Protein mass spectrometry and proteomics

L. Dayon

Spring Semester 2025

EPFL

Course outline

- 1. Introduction

Introduction to protein analysis and proteomics; Reminders in mass spectrometry; Why proteomics and mass spectrometry?; Ionization sources, analysers, and detectors used in proteomics; Latest generation of mass spectrometers used in proteomics

- 2. Proteomic strategy and workflows

Bottom-up versus top-down strategies; Data-dependent acquisition (DDA) and data-independent acquisition (DIA) approaches; Sample preparation

- 3. Separations techniques in proteomics

Gel electrophoresis; Isoelectric focusing; Liquid chromatography (RP, IEX)

- 4. Quantitative proteomic workflows

Label-free methods; Labelling-based techniques; Other quantitative techniques

- 5. Proteomic bioinformatics

Databases; Identification of protein; Quantification of proteins; Bioinformatics tools; Practical examples

- 6. Applications to biology and clinical research

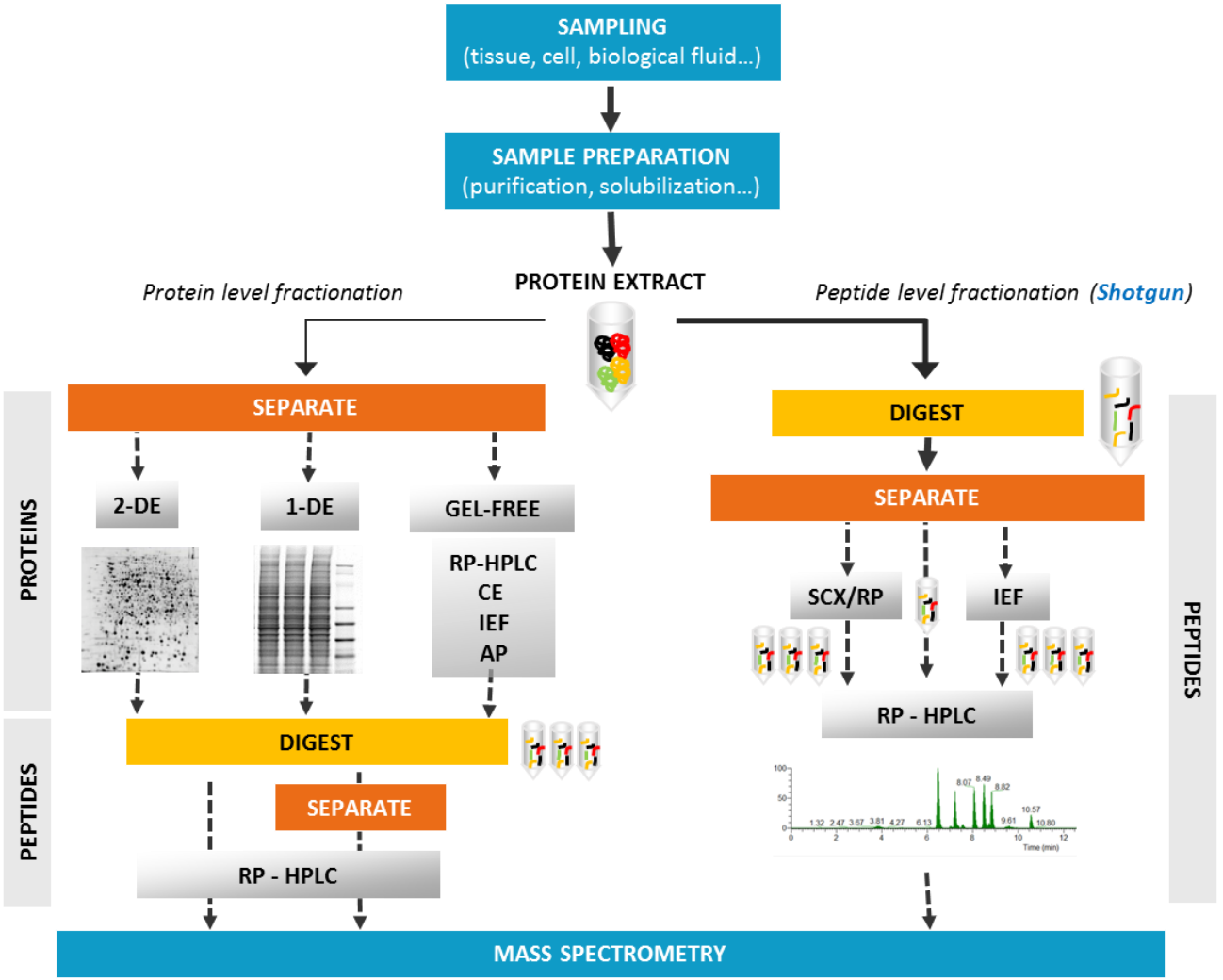
What strategy?; Experimental design; Biomarker discovery; Industrialized and population proteomics; Forensics; Targeted mass spectrometry-based approaches; Other biological applications of mass spectrometry; Advanced innovations (single-cells, 4D proteomics, multi-omics) and emerging technologies; Limitations and ethical consideration; Lab visit

Course outline

- 6. Applications to biology and clinical research

What strategy?; Experimental design; Biomarker discovery; Industrialized and population proteomics; Forensics; Targeted mass spectrometry-based approaches; Other biological applications of mass spectrometry; Advanced innovations (single-cells, 4D proteomics, multi-omics) and emerging technologies; Limitations and ethical consideration; Lab visit

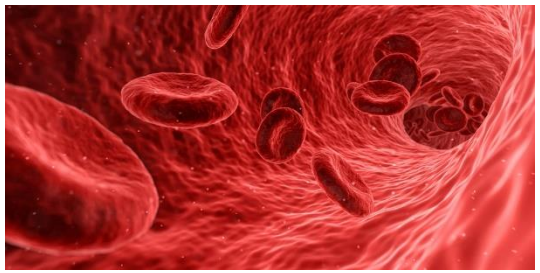
6.1. What strategy?; Experimental design

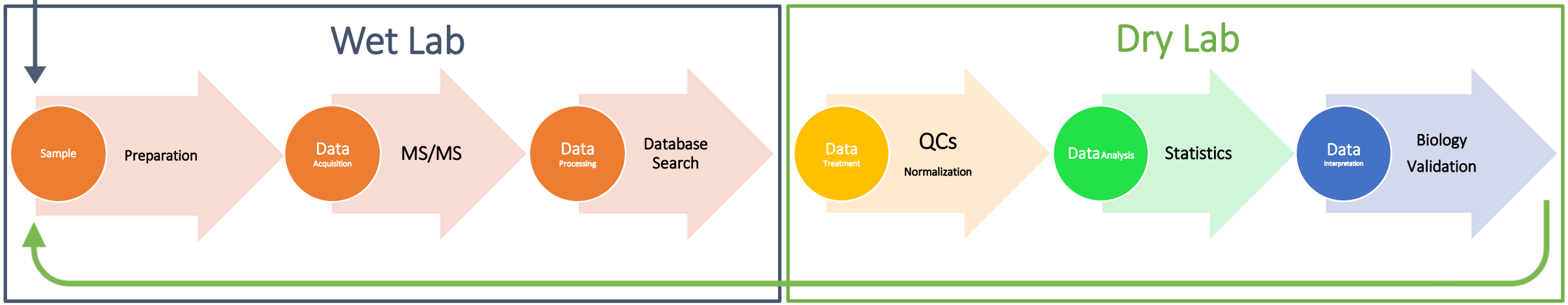


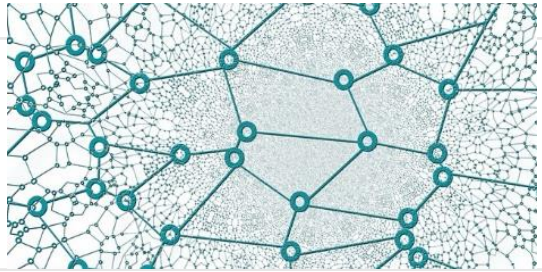
M. Abonnenc and M. Mayr, 2012, *Inflammation and Atherosclerosis*, 249-266, DOI: 10.1007/978-3-7091-0338-8_13

The path to the results

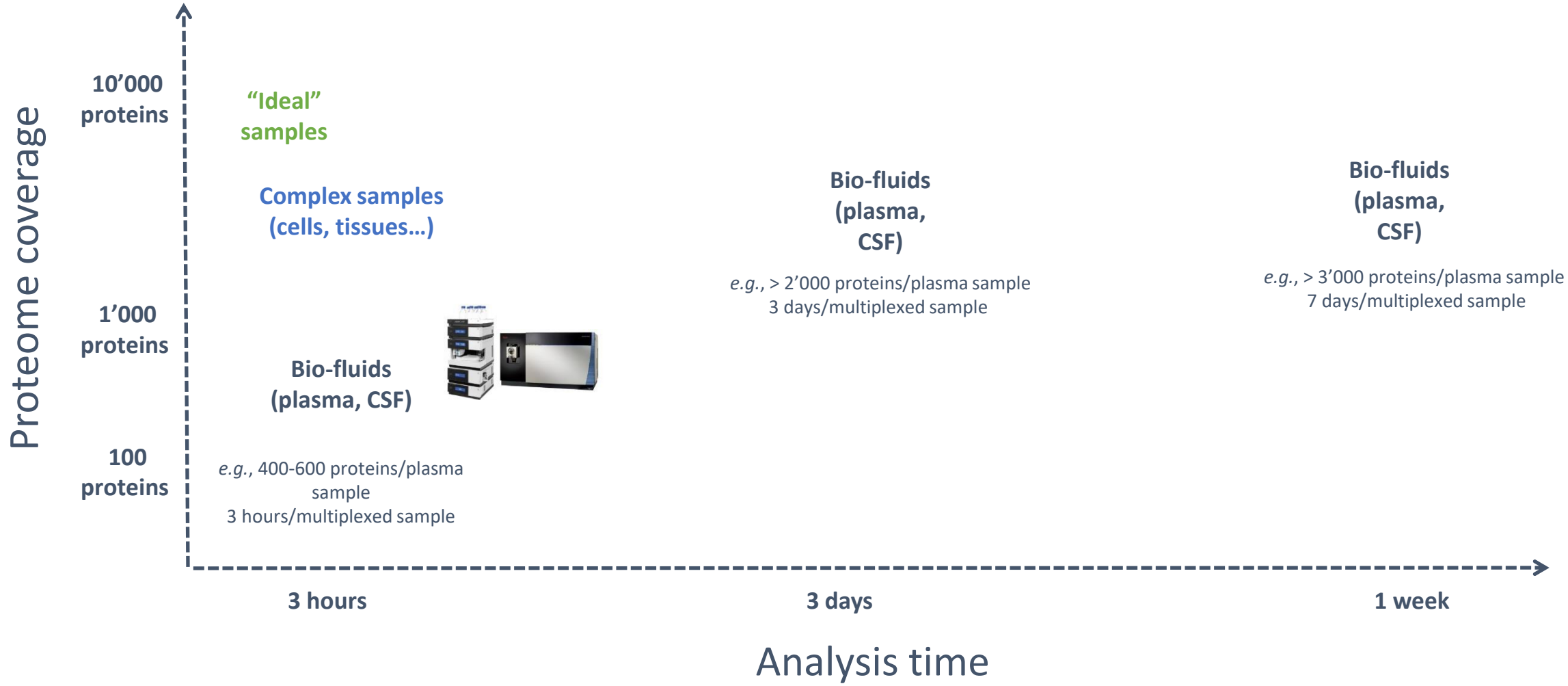
- Plasma
- Other biofluids
- Tissues
- Cells
- Organelles
- …





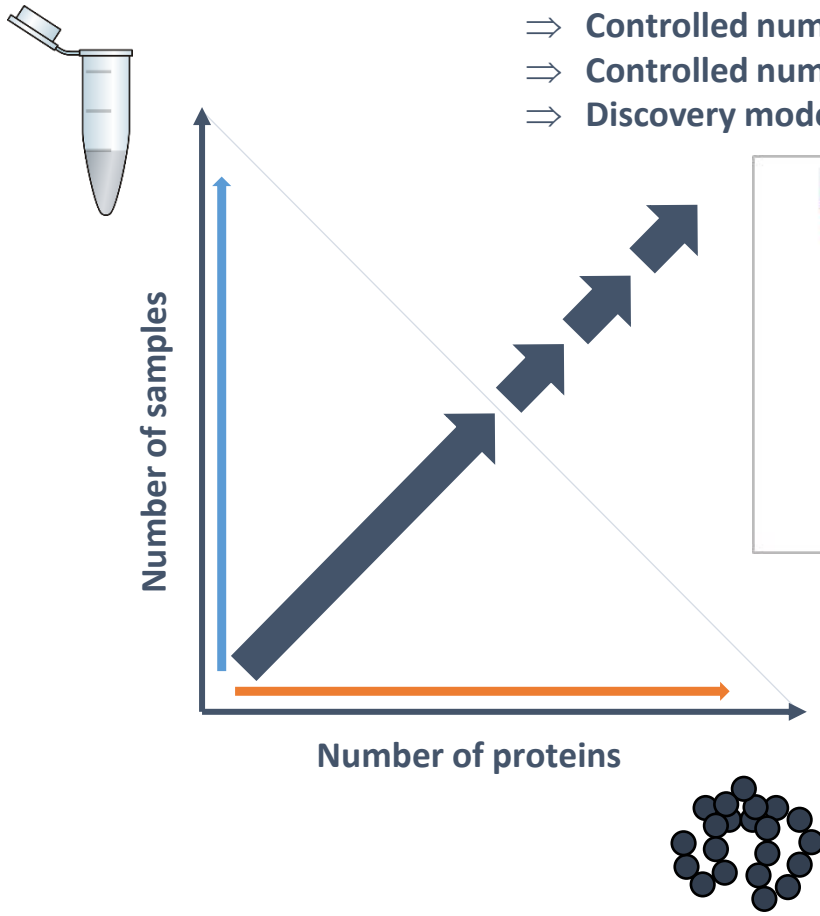


What do you want to get from your analysis?

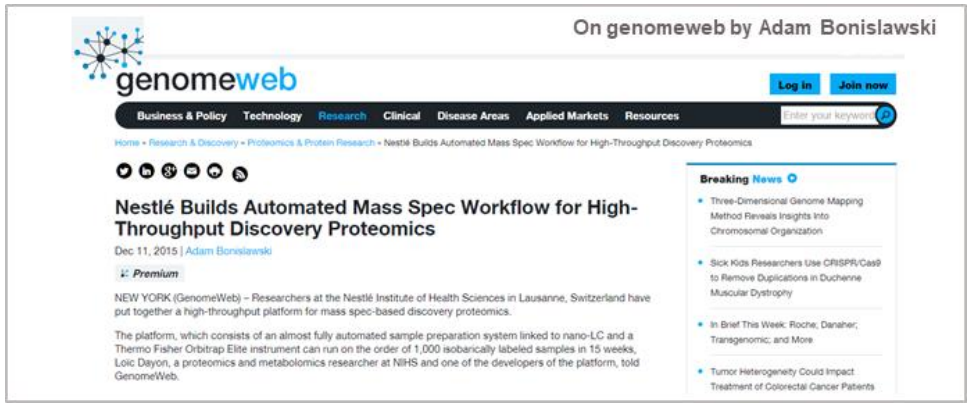


Large-Scale Proteome Profiling with MS

Option 2: *Large* number of samples (large sample-size)
- ⇒ **Short analysis time**
- ⇒ **Low number of proteins**
- ⇒ **Targeted mode**



Our vision
- ⇒ **Controlled analysis time**
- ⇒ **Controlled number of proteins**
- ⇒ **Controlled number of samples**
- ⇒ **Discovery mode**


On genomeweb by Adam Bonislawski

genomeweb

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Nestlé Builds Automated Mass Spec Workflow for High-Throughput Discovery Proteomics

Dec 11, 2015 | Adam Bonislawski

Premium

NEW YORK (GenomeWeb) – Researchers at the Nestlé Institute of Health Sciences in Lausanne, Switzerland have put together a high-throughput platform for mass spec-based discovery proteomics.

The platform, which consists of an almost fully automated sample preparation system linked to nano-LC and a Thermo Fisher Orbitrap Elite instrument can run on the order of 1,000 isobarically labeled samples in 15 weeks, Loic Dayon, a proteomics and metabolomics researcher at NIHS and one of the developers of the platform, told GenomeWeb.

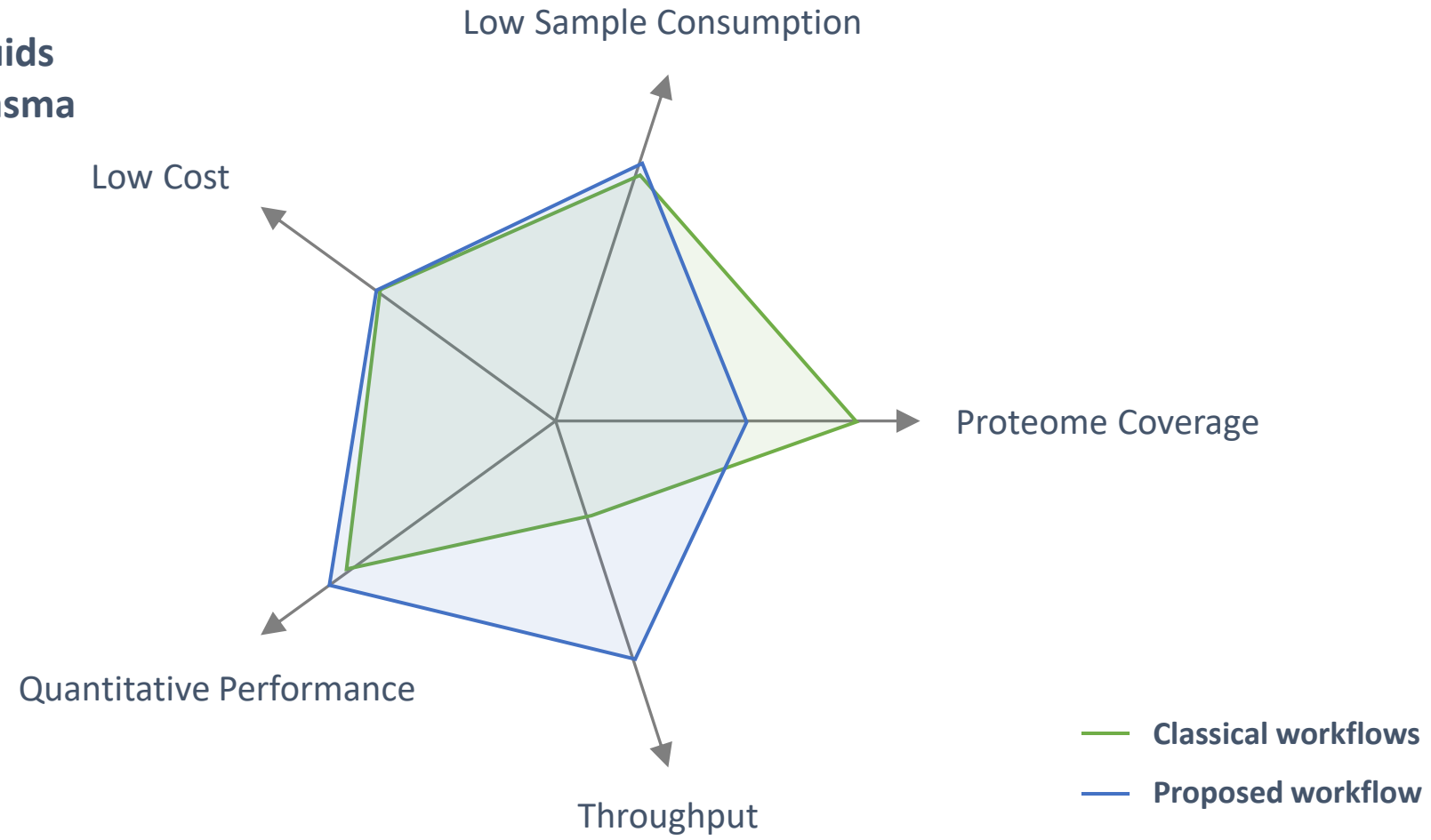
Breaking News

- Three-Dimensional Genome Mapping Method Reveals Insights Into Chromosomal Organization
- Sick Kids Researchers Use CRISPR/Cas9 to Remove Duplications in Duchenne Muscular Dystrophy
- In Brief This Week: Roche; Danaher; Transgenomic; and More
- Tumor Heterogeneity Could Impact Treatment of Colorectal Cancer Patients

Option 1: *Large* number of proteins (deep proteome coverage)
- ⇒ **Long analysis time**
- ⇒ **Low number of samples**
- ⇒ **Discovery mode**

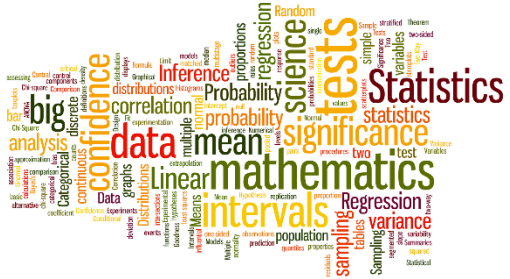
A practical example: Plasma proteomics

In body-fluids such as plasma



Experimental design: Important considerations

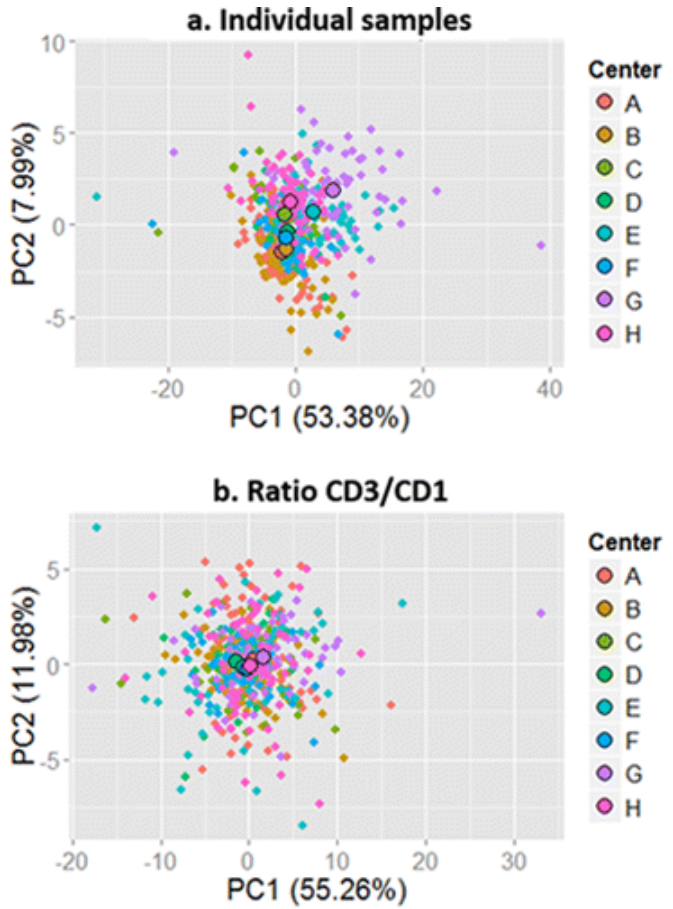
- What is the nature of the sample(s) you will analyze?
- Do you expect important protein changes between samples?
- Do you expect important variability between biological or technical replicates?
- How many replications will you performed?
- Do you need a standard or a sample for quality control?
- What workflow will be the more appropriate to answer your scientific questions?
- How will you analyze the data?
- …



