



PREOMICS GMBH AND BIOGNOSYS AG
v.
THE BRIGHAM AND WOMEN'S HOSPITAL, INC.
IPR2024-01473
U.S. Patent No. 11,435,360

Petitioners' Demonstratives

Oral Argument — January 15, 2026

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

US 11,435,360 (“’360 Patent”)

(12) United States Patent Farokhzad et al.	(10) Patent No.: US 11,435,360 B2 (45) Date of Patent: *Sep. 6, 2022
(54) SYSTEM AND SENSOR ARRAY	(56) References Cited
(71) Applicant: THE BRIGHAM AND WOMEN'S HOSPITAL, INC. , Boston, MA (US)	U.S. PATENT DOCUMENTS
(72) Inventors: Omid Farokhzad , Waban, MA (US); Morteza Mahmoudi , Brookline, MA (US); Claudia Corbo , Milan (IT)	5,498,545 A 3/1996 Vestal 5,928,952 A 7/1999 Hutchins et al. 5,952,653 A 9/1999 Covey et al. 6,291,183 B1 9/2001 Pirnang 6,361,944 B1 3/2002 Mirkin 6,413,780 B1 7/2002 Bach et al. 6,649,419 B1 11/2003 Anderson 6,730,517 B1 5/2004 Koster et al. 6,759,010 B2 7/2004 Lewis 6,969,615 B2 11/2005 Knezevic 7,348,184 B2 3/2008 Rich et al. 7,375,234 B2 5/2008 Sharpless et al. 7,442,921 B2 10/2008 Franzen 7,754,861 B2 7/2010 Boschetti et al. 7,980,184 B2 6/2011 Merozov et al. 8,021,891 B2 9/2011 Bird 8,268,264 B2 9/2012 Lenz 8,357,537 B2 1/2013 Blecka et al. 8,906,608 B2 12/2014 Boschetti et al. 9,005,994 B2 4/2015 Hao 9,234,895 B2 1/2016 Hood 9,689,039 B2 6/2017 Wong 9,758,811 B2 9/2017 Brown et al. 9,945,994 B2 4/2018 Hebnink et al. 10,144,955 B2 12/2018 Fernando 10,866,242 B2* 12/2020 Farokhzad G01N 33/6848 2002/0009394 A1 1/2002 Koster et al. 2002/0127727 A1 9/2002 Bach et al. 2004/0132122 A1 7/2004 Banerjee et al. 2004/0147040 A1 7/2004 Shugget et al. 2004/0153249 A1 8/2004 Zhang et al. 2005/0053999 A1 3/2005 Gough et al. (Continued)
(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days. This patent is subject to a terminal disclaimer.	FOREIGN PATENT DOCUMENTS
(21) Appl. No.: 17/215,923	CN 103874770 6/2014 CN 103703143 12/2016 (Continued)
(22) Filed: Mar. 29, 2021	OTHER PUBLICATIONS
(65) Prior Publication Data US 2021/0311039 A1 Oct. 7, 2021	Hajipour et al. (Biomater. Sci., 2014, 2, 1210-1221) (Year: 2014). Walkey, et al. (ACS Nano, 2014, 8, 2439-2455) (Year: 2014). Hadjidemetriou et al. (vol. 9, No. 8, pp. 8142-8156, 2015). (Year: 2015). Micallef, J. et al. Applying mass spectrometry based proteomic technology to advance the understanding of multiple myeloma. Journal of hematology & oncology 3, 1 (2010). Miller, A., Hoogstraaten, B., Staggel, M. & Winkler, A. Reporting results of cancer treatment. cancer 47, 207-214 (1981). (Continued)
(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.	Primary Examiner — Lisa V Cook (74) Attorney, Agent, or Firm — Quarles & Brady LLP
(60) Provisional application No. 62/435,409, filed on Dec. 16, 2016.	(57) ABSTRACT The present disclosure provides a system comprising a communication interface and computer for assigning a label to the biomolecule fingerprint, wherein the label corresponds to a biological state. The present disclosure also provides a sensor arrays for detecting biomolecules and methods of use. In some embodiments, the sensor arrays are capable of determining a disease state in a subject.
(51) Int. Cl. G01N 31/00 (2006.01) G01N 33/53 (2006.01) G01N 33/68 (2006.01) C01G 49/02 (2006.01) G06K 9/00 (2022.01) G06N 20/20 (2019.01) G06K 9/62 (2022.01) G01N 33/543 (2006.01) G16B 40/20 (2019.01) G01N 33/574 (2006.01) B82Y 35/00 (2011.01)	(58) Field of Classification Search None See application file for complete search history.
(52) U.S. Cl. CPC G01N 33/6848 (2013.01); C01G 49/02 (2013.01); G01N 33/5432 (2013.01); G01N 33/54326 (2013.01); G01N 33/57488 (2013.01); G01N 33/6842 (2013.01); G06K 9/00563 (2013.01); G06K 9/6267 (2013.01); G06N 20/20 (2019.01); G16B 40/20 (2019.02); B82Y 35/00 (2013.01); C01P 2004/64 (2013.01)	29 Claims, 121 Drawing Sheets

Title: System and Sensor Array

Assignee: The Brigham And Women's Hospital, Inc.

