ETH
Eidgenössische Technische Hochschule Zürich
Swiss Federal Institute of Technology Zurich

Reproducible protein sample preparation from small amount of clinical specimens for proteomic studies

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2016.6.28
Dortmund, Germany

Outline

1. The clinical need
2. PCT-assisted sample preparation for proteomics
3. SWATH-mass spectrometry
4. PCT-SWATH
5. Application to drug responsiveness prediction

PBI Pressure BioSciences Inc.

I&L Biosystems

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Protein biomarkers in kidney cancers

Table 1 – World Health Organisation histologic classification of renal cell carcinoma

Clear cell renal cell carcinoma
Multilocular clear cell renal cell carcinoma
Papillary renal cell carcinoma
Chromophobe renal cell carcinoma
Carcinoma of the collecting ducts of Bellini
Renal medullary carcinoma
Xp11 translocation carcinomas
Carcinoma associated with neuroblastoma
Mucinous tubular and spindle cell carcinoma
Renal cell carcinoma, unclassified

Algaba, F. *et al*. Current pathology keys of renal cell carcinoma. *Eur. Urol.* **60**, 634–643(2011).

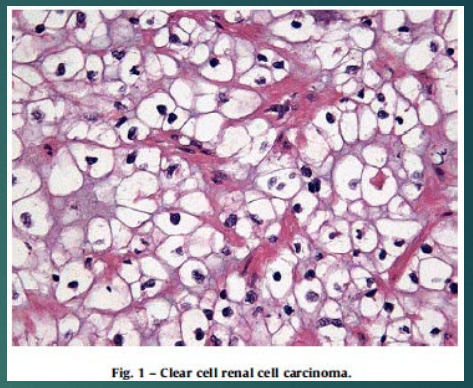


Fig. 1 – Clear cell renal cell carcinoma.

3.1.3. Immunophenotype

The cells express low molecular weight cytokeratins (CKs; CAM 5.2 60%) more frequently [8], with vimentin in 82.6% [9] and CD10 in 94% [10] (Fig. 2). Epithelial membrane antigen (EMA) (MUC-1) appears in 85% [11] and glutathione S-transferase α in 82% [12]. c-kit and α-methylacyl-CoA racemase (AMACR) are negative [13].

53 Swiss-Prot proteins presently used as diagnostic or prognostic biomarkers for renal cancers

Tan, P.H. *et al*. Renal tumors: diagnostic and prognostic biomarkers. *Am. J. Surg. Pathol.***37**, 1518–1531 (2013).

In reality, for most kidney cancer patients, few protein biomarkers are measured.

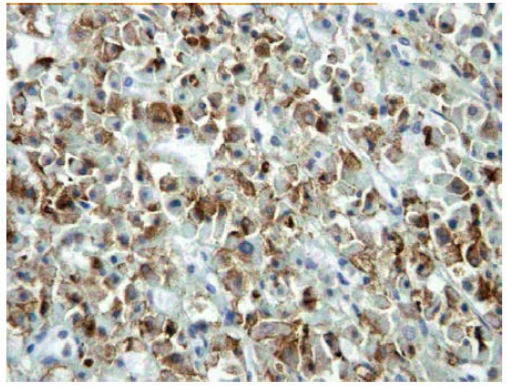
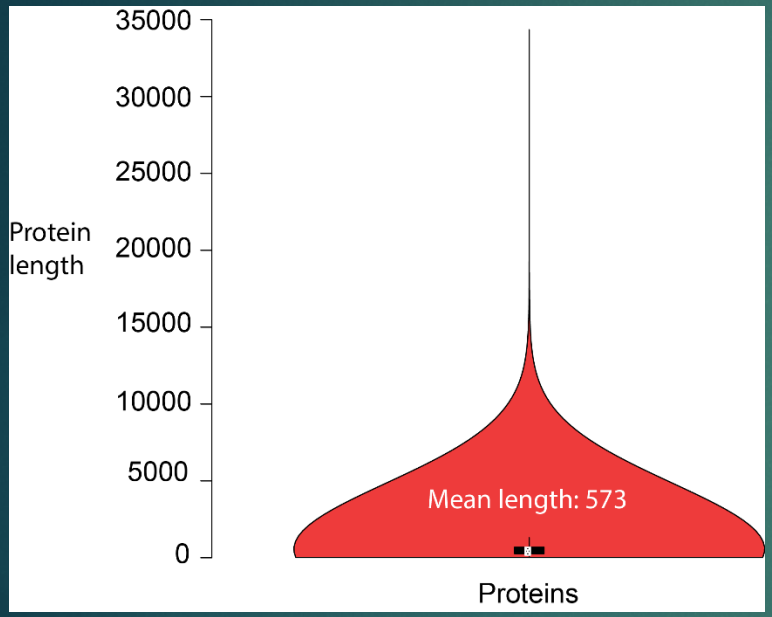


Fig. 2 – Clear cell renal cell carcinoma with CD10 cytoplasmic expression.

The proteins as analytes



Human proteome:

Minimal protein number: 19,815 (SwissProt)

Average length: 573 residues

Average molecular weight: 63,000 Da

1 µg protein sample:

~10 trillion protein molecules

~ 1 billion molecules per protein

The reality

- Small amount of irreplaceable samples;
- Expensive assays
- Dynamics of proteins

