

UNITED STATES PATENT AND TRADEMARK OFFICE

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BEFORE THE PATENT TRIAL AND APPEAL BOARD

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PreOmics GmbH and Biognosys AG,  
Petitioners

v.

The Brigham and Women's Hospital, Inc.,  
Patent Owner

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

**DECLARATION OF JESSICA VARGAS, PHD**

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## **I. Engagement and Compensation**

1. My name is Jessica Vargas, Ph.D. I have been retained by PreOmics GmbH and Biognosys AG. (collectively, “Petitioners” or “PreOmics”) for the purpose of providing my opinion with respect to the patentability of claims 1-4, 6-7, 17, and 22-25 of U.S. Patent No. 11,435,360 (“the ’360 Patent”).

2. I am being compensated for my time in preparing this declaration at my standard hourly rate, and my compensation is not dependent upon my opinions or the outcome of the proceedings. I have no stake in the outcome of this proceeding.

3. I am not an employee of Petitioners or any affiliate or subsidiary thereof.

4. This declaration summarizes the opinions I have formed to date. I reserve the right to modify my opinions, if necessary, based on further review and analysis of information that I receive subsequent to the filing of this declaration, including in response to positions that parties to the *inter partes* review proceeding, or their experts, may take that I have not yet seen.

## **II. Qualifications**

5. I have summarized in this section my educational background, career, publications, and other relevant qualifications. My full curriculum vitae is provided as Exhibit 1004, which I incorporate into this declaration by reference.

6. I am currently a Principal Scientist in the Polymer Science and Materials Chemistry Practice at Exponent. I am a member of the American Chemical Society, and I hold three academic degrees. I was a National Science Foundation Graduate Research Fellow and received my Ph.D. in Chemistry from Stanford University in 2016. I received a Bachelor of Science degree in Biochemistry in 2010 and a Masters degree in Education in 2011, both from Loyola Marymount University. While at Loyola Marymount University, I also received the 2010 Scholar of the Year award, given to the university's top undergraduate. I have previously held a position as an Adjunct Professor in the Department of Chemistry & Biochemistry at Loyola Marymount University.

7. During the pursuit of my doctorate, I successfully completed several graduate courses that specifically focused on experimental tools for molecular characterization, organic chemistry, molecular imaging of biomarkers, diagnostics, and biomaterials. My coursework also included biochemistry, analytical chemistry, medicinal chemistry, immunology, and endocrinology.

8. My graduate research at Stanford University included designing, synthesizing, and characterizing polymeric materials for self-assembly into nanoparticles for drug, gene, and/or diagnostic delivery. As part of my research, I have performed analytical chemistry and biochemical characterization using chromatography and mass spectrometry techniques, as well as protein analysis and

expression assays. I have also characterized the size, charge, and other characteristics of different nanoparticle preparations. I leveraged these and other techniques as I developed biodegradable block copolymers for small molecule, messenger RNA (mRNA) drug delivery and gene therapy applications. I also previously interned at W.L. Gore & Associates, where I developed and qualified analytical testing methods used in coated stent manufacturing.

9. For over seven years at Exponent, I have led numerous investigations related to product and process development, as well as method development, method transfer, and method qualification for products such as pharmaceuticals, proteins, excipients, medical devices, and diagnostics utilizing liquid chromatography-mass spectrometry. I regularly consult on intellectual property matters related to pharmaceutical and medical products and formulations.

10. On the basis of my education and the personal and professional experiences described above and set forth in my curriculum vitae, I believe I am qualified to give the opinions set forth herein.

### III. Summary of Opinions

11. In my opinion, the claims 1, 2, 3, 4, 6, 7, 17, 22, 23, 24, and 25 of the '360 Patent (Ex. 1001) are anticipated and/or obvious in view of the following prior art:

- **Ground 1:** Claims 1-4, 6-7, 17, and 22-25 are obvious in view of Hu;
- **Ground 2:** Claims 1, 4, 6, 17, 22, and 25 are anticipated by Cai;
- **Ground 3:** Claims 1, 4, 6, 17, 22, and 25 are also obvious in view of Cai; and
- **Ground 4:** Claims 1, 2, 4, 6-7, 17, 22, 23, and 25 are obvious in view of Zaccaria.

### IV. Materials Reviewed

12. In forming my opinions, I have relied on my knowledge of the field and my experience in the relevant field (discussed in Section II above and in my CV (Ex. 1004), and I have specifically reviewed the following exhibits. I have considered both journal articles and associated supplemental information files which contain raw data files as part of my analysis (*e.g.*, Ex. 1005-1008). It is my opinion that these supplemental information files are publicly accessible materials that provide or support the basis for findings presented in technical literature.

| Ex. No. | Description                                                       | Abbreviation |
|---------|-------------------------------------------------------------------|--------------|
| 1001    | U.S. Patent No. 11,435,360                                        | '360 Patent  |
| 1002    | File History of U.S. Patent No. 11,435,360<br>( <i>excerpts</i> ) |              |

| <b>Ex. No.</b> | <b>Description</b>                                                                                                                                                                                                                                                      | <b>Abbreviation</b> |
|----------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------|
| 1005           | Cai, X., <i>et al.</i> , Characterization of carbon nanotube protein corona by using quantitative proteomics, <i>NBM</i> , 9(5):583-593 (2013) including supplementary data (Table S2)                                                                                  | Cai                 |
| 1006           | Walkey et al., “Protein Corona Fingerprinting Predicts the Cellular Interaction of Gold and Silver Nanoparticles,” <i>ACS Nano</i> 8 (2014) 2439-2455                                                                                                                   | Walkey              |
| 1007           | Zaccaria, A., <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>Int. J. Nanomedicine</i> , 9(10):1869-1883 (2015), including supplemental materials         | Zaccaria            |
| 1008           | Hu, Z., <i>et al.</i> , Nanoparticle size matters in the formation of plasma protein coronas on Fe <sub>3</sub> O <sub>4</sub> nanoparticles, <i>Colloids Surf., B.</i> , 121:354-361 (2014) including Appendix A (Table SI)                                            | Hu                  |
| 1009           | Bryk et al., “Quantitative Analysis of Human Red Blood Cell Proteome,” <i>Journal of Proteome Research</i> 16 (2017) 2752-2761, including supplemental materials                                                                                                        | Bryk                |
| 1010           | Hajipour et al., “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                                                                                                                                                                                                                                              | Adams Declaration   |
| 1014           | Nanjappa <i>et al.</i> , “Plasma Proteome Database as a Resource for Proteomics Research: 2014 Update,” <i>Nucleic Acids Research</i> 42 (214) 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                 |

| Ex. No. | Description                                                                                                                                                                                                                                                                                                                                                          | Abbreviation                 |
|---------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------|
| 1017    | U.S. Provisional Patent Application No. 62/435,409                                                                                                                                                                                                                                                                                                                   | '409 Provisional Application |
| 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 &amp; Cellular Proteomics</i> 4.9 (2005) 1265-1272                                                                                                           | Ishihama                     |
| 1019    | U.S. Patent No. 8,796,184                                                                                                                                                                                                                                                                                                                                            | Chilkoti                     |
| 1020    | Archival website for <a href="http://www.plasmaproteomedatabase.org/faq.html">http://www.plasmaproteomedatabase.org/faq.html</a> as of Feb. 19, 2015, available at <a href="https://web.archive.org/web/20150219211334/http://www.plasmaproteomedatabase.org/faq.html">https://web.archive.org/web/20150219211334/http://www.plasmaproteomedatabase.org/faq.html</a> |                              |
| 1021    | Dean, L. "Blood groups and red cell antigens [internet]," Chapter 1: Blood and the cells it contains, Bethesda (MD): National Center for Biotechnology Information (US) (2005), available at <a href="https://www.ncbi.nlm.nih.gov/books/NBK2263/">https://www.ncbi.nlm.nih.gov/books/NBK2263/</a> .                                                                 | Dean                         |
| 1022    | Norouzi, N. <i>et al.</i> , "Sorting cells by their density," <i>PLoS ONE</i> , 12(7):e0180520 (2017).                                                                                                                                                                                                                                                               | Norouzi                      |
| 1023    | Archival website for <a href="https://www.ncbi.nlm.nih.gov/books/NBK2261/">https://www.ncbi.nlm.nih.gov/books/NBK2261/</a> as of Mar. 20, 2014, available at <a href="https://web.archive.org/web/20140320071602/https://www.ncbi.nlm.nih.gov/books/NBK2261/">https://web.archive.org/web/20140320071602/https://www.ncbi.nlm.nih.gov/books/NBK2261/</a>             |                              |
| 1024    | Graves, P. R., & Haystead, T. A. (2002). Molecular biologist's guide to proteomics. <i>Microbiology and molecular biology reviews</i> , 66(1), 39-63.                                                                                                                                                                                                                | Graves                       |
| 1025    | Berg, J.M., Tymoczko, J.L., Stryer, L.(2007) <i>Biochemistry, Sixth Edition</i> . W.H. Freeman and Company ( <i>excerpts</i> )                                                                                                                                                                                                                                       | Berg                         |
| 1026    | McDonald, W. H., & Yates Iii, J. R. (2002). Shotgun proteomics and biomarker discovery. <i>Disease markers</i> , 18(2), 99-105                                                                                                                                                                                                                                       | McDonald                     |
| 1027    | Aebersold, R., & Mann, M. (2003). Mass spectrometry-based proteomics. <i>Nature</i> , 422(6928), 198-207                                                                                                                                                                                                                                                             | Aebersold                    |

| <b>Ex. No.</b> | <b>Description</b>                                                                                                                                                                                                    | <b>Abbreviation</b> |
|----------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------|
| 1028           | Yates, J. R., Ruse, C. I., & Nakorchevsky, A. (2009). Proteomics by mass spectrometry: approaches, advances, and applications. Annual review of biomedical engineering, 11(1), 49-79                                  | Yates               |
| 1029           | Feist, P., & Hummon, A. B. (2015). Proteomic challenges: sample preparation techniques for microgram-quantity protein analysis from biological samples. International journal of molecular sciences, 16(2), 3537-3563 | Feist               |
| 1030           | Szabo, Z., & Janaky, T. (2015). Challenges and developments in protein identification using mass spectrometry. TrAC Trends in Analytical Chemistry, 69, 76-87                                                         | Szabo               |
| 1031           | Bantscheff, M., Schirle, M., Sweetman, G., Rick, J., & Kuster, B. (2007). Quantitative mass spectrometry in proteomics: a critical review. Analytical and bioanalytical chemistry, 389, 1017-1031                     | Bantscheff          |
| 1032           | Harris, D. (2007) <i>Quantitative Chemical Analysis, Seventh Edition</i> . W.H. Freeman and Company (excerpts)                                                                                                        | Harris              |

## **V. Understanding of the Relevant Law**

### **A. Anticipation**

13. I have been informed and understand that a patent claim is anticipated when a single piece of prior art describes every element of the claimed invention, arranged in the same way as in the claim, either expressly or inherently. I have been told to consider elements to be inherently described only if they are necessarily present in the piece of prior art.

## **B. Obviousness**

14. I have been informed and understand that a patent claim is unpatentable and invalid if the subject matter of the claim as a whole would have been obvious to a person of ordinary skill in the art in the field of the patent as of the time of the invention at issue.<sup>1</sup> I have been informed and understand that the following factors must be evaluated to determine whether the claimed subject matter is obvious: (i) the scope and content of the prior art; (ii) the difference or differences, if any, between each claim of the patent and the prior art; (iii) the level of ordinary skill in the art at the time the patent was filed; and (iv) any objective indicia of non-obviousness.

15. I have been informed and understand that the objective indicia of non-obviousness (or “secondary considerations”) that should be considered include, for example, the following: (i) commercial success; (ii) long-felt but unresolved needs; (iii) copying of the invention by others in the field; (iv) initial expressions of disbelief by experts in the field; (v) failure of others to solve the problem that the inventor solved; and (vi) unexpected results. I have been informed and understand that evidence of these objective indicia must be commensurate in scope with the

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<sup>1</sup> As I discuss in Section VI, below, I use the shorthand “POSA” (or “POSITA”) to describe this person.

claimed subject matter. I am not aware of any objective indicia of non-obviousness relevant to the claims of the '360 Patent.

16. I have been informed and understand that independently made, simultaneous inventions, made within a comparatively short space of time, are evidence that the claimed apparatus was the product only of ordinary mechanical or engineering skill.

17. In determining whether the subject matter as a whole would have been obvious at the time that the invention was made to a person having ordinary skill in the art, I have been informed of and understand certain principles regarding the combination of elements of the prior art. A combination of familiar elements according to known methods is likely to be obvious when it yields predictable results. Also, if a person of ordinary skill in the art can implement a predictable variation in a prior art device, and would see the benefit from doing so, such a variation would be obvious. In particular, when there is pressure to solve a problem and there are a finite number of identifiable, predictable solutions, it would be reasonable for a person of ordinary skill to pursue those options that fall within his or her technical grasp. If such a process leads to the claimed invention, then the latter is not a patentable invention, but the result of ordinary skill and common sense.

18. I have also been informed and understand that a teaching, suggestion or motivation is a useful guide in establishing a rationale for combining elements of the

prior art. The test poses the question as to whether there is an explicit teaching, suggestion, or motivation in the prior art to combine prior art elements in a way that realizes the claimed invention. Though useful to the obviousness inquiry, I understand that this test should not be treated as a rigid rule. It is not necessary to seek out precise teachings; it is permissible to consider the inferences and creative steps that a person of ordinary skill in the art (who is considered to have an ordinary level of creativity and is not an automaton) would employ.

**C. Priority Date**

19. I have been informed and understand that, when a U.S. patent claims priority to an earlier filed patent application (such as a provisional application), the claims of the patent are not automatically entitled to the filing date of the earlier filed application.

20. I have been informed and understand that, in order for a claim to be entitled to the benefit of an earlier filed patent application's filing date, the specification of the earlier filed application must contain a written description of the invention and the manner and process of making and using it, in such full, clear, concise, and exact terms to enable an ordinarily skilled artisan to practice the invention claimed in the non-provisional application. I have been informed and understand that the written description requirement is satisfied if the specification of the earlier filed application demonstrates to a person of ordinary skill in the art that

the inventor, as of the application's filing date, had possession of the subject matter recited in the claims.

**D. Claim Construction**

21. I have been informed and understand that the United States Patent and Trademark Office interprets claim terms in an *inter partes* review proceeding under the same claim construction standard that is used in a United States federal court. I have been informed and understand that under this standard, the meaning of claim terms is considered from the viewpoint of one of ordinary skill in the art at the time of the invention.

22. I have been informed and understand that claim terms are generally given their plain and ordinary meaning as understood by one of ordinary skill in the art in light of the specification and the prosecution history pertaining to the patent. I have been informed and understand, however, that claim terms are generally not limited by the embodiments described in the specification.

23. I have been informed and understand that in addition to the claims, specification, and prosecution history, other evidence may be considered to ascertain the meaning of claim terms, including textbooks, encyclopedias, articles, and dictionaries. I have been informed and understand that this other evidence is often less significant and less reliable than the claims, specification, and prosecution history.

## **VI. Level of Ordinary Skill in the Art**

24. I have been informed and understand that the disclosure of patents and prior art references are to be viewed from the perspective of a person having ordinary skill in the art at the time of the alleged invention. I use the shorthand “POSA” (or “POSITA”) to describe this person. I have provided my opinions from this perspective. For purposes of my declaration, I have considered the relevant time period as both around December 2016 (the filing date of the provisional application to which the ’360 Patent claims priority) and around December 2017 (the filing date of the PCT application to which the ’360 Patent also claims priority). As I explain below in Section VIII.D, it is my opinion that the claims of the ’360 Patent that I have considered for my declaration (*i.e.*, claims 1-4, 6-7, 17, and 22-25) are not entitled to claim priority to the provisional application. However, my opinions regarding whether these claims of the ’360 Patent that I have considered are anticipated and/or obvious are the same regardless of which date is used.

25. I have been informed and understand that prior art references can provide evidence of the level of ordinary skill in the art, and that factors that may be considered in determining this level of skill can include the educational level of the inventors and active workers in the field, the types of problems encountered in the art, the prior art solutions to those problems, the rapidity with which innovations are made, and the sophistication of the technology.

26. It is my opinion that those of ordinary skill in the art during the relevant period 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. However, I note that this description is approximate, and a higher level of education or skill might make up for less experience, and additional experience could make up for a lower education level.

27. Based on the above description, I consider myself a person of ordinary skill in the art. Given my experience in the field, I am familiar with the knowledge that a person of ordinary skill in the art would have had at the time and how such a person would have viewed the available information at the time. My testimony is offered from this perspective.

## **VII. Overview of the State of the Art**

28. I have provided in this Section VII an overview of the state of the art at the time of the '360 Patent's earliest priority claim (December 2016). I discuss additional state of the art elsewhere in my Declaration (including the prior art that I discuss in Sections IX and XI below).

### **A. Proteomics Fundamentals**

29. Proteomics is the large-scale characterization of the proteins within a living system (*i.e.*, the proteome). Ex. 1024 at 40. Proteomics is used for evaluating

a variety of biological questions (*e.g.*, protein expression, function, structure). Patterns and changes in protein expression can be used to identify biomarkers and assess disease state of a given tissue or organism. The proteomes of different biological systems are composed of unique collections of proteins, although specific individual proteins can often be observed in a variety of systems.

30. Proteins are a diverse class of macromolecules composed of a chain of amino acids. Ex. 1025. Proteins vary in mass and shape depending on the number and sequence of the amino acids that form the full protein. Protein sequences are encoded within genes (*i.e.*, a gene is a sequence of deoxyribonucleic acid (DNA) nucleotides) for that protein. Thus, knowledge of the DNA sequence encoding for a given protein enables accurate predictions of the amino acid sequence of that protein.

31. Biological samples are the predominant sample type for proteomics, although any sample with protein present could theoretically be evaluated. Examples of such systems subject to proteomic investigations include tissues, cells, and biological fluids. Factors such as disease state, external stressors, and unique individual characteristics can influence the proteome complexity of a sample. Ex. 1027 at 202, 205, 206.

32. Proteomic studies can sometimes be described as applying a “bottom-up” approach (also referred to as a “shotgun” approach) or a “top-down” approach. Ex. 1028 at 58-60. The bottom-up or shotgun approach, which will be the focus of

my discussion, evaluates mixtures of proteins that have been broken down into smaller fragments via enzymatic digestion prior to mass spectrometry analysis. The top-down approach, by contrast, uses mass analysis of intact proteins (and protein fragments arising from fragmentation reactions, not enzymatic digestion), typically after purification.

## **B. Protein Characterization via Liquid Chromatography-Mass Spectrometry (LC-MS)**

33. Mass spectrometry-based techniques are a major tool used in proteomics studies. Ex. 1027 at 198. Although several mass spectrometry techniques exist and can be used, I will focus my discussion on liquid chromatography-tandem mass spectrometry (LC-MS/MS).

### **1. Sample Preparation**

34. Generally, LC-MS/MS-based proteomics follows a similar experimental procedure. Ex. 1029 at 3539-3546. The material (*e.g.*, tissues, fluids, cells, etc.) of interest is prepared to facilitate the extraction and isolation of proteins. This may include homogenization of tissues and lysis of cell membranes, among others steps necessary to produce a solution of proteins, which may be further isolated from other compounds, such as lipids, carbohydrates, etc. Often, the concentration of the total extracted protein is quantified using a common total protein concentration assay such as a Bradford assay. Ex. 1007 at 1871. In addition

to mass spectrometry-based techniques, polyacrylamide gel electrophoresis (PAGE or SDS-PAGE) is also widely used for protein separation.

35. The bottom-up approach further prepares the sample of proteins by denaturation (that is, unfolding the protein), disulfide reduction and thiol alkylation, resulting in proteins with simpler, linear structures. Next, the protein is cleaved into short, predictable segments called peptides by enzymatic digestion with one or more enzymes. The enzymes cleave proteins at specific locations in the amino acid sequence. This specificity results in an enzymatically digested protein yielding predictable peptide sequences. The resulting peptides are then treated to remove residual detergents, and the buffered solution is replaced (a process sometimes referred to as “desalting”).

## **2. Liquid Chromatography-Mass Spectrometry (LC-MS/MS)**

36. Following sample preparation, the peptide solution is analyzed by the LC-MS/MS instrument. The general flow of the analysis is injection into the instrument, followed by separation of the peptides via liquid chromatography (LC, or reverse-phase liquid chromatography (RPLC)) and eventual sample ionization and mass spectral evaluation. Ex. 1024 at 43-45.

37. Peptide samples must be ionized (i.e., charged) for detection in the mass spectrometer. Ex. 1027 at 198. In electrospray ionization (ESI), the separated peptides in the sample eluting from the LC column in the mobile phase are sprayed

into spectrometer. Peptides are often in a charged/ionized state and are under an applied voltage when sprayed.

38. Mass spectral evaluation uses a mass analyzer to resolve the mass-to-charge values of the peptide ions within the sample (expressed as a  $m/z$  ratio) and a detector to measure the signal associated with each ion. Ex. 1027 at 198. The signal arising from an individual peptide is related to the peptide's ionizability, charge state, and abundance. The abundance-signal relationship is linear over a range of abundances (i.e., higher abundance results in greater signal). The spectrometer acquires the mass spectra of the ionized peptides, and this process is repeated potentially thousands of times through the course of an experimental run, yielding thousands of acquired spectra.

### **3. Search Algorithms and Database Identification**

39. In most MS-based proteomics studies, proteins are identified from the raw mass spectral data by using computer algorithms and databases. Ex. 1030 at 4. These searches are informed by predictions of all the theoretical peptides possible from the enzymatic cleavage used, and the predicted MS2 spectra that serve as a kind of “fingerprint” for the peptides. These fragmentation predictions are possible due to the known DNA sequences encoding for the proteins.

## **C. Nanoparticle Technology for Proteomic Sensors**

40. Nanoparticles (NPs) are often used for enhancing the proteomic experiments. In essence, NPs are used in this context to selectively “fish for” proteins from a system based on the protein-NP interaction by collecting certain proteins at the NP surface.

### **1. Types and Sizes of Nanoparticles**

41. It is well known in the art that a wide range of nanoparticle (NP) types and sizes form protein coronas upon contact with biological samples. Ex. 1008 at 354. Ex. 1005 at 583. NP types include those produced from metals and metal complexes, polymers, lipids, and carbon nanotubes. Ex. 1008 at 354; Ex. 1005 at 583. The NP size can range from tens to hundreds of nanometers in diameter. NPs have a relatively high surface area to volume ratio; this differentiates NP properties from those of bulk materials with similar composition.

### **2. Protein Corona Formation**

42. Protein corona formation is driven by the physicochemical properties of the NP surface (*e.g.* nanoparticle composition, size, shape, morphology, and chemical functionalization of the NP surface) and its interaction with proteins in the sample. Ex. 1008 at 354. Similarly, the properties of individual proteins in the sample will determine their affinity to form a corona as well as the characteristics of corona formation on a given NP. It has been reported that use of a number of NPs

with varied properties can increase the breadth of proteins captured from a biological sample. Prior to proteomics analysis, proteins need to be desorbed from the NP surface, typically by exposing the coated NPs to a solution such as urea. The desorbed proteins are then isolated from the nanoparticles using a separation step such as centrifugation or filtration.

### **3. Protein Quantification**

43. Methods for quantifying isolated proteins generally known in the art include label-free, label-based, and intact protein. These methods are typically used to determine relative protein abundance, rather than the absolute concentrations of each protein. Ex. 1031 at 1017-1018. The label-free approach to determine relative protein abundance accomplishes quantification by either measuring the MS signal intensity of peptide ions belonging to an individual protein or by counting the number of spectra of peptides from an individual protein. Labeling approaches generally improve quantitation and rely on modification to label proteins with stable isotopes (e.g., nitrogen-15, carbon-13) via metabolic, chemical, or enzymatic approaches, or by exogenously adding (“spiking”) in proteins that have been synthetically labeled. Mass spectrometers can distinguish between labeled and unlabeled forms of a peptide, and quantification can be achieved by comparing the relative intensities of the two forms (labeled and unlabeled).

44. The dynamic range of a method is the concentration range over which the technique has a measurable response to the presence of an analyte, although not necessarily a linear response. Ex. 1032.

### **VIII. Overview of the United States Patent No. 11,435,360**

45. This Section VIII provides an overview of U.S. Patent No. 11,435,360 (“the ’360 Patent”) (Ex. 1001), prosecution history (excerpted in Ex. 1002), and whether any of claims 1-4, 6-7, 17, and 22-25 of the ’360 Patent are entitled to claim priority to the provisional application that may be pertinent to my analysis below in Section XI. This Section VIII also lists claims 1-4, 6-7, 17, and 22-25 of the ’360 Patent. My descriptions and opinions herein are not intended to be a comprehensive discussion of an entire understanding of the ’360 Patent. I include further discussion of the ’360 Patent in Section XI.

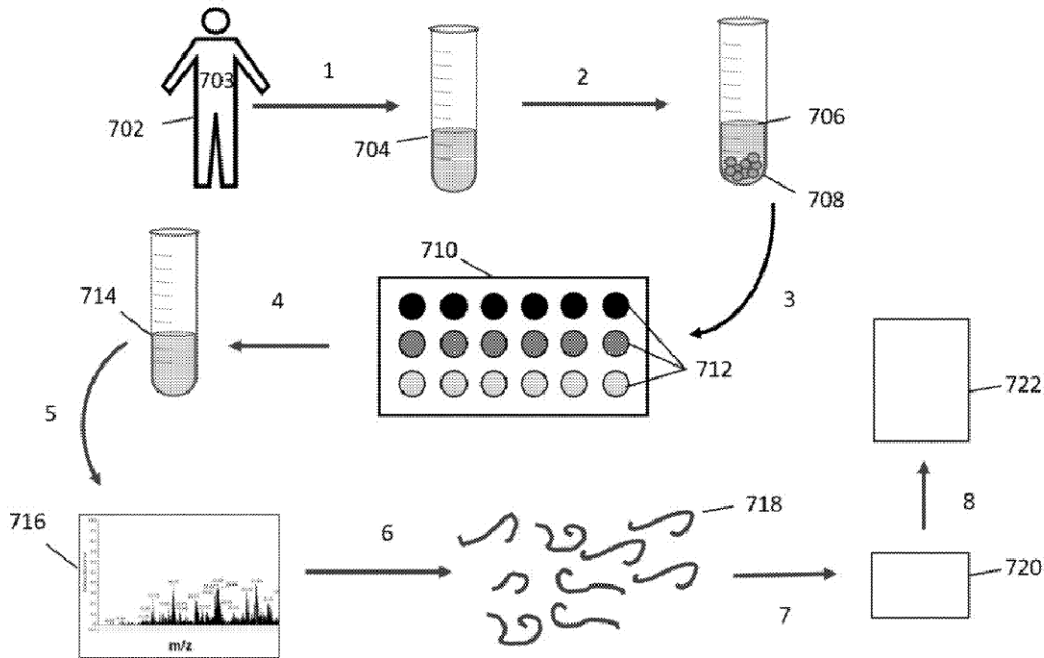
#### **A. Patent Overview**

46. The ’360 Patent (U.S. Patent No. 11,435,360), entitled “System and Sensor Array,” relates to sensor arrays for detecting biomolecular fingerprints of protein coronas from a plurality of sensor elements (*e.g.*, particles) in biological samples to predict disease states in a subject. Ex. 1001 at Cover, 2:11-17.

