

# Protein mass spectrometry and proteomics

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Spring Semester 2026

**EPFL**

# Course outline

## 1. Proteomics and mass spectrometry

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. Mass spectrometry-based proteomic strategy and workflows

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

Lab visit of the Proteomics Laboratory at Nestlé Research (EPFL Innovation Park)

## 3. Quantitative proteomic workflows

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

## 4. Proteomic bioinformatics

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

## 5. Applications to biology, clinical research, and beyond

What strategy?; Experimental design & randomization; 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

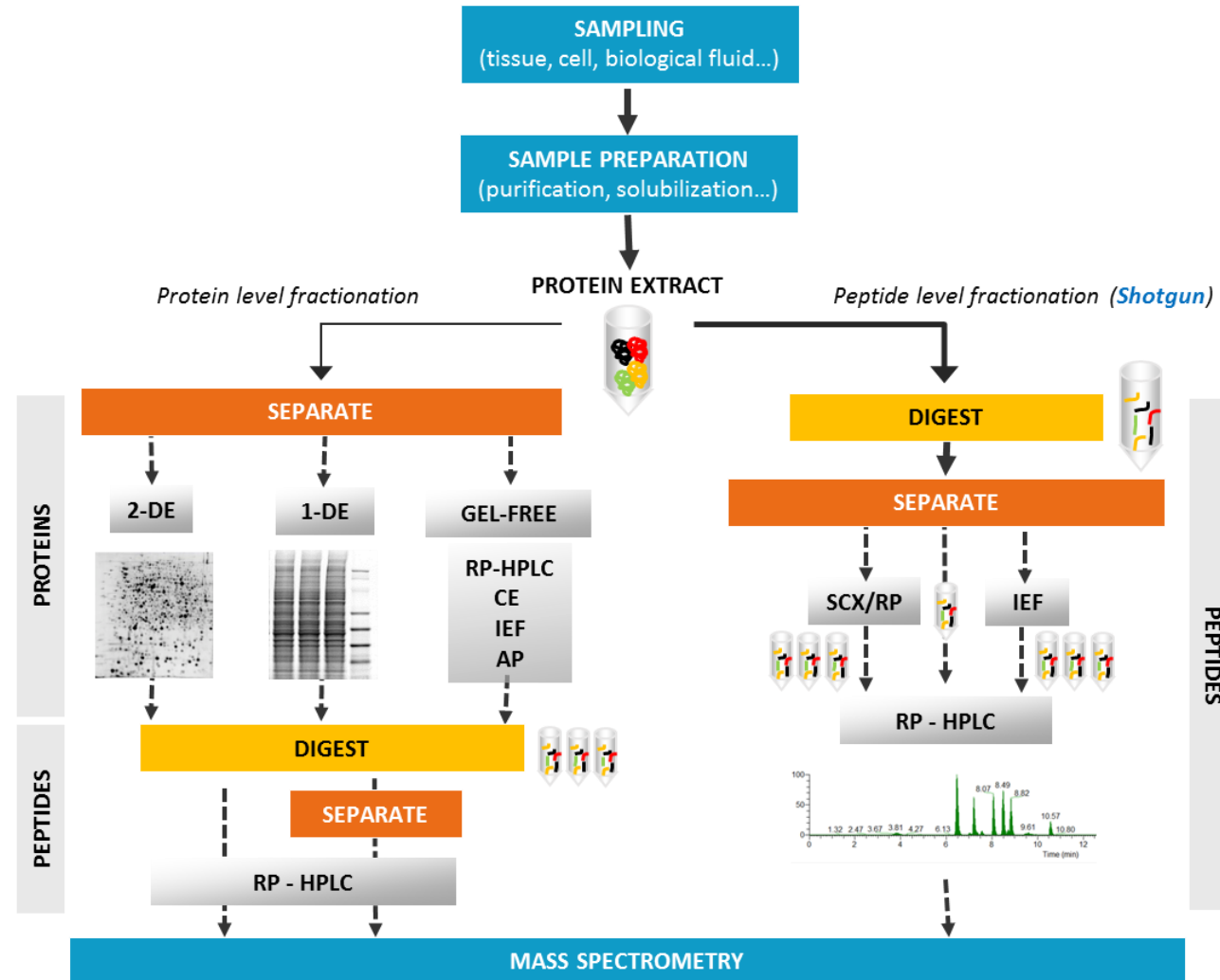
Additional support/information on [Separations techniques in proteomics](#)

