Leiden University
Medical Center

Different technologies used for proteomics measurement

EPI-OMICs Journal Club
9-Feb-2023

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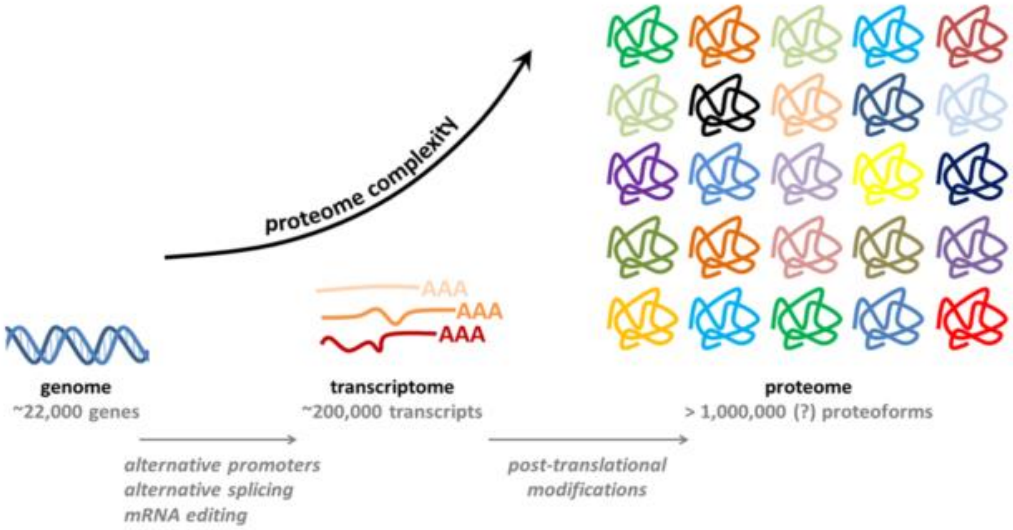
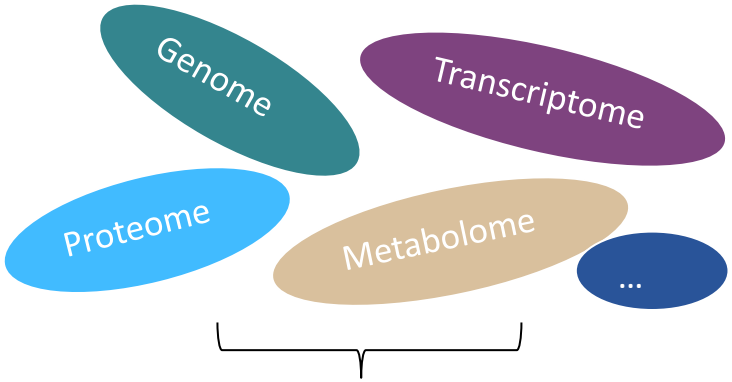
Department of Clinical Epidemiology

Background



Central dogma

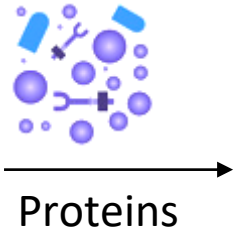


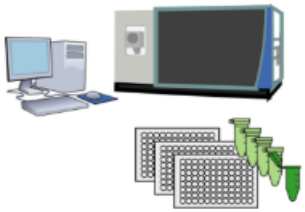
Genome: relatively static

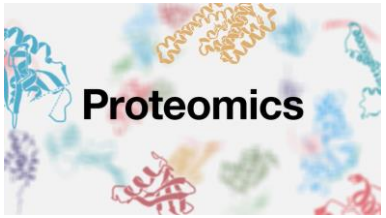
Proteome: dynamic in time and different for each cell type

- ✓ ~30,000 proteins make up the human proteome
- ✓ Medications or lifestyle changes cause shifts in the proteome

What's Proteomics?