Claims under review: 1–4, 6-7, 17, 22–25

EX1001 (’360 Patent) at Cover; Paper 1 (Petition) at 1



Independent Claim 1

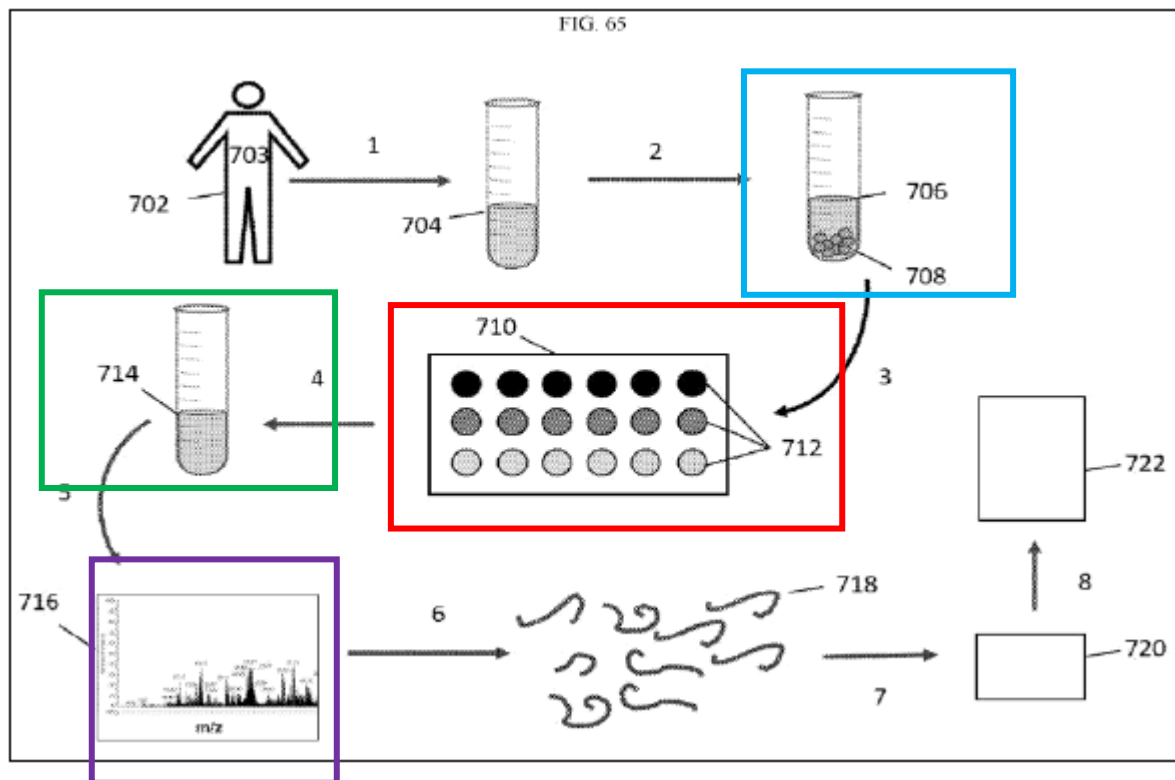
A method for assaying a **biological sample**, comprising:

- 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.

EX1001 ('360 Patent) at cl. 1; Paper 1 (Petition) at viii



The '360 Patent – Exemplary Embodiment



Next, as shown at step 3 of FIG. 65, a complex biological sample (e.g., plasma 706) can be incubated with a sensor array 710 comprising a plurality of particles 712 with different physicochemical properties. The plurality of particles 712 can be incubated with the plasma 706 to allow biomolecules in the plasma 706 (e.g., proteins in the plasma 706) to bind to one or more of the particles 712. Subsequently, as shown at step 4 of FIG. 65, the biomolecules (e.g., proteins) bound to the particles 712 can be isolated in a protein solution 714 for further analysis, for example to determine the compositions of the proteins bound to each type of particle 712 (e.g., anionic, neutral and cationic particles 712).

The protein solution 714 can be characterized by, for example as shown at step 5 of FIG. 65, liquid chromatography-tandem mass spectrometry (LC-MS/MS) 716. Proteins identified using LC-MS/MS 716 can then be analyzed at step 6 of FIG. 65 to determine a biomolecule fingerprint 718 (e.g. representative of proteins, nucleic acids, lipids and polysaccharides that bind to one or more of particles 712) associated with the biological state 703.

EX1001 ('360 Patent) at FIG. 65, 21:30-50; Paper 1 (Petition) at 4-6

The '360 Patent – “dynamic range”

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

EX1001 ('360 Patent) at 20:20-32; Paper 1 (Petition) at 6, 10, 14, 38



The '360 Patent – “dynamic range”

approaches. 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. In other words, the protein corona composition enables sampling across a vast dynamic range of the plasma proteome, which can substantially enhance the depth of protein coverage without protein depletion. Since

EX1001 ('360 Patent) at 72:1-25; Paper 1 (Petition) at 6, 14, 38, 76



Instituted Grounds of Unpatentability

Ground	Reference	Statutory Basis	Claims
1	Hu (EX1008)	§103	1–4, 6, 7, 17, 22–25
2	Cai (EX1005)	§102	1, 4, 6, 17, 22, 25
3	Cai	§103	1, 4, 6, 17, 22, 25
4	Zaccaria (EX1007)	§103	1, 2, 4, 6, 7, 17, 22, 23, 25

Paper 1 (Petition) at 3



Petitioners' Expert – Dr. Vargas



Dr. Vargas is a chemist who... has diverse experience in the fields of polymer science, analytical chemistry, **biochemistry**, and drug and gene delivery.

Her **interdisciplinary training in chemistry and biochemistry** enables her to support clients in applications related to pharmaceuticals, medical devices, wearables, food, cleaning products, cosmetics, and other consumer products. Dr. Vargas is experienced in designing and validating non-standard methods for chemical evaluations, and **she is skilled in the techniques utilized in organic synthesis and small molecule characterization, including NMR, FTIR, and UV-Vis spectroscopy, as well as chromatography and mass spectrometry methods such as GC-MS, HPLC, LC-MS, and MALDI-TOF. She also has practical knowledge characterizing polymeric materials and nanoparticles using size-exclusion chromatography (SEC), dynamic light scattering, and zeta potential measurements.**

Dr. Vargas was a National Science Foundation Graduate Research Fellow in the Department of Chemistry at Stanford University, where **she developed biodegradable block copolymers for small molecule and nucleic acid drug delivery, such as pDNA, siRNA, and mRNA. Her research leveraged organocatalytic polymerization techniques to synthesize functionalized polycarbonates and polyesters. She designed cellular and in vivo assays to measure the delivery efficiency and cytotoxicity of the resulting biomaterials using fluorescence microscopy, flow cytometry, and bioluminescent imaging.** She also previously interned at W.L. Gore & Associates, where she developed and qualified analytical test methods used in coated stent manufacturing.

Academic Credentials & Professional Honors

Ph.D., Chemistry, Stanford University, 2017

M.A., Education, Loyola Marymount University, 2011

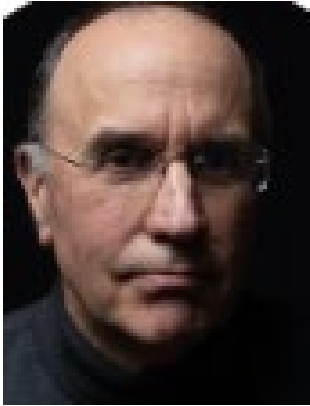
B.S., Biochemistry, Loyola Marymount University, 2010

Stanford Chemistry Safety in Teaching and Research Award, 2016

EX1004 at 1; Paper 1 (Petition) at 11



Patent Owner's Expert – Dr. Cima



MICHAEL J. CIMA

David H. Koch Professor of Engineering
Department of Materials Science and Engineering
Koch Institute for Integrative Cancer Research

3 **Q. You've never been in the biology**
4 **department at MIT; correct?**

5 A. No. I'm at the Koch Institute, which
6 is half cancer biology and half engineering.

7 **Q. And your half is the engineering**
8 **side; right?**

9 A. That's right.

EX1036 at 28:3-9; Paper 27 (Pet. Reply) at 26



CLAIM CONSTRUCTION



Institution Phase: PO “agrees no construction is needed at this stage”

Petitioner “submits that no express construction of terms is necessary.” Pet. 15. Patent Owner agrees no construction is needed at this stage. See Prelim. Resp. 26.

