

Patent Owner's Demonstratives

PreOmics GmbH and Biognosys AG v. The Brigham and Women's Hospital, Inc.

IPR2024-01473

U.S. Patent No. 11,435,360

**WILSON
SONSINI**

Brigham and Women's Hospital - EXHIBIT 2031
PreOmics GmbH and Biognosys AG v. The Brigham and
Women's Hospital, Inc.

January 15, 2026

Grounds

'360 Patent Claims	PGR2021-00088 Challenge
1-4, 6-7, 17, 22-25	Ground 1: Obviousness: Hu (EX1008)
1, 4, 6, 17, 22, 25	Ground 2: Anticipation: Cai (EX1005)
1, 4, 6, 17, 22, 25	Ground 3: Obviousness: Cai
1-2, 4, 6-7, 17, 22-23, 25	Ground 4: Obviousness: Zaccaria (EX1007)

Themes Throughout Petitioners' Challenges

Failure to meet statutory burden.

- All grounds fail to show claimed assaying step (element 1[c]).
 - Undisputed facts are in Patent Owner's favor.
- Grounds 2 and 3 (Cai) fail to show the claimed contacting step (element 1[a]).
- All grounds fail to show claimed separating step (element 1[b]).
- All grounds fail to show claimed “biological sample” (throughout Claim 1).

All Grounds Fail to Show Claim Element 1[c]

concentration in the biological sample across at least six orders of magnitude

All Claims Require Detecting Concentration In The Biological Sample Across 6 Orders of Magnitude

'360 Patent

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.

Undisputed: Cai Fails to Show Concentration in the Sample Across 6 Orders of Magnitude

Ground 2: Petition seizes on Cai's "over 8 orders of magnitude" emPAI statement without critical review.

orders of magnitude." EX1005, 587; EX1003 ¶¶229, 97. A POSA would have understood that the **relative abundance of bound proteins** "spread over 8 orders of magnitude" shows that Cai's MS analysis detected protein concentrations in the subset across a dynamic range comprising the claimed "*at least 6 orders of magnitude,*" which is further confirmed by the concentrations detected in Cai.

Pet., 60.

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).

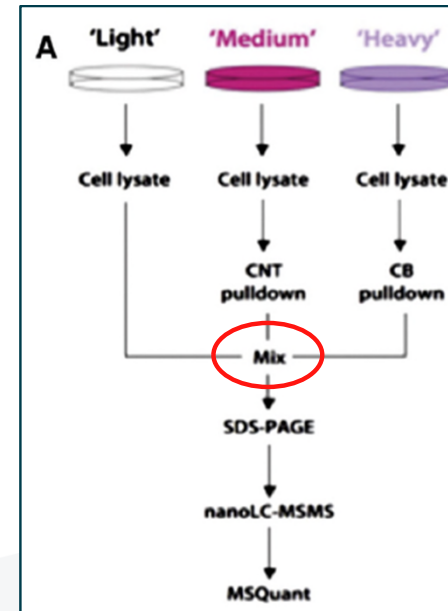
EX1003 (Vargas Decl.), ¶ 229

Undisputed: Cai Fails to Show Concentration in the Sample Across 6 Orders of Magnitude

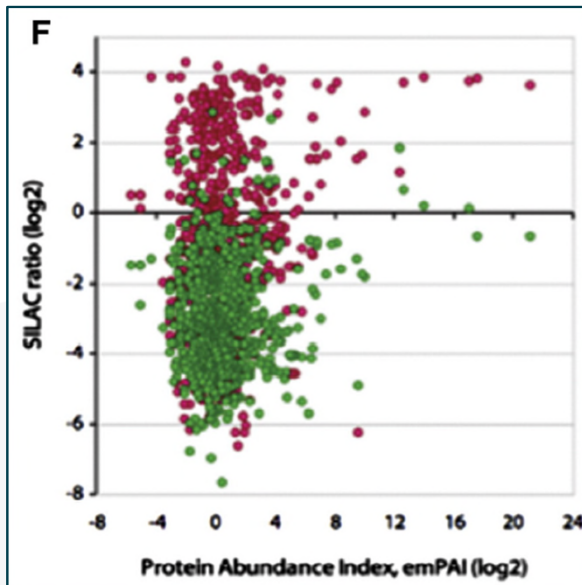
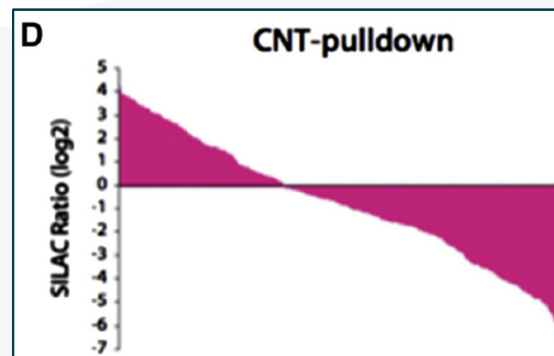
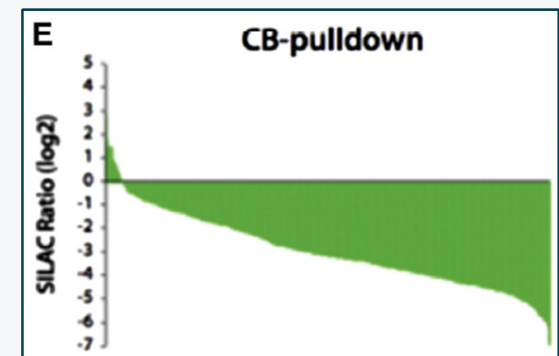
But emPAI at most estimates **abundance** of protein found within the post-pull down mix, not concentration in the biological sample.