# Course outline

- 5. Applications to biology and clinical research

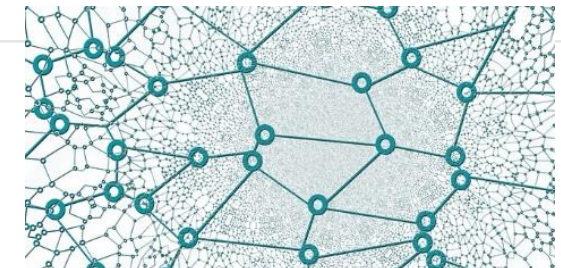
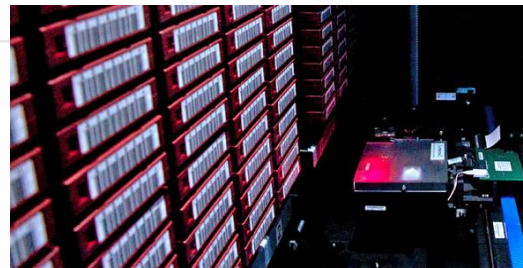
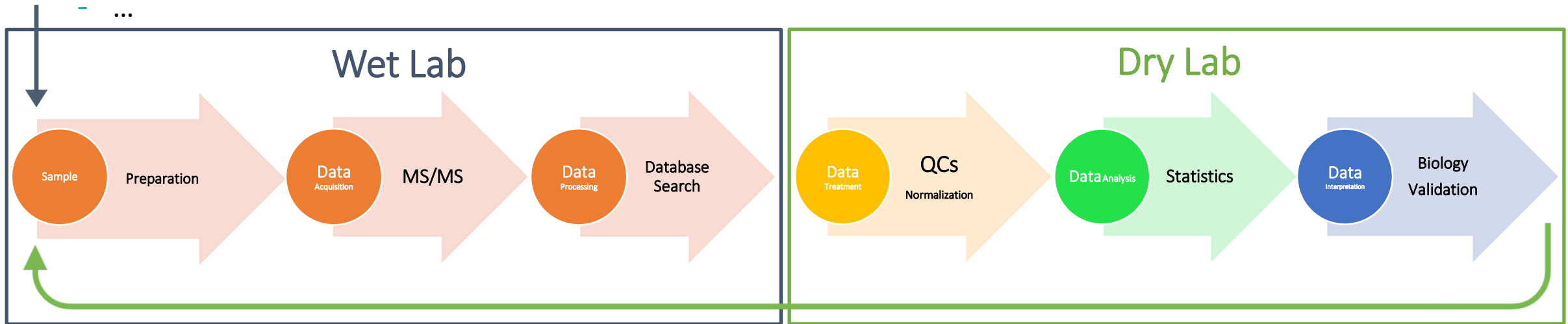
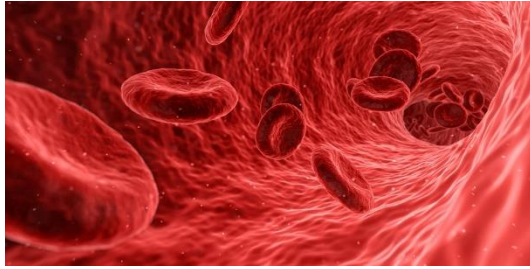
What strategy?; Experimental design; Biomarker discovery; Industrialized and population proteomics; Forensics; Targeted mass spectrometry-based approaches; Other biological applications of mass spectrometry; Advanced innovations (single-cells, 4D proteomics, multi-omics) and emerging technologies; Limitations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