We agree that no formal claim construction is necessary for this decision. *Realtime Data, LLC v. Iancu*, 912 F.3d 1368, 1375 (Fed. Cir. 2019) (“The Board is required to construe ‘only those terms that . . . are in controversy, and only to the extent necessary to resolve the controversy.’”) (quoting *Vivid Techs., Inc. v. Am. Sci. & Eng’g, Inc.*, 200 F.3d 795, 803 (Fed. Cir. 1999)). To the extent we have concerns regarding the parties’ apparent interpretation of the claim language, or in some instances Patent Owner’s attempt to read-in unrecited limitations, we address those arguments below.

POR: “Claim Construction”

V.Claim Construction	13
A. “a biological sample”	13
B. “a subset of the plurality of particles comprising the coronas”	15



“biological sample” – the ’360 Patent

The term “sample” refers to a biological sample or a complex 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

EX1001 (’360 Patent) 20:33-40; Paper 1 (Petition) at 23, 48; Paper 27 (Pet. Reply) at 2-3



“biological sample” – the ’360 Patent

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.

EX1001 (’360 Patent) cl. 22; Paper 1 (Petition) at 43, 61, 79;
EX1003 ¶ 133; Paper 27 (Pet. Reply) at 2-3, 6



“biological sample” – the Prior Art (Cai)

194.

source of the human proteome.” Ex. 1005 at 585. Cai similarly discloses that “Cell lysates were incubated....”. Ex. 1005 at 584.

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).

Protein corona of carbon nanoparticles

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 1A). Both MWCNT and CB formed loose agglomerates that could be collected by low-speed centrifugations after protein binding.

EX1003 ¶¶ 194, 196-197 (citing EX1001 at 20:32-45); Paper 1 (Petition) at 48

EX1005 (Cai) at 585; Paper 1 (Petition) at 48



“biological sample” – the Prior Art (Hu)

130. In my opinion, a POSA would have understood Hu’s method of using Fe₃O₄ 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).

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.

3.3. Identification and quantification of plasma proteins bound on Fe₃O₄ NPs with different sizes

In our study, to guarantee identification accuracy, each sample was analyzed in at least three replicates, and only proteins detected in at least two runs and identified by at least two unique peptides in each analysis were considered as constituents of the protein coronas formed on the Fe₃O₄ NPs. The protein coronas bound on the Fe₃O₄ NPs after 1 h of incubation with human plasma were used to investigate the size effects on the formation of protein coronas on the Fe₃O₄ NPs. In total, there were 117, 133 and 100

“biological sample” – the Prior Art (Zaccaria)

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.

RBC preparation and lysis

Blood samples were collected from five healthy consenting donors, three men and two women aged 44–62 years (mean 55.4 ± 7.7 years) by venipuncture in ethylenediaminetetraacetic acid vacutainer tubes. RBC lysis was performed according to the protocol previously published by Roux-Dalvai et al.³⁷ Briefly, samples were firstly centrifuged at $1,000 \times g$ at $+4^\circ\text{C}$ for 10 minutes to eliminate plasma and buffy coat. To further eliminate leukocytes, a ficoll gradient separation was performed. After removal of the monolayer containing mononuclear cells, erythrocytes were carefully collected, avoiding top RBC layer and polynuclear cells collected in the pellet. Erythrocytes were then washed three times with PBS + PMSF (154 mM NaCl, 10 mM phosphate buffer, pH 7.4 containing 0.1 mM PMSF). At each step, along with supernatant, the upper RBC layer was removed.

EX1003 ¶243 (citing EX1007 at 1869-70, 1878); Paper 1 (Petition) at 64

EX1007 (Zaccaria) at 1870; Paper 1 (Petition) at 64



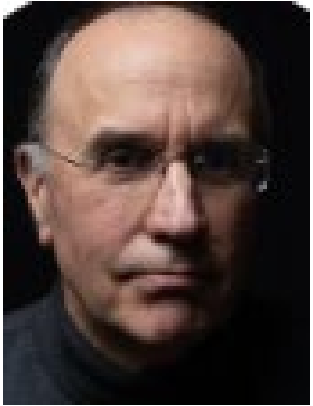
“biological sample” – POR Constructions

EX2022, 36:10-37:8 (Dr. Vargas confirming); EX2023, ¶¶43-44. Thus, the claimed “a biological sample” is limited by definition to what is obtained from an *individual* subject or patient. The definition would exclude a lysate taken from an immortalized cell line that is engineered and grown in a laboratory (e.g., Cai’s HeLa cell populations) rather than obtained from a subject. Additionally, the definition excludes a mixture of materials from different donors for the purpose of eliminating individual differences, thereby precluding the ability to assay a biological sample from any given subject (e.g., pooled blood plasma or RBCs in Hu and Zaccaria, respectively). The petition never proposed nor substantiated any contrary construction.

Paper 18 (POR) at 14; Paper 27 (Pet. Reply) at 3



“biological sample” –Dr. Cima’s Testimony



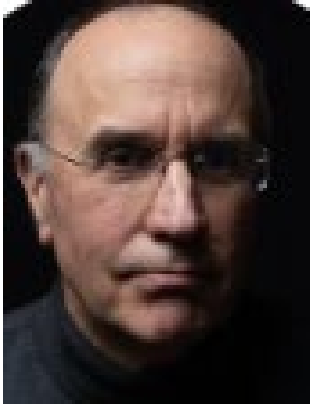
Q. Right. So there is an “or” there’s the phrase “biological sample,” and then there's the word “or,” and then there’s the phrase “complex biological sample.” Right? So doesn’t that mean that a sample can be either a biological sample or complex biological sample?

A. So the second sentence defines biological sample; right. “Suitable biological samples include, but are not limited to, biological fluids, including, but not limited to, systemic blood, plasma, serum, lung.” You know, these are all -- so that's biological sample. Whether you want to refer to those as complex or not, the actual sentence only refers to them as biological samples.

EX1036, 63:4-64:7; Paper 27 (Pet. Reply) at 2



“biological sample” –Dr. Cima’s Testimony



Q. So I take it you don't have an understanding of the plain meaning of biological sample –

A. That’s –

Q. -- is that your testimony?

A. No.

Q. If it's not your testimony, then tell me what your understanding is.

A. It's -- in the context of this patent, it’s a sample derived from a patient.

EX1036, 40:13-41:2; Paper 27 (Pet. Reply) at 6

Q. So Henrietta Lack is the original donor from whom all HeLa cells derive; correct?

A. That's right

EX1036, 57:9-11; Paper 27 (Pet. Reply) at 6

“a subset of the plurality of particles...”

