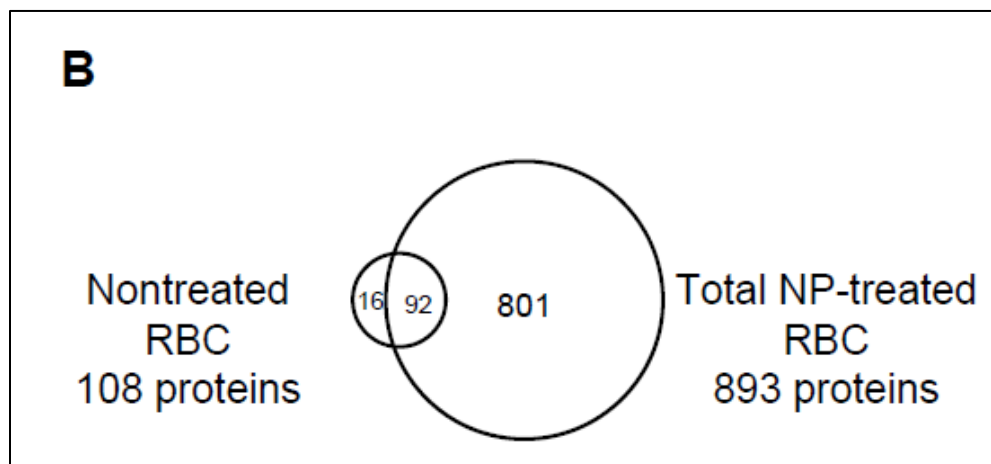
¶251. Zaccaria also discloses “investigat[ing] the capacity of three different kinds of 100nm NPs for protein capture in RBC lysates through the protein identification and quantification of their respective eluted corona.” EX1007, 1872; EX1003 ¶251. Specifically, Zaccaria describes that “500 µg of each kind of NPs” were incubated for “45 minutes with 200 µL of RBC lysates ([i.e.], 1 mg of proteins)” and then “followed by extensive washing to remove all unbound proteins.” EX1007, 1872; EX1003 ¶251. The coronas formed on the nanoparticles are then eluted which indicates that selected binding of proteins occurred on the nanoparticle and “SDS-PAGE analysis of the eluted protein corona revealed a pattern obviously different from nontreated sample.” EX1007, 1872; EX1003 ¶251. Zaccaria also discloses that “[t]o better estimate the protein enrichment offered by the NP treatment, a deeper characterization of each NP corona was performed by nano-LC-MS/MS protein identification.” EX1007, 1873; EX1003 ¶251.

Accordingly, a POSA would have understood that the incubation step permits the proteins in the RBC lysate to bind to the plurality of NPs, and that the elution and analysis of the proteins from the nanoparticles indicates that the proteins in the sample formed coronas around (*e.g.*, “cover,” which was “well-recognized” per Zaccaria) the nanoparticles. EX1003 ¶252.

1[a][3] “wherein the coronas corresponding to the different particle types (i) differ based on particle type, and (ii) comprise overlapping and distinct proteins;”

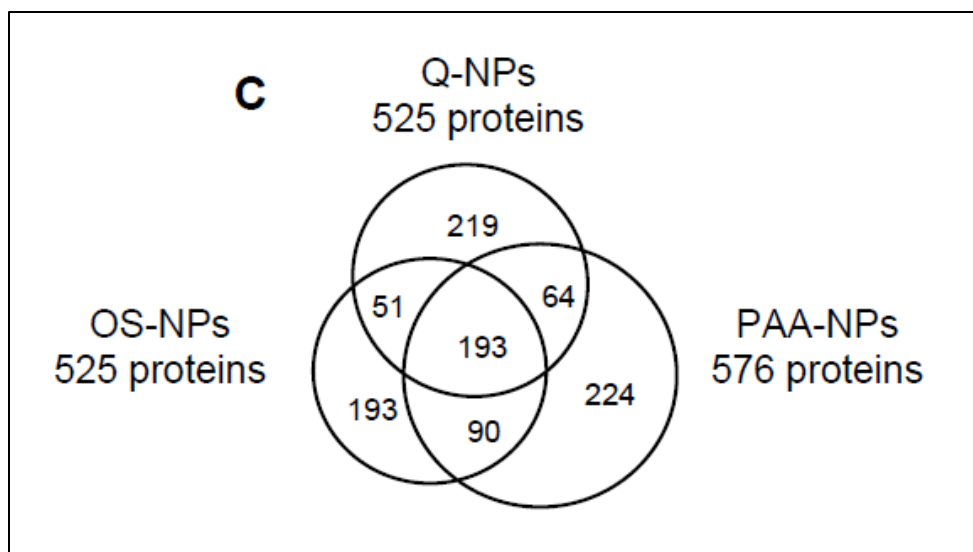
Zaccaria discloses limitation 1[a][3]. EX1003 ¶¶253-260.