47. Specifically, the invention is directed to a method of assaying a biological sample to distinguish its biological states using a plurality of particles having surfaces with different physicochemical properties, wherein the method

comprises: (i) obtaining a biological sample from the subject, (ii) contacting the biological sample to the plurality of particles with different physicochemical properties to permit different patterns of protein binding to the particles, and (iii) determining a biomolecule fingerprint representative of the proteins that bind to the particles. Ex. 1001 at 2:41-51. In other embodiments, the method further comprises (iv) associating the biomolecule fingerprint with a biological state of the subject. Ex. 1001 at 2:51-55, 3:52-64. An exemplary scheme of the disclosed “invention” (Fig. 65) showing biological sample **704** or **706**, plurality of particles **712**, liquid chromatography-tandem mass spectrometry (LC-MS/MS) **716**, biomolecule fingerprint **718**, computer **720** generated association **722** of biological state **703** and the biological fingerprint **718** (using methods known in the art) is reproduced below. Ex. 1001 at 21:14-22:10.

FIG. 65



Ex. 1001, Figure 65.

48. According to the '360 Patent, each sensor element (*e.g.*, a particle with different physicochemical properties) in the sensor array can each sensor element (*e.g.*, a particle with different physicochemical properties) in the sensor array to detect relative changes in the compositions of the biomolecule corona or protein binding patterns that allow determination of unique biomolecule fingerprint and further determine pre-disease states in a subject without the need for specific biomarkers associated with the disease. Ex. 1001 at 14:41-15:6. The '360 Patent describes the ability to not only detect different proteins using different particles but also to compare different levels of the same proteins between different particles. Ex.

1001 at 34:1-4. For example, concentrations of albumin in protein corona signature vary for different particles. Ex. 1001 at 34:20-33.

49. The '360 Patent describes certain terms used in the patent:

a. Suitable biological samples include “biological fluids” such as “systemic blood, plasma, serum, lung lavage, cell lysates, menstrual blood, urine, processed tissue samples, amniotic fluid, cerebrospinal fluid, tears, saliva, semen and the like.” A preferred biological sample is “blood or serum.” Ex. 1001 at 20:34-40.

b. Suitable sensor elements include “particles, such as organic particles, non-organic particles or combinations thereof” that “are able to bind to a plurality of biomolecules when in contact with a sample.” Examples of particles in various embodiments include “nanoparticles, microparticles, micelles, liposomes, iron oxide, graphene, silica, protein-based particles, polystyrene, silver, and gold particles, quantum dots, palladium, platinum, titanium, and combinations thereof.” Ex. 1001 at 15:43, 20:61-21:1.

c. An example of biomolecule corona is “protein corona.” Biomolecule corona signature is “the composition, signature or pattern” of biomolecule corona, wherein signature refers to “the differences in the amount, level or quantity” or “differences in the conformational

state of the biomolecule that is bound to the sensor element.” Ex. 1001 at 17:29-31, 17:66-18:5. Further, biomolecule fingerprint is “the combined composition or pattern of biomolecules of at least two biomolecule corona signatures for the plurality of sensor elements.” Ex. 1001 at 15:37-42.

50. According to the '360 Patent, the plurality of particles have different physicochemical properties that are capable of binding a plurality of biomolecules (*e.g.*, proteins) in a biological sample with different binding patterns to produce a distinct biomolecule corona signature. Ex. 1001 at 2:18-26, 3:65-4:5, 15:29-39. The '360 Patent describes examples of physicochemical properties of the particles as “the composition, [*e.g.*, different types and different chemical/physical properties of materials], size, surface charge, hydrophobicity, hydrophilicity, surface functionality (surface functional groups), surface topography, surface curvature and shape.” Ex. 1001 at 26:29-37.

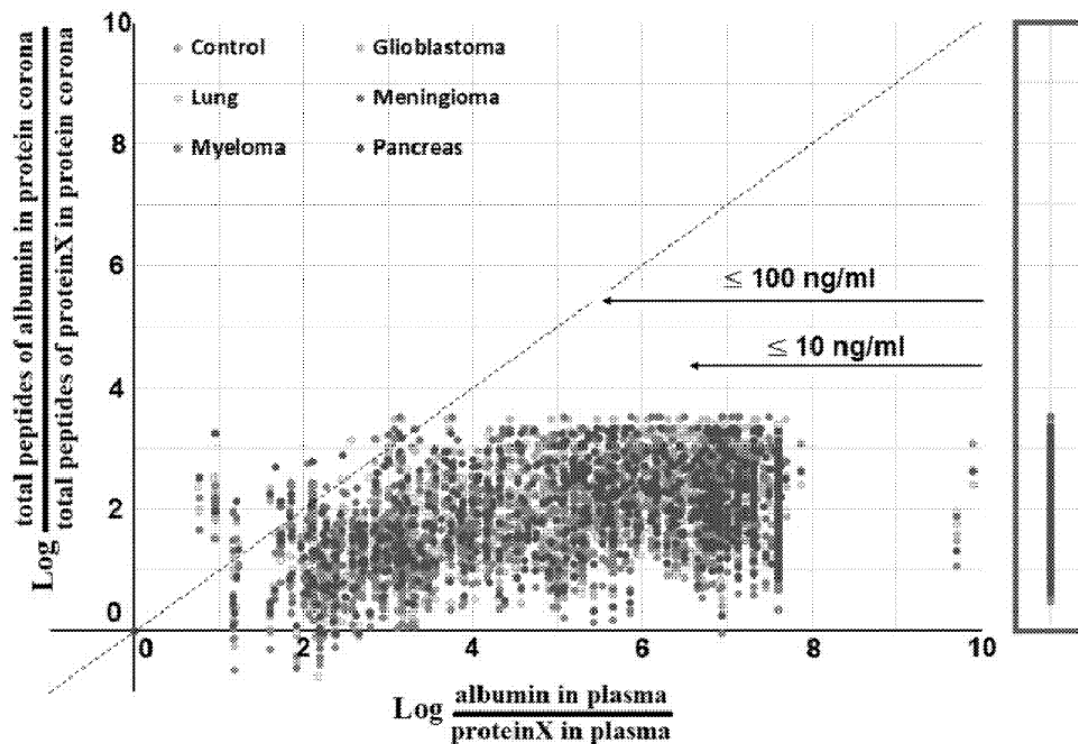
51. The '360 Patent describes alleged limitations on existing proteomic analysis using mass spectrometry. Specifically, the '360 Patent states that “due to high noise created by 10000 of proteins comprising human proteome, with estimated concentrations spanning 35-50 mg/ml for albumin to 1-10 pg/ml for some cytokines,” low sensitivity and specificity of mass spectrometry led to “a trade-off

between depth of coverage and the throughput of processing of plasma proteins.”

Ex. 1001 at 19:19-30.

52. The '360 Patent further states the present sensor array has “a sensitivity and *dynamic range of ten (10) orders of magnitude* [emphasis ended]” for protein detection using mass-spectrometry as demonstrated by Fig. 12 of the '360 Patent reproduced below. Ex. 1001 at 20:20-32, 21:14-50, 39:45-49, 39:59-63, 41:1-37.

FIG. 12



Ex. 1001, Figure 12.

53. While the '360 Patent describes its array resulting in 10 orders of magnitude, it states that known mass spectrometry provides 4-6 orders of magnitude (and I note, and discuss further below, that the claims of the '360 Patent only have a

6-order requirement): “The present sensor array has a sensitivity and dynamic range of ten (10) orders of magnitude in terms of protein detection using mass-spectroscopy [sic] 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 not previously able to be detected.” Ex. 1001 at 20:20-37 (emphasis added).

54. Similar to the '360 Patent's disclosure at 20:20-37 that I discussed in the previous paragraph, the '360 Patent also states that known mass spectrometry techniques such as LC-MS/MS can detect proteins at a dynamic range of 4-6 orders of magnitude: “The role of the protein corona in concentrating a wide range of low-abundance and very rare proteins may go a long way towards overcoming the main reasons behind the limited success **of current mass spectrometry techniques (including LC-MS/MS)** in early detection of cancers, as **these techniques can detect proteins at a dynamic range of 4-6 orders of magnitude.**” Ex. 1001 at 72:1-25 (emphases added).

55. According to the '360 Patent, the present sensor array allows detection of “low abundant and [very] rare proteins” (*e.g.*, concentrations of  $\leq 100$  ng/mg and  $\leq 10$  ng/mg, respectively) as well as proteins with “unknown/unreported plasma concentration.” Ex. 1001, 39:56-40:1 at 69:55-59. To obtain these concentration values, the '360 Patent discloses the use of “the plasma proteome database,” as one of the public websites that contain known concentrations of biological samples, to calculate the concentrations of detected proteins using spectral counting. Examples of concentrations obtained from the public website for detected proteins via LC-MS/MS are listed in Table 5 as reproduced below. Ex. 1001 at 63:52-55, 66:63-67.

| 67<br>TABLE 5                                                                                                       |                                       |                                                                                             |                   |  |                                                                                                                                     |                                          |                                          |
|---------------------------------------------------------------------------------------------------------------------|---------------------------------------|---------------------------------------------------------------------------------------------|-------------------|--|-------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------|------------------------------------------|
| Information about the most important overall proteins, found to be detected on combinations of all three liposomes. |                                       |                                                                                             |                   |  |                                                                                                                                     |                                          |                                          |
| Uniprot entry name                                                                                                  | Protein description                   | Function                                                                                    | Amount in plasma* |  |                                                                                                                                     |                                          |                                          |
| FCN3                                                                                                                | Ficolin-3                             | complement activation, lectin pathway                                                       | 1 µg/ml           |  | KV118                                                                                                                               | Ig kappa chain V-I region WEA            | Antigen binding                          |
| SAA4                                                                                                                | Serum amyloid A-4 protein             | chemoattractant activity                                                                    | 30 µg/ml          |  | CPN2                                                                                                                                | Carboxypeptidase N subunit 2             | regulation of complement activation      |
| CBPN                                                                                                                | Carboxypeptidase N catalytic chain    | protects the body from vasoactive and inflammatory peptides containing C-terminal Arg or Ly | 720 ng/ml         |  | COL11                                                                                                                               | Collectin-11                             | mannose binding, complement activation   |
| APOA2                                                                                                               | Apolipoprotein A2                     | acute inflammatory response, lipid transport                                                | 750 µg/ml         |  | MASP1                                                                                                                               | Mannan-binding lectin serine protease 1  | complement activation, lectin pathway    |
| CO7                                                                                                                 | Complement component C7               | regulator of innate and adaptive immune response                                            | 2.6 µg/ml         |  | FIBB                                                                                                                                | Fibrinogen beta chain                    | hemostasis                               |
| FHR5                                                                                                                | Complement factor H-related protein 5 | complement activation, alternative pathway                                                  | 11 ng/ml          |  | FIBA                                                                                                                                | Fibrinogen alpha chain                   | hemostasis                               |
| COF1                                                                                                                | Cofilin-1                             | actin cytoskeleton organization                                                             | 140 ng/ml         |  | C1S                                                                                                                                 | Complement C1s subcomponent              | regulation of complement activation      |
| HABP2                                                                                                               | Hyaluronan-binding protein 2          | serine-type endopeptidase activity                                                          | 1.1 µg/ml         |  | FGL1                                                                                                                                | Fibrinogen-like protein 1                | hemostasis                               |
| IGHG1                                                                                                               | Immunoglobulin heavy constant gamma 1 | antigen binding                                                                             | N.A.              |  | VWF                                                                                                                                 | von Willebrand factor                    | hemostasis                               |
| IGHG3                                                                                                               | Immunoglobulin heavy constant gamma 3 | antigen binding                                                                             | N.A.              |  | CO6                                                                                                                                 | Complement component C6                  | regulation of complement activation      |
| IGHG2                                                                                                               | Immunoglobulin heavy constant gamma 2 | antigen binding                                                                             | N.A.              |  | IGHA1                                                                                                                               | Immunoglobulin heavy constant alpha 1    | antigen binding                          |
| RET4                                                                                                                | Retinol-binding protein 4             | retinol transporter                                                                         | 580 µg/ml         |  | IGLL5                                                                                                                               | Immunoglobulin lambda-like polypeptide 5 | antigen binding                          |
| VTNC                                                                                                                | Vitronectin                           | cell adhesion                                                                               | 35 µg/ml          |  | CO8A                                                                                                                                | Complement component C8 alpha chain      | regulation of complement activation      |
| GRP78                                                                                                               | 78 kDa glucose-regulated protein      | ATPase activity                                                                             | 100 ng/ml         |  | K1C14                                                                                                                               | Keratin, type I cytoskeletal 14          | structural constituent of cytoskeleton   |
|                                                                                                                     |                                       |                                                                                             |                   |  | CRP                                                                                                                                 | C-reactive protein                       | complement activation, classical pathway |
|                                                                                                                     |                                       |                                                                                             |                   |  | CO8G                                                                                                                                | Complement component C8 gamma chain      | regulation of complement activation      |
|                                                                                                                     |                                       |                                                                                             |                   |  | *The concentrations are values obtained using spectral counting from the plasma proteome database (www.plasmaproteomedatabase.org). |                                          |                                          |

Ex. 1001 at Table 5, 67:1-75.

56. Using the known plasma proteome database public website, the '360 Patent discloses in its Example 1B that detected protein corona compositions comprise both “highly abundant proteins (*e.g.*, albumin, transferrin, complement proteins, apolipoproteins, and alpha-2-macroglobulin) and very rare proteins (defined as <100 ng/ml) such as transforming growth factor beta-I-induced transcript 1 protein (~10 ng/ml), fructose-bisphosphate aldolase A (~20 ng/ml), thioredoxin (~18 ng/ml), and L-selectin (~92 ng/ml)” as well as “388 proteins without a known previous plasma concentrations” based on the plasma proteome database. Ex. 1001 at 64:10-21.

57. As the '360 Patent acknowledges, the publicly accessible database it relies upon to look up the protein concentrations for the identified proteins was already known in the field. The database's purpose and contents are further explained in various literature, including those involved with the database (the Plasma Proteome Database). For example, an article from Nanjappa *et al.* in 2013 (Ex. 1014) provides an overview in its Abstract (Ex. 1014 at D959-D960):

#### ABSTRACT

Plasma Proteome Database (PPD; <http://www.plasmaproteomedatabase.org/>) was initially described in the year 2005 as a part of Human Proteome Organization's (HUPO's) pilot initiative on Human Plasma Proteome Project. Since then, improvements in proteomic technologies and increased throughput have led to identification of a large number of novel plasma proteins. To keep up with this increase in data, we have significantly enriched the proteomic information in PPD. This

database currently contains information on 10 546 proteins detected in serum/plasma of which 3784 have been reported in two or more studies. The latest version of the database also incorporates mass spectrometry-derived data including experimentally verified proteotypic peptides used for multiple reaction monitoring assays. Other novel features include published plasma/serum concentrations for 1278 proteins along with a separate category of plasma-derived extracellular vesicle proteins. As plasma proteins have become a major

thrust in the field of biomarkers, we have enabled a batch-based query designated Plasma Proteome Explorer, which will permit the users in screening a list of proteins or peptides against known plasma proteins to assess novelty of their data set. We believe that PPD will facilitate both clinical and basic research by serving as a comprehensive reference of plasma proteins in humans and accelerate biomarker discovery and translation efforts.

Ex. 1014 at D959-D960.

58. The article also discusses the “wide dynamic range of protein abundance, greater than 10 orders of magnitude” (Ex. 1014 at D590) and use of known “[t]argeted approaches like multiple reaction monitoring (MRM) have enabled quantitation of plasma proteins with high specificity as well as increased throughput.” Ex. 1014 at D962. The article explains how this has been used for the plasma proteome database (“PPD; <http://www.Plasmaproteomedatabase.org/>”) content (Ex. 1014 at D959, D962-D963):

MRM assays use multiple parameters to identify and quantify specific protein/s in complex samples. Proteotypic peptides that uniquely represent a protein are first selected, and assays are developed, which take into account the retention time of the peptides, their m/z values and measurement of specific fragment ions (39). This multi-step process provides superior specificity and allows for accurate quantification of protein levels over a wide dynamic range of abundance (36,40). MRM analysis has thus revolutionized biomarker research and is being widely used to determine altered protein levels across various disease phenotypes. A compilation of proteotypic peptides of plasma proteins that generate good signals in MS experiments along with their transitions will be immensely beneficial in developing MRM assays. In PPD, we have annotated 591 reported peptides corresponding to 279 proteins for MRM analysis. This includes details of precursor m/z, fragment ion m/z, collision energy, charge of the precursor ion and instrument used for the analysis (Figure 1).

Ex. 1014 at D962-D963

59. An archival version from “The Wayback Machine” of the database website’s FAQ as of February 2015 (Ex. 1020) provides further background, including, for example, that by February 2015 there were over 9,000 proteins in plasma and serum that have been identified, the use of mass spectrometry (similar to the article discussed above as well as the ’360 Patent), and the use of the database contents which aligns with the approach taken in the ’360 Patent. For example, FAQs 1-4 from the web archive (Ex. 1020) state:

1. The database deals purely with proteins found in plasma and serum. Why was it necessary to create Plasma Proteome Database (PPD)?  
Plasma is the most commonly diagnosed clinical sample. Clinicians can diagnose pathological conditions by quantification of specific proteins. Over 9,000 proteins in plasma and serum have thus far been reported and are only expected to increase with evolving high-resolution and high-sensitivity protein detection/identification technologies. Documentation of qualitative and quantitative information on broad range of proteins in plasma/serum in a user-friendly and queryable manner is a prerequisite to explore the blood proteome. This is why the Plasma Proteome Database was created.
2. What is the information that one can get from Plasma proteome Database?  
PPD contains proteins reported in plasma and serum from healthy individuals. Experimental method employed to identify each protein is also provided. From studies employing mass spectrometry-based approaches to identify plasma/serum proteins, information pertaining to the type of mass spectrometer employed and the proteotypic peptides reported are available. Quantitative information on plasma/serum proteins from immunoassays such as ELISA and radioimmuno assay, and mass spectrometry-based assays such as single reaction monitoring/multiple reaction monitoring and spectral counting are also available in PPD. All the information is linked to their corresponding PubMed identifiers. Proteins identified in plasma exosomes along with other experimental details are also available in PPD.
3. Is the protein concentration provided only from healthy individuals?  
Yes. Plasma protein levels are provided in healthy conditions as reported in literature.
4. What are the applications of PPD?  
PPD can be used as a reference database to compare and report proteins or peptides novel to experimental results obtained by researchers. The proteotypic peptides cataloged in PPD can be used to design and evaluate proteins using mass spectrometry both qualitatively and quantitatively. The list of proteins in PPD can also be used to analyze proteins from other body fluids. The proteins thus far identified in plasma/serum would also provide a reference for analysis of cellular secretome, proteins shed from cell surface and screen for ligands of orphan receptors. Above all, PPD will enable researchers in biomarker development to screen candidate molecules that are reported in plasma/serum, from a larger set of proteins identified in the discovery phase to pursue further validation.

Ex. 1020 at 1.

## **B. Prosecution History**

60. I have also reviewed the file history for the application leading to the '360 Patent. Exhibit 1002 includes excerpts from the file history. I note that during prosecution, the Examiner found that the prior art of record failed to disclose the limitation "6 orders of magnitude," recited in all claims of the '360 Patent (directly or by dependency). This led to the allowance of rejected claims as described below. I have summarized below certain aspects of the prosecution of the application that led to the '360 Patent.

61. The '360 Patent (Patent No. 11,435,360) was filed as United States Patent Application No. 17/215,923 ("the '360 Application") on March 29, 2021. Ex. 1002 at 2-11. The '360 Application is a division of U.S. Patent Application No. 17/099,331 filed on November 16, 2020, which was a continuation-in-part of U.S. Patent Application No. 15/880,627 filed on January 26, 2018 and now patented as

U.S. Patent No. 10,866,242, which is a continuation of a national stage application of International Application No. PCT/US2017/067013 filed on December 18, 2017. The '360 Application claims priority to U.S. Provisional Patent Application No. 62/435,409 filed on December 16, 2016. Ex. 1002 at 1608-1609.

62. As originally filed, the '360 Application included 30 claims. Claim 1 was an independent claim, while the remaining claims were all dependent claims that depended upon other claims. Ex. 1002 at pp. 136-139. The inventors listed on the '360 Application include Omid Farokhzad, Morteza Mahmoudi, and Claudia Corbo. Ex. 1002 at 5-6.

63. On June 21, 2021, the Examiner issued a Restriction Requirement to elect over four patentably distinct species inventions. Ex. 1002 at 1344-1356. These four patentably distinct species include (1) “dynamic range” recited in claims 2 and 6-7 in view of generic claims 1, 3-5, and 8-30 due to non-obvious variants that require different parameters in units, modes of operation, and method outcome, (2) “protein concentrations” recited in claims 3 and 8 in view of generic claims 1, 2, 4-7, and 9-30 due to non-obvious variants that are different species of measure diverse concentrations that require different search, (3) “detection results/measured component” recited in claims 9-10, 14-18, 21-22, and 28-29 in view of generic claims due to non-obvious variants that require different activity of reagents, instructions, sensitivity, and specificity, and (4) “assay procedures” recited in claims

18-20 in view of generic claims 1-17 and 21-30 due to non-obvious variants that require different parameters in units, modes of operation, and method outcome. Ex. 1002 at 1344-1356.

64. On June 25, 2021, Applicant submitted a preliminary amendment with a substitute specification to amend related U.S. application data. Ex. 1002 at 1359-1605. On August 20, 2021, in response to the Restriction Requirement, Applicants submitted a response to elect the dynamic range parameter of claim 2, the protein concentrations of claim 3 (*i.e.*, greater than 1 mg/ml and a protein of less than 10 ng/ml), the detection results/measured component of claim 10 (*i.e.*, determining relative amounts of different types of protein absorbed onto a particle), and the assay procedures of claim 19 (*i.e.*, dividing the biological sample into volumes). Ex. 1002 at 1623-1630. The claims 6-9, 14-18, 20-22, and 28-29 were withdrawn. Ex. 1002 at 1638.

65. On September 22, 2021, the Examiner issued a non-final Office Action objecting claims 1 and 27 of the '360 Application due to a typographical error and informality issue, respectively, and rejecting claims 1-5, 10-13, 19, 23-27, and 30 of the '360 Application under 35 U.S.C. § 112 as being indefinite including the term "broad dynamic range" recited in claims 1 and 2. Ex. 1002 at 1638-1641. The Examiner further rejected claims 1, 2, 4-5, 10, 23-24, 26, and 30 of the '360 Application under 35 U.S.C. § 103 over Hajipour (Bimater. Sci., 2014, 2, 1210-

1221) (“**Hajipour**”) (Ex. 1010) in view of Walkey et al. (ACS Nano, 2014, 8, 2439-2455) (“**Walkey**”) (Ex. 1006), and rejected claims 3, 11-13, 19, 25, and 27 of the ’360 Application under 35 U.S.C. § 103 over Hajipour in view of Walkey, in further view of Hadjidemetriou et al. (Vol.9, No.8, pages 8142-8156, 2015) (“**Hadjidemetriou**”). Ex. 1002 at 1641-1646.

66. The Examiner stated that Hajipour discloses the identification of proteins including albumin from protein coronas formed on two different nanoparticles (*i.e.*, polystyrene and silica with different surface properties) to correlate biomolecule fingerprint of proteins to types of disease (*e.g.*, breast cancer), and Walkey teaches that protein corona can be characterized by mass spectrometry. Ex. 1002 at 1642-1644. The Examiner further stated that Hadjidemetriou is directed to PEG-coated liposome nanoparticles which discloses concentrations recited in certain claims of the ’360 Application (*i.e.*, proteins at concentrations  $> 1\text{mg/ml}$  and  $< 10\text{ng/ml}$ , measuring ratios and coronas at least 15nm thick) in its Figures. Ex. 1002 at 1644-1646.

67. On December 15, 2021, Applicant initiated a phone interview. During the interview, Applicant argued “identifying the 6 order of magnitude (claim 2) is an art recognized term which encompasses the measurement of low abundant proteins and high abundant proteins in a sample,” and “neither Hajipour ... or Walkey teach a range of at least 6 magnitudes.” Ex. 1002 at 1712-1715.

68. On December 22, 2021, Applicant submitted a response to the non-final Office Action. Ex. 1002 at pp. 1716-1728. Applicant amended specification to overcome informality objections and amended claim 1 to remove “broad” from dynamic range in claim 1 that was subject to the § 112 indefinite rejection. Applicant further amended claim 1 to add “dynamic range comprising at least 6 orders of magnitude” to address the § 103 rejections (which was previously in dependent claim 2). Applicant argued that all of other rejected claims depend from claim 1 and require the limitation of amended claim 1. Additionally, claim 2 was cancelled, claim 27 was amended to include certain surface functionalization, and claims 6-9, 14-18, 20-22, and 28-29 were withdrawn. Ex. 1002 at 1716-1728.

69. On February 10, 2022, the Examiner issued a non-final Office Action rejecting claims 1, 4-5, 10, 23-24, 26, and 30 under 35 U.S.C. § 103 over Hajipour in view of Walkey, in further view of U.S. Patent No. 10,866,242 (“**Farokhzad**”) (Ex. 1011) and rejecting claims 3, 11-13, 19, 25, and 27 under 35 U.S.C. § 103 over Hajipour in view of Walkey and Farokhzad, in further view of Hadjidemetriou. Ex. 1002 at 1731-1743.

70. The Examiner stated that Farokhzad discloses a sensor array that detects low abundant and rare proteins in the presence of highly abundant proteins (*e.g.*, albumin) at dynamic range of 10 orders of magnitude using mass-spectrometry. The Examiner used the same arguments for Hajipour, Walkey, and

Hadjidemetriou and added Farokhzad for claim 1 limitation of 6 orders of magnitude. Ex. 1002 at 1734-1740.

71. On May 6, 2022, Applicant submitted a response to the non-final Office Action. Ex. 1002 at 1755-1757. In the response, Applicant argued Farokhzad is not an eligible prior art reference due to the same priority chain as the '360 Application and submitted a terminal disclaimer to obviate a double patenting rejection. Ex. 1002 at 1758-1759. I note that Farokhzad includes the same three inventors as the '360 Patent, and shares common portions of the specification, figures, and tables with the '360 Patent (including, for example, disclosure concerning the orders of magnitude of the dynamic range and discussion of the plasma proteome database).

72. On May 26, 2022, the Examiner issued a Notice of Allowance for the '360 Application. Ex. 1002 at 1796-1803. The Examiner provided the following reasons for allowance:

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 concentration across a dynamic range of at least 6 orders of magnitude. The closest prior art is US Patent Number 10,866,242. A Terminal Disclaimer has been filed over US Patent Number 10,866,242. Accordingly claims 1, 3-5, 10-13, and 19-30 are allowed.