'360 Patent Independent 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.

EX1001 ('360 Patent) at cl. 1; Paper 1 (Petition) at viii



“a subset of the plurality of particles...” - Board in ID

proteins subsequently isolated from each. Indeed, limitation 1(b) does not require that all of the protein coronas formed in limitation 1(a) be separated, but instead recites “*separating at least a subset* of the plurality of particles comprising the coronas.” Ex. 1001, 86:61–62 (emphasis added). Given its ordinary meaning, this language suggests limitation 1(b) could be met by separating particles from only one of the different particle types in limitation 1(a). Accordingly, to the extent Patent Owner continues this argument at trial, it should explain how it construes claim 1 to support the distinction it attempts to draw with Cai and identify any intrinsic or extrinsic support for that construction.

“a subset of the plurality of particles...” - Board in ID

We say this for several reasons. First, neither claim 1, nor any of the other challenged claims, recite a sensor array or otherwise state that the different particle types must be contacted with a biological sample at the same time. Second, the recitations in claims 13 and 14 (which are not challenged in the Petition) affirmatively suggest that claim 1 is not so limited.¹¹ See *InterDigital Commc'ns, LLC v. Int'l Trade Comm'n*, 690 F.3d 1318, 1324 (Fed. Cir. 2012) (“The doctrine of claim differentiation is at its strongest in this type of case, ‘where the limitation that is sought to be ‘read into’ an independent claim already appears in a dependent claim.’”) (quoting *Liebel-Flarsheim Co. v. Medrad, Inc.*, 358 F.3d 898, 910 (Fed. Cir. 2004)).

Paper 11 (ID) at 25; Paper 27 (Pet. Reply) at 12



“a subset of the plurality of particles...” Claims 13 and 14

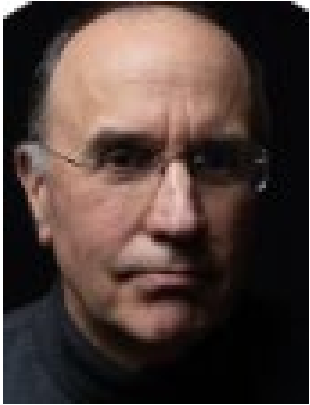
13. The method of claim 1, wherein the assaying comprises detecting a protein from the corona of a first particle from among the plurality of particles and a protein from the corona of a second particle from among the plurality of particles at the same time, wherein the first particle and the second particle are different particle types.

14. The method of claim 13, wherein the detecting is performed on an array.

EX1001 ('360 Patent), claims 13-14; Paper 11 (ID) at 25; Paper 27 (Pet. Reply) at 12



“a subset of the plurality of particles ...” – Dr. Cima’s Testimony



Q. Well, if you have a set of four things A, B, C, D, and you take a subset of it, the subset could be just A; right?

A. So let me just do it this way. Let’s have two types of elements in the set. I have five elements of A and five elements of B. A subset would be -- would include four elements of A and four elements of B.

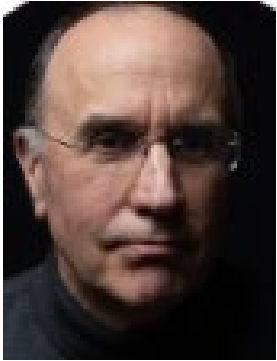
Q. And they could also include just B; right?

A. If I could pick those, **yes.** Because it didn't -- **needn't be all of them.**

EX1036, 70:3-14; Paper 27 (Pet. Reply) at 9-10

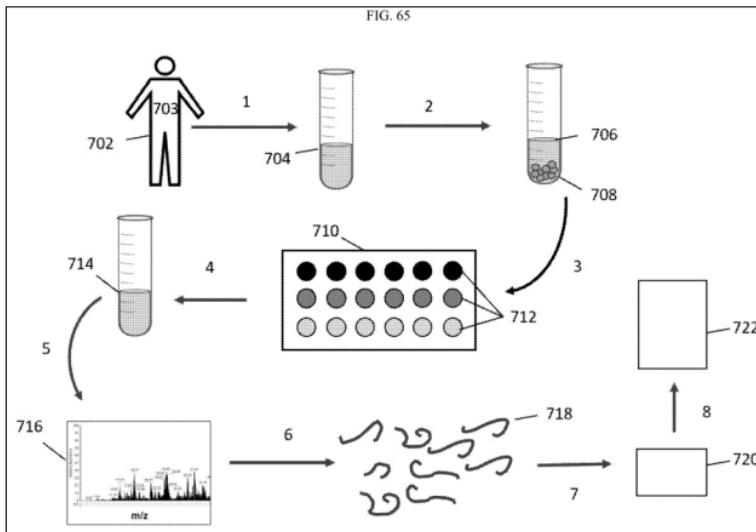


“a subset of the plurality of particles ...” – Dr. Cima’s Testimony



Q. Okay. So how many particle types are within each one of the circles?

A. I'm presuming -- well, I don't -- they don't say. But it could -- it could be two or it could be three. It could be one. They don't say. It's just that each one of those is meant to represent different samples.



EX1001('360 Patent) , Fig. 65; Paper 1
(Petition) at 4-6; Paper 27 (Pet. Reply) at 11

EX1036, 73:12-18; Paper 27 (Pet. Reply) at 11



“a subset of the plurality of particles...” PO Sur-Reply

Yet, both Drs. Vargas and Cima testified that the words “the coronas” in element 1(b) invoke *the coronas* corresponding to different particle types from element 1(a). POR 15-20; EX2023, ¶¶45-46 (Dr. Cima); EX2022, 26:6-27:2 (Dr. Vargas: “Q. You agree that those are both two requirements for what the claim calls ‘the coronas,’ correct? A. Yes.”); *see also* EX2022, 27:21-28:3 (Q. “[D]o you believe that the coronas in element 1B did not have to satisfy the requirements of the coronas in step 1A? A. I think they have to satisfy 1A....”), 28:22-29:7 (Q. “So you’re saying there, there needs to be at least two particle types; is that correct? A. The plurality of particles, operationally, how you do this is that it would be [at least two] types.”). Again, the Reply never squarely addresses the claim language,

Paper 33 (PO Sur-Reply) at 16-17



“a subset of the plurality of particles...” Dr. Vargas’ Testimony

Q. Is it correct then when you were performing your analysis for this case, do you believe that the coronas in element 1B did not have to satisfy the requirements of the coronas in step 1A?