# 5.1. What strategy?; Experimental design

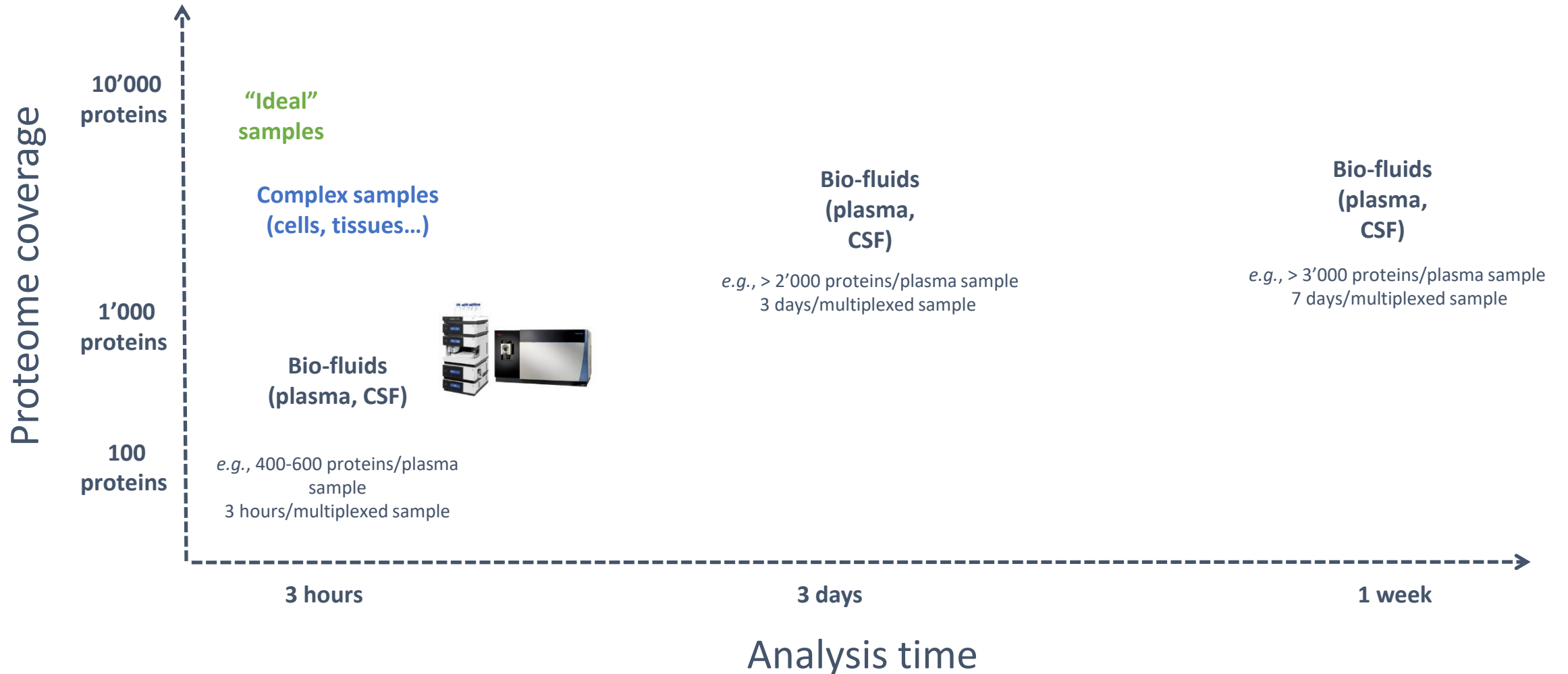


# The path to the results

- Plasma
- Other biofluids
- Tissues
- Cells
- Organelles
- ...



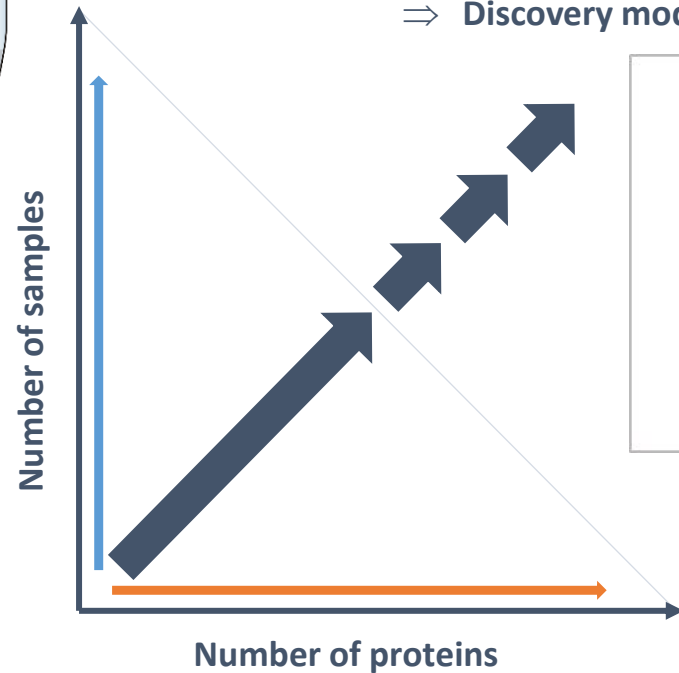
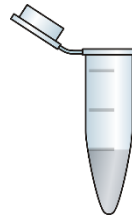
# What do you want to get from your analysis?



# Large-Scale Proteome Profiling with MS

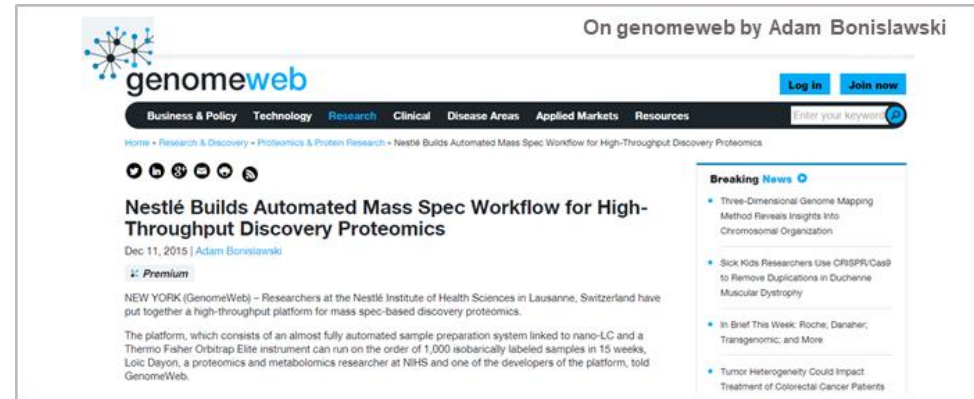
## Option 2: Large number of samples (large sample-size)