Some sources of variability

To minimize **biological variance**, you need:

- Standardized collection protocols
- Appropriate samples (matched controls)
- Time of harvest/collection
- To consider potential batch effect (e.g., collection center, gender) and cope with it using proper randomization for instance
- …

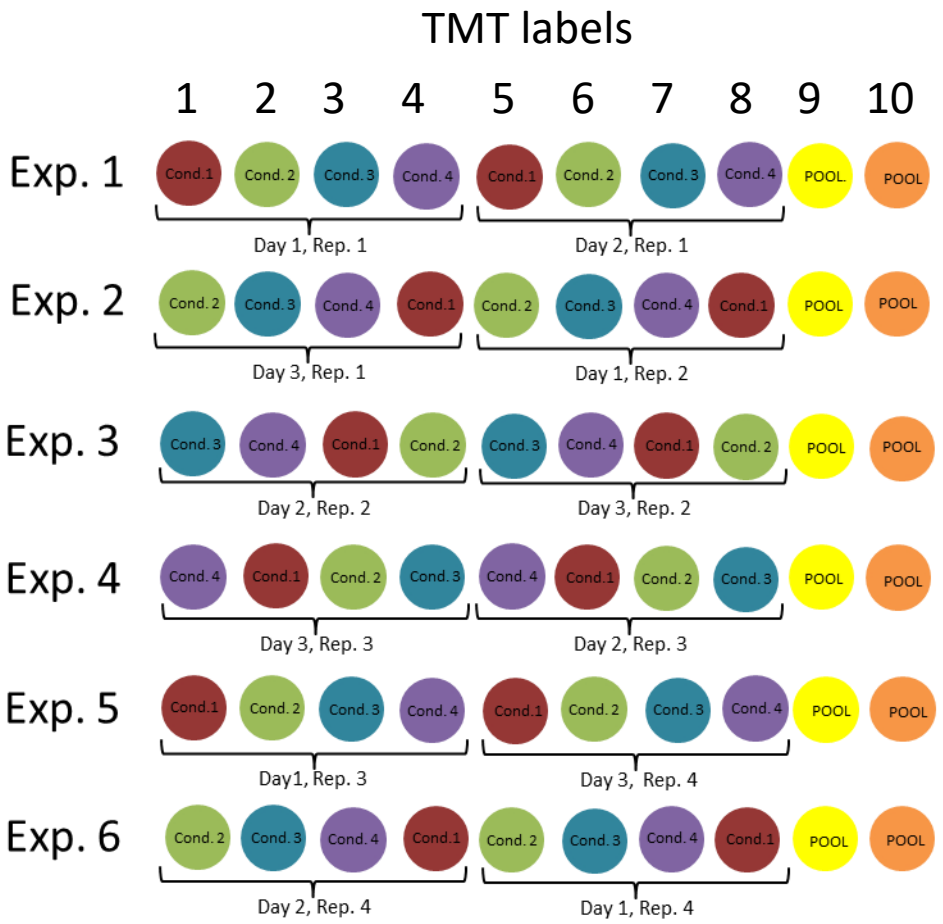


Cominetti *et al.*, *J. Proteome Res.*, **2016**, 15 (2), 389–399

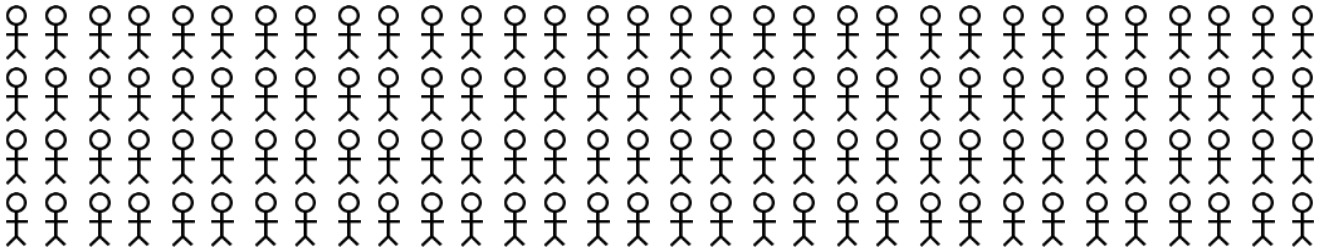
Some sources of variability

To minimize **experimental variance**, you need:

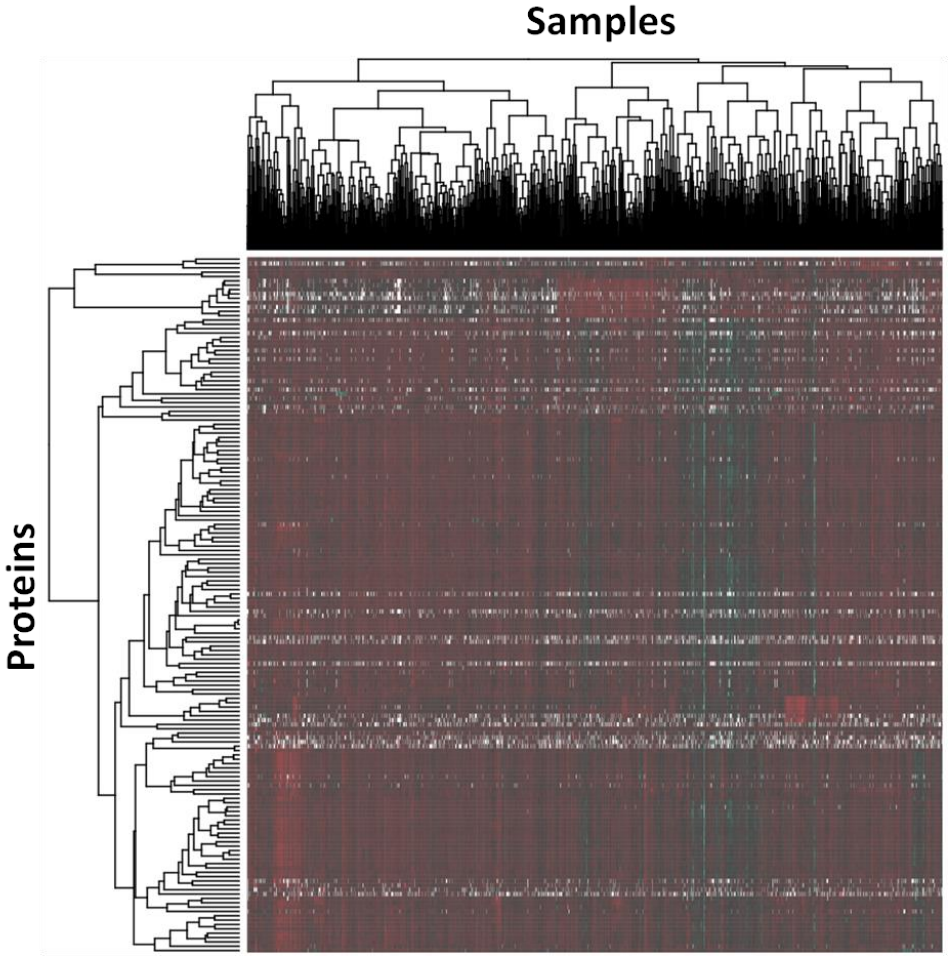
- To well choose your proteomic workflow
- To consider the influence of a change of operator
- To introduce standard and quality control checks
- To randomize your analyses (e.g., random labeling and MS sequence of analysis)
- …



6.2. Biomarker discovery; Population proteomics



DOI: 10.1186/gm364



Cominetti *et al.*, *J. Proteome Res.*, **2016**, 15 (2), 389–399

Biomarkers and proteins (1)

Clinical Proteomic Tumor Analysis Consortium (CPTAC)
https://proteomics.cancer.gov

The White House
Office of the Press Secretary

For Immediate Release — January 30, 2015

FACT SHEET: President Obama's Precision Medicine Initiative

Building on President Obama's announcement in his State of the Union Address, today the Administration is unveiling details about the Precision Medicine Initiative, a bold new research effort to revolutionize how we improve health and treat disease. Launched with a $215 million investment in the President's 2016 Budget, the Precision Medicine Initiative will pioneer a new model of patient-powered research that promises to accelerate biomedical discoveries and provide clinicians with new tools, knowledge, and therapies to select which treatments will work best for which patients.

Most medical treatments have been designed for the "average patient." As a result of this "one-size-fits-all-approach," treatments can be very successful for some patients but not for others. This is changing with the emergence of precision medicine, an innovative approach to disease prevention and treatment that takes into account individual differences in people's genes, environments, and lifestyles. Precision medicine gives clinicians tools to better understand the complex mechanisms underlying a patient's health, disease, or condition, and to better predict which treatments will be most effective.

https://obamawhitehouse.archives.gov

Biomarkers and proteins (2)

- "Biological marker, medical sign that **can be measured** accurately and reproducibly."
- "Any substance, structure, or process that **can be measured** in the body or its products and influence or predict the incidence of outcome or disease."
- "A characteristic that is objectively **measured** and **evaluated** as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention."
- "Almost any **measurement** reflecting an interaction between a biological system and a potential hazard." (WHO).
- "Objective, **quantifiable** characteristics of biological processes."

Diagnostic: allows (early) detection of a disease in a (non-invasive) way. Secondary prevention.

Prognostic: clinical or biological characteristic that provides information on the likely course of the disease. Outcome of the patient.

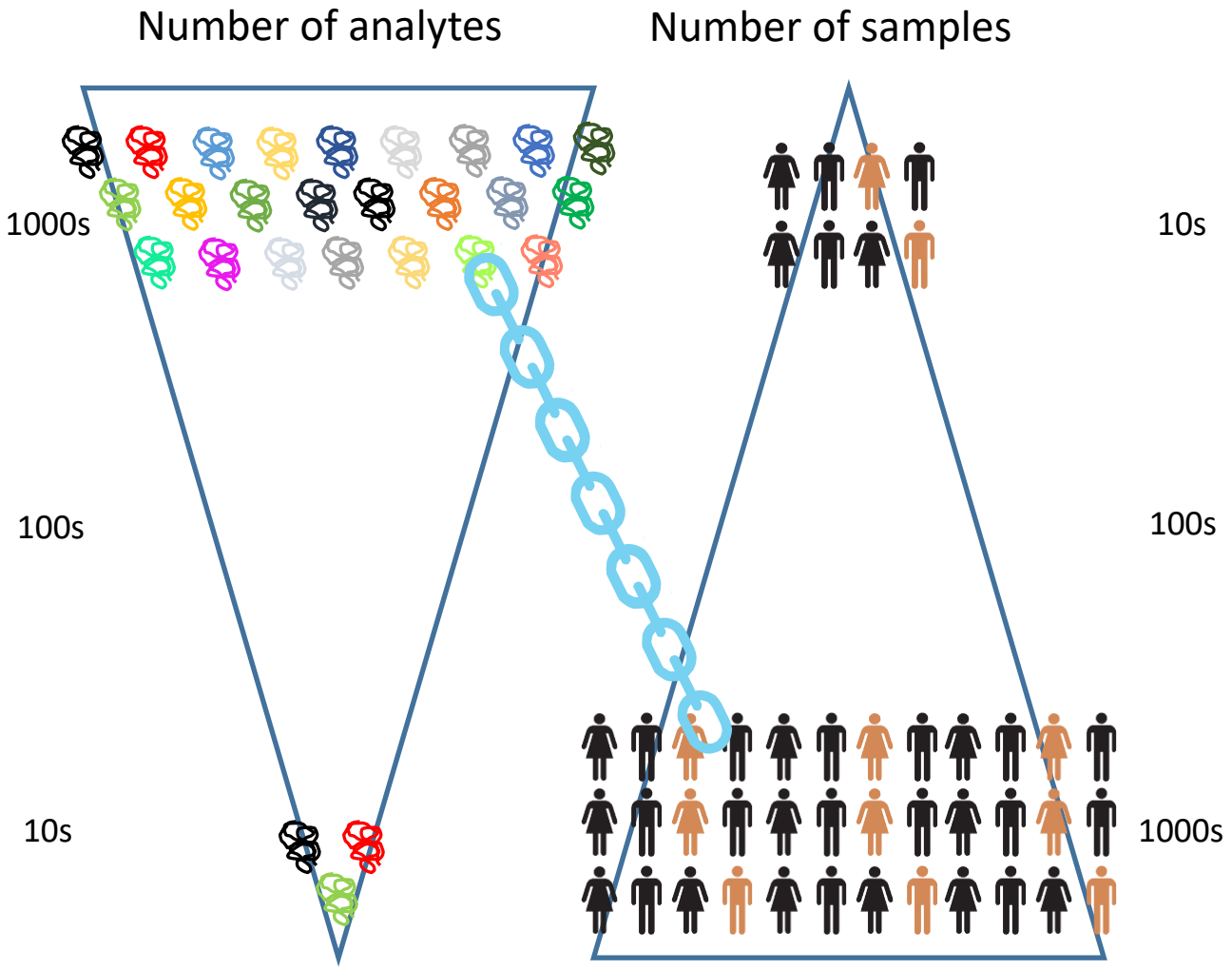
Therapeutic: generally a protein that could be used as target for a therapy.

Predictive: allows predicting the response of the patient to a targeted therapy and so defining subpopulations of patients that are likely to benefit from a specific therapy.

Courtesy of O. Cominetti

Biomarker discovery

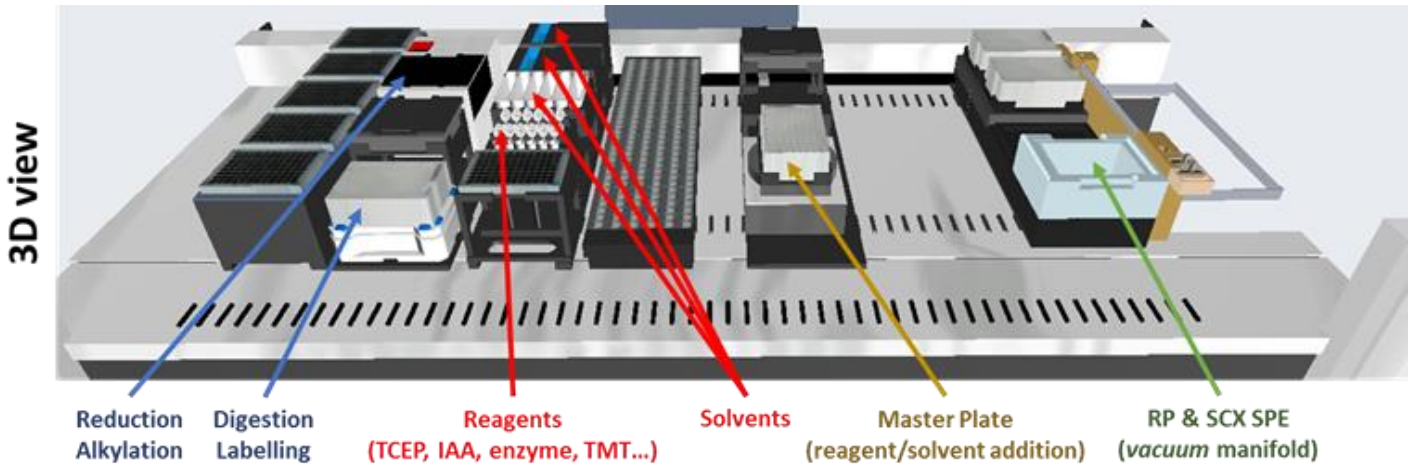
Phase	Samples and process
Initial discovery	Proximal fluids, cell lines, tissues Shotgun LC MS/MS proteomic profiling
Verification	Human plasma Targeted LC MS/MS
Validation	Human plasma Immunoassays



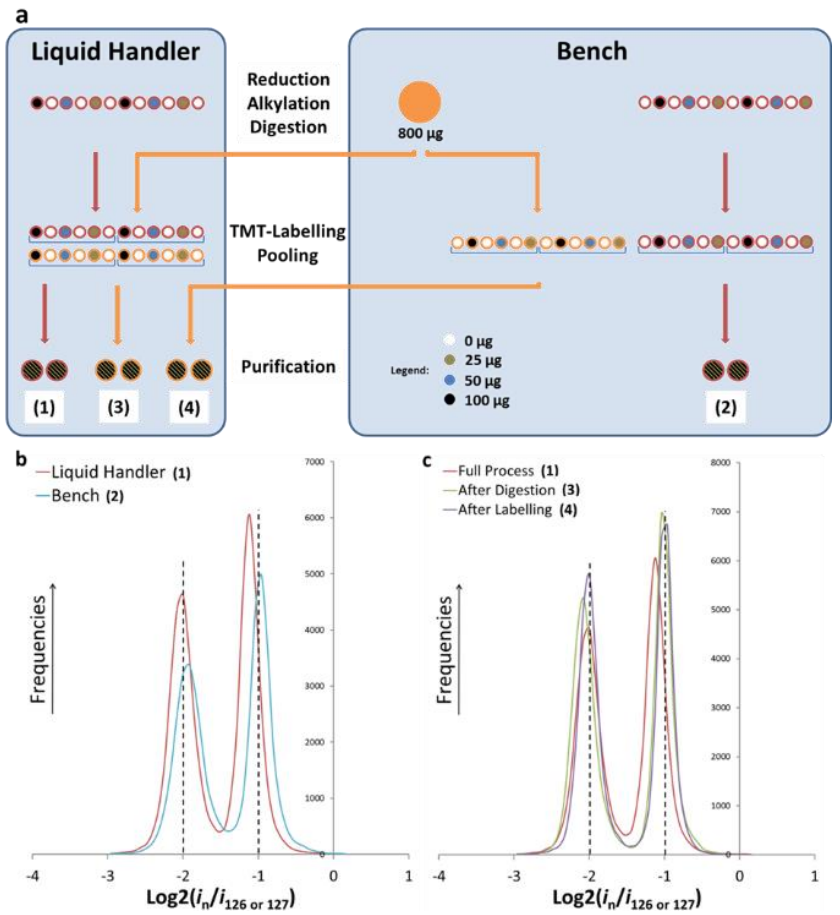
Adapted from Rifai *et al.*, *Nat. Biotechnol.*, **2006**, 24, 971–983

Need of robust and automated workflows

- Reduction/alkylation/digestion
- TMT labeling
- Purification

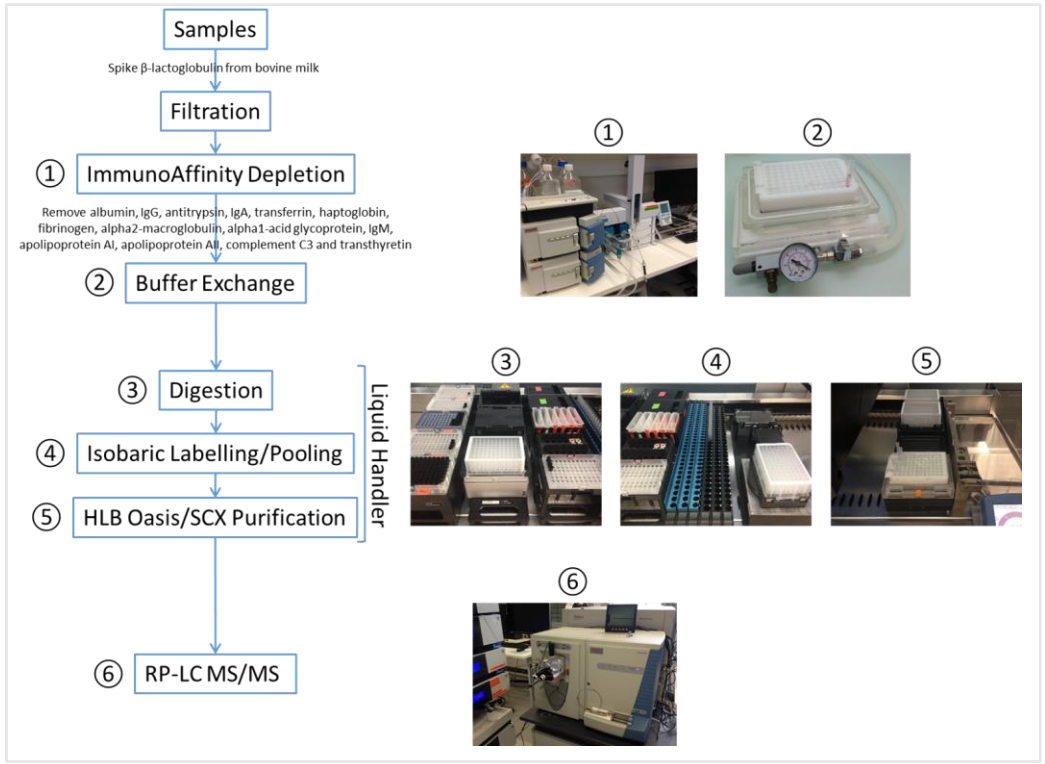


Dayon *et al.*, *Methods Mol. Biol.*, **2017**,1619, 433–449

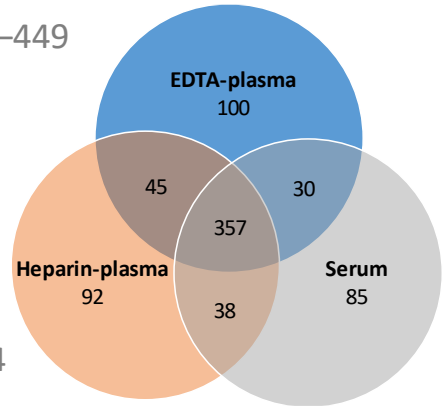


Dayon *et al.*, *J. Proteome Res.*, **2014**, 13 (8), 3837–45

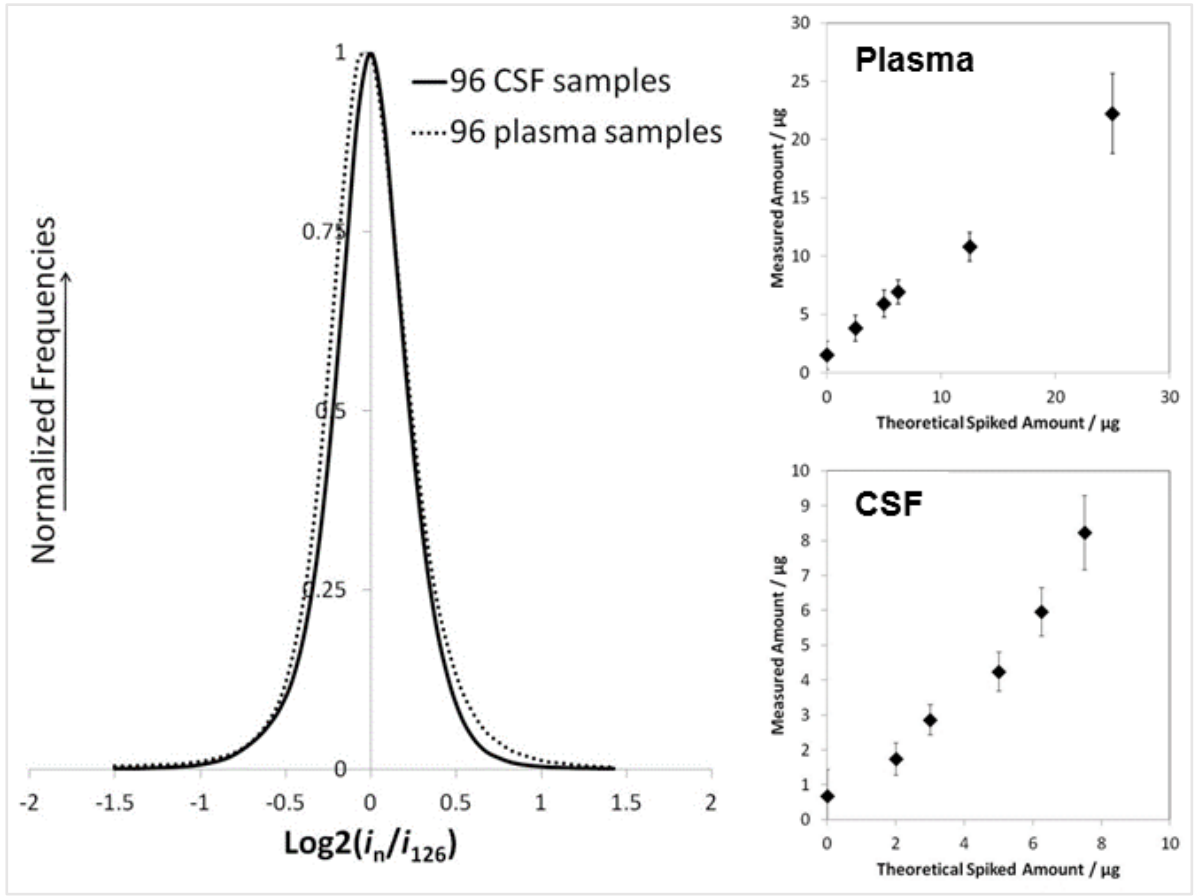
Analysis of plasma and cerebrospinal fluid



Dayon *et al., Methods Mol. Biol.*, **2017**, 1619, 433–449



Lan *et al., J. Proteome Res.*, **2018**, 17 (4), 1426–14



Dayon *et al., J. Proteome Res.*, **2014**, 13 (8), 3837–45
Núñez Galindo *et al., Anal. Chem.*, **2015**, 87(21), 10755–61

Interested? A recent review you can have a look at

EXPERT REVIEW OF PROTEOMICS
https://doi.org/10.1080/14789450.2022.2070477

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REVIEW

Check for updates

Proteomics of human biological fluids for biomarker discoveries: technical advances and recent applications

Loïc Dayon [a,b], Ornella Cominetti [a] and Michael Affolter [a]

[a]Bioanalytics Department, Proteomics, Nestlé Institute of Food Safety & Analytical Sciences, Nestlé Research, Lausanne, Switzerland; [b]Institut des Sciences et Ingénierie Chimiques, Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

ABSTRACT

Introduction: Biological fluids are routine samples for diagnostic testing and monitoring. Blood samples are typically measured because of their moderate invasive collection and high information content on health and disease. Several body fluids, such as cerebrospinal fluid (CSF), are also studied and suited to specific pathologies. Over the last two decades, proteomics has quested to identify protein biomarkers but with limited success. Recent technologies and refined pipelines have accelerated the profiling of human biological fluids.