A. I think they have to satisfy 1A, but it could still be a subset of the total number of the plurality of particles that were contacted in part A.

EX2022 at 27:21-28:3; Paper 33 (PO Sur-Reply) at 16-17



UNPATENTABILITY GROUNDS



Ground 2: Anticipation by Cai

360 Patent Claim 1	Cai
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.	✓

Cai Discloses All Limitations of Claim 1

“In this study, we utilised stable isotope labeling by amino acids in cell culture (SILAC)-based quantitative proteomics to characterise the binding of human cellular proteins to **two forms of carbon nanoparticles: namely multi-walled carbon nanotubes (MWCNTs) and carbon black (CB)**. The relative binding efficiency of over 750 proteins to these materials is measured. The data indicate that MWCNTs and CB bind to vastly different sets of proteins.”

“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.”

“Mass spectrometry was performed on an LTQ-orbitrap mass spectrometer system”

“The relative abundance of the MWCNT-binding proteins spread over 8 orders of magnitude.”

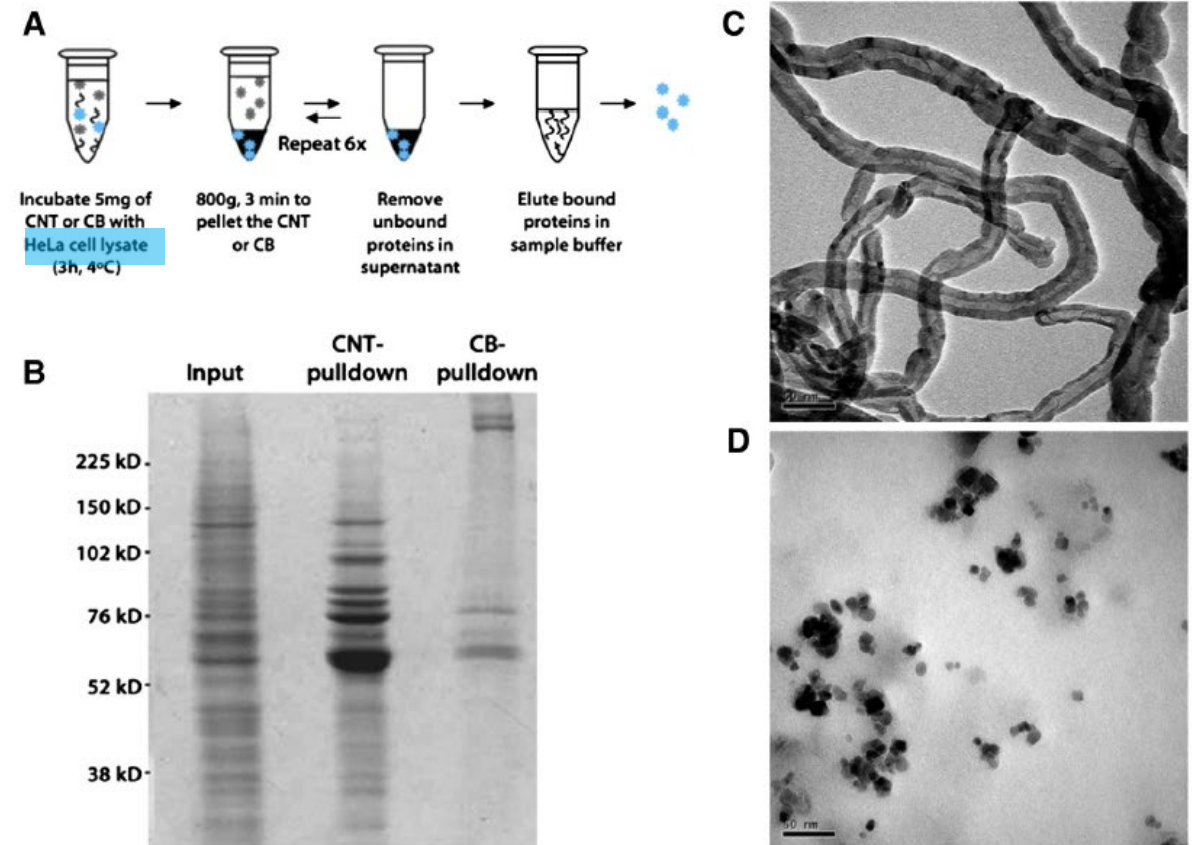


Figure 1. Protein binding assay to study MWCNT- and CB-protein corona. (A) Scheme of carbon nanoparticle pull-down experiment. (B) Proteins from HeLa cell lysate pulled down by MWCNTs and CB were separated by SDS-PAGE and stained by coomassie blue. (C-D) Transmission electron micrographs of the MWCNTs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

EX1005 (Cai) at FIG. 1, Abstract, 585-587; Paper 1 (Petition) at 17-19

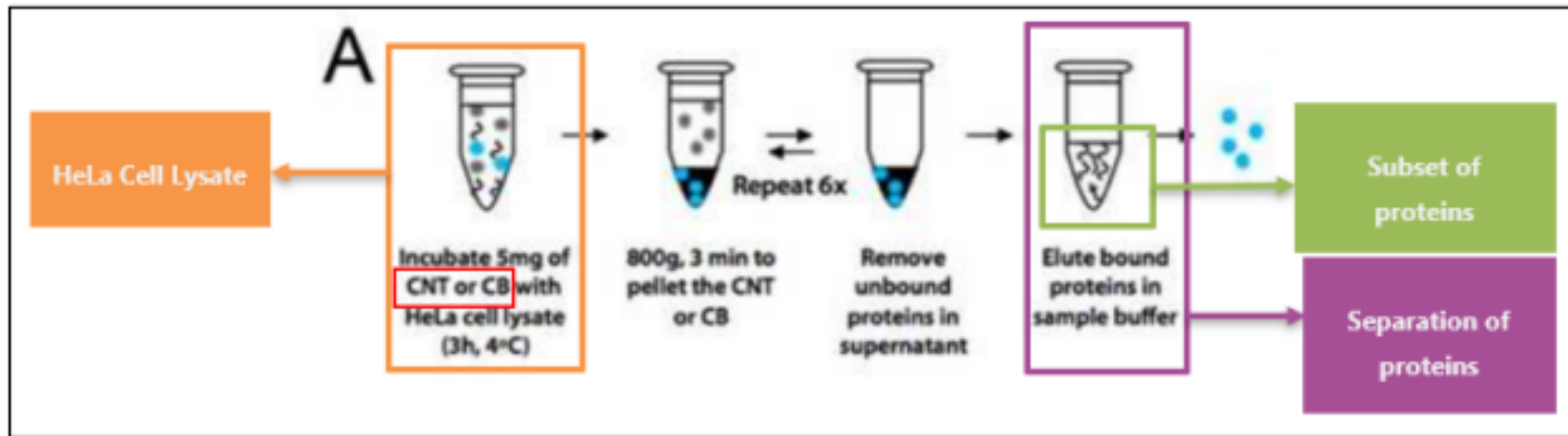
POR Cai Arguments

VI. Ground 2 (Cai Anticipation) Fails	21
A. Cai’s HeLa Cell Lysate Is Not “A Biological Sample” As Claimed.	23
B. Cai Fails to Disclose Pulling Down a Subset of Particles Comprising the Coronas (Element 1(b)).	28
C. Cai Fails to Disclose Element 1(c).	32
D. Dependent Claims	34