Our MS analysis confirmed that the number of proteins enriched by MWCNT-binding is much higher than that enriched by CB (Figures 3, D and E). 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,³⁰ reveals no strong correlation between the MWCNT- and CB-binding efficiencies 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

EX1005 (Cai), 587



EX1005, Fig. 3A
(annotated)



EX1005, Fig. 3D

EX1005, Fig. 3E

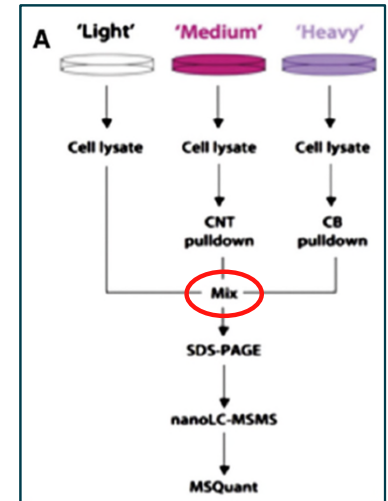
EX1005, Fig. 3F

PO Sur-Reply, 3-4, 6; POR, 22-23, 32-34; Pet., 60

Undisputed: Cai Fails to Show Concentration in the Sample Across 6 Orders of Magnitude

Experts agree: Cai's emPAI is not concentration in the biological sample.

- Dr. Cima: "Cai explains that emPAI is a semi-quantitative measure of abundance of proteins **in the mixture** of proteins created during the SILAC experiment." EX2023, ¶ 65
 - *Id.*, "emPAI does not demonstrate abundance, much less concentration, in the lysate identified by the petition and Dr. Vargas as the 'biological sample.'"
- Dr. Vargas confirmed:



EX1005, Fig. 3 (annotated).

Q. Okay. So the reason that I'm asking about this is, there's a point in time where there's a mix in the workflow in figure 3. Do you see that?

A. Yes.

Q. When we have that mix, by that time, we no longer are -- we're not mixing together the particles, we're just mixing together proteins that have already been desorbed or eluted from the particles; is that correct?

A. Correct.

EX2022 (Vargas Depo. Tr.), 132:18-133:2

Q. And that particle-free mixture of proteins from three populations of HeLa cells, that's what we're loading onto the SDS-PAGE gel; is that correct?

A. Yes.

Q. Okay. And then that is what we're subsequently analyzing using mass spec in Cai; is that correct?

A. Yes.

EX2022 (Vargas Depo. Tr.), 136:11-22

PO Sur-Reply, 3-4, 6; POR, 22-23, 32-33; Pet., 60

Petitioners' "In the Subset" Reply Argument Fails

Petitioners' reply:

corona." POR, 33 (emphasis in original). But the claim language expressly requires "assaying the subset of proteins of (b) with an instrument to detect, *in the subset*" proteins in the sample at the required concentration range. EX1001, cl. 1. Thus the actions claimed in limitation 1(c) occur with respect to the subset, not the biological sample. Patent Owner's attempt to distinguish Cai in this regard only confirms that

Pet. Reply, 19

Petition argument.

- Pet., 60 (**Cai** "assaying the biological sample using MS across a range large enough to detect both low abundant and high abundant proteins **in the biological sample.**")
- Pet., 36 ("**Hu** detects the bound proteins **in the biological sample** at concentrations greater than 10 mg/mL...and less than 10 ng/mL...comprising a dynamic range of at least six orders of magnitude.")
- Pet., 75 ("the NPs in **Zaccaria** are detecting proteins **in the biological sample** at concentrations across a dynamic range comprising at least 6 orders of magnitude.")

Petition anticipation theory misapprehended Cai's disclosure.

PO Sur-Reply, 4-7; Pet. Reply, 19

Ground 3 (Cai Obviousness) Fails to Remedy Ground 2

Petition: convert Cai's emPAI "into more traditional concentration numbers."

But this relies on the same emPAI values the petition advanced in Ground 2.

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

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

Pet., 63.

- Still based on **pulled-down protein abundance** emPAI values, **not concentration in the biological sample**.

$$\text{Protein content (mol \%)} = \frac{\text{emPAI}}{\sum (\text{emPAI})} \times 100 \quad (\text{Eq. 3})$$

EX1018 (Ishihama), 1267 & Equation 3. This is still, at its core, the same emPAI number that is "roughly proportional to protein abundance" with all the caveats and issues I described above. I also note that Ishihama only tested the linearity of

EX2023 (Cima Decl.), ¶ 69

- Incorporates the same deficiencies from Ground 2.

PO Sur-Reply, 4, 10-11; POR, 35-36; Pet., 62-64

Ground 3 (Cai Obviousness) Fails to Remedy Ground 2

Vague “POSA’s knowledge” cannot supply this missing claim limitation.

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

Pet., 62.

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

Pet., 63-64.

Cannot supply missing claim limitation from general knowledge.

- 35 U.S.C. §311(b) (“patents or printed publications”)
- 37 C.F.R. §42.104(b)(4) (same)
- July 31, 2025 USPTO Memo at 1 (“evidence that is not ‘prior art consisting of patents or printed publications’ (collectively, ‘general knowledge’) may not be used to supply a missing claim limitation.”).

PO Sur-Reply, 10-11; POR, 35-36; Pet., 62-64

Indisputable: Ground 1 Failure to Establish Inherency

Ground 1: Petition relied on **inherency** of LTBP-1 and serum albumin from Cao and Ahmed as indicative of protein concentrations in Hu.

certain concentrations in the biological sample being assayed. EX1003 ¶162. A POSA would have understood that serum albumin is normally present in healthy human plasma at concentrations ranging from 35 to 52 mg/mL and LTBP-1 in concentrations ranging from 1.7 to 6.0 ng/mL including because both proteins are well-known components of human plasma. EX1003 ¶¶163-165, 126, 102-108;

Pet., 36

Multiple flaws in Petitioners' argument:

- “Normally present” theory is legally misplaced and factually unsubstantiated.
- Petitioners fail to demonstrate actual LTBP-1 detection.
- Failure to establish concentration in Hu’s mixture.
 - Ahmed (albumin) and Cao (LTBP-1) not reflective of Hu
 - Hu and Cao address different proteins.

'360 Patent

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, Claim 1

PO Sur-Reply, 11-13; POR, 42-49

Indisputable: Ground 1 Failure to Establish Inherency

What is purported “normally present” is insufficient for inherency.

- “Inherency, however, may not be established by probabilities or possibilities. The mere fact that a certain thing *may* result from a given set of circumstances is not sufficient.” *Cont’l Can Co. v. Monsanto Co.*, 948 F.2d 1264, 1269 (Fed. Cir. 1991).
- An “inherent result must inevitably result from the disclosed steps.” *In re Montgomery*, 677 F.3d 1375, 1380 (Fed. Cir. 2021).
- “The mere fact that a certain thing may result from a given set of circumstances is not sufficient.” *Bettcher Indus., Inc. v. Bunzl USA, Inc.*, 661 F.3d 629, 639 (Fed. Cir. 2011).