Areas covered: We review proteomic technologies for the identification of biomarkers. These are based on antibodies/aptamers arrays or mass spectrometry (MS), but new ones are emerging. Advances in scalability and throughput have allowed to better design studies and cope with the limited sample size that has until now prevailed due to technological constraints. With these enablers, plasma/serum, CSF, saliva, tears, urine, and milk proteomes have been further profiled; we provide a non-exhaustive picture of some recent highlights (mainly covering literature from the last 5 years in the Scopus database) using MS-based proteomics.

Expert opinion: While proteomics has been in the shadow of genomics for years, proteomic tools and methodologies have reached certain maturity. They are now better suited to discover innovative and robust biofluid biomarkers.

ARTICLE HISTORY
Received 01 February 2022
Accepted 21 April 2022

KEYWORDS
Automation; biofluid; biomarker; cohort; human; mass spectrometry; MS; plasma; proteomics

1. Introduction

Many diseases face the unmet clinical need for accurate biomarkers in accessible sample biopsies, such as liquids, to properly stratify patients and monitor treatments in a precision medicine approach. Proteome profiling constitutes the missing bridge between genome and phenome determination. Yet, proteomics of human biological fluids has been hampered by several technical constraints that recent developments have partially overcome.

The identification of novel biomarkers with proven clinical utility classically relies on a complex process composed of discovery, qualification, verification, and validation steps (Figure 1). Proteomic biomarker discovery has provided numerous candidates, but only few of those have reached the validation stage and even fewer are used in today's routine clinical practice. While, for instance, more than 100 plasma/serum protein analytes are approved for clinical testing by the United States Food and Drug Administration [1], the rate of introduction of new approved protein-based tests in plasma/serum was reported in 2013 to be below 1.5 per year [2]. In view of the many proteome profiling research studies performed, this pace of translation may appear rather disproportionate, either delayed by long validation, approval and market introduction [3], inadequate study design, or technology constraints [4–6].

One recognized caveat to the discovery of protein biomarkers has been the limited size (subject numbers) of the clinical studies performed at the discovery stage, the findings of which fail to be replicated in (larger) verification and validation trials. Pragmatically, underpowered studies present substantial risk of generating false-positive biomarker candidates, which is counterproductive, but also of producing false negatives that lead to missed opportunities. Compromised study designs and insufficient statistical power are consequences of the up-to-recently limited capacity of proteomic workflows to handle large numbers of samples in a realistic time frame while delivering comprehensive coverages of proteomes – all this ideally with minimally invasive, peripheral sampling. Trade-offs between scale, depth, and throughput of a proteomic study have still to be faced, but many recent improvements have reduced the gaps.

In this review, we describe the recent evolutions of proteomic approaches for the discovery of biomarkers. With a focus on mass spectrometry (MS)-based approaches, technological advances but also considerations on study design are discussed. We provide examples of promising findings obtained in different biological fluid sample matrices, highlighting the characteristics of their proteomes and their relevance for monitoring health and disease.

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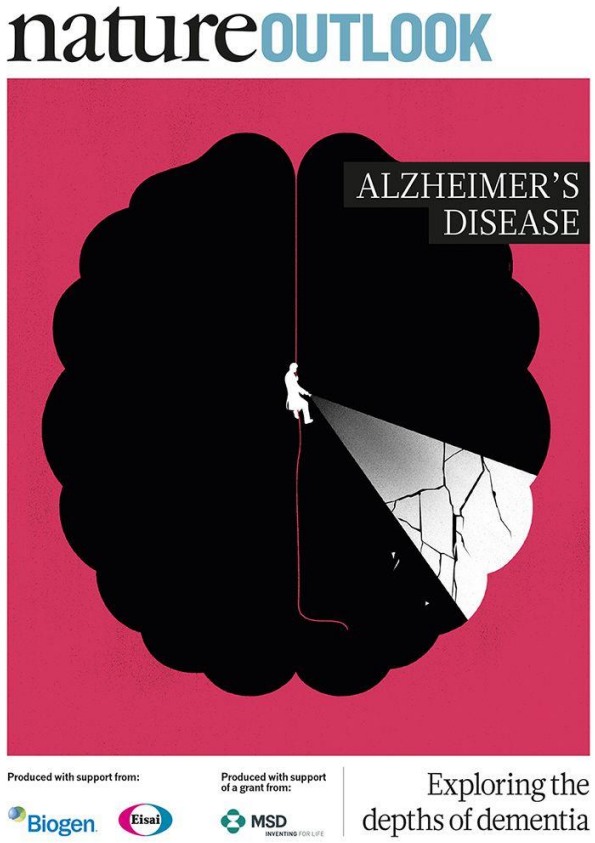
Alzheimer disease and biomarker needs



Nature Outlook, 26 July 2018

Why diagnosing Alzheimer's today is so difficult—and how we can do better

By Bill Gates | July 17, 2018

—

When I announced that I was investing in Alzheimer's research for the first time last fall, I thought I knew what to expect. I knew I would get to engage more deeply with the brilliant scientists and advocates working to stop Alzheimer's—and I haven't been disappointed. The things I've seen over the last seven months make me more hopeful than ever.

What I didn't see coming was the amazing response I got from the Alzheimer's community at large. Because my family didn't talk publicly about my dad's diagnosis before the announcement, I had yet to experience how remarkable the support community is. So many of you have shared your personal experiences with me, both in person and online (including here on TGN). It helps to hear from others who are going through the same thing.

Alzheimer's research is a frontier where we can dramatically improve human life—both the lives of people who have the disease and their loved ones. I'm optimistic that we can substantially alter the course of Alzheimer's if we make progress in several key areas. One of the biggest things we could do right now is develop a reliable, affordable, and accessible diagnostic.

The process of getting diagnosed with Alzheimer's today is less than ideal. It starts with a cognitive test. If you don't perform well, your doctor needs to rule out all other possible causes for memory loss, like stroke or a nutritional deficiency. Then your doctor can order a spinal tap or PET scan to confirm you have Alzheimer's. Although these tests are fairly accurate, the only way to diagnose the disease definitively is through an autopsy after death.

https://www.gatesnotes.com/

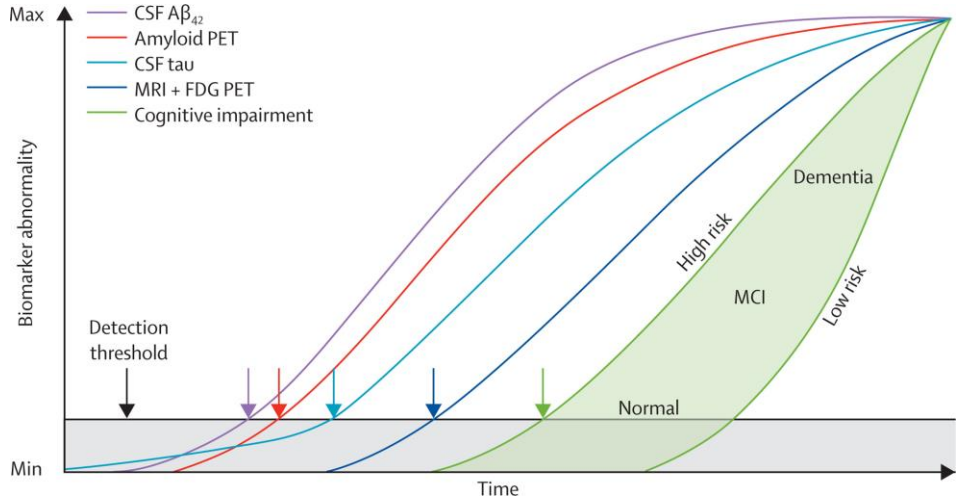
Alzheimer disease and diagnostics

The underlying pathology of AD seems to initiate **10 years or more prior to the diagnosis**

- Clinical evaluation (cognitive testing by specialists)

- Imaging
  - MRI (brain atrophy)
  - PET (Aβ load)

- CSF detection of Aβ peptide, tau and phosphorylated tau proteins
  - ELISA
  - Luminex



Jack *et al., Lancet Neurol.*, **2013**, 12 (2), 207–216

AD and Nutrition

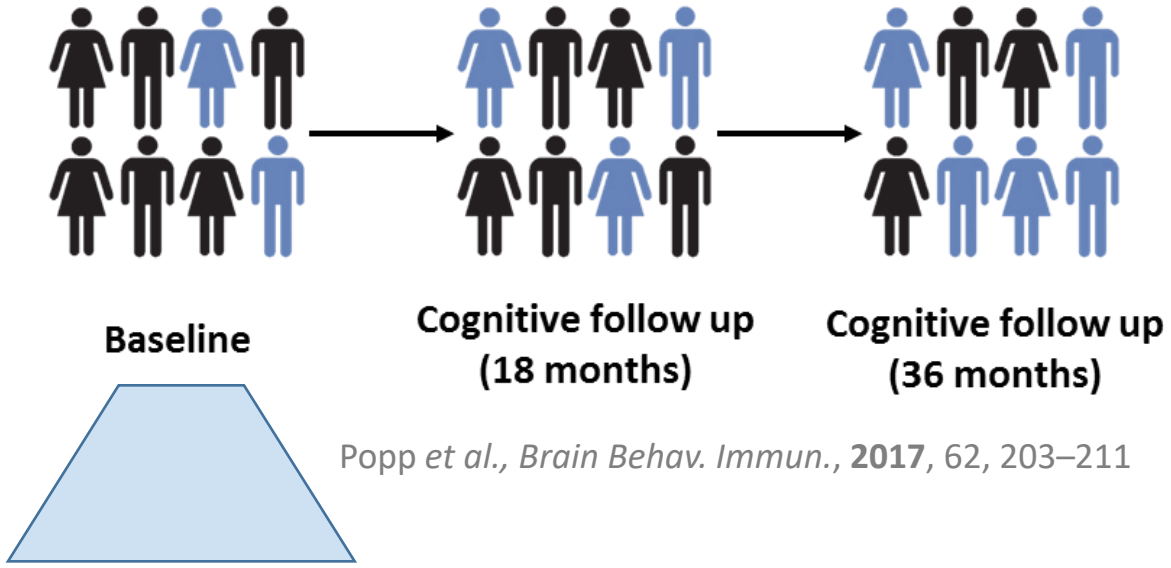
- Still **no drug** can slow down the progression of AD
- **Prevent**ive therapies
- Changes in **lifestyle**:
  - Physical exercise
  - Intellectual stimulation
  - Healthy eating (*e.g.*, Mediterranean diet)

Early detection in the preclinical stage of dementia is essential to identify those at risk and intervene with **preventive therapies**

GOAL

- Identify novel **biomarker** profiles (diagnostic, prognostic and some with therapeutic potential) and **nutrition**al concepts
- Develop new "profiles" that identify **high-risk populations** and improve clinical **trial** design

An example of a study in brain health research

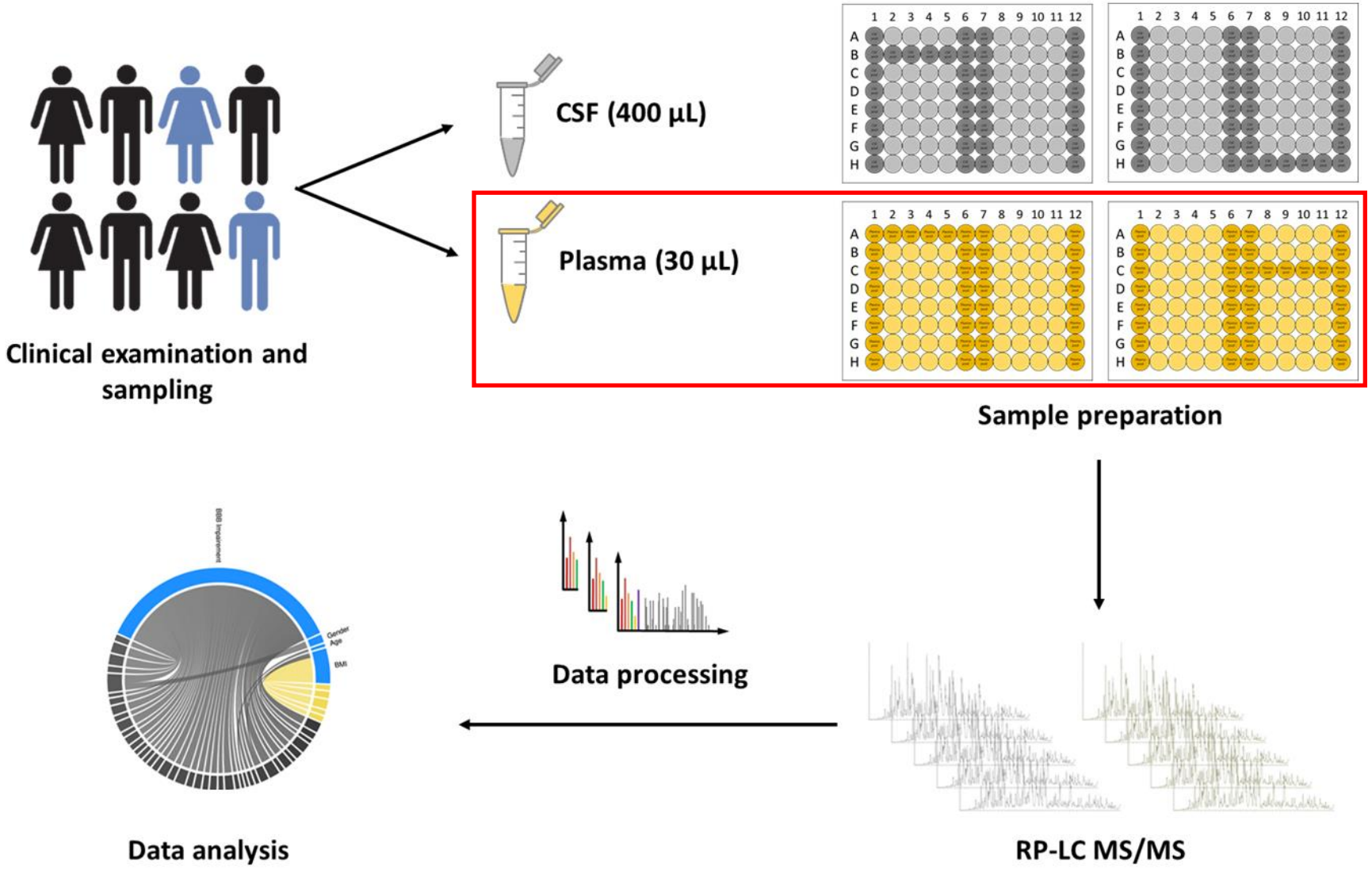


Popp *et al., Brain Behav. Immun.*, **2017**, 62, 203–211

	P-tau181/Aβ1-42 ≤ 0.0779 (*n* = 78)	P-tau181/Aβ1-42 > 0.0779 (*n* = 42)	[Aβ1-42] ≥ 724 pg/mL (*n* = 73)	[Aβ1-42] < 724 pg/mL (*n* = 47)
Age (years), mean (SD)	68.4 (8.3)	74.1 (5.6)*	68.1 (8.2)	73.9 (6.1)*
Gender, No. (%) of Males	25 (32.05%)	18 (42.86%)	26 (35.62%)	17 (36.17%)
Education years, mean (SD)	12.5 (2.7)	12.1 (2.4)	12.6 (2.7)	12.0 (2.5)
MMSE scale, mean (SD)	27.8 (2.3)	25.2 (3.7)*	27.8 (2.2)	25.6 (3.8)*
APOE ε4 carriers, No. (%)	13 (16.67%)	24 (57.14%)*	10 (13.70%)	27 (57.45%)*

Dayon *et al., J. Alzheimers Dis.*, **2017**, 60(4);1641–1652

Study design



Dayon *et al.*,, **2018**, DOI: 10.1021/acs.jproteome.8b00809

Rationale and methodology

Sampling of **blood** is easily available, minimally invasive and repeatable

Endophenotype approach to define preclinical AD and cerebral amyloidosis based on well-accepted **CSF P-tau 181** and **Aβ1-42**

Identification of **plasma-based protein biomarker candidates**

Lasso Logistic Regression

ID clinical variables that best classify each outcome of interest

(**Reference model**)

Lasso Logistic Regression

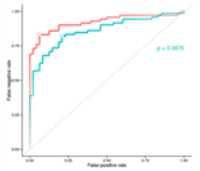
ID clinical + candidate biomarkers that best classify each outcome of interest

(**Best model**)

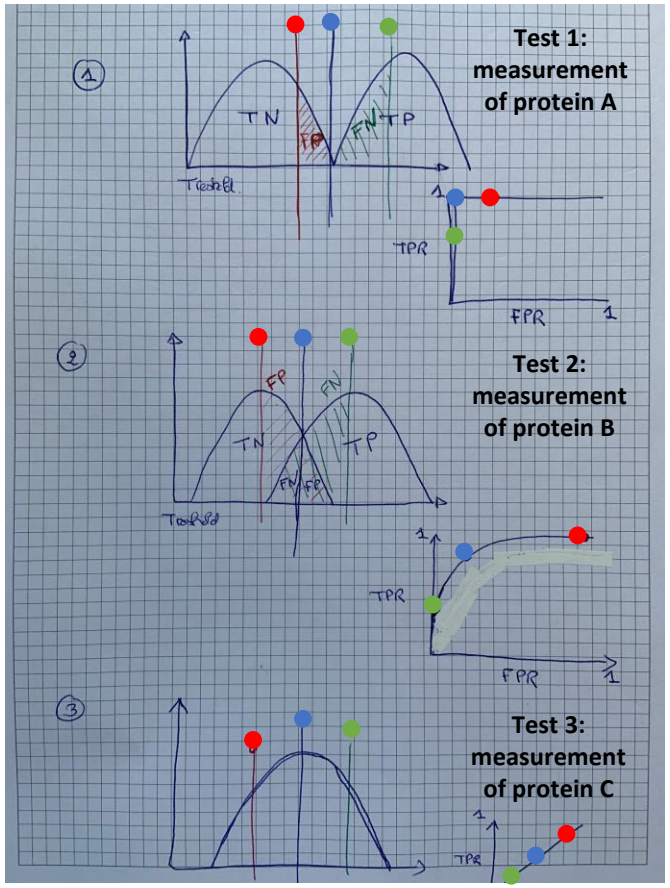
Compare Reference and Best model

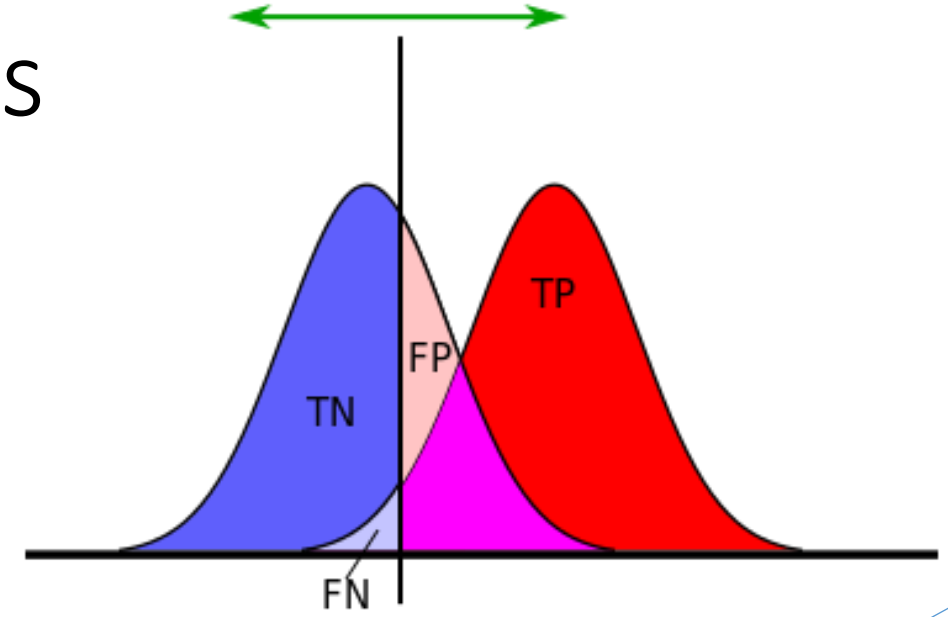
ROC curves

Prediction accuracy



ROC curves





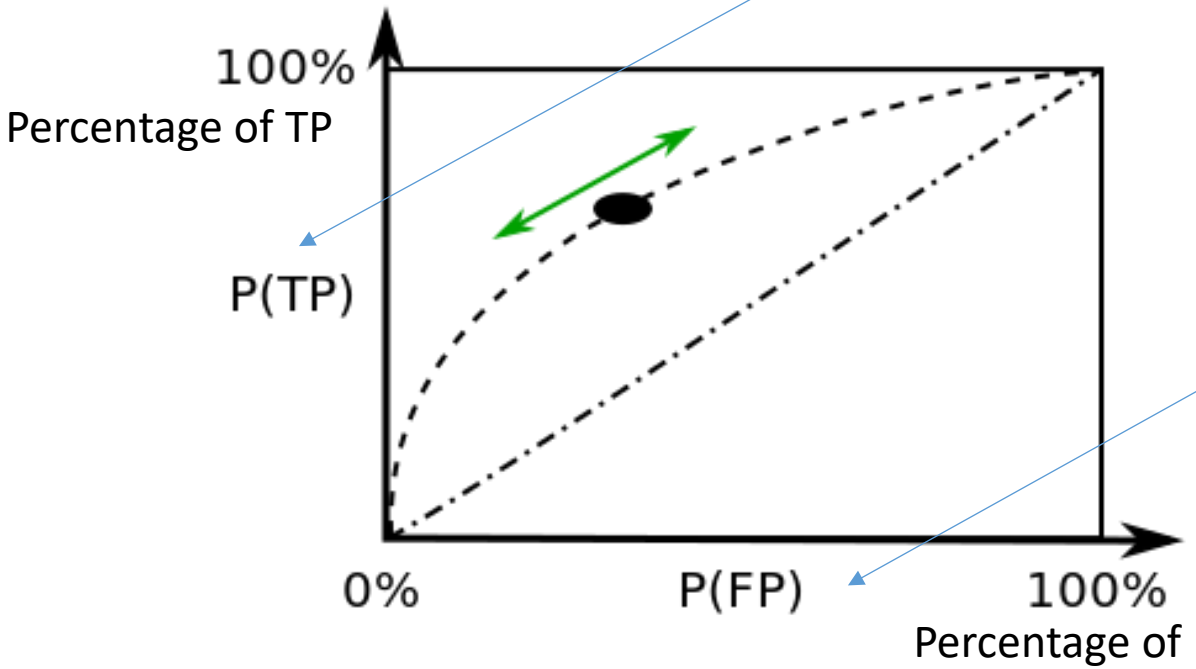
Cases	Controls
TP	FP
FN	TN

Sensitivity = TP/(TP+FN)