Cai – “contacting” and “separating”

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**



EX1003 ¶ 223 (citing EX1005 (Cai), 585), Paper 1 (Petition) at 57-58

Dr. Vargas on Cai CNTs

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).

EX1003 ¶¶ 199-21 (citing EX1005 (Cai), 583-85), Paper 1 (Petition) at 50



Dr. Vargas on Cai CNTs and CBs

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”).

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.

EX1003 ¶¶ 199, 206 (citing EX1005 (Cai), 583-85), Paper 1 (Petition) at 57-58



Dr. Vargas on Cai CNTs and CBs

Q. I want to ask you briefly about panel C and D in figure 1. So in panel D, there's little kind of discreet dots and in panel C there's what looked like tendrils perhaps. Can you explain what we're seeing in panel C and D?

A. Yes. Those are transmission electron micrographs or TEM images of the nanoparticles used in this study. Panel C is a TEM image of the multi-walled carbon nanotubes, which, as you mentioned, appear to be sort of tendril-like. And in panel D, the carbon black particles are smaller and look more punctate, if you will.

EX2022, 107:9-20; Paper 18 (POR) at 25



Dr. Vargas on Cai CNTs and CBs

Q. So I guess what I'm asking is, if I have a million CB particles in the lysate and the first depiction of the tube, and when I centrifuge it I get 400,000 of the particles in the pellet, 600,000 are still floating in the supernate and I decant that supernate with 600,000 of the million particles. At that point in that tube I have a subset of particles comprising the CB corona in that vial; correct?

A. Yes, that's correct.

Q. Okay. And it doesn't matter that I didn't get all of the CB particles. I have the subset -- I have a subset of CB particles comprising the CB corona; correct?

A. Correct.

EX2022, 119:19-120:9; Paper 18 (POR) at 30



Cai's "study"

The protein corona of a nanomaterial is a complex layer of proteins spontaneously and stably formed when the material is exposed to body fluids or intracellular environments. In this study, we utilised stable isotope labeling by amino acids in cell culture (SILAC)-based quantitative proteomics to characterise the binding of human cellular proteins to two forms of carbon nanoparticles: namely multi-walled carbon nanotubes (MWCNTs) and carbon black (CB). The relative binding efficiency of over 750 proteins to these materials is measured. The data indicate that MWCNTs and CB bind to vastly different sets of proteins. The molecular basis of selectivity in protein binding is investigated. This study is the first large-scale characterisation of protein corona on CNT, providing the biochemical basis for the assessment of the suitability of CNTs as biomedical tools, and as an emerging pollutant.

EX1005 (Cai), Abstract; Paper 1 (Petition) at 46



Cai – Institution Decision

First, we do not agree with Patent Owner’s characterization of Cai as disclosing “two separate studies” that Petitioner has improperly pieced together. *See* Prelim. Resp. 22, 42. Rather, Cai describes a single study “to characterize the binding of human cellular proteins to two forms of carbon nanoparticles: namely multi-walled carbon nanotubes (MWCNTs) and carbon black (CB)” involving the use of several analytical techniques. The current record supports that a POSA would have understood that the process depicted in Cai’s Figure 1A was used to isolate the cell lysate proteins bound to the MWCNT and CB particles for subsequent testing, including the LC-MS/MS with SILAC encoding method that produced the results Petitioner relies upon for limitation 1(c).

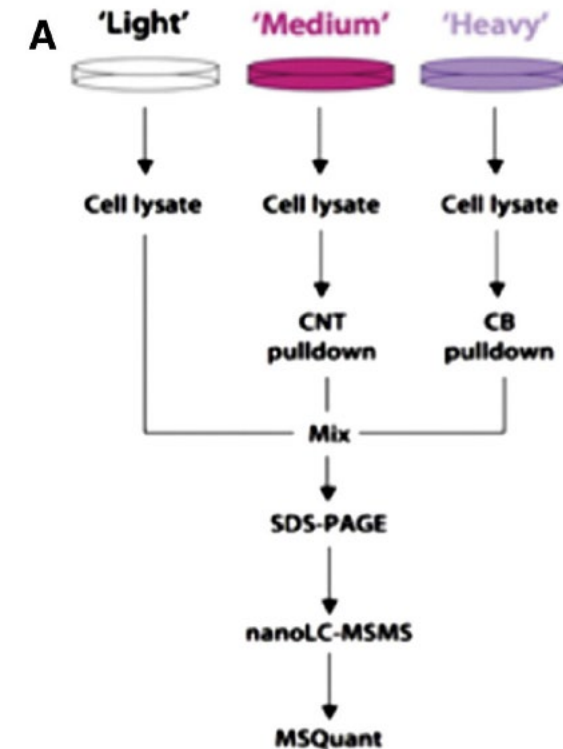
Paper 11 (ID) at 18-19; Paper 27 (Pet. Reply) at 13



Cai – Dr. Vargas Testimony

Q. In figure 3, that is the depiction of the SILAC encoding experiment; correct?

A. In figure 3, it includes both the SILAC encoding and the, what they refer to as CNT pull down, which relies on the general work flow that's depicted in scheme 1A -- or excuse me, figure 1A.