Our hope

- Quantify high number of proteins;
- Analyze high number of samples

Antibody-based protein measurement

- **Market analysis**
  - The 2015 global antibody market: > $80 billion

Biocompare
The Buyer's Guide for Life Scientists

  - In contrast, Mass Spec market: ~$5 billion in 2015, ~7 billion in 2020

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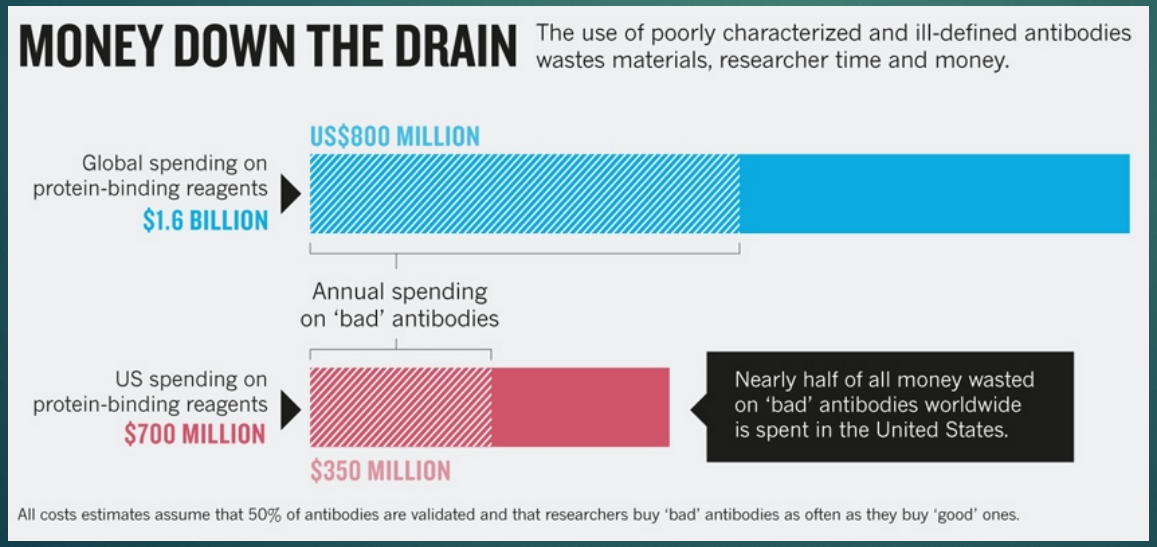
THE WORLD'S LARGEST MARKET RESEARCH STORE

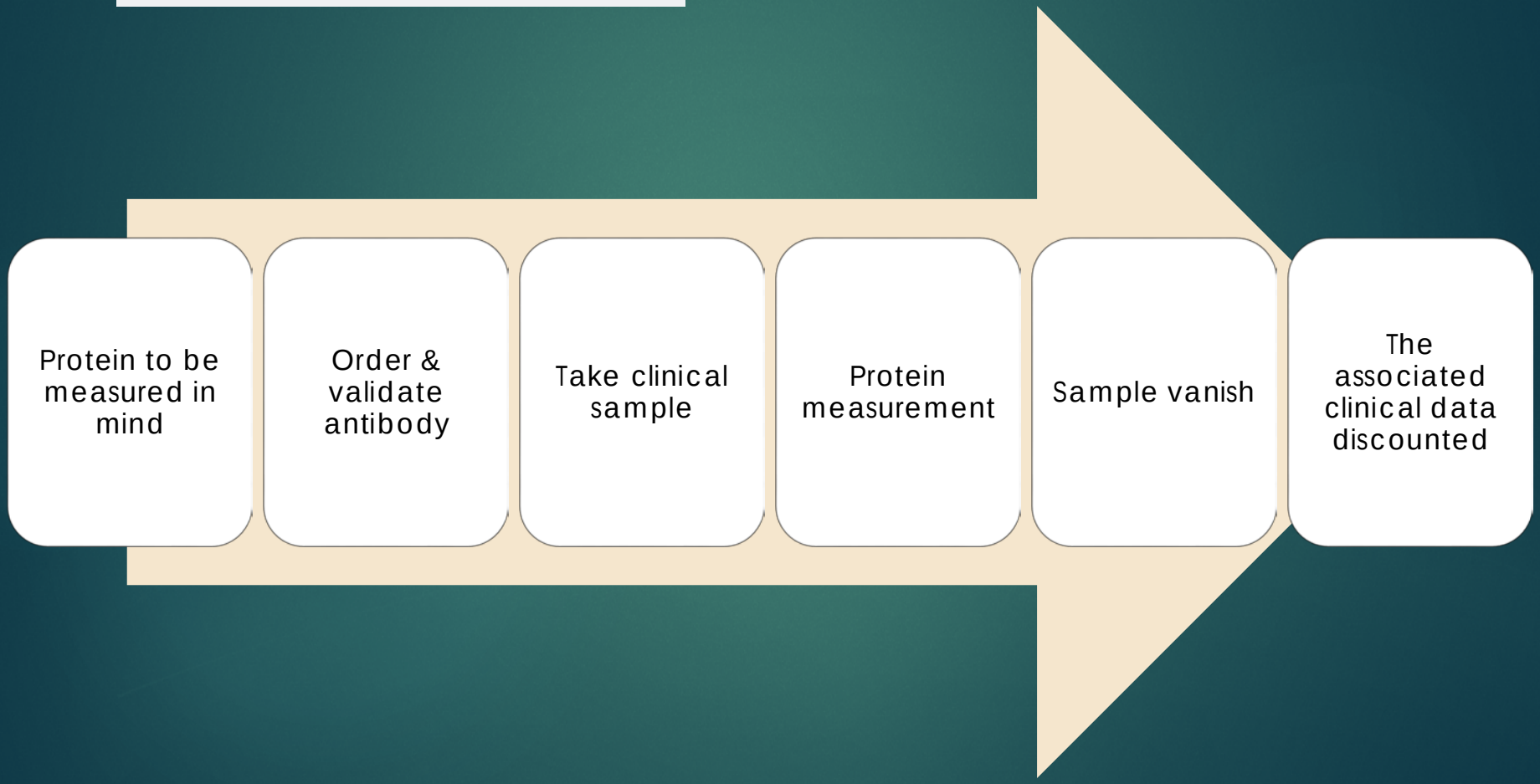
Reproducibility: Standardize antibodies used in research.

Nature. 2015. By Andrew Bradbury& Andreas Plückthun.



The hidden need

SAMPLES **DOWN THE DRAIN**



An ideal methodology

Fast and cheap

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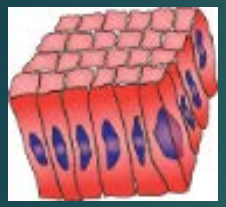
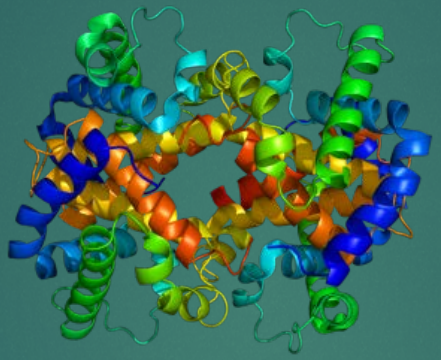
The challenge: reproducible sample preparation

Biopsy-level tissue



Proteins



Peptides

At least 19,815 protein sequences (SwissProt) in human proteome.

~50 % expressed in a tissue.