Proteomics is a high throughput way to measure all proteins in a cell or tissue by using mass spectrometry or other methods

Why do we use Proteomics?

1. Discover potential biomarkers (also those previously unrecorded)
   - prostate-specific antigen (PSA) for cancer diagnosis
   - …
2. Identify druggable targets
3. Identify novel pathways involved in the process of disease

PubMed.gov

Proteomics[Title/Abstract] Search

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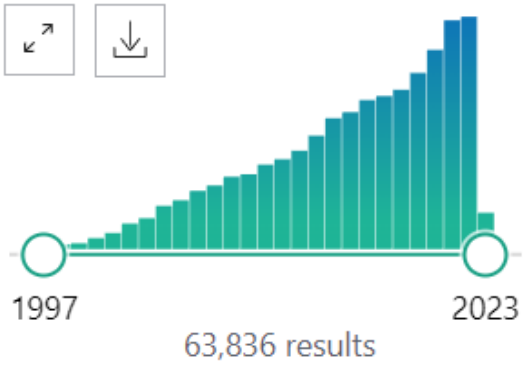
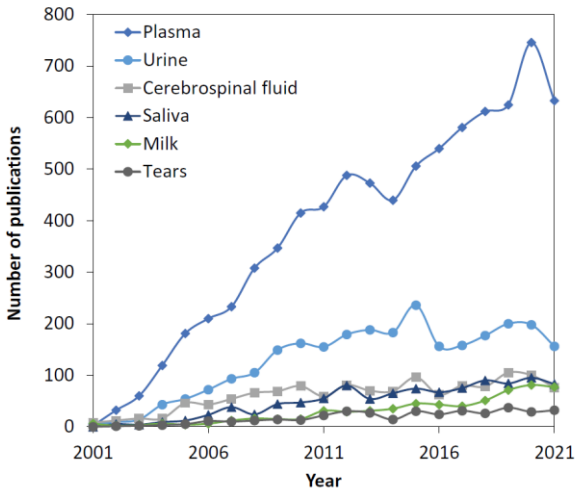
RESULTS BY YEAR





Hepatology. 2023 Feb 6. doi: 10.1097/HEP.0000000000000300. Online ahead of print.

Plasma proteomic signature of fatty liver disease: the Rotterdam Study

Yasir J Abozaid [1], Ibrahim Ayada [2], Laurens A van Kleef [2], Costanza L Vallerga [3], Qiuwei Pan [2], Willem Pieter Brouwer [2], M Arfan Ikram [1], Joyce Van Meurs [3 4], Robert J de Knegt [2], Mohsen Ghanbari [1]

Nature. 2020 Jul;583(7816):469-472. doi: 10.1038/s41586-020-2332-7. Epub 2020 May 14.

Proteomics of SARS-CoV-2-infected host cells reveals therapy targets

Denisa Bojkova [# 1], Kevin Klann [# 2], Benjamin Koch [# 3], Marek Widera [1], David Krause [2], Sandra Ciesek [1 4], Jindrich Cinatl [5], Christian Münch [6 7 8]

nature medicine LETTERS https://doi.org/10.1038/s41591-019-0665-2

Plasma protein patterns as comprehensive indicators of health

Research JAMA. 2016;315(23):2532-2541. doi:10.1001/jama.2016.5951

Original Investigation | INNOVATIONS IN HEALTH CARE DELIVERY

Development and Validation of a Protein-Based Risk Score for Cardiovascular Outcomes Among Patients With Stable Coronary Heart Disease

Peter Ganz, MD; Bettina Heidecker, MD; Kristian Hveem, MD, PhD; Christian Jonasson, PhD; Shintaro Kato, MS; Mark R. Segal, PhD; David G. Sterling, PhD; Stephen A. Williams, MD, PhD

Technology development

Low throughput → High throughput

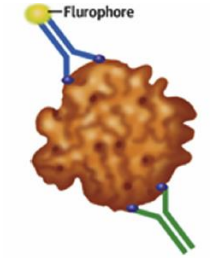
- Western blot
- Enzyme-linked immunosorbent assays (ELISA)
- Immunohistochemistry
- Immunofluorescence