EX2022, 129:14-20; EX1005 (Cai) at 588 (FIG. 3A); Paper 27 (Pet. Reply) at 13-14

Cai - “8 orders of magnitude”

of a protein and its abundance in the lysate (Figure 3, *F*). The relative abundance of the MWCNT-binding proteins spread over 8 orders of magnitude. Some of the proteins with the highest binding efficiencies, as indicated by the SILAC ratios, are low abundance proteins. This suggests that protein binding to MWCNTs is unlikely to be a non-specific aggregation of the highly abundant proteins with MWCNTs. Instead, the binding of proteins to MWCNT and CB appears to be protein-selective. We

EX1005 (Cai), 587; Paper 1 (Petition) at 18



Cai - “Reasons for Allowance” in CIP App. No. 18/644,619

REASONS FOR ALLOWANCE

1. The following is an examiner’s statement of reasons for allowance: The instant method is drawn to protein analysis by assaying a plasma or serum sample utilizing magnetic particles comprising an iron oxide core, a polymer, an ammonium functional group having a positive surface charge wherein an instrument that measures concentration across a dynamic range of at least 6 orders of magnitude is employed. The reference to Cai et al., “Characterization of Carbon Nanotube Protein Corona by Using Quantitative Proteomics,” *Nanomedicine: Nanotechnology, Biology and Medicine* 9 (2013) 583-593, with its supplementary data (Table S2) teaches the dynamic range of measurement

EX1041, 6; Paper 27 (Pet. Reply) at 20-21



The '360 Patent – “dynamic range”

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

EX1001 ('360 Patent) at 20:20-33; Paper 1 (Petition) at 6, 10, 14, 38



The '360 Patent – “dynamic range”

approaches. 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. In other words, the protein corona composition enables sampling across a vast dynamic range of the plasma proteome, which can substantially enhance the depth of protein coverage without protein depletion. Since

EX1001 ('360 Patent) at 72:1-28; Paper 1 (Petition) at 6, 14, 38, 76



Ground 3: Obviousness over Cai

360 Patent Claim 1	Cai
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.	✓

Dr. Vargas on Cai's Dynamic Range

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.

EX1003 ¶¶ 239-40 (citing EX1005 (Cai) at 12-17, 585, 587, 593; EX1018 at 1267), Paper 1 (Petition) at 62-63



Ground 3 – Dr. Vargas

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).

EX1003 ¶ 241; Paper 1 (Petition) at 63-64



Ground 3 – Dr. Vargas

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.

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.

EX1003 ¶¶ 170, 173; Paper 1 (Petition) at 37-38, 63-64



Ground 3 – PO's Primary Argument

Additionally, Petitioners' proposed modification does nothing to remedy the failure of the identified HeLa cell lysate to qualify as the claimed biological sample, the failure of Cai's SILAC experiment to satisfy claim element 1(a), or Cai's failure to disclose pulling down a subset of particles comprising the coronas, as required by claim element 1(b). Petitioners' conclusory obviousness argument thus fails for at least the same reasons as detailed in Ground 2. *Supra*, Section VI; EX2023, ¶70.

Paper 18 (POR) at 36; Paper 27 (Pet. Reply) at 21



Ground 1: Obviousness over Hu

360 Patent Claim 1	Cai
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.	✓

POR Hu Arguments

VIII. Ground 1 (Hu) Fails	36
A. Hu Fails to Disclose Assaying “A Biological Sample” As Claimed.	38
B. Hu Fails to Disclose Separating a Subset of the Plurality of Particles Comprising the Coronas As Required By 1(b).	38
C. Hu Fails to Disclose Detecting Proteins At Concentrations Across a Dynamic Range Comprising At Least 6 Orders of Magnitude As Claimed.	42
1. Petitioners Fail To Demonstrate Hu Actually Detected LTBP1 at a concentration of 1.7-6.0 ng/mL.	43
2. Petitioners’ Inherency Argument is Unsubstantiated and Self- Defeating.	46
3. Petitioners’ Alternate Obviousness Theory Fails.	49



Hu – “contacting...with a plurality of particles”

kept the same for the three different particle sizes. For this purpose, the mass of Fe_3O_4 NPs of different sizes (suspended in PBS with a concentration of 0.2%, w/w) possessing a total surface area ($A = 4\pi R^2$) of 0.01 m^2 was calculated and incubated in a constant amount of human plasma at 37°C with shaking at 1000 rpm for 1 h. The ratio of the plasma volume to the particle surface area was adjusted to a constant value of 5.56 mL/m^2 , as reported previously (PBS was added to obtain a total volume of 1 mL) [23,24].

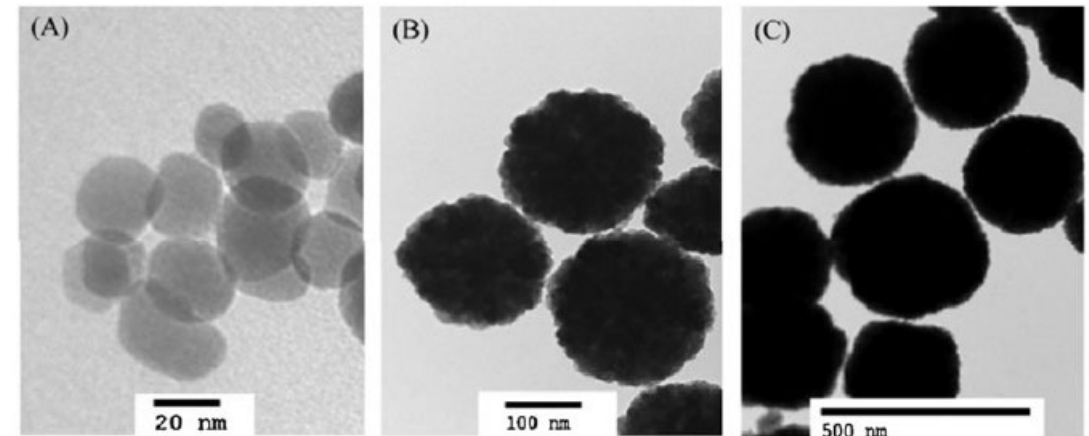


Fig. 1. TEM images of the Fe_3O_4 NPs of different sizes (Fe_3O_4 -30 (A), Fe_3O_4 -200 (B) and Fe_3O_4 -400 (C)).

EX1008 (Hu) at 355, Fig. 1; Paper 1 (Petition) at 23-24; EX1003 ¶¶ 134-35

Hu – “separating at least a subset...”

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.