Tryptic peptides (6~30 residues, cleaved by Trypsin specifically, allowing max. 2 missed cleavages)

779, 467 peptides

Pressure cycling technology (PCT)

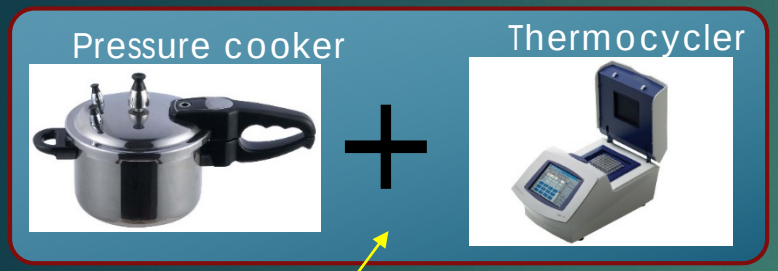


Table 1
Pressure cycling applications

Application	Sample type	Instrument	Comments	References
Native protein isolation, immunoaffinity enrichment	Cells, tissues, whole organisms	Barocycler NEP3229 and NEP2320; Pressure Cycling Technology (PCT) Shredder, Shredder SG3	Extraction of poorly soluble membrane-associated and integral membrane proteins in their native conformation	(71)
Systems biology	Biological fluids, cells, tissues, whole organisms	Barocycler NEP3229 or NEP2320; PCT Shredder, Shredder SG3	Detergent-free concurrent extraction of proteins, nucleic acids, and lipids	(8, 67)
Proteomics: protein extraction	Cells, tissues, whole organisms	Barocycler NEP3229 or NEP2320; PCT Shredder, Shredder SG3	Pressure-enhanced extraction of hydrophobic membrane proteins	(9–11, 41, 72)
Genomics, transcriptomics, DNA sequencing: extraction of nucleic acids	Cells, tissues, whole organisms	Barocycler NEP3229 or NEP2320, PCT Shredder, Shredder SG3	Better recovery of nucleic acids from difficult to lyse samples. Intact or minimally sheared genomic DNA	(73, 74)
Concurrent decontamination and extraction of multiple analytes from highly pathogenic organisms	Samples containing BSL-3 and BSL-4-rated material	Barocycler NEP3229 and NEP2320	Synergy of high temperature, pressure, and chemistry inactivates pathogens	(1)
Pressure-enhanced enzymatic reactions	Samples for proteomics and glycoproteomics	Barocycler NEP3229 and NEP2320	Pressure helps to speed up digestion and decrease the amount of denaturants	(40, 41, 44, 75)
Structural biology, protein conformation studies	Samples of proteins, protein complexes, lyposomes, cells, or microorganisms	Barocycler HUB440	Real-time pressure-perturbation spectroscopy (UV/Vis, fluorescence, EPR, NMR)	(76)

Powell, et al. 2012. Methods Mol Biol. 2012;881:27-62. doi: 10.1007/978-1-61779-827-6_2.

PBI Pressure BioSciences Inc.

I&L Biosystems

PCT-assisted proteomic sample preparation

Tissue biopsy (fresh frozen, FFPE, liquid, etc) (0.2~1mg)

PCT-MicroTube

PCT-MicroPestle and PCT-MicroCap

~4 hours for tissue samples
~2hr for liquid samples

Batch processing (12 ~ 96 samples per batch)

PCT in Barocycler

PCT: Pressure Cycling Technology

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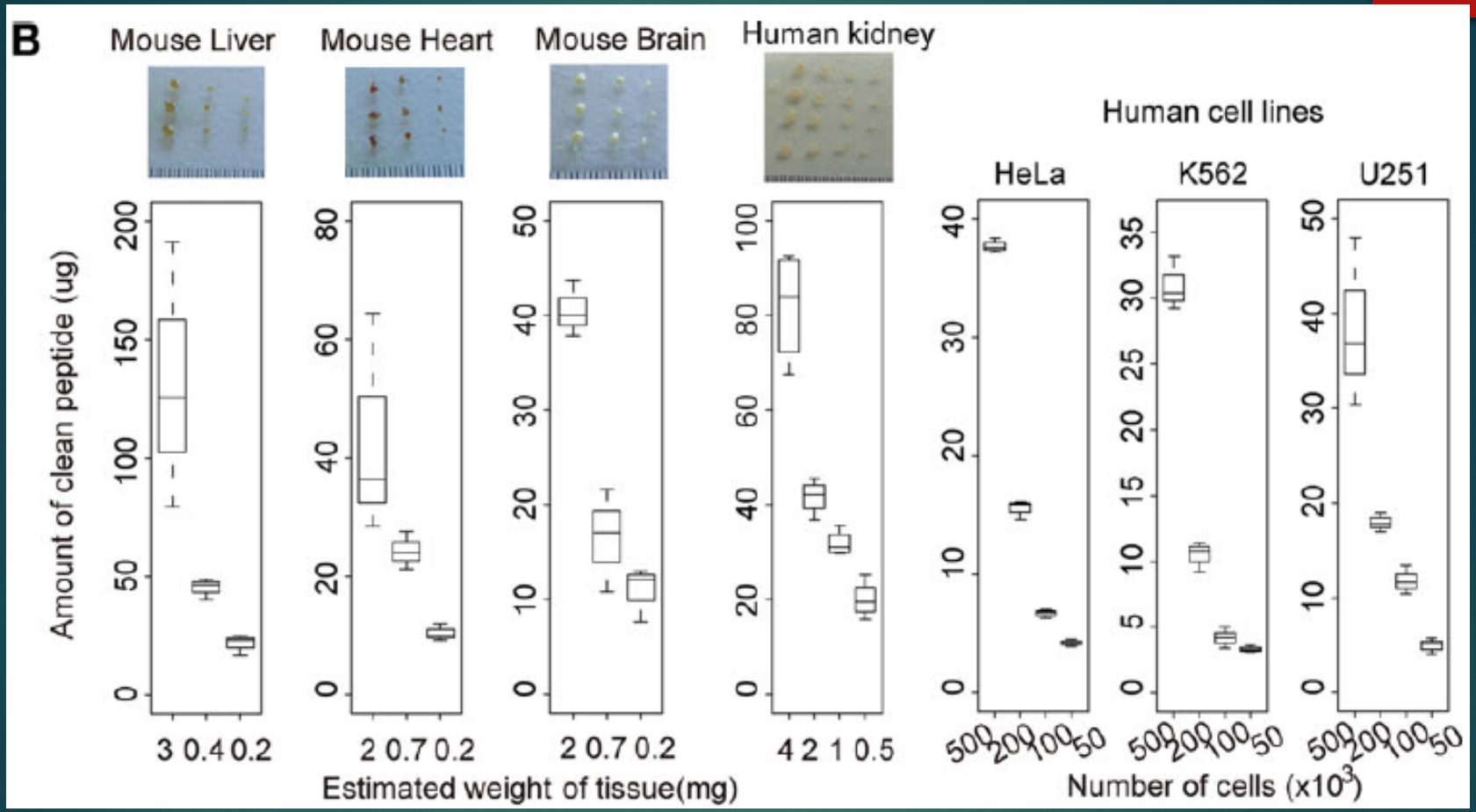
Peptide yield using PCT-MicroCap