- ⇒ Short analysis time
- ⇒ Low number of proteins
- ⇒ Targeted mode



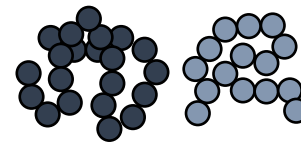
## Our vision

- ⇒ Controlled analysis time
- ⇒ Controlled number of proteins
- ⇒ Controlled number of samples
- ⇒ Discovery mode



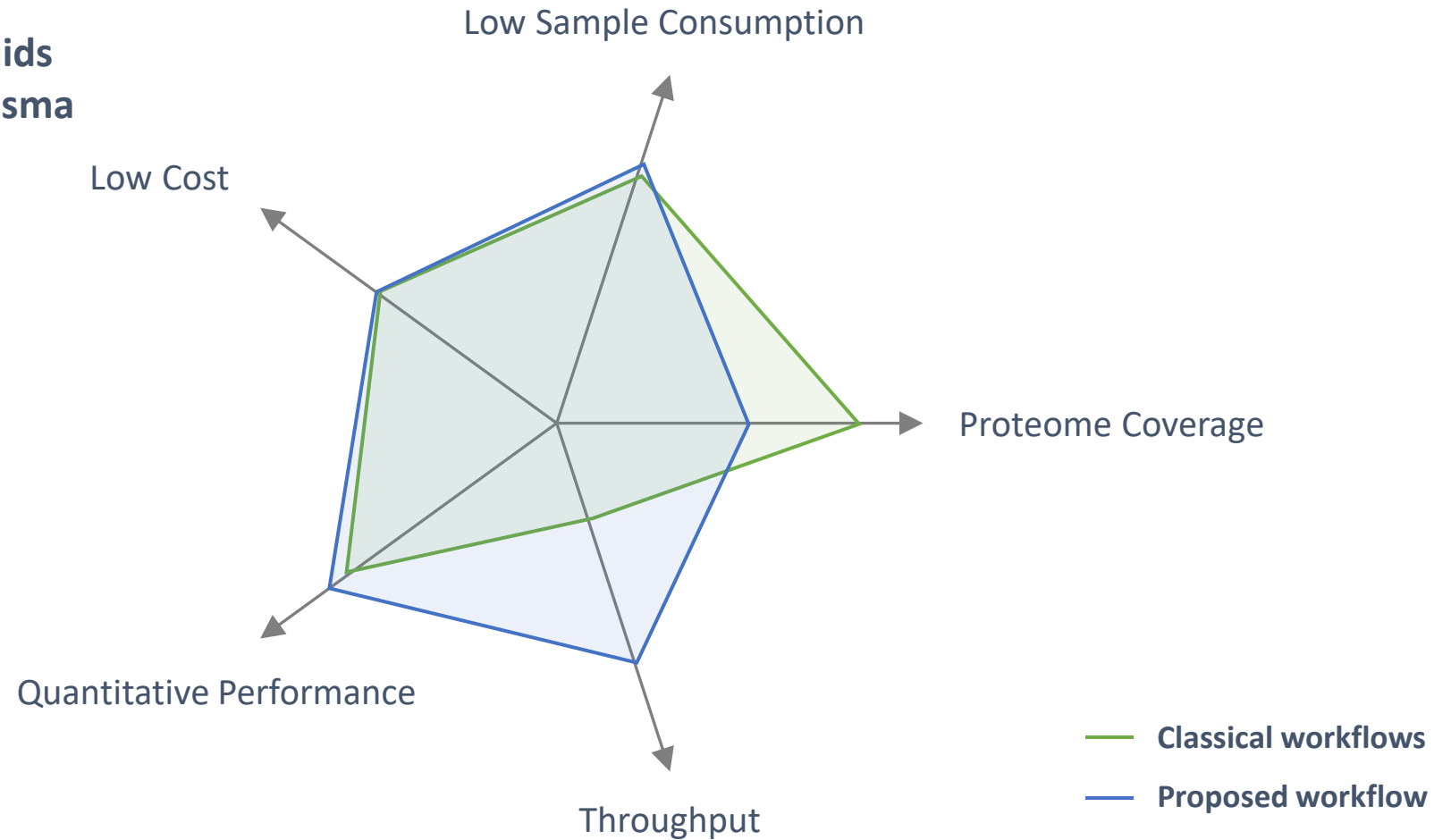
## Option 1: Large number of proteins (deep proteome coverage)