EX1003 ¶ 151 (citing EX1008 (Hu), 355), Paper 1 (Petition) at 31-32

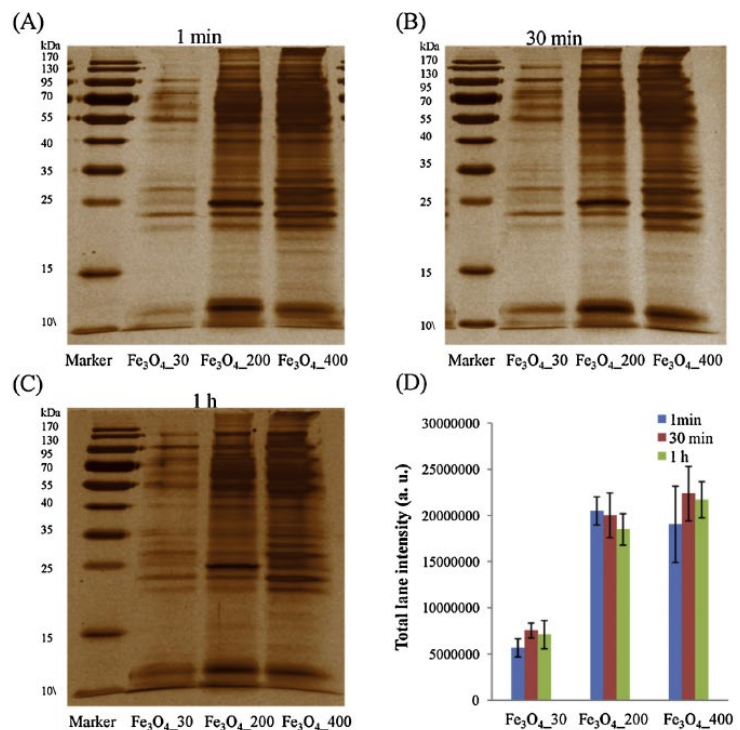


Fig. 2. Silver stained SDS-PAGE gel lanes showing the plasma protein binding profiles on different sizes of Fe₃O₄ NPs after incubation in human plasma for 1 min (A), 30 min (B) and 1 h (C) and the corresponding optical intensities of each gel lanes for the plasma proteins adsorbed on the Fe₃O₄ NPs. The error bars represent the standard deviation from the mean (n=3).

EX1008 (Hu), Fig. 2; Paper 1 (Petition) at 32; EX1003 ¶ 152

Hu - “at least 6 orders of magnitude”

Table 2
The top 20 most abundant plasma proteins bound on the Fe₃O₄ NPs of different sizes.

Rank	Fe ₃ O ₄ -30	Fe ₃ O ₄ -200	Fe ₃ O ₄ -400
1	Platelet factor 4	Apolipoprotein A-I	Apolipoprotein E
2	Apolipoprotein E	Serum albumin	Vitronectin
3	Vitronectin	Apolipoprotein C-III variant 1	Histidine-rich glycoprotein
4	Platelet basic protein	Apolipoprotein C-I	Platelet basic protein
5	IGK@ IGK@ protein	Apolipoprotein E	Platelet factor 4
6	IGKC Anti-RhD monoclonal T125 kappa light chain	Isoform LMW of Kininogen-1	Complement C1q subcomponent subunit C
7	Putative uncharacterized protein	Vitronectin	Complement C1q subcomponent subunit A
8	Complement C1q subcomponent subunit C	Platelet factor 4	Isoform 1 of Gelsolin
9	IGHG1	Histidine-rich glycoprotein	APOC1 Apolipoprotein C-I
10	Apolipoprotein C-III variant 1	Platelet basic protein	Complement C3 (Fragment)
11	IGHG1	Isoform HMW of Kininogen-1	Prothrombin (Fragment)
12	IGHG1	Isoform 1 of Gelsolin	Serum albumin
13	Prothrombin (Fragment)	Apolipoprotein A-II	CIQB 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

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 Fe₃O₄ 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

EX1008 (Hu), 357; Paper 1 (Petition) at 34-35; EX1003 ¶¶ 156-159



Hu - “at least 6 orders of magnitude”

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).

EX1003 ¶ 161; Paper 1 (Petition) at 35-36



The '360 Patent – “dynamic range”

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

EX1001 ('360 Patent) at 20:20-33; Paper 1 (Petition) at 6, 10, 14, 38



The '360 Patent – “dynamic range”

approaches. 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. In other words, the protein corona composition enables sampling across a vast dynamic range of the plasma proteome, which can substantially enhance the depth of protein coverage without protein depletion. Since

EX1001 ('360 Patent) at 72:1-28; Paper 1 (Petition) at 6, 14, 38, 76



Ground 1 – Dr. Vargas

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.

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.

EX1003 ¶¶ 170, 173; Paper 1 (Petition) at 37-38



Ground 4: Obviousness over Zaccaria

360 Patent Claim 1	Cai
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.	✓

POR Zaccaria Arguments

IX. Ground 4 (Zaccaria) Fails 53

 A. Zaccaria Fails to Disclose Assaying “A Biological Sample” As
 Claimed.....53

 B. Zaccaria Fails to Disclose Separating a Subset of the Plurality of
 Particles Comprising the Coronas As Required By 1(b). 54

 C. Zaccaria Fails to Disclose Detecting Proteins At Concentrations
 Across a Dynamic Range Comprising At Least 6 Orders of
 Magnitude As Claimed.....55

 1. The Petition Fails to Prove SBF1 Was Actually Detected by Zaccaria
At The Asserted Concentration.....56

 2. Petitioners’ Inherency Argument is Self-Defeating 60

 D. Dependent Claims.....64



Zaccaria – “contacting...with a plurality of particles”

In this study, we used three kinds of 100 nm super-paramagnetic NPs that strongly differ in their surface chemistry. The magnetic properties of these NPs considerably facilitated their handling during washing and elution steps through the use of an external magnet. We selected a strong anion exchanger (Q-NP), a strong cation exchanger (PAA-NP), and a hydrophobic (OS-NP) surface to increase

EX1007 (Zaccaria) at 1878; Paper 1 (Petition) at 65; EX1003 ¶ 247



Zaccaria – “separating at least a subset...”

RBC proteins–NPs incubation

Five hundred micrograms of each kind of NPs were individually incubated in two replicates for 45 minutes with 200 μ L of hemolysate containing 1 mg of total protein. Then, NPs were attracted to one side of the vial by an external magnet, and supernatant was removed. NPs were washed three times in 500 μ L PBS. Finally, NP-associated proteins were desorbed using 40 μ L of 8 M urea –2% CHAPS, and the protein concentrations were determined by a Bradford protein assay (Bio-Rad Laboratories, Hercules, CA, USA).

EX1007 (Zaccaria) at 1871; Paper 1 (Petition) at 71; EX1003 ¶ 262



Dr. Vargas on Zaccaria's Dynamic Range

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).

EX1003 ¶ 269 (citing EX1007 at 24-31); Paper 1 (Petition) at 72



The '360 Patent – “dynamic range”

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

EX1001 ('360 Patent) at 20:20-33; Paper 1 (Petition) at 6, 10, 14, 38



The '360 Patent – “dynamic range”

approaches. 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. In other words, the protein corona composition enables sampling across a vast dynamic range of the plasma proteome, which can substantially enhance the depth of protein coverage without protein depletion. Since

EX1001 ('360 Patent) at 72:1-28; Paper 1 (Petition) at 6, 14, 38, 76