Specificity = TN/(TN+FP)

Cases

Controls



FPR = 1-specficity

FPR = FP/(TN+FP)

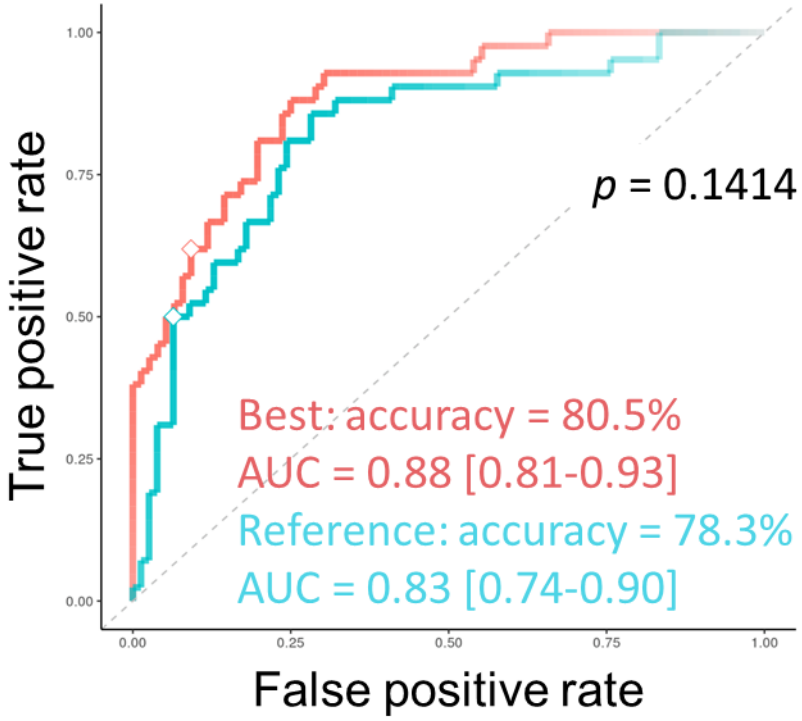
Controls

https://en.wikipedia.org/wiki/Receiver_operating_characteristic

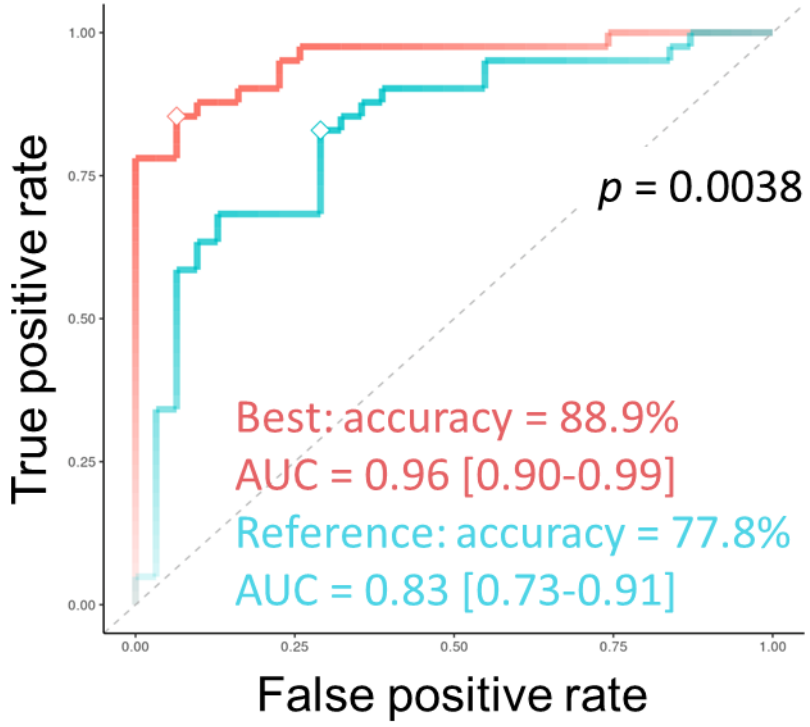
Plasma proteomic profiles of AD

P-tau 181/Aβ1-42 > 0.0779 (positive AD profile)

All subjects

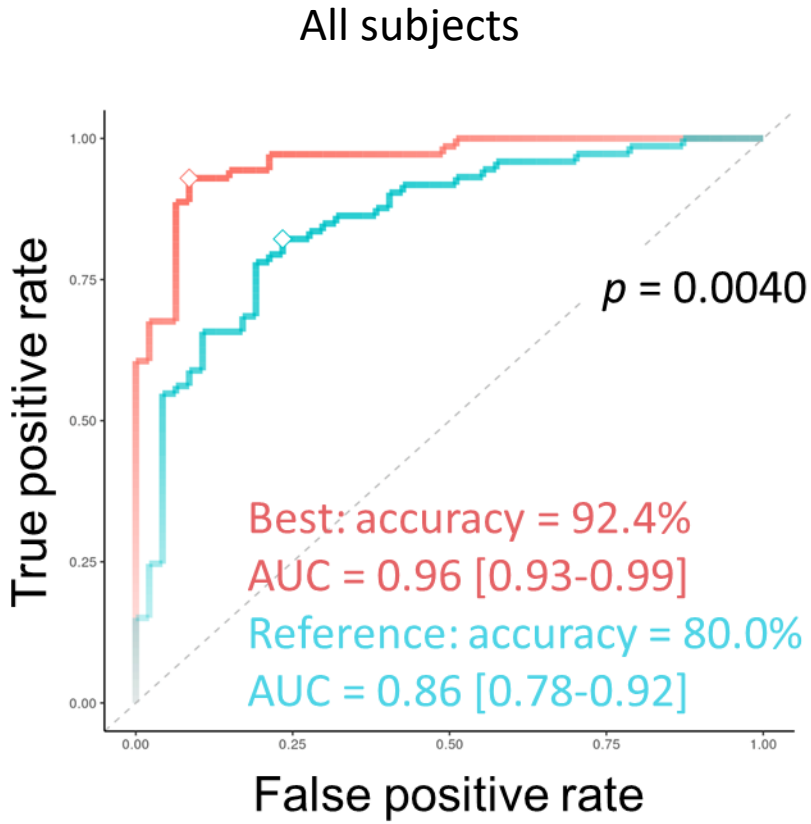


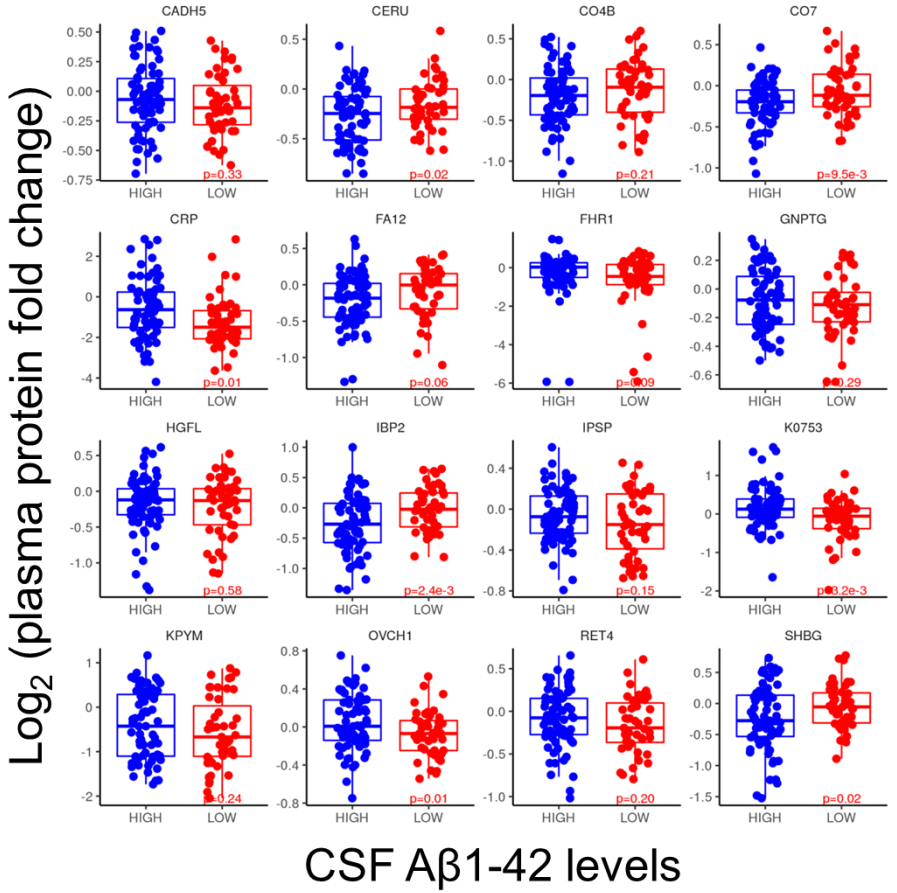
Cognitive impairment



Dayon *et al., J. Alzheimers Dis.*, **2017**, 60(4);1641–1652

Plasma proteomic profiles of amyloid pathology



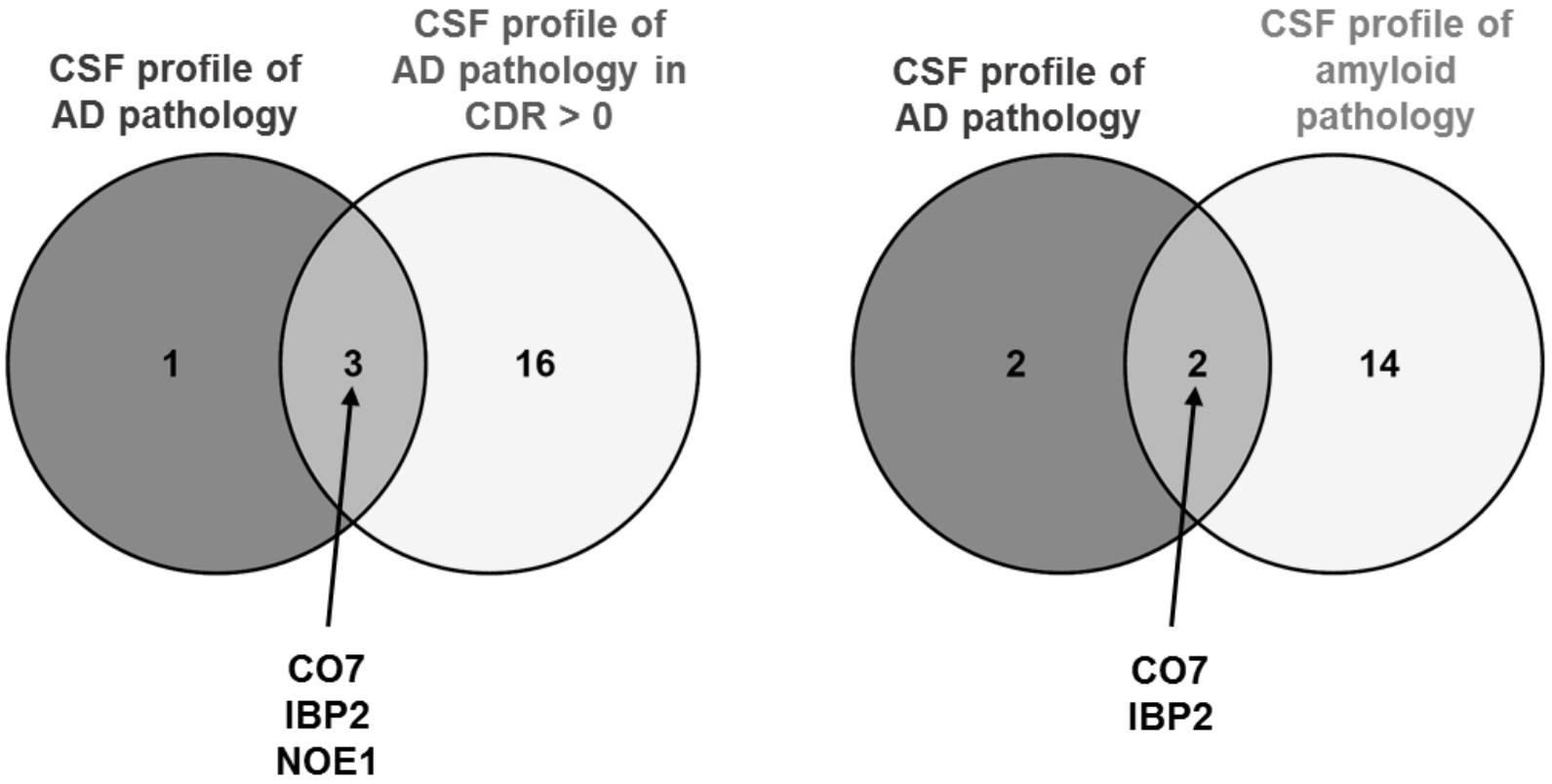


[Aβ1-42] ≥ 724 pg/ml

[Aβ1-42] < 724 pg/ml
(positive profile of amyloid pathology)

Dayon *et al., J. Alzheimers Dis.*, **2017**, 60(4);1641–1652

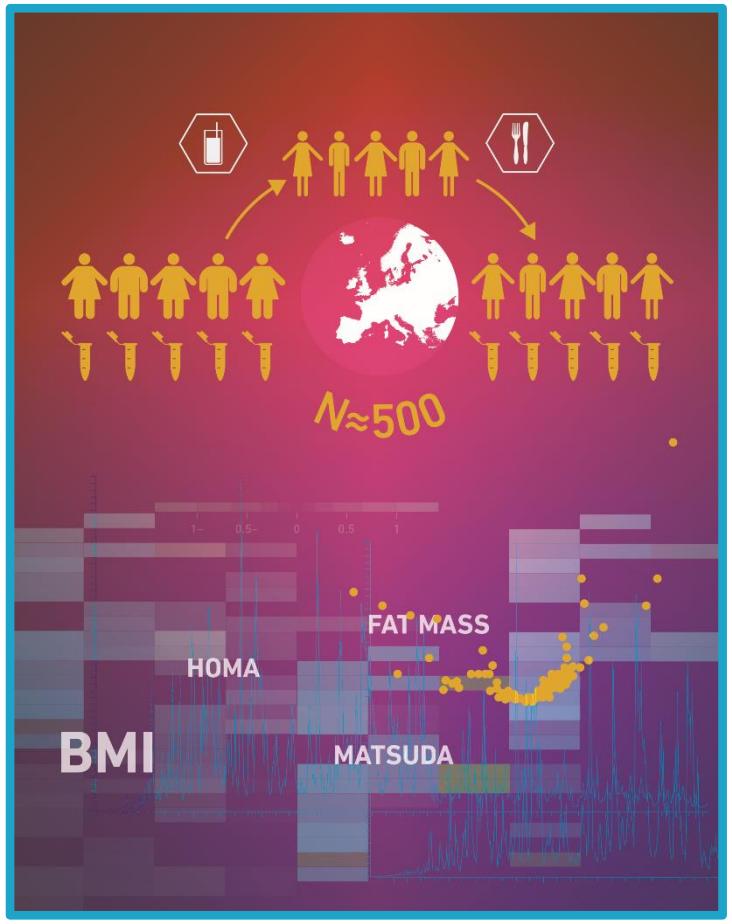
Summary



Dayon *et al., J. Alzheimers Dis.*, **2017**, 60(4);1641–1652

Replication using the same approach before further translation to clinical use…

Another example we can discuss…



Proteomics Clin. Appl. 12, 1, 2018, 1600150 DOI 10.1002/prca.201600150 (1 of 11) 1600150

RESEARCH ARTICLE

The differential plasma proteome of obese and overweight individuals undergoing a nutritional weight loss and maintenance intervention

Sergio Oller Moreno[1,5**], Ornella Cominetti[1**], Antonio Núñez Galindo[1], Irina Irincheeva[2], John Corthésy[1], Arne Astrup[3], Wim H.M. Saris[4], Jörg Hager[2], Martin Kussmann[1*] and Loïc Dayon[1]

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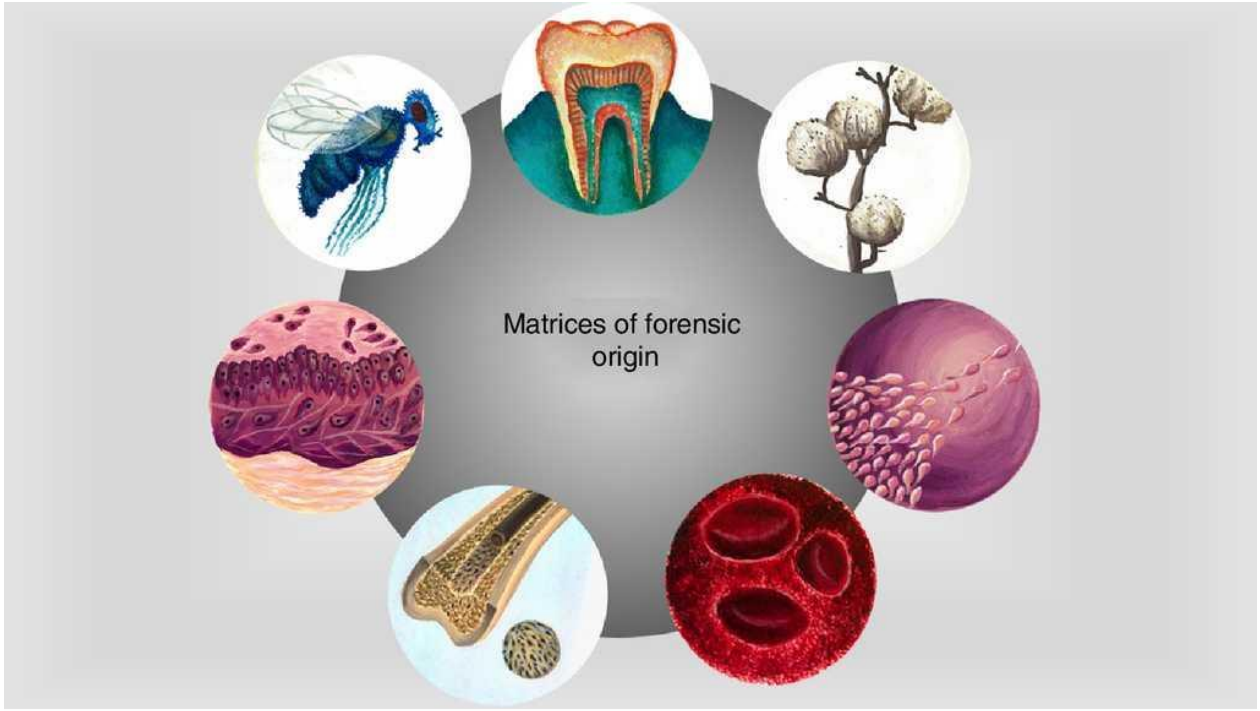
[2] Nutrition and Metabolic Health, Nestlé Institute of Health Sciences, Lausanne, Switzerland
[3] Department of Nutrition, Exercise and Sports, Faculty of Science, University of Copenhagen, Copenhagen, Denmark
[4] Department of Human Biology, NUTRIM, School for Nutrition, Toxicology and Metabolism, Maastricht University Medical Centre, Maastricht, The Netherlands
[5] Signal and Information Processing for Sensing Systems, Institute for Bioengineering of Catalonia, Barcelona, Spain

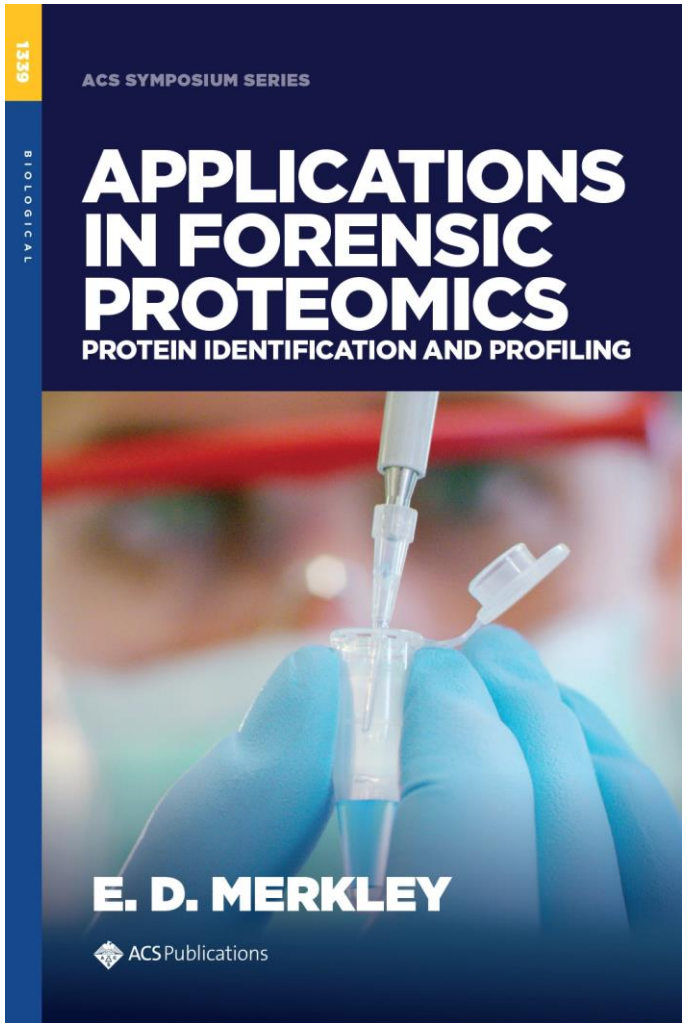
Purpose: The nutritional intervention program "DiOGenes" focuses on how obesity can be prevented and treated from a dietary perspective. We generated differential plasma proteome profiles in the DiOGenes cohort to identify proteins associated with weight loss and maintenance and explore their relation to body mass index, fat mass, insulin resistance, and sensitivity.
Experimental design: Relative protein quantification was obtained at baseline and after combined weight loss/maintenance phases using isobaric tagging and MS/MS. A Welch *t*-test determined proteins differentially present after intervention. Protein relationships with clinical variables were explored using univariate linear models, considering collection center, gender and age as confounding factors.
Results: Four hundred and seventy three subjects were measured at baseline and end of the intervention; 39 proteins were longitudinally differential. Proteins with largest changes were sex hormone-binding globulin, adiponectin, C-reactive protein, calprotectin, serum amyloid A, and proteoglycan 4 (PRG4), whose association with obesity and weight loss is known. We identified new putative biomarkers for weight loss/maintenance. Correlation between PRG4 and proline-rich acidic protein 1 variation and Matsuda insulin sensitivity increment was showed.
Conclusion and clinical relevance: MS-based proteomic analysis of a large cohort of non-diabetic overweight and obese individuals concomitantly identified known and novel proteins associated with weight loss and maintenance.