- ⇒ Long analysis time
- ⇒ Low number of samples
- ⇒ Discovery mode



# A practical example: Plasma proteomics

**In body-fluids  
such as plasma**

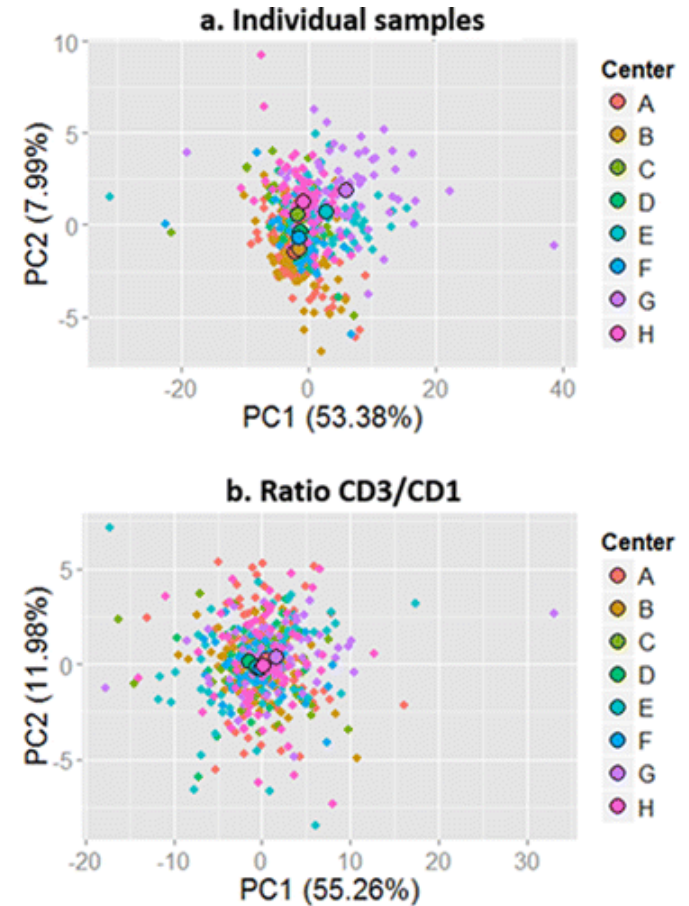




# Some sources of variability

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
- ...