- Mass spectrometry
- Aptamer-based array
- Immunoaffinity array (proximity extension assay-based)

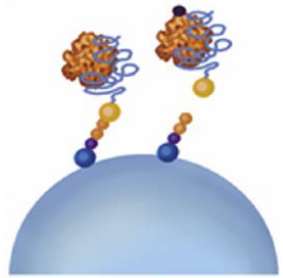
Four common proteomic technologies

- Mass spectrometry (MS)-based
  - LC/MS-MS
  - Fragments proteins and measure Mass-to-charge ratios

- Protein microarray
  - Antibodies with fluorophore

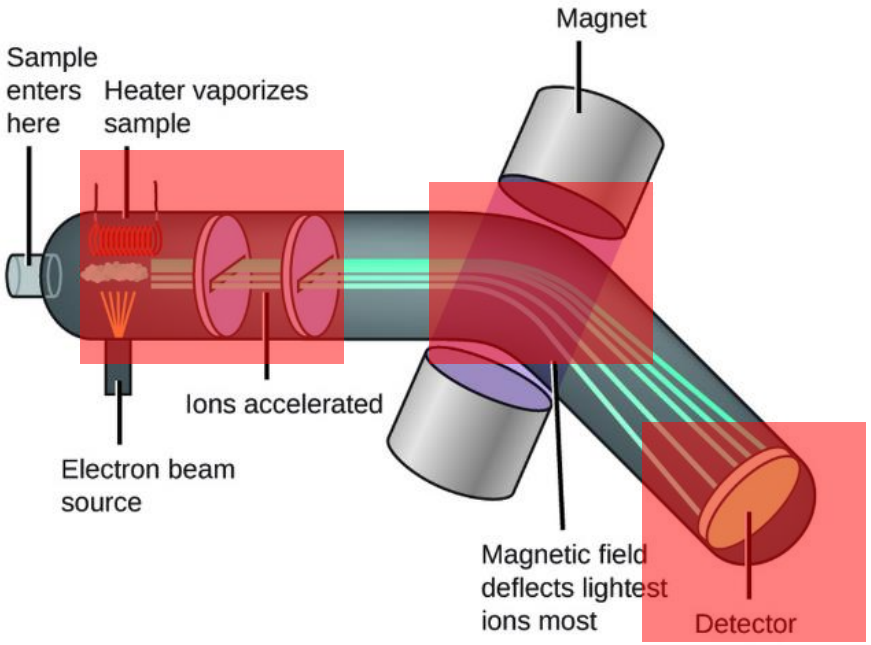


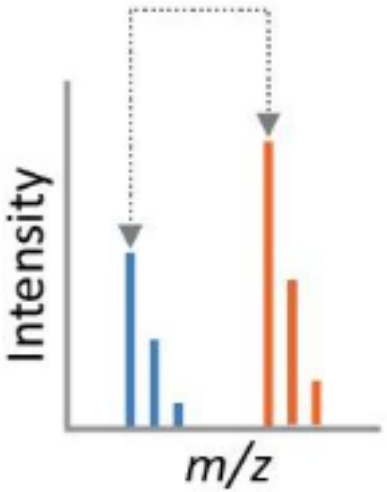
- Aptamer-based
  - SOMA (slow off-rate modified aptamers)scan platform
  - Oligonucleotides increase sensitivity & specificity



- Proximity extension assay (PEA)-based
  - OLINK platform
  - Paired DNA-conjugated antibodies

1. MS-based proteomics





MS has three basic components:

- Ion source
  - Electrospray ionization (ESI)
- Mass analyser: separate ions by their mass-to-charge ratios (m/z)
  - Quadrupoles, time-of-flight (TOF), Orbitrap
- Detector