Ex. 1002 at 1801.

73. On September 6, 2022, Applicant submitted a Certificate of Correction to revise certain embodiments of specification to correct units from  $\mu\text{m}$  to  $\text{nm}$ . Ex. 1002 at 1849-1861.

**C. Claims of the '360 Patent**

74. I understand Petitioners are challenging claims 1, 2, 3, 4, 6, 7, 17, 22, 23, 24, and 25 of the '360 Patent. These claims recite:

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

- (a) contacting the biological sample with a plurality of particles comprising different particle types to permit biomolecules of the biological sample to bind to the plurality of particles and form coronas around the plurality of particles, wherein the coronas corresponding to the different particle types (i) differ based on particle type, and (ii) comprise overlapping and distinct proteins;
- (b) separating at least a subset of the plurality of particles comprising the coronas from the biological sample by removing the subset of the plurality of particles thereby producing a subset of proteins from the biological sample;
- (c) assaying the subset of proteins of (b) with an instrument to detect, in the subset, proteins in the biological sample at concentrations across a dynamic range comprising at least 6 orders of magnitude, thereby assaying the biological sample.

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  $\text{mg/ml}$  and a protein present in the sample at a concentration of less than 10  $\text{ng/ml}$ .

Claim 3: The method of claim 1, wherein the plurality of particles comprise iron oxide.

- 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.
- Claim 6: The method of claim 1, wherein the dynamic range of the proteins detected spans 6 or more orders of magnitude.
- 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.
- Claim 17: The method of claim 1, wherein the assaying further comprises digesting the proteins.
- 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.
- 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.
- 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.
- 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.

#### **D. Priority Date of the Challenged Claims of the '360 Patent**

75. The '360 Patent identifies on its cover page the following “Related U.S. Application Data”. Ex. 1001 at Cover Page.

##### **Related U.S. Application Data**

(60) Division of application No. 17/099,331, filed on Nov. 16, 2020, which is a continuation-in-part of application No. 15/880,627, filed on Jan. 26, 2018, now Pat. No. 10,866,242, which is a continuation of application No. PCT/US2017/067013, filed on Dec. 18, 2017.

(60) Provisional application No. 62/435,409, filed on Dec. 16, 2016.

Ex. 1001 at Cover Page.

76. The '360 Patent provides a “CROSS-REFERENCE TO RELATED APPLICATIONS” in column 1. Ex. 1001 at 1:3-14. The cross-reference states:

This application is a division of U.S. application Ser. No. 17/099,331 filed Nov. 16, 2020; which is a continuation-in-part of U.S. application Ser. No. 15/880,627 filed Jan. 26, 2018 now patented as U.S. Pat. No. 10,866,242; which is a continuation of PCT Application PCT/US2017/067013 filed on Dec. 18, 2017 which claims priority to U.S. Provisional Application 62/435,409 filed Dec. 16, 2016, the contents of which are incorporated by reference in its entirety.

Ex. 1001 at 1:6-14.

77. For purposes of my opinions, I have considered whether any of independent claim 1 or dependent claims 2, 3, 4, 6, 7, 17, 22, 23, 24, or 25 are entitled to claim priority to the provisional application, U.S. Provisional Patent Application No. 62/435,409 (“the '409 Provisional Application”), which was filed on December

16, 2016. The '409 Provisional Application is Exhibit 1017. It is my opinion that no claims are entitled to claim priority to the provisional.

78. I have reviewed the '409 Provisional Application and found no support in the provisional for at least the following limitations of independent claim 1 of the '360 Patent (and by dependency, claims 2, 3, 4, 6, 7, 17, 22, 23, 24, and 25 of the '360 Patent):

| <b>Claim Element</b> | <b>Claim Language</b>                                                                                                                                                                                                                              |
|----------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 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[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” |

79. The '409 Provisional Application includes a specification section (labeled as pages 1-71 (page 71 is blank)), a claims section (with claims 1-54 on pages 72 to 79) and an Abstract (on page 80). Ex. 1017 at 51-130 (a specification section pages 1-80 which starts on the first page of the specification section and continues through the abstract).

80. The '409 Provisional Application also includes Figures 1-44. Ex. 1017 at [0020]-[0097].

81. The '409 Provisional Application does not include certain disclosures that were added to the '360 Patent, including, among others, those that provide the

particular protein concentrations and relative abundance, identification of a database to look up concentrations, orders of magnitude of the protein subset to determine the dynamic range, and that the coronas differing based on particle type and comprising overlapping and distinct proteins as required by the claims of the '360 Patent.

82. The added disclosures in the '360 Patent include, for example, the following that was added to the '360 Patent:

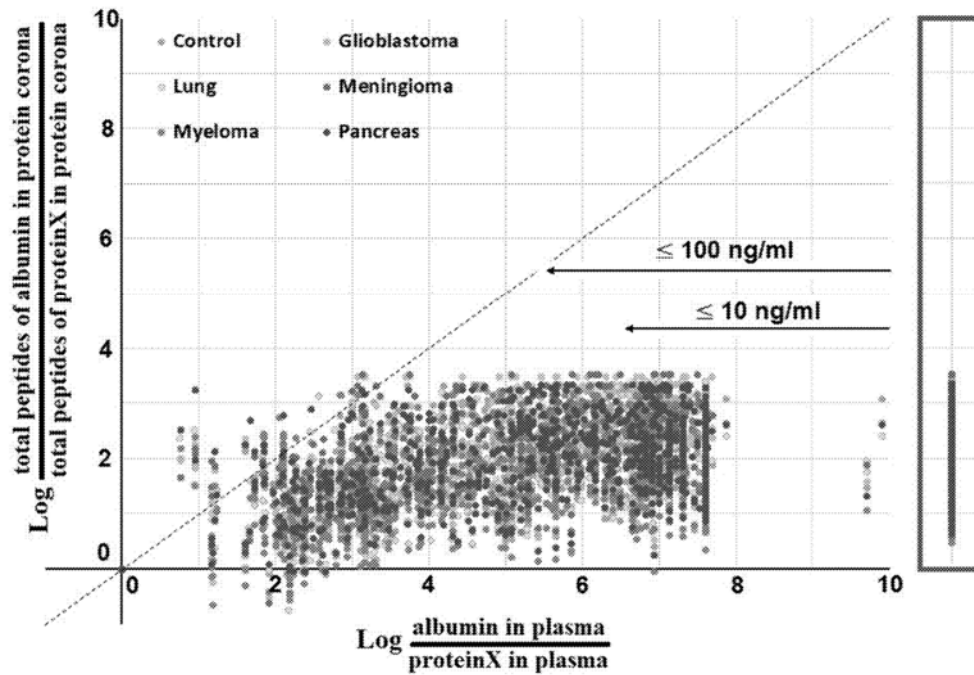
- 8:48-59 (discussing Figure 12);
- 20:20-32 (discussing 10 orders of magnitude of dynamic range and that mass spectrometry was known to allow for 4-6 orders);
- 39:46-65 (discussing detection of plasma concentrations, 10 orders of magnitude of dynamic range and Figure 12);
- 66:63-72:60 (discussing, among other things, protein concentration look ups using known database to determine orders of magnitude of dynamic range, Table 5, Figure 12, that known mass spectrometry techniques (including LC-MS/MS) can detect proteins at a dynamic range of 4-6 orders of magnitude, and overlapping and distinct corona proteins); and
- 8:20-23 (discussing Figure 10B).

83. I also note that the '409 Provisional Application does not mention or discuss the Plasma Proteome Database that is described in the '360 Patent and used to determine protein concentrations (as I summarized above in my patent overview in Section VIII.A). For example, the '409 Provisional Application (Ex. 1017) does not discuss or otherwise describe the disclosure that was added to the '360 Patent

(Ex. 1001) at 66:63-72:60, which the '360 Patent explains is used to look up the concentrations of the detected proteins (and in turn, determine the orders of magnitude for the dynamic range).

84. The '409 Provisional Application also does not include Figure 12 that was added to the '360 Patent. This is notable, as the '360 Patent describes Figure 12 as: "Multi-liposomes concentrate low-abundance and rare proteins. Protein corona contribution vs. known plasma concentration, plotted on log-log scale. Each point represents a single protein and liposome, with corona contribution for each disease and healthy individuals, and both corona contribution and plasma concentration are normalized with respect to albumin. Plasma concentrations vary over 10 orders of magnitude, while the liposome array detects these same proteins over 4-5 orders of magnitude. Corona contributions of proteins whose plasma concentration is unknown/unreported are plotted in the red area to the right." Ex. 1001 at 8:48-59. Figure 12 of the '360 Patent is below:

FIG. 12



Ex. 1001, Figure 12.

85. The '409 Provisional Application also does not include Table 5 that was added to the '360 Patent (found in the '360 Patent in column 67), which the '360 Patent describes as including "Information about the most important proteins, found to be detected on combinations of all three liposomes" where "The concentrations are values obtained using spectral counting from the plasma proteome database (see website at [plasma.proteomedatabase.org](http://plasma.proteomedatabase.org))."

Ex. 1001 at 66:65-67; 67:1-67 (Table 5).

Table 5 of the '360 Patent is below:

TABLE 5

| Information about the most important overall proteins, found to be detected on combinations of all three liposomes. |                                          |                                                                                              |                   |
|---------------------------------------------------------------------------------------------------------------------|------------------------------------------|----------------------------------------------------------------------------------------------|-------------------|
| Uniprot entry name                                                                                                  | Protein description                      | Function                                                                                     | Amount in plasma* |
| FCN3                                                                                                                | Ficolin-3                                | complement activation, lectin pathway                                                        | 1 µg/ml           |
| SAA4                                                                                                                | Serum amyloid A-4 protein                | chemoattractant activity                                                                     | 30 µg/ml          |
| CBPN                                                                                                                | Carboxypeptidase N catalytic chain       | protects the body from vasoactive and inflammatory peptides containing C-terminal Arg or Lys | 720 ng/ml         |
| APOA2                                                                                                               | Apolipoprotein A2                        | acute inflammatory response, lipid transport                                                 | 750 µg/ml         |
| CO7                                                                                                                 | Complement component C7                  | regulator of innate and adaptive immune response                                             | 2.6 µg/ml         |
| FHR5                                                                                                                | Complement factor H-related protein 5    | complement activation, alternative pathway                                                   | 11 ng/ml          |
| COF1                                                                                                                | Cofilin-1                                | actin cytoskeleton organization                                                              | 140 ng/ml         |
| HABP2                                                                                                               | Hyaluronan-binding protein 2             | serine-type endopeptidase activity                                                           | 1.1 µg/ml         |
| IGHG1                                                                                                               | Immunoglobulin heavy constant gamma 1    | antigen binding                                                                              | N.A.              |
| IGHG3                                                                                                               | Immunoglobulin heavy constant gamma 3    | antigen binding                                                                              | N.A.              |
| IGHG2                                                                                                               | Immunoglobulin heavy constant gamma 2    | antigen binding                                                                              | N.A.              |
| RET4                                                                                                                | Retinol-binding protein 4                | retinol transporter                                                                          | 580 µg/ml         |
| VTNC                                                                                                                | Vitronectin                              | cell adhesion                                                                                | 35 µg/ml          |
| GRP78                                                                                                               | 78 kDa glucose-regulated protein         | ATPase activity                                                                              | 100 ng/ml         |
| KV118                                                                                                               | Ig kappa chain V-I region WEA            | Antigen binding                                                                              | N.A.              |
| CPN2                                                                                                                | Carboxypeptidase N subunit 2             | regulation of complement activation                                                          | 2 µg/ml           |
| COL11                                                                                                               | Collectin-11                             | mannose binding, complement activation                                                       | N.A.              |
| MASP1                                                                                                               | Mannan-binding lectin serine protease 1  | complement activation, lectin pathway                                                        | 240 ng/ml         |
| FIBB                                                                                                                | Fibrinogen beta chain                    | hemostasis                                                                                   | 706 µg/ml         |
| FIBA                                                                                                                | Fibrinogen alpha chain                   | hemostasis                                                                                   | 2.5 mg/ml         |
| C1S                                                                                                                 | Complement C1s subcomponent              | regulation of complement activation                                                          | 50 µg/ml          |
| FGL1                                                                                                                | Fibrinogen-like protein 1                | hemostasis                                                                                   | 2.3 ng/ml         |
| VWF                                                                                                                 | von Willebrand factor                    | hemostasis                                                                                   | 110 µg/ml         |
| CO6                                                                                                                 | Complement component C6                  | regulation of complement activation                                                          | 40 µg/ml          |
| IGHA1                                                                                                               | Immunoglobulin heavy constant alpha 1    | antigen binding                                                                              | N.A.              |
| IGLL5                                                                                                               | Immunoglobulin lambda-like polypeptide 5 | antigen binding                                                                              | N.A.              |
| CO8A                                                                                                                | Complement component C8 alpha chain      | regulation of complement activation                                                          | 70-90 µg/ml       |
| K1C14                                                                                                               | Keratin, type I cytoskeletal 14          | structural constituent of cytoskeleton                                                       | 210 ng/ml         |
| CRP                                                                                                                 | C-reactive protein                       | complement activation, classical pathway                                                     | 2 µg/ml           |
| CO8G                                                                                                                | Complement component C8 gamma chain      | regulation of complement activation                                                          | 1.1 µg/ml         |

\*The concentrations are values obtained using spectral counting from the plasma proteome database ([www.plasmaproteomedatabase.org](http://www.plasmaproteomedatabase.org)).

Ex. 1001, Table 5, 67:1-67.

86. I note that the '409 Provisional Application also does not disclose a dynamic range anywhere in the specification, or that the detected coronas corresponding to the different particle types both differ based on particle type and comprise overlapping and distinct proteins. I also note that none of the provisional application claims (*i.e.*, claims 1-54) of the '409 Provisional Application claims or mentions a dynamic range or orders of magnitude, or coronas differing by particle type and comprising overlapping and distinct proteins. Ex. 1017 at 122-129 (or a specification section pages 72-79, application claims 1-54 of the provisional).

87. The '409 Provisional Application mentions orders of magnitude only once, in the context of what a “sample” is when describing the orders of magnitude that exist in blood plasma:

The term “sample” refers to a biological sample obtained from a subject. Suitable biological samples include, but are not limited to, biological fluids, including, but not limited to, systemic blood, plasma, serum, lung lavage, cell lysates, menstrual blood, urine, processed tissue samples, amniotic fluid, cerebrospinal fluid, tears, saliva, semen and the like. In a preferred embodiment, the sample is a blood or serum sample. **Blood plasma contains several thousands of different proteins with twelve orders of magnitude differences in the concentrations of these proteins.** The present sensor array is able to detect changes within these blood samples over time or over disease states of the subject.

Ex. 1017 at [00112] (emphasis added).

88. Paragraph [00112] from the '409 Provisional Application (which I quoted directly above) is describing that there are “several thousands of different

proteins with twelve orders of magnitude differences in the concentrations of these proteins” contained in blood plasma. However, this paragraph [00112] of the ’409 Provisional Application is describing the known concept of the number of proteins that exist in blood plasma, not any detection of those proteins (or the protein subset that is assayed in limitation 1[c]).

89. Accordingly, based on the above and disclosure in the ’409 Provisional Application, a POSA looking at the ’409 Provisional Application would not have been able to calculate the orders of magnitude for assayed protein subsets, and thus the provisional lacks disclosure for limitation 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.”

90. Additionally, the ’409 Provisional Application does not include disclosure of “the coronas corresponding to the different particle types (i) differ based on particle type, and (ii) comprise overlapping and distinct proteins” as required by limitation 1[a][3].

## **IX. Overview of the Exemplary Prior Art**

91. I have provided a brief overview in this Section of three prior art references: Cai (Ex. 1005), Hu (Ex. 1008), Zaccaria (Ex. 1007) as well as three additional references: Cao (Ex. 1016), Ahmed (Ex. 1015), and Bryk (Ex. 1009),

which contain database information on certain biological samples. I discuss these (and other) references in more detail in Sections XI.A-XI.D.

92. As I discuss below, several of these references are journal/research articles that (i) further incorporate supplemental materials (often in an Appendix) that include additional data (often a complete dataset that is otherwise summarized and discussed within the article itself) and (ii) provide a link/reference to obtain those materials. This approach was common in the field when publishing such articles. I am familiar with this process through my own study and research, as well as reviewing the work of others. These supplemental materials are typically made available when the articles themselves are first published. When reviewing the articles, those reviewing the articles (which would include POSAs) would naturally look to that additional supplementary material published with the article and consider it as part of the article for review.

**A. Cai (Ex. 1005)**

93. A journal article entitled “Characterization of carbon nanotube protein corona by using quantitative proteomics,” by Cai *et al.* was published in 2013 (“**Cai**”). Ex. 1005 at 583. It further includes a dataset, identified as “Table S2” in its Appendix A. Supplementary data (which table is also referred to as “Supplementary

Table 2: Data reported in Figure 3”). Ex. 1005 at 587, 592, Appendix Table S2.<sup>2</sup> The publication of Cai and its supplemental materials are further explained in the Adams Declaration (Ex. 1012), which is consistent with article publication as I have experienced in the field.

94. Cai discloses the use of a plurality of nanoparticles, *i.e.*, multi-wall carbon nanotubes (MWCNTs) with varying diameters (<10 nm, 20-40 nm, and 60-100 nm) and lengths (1-2  $\mu$ m and 5-15  $\mu$ m) and carbon black (CB), in HeLa cells as an archetypal human proteome for the study of selective protein binding capacities and identification of protein coronas using mass spectrometry (MS) coupled to liquid chromatography (LC) (*i.e.*, LC-MS/MS). Ex. 1005 at 584-586, 591, Table S2.

95. Cai discloses assaying methods of the protein coronas that include incubating cell lysates with CNTs and CB, separating bound proteins via centrifugation and washing, producing subsets of isolated protein coronas (*e.g.*, peptides) by in-gel digestion, and analyzing the subsets of isolated protein coronas using LC-MS/MS. Ex. 1005 at 584-585. Detected proteins from the LC-MS/MS were quantified using an MS-Quant program and database search of MS spectra (UniProt human database with Mascot search engine) for peptide ratio calculation. Ex. 1005 at 585.

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<sup>2</sup> Supplemental Table S2 of Cai is at page 12-17 of Ex. 1005 (the pages on the bottom right corner under “PREOMICS EXHIBIT 1005”).

96. Cai teaches different dimensions of carbon nanoparticles (*e.g.*, different surface curvatures and diameters) influence the types and the total numbers of proteins bound to the nanoparticles to form protein coronas. Ex. 1005 at 585-587. For example, significantly higher amounts of proteins were identified using MWCNTs with a diameter of 20-40 nm or 60-100 nm compared to multi-wall or single-wall CNTs with diameters of less than 10 nm or 2 nm, respectively. Ex. 1005 at 586. The LC-MS/MS analysis further showed the total number of proteins enriched by MWCNTs was higher than that enriched by CB. More specifically, only a small number of proteins with high CB binding efficiencies were bound to MWCNTs while 44 proteins with high CNT binding efficiencies were enriched over 10-fold in MWCNTs, which demonstrates that different types and sizes of carbon nanoparticles exhibit different protein binding capacities and specificities. Ex. 1005 at 587, 591.

97. The assaying methods of Cai using the LC-MS technique quantified a total of 778 proteins that further include “low abundance proteins” with the highest binding efficiencies<sup>3</sup> Ex. 1005 at 587. Cai further discloses “[t]he relative abundance of MWCNT-binding proteins,” expressed as empirical peptide abundance index (emPAI) from the LC-MS/MS analysis, “spread over 8 orders of magnitude.” Ex. 1005 at 587, 12-17 (Table S2). For example, the emPAI of detected proteins

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<sup>3</sup> Binding efficiencies are based on Stable Isotope Labeling by Amino Acids in Cell Culture (SILAC) ratios calculated from LC-MS/MS analysis.

displayed a wide range of values ranging from 0.02 for Plectin-1 protein (PLEC1) as the lowest value to 2254683 for 25 kDa protein (KRT8) as the highest value. Ex. 1005 at 12-17 (Table S2).

**B. Hu (Ex. 1008)**

98. A journal article entitled “Nanoparticle size matters in the formation of plasma protein coronas on Fe<sub>3</sub>O<sub>4</sub> nanoparticles,” by Hu *et al.* was published in 2014 (“**Hu**”). Ex. 1008 at 354. It further includes a dataset, identified as “Table S1” in its “Appendix A. Supplementary data”. Ex. 1008 at 357-58, 361, 10-19 (Table S1).<sup>4</sup> The publication of Hu and its supplemental materials is further explained in the Adams Declaration (Ex. 1012), which is consistent with article publication as I have experienced in the field.

99. Hu discloses the use of a plurality of nanoparticles, *i.e.*, iron oxide nanoparticles (Fe<sub>3</sub>O<sub>4</sub> NPs) with varying diameters (~30 nm, 200 nm, and 400 nm) in human blood plasma for the study of protein binding patterns on iron oxide nanoparticles in biological systems and quantification of plasma protein coronas using reversed-phase liquid chromatography (RP-LC) and tandem mass spectrometry (MS/MS) (*i.e.*, RPLC-MS/MS). Ex. 1008 at 354-355.

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<sup>4</sup> Supplemental Table S1 of Hu is at page 10-19 of Ex. 1008 (the pages on the bottom right corner under “PREOMICS EXHIBIT 1008”)

100. Hu discloses assaying methods of the protein coronas that include incubating human plasma with  $\text{Fe}_3\text{O}_4$  NPs, collecting the nanoparticles using a magnet, and separating unbound proteins via washing. The bound proteins were desorbed from the nanoparticles with urea, denatured, reduced, and alkylated prior to enzyme digestion, desalting, and analysis using RPLC-MS/MS. Ex. 1008 at 355-356. Proteins detected by RPLC-MS/MS were identified using MS spectra processing software (Paragon Algorithm of Protein Pilot software) and database search (International Protein Index) and then quantified using spectral counting (SpC) method for the relative abundance of detected plasma proteins bound on  $\text{Fe}_3\text{O}_4$  NPs. Ex. 1008 at 356-358.

101. Hu teaches different particle sizes of  $\text{Fe}_3\text{O}_4$  NPs (*e.g.*, diameters) influence the protein composition of the plasma protein coronas on  $\text{Fe}_3\text{O}_4$  NPs and the relative abundance of the plasma proteins in the protein coronas. Ex. 1008 at 355-357, Fig. 2. For example, from the total of 171 plasma proteins detected in the protein coronas of the  $\text{Fe}_3\text{O}_4$  NPs using the RPLC-MS/MS technique, each of the  $\text{Fe}_3\text{O}_4$  NPs with different particle sizes comprises both overlapping proteins (*e.g.*, 71 common plasma proteins including serum albumin) and distinct proteins (*e.g.*, 22, 31, and 11 distinct proteins uniquely identified on 30, 200, and 400 nm  $\text{Fe}_3\text{O}_4$  NPs, respectively). Ex. 1008 at 357; see Fig. 4 as reproduced below.

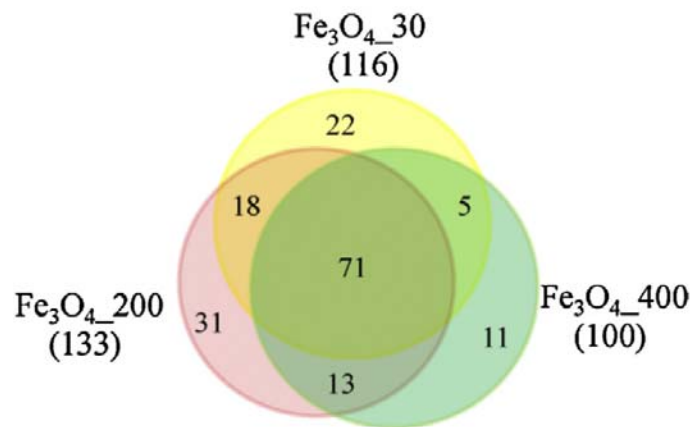


**Fig. 4.** Venn diagram displaying the number of proteins identified in the protein coronas of the different sizes of Fe<sub>3</sub>O<sub>4</sub> NPs.

Ex. 1008, Figure 4.

102. Hu teaches that although serum albumin is the most abundant protein in human plasma that was commonly identified on all three different Fe<sub>3</sub>O<sub>4</sub> NPs, the relative abundance of common plasma proteins varied depending on particle sizes. Ex. 1008 at 358, 360. For example, serum albumin was the second most abundant protein in the protein coronas on 200 nm Fe<sub>3</sub>O<sub>4</sub> NPs while serum albumin was the twenty-seventh most abundant protein in the protein coronas on 30 nm Fe<sub>3</sub>O<sub>4</sub> NPs. Ex. 1008 at 360; see Table 2 as reproduced below.

**Table 2**The top 20 most abundant plasma proteins bound on the Fe<sub>3</sub>O<sub>4</sub> NPs of different sizes.

| Rank | Fe <sub>3</sub> O <sub>4</sub> -30                     | Fe <sub>3</sub> O <sub>4</sub> -200                       | Fe <sub>3</sub> O <sub>4</sub> -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 |

Ex. 1008, Table 2.

103. In addition, Hu teaches the RPLC-MS assaying methods of Hu can detect plasma proteins that are present in low concentrations (*e.g.*, below 100 ng/mL or 10 ng/mL) as well as in high concentrations (*e.g.*, above 1 mg/mL). For example, latent transforming growth factor-beta binding protein 1 (LTBP-1) was uniquely detected on 200 nm Fe<sub>3</sub>O<sub>4</sub> NPs. This protein is known to be present at a low concentration of 2.9 ng/mL in blood plasma in healthy donors. Ex. 1008 at 10-19 (Table S1); Ex. 1016 at 16048. I discuss Exhibit 1016 (Cao) further below.

104. Further, serum albumin was detected as the second most abundant protein on 200-nm Fe<sub>3</sub>O<sub>4</sub> NPs. This protein is known to be present at a high concentration of 35-52 mg/mL (or between 35×10<sup>6</sup> and 52×10<sup>6</sup> ng/mL) in blood

plasma.<sup>5</sup> Ex. 1008 at 360, Table 2; Ex. 1015 at 787, Table 2. I discuss Exhibit 1015 (Ahmed) further below.

# **1. Cao (Ex. 1016)**

105. A journal article entitled “Latent transforming growth factor-beta binding protein-1 in circulating plasma as a novel biomarker for early detection of hepatocellular carcinoma” by Cao *et al.* was published in 2015 (“**Cao**”). Ex. 1016 at 16046.

106. Cao discloses the use of concentrations of latent transforming growth factor-beta binding protein 1 (LTBP-1) present in blood plasma as a biomarker to distinguish hepatocellular carcinoma (HCC) patients with hepatitis or liver cirrhosis. Ex. 1016 at 16046. The concentration of LTBP-1 in blood plasma, collected from 104 healthy individuals, was measured using an enzyme-linked immunosorbent assay (ELISA). Ex. 1016 at 16047; *see* Table 1 as reproduced below.