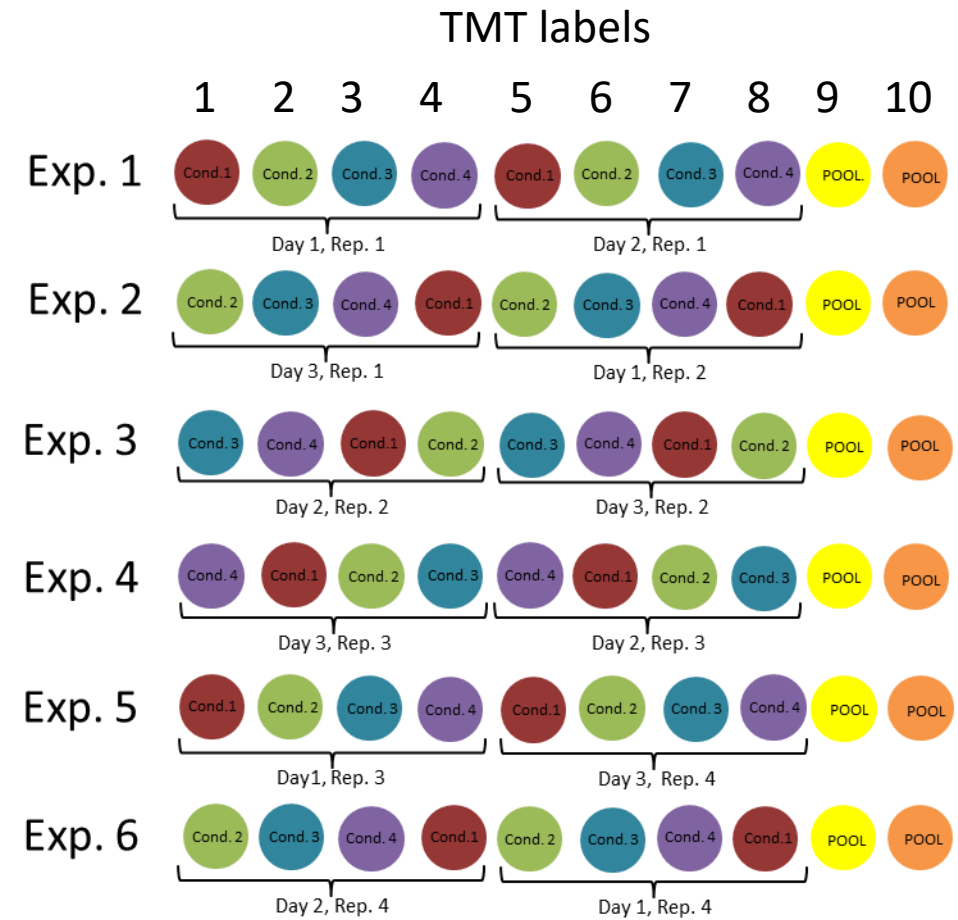


Cominetti et al., *J. Proteome Res.*, 2016, 15 (2), 389–399

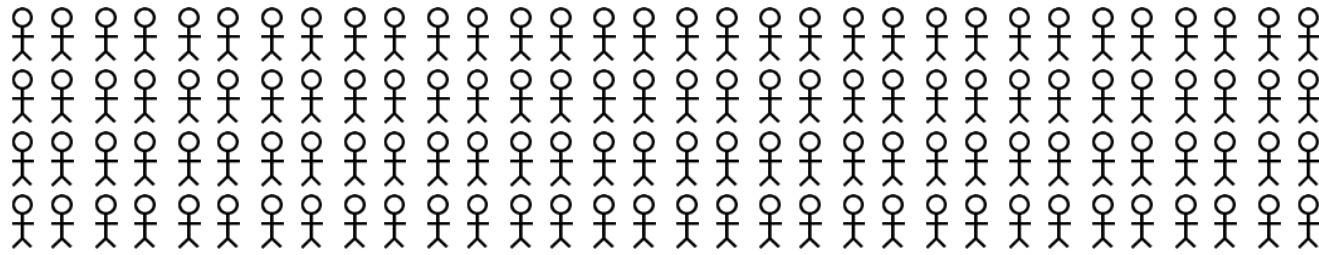
# Some sources of variability

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

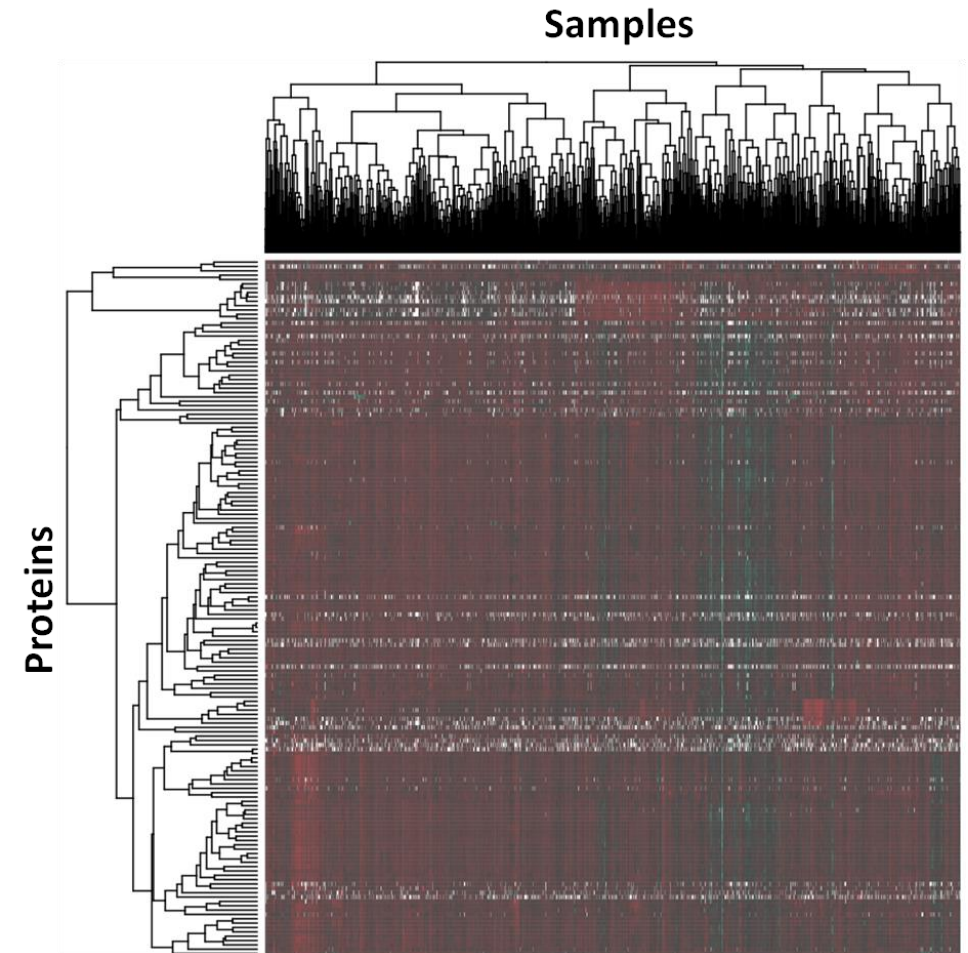
- To well choose your proteomic workflow
- To consider the influence of a change of operator
- To introduce standard and quality control checks
- To randomize your analyses (e.g., random labeling and MS sequence of analysis)
- ...



# 5.2. Biomarker discovery; Population proteomics



DOI: 10.1186/gm364



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

# Biomarkers and proteins (1)



**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.



Clinical Proteomic Tumor Analysis Consortium (CPTAC)

<https://proteomics.cancer.gov>

<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.