“Normally present” is unsubstantiated

[illegible]

EX2027

PO Sur-Reply, 11-13; POR, 42-49

Indisputable: Ground 1 Failure to Establish Inherency

Petitioners do not establish actual LTBP-1 detection in Hu.

No critical analysis in the petition, while Dr. Cima detailed reasons for skepticism.

ID#	Name	pI	MW (kDa)	Fe ₃ O ₄ _30					Fe ₃ O ₄ _400					Fe ₃ O ₄ _400					
				MW/SpC_1	MW/SpC_2	MW/SpC_3	Average	SD	MW/SpC_1	MW/SpC_2	MW/SpC_3	Average	SD	MW/SpC_1	MW/SpC_2	MW/SpC_3	Average	SD	
RP00971477.1	LTBP1 Isoform 4 of 2 (Low transforming growth factor beta-binding protein 1)	5.83	136.74								0.01	0.01	0.01	0.00					
Fe ₃ O ₄ _30 (30 nm)																			
Replicate 1				Replicate 2					Replicate 3										
Fe ₃ O ₄ _400 [sic] (200 nm)																			
Replicate 1				Replicate 2					Replicate 3										
				0.01					0.01										
Fe ₃ O ₄ _400 (400 nm)																			
Replicate 1				Replicate 2					Replicate 3										

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

EX2013

92. Given the weakness of the MWSpC (percent normalized spectral count) and lack of reproducibility, a POSA would more reasonably conclude that any non-replicable signal was more likely experimental artifact rather than an actual detection event. The petition and Dr. Vargas' argument ignores the known sensitivity limitations and experimental error common in shotgun proteomics assays like Hu. Section III, above. They do not meaningfully address the raw data reported in Hu itself—they merely cite an entry without addressing it critically as a POSA would do.

EX2023 (Cima Decl.), ¶ 92; see also *id.*, ¶ 76

- Petitioners opted not to ask Dr. Cima any questions regarding this during deposition.
- Petitioners never offered rebuttal testimony.

PO Sur-Reply, 11-13; POR, 42-49

Indisputable: Ground 1 Failure to Establish Inherency

Undisputed: Hu and Cao address different proteins:

address Cao's samples, and Cao does not address Ahmed. In fact, the spectral count in Hu mapped to LTBP1 is for "Isoform 4." EX1008, 18 (Table S1, row IPI00973177.1); EX2013 (excerpt reproduced below)). But Cao teaches "[t]here are four isoforms of LTBPs (LTBP-1, -2, -3, and -4)" and discusses concentration values from Cao's sample for Isoform 1. EX1016, 16051 (LTBP-1). Dr. Vargas does not appear to recognize that Hu and Cao are addressing different proteins.

EX2023 (Cima Decl.), ¶ 94

Indisputable: Ground 4 Failure to Establish Inherency

Ground 4: Petition relied on **inherency** of Hemoglobin subunit alpha (HBA1) and myotubularin-related protein 5 (SBF1) from Bryk as indicative of protein concentrations in Zaccaria.

A POSA would have understood that HBA1 is normally present in RBC lysate in a concentration of 1.47×10^4 pmol/mg and SBF1 in a concentration of 6.62×10^{-6} pmol/mg including because both proteins are well-known components of RBC.

Pet., 74-75

Multiple flaws in Petitioners' argument:

- “Normally present” theory is legally misplaced and factually unsubstantiated.
- Petitioners fail to demonstrate actual SBF1 detection.
- Failure to establish concentration in Zaccaria's mixture.
 - Bryk not reflective of Zaccaria.
 - Petitioners cherry-picked Bryk's “lowest non-zero detected concentration”; ignored other values.
 - Bryk discarded all SBF1 results.

'360 Patent

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, Claim 1

PO Sur-Reply, 14-15; POR, 55-64

Indisputable: Ground 4 Failure to Establish Inherency

What is purported “normally present” is insufficient for inherency.

- “Inherency, however, may not be established by probabilities or possibilities. The mere fact that a certain thing *may* result from a given set of circumstances is not sufficient.” *Cont’l Can Co. v. Monsanto Co.*, 948 F.2d 1264, 1269 (Fed. Cir. 1991).
- An “inherent result must inevitably result from the disclosed steps” *In re Montgomery*, 677 F.3d 1375, 1380 (Fed. Cir. 2021).
- “The mere fact that a certain thing may result from a given set of circumstances is not sufficient.” *Bettcher Indus., Inc. v. Bunzl USA, Inc.*, 661 F.3d 629, 639 (Fed. Cir. 2011).



“Normally present” is unsubstantiated

SBF1 EX1009 p. 51	Whole erythrocyte			
	Concentra tion (pmol/mg)	Concentra tion (pmol/mg)	Concentra tion (pmol/mg)	Concentra tion (pmol/mg)
	Donor 1	Donor 2	Donor 3	Donor 4
	1.56E-03	0.00E+00	6.62E-06	2.80E-04
PIK3R4 EX1009 p. 44	3.17E-04	0.00E+00	0.00E+00	0.00E+00

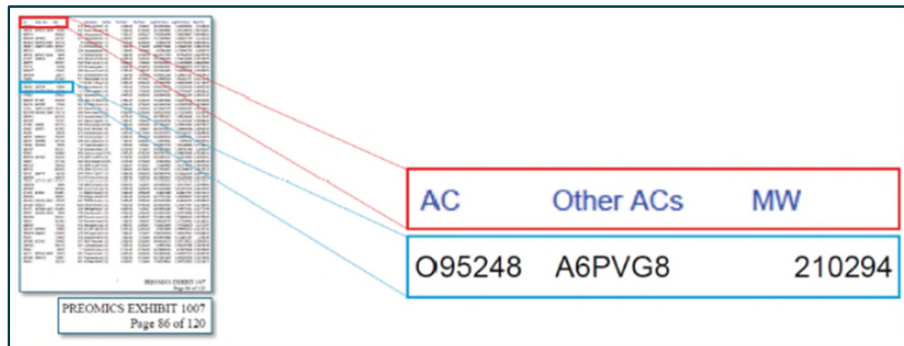
EX2011;
see also EX2023 (Cima Decl.), ¶121

PO Sur-Reply, 14-15; POR, 46-47, 55-64

Indisputable: Ground 4 Failure to Establish Inherency

No demonstration of actual SBF1 detection.