Shao, et al. Proteomics 2015, 15, 1–11

PCT: Pressure Cycling Technology

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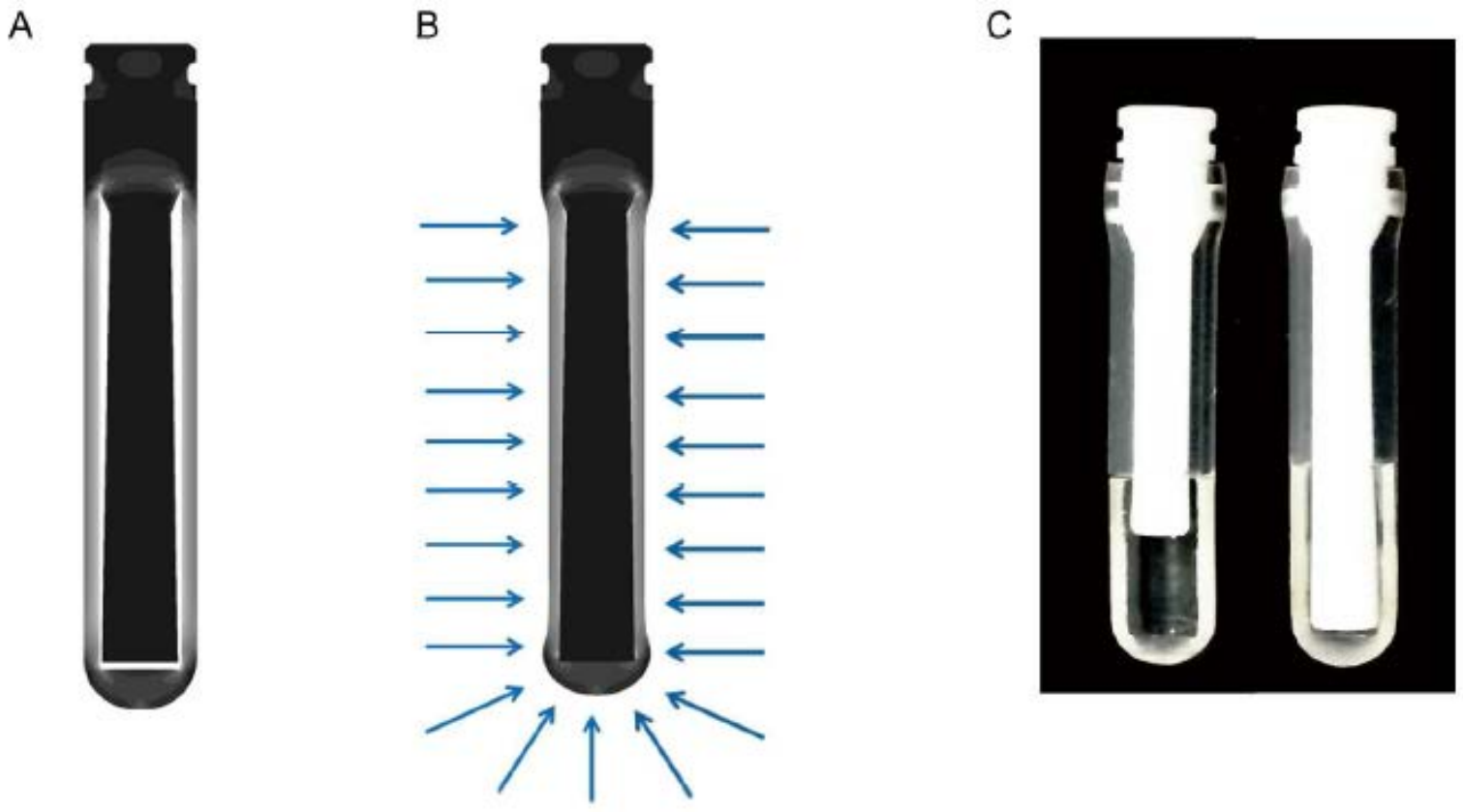
PCT-MicroPestle



Shao, et al. J Prot Res. 2016. accepted

PCT: Pressure Cycling Technology

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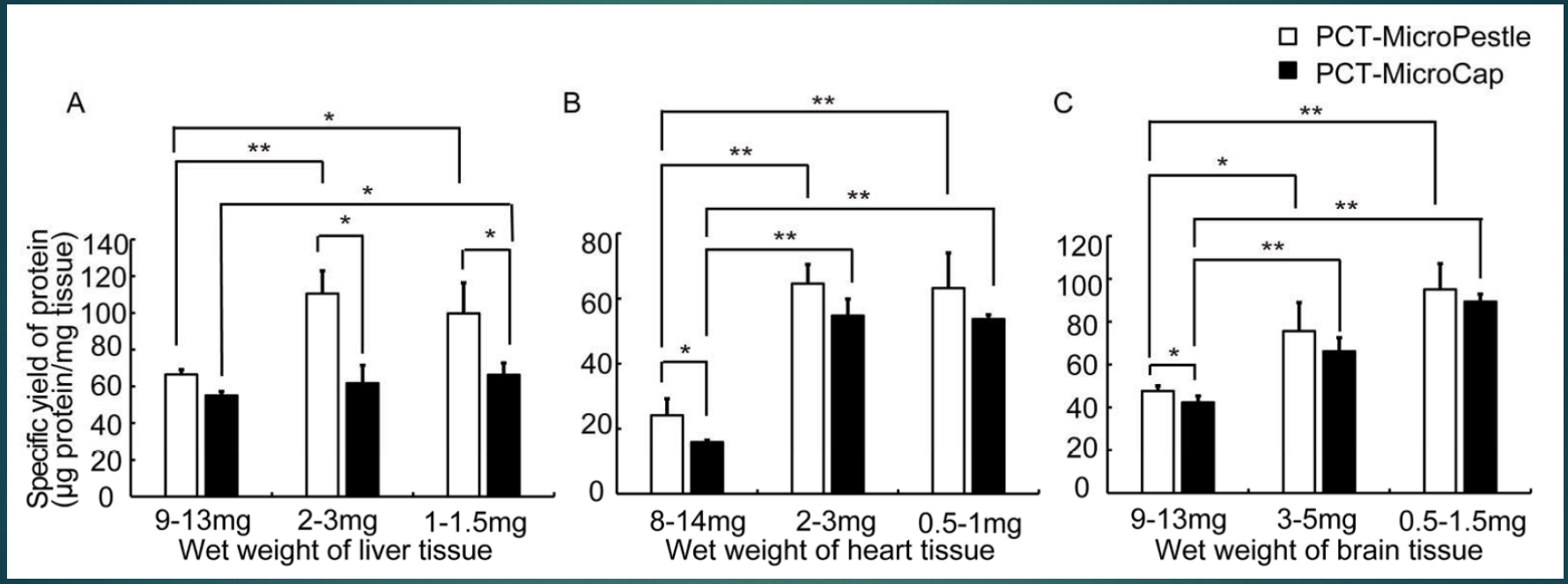
Increased peptide extraction efficiency