**Table 1.** Plasma concentrations of AFP and LTBP-1 in HCC (positive of CHB and cirrhosis) and three other cohorts

| †Cohorts  | N   | AFP (ng/ml)      |          | LTBP-1 (ng/ml) |          |
|-----------|-----|------------------|----------|----------------|----------|
|           |     | Median (range)   | ‡P       | Median (range) | ‡P       |
| Healthy   | 104 | 3.0 (1.1-12.5)   | 7.09E-12 | 2.9 (1.7-6.0)  | 2.14E-24 |
| CHB       | 50  | 4.5 (1.6-638.0)  | 4.34E-4  | 3.0 (1.8-10.7) | 2.7E-10  |
| Cirrhosis | 50  | 9.8 (1.8-282.0)  | 0.199    | 3.4 (1.1-11.8) | 1.74E-4  |
| HCC       | 120 | 9.5 (1.2-1674.0) | -        | 5.0 (1.6-14.3) | -        |

†CHB, chronic hepatitis B; HCC, hepatocellular carcinoma; ‡P values were assessed by comparison between HCC (positive of CHB and cirrhosis) and each of the other cohorts, two-sided Mann-Whitney test.

<sup>5</sup> See Sections IX.B.1 and IX.B.2 for known concentrations of LTBP-1 and serum albumin in blood plasma as disclosed in Cao and Ahmed, respectively.

Ex. 1016 at 16048.

**2. Ahmed (Ex. 1015)**

107. A review journal article entitled “Sample preparation and fractionation for proteome analysis and cancer biomarker discovery by mass spectrometry” by Ahmed was published in 2009 (“**Ahmed**”). Ex. 1015 at 771.

108. Ahmed discloses in the review the challenges and strategies in preparation and fractionation of samples in various states, and how these strategies impact detection with mass spectrometry. Ex. 1015 at 771. Ahmed discloses reference concentration ranges of the 30 most abundant proteins in the plasma from healthy individuals which include a concentration range of albumin as 35000-52000 mg/L. Ex. 1015 at 787; *see* Table 2 as reproduced below.

**Table 2.** Ranking of plasma proteins in healthy persons in order of molar or mass abundance, with examples of half lives<sup>a)</sup>

| No. | Protein rank                      | Concentration |               | Molecular mass<br>(M <sub>r</sub> ) in Da | Half life  |
|-----|-----------------------------------|---------------|---------------|-------------------------------------------|------------|
|     |                                   | M · mol/L     | mg/L          |                                           |            |
| 1   | Albumin                           | 500–800       | 35 000–52 000 | 66 438                                    | 15–10 days |
| 2   | IgG                               | 40–100        | 7 000–16 000  | 160 000                                   |            |
| 3   | Apolipoprotein A-I                | 36–72         | 1 000–2 000   | 28 079                                    | 5 days     |
| 4   | Transferrin                       | 25–45         | 2 000–3 600   | 79 600                                    | 7 days     |
| 5   | Apolipoprotein A-II               | 22–60         | 200–550       | 8 691                                     | 5 days     |
| 6   | $\alpha_1$ -Proteinase inhibitor  | 18–40         | 900–2 000     | 55 000                                    |            |
| 7   | $\alpha_1$ -Acid glycoprotein     | 12–30         | 500–1 200     | 40 000                                    |            |
| 8   | Transthyretin                     | 15–30         | 200–400       | 54 000                                    | 1–2 days   |
| 9   | Hepatoglobin                      | 3–20          | 300–2 000     | 104 000                                   |            |
| 10  | Hemopexin                         | 15            | 900           | 57 000                                    |            |
| 11  | IgA                               | 4–24          | 700–4 000     | 170 000                                   |            |
| 12  | Apolipoprotein C-III              | 6–20          | 60–180        | 8 765                                     |            |
| 13  | $\alpha_2$ -Macroglobulin         | 7–17          | 1 300–3 000   | 30 000                                    |            |
| 14  | $\alpha_2$ -HS glycoprotein       | 12            | 600           | 50 000                                    |            |
| 15  | Gc globulin                       | 8–14          | 400–700       | 51 000                                    |            |
| 16  | Apolipoprotein C-I                | 6–12          | 40–80         | 6 631                                     |            |
| 17  | Fibrinogen                        | 6–12          | 2 000–4 000   | 340 000                                   | 2.5 days   |
| 18  | $\alpha_1$ -Antichymotrypsin      | 7             | 500           | 68 000                                    | 4 days     |
| 19  | C3                                | 5–10          | 900–1 800     | 180 000                                   |            |
| 20  | b <sub>2</sub> -Glycoprotein I    | 4–8           | 150–300       | 40 000                                    |            |
| 21  | Vitronectin                       | 4–8           | 250–450       | 55 000                                    |            |
| 22  | $\alpha_1$ -B Glycoprotein        | 3–6           | 150–300       | 63 000                                    |            |
| 23  | Apolipoprotein A-IV               | 3–6           | 130–250       | 43 375                                    |            |
| 24  | Apolipoprotein C-II               | 2–7           | 20–60         | 8 915                                     |            |
| 25  | $\beta_2$ -Glycoprotein II        | 2–5           | 12–30         | 63 000                                    |            |
| 26  | Antithrombin III                  | 3             | 200           | 65 000                                    |            |
| 27  | Inter $\alpha$ -trypsin inhibitor | 2–4           | 400–700       | 160 000                                   |            |
| 28  | Plasminogen                       | 2–4           | 150–350       | 81 000                                    |            |
| 29  | Ceruloplasmin                     | 1.5–5         | 200–600       | 135 000                                   |            |
| 30  | Retinol-binding protein           | 1.5–3         | 30–60         | 21 000                                    | 10–12 h    |

<sup>a)</sup> Modified from ref. [135].

Ex. 1015, Table 2.

**C. Zaccaria (Ex. 1007)**

109. A journal article entitled “Accessing to the minor proteome of red blood cells through the influence of the nanoparticle surface properties on the corona composition,” by Zaccaria *et al.* was published in March 2015 (“Zaccaria”). Ex. 1007 at 1869.

110. Like the other references such as Cai and Hu (discussed above) and Bryk (discussed below), Zaccaria also includes “Supplementary materials” which are referenced and linked to throughout the reference (for example on pages 1871,

1873, 1874, 1878, 1880. Among other things, Zaccaria identifies that “The different protein lists are detailed in the Supplementary materials.” Ex. 1007 at 1873.

111. I note that the website link provided in the article itself is no longer active with materials. Accordingly, to obtain the materials that were originally published along with the Zaccaria article in March 2015, I directly contacted the first-named author (Affif Zaccaria) and requested a copy of the Supplementary Materials as they were made publicly available along with the original article publication. In response, the author provided the Supplementary Materials, which have been included as part of Ex. 1007. These materials included the following nine files (including the data index which summarizes the content of supplemental data files 1-8). That index is at page 17-18 of Ex. 1007 (the pages on the bottom right corner under “PREOMICS EXHIBIT 1007”).

112. I have also reviewed these materials and they appear to accurately reflect the content that is described in the Zaccaria article, further supporting that these are the materials made publicly available along with the publication of the Zaccaria article in March 2015. For example, Zaccaria explains on page 1873 that “The different protein lists are detailed in the Supplementary Materials” and further that “Altogether, 909 different proteins were identified from our RBC samples....” Ex. 1007 at 1873. This is consistent with supplementary data 2 excel file that includes sheet titled “All 909 proteins” as identified on the index (page 17 of Exhibit

1007 using the bottom right page numbering) and the sheet itself titled “All 909 Proteins” (slip sheet at page 24 and “All 909 Proteins” at pages 25-31).

113. I note that, for ease of review and understanding (including because certain files are Excel spreadsheets with tabs) slip sheet labels have been added.

114. Zaccaria discloses the use of a plurality of 100 nm superparamagnetic nanoparticles (NPs) grafted with three different functional groups (*i.e.*, two negatively charged with either an oleic acid (OS-NP) or aspartic acid (PAA-NP) functionalization, and one positively charged quaternary amine-functionalized polydiallyldimethylammonium chloride (Q-NP)) in hemolysate prepared from a sample of red blood cells (RBCs) for the identification and quantification of erythrocyte minor proteome using nano-liquid chromatography (LC) and tandem mass spectrometry (MS/MS) (*i.e.*, LC-MS/MS). Ex. 1007 at 1869-1872.

115. Zaccaria discloses assaying methods of the protein coronas that include incubating hemolysate with NPs, collecting the NPs using a magnet and washing, desorbing proteins forming the corona, producing peptides from the proteins using in-gel digestion, and analyzing the subsets of the isolated protein coronas using LC-MS/MS. Ex. 1007 at 1871. Detected proteins from the LC-MS/MS were identified using MS spectra processing software and database search results, and then analyzed using protein abundance index (PAI) and MS/MS queries to quantify the relative protein abundance. Ex. 1007 at 1871-1872.

116. Zaccaria teaches different surface treatments of NPs influence protein binding specificity and relative protein concentration in the RBC protein coronas. Ex. 1007 at 1873-1874. For example, from the total of 893 proteins detected in protein coronas on all three surface-treated NPs via the LC-MS/MS, each of the NPs with different surface treatments comprises both overlapping proteins (*e.g.*, 193 common RBC proteins) and distinct proteins (*e.g.*, 193, 219, and 224 distinct proteins uniquely identified on OS-NPs, Q-NPs, and PAA-NPs, respectively). Ex. 1007 at 1873; see Fig. 2C as reproduced below.

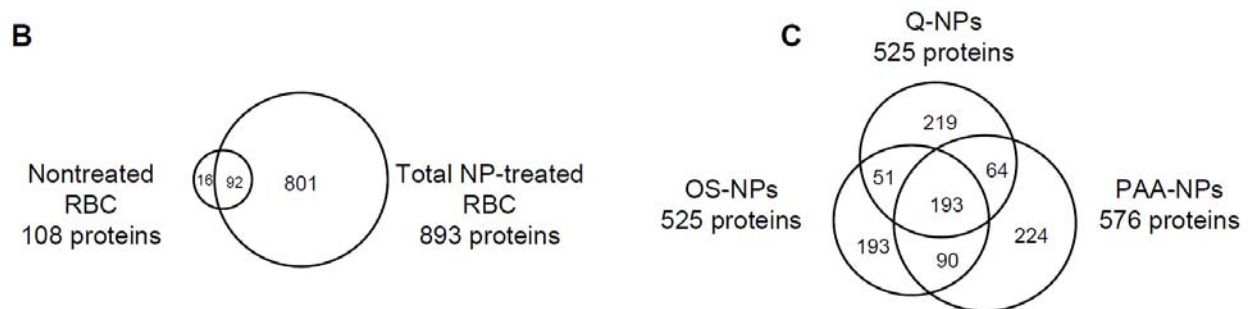


Figure 2 Protein identification results and NP comparison.

Ex. 1007, Figures 2B and 2C.

117. Zaccaria further teaches that the assaying methods using both the surface-treated NPs and the LC-MS/MS technique enhance the number of identified proteins as well as the detection of “low abundant proteins” present in RBCs. Ex. 1007 at 1873-1874. For example, the LC-MS analysis discloses more than 500 proteins were detected on each of the surface-treated NPs while only 108 proteins were detected in crude RBC lysate. Among all detected proteins, 801 proteins were exclusively identified from protein coronas on the surface-treated NPs, and only 92

proteins were commonly identified in both the surface-treated NPs and the crude RBC lysate. Ex. 1007 at 1873, *see* Fig. 2B as reproduced above. Additionally, the LC-MS/MS analysis discloses low abundant proteins identified in crude RBC lysate were present in higher abundance on surface-treated nanoparticles, and “through the respective decrease of high-abundant proteins and increase of low-abundant proteins, NP treatment greatly reduces the range of concentration in the sample, making detectable more peptides by the MS instruments.” Ex. 1007 at 1872-1873, 1881.

118. This decrease in the proportion of high abundant proteins (*e.g.*, hemoglobin (Hb), which represents 98% of the total RBC soluble protein content Ex. 1007 at 1870) and the subsequent increase in the proportion of low abundant proteins on surface-treated NPs are described using MS/MS queries as follows:

[T]he number of MS/MS queries assigned to these 20 most abundant proteins was greatly lower in NP-treated condition than in control [*i.e.*, crude RBC lysate]. In control, 94% of the total MS/MS queries that were generated during the run were assigned to these 20 proteins, of which 91% to the Hb-related subunits. In the NP treated sample, the 20 most abundant proteins only generated 42%–45% of the total MS/MS queries, of which 20%–29% were assigned to Hb-related subunits, according to the kind of NPs. This observation revealed that NP treatment greatly decreased the proportion of abundant proteins.

Ex. 1007 at 1874

To determine whether NP treatment increased the proportion of low-abundant proteins in RBC samples, the MS/MS queries, the peptide number, and the sequence coverage of ten low-abundant proteins in nontreated sample (*ie*, proteins with low MS/MS queries) were determined in OS-NPs sample (Table 3). In nontreated sample, these

ten low-abundant proteins generated from one to three MS/MS queries, with a low coverage of each protein sequence (from 1.4% to 17.1%). They represented 0.1% of the total MS/MS queries generated by the sample. In OS-NP samples, the same proteins generated from five to 63 MS/MS queries, allowing a greater coverage of the protein sequence (from 11.9% to 79.6%) through the detection and identification of different peptides for each protein. These ten proteins represented 2% of the total MS/MS queries generated by the NP-treated sample. This enhanced detection of low-abundant proteins was also observed in Q- and PAA-NPs (Supplementary materials). These observations clearly demonstrated that NP treatment also increased the proportion of low-abundant proteins.

Ex. 1007 at 1874.

119. Zaccaria additionally discloses a total of 909 proteins detected from LC-MS/MS which includes the name of each protein, molecule weight and MS/MS queries for each identified protein. Ex. 1007 at 1873 and supplementary materials (particularly “All 909 Proteins” found on pages 25-31 of Ex. 1007, using the bottom right page numbering below “PREOMICS EXHIBIT 1007”). For example, Zaccaria’s list of detected proteins contained high abundance proteins (*e.g.*, MS/MS queries of at least 35) including but not limited to “Hemoglobin subunit alpha” (“HBA1”) and “carbonic anhydrase 1” (“CA1”) which are reported with molecular weights of 15304.95 and 28909.41, respectively, as well as low abundance proteins (*e.g.*, MS/MS queries of less than 13) including but not limited to “phosphoinositide-3-kinase, regulatory subunit 4” (“PIK3R4”) and “myotubularin-related protein 5” (“SBF1”) which are reported with molecular weights of 154373.14 and 210294.08,

respectively. Ex. 1007 at Supplementary Materials, “All 909 Proteins” sheet on pages 25-31 of the exhibit.

### 1. Bryk (Ex. 1009)

120. A journal article entitled “Quantitative analysis of human red blood cell proteome,” by Bryk *et al.* was published on July 8, 2017 (“**Bryk**”). Ex. 1009 at 2752. It further includes supporting information, identified in Bryk on page 2759 as follows:

#### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/acs.jproteome.7b00025](https://doi.org/10.1021/acs.jproteome.7b00025).

Figure S1. Western blot analysis of selected proteins. (PDF)

Table S1. Stable isotope labeled peptide based protein quantification. Table S2. Enrichment of membrane proteins in the white ghost fraction. Table S3. List of identified proteins and their concentrations in the whole red cells and white ghosts. (XLSX)

121. The publication of Bryk and its supporting information is further explained in the Adams Declaration (Ex. 1012), which is consistent article publication as I have experienced in the field.

122. Bryk discloses the LC-MS/MS detected hemoglobin subunit alpha (HBA1) and carbonic anhydrase 1 (CA1) in high concentrations expressed as pmol of proteins in mg of RBCs (*i.e.*, averages across the four donors of  $1.47 \times 10^4$  and

1.80×10<sup>3</sup> pmol/mg, respectively), and phosphoinositide 3-kinase regulatory subunit 4 (PIK3R4) and myotubularin-related protein 5 (SBF1) proteins in low concentrations expressed as pmol of proteins in mg of RBCs (*i.e.*, lowest non-zero detected concentrations of 3.17×10<sup>-4</sup> and 6.62×10<sup>-6</sup> pmol/mg, respectively). Ex. 1009 at 16-61 (Table S3).

123. These concentrations reported in pmol/mg can be converted to concentrations in ng/mL using the molecular weight of the same protein reported for detected proteins disclosed by Zaccaria (Ex. 1007 at 25-31 Table S2) and publicly known information on RBCs, including reference ranges of density, volume, protein content, and cell counts of RBCs as described below:

- Bryk discloses a reference range for the mean volume of RBCs (*i.e.*, 90 fL (femtoliter)) and the average total protein content per cell (*i.e.*, 20% of RBCs mass). Ex. 1009 at 2755.
- An online book entitled “Blood groups and red cell antigens” by Dean, published in 2005, discloses a reference range for the number of RBC counts per volume of human blood. Specifically, 4-6 million RBCs are present in 1 mm<sup>3</sup> of male blood, and 3.5-5.5 million RBCs are present in 1 mm<sup>3</sup> of female blood. Additionally, Dean discloses a reference range for mean corpuscular volume (MCV) (*i.e.*, average size of RBC) of 80-100 fL (femtoliter) per cell. Ex. 1021 at 1, 5. As further evidence of publication Exhibit 1023 at least prior to the earliest possible priority date of the '360 Patent, includes an Internet Archival version of the website on which Dean is available as of March 20, 2014.
- A journal article entitled “Sorting cells by their density” by Norouzi et al., published on July 19, 2017, discloses a reference range for density of RBC (*i.e.*, 1.110 g/mL). Ex. 1022 at 1, 3.

124. Using these reference ranges, the concentrations of HBA1 and CA1 disclosed in Bryk are converted to 27 and 6 mg/mL (or  $27 \times 10^6$  and  $6 \times 10^6$  ng/mL), respectively, and the concentrations of PIK3R4 and SBF1 disclosed in Bryk are converted to 5.86 and 0.17 ng/mL, respectively.

## **X. Claim Construction**

125. In comparing the claims of the '360 Patent to the prior art, I have carefully considered the '360 Patent and its file history as a POSA would understand them at the time of the invention of the '360 Patent. As relevant to determining the patentability of the claims of the '360 Patent in this proceeding, the proper interpretation of the claim terms of the '360 Patent is consistent with the terms' plain and ordinary meaning in view of the evidence of record (including the intrinsic evidence of record, *i.e.*, the patent and file history, as well as any extrinsic evidence), as a POSA would understand them, as of the time of the invention of the '360 Patent.

## **XI. Analysis of the Patentability of the '360 Patent Claims**

### **A. Ground 1: Claims 1-4, 6-7, 17, and 22-25 are Obvious in View of Hu**

126. As I explain below, in my opinion, Hu (Ex. 1008) renders obvious the limitations of claims 1-4, 6-7, 17, and 22-25. Hu discloses all of the limitations, except it does not numerically state the protein concentrations for assessing the

orders of magnitude (which concentrations were well known in the field). As I described in Sections IX.B.1-IX.B.2, Ahmed (Ex. 1015) and Cao (Ex. 1016) disclose particular reference concentration values for proteins in various biological samples. I have considered the concentration values reported by Ahmed and Cao in reviewing Hu. Hu, Ahmed, and Cao are directed to the same field of art as each other and the '360 Patent, *e.g.*, the detection of proteins as biomarkers for diseases, and Ahmed and Hu provide additional details of particular concentrations for proteins described in Hu. As I explain further below, a POSA logically would have looked up concentrations as well known and typically done in the field (and admitted by the '360 Patent (see Section VIII.A).

**1. Claim 1**

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

127. Regardless of whether the preamble of claim 1 is found to be limiting, Hu discloses “*a method of assaying a biological sample*” as claimed in claim 1. As for the “biological sample” in claim 1[pre], Hu discloses human blood plasma, Ex. 1008 at 355, which I note is also discussed in the '360 Patent as being a “biological sample.” Ex. 1001 at 4:45-50, 5:5-11, 8:49-56, 52:42-56.

128. Hu provides several disclosures concerning its method. For example, Hu discloses that, in the method, they “quantified the plasma protein coronas bound on three different sizes of pristine Fe<sub>3</sub>O<sub>4</sub> NPs (~30 nm, 200 nm and 400 nm) by

means of label-free quantitative proteomic strategy.” Ex. 1008 at 355. Hu also discloses that “[i]n this study, the plasma protein coronas formed on pristine Fe<sub>3</sub>O<sub>4</sub> NPs of three different particle sizes were investigated for the first time.” Ex. 1008 at 360. “The protein coronas bound on the Fe<sub>3</sub>O<sub>4</sub> NPs after 1 h of incubation with human plasma were used to investigate the size effects on the formation of protein coronas on the Fe<sub>3</sub>O<sub>4</sub> NPs.” Ex. 1008 at 356-357. Figure 2 shows the plasma protein binding profiles:

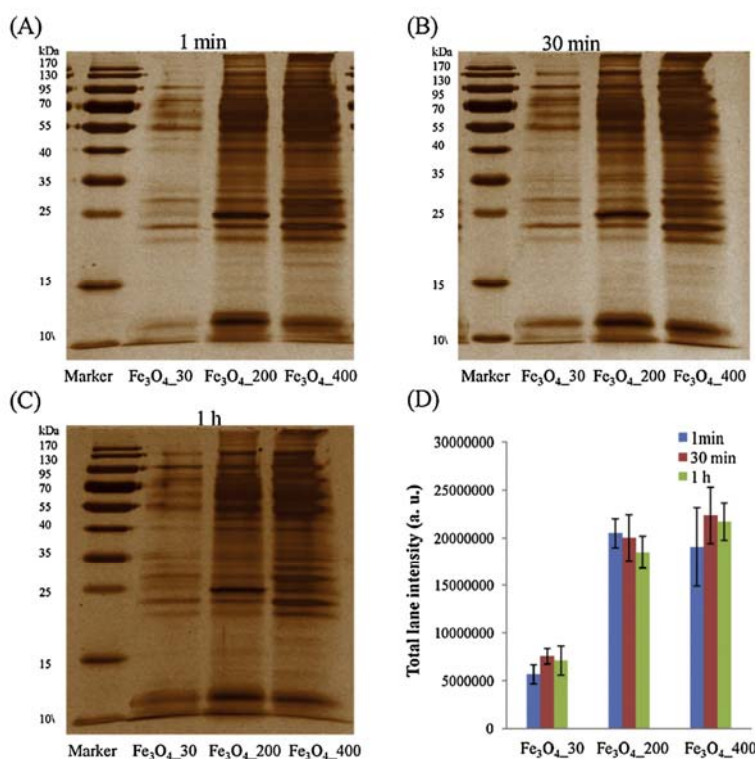


Fig. 2. Silver stained SDS-PAGE gel lanes showing the plasma protein binding profiles on different sizes of Fe<sub>3</sub>O<sub>4</sub> NPs after incubation in human plasma for 1 min (A), 30 min (B) and 1 h (C) and the corresponding optical intensities of each gel lanes for the plasma proteins adsorbed on the Fe<sub>3</sub>O<sub>4</sub> NPs. The error bars represent the standard deviation from the mean (n = 3).

Ex. 1008, Figure 2.

129. Hu discloses that “based on the spectral counting (SpC) method, we applied the label-free quantitative proteomic strategy to quantitatively analyze the

plasma proteins bound.” Ex 1008 at 357. Hu further discloses searching its identification results against the International Protein Index. Ex. 1008 at 356. This is similar to the ’360 Patent’s counting approach. Ex. 1001 at 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. As I explained, above in Section VIII.A (including with reference to Exhibits 1014 and 1020), the ’360 Patent relied on the use of a known database.

130. In my opinion, a POSA would have understood Hu’s method of using Fe<sub>3</sub>O<sub>4</sub> NPs (*i.e.*, iron oxide nanoparticles) to analyze human blood plasma proteins to be a “method for assaying a biological sample.” I also note that Hu’s use of a biological sample consisting of human blood plasma is consistent with the ’360 Patent description of “biological samples” that “include, but are not limited to, systemic blood, plasma, serum, lung lavage, cell lysates, menstrual blood, urine, processed tissue samples, amniotic fluid, cerebrospinal fluid, tears, saliva, semen and the like” (Ex. 1001 at 20:32-45), and “a complex biological sample” may be “e.g., plasma, or any sample having a complex mix of biomolecules such as proteins and a nucleic acid and at least one of a polysaccharide and lipid” (Ex. 1001 at 28:11-18).

131. I further explain Hu’s method in connection with the claim limitations below.

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

132. Hu discloses a method for assaying a biological sample that involves “*contacting the biological sample with a plurality of particles comprising different particle types.*” As I discussed above in 1[pre] and further below, the biological sample in Hu is human blood plasma and the various particle types are Fe<sub>3</sub>O<sub>4</sub> NPs (iron oxide nanoparticles) of various sizes.

133. I note that Hu’s disclosure is consistent with the ’360 Patent, as the ’360 Patent discusses that a biological sample may be human blood plasma (Ex. 1001 at 20:2-7) and that the particles may be “iron oxide nanoparticles.” Ex. 1001 at 75:24-52, 20:33-45, 21:14-33, dependent claim 3, dependent claim 22.

134. Hu also discloses the protocol for how the different particle types and a biological sample were placed in contact by describing that the Fe<sub>3</sub>O<sub>4</sub> NPs were “incubated in a constant amount of human plasma at 37°C with shaking at 1000 rpm for 1 h.” Ex. 1008 at 355. Hu further discloses that “to investigate the plasma protein adsorption dynamics of the Fe<sub>3</sub>O<sub>4</sub> NPs, Fe<sub>3</sub>O<sub>4</sub> NPs of different sizes and with a total surface area of 0.005 m<sup>2</sup> were also incubated with the same amount of human plasma at 37°C with shaking at 1000 rpm for 1 min and 30 min.” Ex. 1008 at 355. Hu also discloses that “Fe<sub>3</sub>O<sub>4</sub>\_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)...” and that “Fe<sub>3</sub>O<sub>4</sub> NPs with sizes of ~200 and ~400 nm were synthesized according to previous literature.” Ex. 1008 at 355.

135. In Figure 1, the different sizes of Fe<sub>3</sub>O<sub>4</sub> NPs using transmission electron microscopy (TEM) imaging are shown. Ex. 1008 at 356.