Received: October 27, 2016
Revised: March 1, 2017
Accepted: March 27, 2017

6.3. Forensics

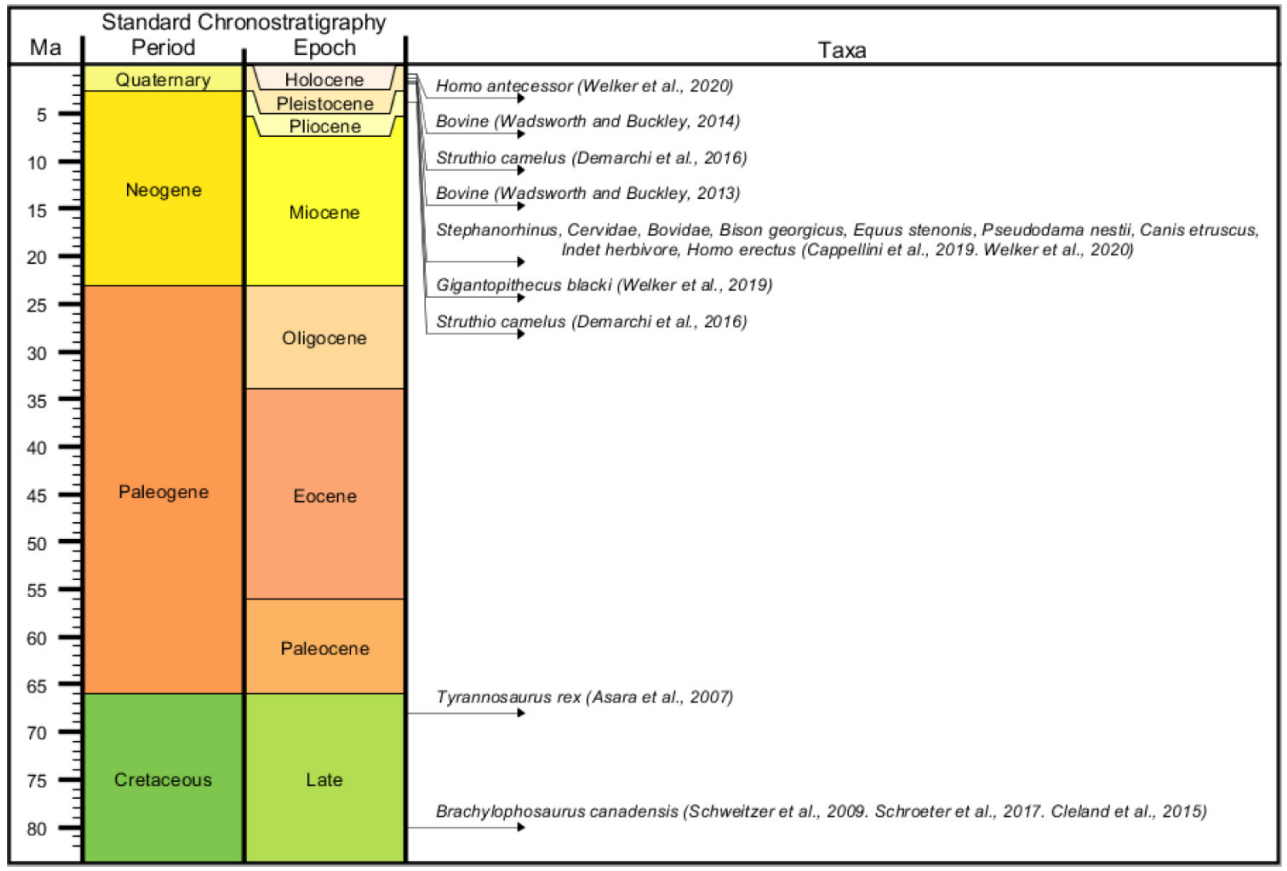


Spanish Journal of Legal Medicine. 2019;45:114–22



DOI: 10.1021/bk-2019-1339

Proteins are more stable than DNA



Schroeter *et al.*, *J. Proteome Res.* 2022, 21, 1, 9–19

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Proteomics Offers New Clues for Forensic Investigations

Cite This: *ACS Cent. Sci.* 2021, 7, 1595–1598

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Carolyn Wilke

Analyzing proteins in bones, blood, and other biological samples can answer questions that DNA can't.

In May 2014, 2-year-old Aleka Gonzales of North Vancouver, British Columbia, died under mysterious circumstances. She had been with a babysitter the day before, and bruising on her body led the coroner to initially think that the toddler had been beaten. But the uniformity of Gonzales's injuries hinted at a different cause: The babysitter kept exotic pets, and bites from venomous animals can turn skin black and blue. Unable to search the babysitter's home, police requested help from the local scientific community to identify potential toxins in blood and urine samples collected from the child's body.

Leonard J. Foster, a biochemist at the University of British Columbia, answered the call. He set about analyzing the population of proteins in the samples, aiming to capture any peptide or protein toxins present. With information from the police investigators, Foster's team started to focus its search on snake venom. After removing some of the most abundant proteins from the blood, the researchers zeroed in on nonhuman proteins, comparing them against a set of known proteins in snake venom. "We got what we felt was unequivocal proof that there were snake proteins in this girl's blood," Foster says, and the analysis suggested venom from a rattlesnake.

DNA is the reigning forensics biomolecule, allowing inspectors to catch suspects through traces left at a crime scene. "DNA in forensics is the gold standard for a reason," says Queenie W.T. Chan, a biochemist on the UBC team that found the snake proteins. Genetics and its statistical backing are well understood. But in investigating Gonzales's death, DNA wouldn't have helped. Reptilian DNA, if

Proteins in evidence collected from a crime scene can reveal information from before and after death. Credit: Darko Cacic/ Shutterstock

present, might have suggested that the child came in contact with a snake but couldn't have confirmed a snakebite.

DNA can unveil its donor's species or even specific identity. Proteins can do that too, which may be useful in cases where there isn't much DNA or where the DNA is highly degraded. And proteins can answer some questions that DNA can't. Nucleic acids don't reveal the type of tissue or fluid that was their source, for example. A population of proteins is not only characteristic of that biological source but also carries clues from the environment, inside the body and out.

The word *proteomics* encompasses a range of methods for identifying and measuring the abundance of all the proteins in a sample. The field is yielding big data for biology, even at the single-cell level, and researchers around the world are looking into ways that proteins could help solve crimes. "In forensics, it's cutting edge," says Donald Siegel, a

Published: October 18, 2021

ACS Publications

https://doi.org/10.1021/acscentsci.1c01252
ACS Cent. Sci. 2021, 7, 1595–1598

Some specific examples

Identification of body fluids (specific combination of proteins; DNA do not reveal the type of tissue or fluid; biochemical tests or immunoassays can suffer limitations such as cross-reactivity, specificity, degradation)

–> demonstrate the source of DNA (*e.g.*, snake proteins in the blood (2-year-old Aleka Gonzales' case))

–> Several tested at a time (blood, saliva, semen…) with characteristic peptides (MS-based multiplexed assays – *e.g.*, predict path of a bullet)

"Temporal & environmental aspect" (changes over time)

–> aging or life events such as pregnancy

–> age at death (*e.g.*, fetuin A amount in buried bones)

–> after death, protein or their modifications may serve as "molecular timers"

When DNA is not in sufficient amount (*e.g.*, hair shafts) or degraded (bone samples)

–> genetic information in the amino acid sequences of proteins

–> sex determination (*e.g.*, amelogenin in teeth in archaeological context)

–> species determination

N.B: in MS-based proteomics, often the lack of detection cannot be taken as evidence of absence

ACS Cent. Sci. 2021, 7, 10, 1595–1598

Forensic Science International: Genetics 54 (2021) 102529

6.4. Targeted mass spectrometry-based approaches

Targeted proteomics

Analysis of a preselected group of proteins delivers more precise, quantitative, sensitive data to more biologists. Vivien Marx reports.

Although the number and identity of protein-coding genes in humans and many other organisms are known to a certain level of approximation, the numbers of proteins produced by each of these genes remains a mystery. Further complicating matters, given the many possible splice forms and post-translational modifications, the potential number of proteins is "staggering," says Arizona State University researcher Josh LaBaer, who is also president-elect of the US Human Proteome Organization. A protein is also dynamic. "It's phosphorylated this minute; it's not phosphorylated the next minute," he says. This is fascinating science, but it makes proteins in a complex, dynamic sample hard to precisely measure.

Understanding disease-related changes, for example, calls for reliable, quantitative ways of assessing protein levels, and mass spectrometers are instruments able to nail that task. But the data from so-called discovery proteomics experiments in which mass spectrometry is used to identify a large number of proteins in a sample are not always useful to biologists. Enter tar-

"I personally can't wait until we stop hearing about someone describing how big of a list of proteins, peptides or phosphopeptides they detected," says one researcher critical of discovery proteomics who did not wish to be identified. Proteomics has been doing "my list is bigger than your list" for far too long. "It is way more important to measure the one right protein than 10,000 wrong ones."

Scientists wanting to follow well-founded hunches about dozens or hundreds of proteins seek a focused, reproducible, quantitative view of a small subset of the whole proteome in their lab vials. High-throughput biology experiments, which include DNA sequencing, genome analysis and gene expression analysis, are generating massive data sets pertaining to particular genes


ETH Zurich

Ruedi Aebersold hopes many laboratories will adopt targeted proteomics.

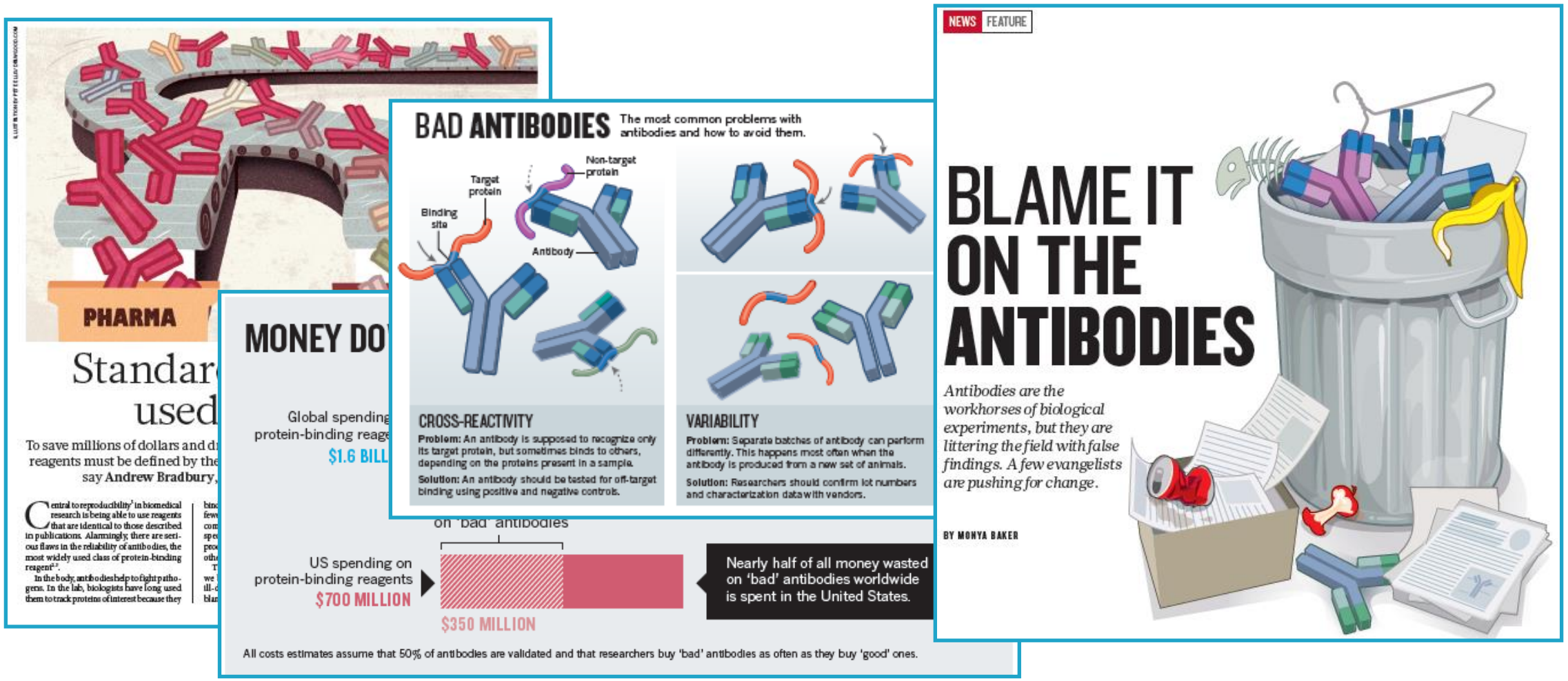

Katie Vicari

Targeted proteomics detects proteins of interest with high sensitivity, quantitative accuracy and reproducibility.

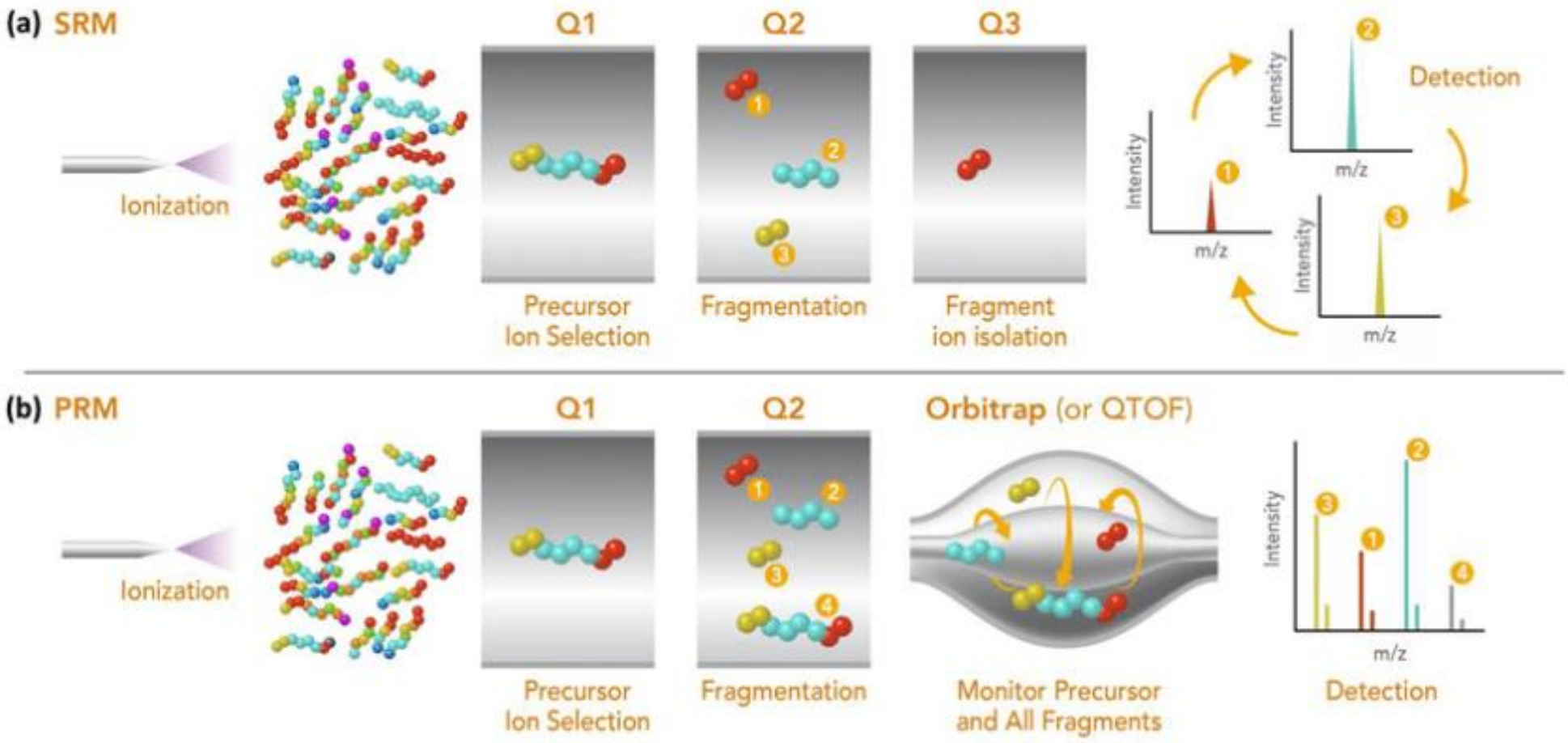
are not inherently the same," says Ruedi Aebersold, from the Institute of Molecular Systems Biology at the Swiss Federal Institute of Technology in Zurich. Neither person is necessarily wrong: the contradiction stems from their measurement of different subsets of the whole proteome,

DOI:10.1038/nmeth.2285

Why targeted MS?

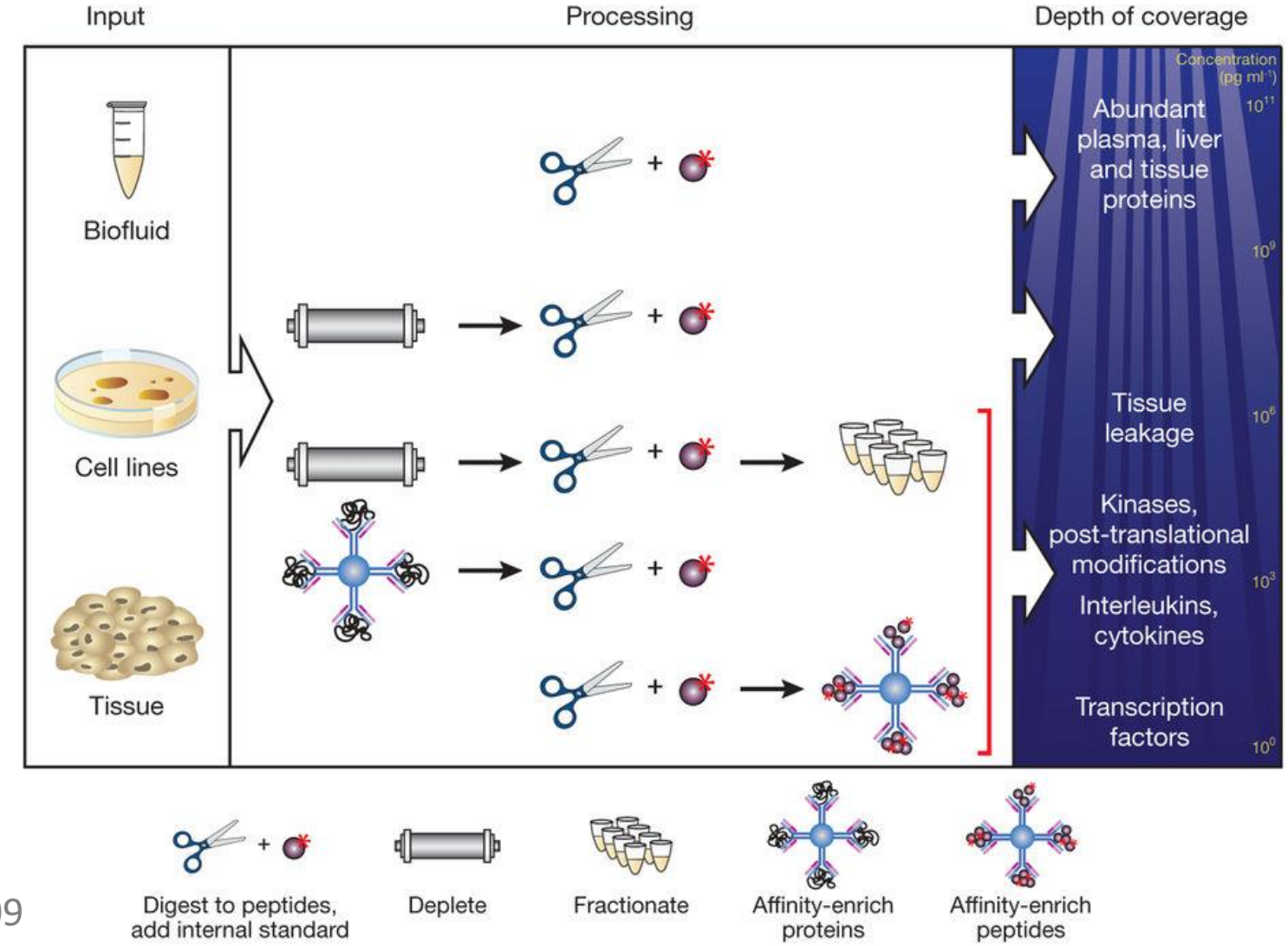


Selected reaction monitoring (SRM) and parallel reaction monitoring (PRM)