Zaccaria discloses that “909 different proteins were identified from our RBC samples of which 893 were present in the list of NP-treated samples (Figure 2B).” EX1007, 1873; EX1003 ¶253. Figure 2(B), below, depicts that “[a]mong these 893 proteins, 801 were exclusively identified from NP-eluted coronae, and 92 were shared with crude RBC lysate.” EX1007, 1873; EX1003 ¶¶253-254.



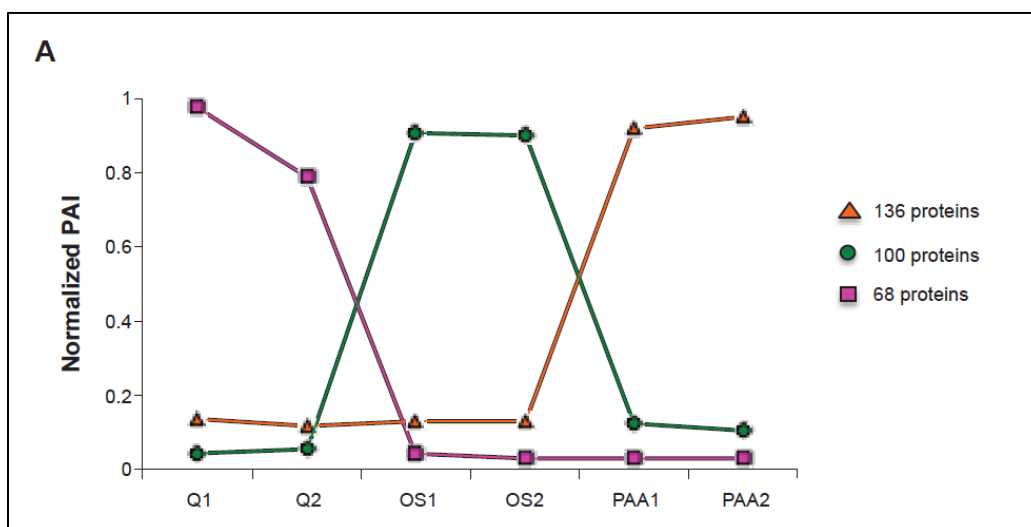
EX1007, FIG. 2(B).

From the NP-eluted coronae, Zaccaria discloses that the three different NP samples had both distinct and overlapping proteins, as shown, for example, in Figure 2(C), below. EX1007, 1873; EX1003 ¶¶255-256.



EX1007, FIG. 2(C).

Zaccaria discloses further analysis that shows different proteins that specifically constituted the corona of the different nanoparticle types. EX1003 ¶¶257-259. As seen in Figure 4(A), below, protein abundance index (“PAI”) values of each protein in each condition “revealed 60, 100, and 136 different proteins that specifically constituted the respective corona of Q-, OS-, and PAA-NPs.” EX1007, 1878; EX1003 ¶¶257-258.



EX1007, FIG. 4(A).

Zaccaria also discloses protein capture specificity based on molecular weight and isoelectric point of the proteins in the three nanoparticle types. EX1007, 1878-80; EX1003 ¶259. For example, “PAA-specific proteins revealed significantly higher MWs than OS-NPs,” and regarding pI, “[t]he positively charged Q-NPs revealed 90% of specific proteins that were negatively charged at pH 7.4 (ie, with a pI <7.4), whereas negatively charged OS- and PAA-NPs harvested less than 70% of specific protein with pI <7.4, confirming previous reports that suggested NP surface charge would influence the protein corona.” EX1007, 1878; *see also* 1880 (“These results confirm that NPs modify the range of protein concentration at their surface. Interestingly, the 20 most abundant proteins harvested by NPs were also different according to the NP chemical functionalization (Supplementary materials). This observation confirms that the affinity of each protein may be different according to the NP properties, influencing the protein concentration on the NP surface.”); EX1003 ¶259.