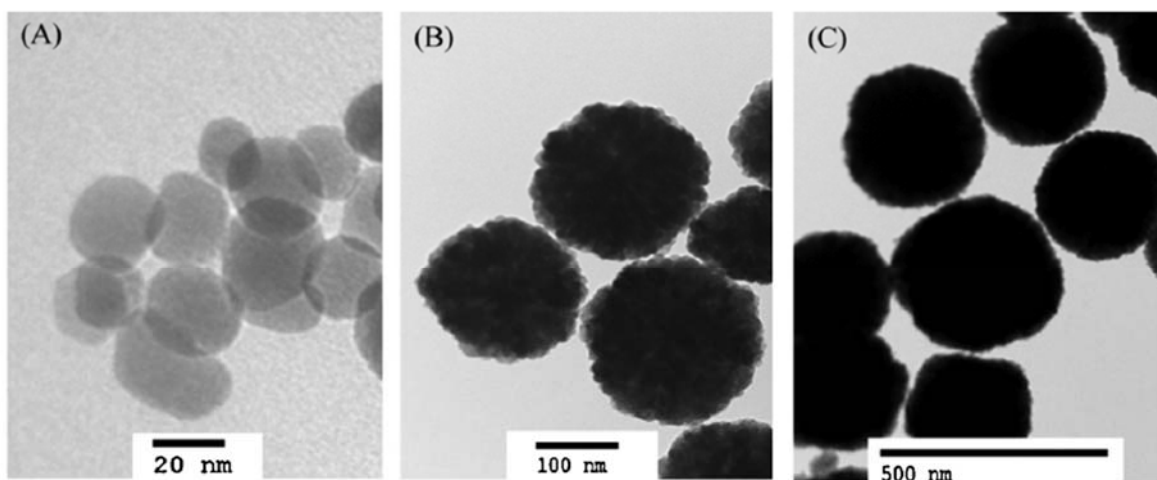


Fig. 1. TEM images of the Fe<sub>3</sub>O<sub>4</sub> NPs of different sizes (Fe<sub>3</sub>O<sub>4</sub>\_30 (A), Fe<sub>3</sub>O<sub>4</sub>\_200 (B) and Fe<sub>3</sub>O<sub>4</sub>\_400 (C)).

Ex. 1008, Figure 1.

136. In my opinion, based on the above, a POSA would thus have understood the human blood plasma disclosed by Hu to be a biological sample that is contacted with “Fe<sub>3</sub>O<sub>4</sub> NPs of different sizes” including 30, 200, and 400 nm. Ex. 1008 at 355. These different sizes of Fe<sub>3</sub>O<sub>4</sub> NPs in Hu encompass the claimed “*different particle types*” in the ’360 Patent. Ex. 1001 at 33:27-67, 86:10-22, Fig. 13. This is further confirmed by dependent claim 25 of the ’360 Patent where “different particle types” may differ by “size,” etc. I further note that Hu also discloses that

that “[a]ccording to the results, it could be concluded that the particle size induced different surface curvature and aggregation state of the Fe<sub>3</sub>O<sub>4</sub> NPs, both of which may have played important roles in the formation of plasma protein coronas on these Fe<sub>3</sub>O<sub>4</sub> NPs.” Ex. 1008 at 360.

137. In my opinion, based on the above, a POSA would also have understood that the incubation protocol disclosed by Hu necessarily involves “*contacting*” the biological sample with a plurality of particles comprising different particle types, as the incubation inherently encompasses the NPs coming into contact with the sample (otherwise, *e.g.*, there could be no binding). This is also consistent with the ’360 Patent, which similarly describes incubating. Ex. 1001 at 9:3-5, 21:30-42, 76:22-35, 83:1-63. It is further confirmed by dependent claims of the ’360 Patent, such as dependent claims 18 and 19, which describe incubating as part of contacting.

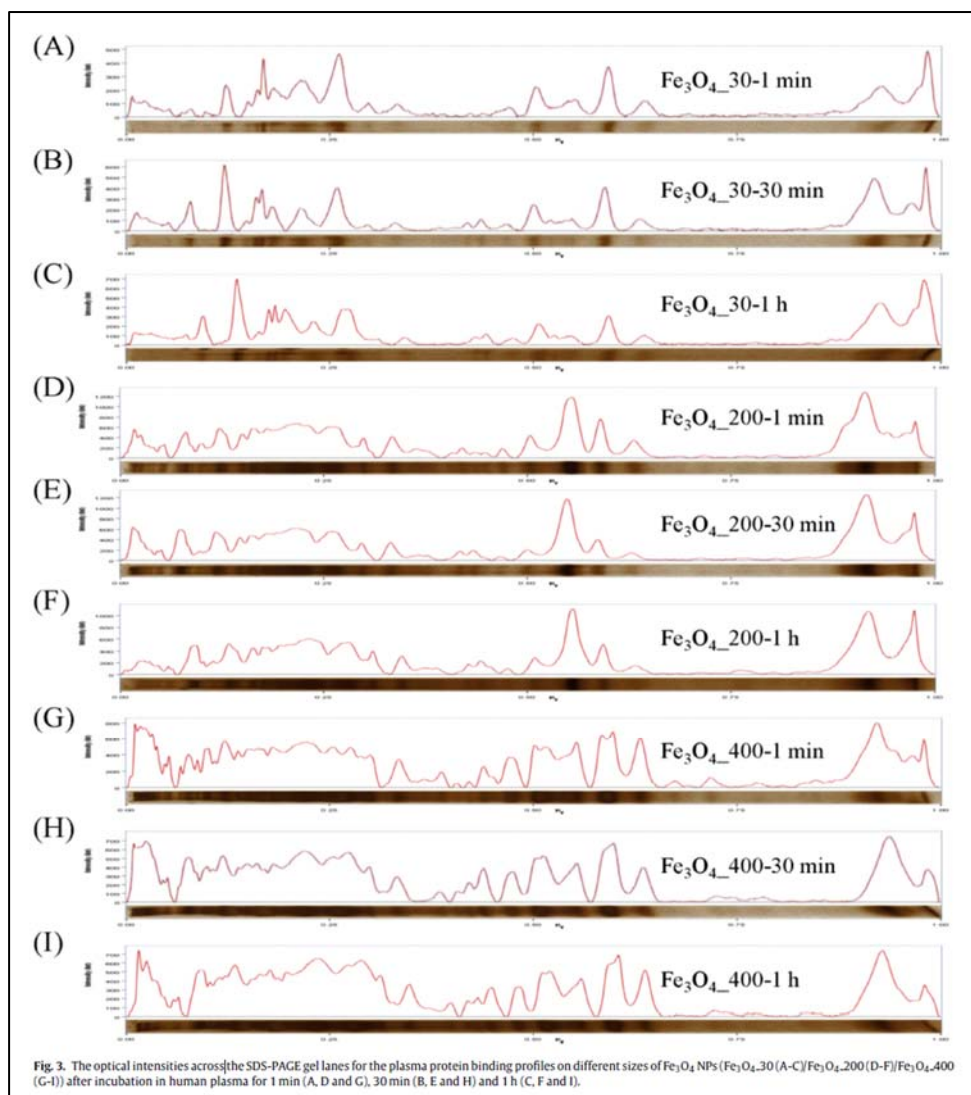
***1[a][2] “to permit biomolecules of the biological sample to bind to the plurality of particles and form coronas around the plurality of particles,”***

138. Hu discloses a method in which the NPs contact the biological sample “to permit biomolecules of the biological sample to bind to the plurality of particles and form coronas around the plurality of particles.” As explained above with respect to limitation 1[a][1], it is contact between NPs and the biological sample (i.e., human blood plasma) that allows the proteins in said human blood plasma (the claimed “biomolecules”) to bind to the Fe<sub>3</sub>O<sub>4</sub> NPs and form coronas around them (as I discuss

below). Hu discloses an investigation into the influence of NP sizes on protein corona formation and concluded that “different surface curvature and aggregation state” of the particles “may have played important roles in the formation of plasma protein coronas” on the particles. Ex. 1008 at 354, 360.

139. As I explained above for limitation 1[a][1], Hu discloses that 30, 200, and 400 nm  $\text{Fe}_3\text{O}_4$  NPs were incubated with human blood plasma for various intervals, including 1 min, 30 min, and 1 hour. Ex. 1008 at 355-56.

140. In Figure 3, “[t]he optical intensities across the SDS-PAGE gel lanes for the plasma protein binding profiles on different sizes of  $\text{Fe}_3\text{O}_4$  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)” are shown. Ex. 1008 at 358.



Ex. 1008, Figure 3

141. Hu further discloses “[quantifying] the plasma protein coronas bound on three different sizes of pristine  $\text{Fe}_3\text{O}_4$  NPs of different sizes (~30 nm, 200 nm and 400 nm) by means of label-free quantitative proteomic strategy” and further the formation of protein coronas on the  $\text{Fe}_3\text{O}_4$  NPs (that is, the protein coronas that formed around the  $\text{Fe}_3\text{O}_4$  NPs during that incubation period resulted in the NPs being bound to the human blood plasma proteins. Ex. 1008 at 355. Indeed, formation of

coronas around NPs is “well recognized”: “[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.” Ex. 1007 at 1869.

142. Hu’s teachings and the knowledge of a POSA regarding coronas are 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.” Ex. 1001 at 17:29-59, 18:26-39.

143. In my opinion, based on the above, a POSA would have understood that the incubation step described in Hu permits proteins (*i.e.* “*biomolecules*”) in the human blood plasma (*i.e.* “*biological sample*”) to bind to the different sizes of Fe<sub>3</sub>O<sub>4</sub> particles to form protein coronas around the 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;”***

144. Hu discloses a method “*wherein the coronas corresponding to the different particle types (i) differ based on particle type, and (ii) comprise overlapping and distinct proteins.*” Hu discloses that the size and composition of the protein coronas formed on the Fe<sub>3</sub>O<sub>4</sub> NPs are affected by the diameter of the

nanoparticle, which resulted in different protein abundance profiles as provided by Molecular Weighted Spectral Count (MwSpC) as a measure of abundance for which Hu describes an equation for calculating. Ex. 1008 at 357. For example, Hu discloses that “serum albumin was observed in the protein corona of the Fe<sub>3</sub>O<sub>4</sub> 200 NPs with an average MWSpC of 9.01, while the average MWSpC for serum albumin in the protein coronas of the Fe<sub>3</sub>O<sub>4</sub> 30 and Fe<sub>3</sub>O<sub>4</sub> 200 NPs were 1.27 and 2.50, respectively, indicating the selective binding of plasma proteins on different sizes of Fe<sub>3</sub>O<sub>4</sub> NPs.” Ex. 1008 at 358.

145. In Table 2, the top twenty most abundant plasma proteins bound to the 30 nm, 200 nm, and 400 nm Fe<sub>3</sub>O<sub>4</sub> nanoparticles are shown, which present overlapping proteins across certain sizes of NPs, as well as proteins that appear in only one. Ex. 1008 at 359.

**Table 2**  
The top 20 most abundant plasma proteins bound on the Fe<sub>3</sub>O<sub>4</sub> NPs of different sizes.

| Rank | Fe <sub>3</sub> O <sub>4</sub> _30                     | Fe <sub>3</sub> O <sub>4</sub> _200                       | Fe <sub>3</sub> O <sub>4</sub> _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 |

Ex. 1008, Table 2

146. Hu also discloses in Figure 4, a Venn diagram comparing the number of distinct and overlapping proteins found in the coronas of 30 nm, 200 nm, and 400 nm Fe<sub>3</sub>O<sub>4</sub> NPs. Ex. 1008 at 357, 359.

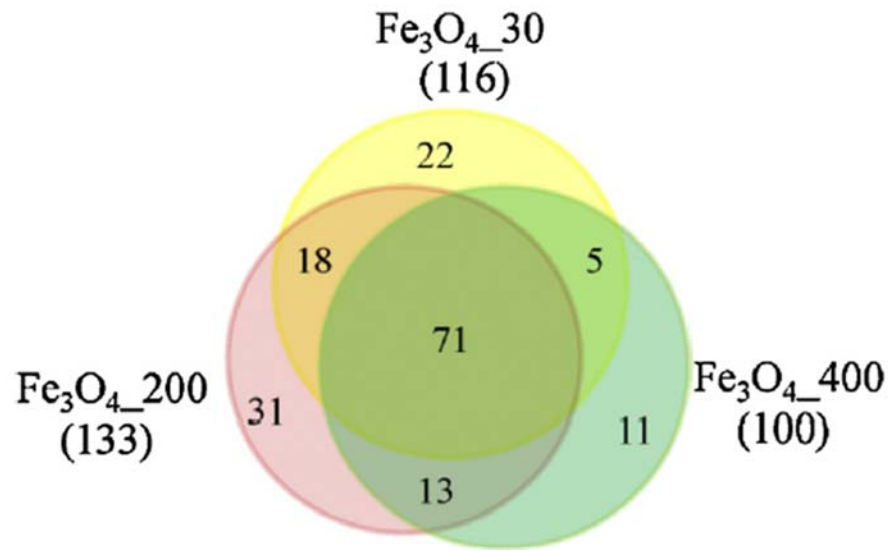


**Fig. 4.** Venn diagram displaying the number of proteins identified in the protein coronas of the different sizes of Fe<sub>3</sub>O<sub>4</sub> NPs.

Ex. 1008, Figure 4

147. With respect to Figure 4, Hu describes that “[i]n total, there were 117, 133, and 100 plasma proteins identified in the protein coronas of the Fe<sub>3</sub>O<sub>4</sub>\_30, Fe<sub>3</sub>O<sub>4</sub>\_200 and Fe<sub>3</sub>O<sub>4</sub>\_400 NPs, respectively.... A total of 71 plasma proteins were detected in the protein coronas of Fe<sub>3</sub>O<sub>4</sub> NPs of the three different sizes, with 22, 31 and 11 distinct proteins for the Fe<sub>3</sub>O<sub>4</sub>\_30, Fe<sub>3</sub>O<sub>4</sub>\_200 and Fe<sub>3</sub>O<sub>4</sub>\_400 NPs, respectively (shown in Fig. 4).” Ex. 1008 at 357. I note that there may be a typographical error, as Figure 4 identifies 116 for Fe<sub>3</sub>O<sub>4</sub>\_30 instead of 117, where

the text describes 117; however, that difference (between 116 and 117 plasma proteins) does not impact my analysis here regarding Hu's teachings.

148. The above disclosure also evidences to a POSA that the differences in the types and amounts of proteins bound to the three different sizes of Fe<sub>3</sub>O<sub>4</sub> NPs result in different coronas formed on each of the different types of particles.

149. Figure 6 shows relative protein abundance for a subset of proteins from the sample, which demonstrates the distinct and overlapping proteins bound to the different nanoparticle coronas. Ex. 1008 at 360.

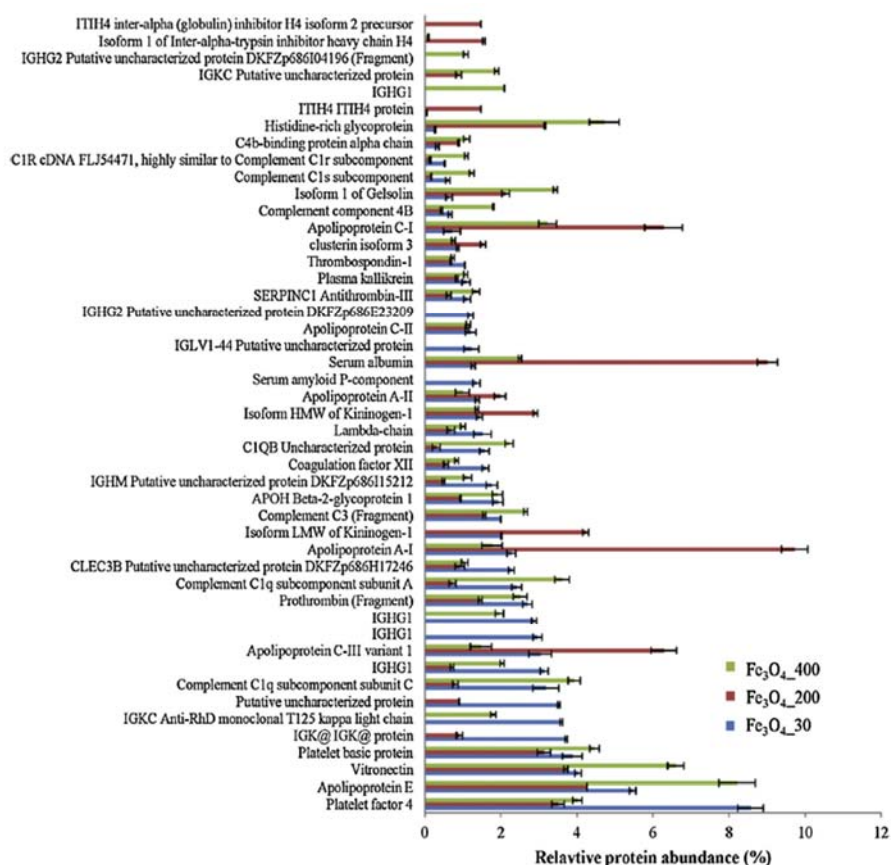


Fig. 6. Relative protein abundance of proteins (normalized to molecular weight, MWSpC) satisfying the criterion that MWSpC ≥ 1 in the coronas of the different sizes of Fe<sub>3</sub>O<sub>4</sub> NPs. As the proteins were detected in more than 2 replicates, the error bars represent the standard deviation from the mean (n = 2 or n = 3).

Ex. 1008, Figure 6

150. Beyond the above, Hu provides additional disclosure that its coronas corresponding to the different particle types have different protein compositions depending on particle type (*e.g.*, size) as shown by overlapping and distinct proteins.

In particular, Hu explains this in the following paragraph:

It was found that although there were 71 common plasma proteins bound on the different sizes of Fe<sub>3</sub>O<sub>4</sub> NPs, the relative protein abundances for these proteins differed. For instance, serum albumin was observed in the protein corona of the Fe<sub>3</sub>O<sub>4</sub>\_200 NPs with an average MWSpC of 9.01, while the average MWSpC for serum albumin in the protein coronas of the Fe<sub>3</sub>O<sub>4</sub>\_30 and Fe<sub>3</sub>O<sub>4</sub>\_200 NPs were 1.27 and 2.50, respectively, indicating the selective binding of plasma proteins on different sizes of Fe<sub>3</sub>O<sub>4</sub> NPs. Additionally, platelet factor 4 was observed in the corona of Fe<sub>3</sub>O<sub>4</sub>\_30 with an average MWSpC of 8.57, which accounted for 3.51% and 4.01% in the protein coronas of the Fe<sub>3</sub>O<sub>4</sub>\_200 and Fe<sub>3</sub>O<sub>4</sub>\_400 NPs, respectively. Furthermore, the plasma proteins with an average MWSpC  $\geq 1$  in the protein corona on the Fe<sub>3</sub>O<sub>4</sub> NPs are illustrated in Fig. 6, which also reveals the distinctive properties of the plasma protein coronas formed on the different sizes of Fe<sub>3</sub>O<sub>4</sub> NPs.

Ex. 1008 at 358-359.

***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;”***

151. Hu discloses a method for assaying a biological sample that further involves “*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.*” For

example, Hu discloses separating out the nanoparticles comprising the coronas from the biological sample using a magnet, 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 (i.e., those that are bound and not removed during washing, thereby making up the subset of proteins). Ex. 1008 at 355.

152. Figure 2 shows “the adsorption dynamics of the human plasma protein coronas on the  $\text{Fe}_3\text{O}_4$  NPs” (as explained in the prior paragraph above, the subset that is bound to the nanoparticles). Ex. 1008 at 356-357.

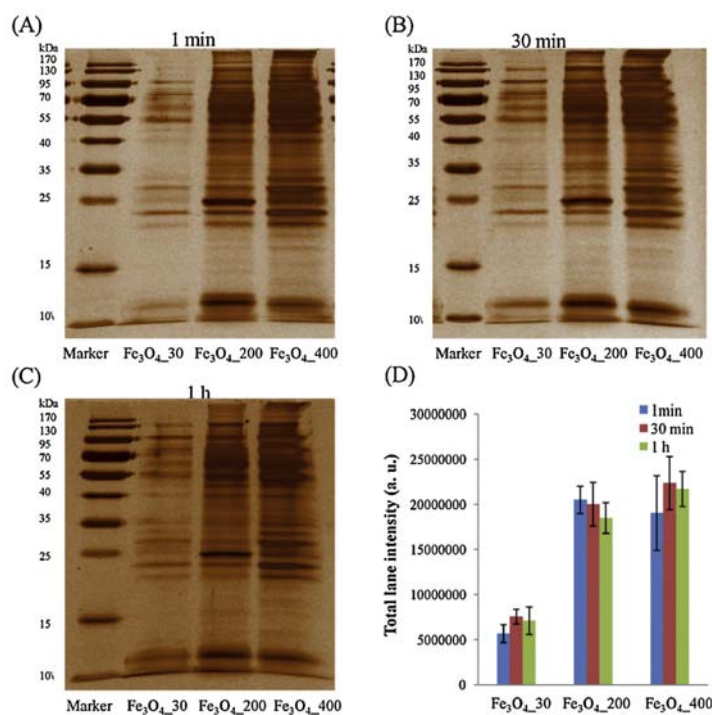


Fig. 2. Silver stained SDS-PAGE gel lanes showing the plasma protein binding profiles on different sizes of  $\text{Fe}_3\text{O}_4$  NPs after incubation in human plasma for 1 min (A), 30 min (B) and 1 h (C) and the corresponding optical intensities of each gel lanes for the plasma proteins adsorbed on the  $\text{Fe}_3\text{O}_4$  NPs. The error bars represent the standard deviation from the mean (n=3).

Ex. 1008, Figure 2

153. In my opinion, a POSA would have understood that separating these particles and their coronas out from the biological sample as explained in Hu would produce a subset of proteins from the biological sample based on the binding affinities of proteins with Fe<sub>3</sub>O<sub>4</sub> NPs disclosed by Hu.

***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.”***

154. It would have been obvious to include as part of Hu, the claimed “*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.*” In fact, Hu discloses all but particularly 6 orders of magnitude in a numerical form. However, as I explain below, a dynamic range of at least 6 orders of magnitude amounts to looking up the known concentrations of the proteins provided in Hu and a calculation using the information provided in Hu and the state of the art.

155. Initially, Hu discloses assaying the subset of proteins using Reverse Phase Liquid Chromatography (RPLC) in conjunction with tandem mass spectrometry (RPLC-MS/MS). Ex. 1008 at 355-56. Hu explains in Section 2.5 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.” Ex. 1008 at 355. In Section 2.6, Hu

explains using known software (“Protein Pilot software (AB SCIEX, USA)”) to analyze and search a known index (International Protein Index, ipi.human.3.80.fasta) for MS/MS spectral data protein matches. Ex. 1008 at 356. Hu explains:

The MS/MS spectral data acquired by the triple TOF 5600 mass spectrometer were searched by the “Paragon Algorithm” of the Protein Pilot software (AB SCIEX, USA) with trypsin specificity in “Thorough ID” mode. The peptide false discovery rate (FDR) was controlled <1% by setting the criterion for the corresponding confidence of global FDR of hit. The results from the human plasma protein coronas were searched against the International Protein Index (ipi.human.3.80.fasta). To confirm the identification results for the plasma proteins bound on Fe<sub>3</sub>O<sub>4</sub> NPs, each sample was analyzed in at least three replicates, and only proteins that were both detected at least 2 times in triplicate analysis and identified by at least 2 unique peptides in each run were considered as positively identified.

Ex. 1008 at 356.

156. Table 2 shows the “top 20 most abundant plasma proteins bound on the Fe<sub>3</sub>O<sub>4</sub> NPs of different sizes.” Ex. 1008 at 359. As shown in Table 2 and explained in Hu, there are several proteins, among them is serum albumin. Hu explains: “we list the top 20 most abundant plasma proteins comprising the protein coronas formed on the Fe<sub>3</sub>O<sub>4</sub> in Table 2 to further illustrate the different binding patterns of the plasma proteins and understand the following potential biological impacts. It 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 Fe<sub>3</sub>O<sub>4</sub>\_200 NPs, while it

was the 12th most abundant protein in the protein corona on the Fe<sub>3</sub>O<sub>4</sub>\_400 NPs and was not among the top 20 most abundant proteins for the Fe<sub>3</sub>O<sub>4</sub>\_30 NPs (27th).” Ex. 1008 at 360. Table 2 shows:

**Table 2**  
The top 20 most abundant plasma proteins bound on the Fe<sub>3</sub>O<sub>4</sub> NPs of different sizes.

| Rank | Fe <sub>3</sub> O <sub>4</sub> _30                     | Fe <sub>3</sub> O <sub>4</sub> _200                       | Fe <sub>3</sub> O <sub>4</sub> _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 |

### Ex. 1008, Table 2

157. While Table 2 includes the top 20, Hu includes in “Table S1” all of the proteins identified. Ex. 1008 at 357-358. Hu summarizes the information included in Table S1 (part of its Appendix):

In total, there were 117, 133 and 100 plasma proteins identified in the protein coronas of the Fe<sub>3</sub>O<sub>4</sub>\_30, Fe<sub>3</sub>O<sub>4</sub>\_200 and Fe<sub>3</sub>O<sub>4</sub>\_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 Fe<sub>3</sub>O<sub>4</sub> NPs of the three different sizes, with 22, 31 and 11 distinct proteins for the Fe<sub>3</sub>O<sub>4</sub>\_30, Fe<sub>3</sub>O<sub>4</sub>\_200 and Fe<sub>3</sub>O<sub>4</sub>\_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 Fe<sub>3</sub>O<sub>4</sub> NPs, while ceruloplasmin and serum amyloid P-component, among

others, were found to be uniquely bound on the surface of only the Fe<sub>3</sub>O<sub>4</sub>\_30 NPs. Fibronectin, serotransferrin and serum paraoxonase/arylesterase 1 were observed to only be adsorbed on the Fe<sub>3</sub>O<sub>4</sub>\_200 NPs, and coagulation factor XIII A chain and inter-alpha (Globulin) inhibitor H2 were only bound on the Fe<sub>3</sub>O<sub>4</sub>\_400 NPs (Table S1).

Ex. 1008 at 357.

158. In Table S1 of Hu, the plasma proteins identified in the various-sized protein coronas included, for example, “Latent Transforming Growth Factor-Beta Binding Protein-1” (LTBP-1). Ex. 1008 at 10-19 (Table S1).

159. Hu further explains its equation for MWSpC:

On the other hand, based on the spectral counting (SpC) method [46], we applied the label-free quantitative proteomic strategy to quantitatively analyze the plasma proteins bound on the surfaces of the Fe<sub>3</sub>O<sub>4</sub> NPs of different sizes. Here, the SpC of each protein identity was normalized to the protein mass and described as the relative protein quantity by applying the following equation:

$$MWSpC_k = \left( \frac{(SpC/Mw)_k}{\sum_{i=1}^n (SpC/Mw)_i} \right) \times 100$$

where MWSpC is the percentage normalized spectral count for protein *k*, SpC is the spectral count identified and Mw is the molecular weight in kDa for protein *k*. This correction takes into account the protein size and evaluates the real contribution of each protein to the hard corona composition [20,21]. By using this equation, the

Ex. 1008 at 357.

160. According to Hu, “[b]y using this equation, the relative protein abundances for the plasma proteins bound on the Fe<sub>3</sub>O<sub>4</sub> NPs were calculated and listed in Table S1.” Ex. 1008 at 357-358.