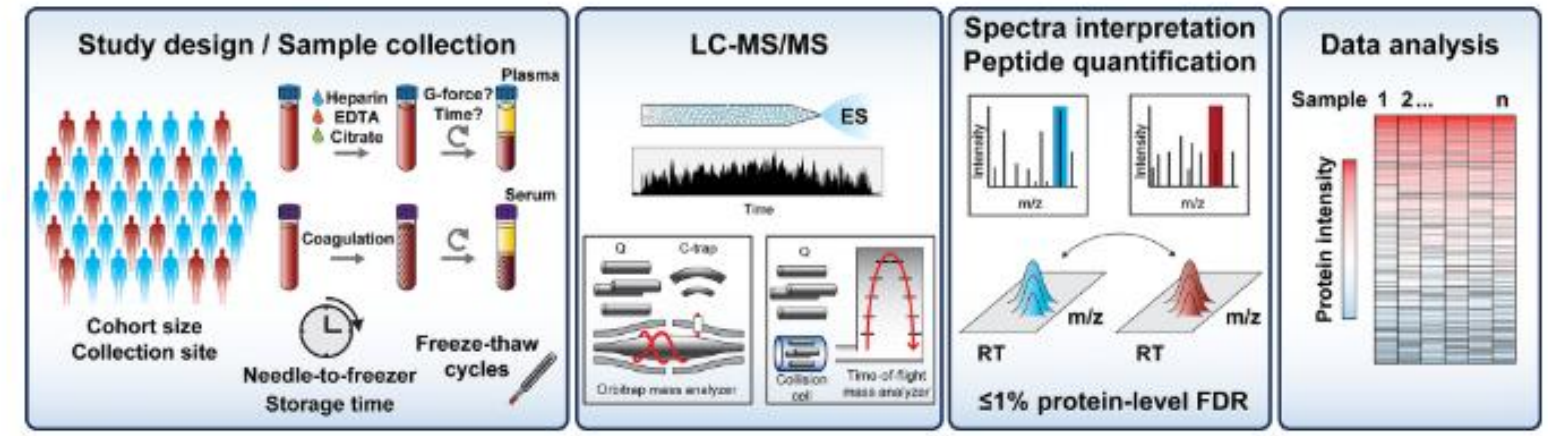
1. MS-based proteomics

Top-down strategy

- Full-length protein -> problems: heterogeneity of proteins

Bottom-up strategy

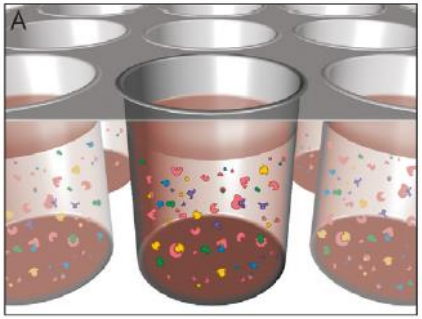
- Peptides (digested protein)
- Coverage more than 10,000 preteins



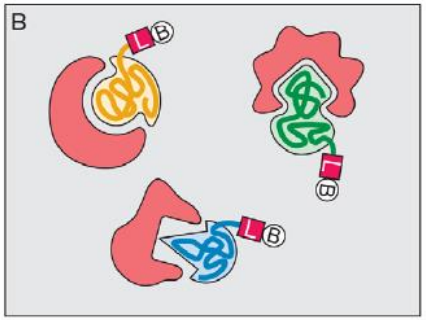
Protein digestion -> peptide purification -> separated by Liquid chromatography -> Ionized by electrospray (ES) -> Analysis in MS

2. Aptamer-based proteomics

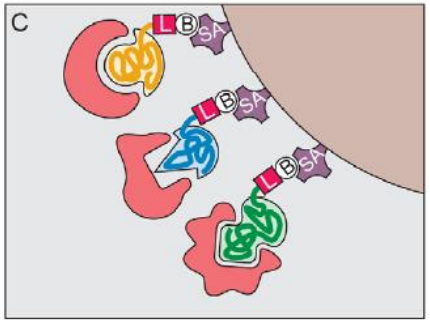
- Aptamers are short, single-stranded oligonucleotides (DNA) that can selectively bind to a specific protein target