(Alleged SBF1)

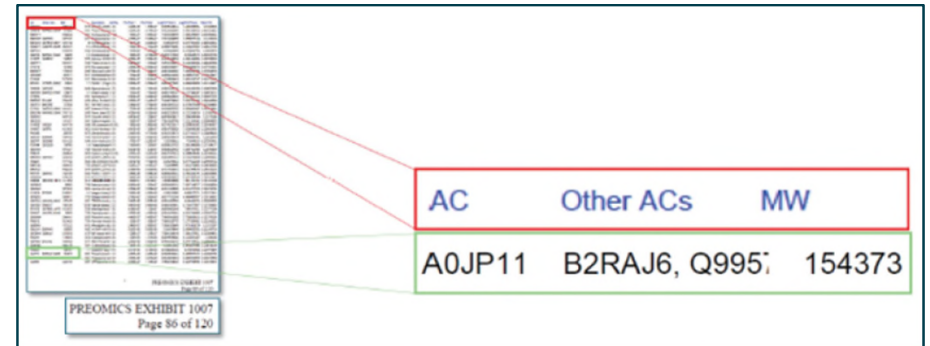


AC	Other ACs	MW
O95248	A6PVG8	210294

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EX2014

(Alleged PIK3R4)



AC	Other ACs	MW
A0JP11	B2RAJ6, Q995	154373

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EX2015

AC/ID: accession number in Uniprot database. For more clarity, if several proteins match the same set of peptides, only one member of the protein group is indicated in these concise lists.

EX1007 (Zaccaria), 17

- Dr. Cima described: multiple accession numbers were associated with the signal. EX2023, ¶¶ 117-118.
- Petitioners opted to ignore Dr. Cima's testimony in Reply; never offered rebuttal testimony.
- Dr. Vargas did not know what AC stood for.

Q. So the first column says what?
A. The column -- the first column says AC.
Q. Okay. And do you know what AC stands for?
A. I don't offhand.

EX2015 (Vargas Depo. Tr.), 58:21-24.

PO Sur-Reply, 14-15; POR, 55-64

Indisputable: Ground 4 Failure to Establish Inherency

No demonstration of actual SBF1 detection.

- Dr. Cima explained: skepticism of weak, non-reproduced signals in **raw supplemental** mass spectrometry data.
- Petitioners seized on Zaccaria's supplemental data entry without critical review.

AC	ID	MW	pI	Description	Fractions	Score	#Peptides	#Sequences	Coverage (%)	#MS/MS	
O95248	MTMR5_HUMAN	210294.08	6.46	Myotubularin-related protein 5 OS=Homo sapiens GN=SBF1 PE=1 SV=3							
Q-Treated											
Replicate 1					Replicate 2						
Fractions	Score	#Peptides	#Sequences	Coverage (%)	#MS/MS	Fractions	Score	#Peptides	#Sequences	Coverage (%)	#MS/MS
GR_Q1	223.78	2	2	1	10						
OS-Treated											
Replicate 1					Replicate 2						
Fractions	Score	#Peptides	#Sequences	Coverage (%)	#MS/MS	Fractions	Score	#Peptides	#Sequences	Coverage (%)	#MS/MS
PAA-Treated											
Replicate 1					Replicate 2						
Fractions	Score	#Peptides	#Sequences	Coverage (%)	#MS/MS	Fractions	Score	#Peptides	#Sequences	Coverage (%)	#MS/MS
						GR_PAA2	149.65	7	7	3.4	13

EX2009

predictions”). POSAs viewed non-replicable #MS/MS and corresponding matches to a database as **likely experimental error or artifact**, rather than a reliable detection event. EX2022 (Vargas Depo. Tr.), 43:20-44:7. A POSA would more reasonably view Zaccaria's mention of SBF1 as indicating a level of detection/specificity that was **inadequate to detect (and did not in fact detect) this protein**. A POSA would be **highly skeptical that Zaccaria had actually detected SBF1**. As explained in further

EX2023 (Cima Decl.), ¶ 114; *see also id.*, ¶¶ 113-116.

PO Sur-Reply, 14-15; POR, 55-64

Indisputable: Ground 4 Failure to Establish Inherency

Unrebutted: Bryk does not establish SBF1 concentration in Zaccaria.

Dr. Cima explained:

- Preparation and analysis methods materially alter concentration values.
- Zaccaria uses materially distinct approaches as compared to Bryk.
- Bryk does not establish concentrations for proteins ***in Zaccaria***. EX2023, ¶123.

123. Bryk does not establish protein concentrations in Zaccaria's tested material because it is a different reference that employed different methodologies on different samples. EX1015 (Ahmed), 786 (discussing that even "slight changes in a given sample preparation could lead to very different protein profiles"). Dr. Vargas confirmed at her deposition that numerous differences existed in the preparation protocol employed in Zaccaria as compared to Bryk. EX2022 (Vargas Depo. Tr.), 51:11-54:4. I agree. A POSA could thus not read Bryk's supplemental materials as corresponding to results expected from Zaccaria's hemolysate.

EX2023 (Cima Decl.), ¶123

- Petitioners **did not dispute or rebut** this deficiency in Reply.

PO Sur-Reply, 14-15; POR, 55-64

Indisputable: Ground 4 Failure to Establish Inherency

Undisputed: petition cherry-picked “normally present” data, ignored other values.

Dr. Vargas: “the lowest non-zero detected concentration” from Bryk as purportedly “normally present.”

⁶ As I explained in Section IX.C.1, the concentration of HBA1 is an average across the four donors ranging from 1.44×10^4 pmol/mg to 1.55×10^4 pmol/mg. The concentration of SBF1 is the lowest non-zero detected concentration.

EX1003 (Vargas Decl.), ¶271n.6

Petitioners and Dr. Vargas ignored Bryk’s other values (including **zero** in some donors).

	Whole erythrocyte			
	Concentra	Concentra	Concentra	Concentra
	tion	tion	tion	tion
	(pmol/mg)	(pmol/mg)	(pmol/mg)	(pmol/mg)
	Donor 1	Donor 2	Donor 3	Donor 4
SBF1 EX1009 p. 51				
	1.56E-03	0.00E+00	6.62E-06	2.80E-04
PIK3R4 EX1009 p. 44				
	3.17E-04	0.00E+00	0.00E+00	0.00E+00

EX2011; *see also*
EX2023 (Cima Decl.), ¶121

No Rebuttal - “Bryk confirms the relied-upon proteins may not be detectable or present at all in a sample—including Zaccaria’s sample.” EX2023 (Dr. Cima Decl.), ¶ 121.