# Biomarker discovery

Phase

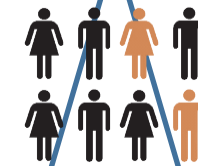
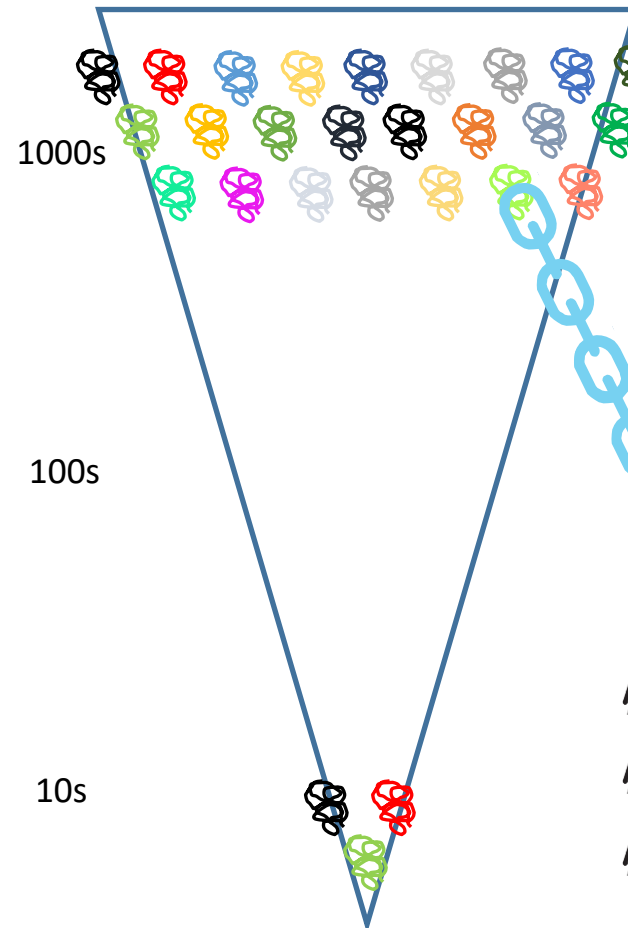
Samples and process