161. As I discussed above, Hu does not state (in numerical form) the orders of magnitude of the dynamic range for the identified proteins. Although Hu does not explicitly disclose “assaying the subset of ... proteins in the biological sample at concentrations across a dynamic range comprising at least 6 orders of magnitude,” this is inherently disclosed by Hu, or at least it would have been obvious to a POSA looking at Hu (including when Hu’s supplemental materials are considered). A POSA seeking to understand the dynamic range of protein concentrations in Hu’s method would have found it obvious to turn to additional references as a source of concentration values for proteins detected by Hu. This amounts to routine look-up and calculation to make that determination using the information provided in Hu and a POSA’s knowledge (including utilizing known protein concentration data from the same field of the art) such that, when the information is put together, a POSA would have understood that the proteins in the subset in Hu are detected with the instrument are present at concentrations across a dynamic range comprising more than six orders of magnitude (*see* Section IX.B for known concentrations of serum albumin and LTBP-1 disclosed by Cao and Ahmed as well as below for my analysis).

162. Consistent with the disclosure in Hu when proteins are bound to the iron oxide nanoparticles, a POSA would have understood that those proteins existed in certain concentrations in the biological sample being assayed. Two of those proteins detected from Hu are serum albumin and LTBP-1 (discussed above).

163. As I explained above in Sections IX.B.1-IX.B.2 and also above in this section, a POSA would have understood and sought out typical concentrations for the well-known identified proteins in human blood plasma (the biological sample in Hu), such that the dynamic range calculation could be performed.

164. A POSA would have turned to references such as Ahmed to ascertain that serum albumin is normally present in healthy human plasma at concentrations ranging from 35 to 52 mg/mL. Ex. 1015 at 786-87, Table 2. Ahmed explains its process, including that the “Human Proteome Consortium has recommended that blood be examined as plasma rather than serum and established standard sample collection protocols.” Ex. 1015 at 787. Table 2 of Ahmed (Ex. 1015) shows known reference values for the concentration of serum albumin in healthy human blood (which a POSA would have understood apply to serum albumin in Hu):

**Table 2.** Ranking of plasma proteins in healthy persons in order of molar or mass abundance, with examples of half lives<sup>a)</sup>

| No. | Protein rank                         | Concentration |               | Molecular mass<br>(M <sub>r</sub> ) in Da | Half life          |
|-----|--------------------------------------|---------------|---------------|-------------------------------------------|--------------------|
|     |                                      | M · mol/L     | mg/L          |                                           |                    |
| 1   | Albumin                              | 500–800       | 35 000–52 000 | 66 438                                    | 15–10 days         |
| 2   | IgG                                  | 40–100        | 7 000–16 000  | 160 000                                   |                    |
| 3   | Apolipoprotein A-I                   | 36–72         | 1 000–2 000   | 28 079                                    | 5 days             |
| 4   | Transferrin                          | 25–45         | 2 000–3 600   | 79 600                                    | 7 days             |
| 5   | Apolipoprotein A-II                  | 22–60         | 200–550       | 8 691                                     | 5 days             |
| 6   | α <sub>1</sub> -Proteinase inhibitor | 18–40         | 900–2 000     | 55 000                                    | 1–2 days           |
| 7   | α <sub>1</sub> -Acid glycoprotein    | 12–30         | 500–1 200     | 40 000                                    |                    |
| 8   | Transthyretin                        | 15–30         | 200–400       | 54 000                                    |                    |
| 9   | Hepatoglobulin                       | 3–20          | 300–2 000     | 104 000                                   |                    |
| 10  | Hemopexin                            | 15            | 900           | 57 000                                    |                    |
| 11  | IgA                                  | 4–24          | 700–4 000     | 170 000                                   | 2.5 days<br>4 days |
| 12  | Apolipoprotein C-III                 | 6–20          | 60–180        | 8 765                                     |                    |
| 13  | α <sub>2</sub> -Macroglobulin        | 7–17          | 1 300–3 000   | 30 000                                    |                    |
| 14  | α <sub>2</sub> -HS glycoprotein      | 12            | 600           | 50 000                                    |                    |
| 15  | Gc globulin                          | 8–14          | 400–700       | 51 000                                    |                    |
| 16  | Apolipoprotein C-I                   | 6–12          | 40–80         | 6 631                                     |                    |
| 17  | Fibrinogen                           | 6–12          | 2 000–4 000   | 340 000                                   |                    |
| 18  | α <sub>1</sub> -Antichymotrypsin     | 7             | 500           | 68 000                                    |                    |
| 19  | C3                                   | 5–10          | 900–1 800     | 180 000                                   |                    |
| 20  | b <sub>2</sub> -Glycoprotein I       | 4–8           | 150–300       | 40 000                                    |                    |
| 21  | Vitronectin                          | 4–8           | 250–450       | 55 000                                    | 10–12 h            |
| 22  | α <sub>1</sub> -B Glycoprotein       | 3–6           | 150–300       | 63 000                                    |                    |
| 23  | Apolipoprotein A-IV                  | 3–6           | 130–250       | 43 375                                    |                    |
| 24  | Apolipoprotein C-II                  | 2–7           | 20–60         | 8 915                                     |                    |
| 25  | β <sub>2</sub> -Glycoprotein II      | 2–5           | 12–30         | 63 000                                    |                    |
| 26  | Antithrombin III                     | 3             | 200           | 65 000                                    |                    |
| 27  | Inter α-trypsin inhibitor            | 2–4           | 400–700       | 160 000                                   |                    |
| 28  | Plasminogen                          | 2–4           | 150–350       | 81 000                                    |                    |
| 29  | Ceruloplasmin                        | 1.5–5         | 200–600       | 135 000                                   |                    |
| 30  | Retinol-binding protein              | 1.5–3         | 30–60         | 21 000                                    |                    |

<sup>a)</sup> Modified from ref. [135].

## Ex. 1015, Table 2

165. A POSA would also have turned to references such as Cao to ascertain that LTBP-1 is normally present in healthy human plasma at concentrations ranging from 1.7 to 6.0 ng/mL. Ex. 1016 at 16048, Table 1. Table 1 of Cao (Ex. 1016) shows known reference values for the concentration of LTBP-1 in healthy human blood (which a POSA would have understood apply to LTBP-1 in Hu):

**Table 1.** Plasma concentrations of AFP and LTBP-1 in HCC (positive of CHB and cirrhosis) and three other cohorts

| †Cohorts  | N   | AFP (ng/ml)      |          | LTBP-1 (ng/ml) |          |
|-----------|-----|------------------|----------|----------------|----------|
|           |     | Median (range)   | ‡P       | Median (range) | ‡P       |
| Healthy   | 104 | 3.0 (1.1-12.5)   | 7.09E-12 | 2.9 (1.7-6.0)  | 2.14E-24 |
| CHB       | 50  | 4.5 (1.6-638.0)  | 4.34E-4  | 3.0 (1.8-10.7) | 2.7E-10  |
| Cirrhosis | 50  | 9.8 (1.8-282.0)  | 0.199    | 3.4 (1.1-11.8) | 1.74E-4  |
| HCC       | 120 | 9.5 (1.2-1674.0) | -        | 5.0 (1.6-14.3) | -        |

†CHB, chronic hepatitis B; HCC, hepatocellular carcinoma; ‡P values were assessed by comparison between HCC (positive of CHB and cirrhosis) and each of the other cohorts, two-sided Mann-Whitney test.

Ex. 1016, Table 1

166. Using these typical protein concentrations for the human blood plasma, a POSA would have understood that the methods disclosed by Hu are detecting 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), which result in a dynamic range of more than 6 orders of magnitude (thereby meeting the claim limitation of “*at least 6 orders of magnitude*”).

167. As I explained above in Section VIII.A (my overview of the '360 Patent), the '360 Patent confirms to use a known reference database in order to determine the concentrations of the detected proteins. Ex. 1001 at 66:63-69:67, Table 5, Fig. 12. As I also explained above and further here, it was typical in the field to use such reference materials for protein concentration calculations for

determining orders of magnitude of the dynamic range. As I also explained, Nanjappa explains this known database and its purpose (including to provide a reference that can be looked up for identified proteins). For example, Nanjappa in 2013 (Ex. 1014) explained in its Abstract (on pages D959-D960):

**ABSTRACT**

Plasma Proteome Database (PPD; <http://www.plasmaproteomedatabase.org/>) was initially described in the year 2005 as a part of Human Proteome Organization's (HUPO's) pilot initiative on Human Plasma Proteome Project. Since then, improvements in proteomic technologies and increased throughput have led to identification of a large number of novel plasma proteins. To keep up with this increase in data, we have significantly enriched the proteomic information in PPD. This

database currently contains information on 10 546 proteins detected in serum/plasma of which 3784 have been reported in two or more studies. The latest version of the database also incorporates mass spectrometry-derived data including experimentally verified proteotypic peptides used for multiple reaction monitoring assays. Other novel features include published plasma/serum concentrations for 1278 proteins along with a separate category of plasma-derived extracellular vesicle proteins. As plasma proteins have become a major

thrust in the field of biomarkers, we have enabled a batch-based query designated Plasma Proteome Explorer, which will permit the users in screening a list of proteins or peptides against known plasma proteins to assess novelty of their data set. We believe that PPD will facilitate both clinical and basic research by serving as a comprehensive reference of plasma proteins in humans and accelerate biomarker discovery and translation efforts.

Ex. 1014 at D959-660

168. Nanjappa also discusses the “wide dynamic range of protein abundance, greater than 10 orders of magnitude” (Ex. 1014 at D590) and use of known “[t]argeted approaches like multiple reaction monitoring (MRM) have enabled quantitation of plasma proteins with high specificity as well as increased throughput.” (Ex. 1014 at D962). It also explains how this has been used for the database content (at pages D962-D963):

MRM assays use multiple parameters to identify and quantify specific protein/s in complex samples. Proteotypic peptides that uniquely represent a protein are first selected, and assays are developed, which take into account the retention time of the peptides, their m/z values and measurement of specific fragment ions (39). This multi-step process provides superior specificity and allows for accurate quantification of protein levels over a wide dynamic range of abundance (36,40). MRM analysis has thus revolutionized biomarker research and is being widely used to determine altered protein levels across various disease phenotypes. A compilation of proteotypic peptides of plasma proteins that generate good signals in MS experiments along with their transitions will be immensely beneficial in developing MRM assays. In PPD, we have annotated 591 reported peptides corresponding to 279 proteins for MRM analysis. This includes details of precursor m/z, fragment ion m/z, collision energy, charge of the precursor ion and instrument used for the analysis (Figure 1).

Ex. 1014 at D962-D963.

169. Further as I discussed in Section VIII.A (discussing the '360 Patent and its reliance on the publicly available Plasma Proteome Database website), an archival version from "The Wayback Machine" of the database website's FAQs as of February 2015 (Ex. 1020) provides further background, including, for example, that by February 2015 there were over 9,000 proteins in plasma and serum that have been identified, the use of mass spectrometry (similar to the article discussed above as well as the '360 Patent), and the use of the database contents (which aligns with

the approach taken in the '360 Patent. For example, FAQs 1-4 from the web archive (Ex. 1020) state:

1. The database deals purely with proteins found in plasma and serum. Why was it necessary to create Plasma Proteome Database (PPD)?  
Plasma is the most commonly diagnosed clinical sample. Clinicians can diagnose pathological conditions by quantification of specific proteins. Over 9,000 proteins in plasma and serum have thus far been reported and are only expected to increase with evolving high-resolution and high-sensitivity protein detection/identification technologies. Documentation of qualitative and quantitative information on broad range of proteins in plasma/serum in a user-friendly and queryable manner is a prerequisite to explore the blood proteome. This is why the Plasma Proteome Database was created.
2. What is the information that one can get from Plasma proteome Database?  
PPD contains proteins reported in plasma and serum from healthy individuals. Experimental method employed to identify each protein is also provided. From studies employing mass spectrometry-based approaches to identify plasma/serum proteins, information pertaining to the type of mass spectrometer employed and the proteotypic peptides reported are available. Quantitative information on plasma/serum proteins from immunoassays such as ELISA and radioimmuno assay, and mass spectrometry-based assays such as single reaction monitoring/multiple reaction monitoring and spectral counting are also available in PPD. All the information is linked to their corresponding PubMed identifiers. Proteins identified in plasma exosomes along with other experimental details are also available in PPD.
3. Is the protein concentration provided only from healthy individuals?  
Yes. Plasma protein levels are provided in healthy conditions as reported in literature.
4. What are the applications of PPD?  
PPD can be used as a reference database to compare and report proteins or peptides novel to experimental results obtained by researchers. The proteotypic peptides cataloged in PPD can be used to design and evaluate proteins using mass spectrometry both qualitatively and quantitatively. The list of proteins in PPD can also be used to analyze proteins from other body fluids. The proteins thus far identified in plasma/serum would also provide a reference for analysis of cellular secretome, proteins shed from cell surface and screen for ligands of orphan receptors. Above all, PPD will enable researchers in biomarker development to screen candidate molecules that are reported in plasma/serum, from a larger set of proteins identified in the discovery phase to pursue further validation.

Ex. 1020 at 1.

170. In short, it was well-known and desirable in the field (and in fact, a necessity when assessing datasets from research such as the proteins detected in Hu's method) to look up the protein concentrations to determine the orders of magnitude of the dynamic range for the detected low abundant and high abundant proteins, and in my opinion, a POSA would have understood the need and desirability of an analysis method that could achieve dynamic ranges including 6 orders and beyond. Ex. 1014 at D959-D962 (discussed above); Ex. 1020 at 1 (discussed above), Ex. 1005 at 587 (Cai discussing orders of magnitude); Ex. 1007 at 1869-1870, 1881 (Zaccaria explaining desirability of increased range of low and high abundant proteins detected, which I discuss further in Section IX.C-XI.D). Moreover, the detection could readily be accomplished by known techniques on known instruments

(such as LC-MS/MS disclosed in both Hu and the '360 Patent, (Ex. 1008 at 355, Ex. 1001 at 72:1-25) and admitted by the '360 Patent that "...current mass spectrometry techniques (including LC-MS/MS)... can detect proteins at a dynamic range of 4-6 orders of magnitude." Ex. 1001 at 72:1-25.

171. I further note that the '360 Patent admits that mass spectrometry already provided a dynamic range of 4-6 orders of magnitude but that its invention increased that to 10 orders:

The present sensor array has a sensitivity and dynamic range of ten (10) orders of magnitude in terms of protein detection using mass-spectroscopy [sic] 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 not previously able to be detected.

Ex. 1001 at 20:20-32.

172. The '360 Patent similarly discloses (as I discussed in Paragraph 170 and in Section VIII.A) that known mass spectrometry techniques enabled detection of proteins to 6 orders of magnitude (*i.e.*, the range claimed in claim 1): "The role of the protein corona in concentrating a wide range of low-abundance and very rare proteins may go a long way towards overcoming the main reasons behind the limited success of current mass spectrometry techniques (including LC-MS/MS) in early

detection of cancers, as these techniques can detect proteins at a dynamic range of 4-6 orders of magnitude.” Ex. 1001 at 72:1-25.

173. In other words, consistent with the '360 Patent's admissions and the knowledge of a POSA (as I have discussed), claim 1 of the '360 Patent simply claims what was already known in the art (and to which the '360 Patent does not put any significance on), not the alleged 10-orders of magnitude advancement. Further, consistent with the '360 Patent and the knowledge of a POSA, broad dynamic ranges for low and high abundant proteins (including those encompassing the claimed range) were well known and understood to be typically and advantageously present when assaying proteins of various abundance in biological samples, including using known techniques such LC-MS/MS and others. Ex. 1014 at D959-D962; Ex. 1005 at 587; Ex. 1007 at 1869-1870, 1881; Ex. 1019 at 12:46-49 (discussing usefulness of wide dynamic range); Ex. 1008 at 355; Ex. 1001 at 72:1-25.

## **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.”***

174. It would have been obvious to include as part of Hu a method “*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*” for essentially the same reasons as claim 1 in Section XI (namely, serum albumin and LTBP-1 detected in Hu exist in the concentrations as claimed in claim 2). Hu also renders obvious claim 1, from which claim 2 depends, as I explained above.

175. As I explained for claim 1, Hu discloses using samples of human plasma from healthy donors to form protein coronas on different types of iron-oxide nanoparticles. Ex. 1008 at 355-56. I also discussed in claim 1 that Hu discloses that after isolation and digestion, the digested peptides were analyzed using reversed phase liquid chromatography and tandem mass spectrometry (RPLC-MS/MS). Ex. 1008 at 355.

176. I also explained previously for claim 1 that Hu discloses that various proteins in different abundances were found to be bound to the various iron oxide nanoparticles (such as serum albumin and LTBP-1), and their typical concentrations (35 to 52 mg/mL for serum albumin and 1.7 to 6 ng/mL for LTBP-1). Ex. 1008 at 355, 357, 359, 10-19 (Table S1); Ex. 1015 at 786-787, Table 2; Ex. 1016 at 16046-16048, Table 1. In my opinion, a POSA would have understood that proteins (*i.e.*, serum albumin and LTBP-1) found to be bound to the iron oxide nanoparticles would have come from the biological sample being assayed. Both proteins are well-known components of human plasma, and a POSA would have known to turn to references such as Ahmed and Cao to look up the relevant concentrations.

Accordingly, a POSA would have understood—using the relevant concentrations looked up from Ahmed and Cao—that Hu discloses a method 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 (serum albumin) and a protein at less than 10 ng/mL (LTBP-1), as in claim 2.

### 3. Claim 3

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

177. Hu discloses a method “*wherein the plurality of particles comprise iron oxide.*” Hu discloses the use of Fe<sub>3</sub>O<sub>4</sub> (also known as iron oxide) nanoparticles of various diameters. Ex. 1008 at 355-357, Table 1, Fig. 2. Hu also renders obvious claim 1, from which claim 3 depends, as I explained above.

### 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.”***

178. Hu discloses a method “*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*” (including, as I discussed in claim 1 and below, gel-electrophoresis, liquid chromatography, mass

spectrometry). Hu also renders obvious claim 1, from which claim 4 depends, as I explained above.

179. Hu discloses using Sodium Dodecyl Sulfate-Polyacrylamide Gel-Electrophoresis (SDS-PAGE) to characterize the proteins separated from the coronas of the Fe<sub>3</sub>O<sub>4</sub> nanoparticles. Ex. 1008 at 357 (Fig. 2(A)-(C) “Silver stained SDS-PAGE gel lanes showing the plasma protein binding profiles on different sizes of Fe<sub>3</sub>O<sub>4</sub> nanoparticles”).

180. Hu also discloses “[t]he 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.” Ex. 1008 at 355. Here, Hu discloses using liquid chromatography (LC) and mass spectrometry (MS) to perform the assaying of the protein coronas.

## **5. Claim 6**

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

181. It would have been obvious to include as part of Hu a method “*wherein the dynamic range of proteins detected spans 6 or more orders of magnitude*” for the same reasons as I discussed above for limitation 1[c]. That is, as I explained above in claim 1, it would have been obvious that Hu’s method detected 6 or more

orders of magnitude. Hu also renders obvious claim 1, from which claim 6 depends, as I explained above.

**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.”***

182. It would have been obvious to include as part of Hu a method “*wherein the detected proteins comprise proteins present in concentrations of 100 ng/ml or less in the biological sample*” for the same reasons as I discussed above for claim 1. As I explained in claim 1, LTBP-1 proteins are present in healthy human plasma at concentrations between 1.7 to 6.0 ng/mL (*i.e.*, “100 ng/ml or less”). Hu also renders obvious claim 1, from which claim 7 depends, as I explained above.

**7. Claim 17**

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

183. Hu discloses a method “*wherein the assaying further comprises digesting the proteins*.” Hu also renders obvious claim 1, from which claim 17 depends, as I explained above.

184. Hu discloses digesting the proteins from the nanoparticle protein corona with the enzyme trypsin as part of the assaying method: “the solution was diluted with 100 mM  $\text{NH}_4\text{HCO}_3$  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.” Ex. 1008 at 355. This method is consistent with the digestion with trypsin described in the ’360 Patent. Ex. 1001, 59:20-21 (“...digested by adding 2 micrograms of trypsin”), 59:58-59 (“For the database search, trypsin was specified as the proteolytic enzyme...”).

## 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.”***

185. Hu discloses a method “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” for the same reasons as it discloses 1[pre] and limitation 1[a] (i.e., Hu discloses human blood plasma), as I explained above. Hu also renders obvious claim 1, from which claim 22 depends, as I explained above.

## 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.”***

186. Hu discloses a method “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 also renders obvious claim 1, from which claim 23 depends, as I explained above.

187. Hu discloses coronas formed around nanoparticles that comprise lipoprotein (such proteins in Hu include Apolipoproteins such as “Apolipoprotein A-1,” “Apolipoprotein C-1”). Ex. 1008 at 360 (Table 2 “Apolipoprotein A-1,” “Apolipoprotein C-1,” etc.).

**Table 2**

The top 20 most abundant plasma proteins bound on the Fe<sub>3</sub>O<sub>4</sub> NPs of different sizes.

| Rank | Fe <sub>3</sub> O <sub>4</sub> _30                     | Fe <sub>3</sub> O <sub>4</sub> _200                       | Fe <sub>3</sub> O <sub>4</sub> _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 |

Ex. 1008, 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.”***

188. Hu discloses a method “*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 also renders obvious claim 1, from which claim 24 depends, as I explained above.

189. As I explained above regarding claim 1, Hu discloses using three different sizes of Fe<sub>3</sub>O<sub>4</sub> nanoparticles to detect proteins in human plasma. Ex. 1008 at 355. Hu further discloses the relative abundance of serum albumin: “It 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 Fe<sub>3</sub>O<sub>4</sub>\_200 NPs, while it was the 12th most abundant protein in the protein corona on the Fe<sub>3</sub>O<sub>4</sub>\_400 NPs and was not among the top 20 most abundant proteins for the Fe<sub>3</sub>O<sub>4</sub>\_30 NPs (27th).” Ex. 1008, at 360, Table 2. This is further illustrated in Table 2, which shows that all three sizes of nanoparticles enrich for certain Apolipoproteins (*i.e.*, Apolipoprotein E or Apolipoprotein A-1) over and above serum albumin:

**Table 2**  
The top 20 most abundant plasma proteins bound on the Fe<sub>3</sub>O<sub>4</sub> NPs of different sizes.

| Rank | Fe <sub>3</sub> O <sub>4</sub> _30                     | Fe <sub>3</sub> O <sub>4</sub> _200                       | Fe <sub>3</sub> O <sub>4</sub> _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 |

Ex. 1008, Table 2.

190. In my opinion, based on the above, a POSA would have understood that serum albumin is known to be the most abundant protein in human plasma (as Hu states) and that the methods described in Hu 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 Fe<sub>3</sub>O<sub>4</sub> nanoparticles disclosed in Hu is less than a ratio of an amount of albumin to that Apolipoprotein in the biological sample (human plasma, as Hu discloses). A POSA would further understand that the methods in Hu disclose protein-bound particles with different relative amounts of serum albumin (as provided by different rankings of relative abundance in Table 2 of Hu) that would directly affect the ratio of albumin to these enriched proteins X relative to the ratio of albumin to the same proteins X in the biological sample.

## 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.”***

191. Hu discloses a method “*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*” for the same reasons as I explained above in claim 1 (such as size and surface curvature). For example, Hu discloses that “[i]n this study, the plasma protein coronas formed on pristine Fe<sub>3</sub>O<sub>4</sub> NPs of three different particle sizes were investigated for the first time.” Ex. 1008 at 360. Hu further discloses “according to the results, it could be concluded that *the particle size induced different surface curvature* and aggregation state of the Fe<sub>3</sub>O<sub>4</sub> NPs, both of which may have played important roles in the formation of plasma protein coronas on these Fe<sub>3</sub>O<sub>4</sub> NPs” (emphasis added). Ex. 1008 at 360. Hu also renders obvious claim 1, from which claim 25 depends, as I explained above.

**B. Ground 2: Claims 1, 4, 6, 17, 22, and 25 are Anticipated by Cai.**

192. As I explain below, in my opinion, Cai (Ex. 1005) discloses all of the limitations of claims 1, 4, 6, 17, 22, and 25, arranged as in the claims. Thus, it is my opinion that Cai anticipates these claims.

**1. Claim 1**

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

193. Regardless of whether the preamble of claim 1 is found to be limiting, Cai discloses “*a method of assaying a biological sample,*” as claimed by claim 1.

194. 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).” Ex. 1005 at 583 (Abstract). Cai further discloses: “In the present study we exposed CNT’s 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.” Ex. 1005 at 591. Cai also discloses “To test the binding of complex mixtures of proteins to CNTs, 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.” Ex. 1005 at 585. Cai similarly discloses that “Cell lysates were incubated....”. Ex. 1005 at 584.

195. Figure 1 depicts “Protein binding assay to study MWCNT- and CB-protein corona”:

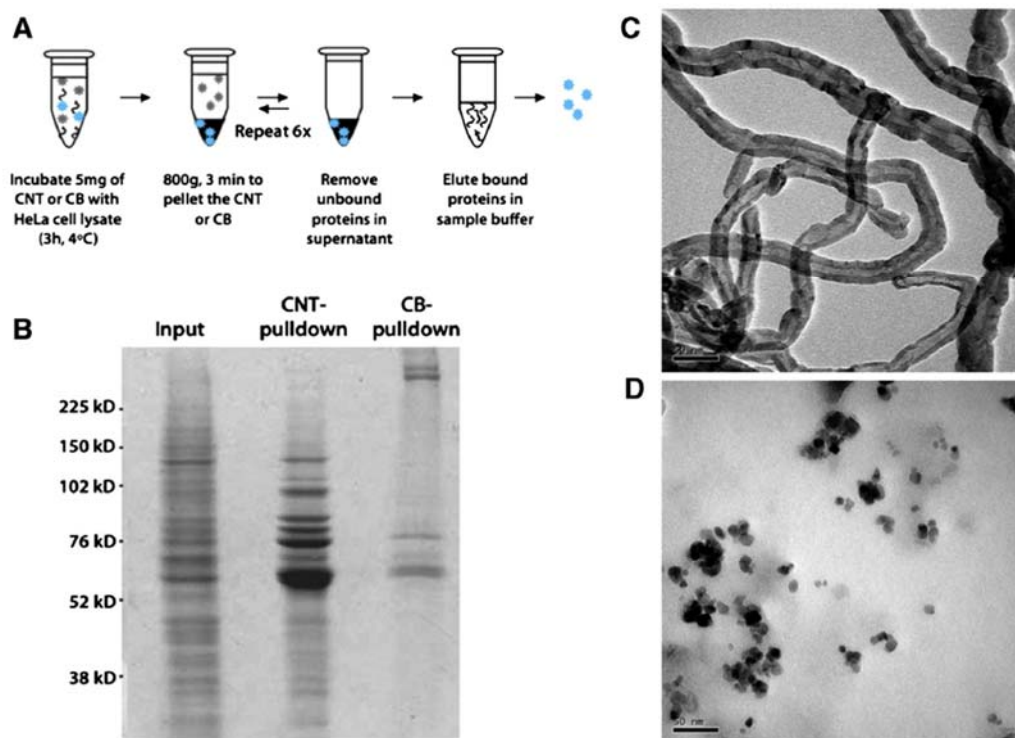


Figure 1. Protein binding assay to study MWCNT- and CB-protein corona. (A) Scheme of carbon nanoparticle pull-down experiment. (B) Proteins from HeLa cell lysate pulled down by MWCNTs and CB were separated by SDS-PAGE and stained by coomassie blue. (C-D) Transmission electron micrographs of the MWCNTs (C) and CNTs (D) used in this study (Bars: 50 nm).