Accordingly, a POSA would have understood that the different nanoparticle types had different coronas based on the different physiochemical properties of each particle type, which included overlapping and distinct proteins, as shown, for example, in Figures 2 and 4 and as explained above. EX1003 ¶260.

1[b] “(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;”

Zaccaria discloses limitation 1[b]. EX1003 ¶¶261-266.

As described above for limitation 1[a][1] and 1[a][2], Zaccaria discloses incubating nanoparticles with RBC lysates to form protein coronas around the nanoparticles. *See also* EX1003 ¶261.

After the incubation step, Zaccaria discloses that “NPs were attracted to one side of the vial by an external magnet, and supernatant was removed,” the “NPs were washed,” and the “NP-associated proteins were desorbed.” EX1007, 1871; EX1003 ¶262. Zaccaria further discloses that “[a]s particles fulfilled critical quality criteria, 500 µg of each kind of NPs, developing 280 cm² surface area, were separately incubated in duplicates for 45 minutes with 200 µL of RBC lysates ([i.e.], 1 mg of proteins), followed by extensive washing to remove all unbound proteins,” thereby leaving only bound proteins. EX1007, 1872; EX1003 ¶¶262-263.

A POSA would have understood that isolating the particles with a magnet and removing the supernatant separated the particles with coronas from the rest of the biological sample, as well as washing to remove the unbound proteins, leaves only the subset of particles for further protein analysis (namely, those that are bound to the nanoparticles). EX1003 ¶264.

Zaccaria further discloses that “the Bradford assays revealed 19 ± 1 μg of proteins eluted from the surface of each kind of NPs, which represented 2% of the initial protein input,” EX1007, 1872, which a POSA would also have understood is a subset of proteins from the biological sample that were bound to the nanoparticles. EX1003 ¶265.

Zaccaria further discloses that “the high-abundant Hb was considerably reduced, and the protein profiles of NP-treated samples revealed a great enrichment between 20 kDa and 250 kDa with the appearance of numerous bands, which were not detected in the control condition,” indicating that a subset of proteins was separated from the biological sample, EX1007, 1872-73; EX1003 ¶266, and a POSA would have understood that the detection of additional proteins between the 20 kDa and 250 kDa range from the NP-treated samples shows that a subset of proteins were removed by the nanoparticles from the biological sample. EX1003 ¶266.

1[c] “(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.”

It would have been obvious to include as part of Zaccaria, limitation 1[c]. EX1003 ¶¶267-279, 242, 119-124.

Zaccaria discloses that “[t]o better estimate the protein enrichment offered by the NP treatment, a deeper characterization of each NP corona was performed by

nano-LC-MS/MS protein identification.” EX1007, 1873; *see also* EX1007, 1871. In

Table 2, below, Zaccaria discloses the twenty most abundant proteins identified from

NPs corona and crude RBC extract. EX1007, 1875-76; EX1003 ¶268.