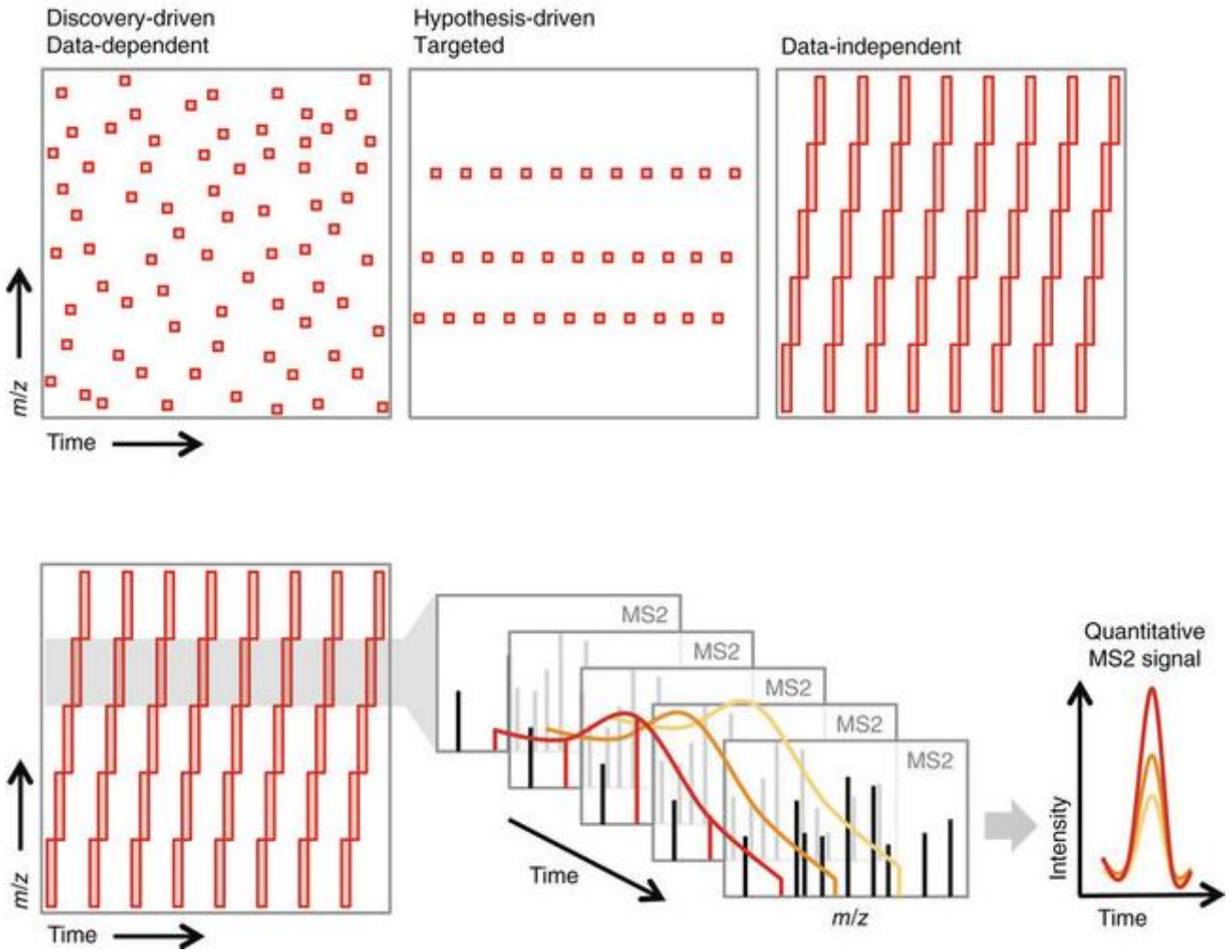
DOI: 10.1074/mcp.M116.061143

Enrichment strategies to increase sensitivity and specificity of analyte detection in SID-MRM-MS



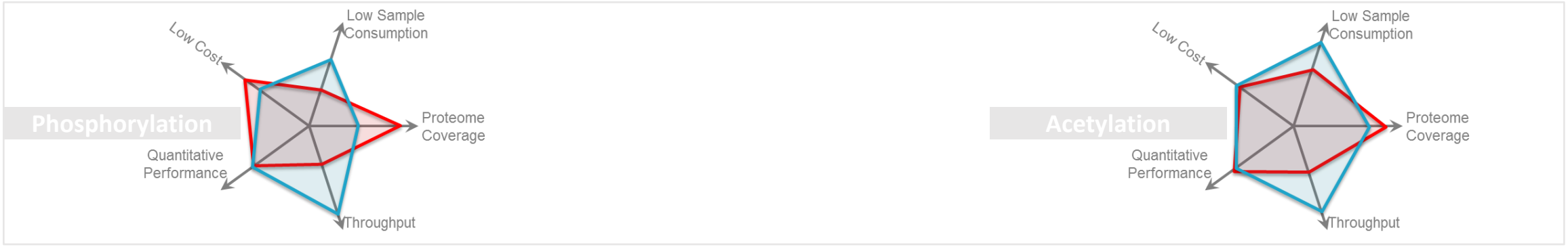
DOI:10.1038/nmeth.2309

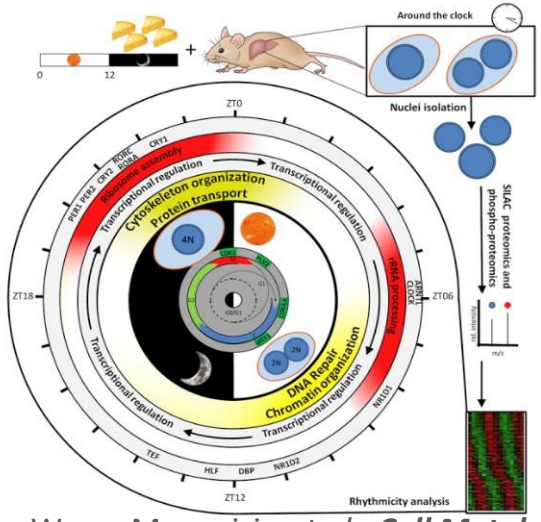
Untargeted MS approaches with targeted data analysis



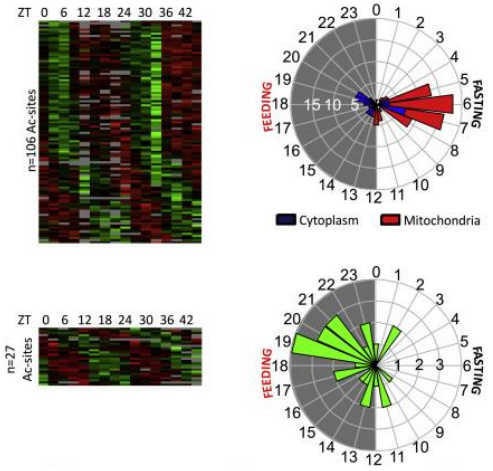
doi:10.1038/nprot.2017.040

Targeted sample preparation with untargeted MS approaches





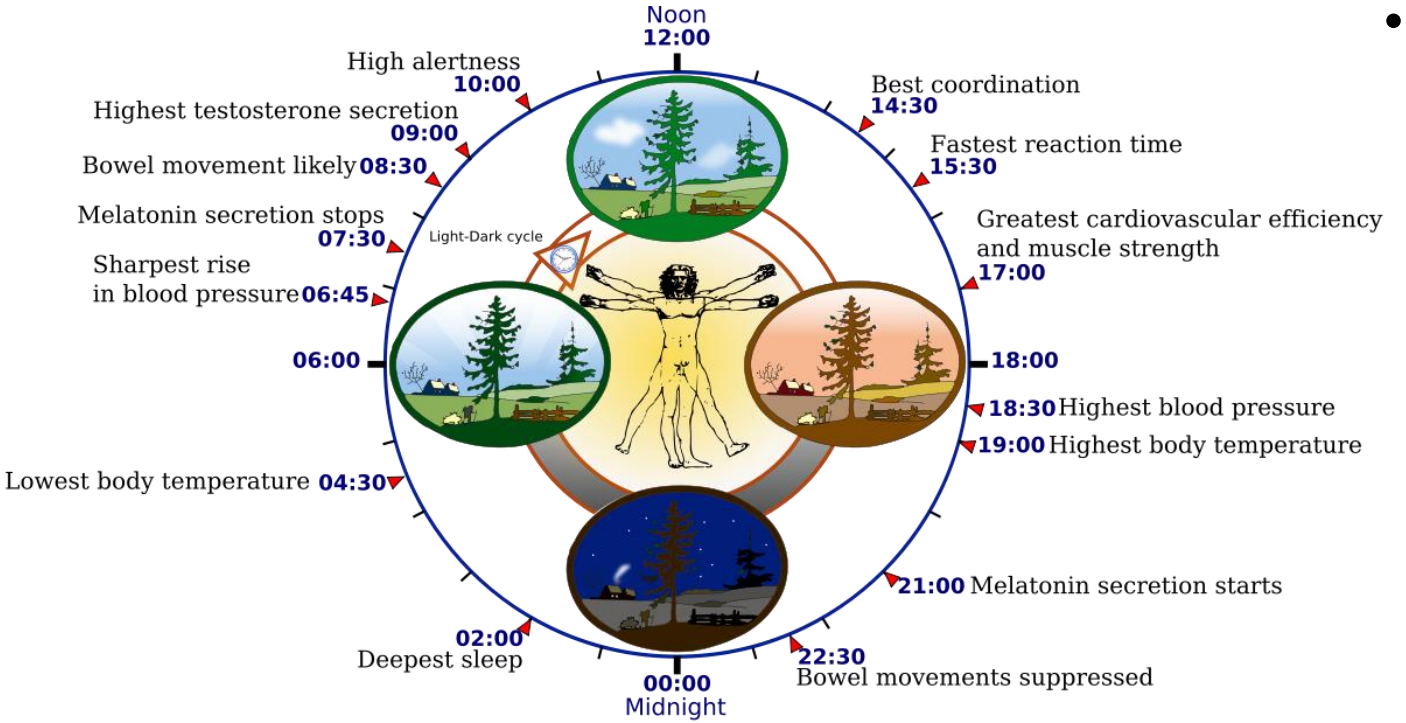
Wang, Mauvoisin *et al.*, ***Cell Metab.*** 2017, 25(1), 102–117.



Mauvoisin *et al.*, ***Cell Reports*** 2017, 20 (7), 1729–1743

Courtesy of A. Núñez Galindo

An example of study about circadian clock



- Perturbation of the circadian clock can lead to diverse pathologies:
  - Sleep disorders
  - Depressive disorders
  - Cardiovascular diseases
  - Metabolic syndrome – Type 2 Diabetes
  - Increase tumour progression
  - Aging

Shift work (15-20% of the working population) has been recently selected as a risk factor for the development of cancer by the WHO.

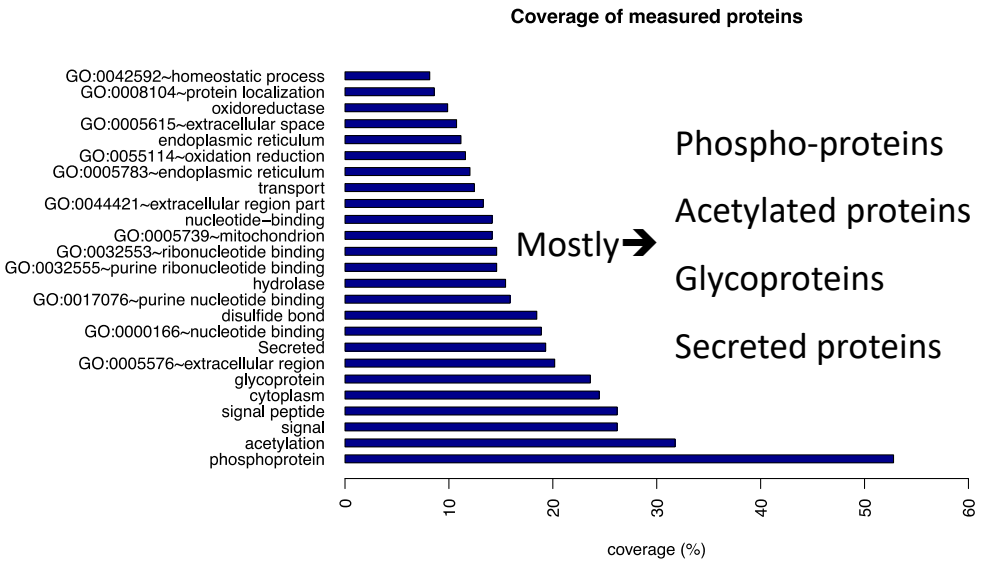
Courtesy of A. Núñez Galindo

Full circadian proteome

- Most work done uses genomic approaches
- Proteome-wide study of rhythmicity

5827 proteins identified

4016 proteins quantified (in at least 8/16 HL ratios)



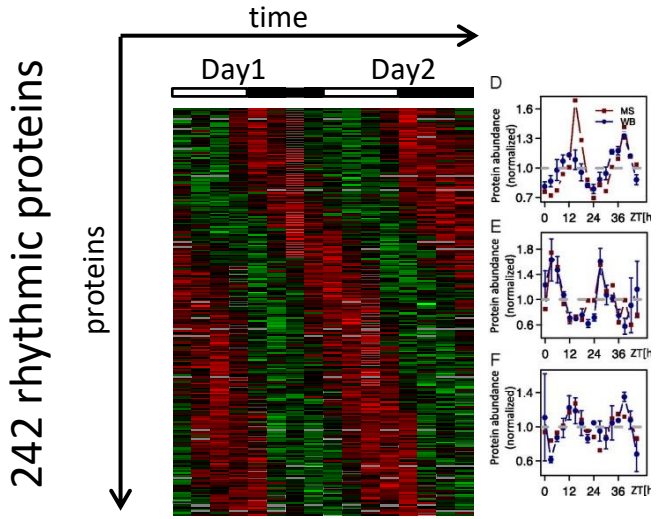
Mostly➔

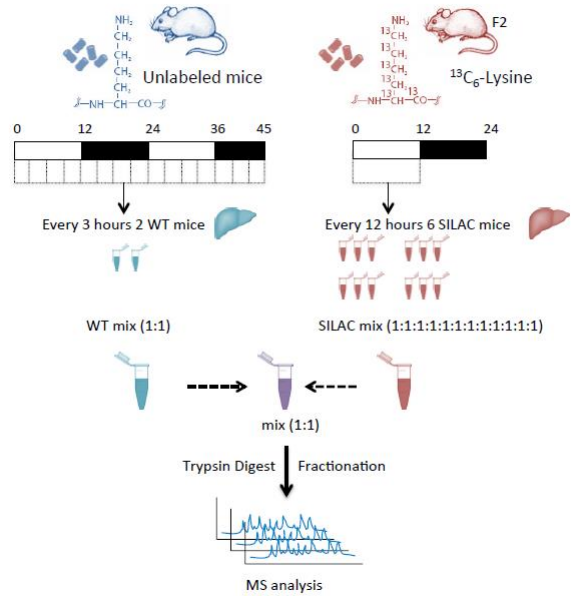
Phospho-proteins

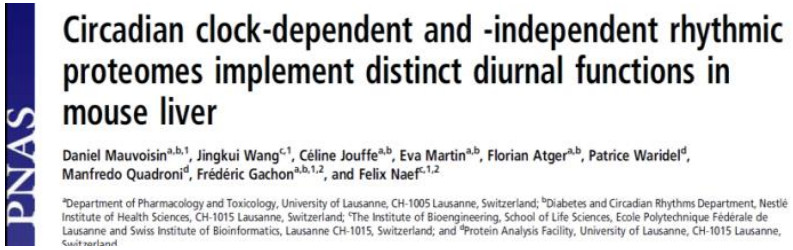
Acetylated proteins

Glycoproteins

Secreted proteins



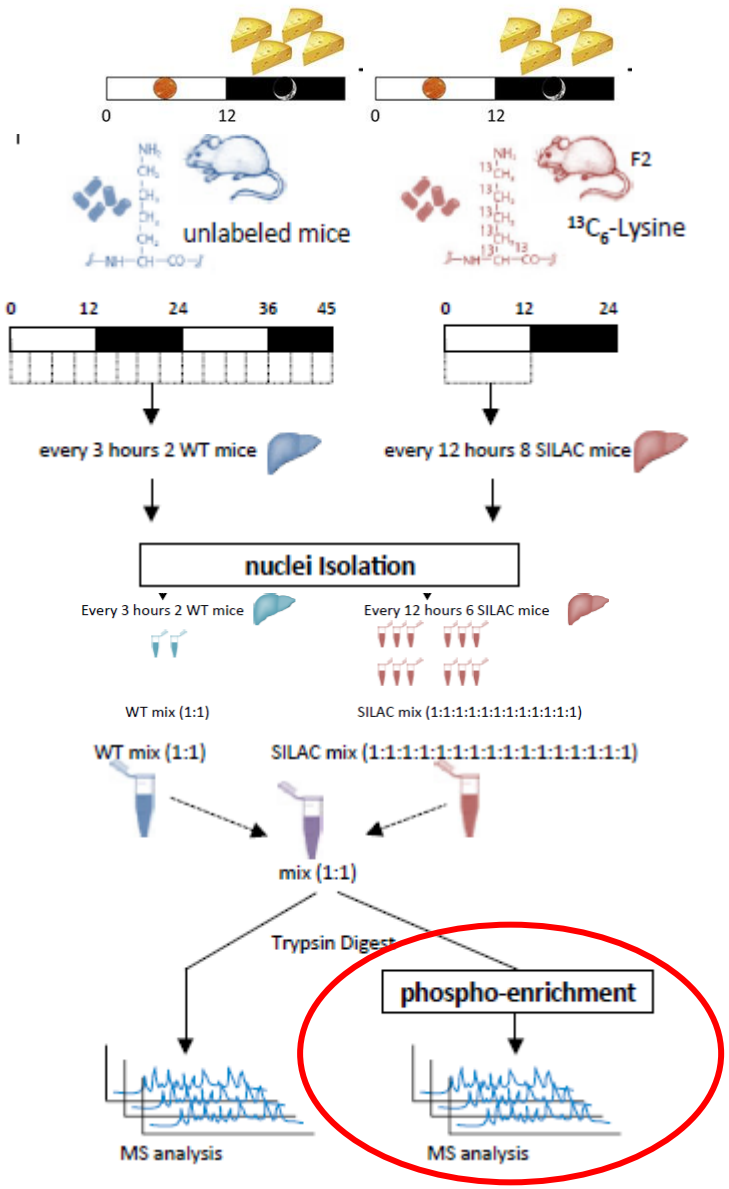




DOI: 10.1073/pnas.1314066111

Courtesy of A. Núñez Galindo

Circadian phosphoproteome

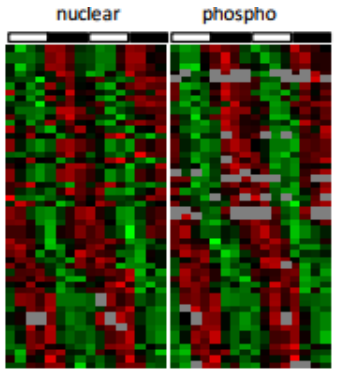


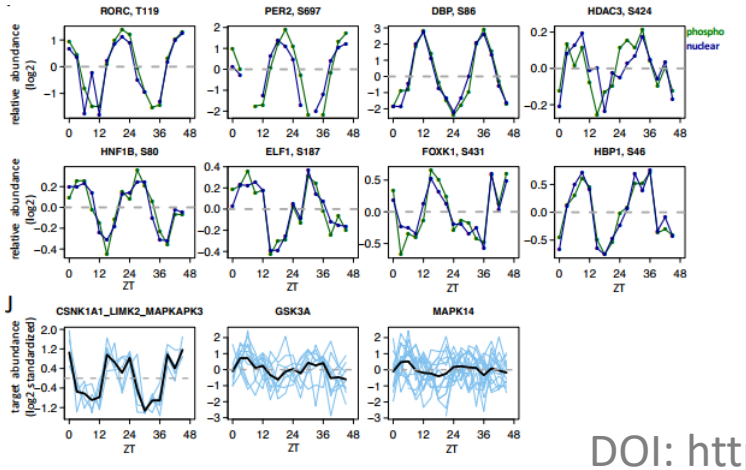
Almost 5000 phosphopeptides identified

1448 quantified (in at least 8/16 HL ratios)

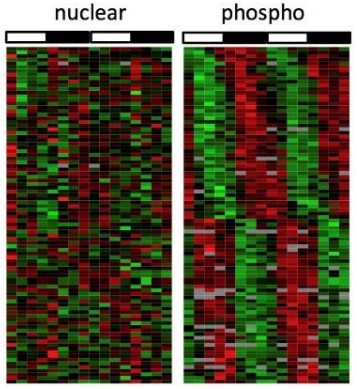
113 phosphoproteins presented rhythmicity