Ex. 1005, Figure 1

196. In my opinion, a POSA would have understood that Cai’s method of using various CNTs alone or with CB to analyze the protein lysate from HeLa cells discloses the claimed “*method for assaying a biological sample.*”

197. I also note that Cai’s disclosure is consistent with the ’360 Patent as the ’360 Patent also describes that “biological samples include, but are not limited to,

systemic blood, plasma, serum, lung lavage, cell lysates, menstrual blood, urine, processed tissue samples, amniotic fluid, cerebrospinal fluid, tears, saliva, semen and the like” (Ex. 1001 at 20:32-45), and “a complex biological sample” may be “e.g., plasma, or any sample having a complex mix of biomolecules such as proteins and a nucleic acid and at least one of a polysaccharide and lipid” (Ex. 1001 at 28:11-18).

198. I further explain Cai’s method in connection with the claim limitations below.

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

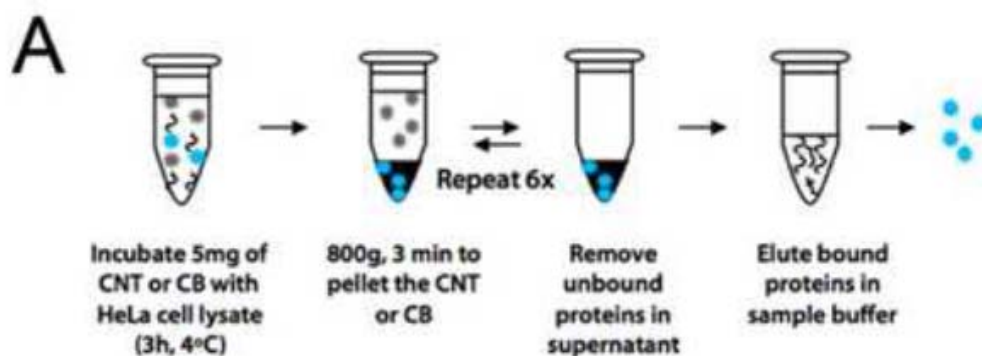
199. Cai discloses a method for assaying a biological sample that involves “*contacting the biological sample with a plurality of particles comprising different particle types.*” As I explain below, by contacting HeLa cell lysate (the claimed “*biological sample*”) with CNTs with different sizes or CNTs and CB (CNTs alone or CNTs together with CB meet the claimed “*plurality of particles comprising different particle types*”).

200. Cai discloses that “[c]ell lysates were incubated with 5 mg of CNTs or CB for 3 hrs at 4 °C.” Ex. 1005 at 584. Cai further discloses that HeLa cells were harvested by trypsinization and then the lysates were collected for further proteomics analysis. Ex. 1005 at 584. Cai further discloses that “[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.” Ex. 1005 at 585. Cai also discloses that “[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).” Ex. 1005 at 584.

201. 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.” Ex. 1005 at 590. Cai also states that “[p]roperties of the CNTs used in this study are summarized in SI Table 1.” Ex. 1005 at 584. A POSA would have understood that the Nanotech CNTs are the CNTs used in the method described in Cai (that is, Cai explains the acquisition of the CNTs for the purpose of using them in its study).

202. 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.” Ex. 1005 at 585. Figure 1, A shows a “[s]cheme of carbon nanoparticle pull-down experiment.”



Ex. 1005, Figure 1, A

203. Cai also discloses:

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 (Table S1 and Figure 2, A) and the recovery of proteins in the CNT pull-downs was studied.

Ex. 1005 at 585-586.

204. In my opinion, a POSA would have understood that HeLa cell lysate disclosed in Cai is a biological sample that is contacted with “[d]ifferent types of CNTs” of various diameters and lengths constitute the claimed “*a plurality of particles comprising different particle types*,” which is consistent with the ’360 Patent (as I explained above in Section XI.A.1 (limitation 1[a][1] discussing Hu)).

Ex. 1001 at 33:27-67, 86:10-22, Fig. 13, dependent claim 25.

205. As I explained above in my discussion of Hu in Section XI.A.1 (limitation 1[a][1]), a POSA would further have understood that incubation, as

disclosed in Cai, covers the claimed “*contacting*,” consistent with the ’360 Patent. Ex. 1001, 9:3-5, 21:30-42, 76:22-35, 83:1-63, dependent claims 18 and 19.

206. In addition to CNTs alone meeting the claimed “*a plurality of particles comprising different particle types*,” the combination of CNTs and CB in Cai also meets the claimed “*a plurality of particles comprising different particle types*.” That is, in my opinion, a POSA would also have understood that CB is a different type of nanoparticle from CNTs with a different size, surface topography, surface curvature, and shape. This is explained throughout Cai. Ex. 1005 at 583 (Hu’s Abstract describing MWCNTs and CBs are “two forms of carbon nanoparticles”), 583-584 (describing properties of CNTs), 585 (describing MWCNTs and CBs, that “[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”). Therefore, a POSA would have understood that the HeLa cell lysate (the claimed “*biological sample*”) is in contact with different particle types (CNTs and CB) as well.

***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,”***

207. Cai discloses a method “*to permit biomolecules of the biological sample to bind to the plurality of particles and form coronas around the plurality of particles*.” The contacting I explained above for limitation 1[a][1] permits

biomolecules (e.g., proteins) of the sample to bind to the CNTs and CB and form coronas around the particles.

208. As I explained above for limitation 1[a][1], Cai discloses various sized CNTs, for example, 20-40 nm length MWCNTs that were incubated with cell lysate prepared from HeLa cells, and the same samples were incubated with CB. Ex. 1005 at 585.

209. Cai also discloses sections entitled “Protein corona of carbon nanoparticles,” “Systematic characterization of carbon nanoparticle-protein corona,” “Functional classification of the carbon nanotube-protein corona,” and “Physiochemical properties of CNT-binding proteins,” which, as I explain further below, shows 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. Ex. 1005 at 583-589.

210. 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.” Ex. 1005 at 586.

211. 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. The same amount of HeLa cell lysate proteins was used in the binding assay (Figure 1,A). 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 SDSPAGE. Remarkably, 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). 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 after 3 hrs of incubation, but only less than 0.1% of these proteins are able to bind to the same amount of CB. The efficient interaction of proteins with CNTs is not restricted to proteins from cultured human carcinoma cells, protein extracts prepared from zebrafish, mouse, drosophila and algae also demonstrated a substantial affinity to CNTs (Figure S1).

Ex. 1005 at 585.

212. Cai includes additional disclosure such as “MWCNT (diameter 20-40 nm)-binding proteins” and “CB-binding proteins” (Ex. 1005 at 584); “protein corona on CNT” (Ex. 1005 at 583); “The binding of cellular proteins to CNTs...” (Ex. 1005 at 583); and “protein corona of CNTs and carbon black (CB)” (Ex. 1005 at 584).

213. Cai also discloses that CNTs—such as those used in its process—“are known to absorb a mixture of proteins to form a ‘protein corona’,” and “the formation of protein corona” as a result of the protein binding. Ex. 1005 at 583, 585. This is further reflected elsewhere in Cai. Ex. 1005 at 584, 586, 589, Figs. 1B, 3.

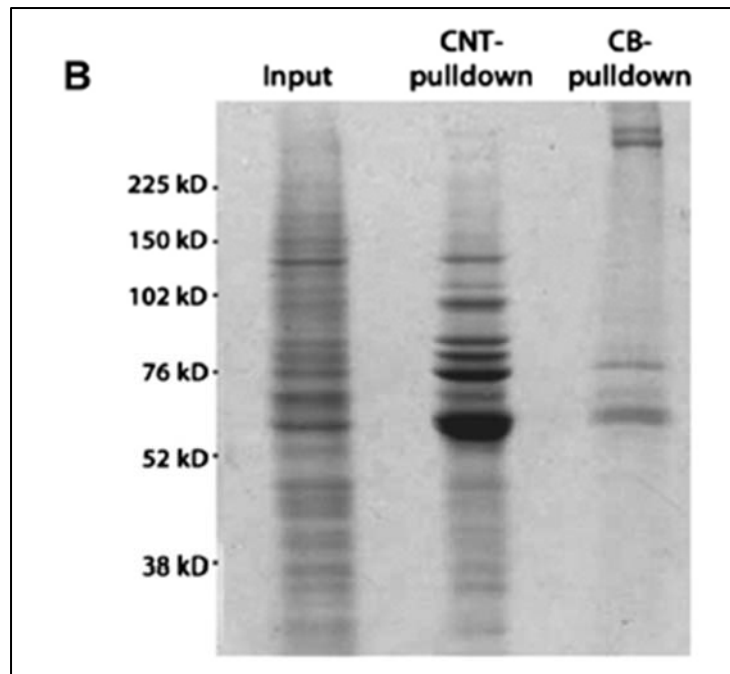
214. I note that the '360 Patent discloses the well-known phenomena that “biomolecule coronas” such as “protein corona[s]” are formed as biomolecules such as proteins “bind nanoparticles when they come into contact with biological samples or biological systems.” Ex. 1001 at 17:29-59, 18:26-39. This concept is consistent with results described in Cai.

215. Accordingly, in my opinion, a POSA would have understood that Cai’s incubation step allows 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.

***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;”***

216. Cai discloses a method “*wherein the coronas corresponding to the different particle types (i) differ based on particle type, and (ii) comprise overlapping and distinct proteins.*”

217. For example, 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.” Ex. 1005 at 586. Figure 1, B shows “[p]roteins from HeLA cell lysate pulled down by MWCNTs and CB were separated by SDS-PAGE and stained by coomassie blue.” Ex. 1005 at 586.



Ex. 1005, Figure 1, *B*

218. Cai also discloses that “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).” Ex. 1005 at 585. 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.” Ex. 1005 at 585.

219. In my opinion, a POSA would have understood that the SDS-PAGE gel in Figure 1, *B* shows that CNT and CB bound different proteins based on the band

intensities at different molecular weights. Specifically, as reflected in Figure 1, B, CNT has dark bands between 150 kD and above 76 kD that CB does not have, which indicates that there are essentially no proteins with the same molecular weights that bound to CB. The CB sample also has bands above 225 kD that CNT does not have, which shows that CNT and CB bind distinct proteins. CB and CNT do have bands that overlap in the 52-76 kD range, which suggests that there are overlapping proteins that bind to both CNT and CB in this molecular weight range.

220. Cai further discloses that its process used “MWCNTs of diameters of 20-40 nm or above [which] could pull-down a significant amount of proteins (Figure 2B, lanes 5-8), whereas those with diameters less than 10 nm bound to virtually no proteins (Figure 2B lanes 3-4).” Ex. 1005 at 586; *see also* 585 (“To 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.”). Cai also discloses that “[s]ingle walled CNT, typically with diameters less than 2 nm, also show no significant protein binding (Figure 2B, Lane 2).” Ex. 1005 at 586. Cai discloses that “[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 2B, Lanes 6, 8).” Ex. 1005 at 586.

221. Figure 2 shows “CNTs of different sizes exhibit different protein-binding capacities.” Ex. 1005 at 587. As described above, different diameters of multiwalled and single walled carbon nanotubes result in different effects on protein binding affinity. Ex. 1005 at 586-587.

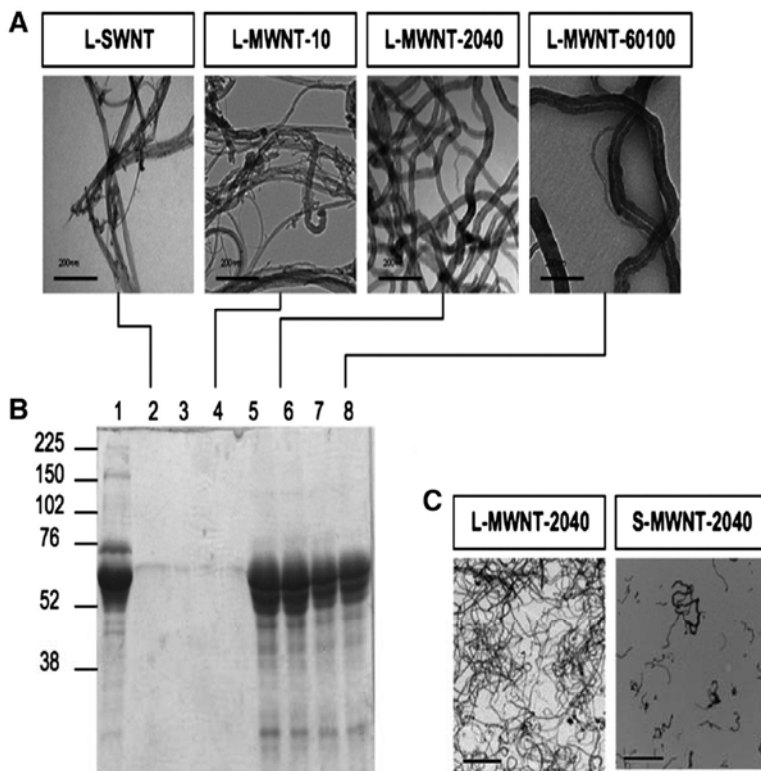


Figure 2. CNTs of different sizes exhibit different protein-binding capacities with. (A) TEM images of CNTs (length: 5–15 μm) with different diameters (Magnification 87000×). From left to right: L-SWNT with diameter <2 nm; L-MWNT-10 with diameter <10 nm; L-MWNT-2040 with diameter in range of 20–40 nm; L-MWNT-60100 with diameter in range of 60–100 nm. (B) FBS pulled down by CNTs of different sizes. Lane1: FBS; Lane2: SWNT; Lane3: S-MWNT-10; Lane4: L-MWNT-10; Lane5: S-MWNT-2040; Lane6: L-MWNT-2040; Lane7: S-MWNT-60100; Lane8: L-MWNT-60100. (C) MWNTs-2040 of different length. L-MWNT-2040, length: 5–15 μm; S-MWNT-2040, length: 1–2 μm.

Ex. 1005, Figure 2.

222. In my opinion, a POSA also would have understood that 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. A

POSA would also have understood that CNTs with diameters 20-100 nm have similar protein content and include overlapping corona proteins. 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.” Ex. 1005 at 587. 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.” Ex. 1005 at 587. In other words, based on the above, a POSA would have understood Cai discloses that the MWCNTs and CB particles have different coronas based upon these different particle types and also have overlapping and distinct proteins.

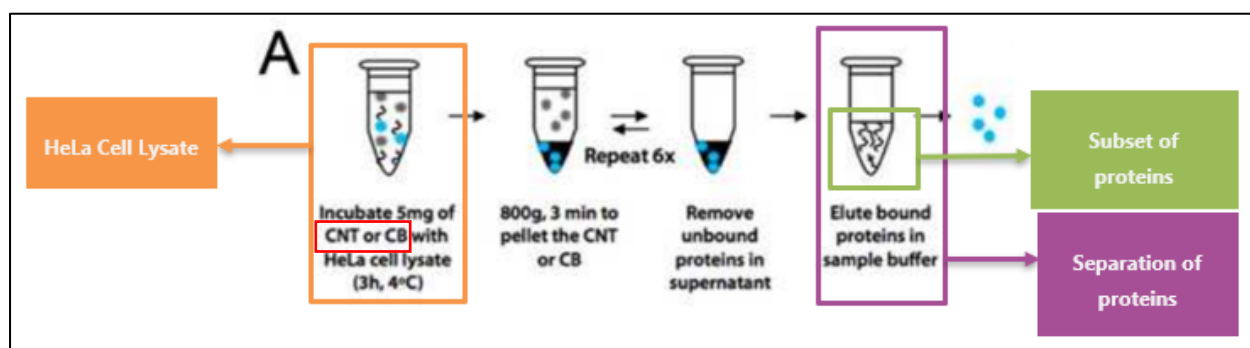
***1[b] “(b) separating at least a subset of the plurality of particles comprising the coronas from the biological sample by removing the subset of the plurality of particles thereby producing a subset of proteins from the biological sample;”***

223. Cai discloses a method that entails “*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.*” Figure 1, A (annotated, below where the

coloring and labeling with white text has been added except for the blue dots) of Cai, below, shows 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**. Ex. 1005 at 585. Cai describes the following:

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 after 3 hrs of incubation, but only less than 0.1% of these proteins are able to bind to the same amount of CB.

Ex. 1005 at 585.



Ex. 1005, Figure. 1A (annotated with coloring and boxes with white wording; blue dots in original).

224. In my opinion, a POSA would have understood that based on the different protein binding affinities for CNTs and CBs described in Cai, separating these particles and their protein coronas out from the HeLa cell lysate (the claimed

“*biological sample*”) would produce a subset of proteins that were bound to the particles from the lysate.

***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.”***

225. Cai discloses a method that entails “*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.*”

226. 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.” Ex. 1005 at 586. As I explained above for limitation 1[b], the proteins that selectively bound to MWCNT of diameters of 20-40 nm are a subset of proteins that were removed from the HeLa cell lysate (the claimed “*biological sample*”).

227. Cai further discloses that “[t]he mixtures were then separated by SDS-PAGE, and analyzed with the ‘gel-LCMS/MS’ approach.” Ex. 1005 at 586-587. 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. Ex. 1005 at 586-587, Fig. 1, B. Cai further

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)....” Ex. 1005 at 585. In my opinion, a POSA would have understood that the various disclosed methods are performed using devices to detect, assay, and characterize proteins. I further note that this is consistent with what is described in the ’360 Patent. Ex. 1001 at 21:43-50, 41:29-38, 75:20-77:23.

228. 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.” Ex. 1005 at 587. A POSA would have understood that these results were from assaying the biological sample using MS across a dynamic range large enough to detect both low abundant and high abundant proteins in the biological sample, as disclosed in Cai.

229. 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.**” Ex. 1005 at 587. (emphasis added) 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.*” See also Section VIII.A. As I described in Section VIII.A, the ’360 Patent relied on the use of a public database (“the plasma proteome database”) to look up known protein concentrations in biological samples to calculate the concentrations of detected proteins to arrive at detections of both high abundant and low or rare proteins (*e.g.*, concentrations of  $\leq 100$  or  $\leq 10$  ng/mg). Ex. 1001 at 39:56-40:1, 66:63-67, 67:1-75, 69:52-59, Table 5, Example 1B. A POSA contemplating the invention of the ’360 Patent would have understood a dynamic range of at least 6 orders of magnitude is a relative concentration range between high abundant proteins and low or rare abundant proteins detected from, *e.g.*, LC/MS-MS. Further, a POSA would have understood that the emPAI values of detected proteins disclosed by Cai that span from 0.02 to 2254683 are relative concentration ranges that are at least 6 orders of magnitude. Ex. 1005 at 12-17 (Table S2).

## 2. 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.”***

230. Cai discloses a method “*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.*” In particular, Cai discloses this limitation for the same reasons it discloses limitation 1[c] (such as mass spectrometry, liquid chromatography, and gel electrophoresis). Cai also discloses claim 1, from which claim 4 depends, as I explained above.

### 3. Claim 6

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

231. Cai discloses a method *“wherein the dynamic range of proteins detected spans 6 or more orders of magnitude”* for the same reasons as I discussed above for limitation 1[c]. That is, as I explained above in claim 1, Cai’s method detects a dynamic range spanning 8 orders of magnitude. Cai also discloses claim 1, from which claim 6 depends, as I explained above.

### 4. Claim 17

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

232. Cai discloses a method *“wherein the assaying further comprises digesting the proteins.”* Cai also discloses claim 1, from which claim 17 depends, as I explained above. Regarding the claim 17 limitation, Cai discloses:

Cell lysates were incubated with 5 mg of CNTs or CB for 3 hrs at 4 °C. After centrifugation at 800 g (3 min, 4 °C), the pellets were thoroughly washed with washing buffers (100 mM NaCl; 25 mM Tris–HCl pH 7.3; 1 mM EDTA, complete protease

inhibitors) until the OD280 of the supernatant was less than 0.1. The bound proteins were eluted by heating the pellets in 50 µl of LDS sample buffer (Invitrogen) to 70 °C for 10 min. In 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), and then followed a standard pull-down experiment. In some experiments, lysates were adjusted to pH 7.2 and pH 11, and then incubated with CNTs, and washed with a washing buffer of pHs 7.2 and 11, respectively. Three-way SILAC encoding of HeLa cells was done as described. Equal protein amounts (as measured by a Bradford assay) of the control cell lysate (light) and MWCNT (diameter 20–40 nm)-binding proteins (medium) were mixed together, CB-binding proteins (heavy) were then added into the mixture with the same volume as CNT-binding proteins. **The mixed protein samples were electrophoresed, gel-sliced and digested as described previously.**

Ex. 1005 at 584-585 (emphasis added).

233. A POSA would also have understood that Cai discloses digesting proteins with the enzyme trypsin, for example, through its disclosure of “trypsin as the enzyme.” Ex. 1005 at 585. This method is consistent to the digestion with trypsin described in the '360 Patent. Ex. 1001 at 59:20-21 (“digested by adding 2 micrograms of trypsin”), 59:58-59 (“For the database search, trypsin was specified as the proteolytic enzyme....”).

## 5. 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.”***

234. Cai discloses a method “*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*” for the same reasons as it discloses 1[pre] and limitation 1[a] (i.e., Cai discloses HeLa cell lysates), as I explained above. Cai also discloses claim 1, from which claim 22 depends, as I explained above.

**6. 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.”***

235. Cai discloses a method “*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*” for the same reasons as I explained above in claim 1 regarding the different sizes, surface topography, surface curvature, and shape of carbon black and carbon nanotube-

based nanoparticles. Cai also discloses claim 1, from which claim 25 depends, as I explained above.

**C. Ground 3: Claims 1, 4, 6, 17, 22, and 25 are Obvious in View of Cai**

236. As I have explained above, Cai discloses limitation 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,*” but if Cai is found not to disclose limitation 1[c] regarding the orders of magnitude, in my opinion, Cai would still render obvious claims 1, 4, 6, 17, 22, and 25.

237. In my opinion, as I explained above and as I explain below, 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 more than six orders a magnitude.

238. As I explained above for 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. 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)”:

More than 9,000 unique peptides were unambiguously mapped to more than 1,750 human proteins. The abundance distribution of each identified peptide among the “light”, “medium” and “heavy” categories was quantified by the MSQuant programme. In total, 778 proteins were accurately quantified (Table S2).

Ex. 1005 at 587.

239. Cai also discloses protein concentrations in the form of empirical peptide abundance index data (emPAI). Ex. 1005 at 12-17 (Table S2). Cai further refers to various other literature as part of its disclosure, including for example, Ishihama (Ex. 1018) for its emPAI conversion formula. Ex. 1005 at 587 (“An examination of the SILAC ratio of each quantified protein and its empirical peptide abundance index (emPAI), a semi-quantitative indication of the overall abundance of protein in the mixture, [note reference 30] reveals no strong correlation between the MWCNT- and CB-binding efficiencies of a protein and its abundance in the lysate (Figure 3, F),”), 593 (note reference 30 identifying Ishihama). In Ishihama, the formula for converting emPAI values into more traditional concentration numbers using a formula provided in a citation. Ex. 1005 at 587, 593 (n.30). Ishihama provides the following formula:

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

Ex. 1018 at 1267, Equation 3.

240. Looking at Cai's disclosure and discussion of Ishihama, 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). I again note that this known approach to looking up values in the prior art is consistent with the '360 Patent's reliance on the known database or other complementary techniques for protein quantification (*e.g.* a Bradford assay). Ex. 1001 at 66:63-69:67, 82:16-21, Fig. 12; Ex. 1014 at D960-D962; Ex. 1020 at 1.

241. Further, as I explained above for limitation 1[c], in Section XI.A.1 (regarding Hu), it was well-known and desirable in the field (and in fact, a necessity when assessing datasets from research such as the proteins detected in Cai's method) to look up the proteins concentration to determine the orders of magnitude of the dynamic range for the detected low abundant and high abundant proteins, and in my opinion, a POSA understood the need, desirability and routine analysis resulting in broader dynamic ranges including 6 orders and beyond (which was calculable from Cai's data and the state of the art and detectable using known techniques such as LC-MS/MS and others described above).

**D. Ground 4: Claims 1, 2, 4, 6-7, 17, 22, 23, and 25 are Obvious in view of Zaccaria**

242. In my opinion, Zaccaria (Ex. 1007) renders obvious claims 1-2, 4, 6-7, 17, 22, 23 and 25 of the '360 Patent. Zaccaria discloses all of the limitations, except it does not numerically state protein concentrations for assessing the orders of magnitude (which concentrations were well known in the field). I have considered Bryk (Ex. 1009), Dean (Ex. 1021) and Norouzi (Ex. 1022) as representative of POSA knowledge in analyzing how a POSA would interpret the disclosure of Zaccaria. As I described in Section IX.C.1, Bryk, Dean, and Norouzi disclose publicly known information on RBCs including reference ranges of density, size, protein content, and cell counts of RBCs, which a POSA would have naturally sought out to determine the numerical value for the orders of magnitude of the dynamic range detected using Zaccaria's process. I have considered the reference ranges reported by Dean and Norouzi to perform "unit conversions" on concentration values reported in Bryk in order to directly compare them to what is claimed by the '360 Patent.

**1. Claim 1**

***1[pre] "A method for assaying a biological sample, comprising:"***

243. To the extent the preamble of claim 1 is found to be limiting, it is my opinion that Zaccaria discloses "*a method for assaying blood samples*" as claimed

in claim 1, specifically, RBC lysates or hemolysates, using superparamagnetic nanoparticles to analyze the erythrocyte minor proteome. Ex. 1007 at 1869, 1878. Zaccaria further discloses “the potential capacity of three kinds of NPs [*i.e.*, nanoparticles] to capture RBC proteins was determined by qualitative and quantitative proteomics analysis.” Ex. 1007 at 1870.

244. Zaccaria also discloses “[f]or the three types of NPs, SDS-PAGE analysis of the eluted protein corona revealed a pattern obviously different from nontreated sample,” and “[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.” Ex. 1007 at 1872-73.

245. I further explain Zaccaria’s method in connection with the claim limitations below.

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

246. Zaccaria discloses a method including “*contacting the biological sample with a plurality of particles comprising different particle types*,” for example, contacting RBC lysate or hemolysate (the claimed “*biological sample*”) with three kinds of superparamagnetic nanoparticles (the claimed “*plurality of particles comprising different particle types*”).

247. 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.” Ex. 1007 at 1878-1880. Zaccaria also discloses that “[f]ive hundred micrograms of each kind of NPs were individually incubated in two replicates for 45 minutes with 200  $\mu$ L of hemolysate containing 1 mg of total protein.” Ex. 1007 at 1871.