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Data-Independent Acquisition (DIA), SWATH MS

Method name	Instrument	Description	LC resolution / duty cycle to cover the full m/z range	Dynamic range	Specificity / % peptides observable[1]	Ref
Shotgun CID	Q-TOF	1 low energy nozzle-skimmer full scan (MS1) 1 high energy nozzle-skimmer full scan (MS2) ⇔ 1 swath of 800 Da for 1 s	+ + + (~2 sec covering 400-1200 m/z)	- (n/a)	- - (7%)	(17)
MS^E (® Waters)	QqTOF	1 low energy full scan (MS1) 1 high energy full scan (MS2) ⇔ 1 swath of 800 Da for 1 s	+ + + (~2 sec covering 400-1200 m/z)	+ (3-4 logs across LC (38))	- - (7%)	(19)
All Ion Fragmentation (AIF)	Orbi-Trap	1 low energy full scan (MS1) 1 high energy full scan (MS2) ⇔ 1 swath of 800 Da for 1 s	+ + + (~2 sec covering 400-1200 m/z)	+ (4 logs across LC)	- - (7%)	(21)
SWATH-MS	QqTOF	Sequential windows (25 Da) scans ⇔ 32 swaths of 25 Da (1 Da overlap) @ 100 ms / swath	+ + (3.2 sec covering 400-1200 m/z)	+ + (4 logs intra-scan)	+ + (89%)	This study
Data Independent Acquisition	Ion Trap	Sequential windows (10 or 20 Da) scans ⇔ 20 swaths of 10 Da (no overlap) @ 200 ms / swath	+ (~4 sec covering 200 Da per sample injection)	+ >2 log? (10:1 ratio mixtures)	- (16%)	(18) (22)
Data Independent Acquisition – ETD-CAD	Ion Trap – ETD-CAD	Sequential windows (20 Da) scans ⇔ swaths of 20 Da (1 Da overlap), rate not disclosed	+ (not disclosed)	- (n/a)	- - (6%)	(23)
Precursor acquisition independent from ion count (PAcIFIC)	Ion Trap	Sequential windows (2.5 Da) scans – 20 Da at a time per LC injection ⇔ 30 swaths of 2.5 Da (1.5 Da overlap) @ 250 ms / swath	- (5.4 sec covering 45 Da per sample injection)	+ + + (7 logs across LC)	+ (68%)	(20) (24)

Gillet, et al. MCP. 2012

17. Purvine S., Eppel J. T., Yi E. C., Goodlett D. R. (2003) Shotgun collision-induced dissociation of peptides using a time of flight mass analyzer. Proteomics 3, 847–850
18. Venable J. D., Dong M. Q., Wohlschlegel J., Dillin A., Yates J. R. (2004) Automated approach for quantitative analysis of complex peptide mixtures from tandem mass spectra. Nat. Methods 1, 39–45
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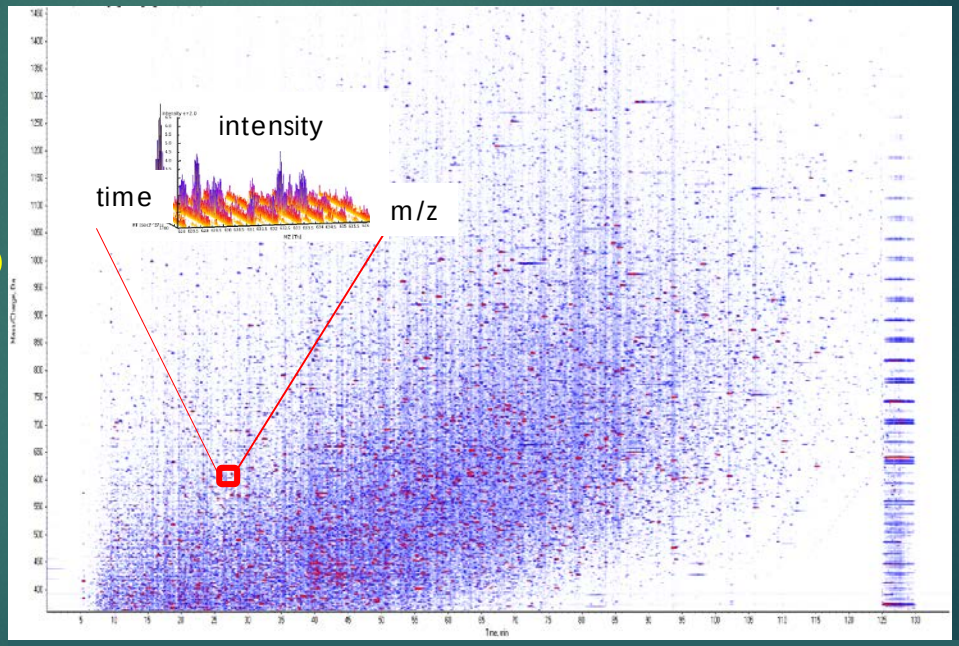
24. Panchaud A., Jung S., Shaffer S. A., Aitchison J. D., Goodlett D. R. (2011) Faster, quantitative, and accurate precursor acquisition independent from ion count. Anal. Chem. 83, 2250–2257 MedlineGoogle Scholar