Table 2 List of the 20 most abundant proteins (decreasing from 1 to 20) identified from NPs corona and crude RBC extract												
Total MS/MS number	MW	pI	Description	#total MS/MS	%total MS/MS	Rank	%total MS/MS	#total MS/MS	Description	pI	MW	Total MS/MS number
Control RBC												
18,796	16,102	6.75	Hemoglobin subunit beta	2,654	14.1	1	4.4	795	Hemoglobin subunit beta OS	6.75	16,102	18,096
	16,101	7.86	Beta-globin	2,424	12.9	2	4.4	788	Spectrin, beta, erythrocyte	5.15	247,271	
	11,534	5.9	Beta globin (fragment)	1,921	10.2	3	4.3	787	Spectrin beta chain, erythrocyte	5.15	247,171	
	11,496	6.17	Hemoglobin beta (fragment)	1,731	9.2	4	4.2	759	Spectrin alpha chain, erythrocyte	4.95	281,039	
	15,305	8.72	Hemoglobin subunit alpha	1,563	8.3	5	3.3	590	Hemoglobin beta (fragment)	6.17	11,496	
	11,532	5.46	Hemoglobin beta (fragment)	1,499	8.0	6	3.1	568	Hemoglobin subunit alpha	8.72	15,305	
	10,979	6.17	Hbbm fused globin protein (fragment)	1,480	7.9	7	3.1	555	Hemoglobin beta (fragment)	5.46	11,532	
	16,159	7.85	Delta globin	1,429	7.6	8	2.7	495	Hbbm fused globin protein (fragment)	6.17	10,979	
	8,386	6.63	Hemoglobin alpha 2 (fragment)	1,242	6.6	9	2.4	443	Delta globin	7.85	16,159	
	4,263	4.96	Truncated beta globin	968	5.2	10	2.0	358	Solute carrier family 4, anion exchanger, member 1	5.1	102,150	
	16,187	6.64	Hemoglobin subunit gamma-1	242	1.3	11	1.8	330	Transitional endoplasmic reticulum ATPase	5.14	89,950	
	28,909	6.59	Carbonic anhydrase I	113	0.6	12	1.5	272	Proteasome subunit alpha type-6	6.34	27,838	
	59,947	6.9	Catalase	103	0.5	13	1.4	245	Acyloxy-acid-releasing enzyme	5.29	82,142	
	247,171	5.15	Spectrin beta chain, erythrocyte	84	0.4	14	1.3	239	Cue1in heavy chain I	5.48	193,260	
	102,150	5.1	Solute carrier family 4, anion exchanger, member 1	72	0.4	15	1.3	236	Hemoglobin subunit gamma-1	6.64	16,187	
	32,325	6.45	Purine nucleoside phosphorylase	59	0.3	16	1.3	228	Myosin-9	5.5	227,646	
	22,049	5.66	Peroxiredoxin-2	57	0.3	17	0.9	171	Delta-aminolevulinic acid dehydratase	6.32	36,728	
	25,133	6	Peroxiredoxin-6	54	0.3	18	0.8	152	Ankyrin-1	5.65	207,334	
	29,285	6.87	Carbonic anhydrase 2	50	0.3	19	0.8	140	Importin-5	4.83	125,032	
	281,039	4.95	Spectrin alpha chain, erythrocyte	49	0.3	20	0.8	136	E3 ubiquitin-protein ligase UBR4	5.7	580,547	
			The 20 most abundant proteins Related to hemoglobin (among the 20 most abundant)	94.7			45.8		The 20 most abundant proteins Related to hemoglobin (among the 20 most abundant)			
				91.3			20.3					
OS-NPs												
13,101	281,039	4.95	Spectrin alpha chain erythrocyte	659	5.0	1	4.3	779	Hemoglobin subunit beta	6.75	16,102	18,073
	247,171	5.15	Spectrin beta chain erythrocyte	468	3.6	2	3.8	688	Beta globin (fragment)	5.9	11,534	
	247,271	5.15	Spectrin beta, erythrocyte	465	3.5	3	3.6	656	Beta-globin	7.86	16,101	
	102,150	5.1	Solute carrier family 4, anion exchanger, member 1	459	3.5	4	3.3	596	Hemoglobin beta (fragment)	6.17	11,496	
	16,102	6.75	Hemoglobin subunit beta	428	3.3	5	3.1	565	Hemoglobin beta (fragment)	5.46	11,532	
	11,532	5.46	Hemoglobin beta (fragment)	351	2.7	6	2.7	484	Hemoglobin subunit alpha	8.72	15,305	
PAA-NPs												
(Continued)												
Table 2 (Continued)												
Total MS/MS number	MW	pI	Description	#total MS/MS	%total MS/MS	Rank	%total MS/MS	#total MS/MS	Description	pI	MW	Total MS/MS number
	14,069	6.46	Hemoglobin beta subunit variant (fragment)†	333	2.5	7	2.6	475	Hbbm fused globin protein (fragment)	6.17	10,979	
	11,496	6.17	Hemoglobin beta (fragment)†	331	2.5	8	2.4	442	Delta globin	7.85	16,159	
	16,159	7.85	Delta globin	283	2.2	9	2.0	363	Spectrin beta chain, erythrocyte	5.15	247,171	
	15,305	8.72	Hemoglobin subunit alpha	272	2.1	10	2.0	360	Spectrin, beta, erythrocyte	5.15	247,271	
	16,187	6.64	Hemoglobin subunit gamma-1	212	1.6	11	1.6	286	Ankyrin-1	5.65	207,334	
	16,173	6.64	Hemoglobin subunit gamma-2	212	1.6	12	1.5	263	Spectrin alpha chain, erythrocyte	4.95	281,039	
	8,386	6.63	Hemoglobin alpha 2 (fragment)	199	1.5	13	1.3	230	Truncated beta globin	4.96	4,263	
	207,334	5.65	Ankyrin-1	196	1.5	14	1.2	217	Uncharacterized protein	7.04	98,190	
	580,547	5.7	E3 ubiquitin-protein ligase UBR4	133	1.0	15	1.2	212	Hemoglobin subunit gamma-1	6.64	16,187	
	271,766	5.77	Talin-1	126	1.0	16	1.2	210	Hemoglobin subunit gamma-2	6.64	16,173	
	57,794	6.01	T-complex protein 1 subunit beta	100	0.8	17	1.1	200	E3 ubiquitin-protein ligase UBR4	5.7	580,547	
	82,465	5.49	Calpain-1 catalytic subunit	86	0.7	18	1.1	191	Solute carrier family 4, anion exchanger, member 1	5.1	102,150	
	100,877	5.08	26S proteasome non-ATPase regulatory subunit 2	84	0.6	19	1.0	178	T-complex protein 1 subunit gamma	6.1	61,066	
	51,296	5.49	RuvB-like 2	84	0.6	20	0.9	162	T-complex protein 1 subunit beta	6.01	57,794	
			The 20 most abundant proteins Related to hemoglobin (among the 20 most abundant)		41.8		41.8		The 20 most abundant proteins Related to hemoglobin (among the 20 most abundant)			
					20.0		29.5					
Notes: Information about protein description, MW, pI, number, and proportion of MS/MS queries are provided. The % MS/MS has been normalized to the total MS/MS queries per run. Bold proteins are hemoglobin-related subunits or fragments.												
Abbreviations: NPs, nanoparticles; RBC, red blood cell; MS/MS, tandem mass spectrometry; MW, molecular weight; pI, isoelectric point.												