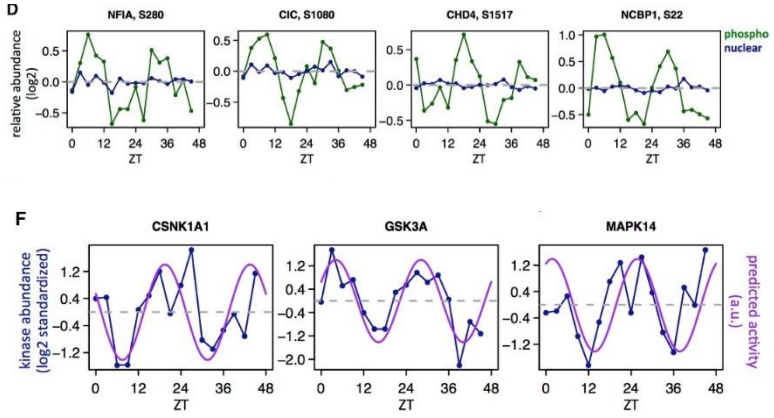
Corresponding rhythmicity





Non Corresponding rhythmicity

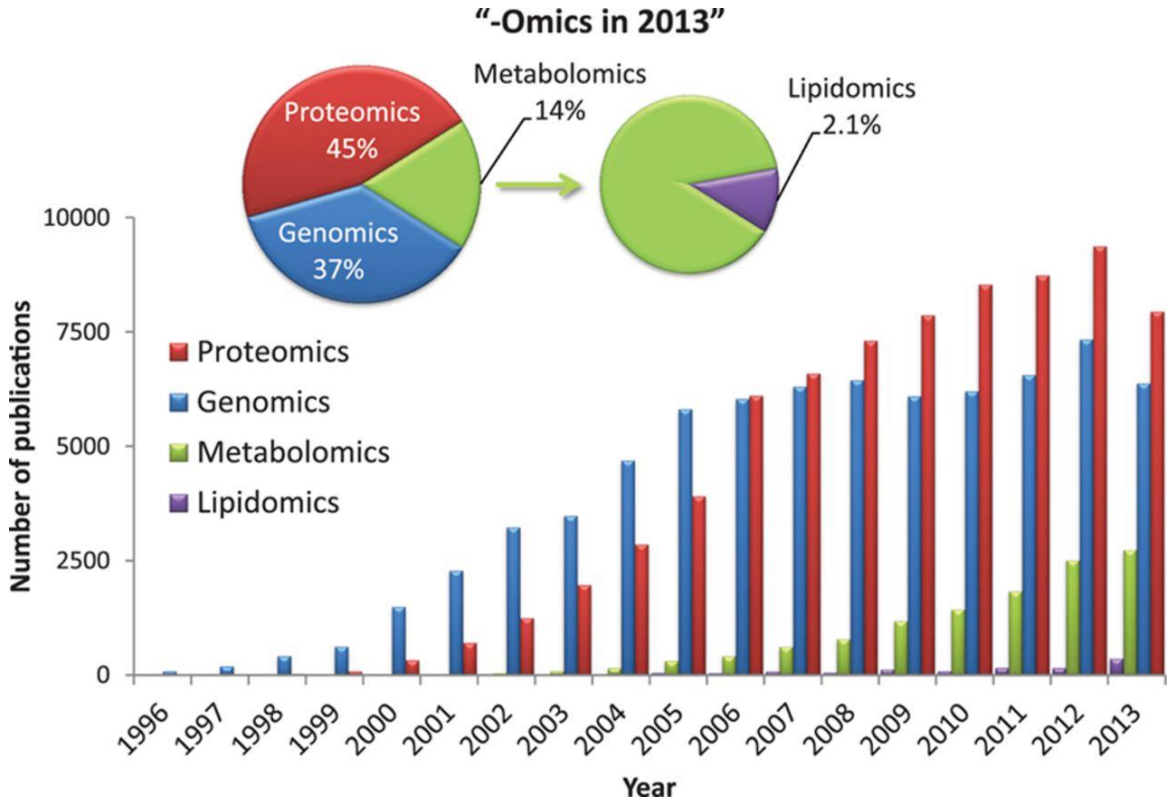




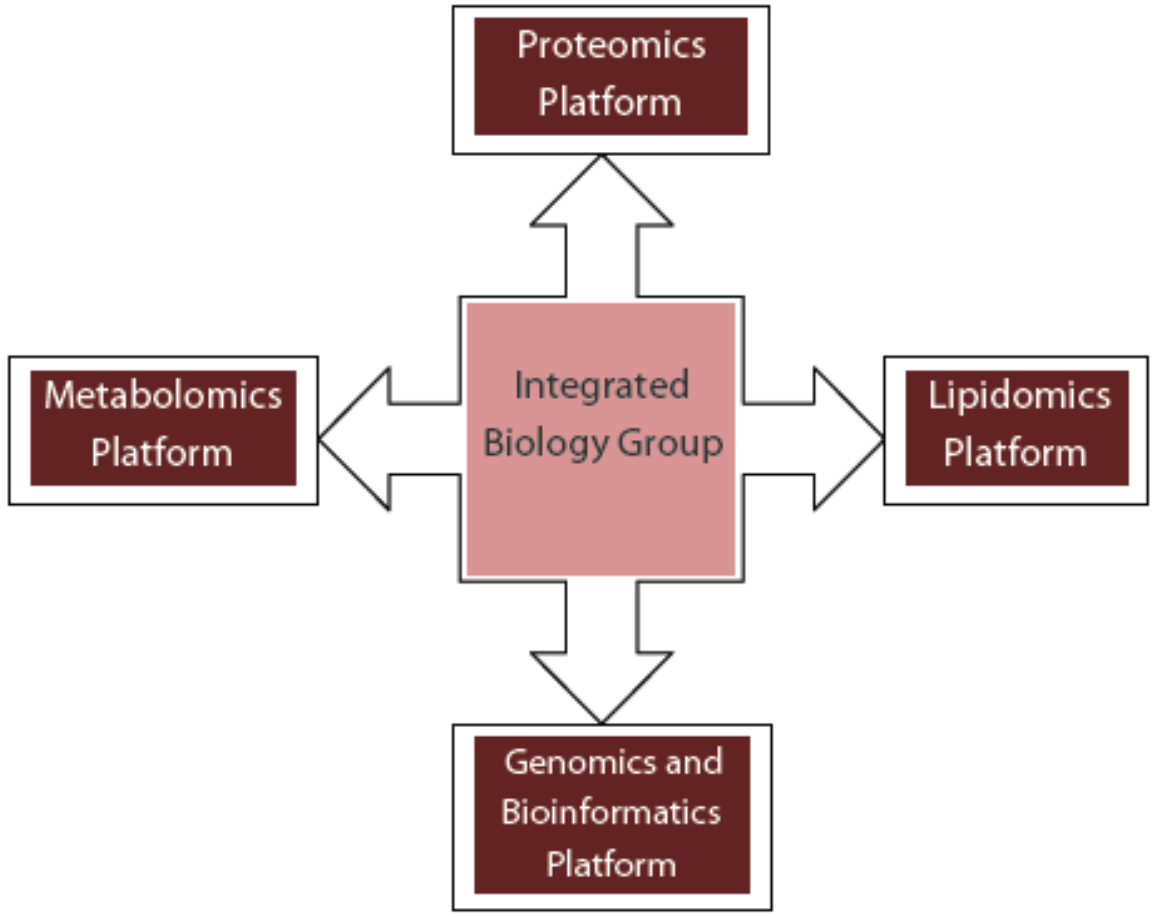
DOI: https://doi.org/10.1016/j.cmet.2016.10.003

Courtesy of A. Núñez Galindo

6.5. Other biological applications of mass spectrometry

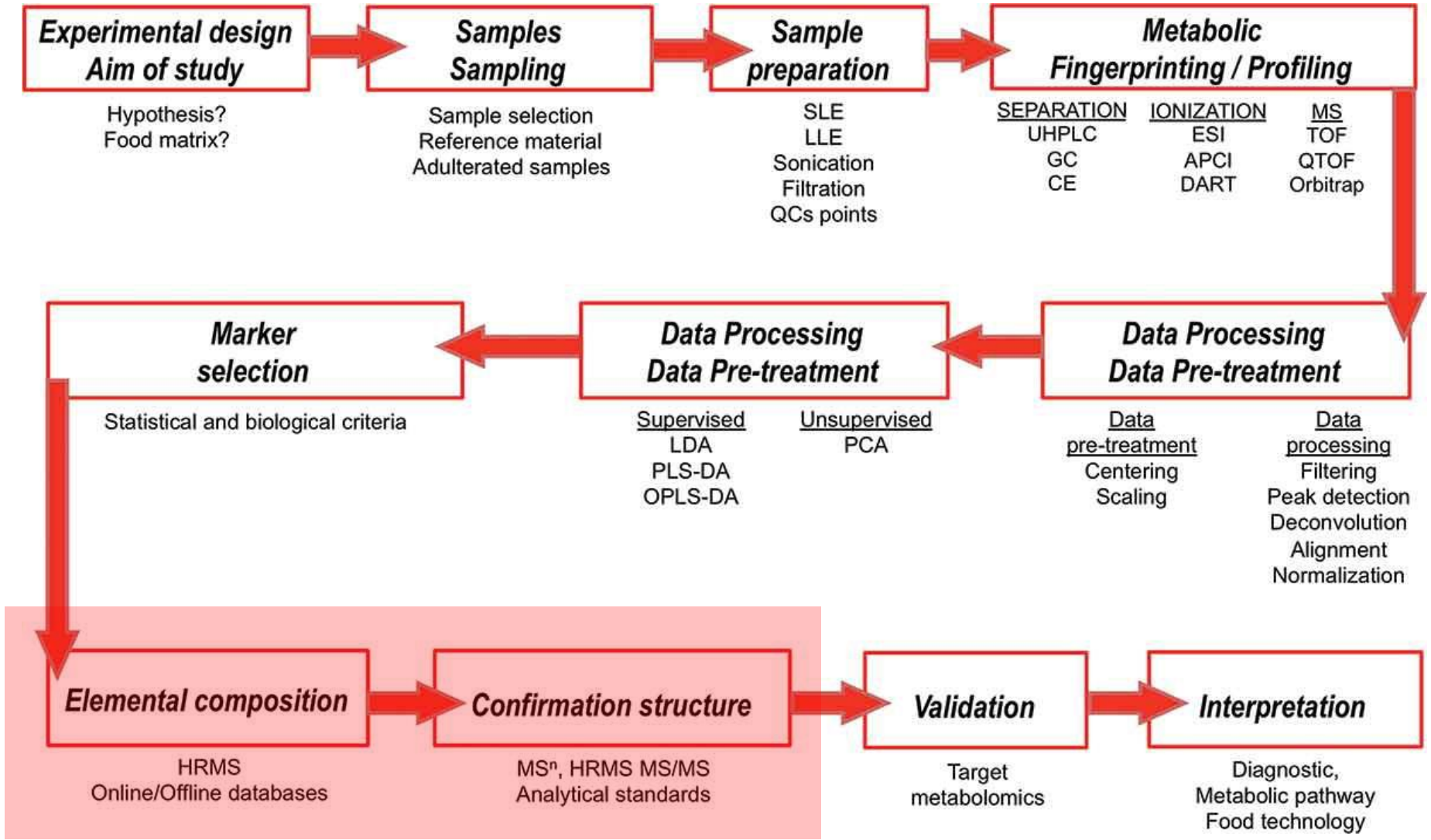


https://doi.org/10.1161/CIRCGENETICS.114.000550



DOI:10.4172/jcsb.1000082

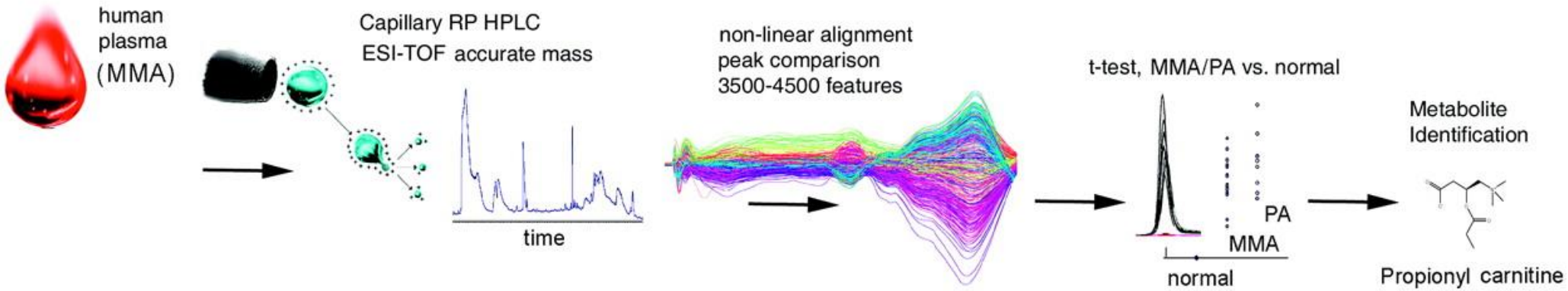
Mass spectrometry-based metabolomics (1)



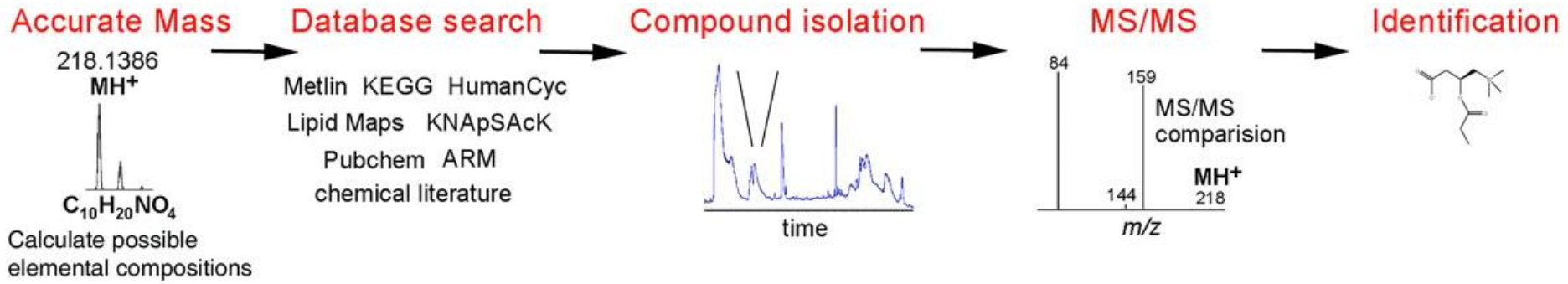
https://doi.org/10.1080/19440049.2015.1084539

Mass spectrometry-based metabolomics (2)

A. Finding Metabolomic Differences (untargeted)

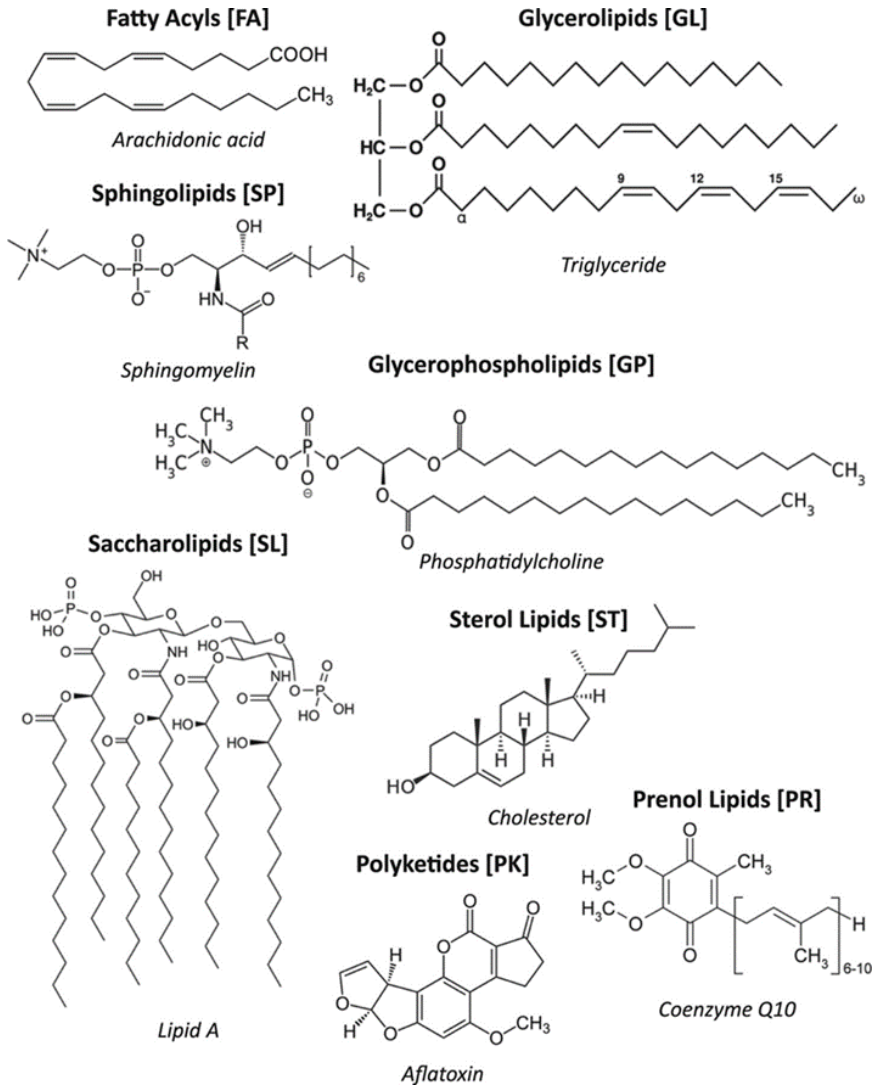


B. Identification of Unknown Metabolites (targeted)



DOI: 10.1373/clinchem.2007.089011

Mass spectrometry-based lipidomics (1)



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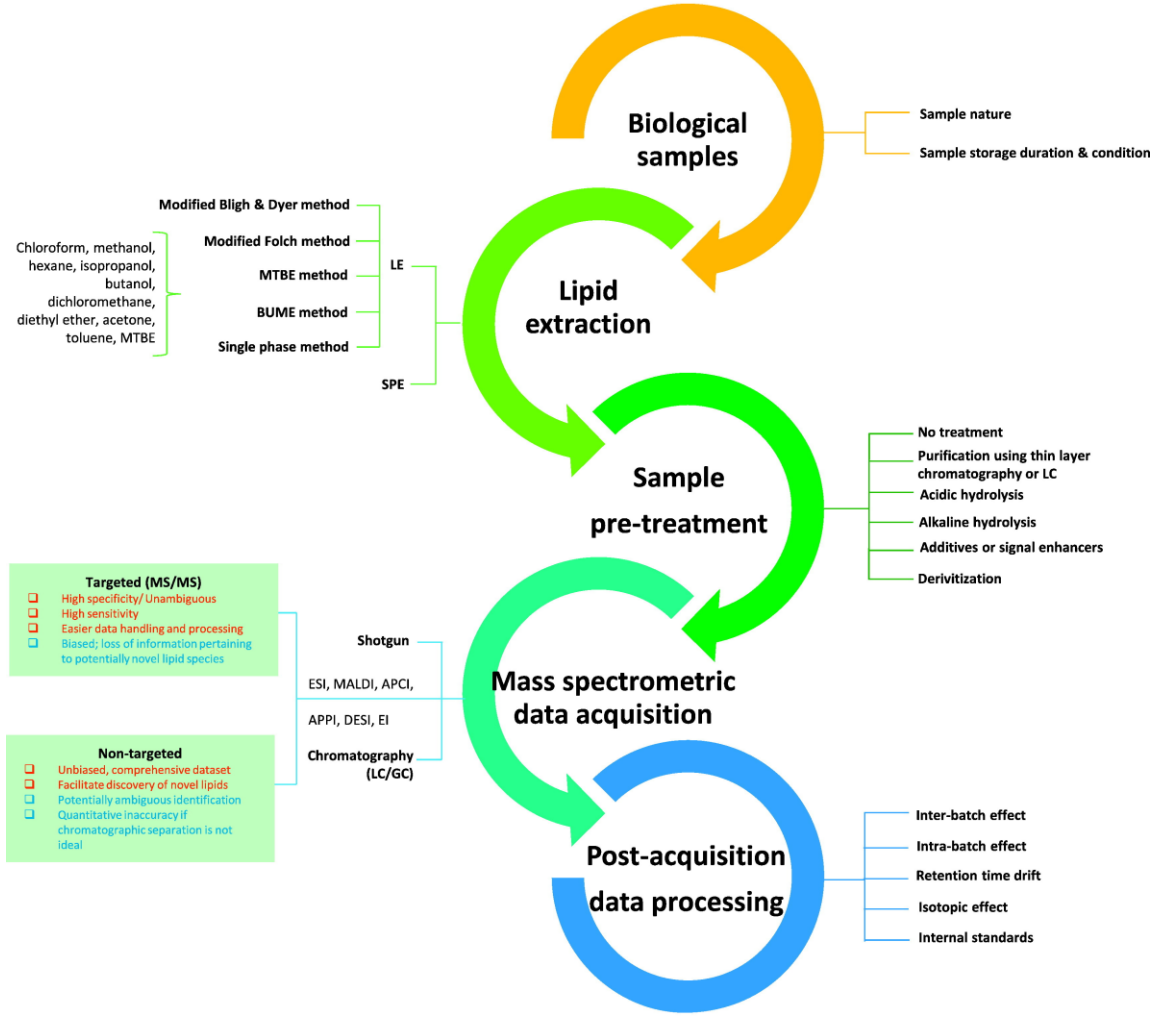
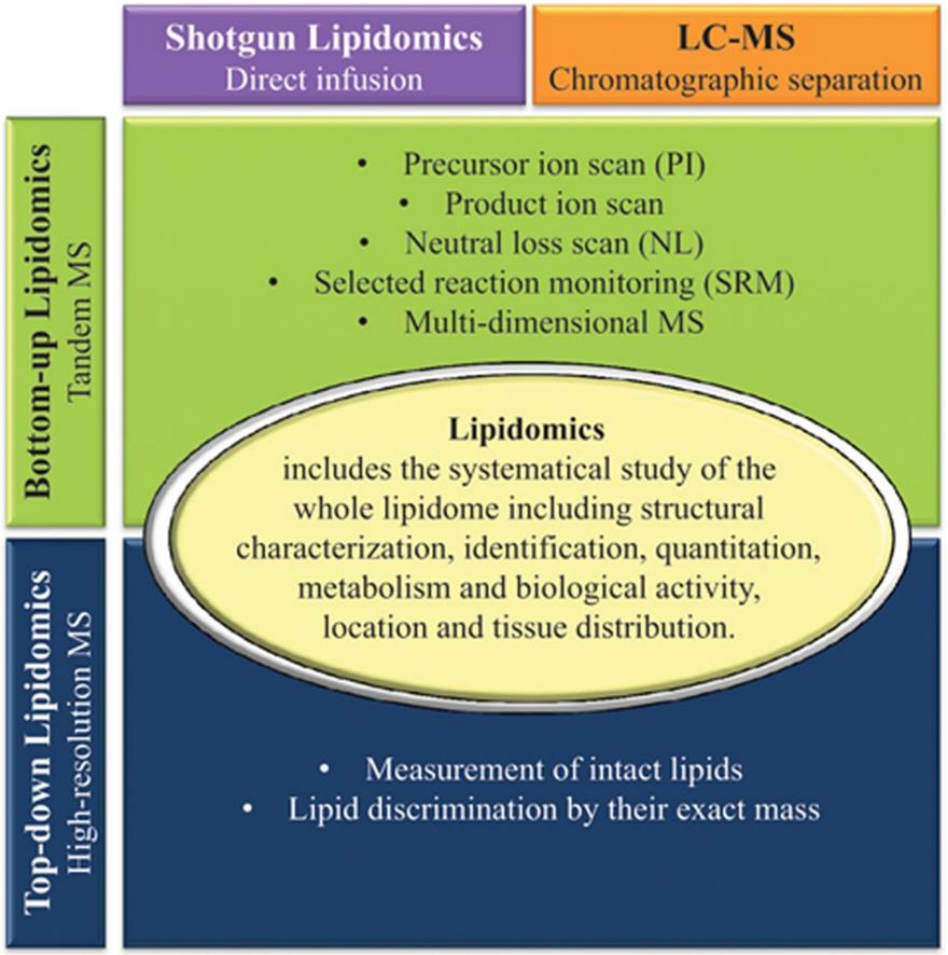
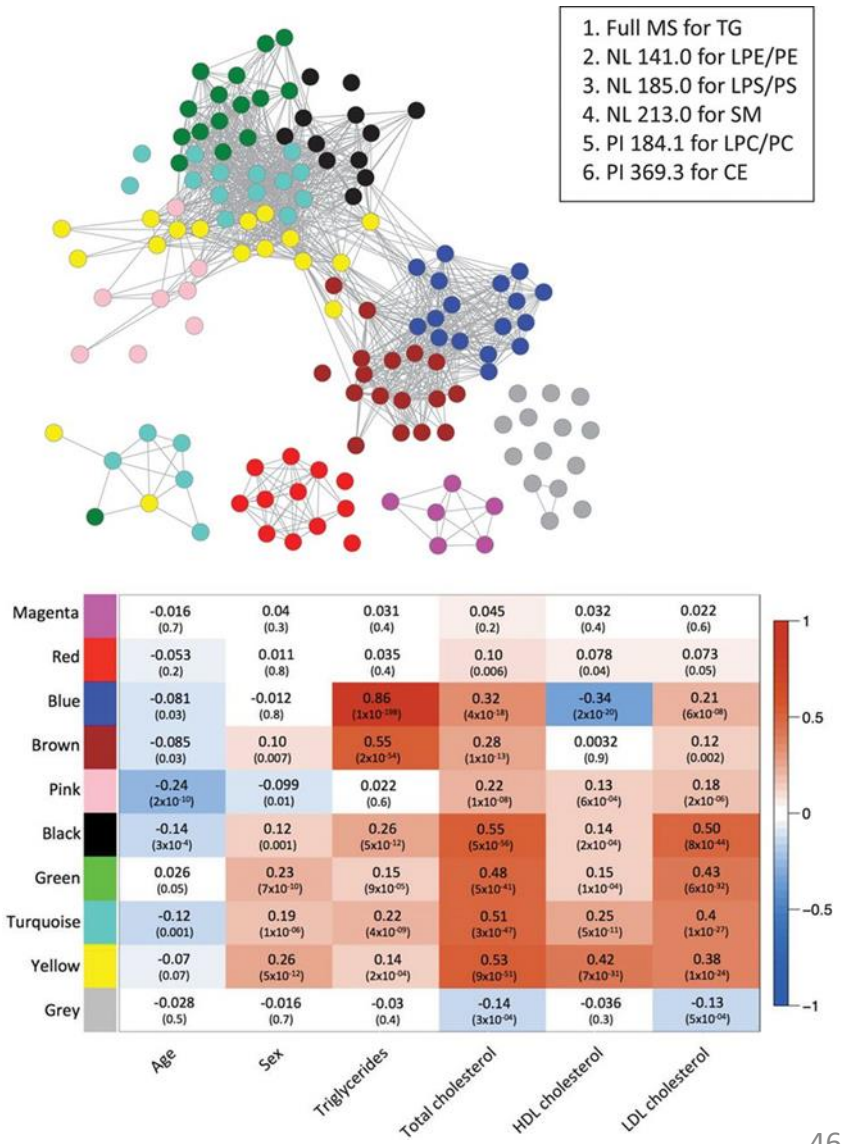


https://doi.org/10.1016/j.bbalip.2017.02.008

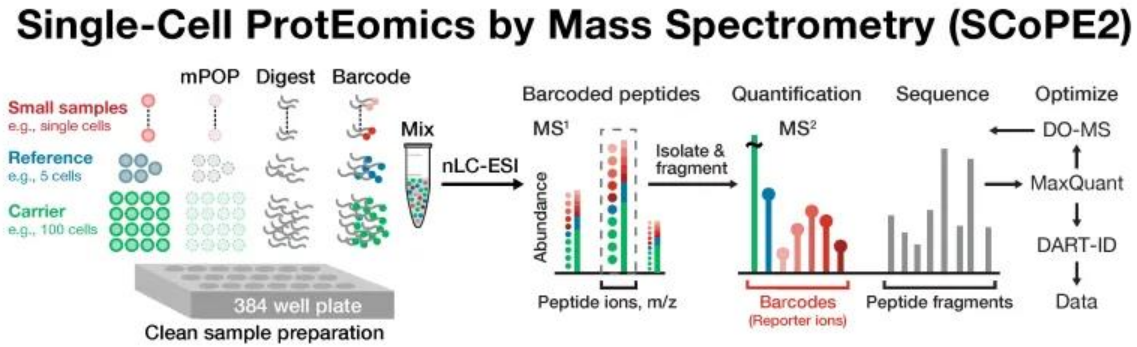
Mass spectrometry-based lipidomics (2)