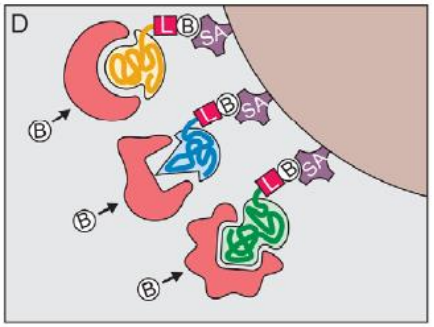
Binding (SOMAmers + samples)



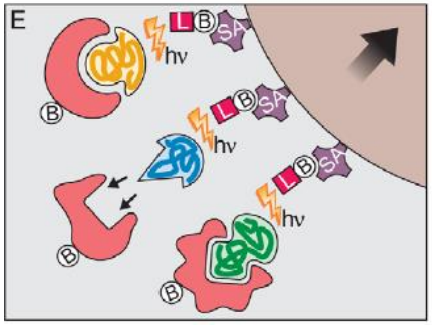
SOMAmers contain biotin (B) and photo-cleavable linker (L) and **fluorescent tag** at 5' end, Binding with proteins



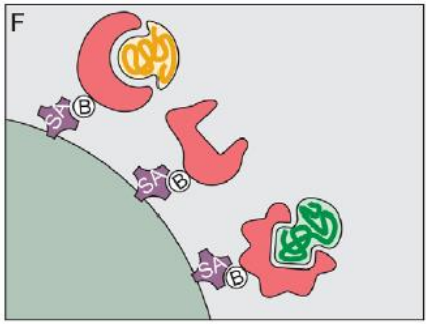
SOMAmers captured onto a bead coated with streptavidin (SA) which binds biotin



Proteins are tagged with NHS-biotin

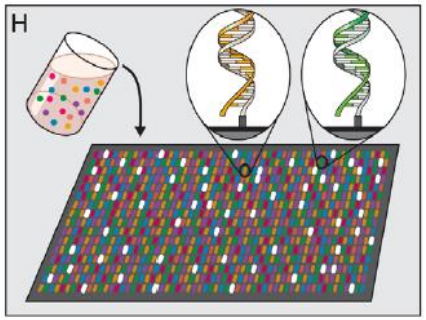


UV light cleaves the linker and SOMAmers released



SOMAmer-protein complexes captured onto new SA coated beads by protein biotin tag

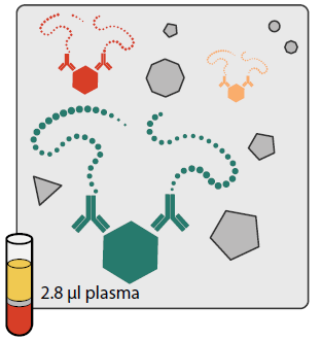
SOMAmers released from complexes into solution at high pH



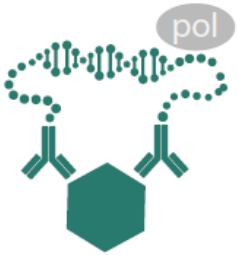
Remaining SOMAmers are quantified by hybridization to microarray

3. Proximity extension assay (PEA)-based proteomics

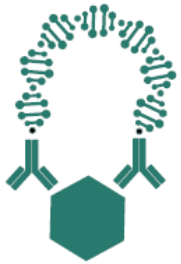
Using paired, nucleotide-labelled antibody probes for multiplexing of proteins at high sample throughput



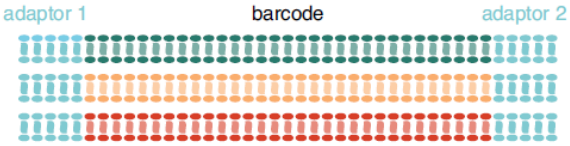
DNA-conjugated **antibodies** bind to their target protein