POR, 55, 61-64; PO Sur-Reply, 14-15

Indisputable: Ground 4 Failure to Establish Inherency

Undisputed: Bryk discarded all SBF1 results

Bryk had a “sample purity threshold” of 100 copies per cell sample to account for contamination.

from the RBC samples. Because a minute contamination of the erythrocyte preparations by other blood cells cannot be avoided we consider proteins above a threshold of 100 copies as those occurring in erythrocytes. However, we do not rule out the possibility that some proteins with abundances below this limit may also originate from RBCs. In addition we cannot exclude contamination of the RBC and their membrane preparations by plasma proteins. For example, the most abundant plasma protein, albumin, was present at several thousand copies.

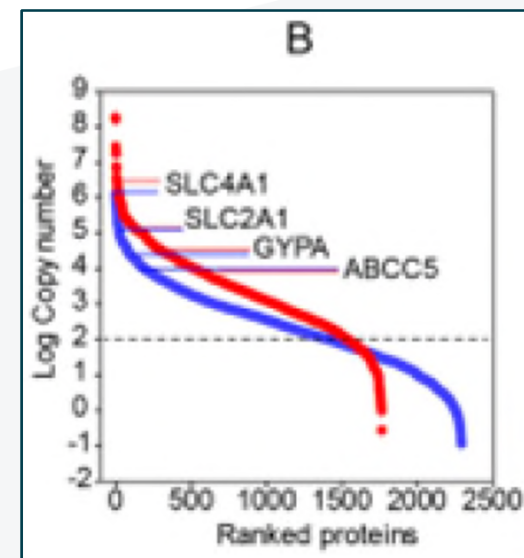
EX1009, 2755

Dr. Cima explained:

122. Additionally, Bryk discarded all the SBF1 results because the number of copies per cell (16.9, 0.0, 0.1, and 3.0) always fell well below its 100-copies “sample purity threshold.” EX1009, 51, 2756

EX2023 (Cima Decl.), ¶122

Reply did not dispute.



EX1009, Fig. 3

POR, 62-64; PO Sur-Reply, 14-15

Additional Reasons Petitioners' Grounds Fail

Cai Fails at the Claimed Contacting Step

'360 Patent

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.

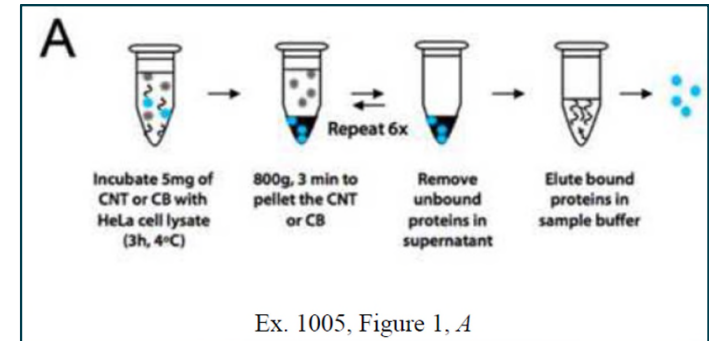
EX1001, Claim 1

Neither Cai Embodiment Anticipates the Claim

Petition relied on Cai's Fig. 1 experiment for "contacting" element.

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

Pet., 48



EX1003 (Vargas Decl.), ¶ 202

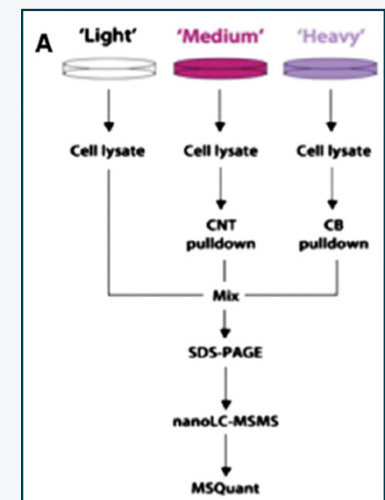
Petitioners acknowledge: Fig. 1 experiment **does not disclose** assaying across a dynamic range comprising "at least 6 orders of magnitude."

- For that element, Petitioners rely on Cai's **Fig. 3 experiment**. Pet., 58-60.

Dr. Vargas attempted **during deposition** to pivot to Cai's Fig. 3 for the contacting element. POR, 21-24.

Neither of Cai's Figs. 1 or 3 experiments anticipate the claims.

- Fig. 1:** indisputably does not disclose assaying across a dynamic range comprising "at least 6 orders of magnitude."
- Fig. 3:** three separate populations; no single population contacted with multiple particle types (CBs vs MWCNTs). **Does not disclose claimed "contacting" step.**



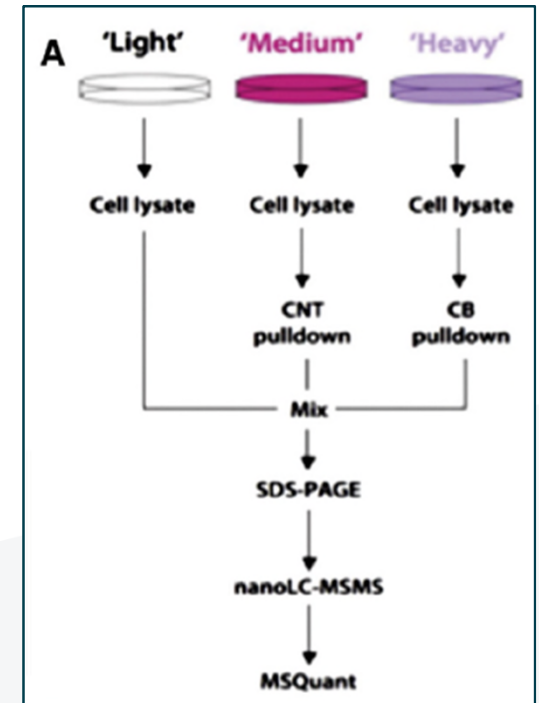
PO Sur-Reply, 7-10; POR, 21-28

Cai Fails at the Claimed Contacting Step

Cai Figure 3: different particle types incubated with **different** HeLa populations (alleged “samples”)

preparation of MWCNT with those that bound to CB. Cell lysates were prepared from **three populations of HeLa cells** that had been metabolically encoded using three different SILAC media (“light”, “medium” and “heavy”, Figure 3, A and Methods). A CNT pull-down was performed on the “medium” lysate, and a CB pull-down was performed on the “heavy” lysate. Equal volumes of proteins eluted from these pull-down experiments were mixed. The mixture was also spiked with an equal amount of unfractionated “light” lysate. The mixtures were then separated by SDS-PAGE, and

EX1005, 587.