EX1007, Table 2.

Among the most abundant proteins, Table 2 identifies Hemoglobin subunit beta and Hemoglobin subunit alpha (HBA1). EX1007, 1875-76; EX1003 ¶269; *see also* EX1007, 1870 (“Because of the presence of hemoglobin (Hb), which represents 98% of the total RBC soluble protein content and masks the 2% of biologically interesting low-abundant proteins, it is difficult to investigate the deep RBC proteome in clinical and large-scale studies.”). While Table 2 shows the most abundant proteins, Zaccaria further discloses in its “Supplementary materials” all the proteins identified from the RBC lysate, including much lower abundant proteins. EX1007, 1873; 24-31 (supplementary materials), which result in the claimed dynamic range. EX1003 ¶¶269, 110-112, 119-124. For example, one of the lower abundant proteins identified was myotubularin-related protein 5 (SBF1). EX1007, 58 (supporting information); EX1003 ¶¶270, 119.

Zaccaria does not specifically state the dynamic range in numerical form, but a POSA would have understood, or at least found it obvious, from Zaccaria’s disclosure and the knowledge of a POSA that proteins in the subset are detected with the instrument at concentrations across a dynamic range comprising more than six orders a magnitude. EX1003 ¶¶271-272, 242, 119-124.

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 including because both proteins are well-known components of RBC.

EX1009, 33, 44 (supplementary materials); EX1012 §II.D & ¶¶34-42 (explaining publication of Bryk and its supplemental information (EX1009)); EX1003 ¶¶270-273, 120-123, 242. The same is true for other detected low-abundant proteins. EX1003 ¶¶122-124. Accordingly, the NPs in Zaccaria are detecting proteins in the biological sample at concentrations across a dynamic range comprising at least 6 orders of magnitude. EX1003 ¶278.

Further confirming the obviousness of claim 1 in view of Zaccaria, Zaccaria is consistent with the '360 Patent and prosecution history regarding the “dynamic range” and orders of magnitude. EX1003 ¶¶274-277, 117-119. Both Zaccaria and the '360 Patent are directed to determining biomarkers for disease detection by analyzing the proteome of human blood samples. EX1003 ¶¶277. Like the '360 Patent, Zaccaria describes how the identification of low-abundant proteins in the disclosed method allows for a greater range of protein concentrations to be detected using mass spectrometry:

In fact, we observed that low-abundant proteins identified in nontreated sample were present in higher abundance on NPs. Here also, the higher affinity of several low-abundant proteins for the different kinds of chemical functionalization allowed their accumulation on the NP surfaces. Thus, 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.