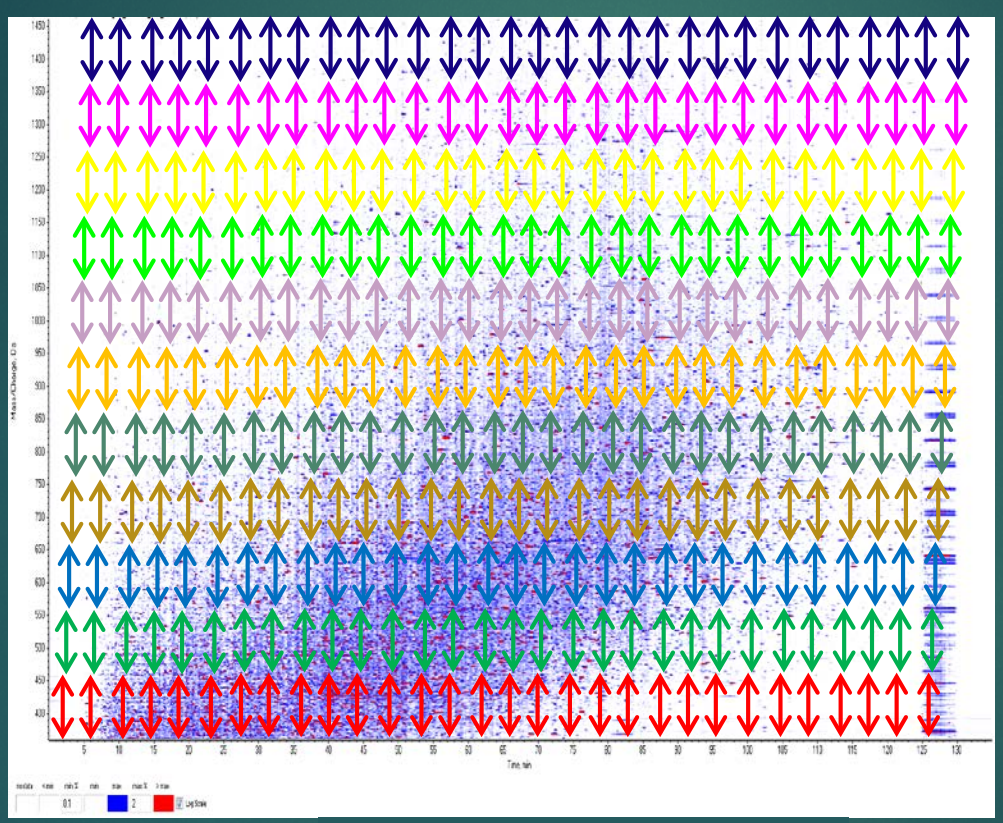
The digital representation of a human MS-measurable proteome

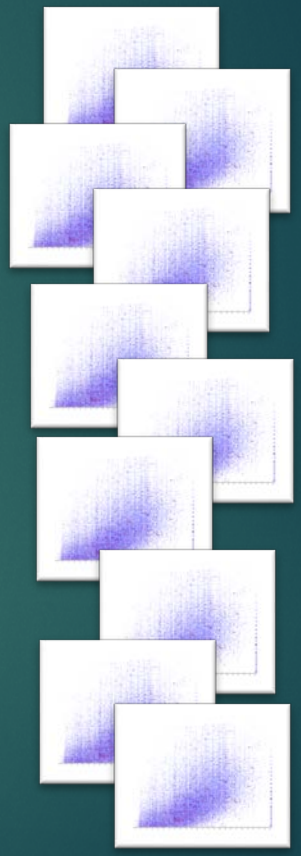
Mass-to-charge ratio (m/z)
(MS behavior)



Retention time (Liquid Chromatography behavior)

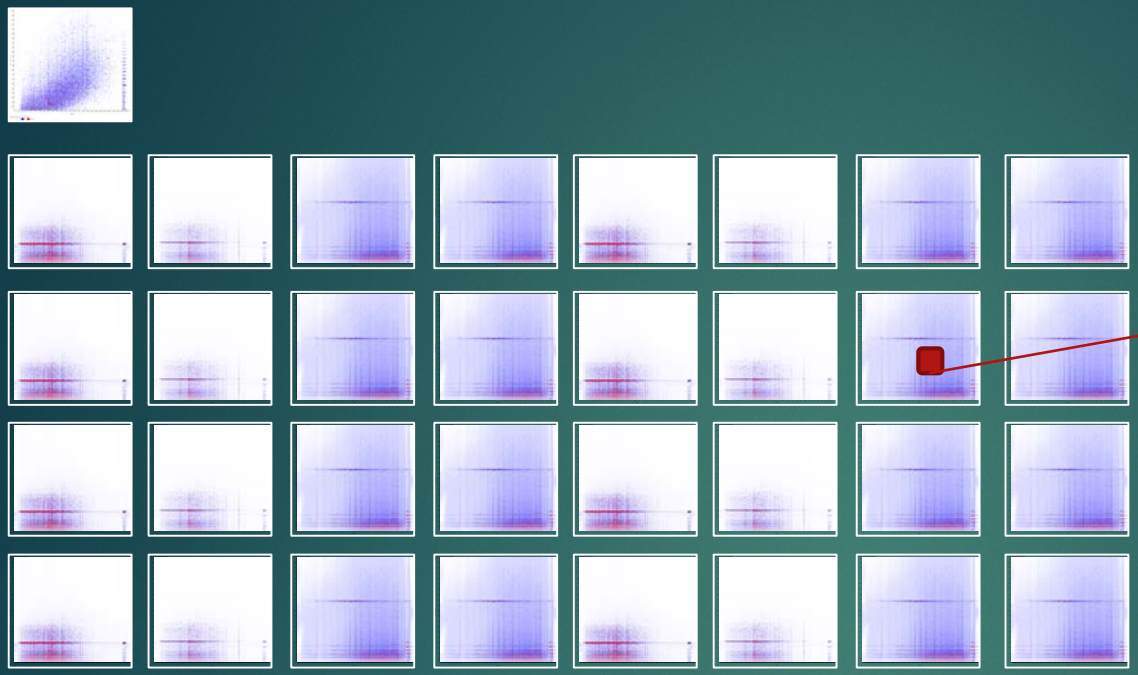
SWATH-MS:
digitization of MS-measurable human proteome

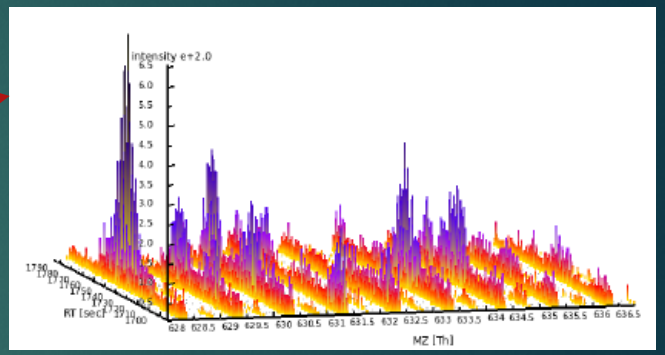




Gillet, et al. Mol Cell Proteomics. 2012.