EX1005, Fig. 3.

- Dr. Cima explained: “in the SILAC experiment depicted in Fig. 3...each different population was contacted by **at most one single particle type**—not by the multiple particle types required by the claims.” EX2023, ¶54.
 - Unrebutted in Reply.
- The MWCNTs do not provide the different particle types claimed.

challenged claims and certainly fails to show anticipation by Cai. First, the experiment relied upon by Dr. Vargas and the petition to generate the alleged “subset of proteins” (Cai’s Figure 1) never employed MWCNTs with diameters less than 10 nm. Second, Cai describes that MWCNTs having diameters less than 10 nm “bound to virtually no proteins.” EX1005 (Cai), 586. In contrast to MWCNTs of diameters less than 10 nm, Cai teaches that “the amount of proteins pulled down by CNTs of diameters from 20-100 nm is essentially the same.”

protein binding amount. EX1005 (Cai), 586. Cai’s disclosure of 20-40 nm and 10 nm CNTs thus did not describe separating a subset of particles comprising “the coronas” of different particle types, and this disclosure instead merely indicates the absence of a corona from a different particle type in that experiment. Accordingly, this argument fails to establish that Cai anticipates the claims.

EX2023 (Dr. Cima Decl.), ¶ 61

PO Sur-Reply, 7-10; POR, 21-27

Cai Fails at the Claimed Contacting Step

Petitioners' vague "at once envisage" theory: not in petition.

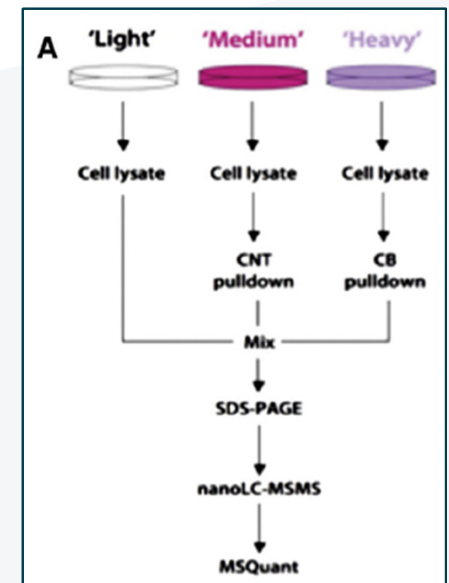
- Reply: Petitioners argue "a POSA would 'at once envisage' the patented method" from Cai's Figs. 1 and 3 experiments.

Even if Cai did comprise wholly distinct experiments as Patent Owner contends (it does not), the Board was correct that a POSA would "at once envisage" the patented method from Cai's disclosure. ID, 20 (case cites omitted). As Dr. Vargas

Pet. Reply, 14.

Even if true that a skilled artisan would at once envisage that the pull-down referenced in Cai Fig. 3 employs the same low-speed centrifugation pulldown procedure as in Fig. 1, that does not solve any of the deficiencies discussed above... Petitioners rely on the Fig. 3 experiment for the "at least six orders of magnitude" assaying step, but this experiment used only a single particle type (or no particle) in each different HeLa cell population.

POR, 27.



EX1005, Fig. 3.

PO Sur-Reply, 7-10; POR, 21-27

All Grounds Fail to Show Separation Step 1[b]

'360 Patent

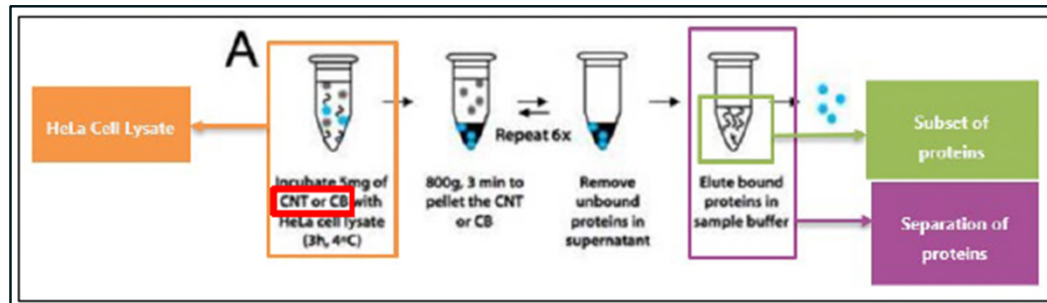
1. A method for assaying a biological sample, comprising:
 - (a) contacting the biological sample with a plurality of particles comprising different particle types to permit biomolecules of the biological sample to bind to the plurality of particles and form coronas around the plurality of particles, 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 Fails to Show Separation Step 1[b]

Ground 2: Petition relies **exclusively** on Cai's Fig. 1A, depicting single particle types in HeLa cell lysate, for this element.

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

Pet., 57.



Pet., 58.

Petition argument: contrary to the claim language.

- “[T]he coronas” of claim element 1[b] invoke the coronas of claim element 1[a].
 - The experts agree.
- Petitioners do not (and could not) allege Cai Fig. 1A comprises “the coronas” of element 1[a].

PO Sur-Reply, 15-18; POR, 28-32

Element 1[b] Requires “The Coronas”

Separating “a subset” comprising “the coronas” of claim element 1[b] invokes the coronas of claim element 1[a].

- Affirmative requirement from the claim language: “a subset” comprises “the coronas.” EX1001, Claim 1.
- Element 1[a] describes: “the coronas correspond[] to the different particle types” and “(i) differ based on particle type, and (ii) comprise overlapping and distinct proteins.” EX1001, Claim 1.
- **Petitioners’ argument otherwise would render “comprising the coronas” a nullity.**

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

EX1001, Claim 1

PO Sur-Reply, 15-17; POR, 15-20, 28-32, 36, 38-42, 54-55.