EX1007, 1881; EX1003 ¶274.

Zaccaria's teachings are also consistent with the problem that the purported invention of the '360 Patent is trying to solve regarding detecting a broad range of protein concentrations, and specifically low-abundant and rare proteins. *See, e.g.*, EX1001, 72:1-24, 20:26-27; EX1003 ¶¶277, 51-57.

Zaccaria further discloses that NPs can be used to help improve the dynamic range of protein concentration detection using mass spectrometry techniques:

This capacity of NPs to concentrate at their surface different subsets of proteins according to their physiochemical properties may be useful in proteomics to improve protein detection and identification, and detect potential disease-associated species. In fact, despite the constant technological advances in mass spectrometry (MS) and sample fractionation methods, the limited dynamic range of current mass spectrometers hinders the entire proteome coverage of biological fluids in which the protein concentration range spans more than ten order of magnitude. Thus, the low-abundant protein compartment where disease-associated biomarkers are thought to reside is still unattainable. Various nanomaterial-associated strategies have been integrated in MS in order to detect disease-associated biomarkers, and have been found extremely useful to improve protein enrichment and sample preparation, particularly NP-associated technology.

EX1007, 1869-70; *see also* 1872 (“For the three types of NPs, SDS-PAGE analysis of the eluted protein corona revealed a pattern obviously different from nontreated

sample.”), 1873 (“To better estimate the protein enrichment offered by the NP treatment, a deeper characterization of each NP corona was performed by nano-LC-MS/MS protein identification.”); EX1003 ¶¶275-276.

Accordingly, Zaccaria also discloses the utility of an instrument to measure a dynamic range of ten orders of magnitude to be able to capture the entire proteome of biological fluids, providing further evidence that Zaccaria renders obvious claim 1. EX1003 ¶278. And as explained above in Section V.B.1 (regarding limitation 1[c]), there is nothing inventive in claim 1 by claiming a dynamic range that was admittedly already well known and understood (and calculable from Zaccaria’s data and a POSA’s knowledge and detectable using known techniques). *See* §§V.B.1, V.D; EX1003 ¶279; *see also, e.g., Iron Grip*, 392 F.3d at 1322; *In re Peterson*, 315 F.3d at 1329-31; *Pfizer*, 94 F.4th at 1347-48.

2. Claim 2

Zaccaria renders obvious claim 1. §V.E.1. Zaccaria discloses or renders obvious claim 2. EX1003 ¶¶280-281. Zaccaria discloses a method of assaying blood samples (specifically, RBC lysates or hemolysates) using superparamagnetic nanoparticles to analyze the erythrocyte minor proteome. EX1007, 1869-70, 1873, 1878; EX1003 ¶¶243, 281. Zaccaria further discloses analyzing the protein corona from the NPs using SDS-PAGE and nano-LC-MS/MS. EX1007, 1872-73; EX1003 ¶¶244, 114-115.

As explained above regarding claim 1, Zaccaria discloses various proteins in different abundances found bound to the three different types of nanoparticles, which include, *e.g.*, Hemoglobin subunit alpha (HBA1) and myotubularin-related protein 5 (SBF1). EX1007, 1875-76, 24-31 (supplementary materials); EX1003 ¶¶281, 119-124. Also as explained in limitation 1[c], 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 including because both proteins are well-known components of RBC. EX1003 ¶281. A POSA would be able to perform a unit conversion calculation on these concentrations and determine that they are 27 mg/ml for HBA1 and 0.17 ng/ml for SBF1. EX1003 ¶281. Accordingly, the detecting includes detecting a protein in the sample at a concentration greater than 1 mg/mL (HBA1) and a protein at less than 10 ng/mL (SBF1). EX1003 ¶281.

3. Claim 4

Zaccaria renders obvious claim 1. §V.E.1. Zaccaria discloses claim 4 for the same reasons as 1[pre] and 1[c] (such as assaying using SDS-PAGE and nano-LC-MS/MS). EX1003 ¶282; §V.E.1.

4. Claim 6

Zaccaria renders obvious claim 1. §V.E.1. Zaccaria also discloses, or it would have been obvious to include as part of Zaccaria, the dynamic range of claim 6 for the same reasons as explained for 1[c]. EX1003 ¶283; §V.E.1.