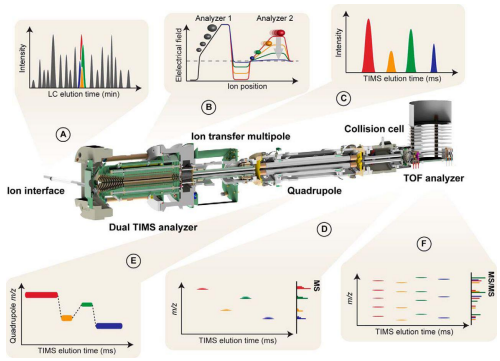


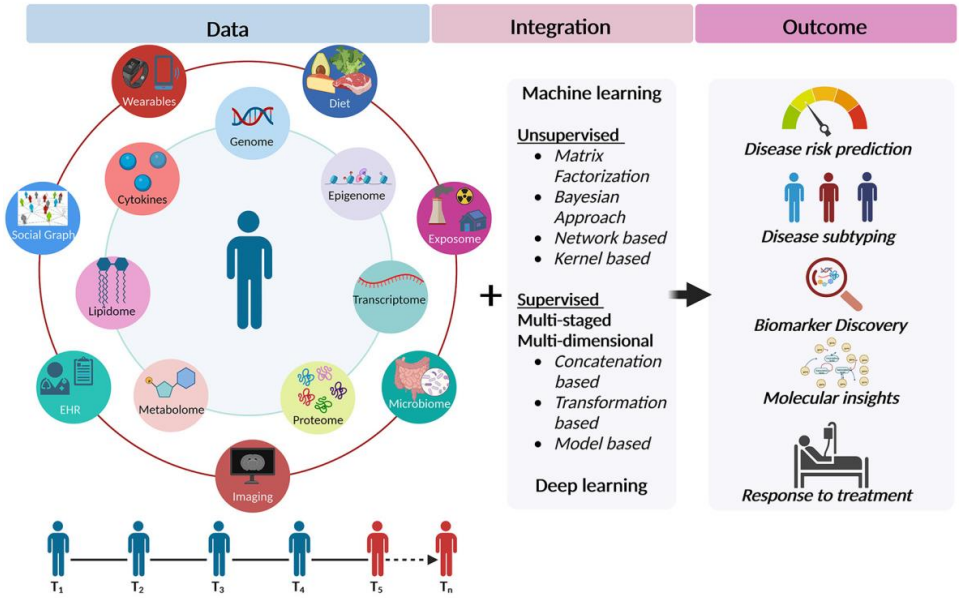
https://doi.org/10.1161/CIRCGENETICS.114.000550

6.6. Advanced innovations (single-cells, 4D proteomics, multi-omics) and emerging technologies



https://web.northeastern.edu/slavovlab/blog/single-cell-proteomics/

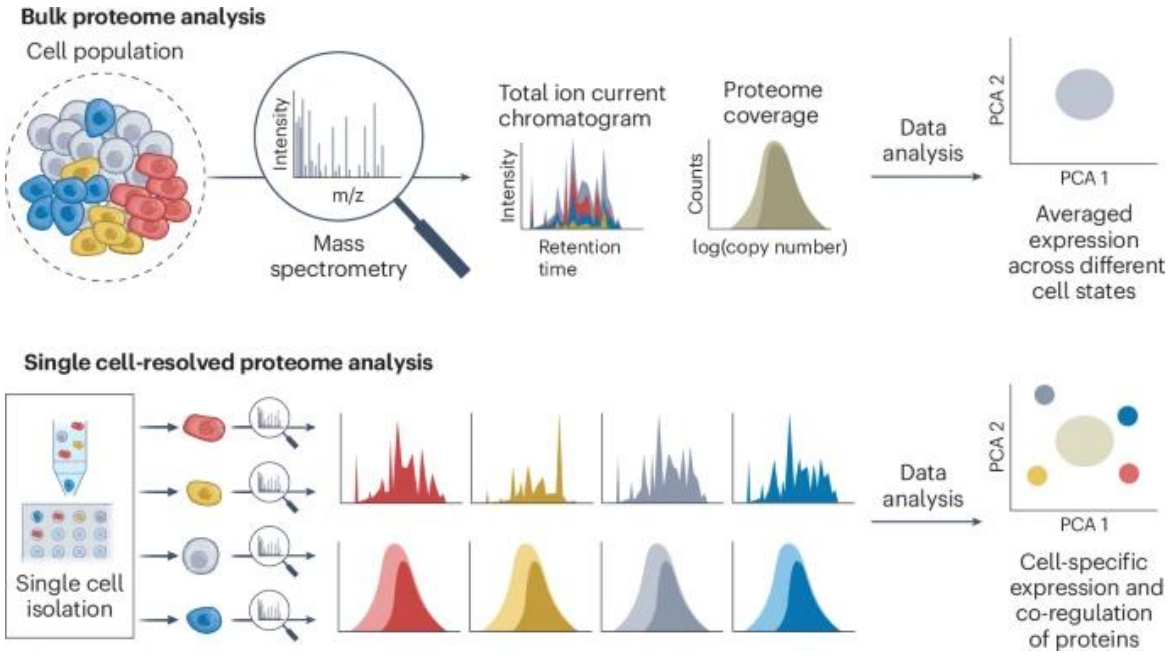




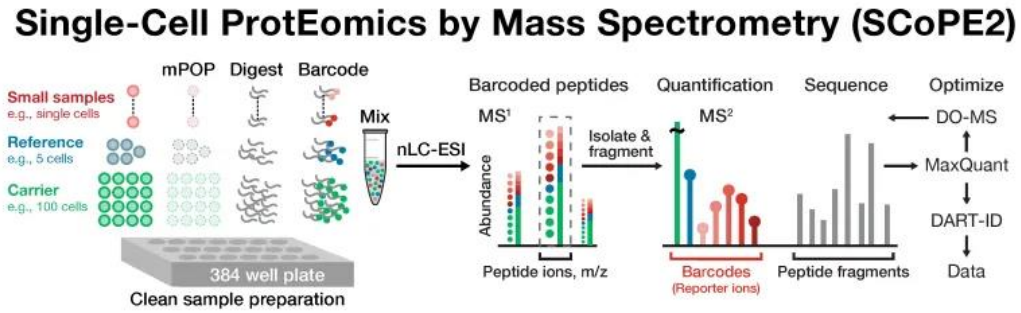
https://doi.org/10.1016/j.mcpro.2023.100561

Molecular & Cellular Proteomics 2018 172534-2545DOI: (10.1074/mcp.TIR118.000900)

Single-cell proteomics



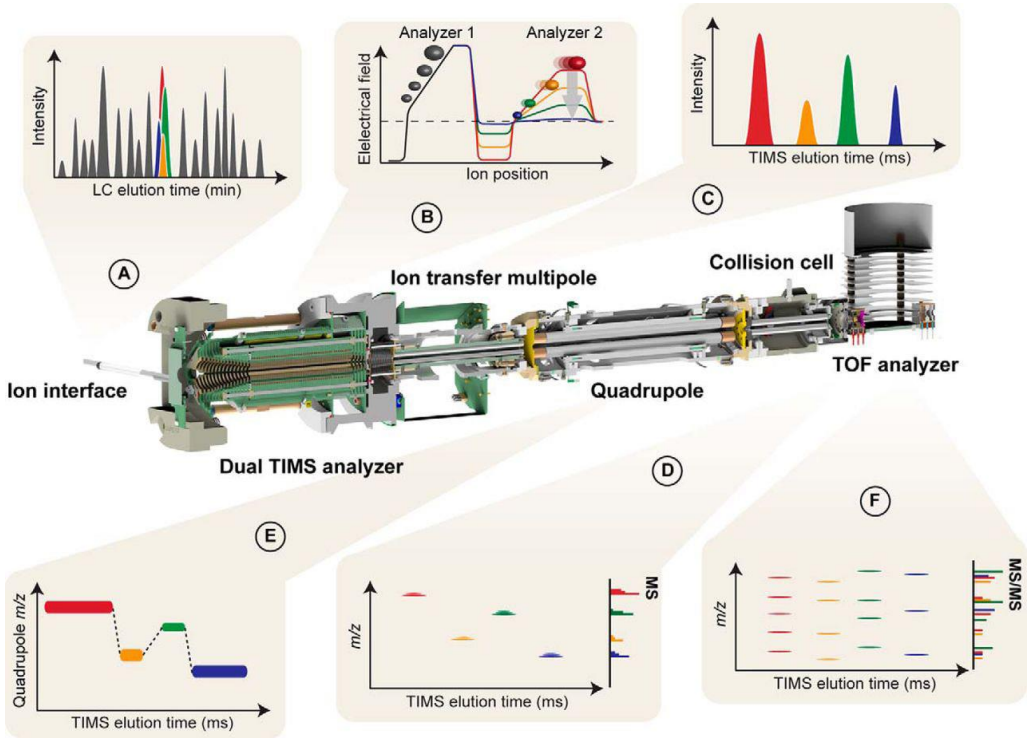
https://doi.org/10.1038/s41592-025-02620-7



https://web.northeastern.edu/slavovlab/blog/single-cell-proteomics/

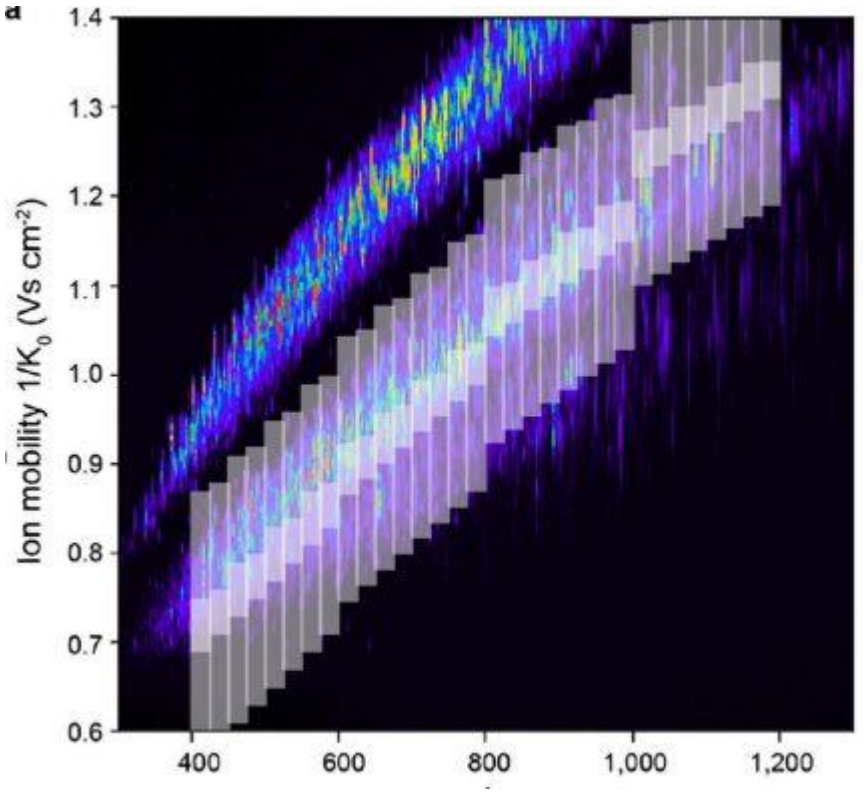
- Sensitivity challenge
- Sample preparation
- Use of isobaric labelling

Ion-mobility separation (IMS)… another dimension to proteome analysis



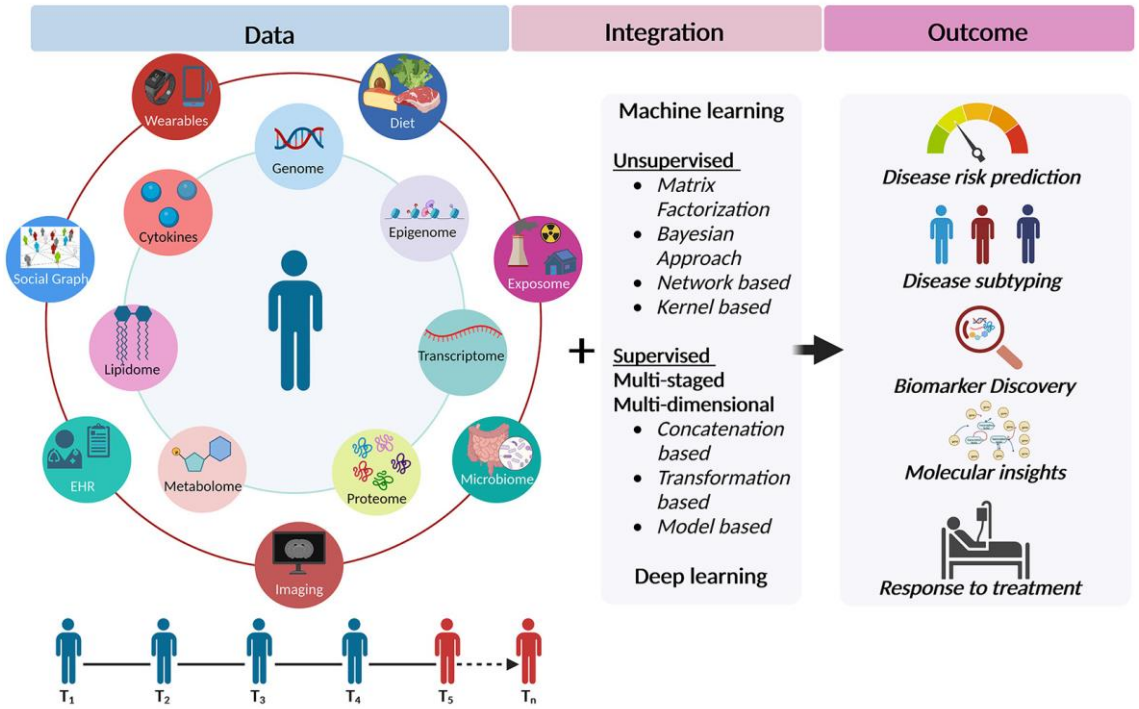
Molecular & Cellular Proteomics 2018 172534-2545DOI: (10.1074/mcp.TIR118.000900)

- 4D proteomics



https://www.bruker.com/fr/applications/academia-life-science/proteomics/4d-proteomics.html

Multi-omics



https://doi.org/10.1016/j.mcpro.2023.100561

EXPERT REVIEW OF PROTEOMICS
2025, VOL. 22, NO. 4, 149–162
https://doi.org/10.1080/14789450.2025.2491357

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REVIEW

Check for updates

Unravelling disease complexity: integrative analysis of multi-omic data in clinical research

Ornella Cominetti [a] and Loïc Dayon [a,b]

[a]Proteomics, Nestlé Institute of Food Safety & Analytical Sciences, Nestlé Research, Lausanne, Switzerland; [b]Institut des Sciences et Ingénierie Chimiques, Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

ABSTRACT

Introduction: A holistic view on biological systems is today a reality with the application of multi-omic technologies. These technologies allow the profiling of genome, epigenome, transcriptome, proteome, metabolome as well as newly emerging 'omes.' While the multiple layers of data accumulate, their integration and reconciliation in a single system map is a cumbersome exercise that faces many challenges. Application to human health and disease requires large sample sizes, robust methodologies and high-quality standards.

Areas covered: We review the different methods used to integrate multi-omics, as recent ones including artificial intelligence. With proteomics as an anchor technology, we then present selected applications of its data combination with other omics layers in clinical research, mainly covering literature from the last five years in the Scopus and/or PubMed databases.

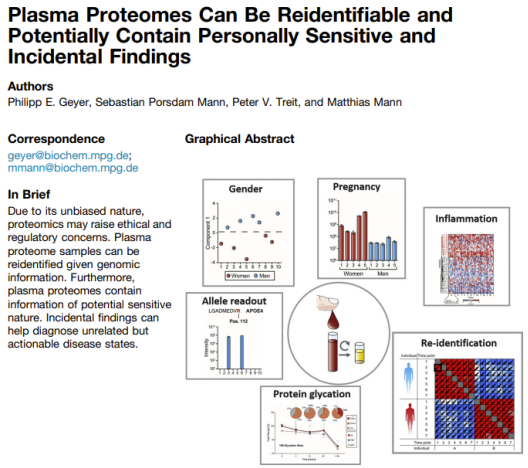
Expert opinion: Multi-omics is powerful to comprehensively type molecular layers and link them to phenotype. Yet, technologies and data are very diverse and still strategies and methodologies to properly integrate these modalities are needed.

ARTICLE HISTORY
Received 24 January 2025
Accepted 6 April 2025

KEYWORDS
Multiomics; multi-omics; integrative omics; omic clinical research; clinical proteomics; proteomics

https://doi.org/10.1080/14789450.2025.2491357

6.7. Opportunities, limitations and ethical consideration



Plasma Proteomes Can Be Reidentifiable and Potentially Contain Personally Sensitive and Incidental Findings

Authors
Philipp E. Geyer, Sebastian Porsdam Mann, Peter V. Treit, and Matthias Mann

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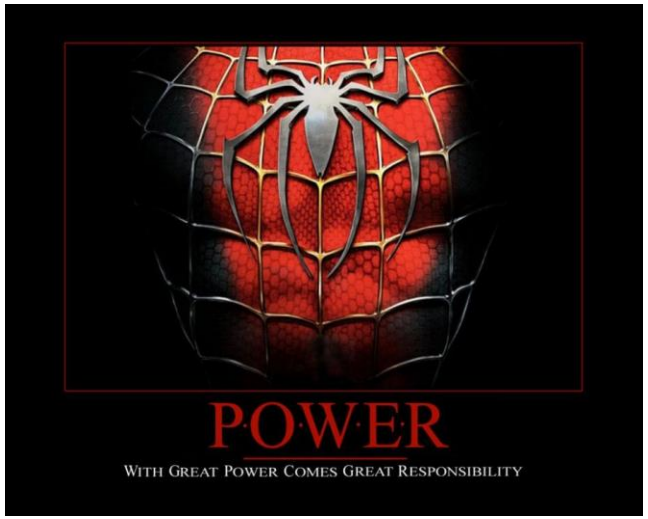
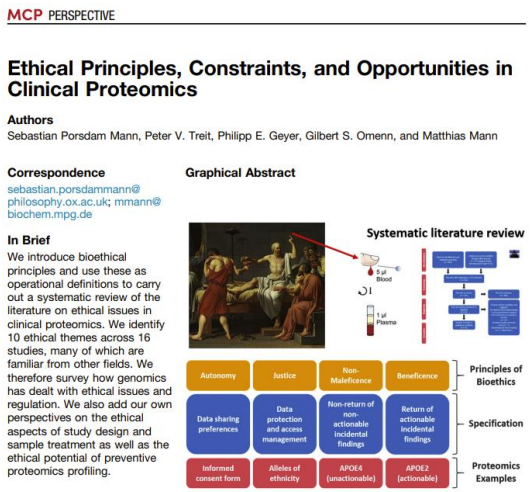
In Brief
Due to its unbiased nature, proteomics may raise ethical and regulatory concerns. Plasma proteome samples can be reidentified given genomic information. Furthermore, plasma proteomes contain information of potential sensitive nature. Incidental findings can help diagnose unrelated but actionable disease states.

Graphical Abstract

https://doi.org/10.1074/mcp.RA120.002359





MCP PERSPECTIVE

Ethical Principles, Constraints, and Opportunities in Clinical Proteomics

Authors
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Correspondence
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In Brief
We introduce bioethical principles and use these as operational definitions to carry out a systematic review of the literature on ethical issues in clinical proteomics. We identify 10 ethical themes across 16 studies, many of which are familiar from other fields. We therefore survey how genomics has dealt with ethical issues and regulation. We also add our own perspectives on the ethical aspects of study design and sample treatment as well as the ethical potential of preventive proteomics profiling.

Graphical Abstract

https://doi.org/10.1016/j.mcpro.2021.100046

Unbiased nature and increasing power of MS-based proteomics has increased not only the overall amount but also the proportion of particularly ethically sensitive data

Individuals can be identified by protein levels in blood plasma; Individuals can be Identified by allelic Information

Untargeted plasma proteomics delivers incidental diagnostic findings; Untargeted plasma proteomics delivers personally sensitive findings

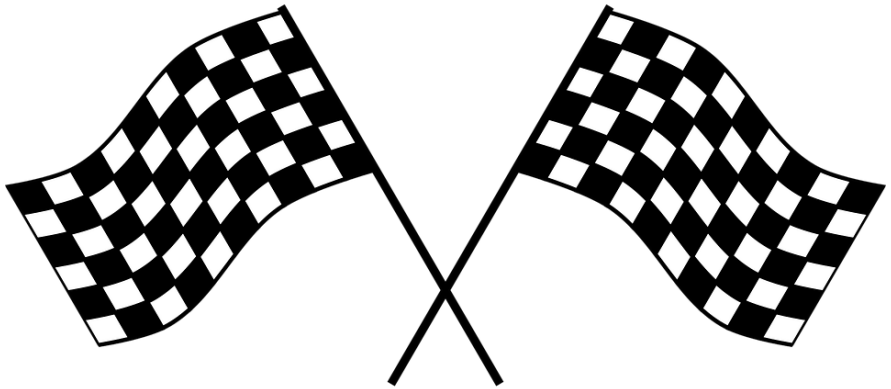
Numerous opportunities, but also responsibilities, including data privacy, consent, and sharing, that must be fulfilled

Summary

- Wide ranges of biological and clinical applications as shown here!

- Proteomics is also used for quality control and safety (e.g., biosimilars, food matrices…)

Finished! Well done!



Any Remaining Questions?