Element 1[b] Requires “The Coronas”

Experts agree: “the coronas” of 1[b] invoke coronas corresponding to different particle types from 1[a].

assayed in step 1(c). Further, the term “the coronas” is introduced and defined in step 1(a), which requires that “the coronas” correspond “to different particle types” and “differ based on particle type” and “comprise overlapping and distinct proteins.” EX1001 (’360 Patent), Claim 1, 86:52-87:3; *see also* EX2022 (Vargas Depo. Tr.), 30:21-31:13, 48:1-49:25 (describing “at least”).

EX2023 (Cima Decl.), ¶ 45-46.

Dr. Vargas: at least two particle types are needed.

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 (Vargas Depo. Tr.), 27:21-28:3.

Q. Okay. All right. I think there was a disconnect a moment ago. 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 types.

EX2022 (Vargas Depo. Tr.), 28:22-29:7.

PO Sur-Reply, 15-17; POR, 15-20, 28-32, 36, 38-42, 54-55.

Cai Fails to Show Separation Step 1[b]

Reply: No Response

“comprising the coronas.” The Reply never squarely addresses “the coronas” language and provides no justification for rendering these claim words a nullity.

PO Sur-Reply, 16

two] types.”). Again, the Reply never squarely addresses the claim language, including “the coronas,” its own expert’s admission, or the corresponding argument in the POR. The petition challenge can be rejected for this independent reason alone.

PO Sur-Reply, 17

PO Sur-Reply, 15-18

Hu Fails to Show Separation Step 1[b]

Ground 1: Petition relies on Hu's SDS-PAGE gel (Fig. 2) for this element. Pet., 31-32.

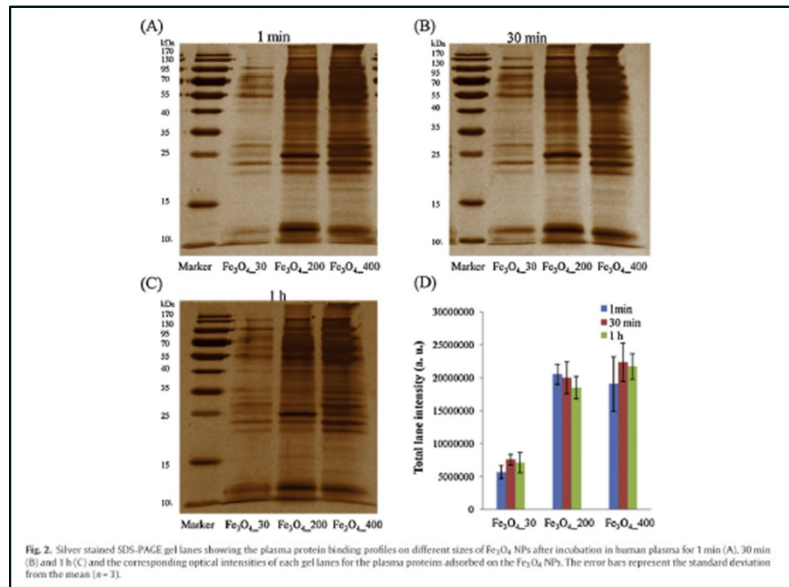


Fig. 2. Silver stained SDS-PAGE gel lanes showing the plasma protein binding profiles on different sizes of Fe₃O₄ NPs after incubation in human plasma for 1 min (A), 30 min (B) and 1 h (C) and the corresponding optical intensities of each gel lanes for the plasma proteins adsorbed on the Fe₃O₄ NPs. The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

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;

EX1001, Claim 1

EX1008, Fig. 2.

Petitioners' reliance fails:

- The SDS-PAGE gel is not shown to contain *any* particles comprising coronas. EX2022 (Vargas Depo. Tr.), 99:3-15.
- Each of Hu's subsets of particles comprises only one corona—not “the coronas” of 1[a].
- Petitioners' Reply does not dispute.

PO Sur-Reply, 15-18; POR, 38-42

Zaccaria Fails to Show Separation Step 1[b]

Ground 4: Zaccaria's separation step generates a subset of particles comprising only the corona of a *single* particle type.

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

Pet., 71.

Petitioners' reliance fails.

- Zaccaria's subset of particles comprise only the corona from a single particle type—not multiple coronas with overlapping and distinct proteins required by 1[b].

("Three separate containers."), 41:5-17 (separately incubated in duplicates). I agree with Dr. Vargas that the separation step in Zaccaria generates a subset of particles comprising only the corona of a single particle type. EX2022 (Vargas Depo. Tr.), 42:16-43:9, 45:10-50:12, 41:8-12. Zaccaria never describes separating a subset of particles comprising coronas from multiple particle types. The subset of particles

EX2023 (Cima Decl.), ¶ 110.

- Petitioners' Reply does not rebut.

PO Sur-Reply, 15-18; POR, 54-55

All Grounds Fail to Show Claimed “Biological Sample”

'360 Patent

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**.

Expressly Defined: “Obtained from a Subject”

Dr. Vargas: the '360 Patent defines “biological sample” to be “obtained from a subject.”

Experts agree “biological sample” defined in patent:

Q. So as you sit here today, is there any reason that you want to omit lines 32 and 33 of column 20 from what you say is consistent with the specifications description of the biological sample?

A. There's no specific reason. I'll say that 32 and 33 sort of set up the definition and after that there are specific examples. So plus or minus the -- that setup I think is a question of, you know, whether or not to include that when those examples are being listed.

EX2022 (Vargas Depo. Tr.), 35:25-36:9

The '360 Patent expressly defines:

The term “sample” refers to a biological sample or a complex biological sample obtained from a subject. Suitable

The terms “individual,” “subject,” and “patient” are used interchangeably herein irrespective of whether the subject has or is currently undergoing any form of treatment. As

EX1001, 20:32-33.

EX1001, 50:26-36.

PO Sur-Reply, 18-24; POR, 13-14, 23-28, 38, 53.

Q. What's identified in the next sentence of your declaration there in paragraph 43?

* * *

THE WITNESS: Well, specifically, it -- the specification describes that the term “sample” refers to a biological sample or a complex biological sample and came from a subject. And that -- in the Vargas deposition that was explored and she agreed that it's a subject or a patient and the terms are used interchangeably.