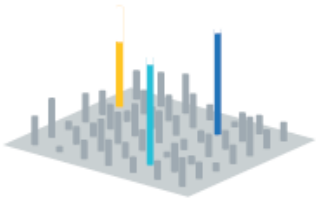
Antibodies with correct matched pairs hybridize and extended by DNA polymerase

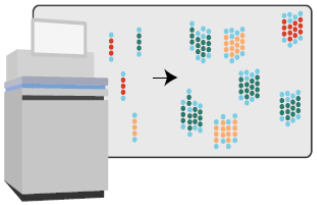


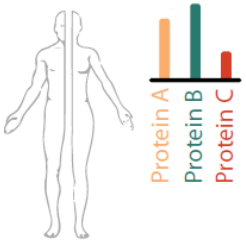
DNA tags extended & amplified



Unique barcode-regions identify each sample and protein and adaptors







Hybridized DNA strands: amplified and measured using qPCR or NGS

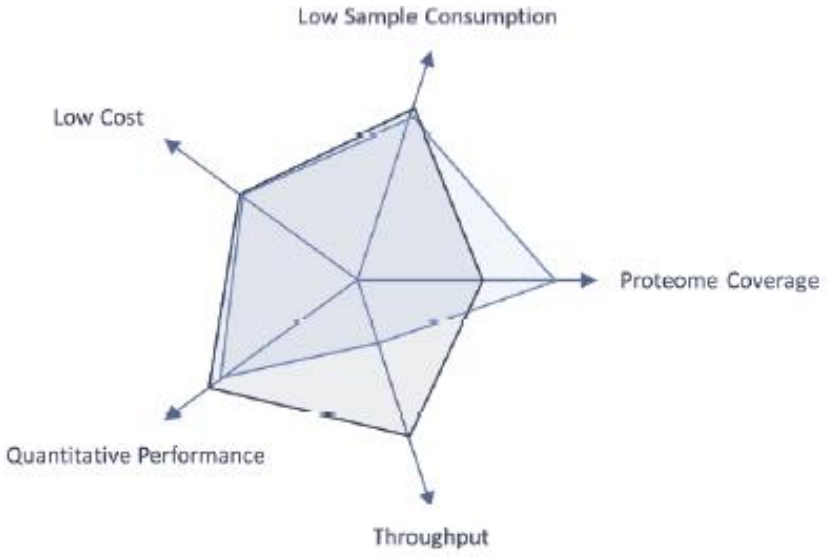
Which one should we choose?

Q: What are important factors?

It depends on

1. Budgets! ($.$, always important)
2. Sample size or power calculation
3. Research question (if focus on posyttranslational modifications)
4. Sample consumption (if difficult to get)
5. Dynamic range (if huge or not)

…



Technical factors influence selection

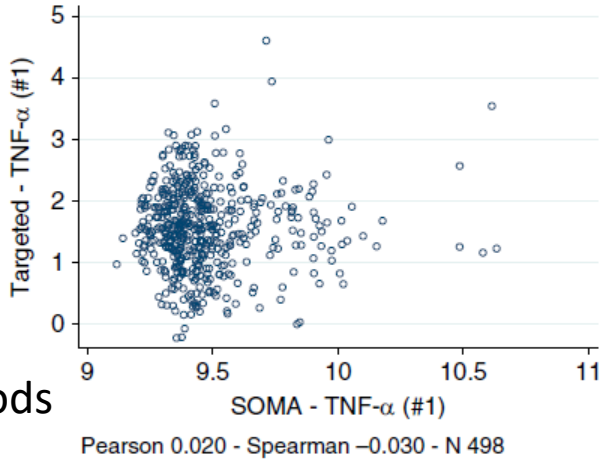
	MS	Aptamer	PEA
Sample throughput	Low (untargeted) to moderate (targeted)	High	High
Analyte multiplex	All proteins in sample: 10 to >5000	>1300 proteins	Depends on different panels
Sample volume	30ul (targeted) to 100s of ul	65ul	1ul to 100s of ul
Dynamic range	High and medium-abundance proteins	Wide (mg/mL to pg/mL)	Wide (down to fg/mL)
Reproducibility	Modest	Good	Good
Quantification	Relative, targeted labeling for absolute	Relative	Absolute or relative
Characterization of PTMs	Yes	NO	NO
Bioinformatics	Multiple software available	Easy	Easy

What will happen while using different methods?