248. Zaccaria also discloses that “[a]s particles fulfilled critical quality criteria, 500  $\mu$ g of each kind of NPs, developing 280  $\text{cm}^2$  surface area, were separately incubated in duplicates for 45 minutes with 200  $\mu$ L of RBC lysates ([i.e.], 1 mg of proteins), followed by extensive washing to remove all unbound proteins.” Ex. 1007 at 1872.

249. In my opinion, a POSA would have understood that the incubation step in Zaccaria with the RBC lysate or hemolysate and NPs corresponds to “contacting the biological sample with a plurality of particles.” Ex. 1007 at 1880. Again, this is consistent with the ’360 Patent as I explained above in Sections XI.A.1 and XI.B.1 for this limitation. Ex. 1001 at 9:3-5, 21:30-42, 76:22-35, 83:1-63, dependent claims 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,”***

250. Zaccaria discloses the claimed *“to permit biomolecules of the biological sample to bind to the plurality of particles and form coronas around the plurality of particles,”* for example, the contacting between RBC lysate and NPs that I explained above in limitation 1[a][1] allows proteins in the RBC lysate (i.e. biomolecules) to bind to the NPs and form coronas that cover the NPs. The '360 Patent supports that biomolecules can be proteins and protein coronas bind nanoparticles. Ex. 1001 at 15:7-11, 17:29-39, 18:26-38.

251. 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.” Ex. 1007 at 1869. Zaccaria also discloses “investigat[ing] the capacity of three different kinds of 100 nm NPs for protein capture in RBC lysates through the protein identification and quantification of their respective eluted corona.” Ex. 1007 at 1872. 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.” Ex. 1007 at 1872. Zaccaria further discloses that the proteins forming the coronas on the nanoparticles are then

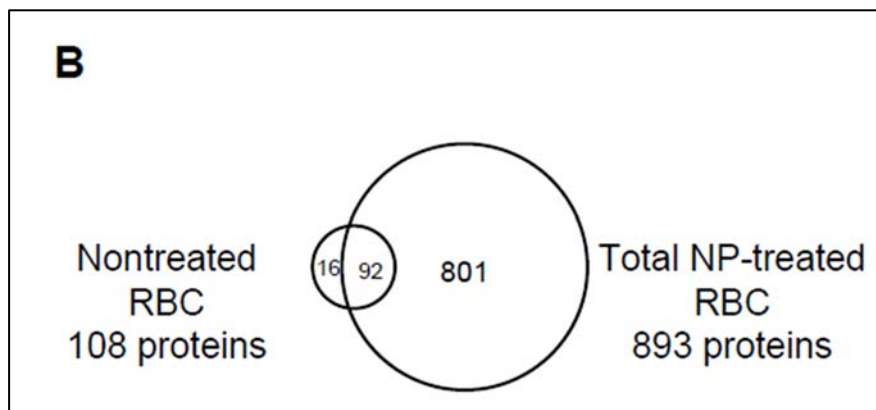
eluted, indicating that selected binding of proteins occurred on the nanoparticle: “the Bradford assays revealed  $19 \pm 1$   $\mu$ g of proteins eluted from the surface of each kind of NPs, which represented 2% of the initial protein input,” and “SDS-PAGE analysis of the eluted protein corona revealed a pattern obviously different from nontreated sample.” Ex. 1007 at 1872. 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.” Ex. 1007 at 1873.

252. In my opinion, based on the above, a POSA would have understood that the incubation step described in Zaccaria 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 the NPs, which as Zaccaria notes, is a well-recognized phenomenon that occurs when NPs are introduced to a solution or fluid containing proteins.

***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;”***

253. Zaccaria discloses “*wherein the coronas corresponding to the different particle types (i) differ based on particle type, and (ii) comprise overlapping and distinct proteins,*” for example, that “909 different proteins were identified from our RBC samples of which 893 were present in the list of NP-treated samples (Figure

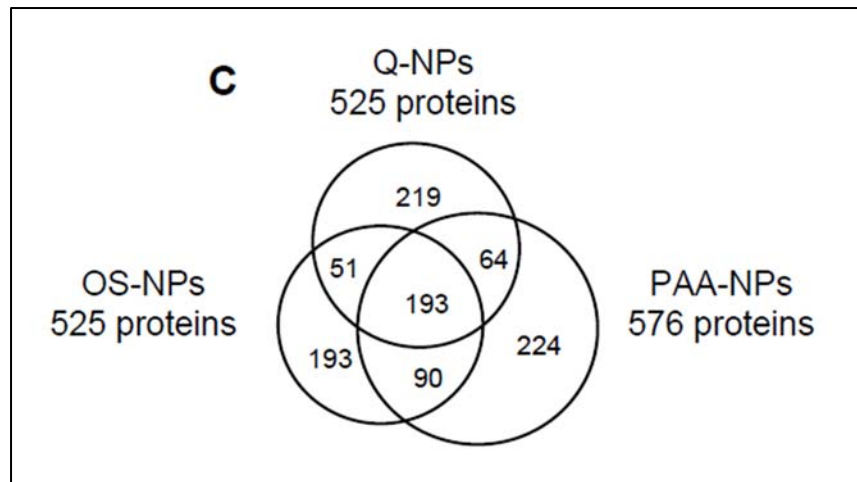
2B).” Ex. 1007 at 1873. Figure 2B shows the “[c]omparison between nontreated RBC extract (108 proteins) and all NP-treated samples (894 proteins).” Ex. 1007 at 1873. I note that there appears to be a typographical error between the text and Figure 2 in Zaccaria, identifying 893 or 894 proteins. This difference, however, does not impact my analysis or Zaccaria’s teachings.



Ex. 1007, Figure 2.

254. The Venn diagram in Figure 2B (above) shows that “[a]mong these 893 proteins, 801 were exclusively identified from NP-eluted coronae, and 92 were shared with crude RBC lysate.” Ex. 1007 at 1873.

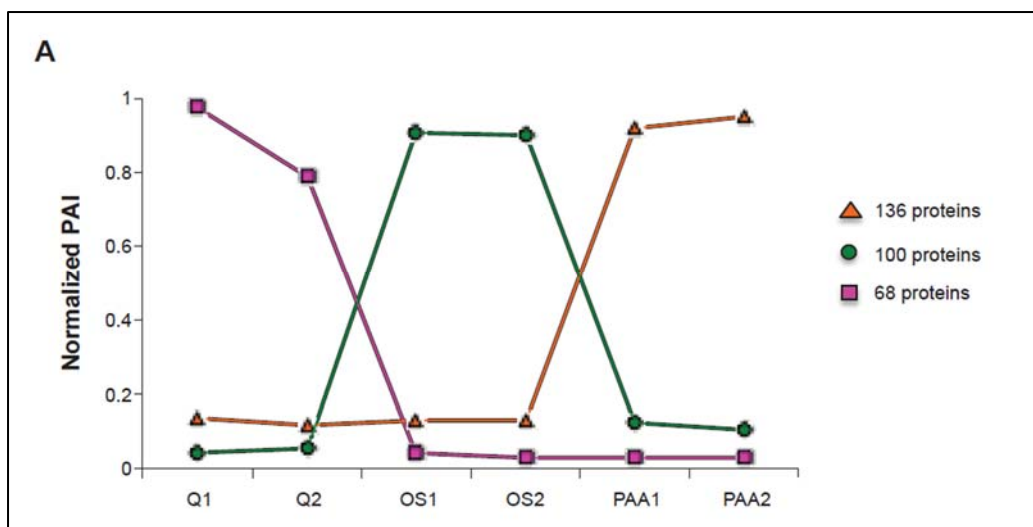
255. Figure 2C shows the “[c]omparison among the three NP-treated samples (PAA-NP, 576 proteins; OS-NP, 525 proteins; Q-NP, 525 proteins).” Ex. 1007 at 1873.



Ex. 1007, Figure 2C.

256. The Venn diagram in Figure 2C (above) shows that the three different NP samples had both distinct and overlapping proteins from the NP-eluted coronae. Ex. 1007 at 1873.

257. Zaccaria also discloses other analysis methods to evaluate the different proteins that constituted the coronae of the different nanoparticles. For example, Figure 4A shows a “[g]raphical representation of the mean normalized PAI value of three protein groups (obtained by hierarchical clustering) specific to NP surface.” Ex. 1007 at 1879.



Ex. 1007, Figure 4.

258. Figure 4A (above) shows that protein abundance index (“PAI”) values of each protein in each condition “revealed 68, 100, and 136 different proteins that specifically constituted the respective corona of Q-, OS-, and PAA-NPs.” Ex. 1007 at 1878.

259. Zaccaria also discloses “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.” Ex. 1007 at 1878. Zaccaria further discloses that “[t]hese 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.” Ex. 1007 at 1880. This demonstrates that the three types of nanoparticles exhibit protein capture specificity based on molecular weight and isoelectric point of the proteins. Ex. 1007 at 1878-80.

260. Accordingly, in my opinion, a POSA would have understood that the three different nanoparticle types in Zaccaria had different protein coronae based on the different physiochemical properties of each type of particle, which included overlapping and distinct proteins, as shown, for example, in Figures 2 and 4.

***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;”***

261. Zaccaria discloses a method that includes “*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.*” For example, as explained above for limitation 1[a][1] and 1[a][2], Zaccaria discloses incubating a plurality of nanoparticles (*e.g.*, three kinds of superparamagnetic NPs) with RBC lysates to form protein coronas around the nanoparticles.

262. More specifically, Zaccaria discloses that “[f]ive hundred micrograms of each kind of NPs were individually incubated in two replicates for 45 minutes with 200  $\mu$ L of hemolysate containing 1 mg of total protein.” Ex. 1007 at 1871. After this 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 three times in 500  $\mu$ L PBS,” and the “NP-associated proteins were desorbed using 40  $\mu$ L of 8 M urea-2% CHAPS, and the protein concentrations were determined by a Bradford protein assay.” Ex. 1007 at 1871.

263. Zaccaria further discloses that “[a]s particles fulfilled critical quality criteria, 500  $\mu$ g of each kind of NPs, developing 280  $\text{cm}^2$  surface area, were separately incubated in duplicates for 45 minutes with 200  $\mu$ L of RBC lysates ([i.e.], 1 mg of proteins), followed by extensive washing to remove all unbound proteins.” Ex. 1007 at 1872. This washing step allows for only proteins bound to the nanoparticles to remain from the RBC lysate sample.

264. In my opinion, a POSA would have understood that these steps described in Zaccaria, specifically isolating the particles with a magnet, removing the supernatant, and washing to remove unbound proteins, would result in separation of the particles, including particles with coronas, from the rest of the biological sample. Further, a POSA would understand the subsequent washing step would result in removal of unbound protein, leaving behind a subset of particles, including

particles with coronas isolated from the biological sample, for further analysis of the remaining bound proteins.

265. This is further confirmed by Zaccaria's disclosure that "the Bradford assays revealed  $19 \pm 1$   $\mu\text{g}$  of proteins eluted from the surface of each kind of NPs, which represented 2% of the initial protein input." Ex. 1007 at 1872. A POSA would have understood that 2% of the initial protein input that is described in Zaccaria is a subset of total protein from the RBC lysate (*i.e.*, biological sample) that was bound to the nanoparticles.

266. Zaccaria also 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." Ex. 1007 at 1872-1873. 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 from the RBC lysate were removed from the biological sample through binding to the nanoparticles.

***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.”***

267. It would have been obvious to include as part of Zaccaria the claimed “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.” In fact, Zaccaria discloses all but the particular 6 orders of magnitude in a numerical form. However, as I explain below, the 6 orders of magnitude amount to looking up known concentrations of the proteins provided in Zaccaria and a calculation using the information provided in Zaccaria and the state of the art.

268. 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.” Ex. 1007 at 1873; *see also* Ex. 1007 at 1871. Table 2 shows a comparison of “the 20 most abundant identified proteins (ie, the 20 proteins with the higher MS/MS queries) in nontreated and NP-treated RBCs.” Ex. 1007 at 1874.

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, erythrocytic                       | 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 1                                                             | 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          | Acylamino-acid-releasing enzyme                    | 5.29                       | 82,142  |        |                    |
| 247,171            | 5.15    | Spectrin beta chain, erythrocyte                                                 | 84                                                 | 0.4          | 14           | 1.3  | 239          | Claudin heavy chain 1                              | 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          | Imperidin-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 |              |                                                    |                            |         |        |                    |
|                    |         |                                                                                  |                                                    | 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 |                    |

(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, erythrocytic                       | 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                      |      |    |                                                |              | 41.8         |      | 41.8         |              | The 20 most abundant proteins                      |      |         |                    |
| Related to hemoglobin (among the 20 most abundant) |      |    |                                                |              | 20.0         |      | 29.5         |              | Related to hemoglobin (among the 20 most abundant) |      |         |                    |

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.

Ex. 1007, Table 2.

269. Notably, Table 2 identifies different types of Hemoglobin, such as Hemoglobin subunit beta and Hemoglobin subunit alpha, as some of the most

abundant proteins. Ex. 1007 at 1875-76. Zaccaria confirms the well-known knowledge that Hemoglobin is the most abundant protein found in RBC. Ex. 1007 at 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.”). Zaccaria also discloses all the proteins identified from the RBC lysate in its supplemental data, which includes lower abundant proteins, a subset of the biological sample that was assayed with an instrument to show their concentrations across a dynamic range comprising at least 6 orders of magnitude. Ex. 1007 at 24-31 (data for “All 909 Proteins”; pages 24-31 are the page numbers in the bottom corner under PREOMICS EXHIBIT 1007).

270. One example of a lower abundant protein identified in Zaccaria was myotubularin-related protein 5 (SBF1) (MS/MS queries reported to be 10). As mentioned earlier, an example of a higher abundance protein identified was Hemoglobin subunit alpha (HBA1) (MS/MS queries reported to be in a range of 244 and 1563). Ex. 1007 at 24-31 (Table S2).

271. These two identified proteins were also detected in RBC samples using an LC-MS/MS technique disclosed by Bryk. As I described in Section IX.C.1, Bryk discloses concentrations of these two proteins as  $1.47 \times 10^4$  pmol/mg for high

abundance protein, HBA1 and  $6.62 \times 10^{-6}$  pmol/mg for low abundance protein, SBF1.<sup>6</sup> These two concentrations differ by at least six orders of magnitude.

272. In other words, while Zaccaria does not specifically state the dynamic range in numerical form, in my opinion, a POSA would have understood, or at least found it obvious, from Zaccaria's disclosure and the knowledge of a POSA as exemplified in the disclosure of Bryk and explained above, that proteins in the subset are detected with the instrument at concentrations across a dynamic range comprising at least six orders a magnitude.

273. A POSA contemplating Zaccaria and seeking to understand the concentration range differentiating a relatively low abundance protein (*e.g.*, SBF1) in comparison to that of a relatively high abundance protein (*e.g.*, HBA1) would turn to other publicly available information to determine the expected concentration of these species in hemolysate (the biological sample assayed by Zaccaria). A POSA would have been easily able to determine from publicly available information, such as the disclosure of Bryk, that HBA1 is normally present in RBC lysate in a high

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<sup>6</sup> As I explained in Section IX.C.1, the concentration of HBA1 is an average across the four donors ranging from  $1.44 \times 10^4$  pmol/mg to  $1.55 \times 10^4$  pmol/mg. The concentration of SBF1 is the lowest non-zero detected concentration. Detected concentrations of SBF1 from the four donors range from  $6.62 \times 10^{-6}$  pmol/mg to  $1.56 \times 10^{-3}$  pmol/mg. Using the lowest detected concentration of HBA1 ( $1.44 \times 10^4$  pmol/mg) and the highest non-zero detected concentration of SBF1 ( $1.56 \times 10^{-3}$  pmol/mg), the two concentrations also differ by at least six orders of magnitude.

concentration (*e.g.*,  $1.47 \times 10^4$  pmol/mg) and SBF1 in a low concentration (*e.g.*,  $6.62 \times 10^{-6}$  pmol/mg). Both proteins are well-known components of RBC, and Bryk represents one example of a publicly available value or peer-reviewed study in which these proteins were assayed. Ex. 1009 at 16-61 (Table S3). Accordingly, the NP-based method disclosed by Zaccaria detected proteins in the biological sample at concentrations across a dynamic range comprising at least 6 orders of magnitude.

274. Zaccaria discloses 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.

Ex. 1007 at 1881.

275. Zaccaria further describes how 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.

Ex. 1007 at 1869-70.

276. Zaccaria also discloses that “[f]or the three types of NPs, SDS-PAGE analysis of the eluted protein corona revealed a pattern obviously different from nontreated sample.” (Ex. 1007 at 1872); and “[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.” (Ex. 1007 at 1873).

277. A POSA would have understood that Zaccaria’s teachings that I describe above are consistent with the problem of detecting a broad range of protein concentrations, and specifically low-abundant and rare proteins, that the ’360 Patent is trying to solve. See, *e.g.*, Ex. 1001 at 72:1-24, 20:26-27.

278. Accordingly, in my opinion, Zaccaria is consistent with the ’360 Patent and prosecution history regarding the “dynamic range” and orders of magnitude by also disclosing the utility of an instrument to measure a dynamic range of ten orders

of magnitude to capture the entire proteome of biological fluids, providing more support that Zaccaria renders obvious claim 1.

279. Lastly, as I explained above for limitation 1[c] in Section XI.A.1 (regarding Hu) and in Section XI.C (regarding Cai), it was well-known and desirable in the field (and in fact, a necessity when assessing datasets from research such as the proteins detected in Zaccaria's method) to look up the protein concentrations to determine the orders of magnitude of the dynamic range for the detected low- and high-abundant proteins, and in my opinion, a POSA understood the need, desirability and routine analysis to evaluate the broader dynamic ranges including 6 orders and beyond (which was calculable from Zaccaria's data and the state of the art and detectable using known techniques as I described above in Sections XI.B and XI.C).

## **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.”***

280. It would have been obvious to include as part of Zaccaria the claimed *“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.”* As discussed in Section XI.D.1, Zaccaria renders obvious claim 1, from which claim 2 depends.

281. For example, as discussed above, Zaccaria reported detection of hemoglobin subunit beta (HBA1) and carbonic anhydrase 1 (CA1), high abundance proteins disclosed by Bryk as being present in hemolysate at concentrations as high as  $1.47 \times 10^4$  pmol/mg and  $1.80 \times 10^3$  pmol/mg, respectively. A POSA would be able to perform a unit conversion calculation on these concentrations and find that they are in excess of 1 mg/ml (*i.e.*, 27 mg/ml and 6 mg/ml for HBA1 and CA1, respectively).<sup>7</sup> Zaccaria also reported detections of phosphoinositide 3-kinase regulatory subunit 4 (PIK3R4) and myotubularin-related protein 5 (SBF1), low abundance proteins disclosed by Bryk as being present in hemolysate at concentrations as low as  $3.17 \times 10^{-4}$  pmol/mg and  $6.62 \times 10^{-6}$  pmol/mg, respectively (*see* Sections IX.C.1). A POSA would be able to perform a unit conversion

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<sup>7</sup> As I described in Section IX.C.1, concentrations of high abundance proteins (HBA1 and CA1) disclosed by Bryk in units of pmol/mg were converted to ng/ml using the molecular weight of the same protein reported in Zaccaria and publicly available information on RBCs (from Bryk, Dean, and Norouzi) including reference ranges of the mean volume of RBCs (*i.e.*, 90 fl), the average total protein content per cell (*i.e.*, 20% of RBC mass), the density of RBC (*i.e.*, 1.110 g/ml), and the number of RBC counts per volume of human blood (*e.g.*, 4-6 million/mm<sup>3</sup>). The unit conversion uses 6 million/mm<sup>3</sup> for the number of RBC counts per volume of human blood. Using any value from a reference range disclosed by Bryk, the converted concentrations are above 1 mg/ml.

calculation on these concentrations and find that they are below 10 ng/ml (i.e., 5.86 ng/ml and 0.17 ng/ml for PIK3R4 and SBF1, respectively).<sup>8</sup>

### 3. 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”.***

282. Zaccaria discloses “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,” for example, assaying using SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and nano-liquid chromatography tandem mass spectrometry (nano-LC-MS/MS) as I discussed regarding claim 1. Ex. 1007 at 1872-1873. Zaccaria also renders obvious claim 1, from which claim 4 depends, as I explained above.

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<sup>8</sup> As I described in Section IX.C.1, concentrations of low abundance proteins (PIK3R4 and SBF1) disclosed by Bryk in units of pmol/mg were converted to ng/ml using the same unit conversion described previously for high abundance proteins. The unit conversion also uses 6 million/ mm<sup>3</sup> for the number of RBC counts per volume of human blood. Using any value from a reference range disclosed by Bryk, the converted concentrations are below 10 ng/ml.

4. **Claim 6**

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

283. It would have been obvious to include as part of Zaccaria the claimed “*wherein the dynamic range of proteins detected spans 6 or more orders of magnitude*” for the same reasons as I discussed above for limitation 1[c]. That is, as I explained above in claim 1, it would have been obvious to that Zaccaria’s method detected 6 or more orders of magnitude. Zaccaria also renders obvious claim 1, from which claim 6 depends, as I explained above.

5. **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.”***

284. It would have been obvious to include as part of Zaccaria “*wherein the detected proteins comprise proteins present in concentrations of 100 ng/ml or less in the biological sample*” for the same reasons as I discussed above for claim 1, specifically phosphoinositide 3-kinase regulatory subunit 4 (PIK3R4) and myotubularin-related protein 5 (SBF1), which were detected at concentrations of  $3.17 \times 10^{-4}$  pmol/mg and  $6.62 \times 10^{-6}$  pmol/mg, respectively, in the biological sample, which are equal to 5.86 ng/ml and 0.17 ng/ml for PIK3R4 and SBF1, respectively. Zaccaria also renders obvious claim 1, from which claim 7 depends, as I explained above.

6. **Claim 17**

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

285. Zaccaria discloses “*wherein the assaying further comprises digesting the proteins.*” Zaccaria also renders obvious claim 1, from which claim 17 depends, as I explained above. For example, Zaccaria discloses that to analyze the RBC proteins from the NP assay, “10 µg of each duplicated eluate and crude RBC lysate were separately diluted in Laemmli buffer and stacked in one band after a short and low-voltage electrophoretic migration on SDS-polyacrylamide gel electrophoresis (SDS-PAGE) gel.” Ex. 1007 at 1871. Zaccaria further discloses that “[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...” Ex. 1007 at 1871.

7. **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.”***

286. Zaccaria discloses “*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*” for the same reasons as it discloses 1[pre] and limitation 1[a] (human blood and RBC

lysate), as I explained above. Zaccaria also renders obvious claim 1, from which claim 22 depends, as I explained above. For example, Zaccaria discloses assaying RBC lysates (or hemolysates) of blood samples using superparamagnetic nanoparticles to analyze the erythrocyte minor proteome. Ex. 1007 at 1869-70, 1873, 1878. Zaccaria further discloses that “[b]lood samples were collected from five healthy consenting donors, three men and two women aged 44-62 years,” “RBC lysis was performed,” and “erythrocytes were carefully collected.” Ex. 1007 at 1870.

#### **8. 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.”***

287. Zaccaria discloses “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.” Zaccaria also renders obvious claim 1, from which claim 23 depends, as I explained above.

288. As discussed above with respect to claim 1, Zaccaria discloses a method for detecting biomolecules. In addition to the proteins detected by this method and discussed above with respect to claim 1, Zaccaria also detected Apolipoprotein A-I (APOA1) which is a lipoprotein species. Ex. 1007 at 24-31 (Table S2).

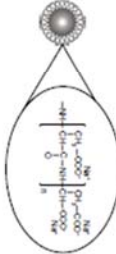
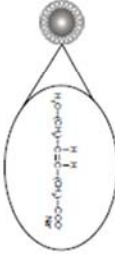
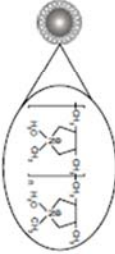
9. Claim 25

***“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.”***

289. Zaccaria discloses “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.” Zaccaria also renders obvious claim 1, from which claim 25 depends, as I explained above.

290. For example, as shown in Table 1, Zaccaria discloses that there are “[t]hree different kinds of NPs grafted with different matrices and functional groups... fluidMAG-OS (OS-NP), fluidMAG-PAA (PAA-NP), and fluidMAG-Q (Q-NP).” Ex. 1007 at 1870. Zaccaria further discloses “according to dynamic light scattering and zeta potential measurements, the respective NPs were homogeneous in size and monodispersed Q-NPs displayed a positive charge, and PAA-NPs and OS-NPs both displayed a negative charge (Table 1).” Ex. 1007 at 1872.

| Table 1 NP properties |                    |                    |                        |
|-----------------------|--------------------|--------------------|------------------------|
|                       | FluidMAG-PAA       | FluidMAG-OS        | FluidMAG-Q             |
| Matrix                | Aspartic acid      | Oleic acid         | PolyDADMAC             |
| Functional group      | Sodium carboxylate | Sodium carboxylate | Quaternary amine group |
| Diameter (nm)         | 112.4              | 107.7              | 106.9                  |
| PDI                   | 0.11               | 0.12               | 0.19                   |
| Zeta potential (mV)   | -37                | -26                | +41.0                  |

Notes: The manufacturer provides information about the matrix and the functional group. The mean diameter, the PDI, and the zeta potential have been determined with a Malvern Zetasizer NanoZS. NPs were diluted in PBS (pH 7.3) before measurement, and the measurement was conducted at 25°C using 0.5 mg/mL NP concentrations. Abbreviations: NP, nanoparticle; PDI, polydispersity index; PBS, phosphate-buffered saline; PolyDADMAC, polydiallyldimethylammonium chloride.

Ex. 1007, Table 1.

291. Zaccaria further discloses using “three kinds of 100 nm 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.” Ex. 1007, 1878-1880. Zaccaria teaches “[t]he positively charged Q-NPs revealed 90% of specific proteins that were negatively charged at pH 7.4 ... whereas negatively charged OS- and PAA-NPs harvested less than 70% of specific protein ... confirming previous reports that suggested NP surface charge would influence the protein corona” Ex. 1007 at 1878. Accordingly, a POSA would have understood that Zaccaria’s particles differ by, *e.g.*, surface charge and surface functionality.

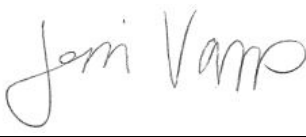
## XII. Concluding Statements

292. In signing this declaration, I understand that the declaration will be filed as evidence in a contested case before the Patent Trial and Appeal Board of the United States Patent and Trademark Office. I acknowledge that I may be subject to cross-examination in the case and that cross-examination will take place within the United States. If cross-examination is required of me, I will appear for cross-examination within the United States during the time allotted for cross-examination.

293. I declare that all statements made herein of my knowledge are true and correct. I also declare that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 35 U.S.C. § 1001.

294. I declare under the penalty of perjury that the foregoing is true and correct.

Executed on October 6, 2024

A handwritten signature in cursive script, appearing to read "Jerri Vargo", written in dark ink. The signature is positioned above a horizontal line.

Jessica Vargas, PhD