One swath file contains ***33*** maps: SWATH maps





4 dimensional images of > 20 billion pixels

Current analysis makes use of only a minor part of the BIG DATA

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PCT-SWATH

Biopsy tissue (0.2~2mg) → MicroTube → MicroPestle → Batch processing → PCT → SWATH/ DIA-MS

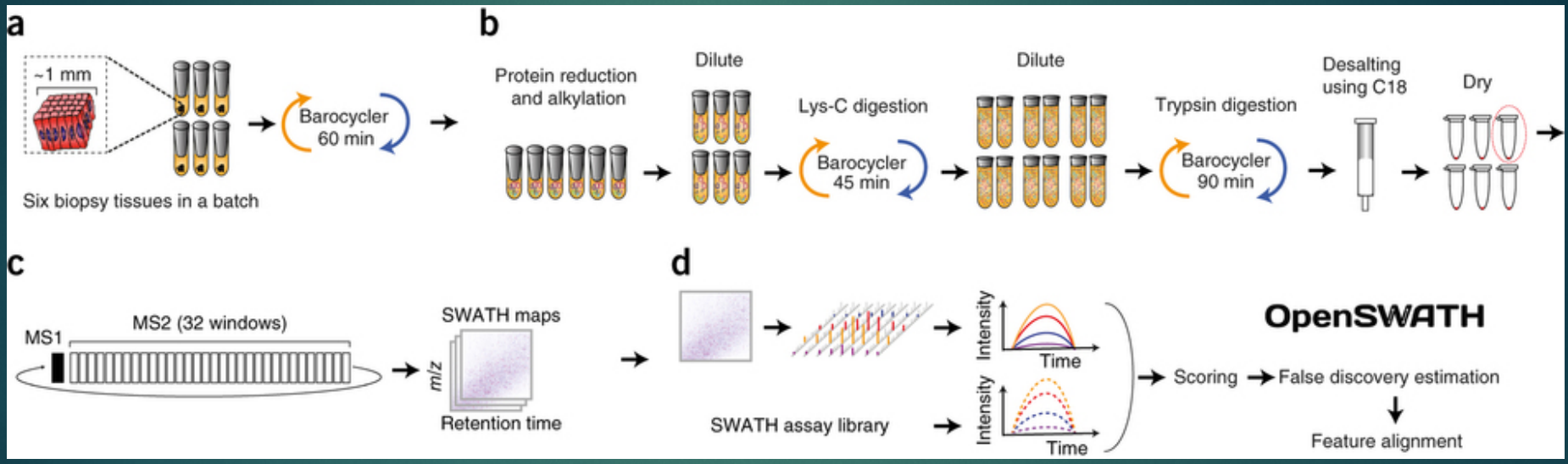
Barocycler (Pressure Biosciences)

< 6 hrs per batch of samples

0.5 ~ 2 hrs per sample

Guo, et al. ***Nature Medicine*** 21, 407–413 (2015)

PCT-SWATH



Guo, et al. ***Nature Medicine*** 21, 407–413 (2015)

Digital biobanking using PCT-SWATH

A tissue biopsy sample

A dry blood spot (< 1 ul)

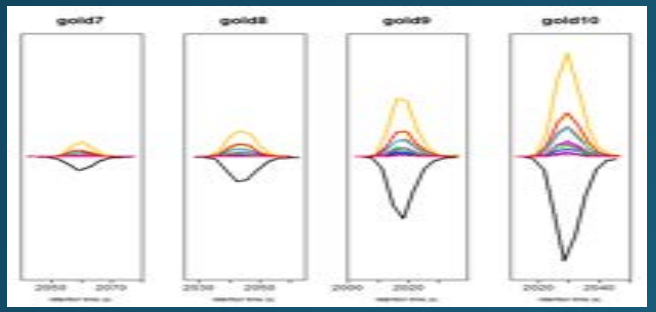
Any samples containing proteins

< 12 hours

Single digital file (3~4 Gb)

In silico experiments

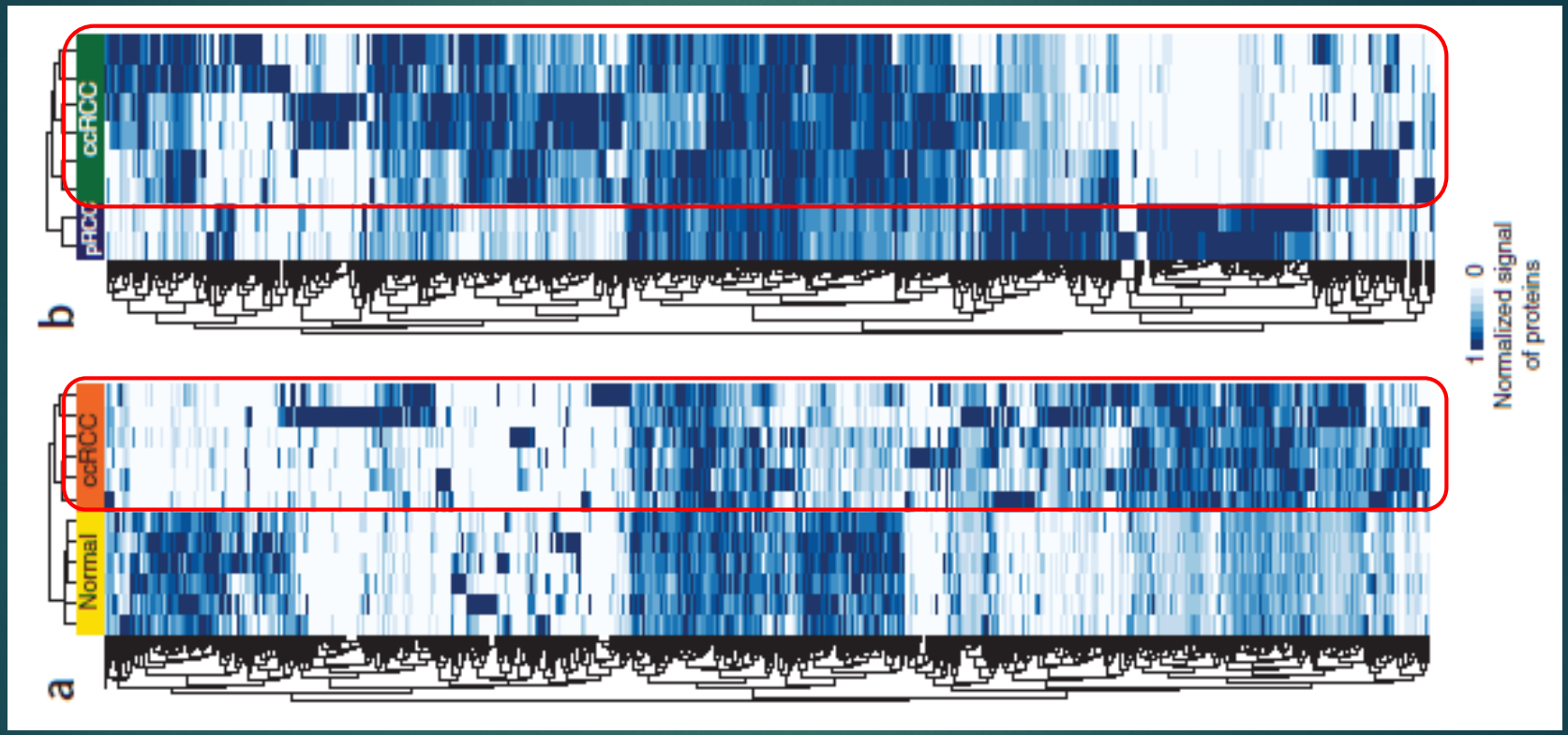
Protein quantification



Digital archive of MS-measurable proteome

Guo, et al. ***Nature Medicine*** 21, 407–413 (2015)