BY MR. ROSATO:

Q. Do you disagree with Dr. Vargas?

A. No. As I state here, I thought we were in agreement. I don't see a difference, any light between our interpretations.

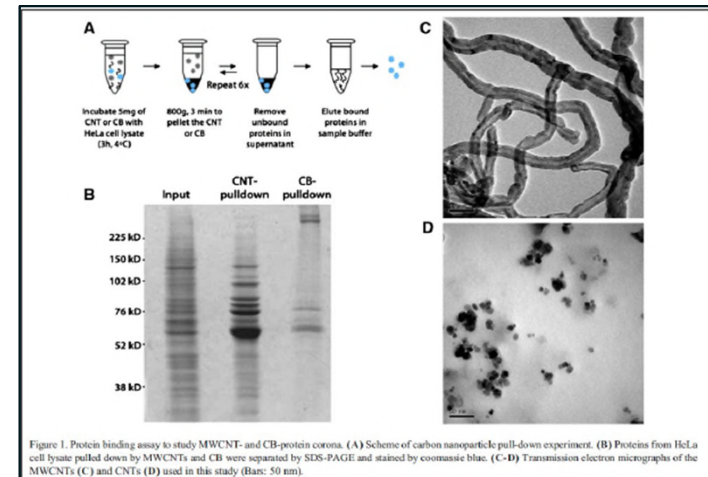
EX1036
(Cima Depo. Tr.),
78:19-79:14

Cai Fails to Show Claimed “Biological Sample”

Ground 2: Petition relies **exclusively** on a lysate of cells from an immortalized HeLa cell line. **Not a claimed “biological sample.”**

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

Pet., 48.



Pet., 47 (citing Cai, Fig. 1).

Cai’s HeLa cell lysate is not a “biological sample.”

- Indisputable: claimed “biological sample” is obtained from an individual subject. HeLa cells are instead obtained from highly-mutated, immortalized cell line.

Cai Fails to Show Claimed “Biological Sample”

Cai's HeLa cell lysate is not a “biological sample” as claimed.

HeLa cells are a highly-mutated, immortalized cell line purchased from a vendor rather than obtained from an individual subject.

Q. Okay. And you previously worked with HeLa cells; is that correct?

A. Yes.

Q. When you worked with HeLa cells, how did you obtain your HeLa cells?

A. We purchased them.

Q. From where did you purchase them?

A. I don't recall specifically, but a commercial vendor. It could have been ATCC or ATTC. It's been a long time, but they're commercially available.

Q. Okay. So it was a commercial product that you were using?

A. Yes.

EX2022 (Vargas Depo. Tr.), 124:4-16

52. HeLa cells are not obtained from an individual subject. I understand Dr. Vargas confirmed at deposition that HeLa cells are vendor-purchased immortalized cells from cell lines cultured in labs. EX2022 (Vargas Depo. Tr.), 117:11-21, 124:4-16, 136:19-137:18. I agree. In fact, HeLa cells differ quite significantly compared to cells that would be obtained from any subject. EX2026 (Heng) (“it might even be argued that the HeLa genome is no longer a human genome.”).

EX2023 (Cima Decl.), ¶ 52.

POR, 23-28; PO Sur-Reply, 19-20.

Cai Fails to Show Claimed “Biological Sample”

Reply:

whom *all* “HeLa” cells derive—Ms. Henrietta Lacks. Every so-called “HeLa cell” is a direct, genetically identical copy of the original cells that were taken directly from Ms. Lacks. EX1037, 3. HeLa cells are thus not engineered or human-created

Pet. Reply, 5.

The Reply argument is demonstrably incorrect:

The HeLa genome is no longer Henrietta Lacks’s personal genome. Although the two share some DNA sequences, the similarity ends there. Lacks’s genome had the usual number of 46 normal chromosomes, whereas most HeLa cells have 70–90 chromosomes and more than 20 translocations, some of which are highly complex.

EX2026 (Heng)

Changes in the HeLa genome in the past few decades have resulted from multiple cycles of genome reorganization during the cancer process and from the initial cell-culture experiments. Considering that chromosomes provide the genome identity and blueprint, it might even be argued that the HeLa genome is no longer a human genome.

EX2026 (Heng)

Q. Are you aware of any human being that has 70 to 90 chromosomes?

A. No. This kind of thing with extra chromosomes is only representative of like cancer cells and things like that. So it's very different than the -- than the original cell.

EX1036 (Cima Depo. Tr.), 83:9-20

PO Sur-Reply, 19-20.

Cai Fails to Show Claimed “Biological Sample”

Reply: Improper New Argument:

Patent Owner’s other arguments simply restate its claim construction arguments and other arguments addressed above. Even if Patent Owner’s extraordinarily narrow claim construction for “biological sample” were adopted, however, and HeLa cells were somehow excluded, the disclosures relied upon in Cai would still render the claims obvious, since it would be trivially easy to apply the same methodology to other types of samples.

Pet. Reply, 21-22.

Hu and Zaccaria Fail to Show Claimed “Biological Sample”

For **Hu** and **Zaccaria** (Grounds 1 and 4), Petitioners map “the biological sample” to ***mixtures*** rather than to a biological sample obtained from an individual subject.

Decl.), ¶¶127-131. Hu’s study combines blood taken from 20 different individuals that are mixed prior to assay. EX1008 (Hu), 355 (“Human blood was *taken from 20 different healthy donors*” and processed, then the resultant plasma was “*mixed together in order to eliminate individual differences.*”). As indicated above, Dr. Vargas identified a definition in the challenged patent, but does not explain how Hu’s mixture is “a biological sample” as defined by the ’360 Patent.

EX2o23 (Cima Decl.), ¶ 78.

(Vargas Decl.), ¶¶243-245. Zaccaria’s study (like Hu’s) combines samples taken from multiple different individuals that are mixed prior to assay. EX1007 (Zaccaria), 1870-1871. As indicated above, Dr. Vargas identified a definition in the challenged patent, but does not explain how Zaccaria’s mixture is “a biological sample” as defined by the ’360 Patent.

EX2o23 (Cima Decl.), ¶ 108.

No factual dispute: Petitioners mapped to a **mixture** rather than a biological sample obtained from an individual subject, as required by the ’360 Patent claims.

PO Sur-Reply, 19; POR, 13-14, 38, 53.