5. Claim 7

Zaccaria renders obvious claim 1. §V.E.1. Zaccaria discloses or renders obvious claim 7 for the same reasons as discussed in §§V.E.1-2 (namely, SBF1 and PIK3R4 are present in RBC lysate at concentrations of 0.17 ng/ml and 5.86 ng/ml, respectively). EX1003 ¶¶284, 119-124.

6. Claim 17

Zaccaria renders obvious claim 1. §V.E.1. Zaccaria discloses claim 17. EX1003 ¶285. Zaccaria discloses that to analyze the RBC proteins from the NP assay, the “eluate and crude RBC lysate were... stacked in one band after a short and low-voltage electrophoretic migration on SDS-polyacrylamide gel electrophoresis (SDS-PAGE) gel” and “[t]he stacking bands were cut, proteins were in-gel digested by trypsin, and resulting peptides were extracted from the gel and analyzed by nano-LC-MS/MS....” EX1007, 1871; EX1003 ¶285.

7. Claim 22

Zaccaria renders obvious claim 1. §V.E.1. Zaccaria discloses claim 22 for the same reason as 1[pre] and 1[a] (namely, RBC lysates or hemolysates). EX1003 ¶286.

8. Claim 23

Zaccaria renders obvious claim 1. §V.E.1. Zaccaria discloses claim 23, *e.g.*, Apolipoprotein A-I (APOA1) (a lipoprotein). Ex. 1007, 26 (Table S2); EX1003 ¶¶287-288.

9. Claim 25

Zaccaria renders obvious claim 1. §V.E.1. Zaccaria discloses claim 25 for the same reasons as claim 1 (*e.g.*, composition, surface charge, and surface functionality). EX1003 ¶¶289-291.

VI. The Board Should Not Deny Institution Under Section 325(d)

The Board should not exercise its discretion under 35 U.S.C. §325(d) to deny institution because this Petition presents new arguments, art, and evidence not previously before the Office.

Neither Hu nor Cai were before the Patent Office during prosecution. *See Apple Inc. v. Omni MedSci, Inc.*, IPR2020-00175, Paper 11, 11 (June 17, 2020); *Zip Top, LLC v. Stasher, Inc.*, IPR2018-01216, Paper 14, 35 (Jan. 17, 2019); *Amazon.com, Inc. v. M2M Sols. LLC*, IPR2019-01205, Paper 14, 16 (Jan. 27, 2020). Zaccaria appears as one of many references cited, but was not relied upon or discussed by the Examiner. *See Advanced Energy Industries v. Reno Techs.*, IPR2021-01397, Paper 7, 7 (Feb. 16, 2022); *Lyft, Inc. v. RideShare Displays, Inc.*, IPR2021-01602, Paper 7, 14-15 (Apr. 11, 2022); *Samsung Elecs. Co., Ltd. v. Power2B, Inc.*, IPR2021-01190, Paper 11, 19 (Jan. 6, 2022). New testimony of Dr. Vargas (EX1003) explaining how a POSA would have understood the prior art's teachings and state of the art (including the allegedly novel “dynamic range”), and

why a POSA would have found the claims invalid, is also new evidence and argument that was never before the Office.

VII. Conclusion

The challenged claims are unpatentable. Petitioner requests cancellation.

Respectfully submitted,

REICHMAN, JORGENSEN, LEHMAN &
FELDBERG LLP

Dated: October 7, 2024

/Patrick Colsher/

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Lead Counsel for Petitioner

CERTIFICATE OF COMPLIANCE

I hereby certify that this paper complies with the type-volume limitation of 37 C.F.R. §42.24 (as determined by the Microsoft Word word-processing system used to prepare this paper) because it contains 13,966 words, excluding the parts of the paper exempted by 37 C.F.R. §42.24(a).

REICHMAN, JORGENSEN, LEHMAN &
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CERTIFICATE OF SERVICE

I hereby certify that the foregoing Petition for *Inter Partes* Review, the Exhibits listed on the List of Exhibits, and the accompanying Powers of Attorney for Petitioners were served on October 7, 2024 pursuant to 37 C.F.R. §§42.6 and 42.105 via Federal Express, upon the following at the correspondence address of record with the Patent Office for Patent Owner:

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