Comparison of Aptamer-Based and Antibody-Based Assays for Protein Quantification in Chronic Kidney Disease

Carolina Lopez-Silva,[1] Aditya Surapaneni,[2,3] Josef Coresh,[1,2,3] Jochen Reiser,[4] Chirag R. Parikh,[1,2,3] Wassim Obeid,[1] Morgan E. Grams,[1,2,3] and Teresa K. Chen[1,2]

Aptamer-based VS immunoassay



Pearson 0.020 - Spearman –0.030 - N 498

- Some proteins are not correlated when using these methods

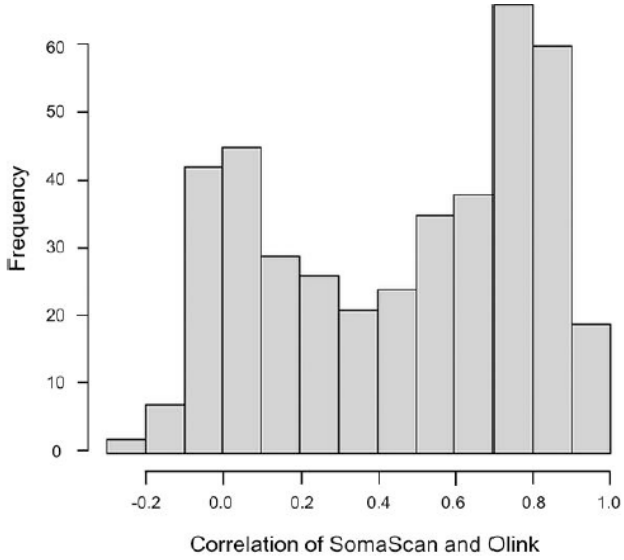
Clinical Chemistry 69:1
68–79 (2023)

Proteomics and Protein Markers

Comparison of Proteomic Measurements Across Platforms in the Atherosclerosis Risk in Communities (ARIC) Study

Mary R. Rooney,[a,*] Jingsha Chen,[a] Christie M. Ballantyne,[b] Ron C. Hoogeveen,[b] Olive Tang,[a,c] Morgan E. Grams,[a,c] Adrienne Tin,[d] Chiadi E. Ndumele,[c] Faiez Zannad,[e] David J. Couper,[f] Weihong Tang,[g] Elizabeth Selvin,[a] and Josef Coresh[a,*]

Aptamer-based VS Olink



- A substantial number of proteins are not correlated across platforms

Conclusion

	Advantage 😃	Disadvantage ☹
Mass spectrometry (MS)-based	• Results can be compared with databases available online • Allows for discovery of new proteins • Allows for analysis of post-translational modifications	• Methods may result in damage to analyte protein of interest (fragmentation by ionization source) • Sensitivity affected by presence of more abundant proteins (albumin)
Aptamer-based	• Strong affinity for target proteins even at low concentrations • Growing aptamer library increases number of proteins in the proteome	• Involving multiple steps (aptamer design, multiple washings etc.,) may introduce human error • Limited by the aptamers in libraries • **Limited ability to detect proteins with post-translational modifications**
PEA-based (OLINK)	• Minimal cross reactivity • Just require a little volume of sample (such as 1ul)	• Limited to currently developed panels • **Limited ability to detect proteins with post-translational modifications** • Limited by oligonucleotide libraries

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Thanks

Q&A