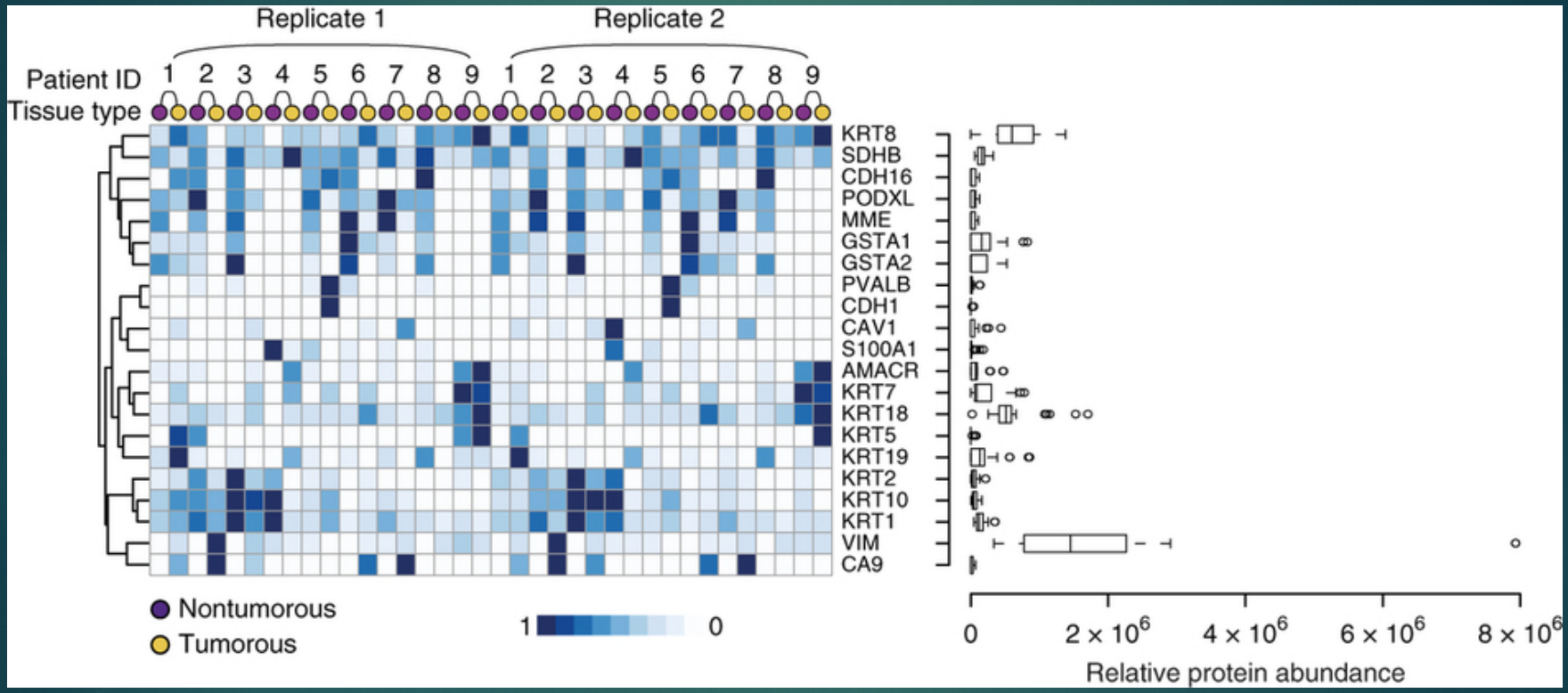
Relative Quantification of ~ 2000 SwissProt proteins from kidney biopsy samples using OpenSWATH



OpenSWATH by ETH zürich

Roest & Rosenberger, et al. Nature Biotech. 2014
Guo, et al. Nature Medicine. 2015

21 of 53 known protein biomarkers for kidney cancers



Guo, et al. Nature Medicine. 2015

Summary

- 1, We present a fast method to digitize the entire proteome of a biopsy-level samples.
- 2, 2000-5000 proteins can be quantified with high degree of accuracy.
- 3, Proof-of-principle application to drug responsiveness prediction
- 4, More proteins can be potentially identified and quantified from the digital proteomic maps.

Acknowledgements

ETH Zurich: Ruedi Aebersold, Alex Leitner, Ching Chiek Koh, Shiying Shao, Petri Kouvonen, Ludovic Gillet, Ben Collins, Hannes Rost, George Rosenberger, Zhu Yi, Vincen Wu, Luxi Zhang, Tatjana Sajic, Peter Blattmann, Uwe Schmitt, Lorenz Blum, Witold Wolski, Marija Buljan, Yasuo Uchida, Ariel Bensimon, Patrick Pedrioli, Sebastian Salzmann, Wen Yan,

Funding

SystemsX: PhosphosNET Precision & Personalized Medicine; Swiss National Science Foundation; European Research Council, PrECISE

University Hospital Zurich
Peter Wild, Christine Fritz, Qing Zhong, Ulrich Wagner, Markus Rechsteiner, Nadejda Valtcheva, Ailsa Christiansen, Niels Rupp, Christian D. Fankhauser, Nora Toussaint

Kantonsspital St. Gallen
Silke Gillessen, Markus Joerger, Wolfram Jochum

EMBL-EBI
Julio Saez-Rodriguez, Michael Menden, Emanuel Gonçalves

University of Cologne
Andreas Beyer, Li Li, Konstantina Charmpi

Pressure Biosciences:
Alexander (Sasha) Lazarev, Vera Gross

National Cancer Institute
Yves Pommier, William Bill Reinhold, Augustin Luna, Vinodh N Rajapakse,

Dana-Farber Cancer Institute
Chris Sander, Augustin Luna

EMBL Heidelberg
Martin Beck, Alessandro Ori, Murat Iskar

Leibniz Institute on Aging
Alessandro Ori

Institut Curie
Laurence Calzone

Presicion Pictures
Guo An

IUL

Thank you for your attention!

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