Number of analytes

Number of samples

Initial discovery

Proximal fluids, cell lines,  
tissues  
Shotgun LC MS/MS  
proteomic profiling



Verification

Human plasma  
Targeted LC MS/MS

Validation

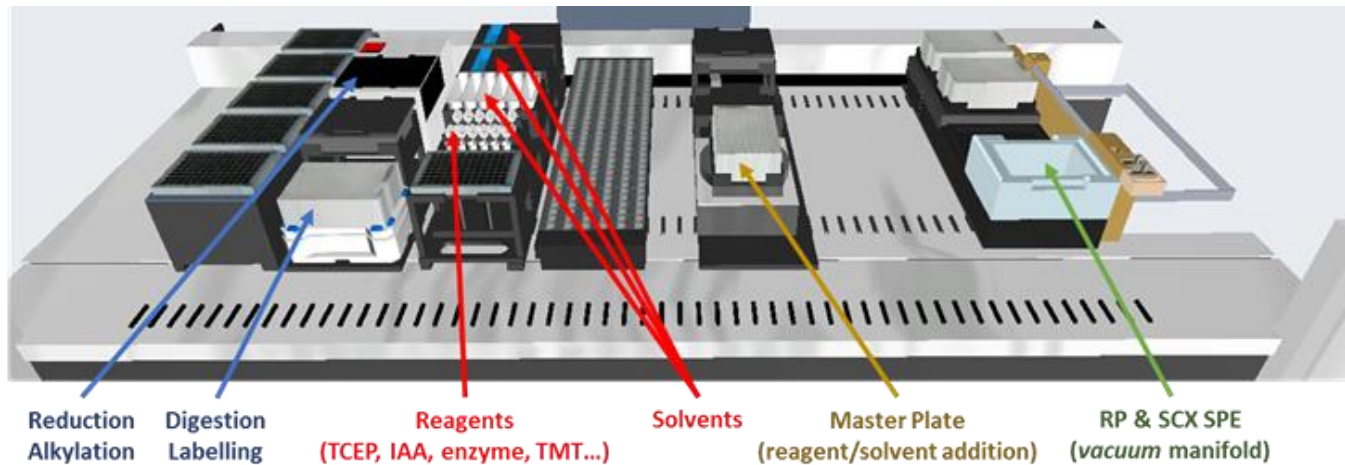
Human plasma  
Immunoassays

# Need of robust and automated workflows

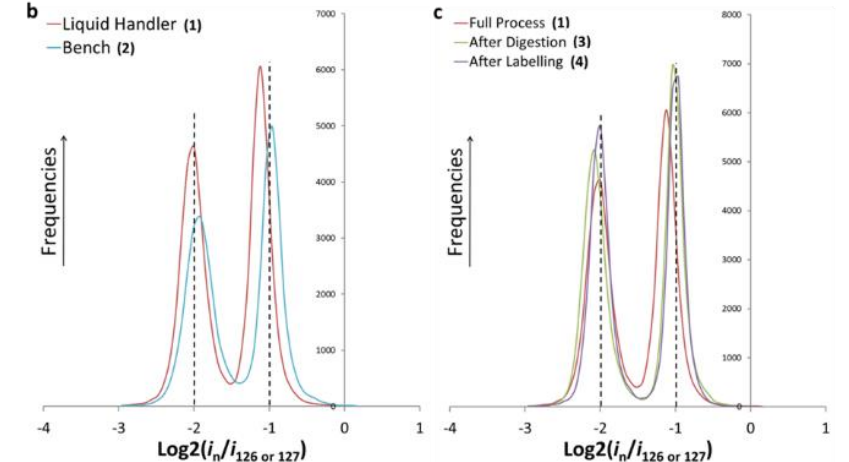
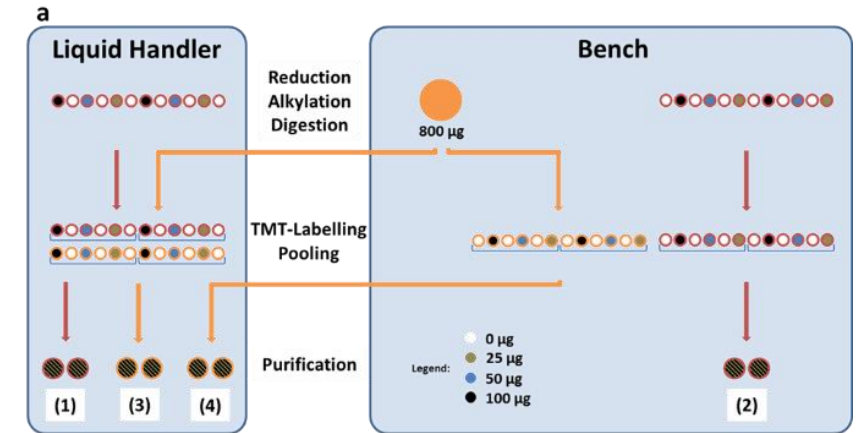
- Reduction/alkylation/digestion
- TMT labeling
- Purification



3D view

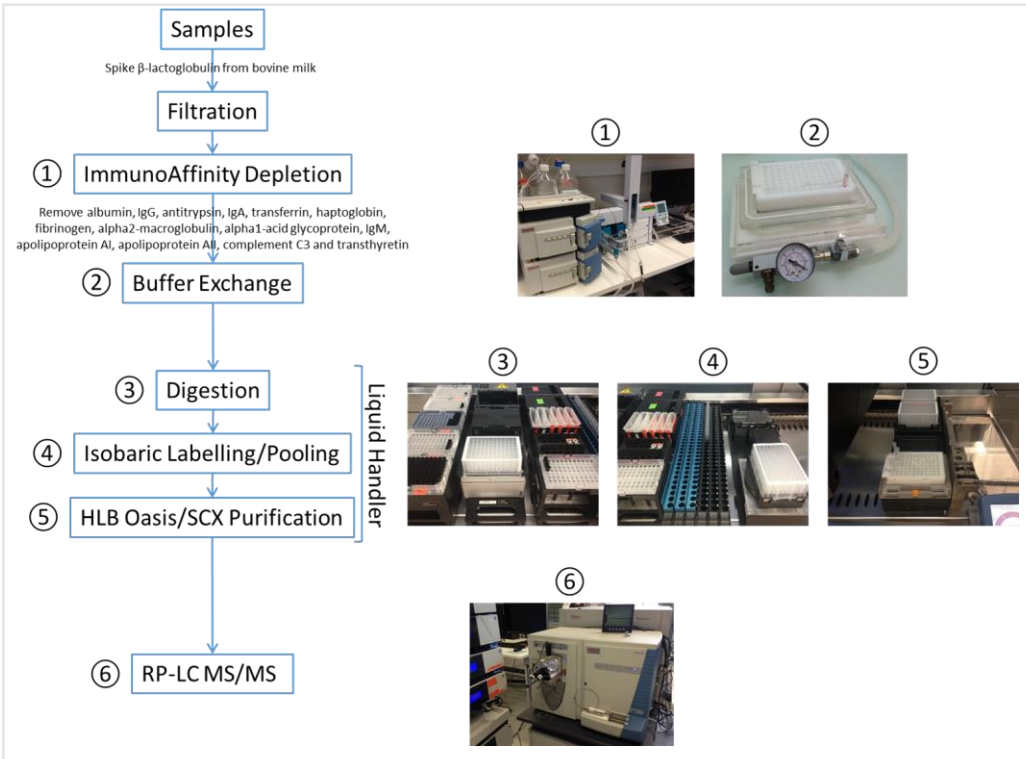


Dayon et al., *Methods Mol. Biol.*, 2017,1619, 433–449

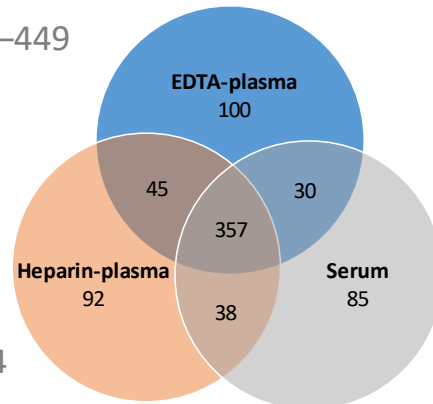


Dayon et al., *J. Proteome Res.*, 2014, 13 (8), 3837–45

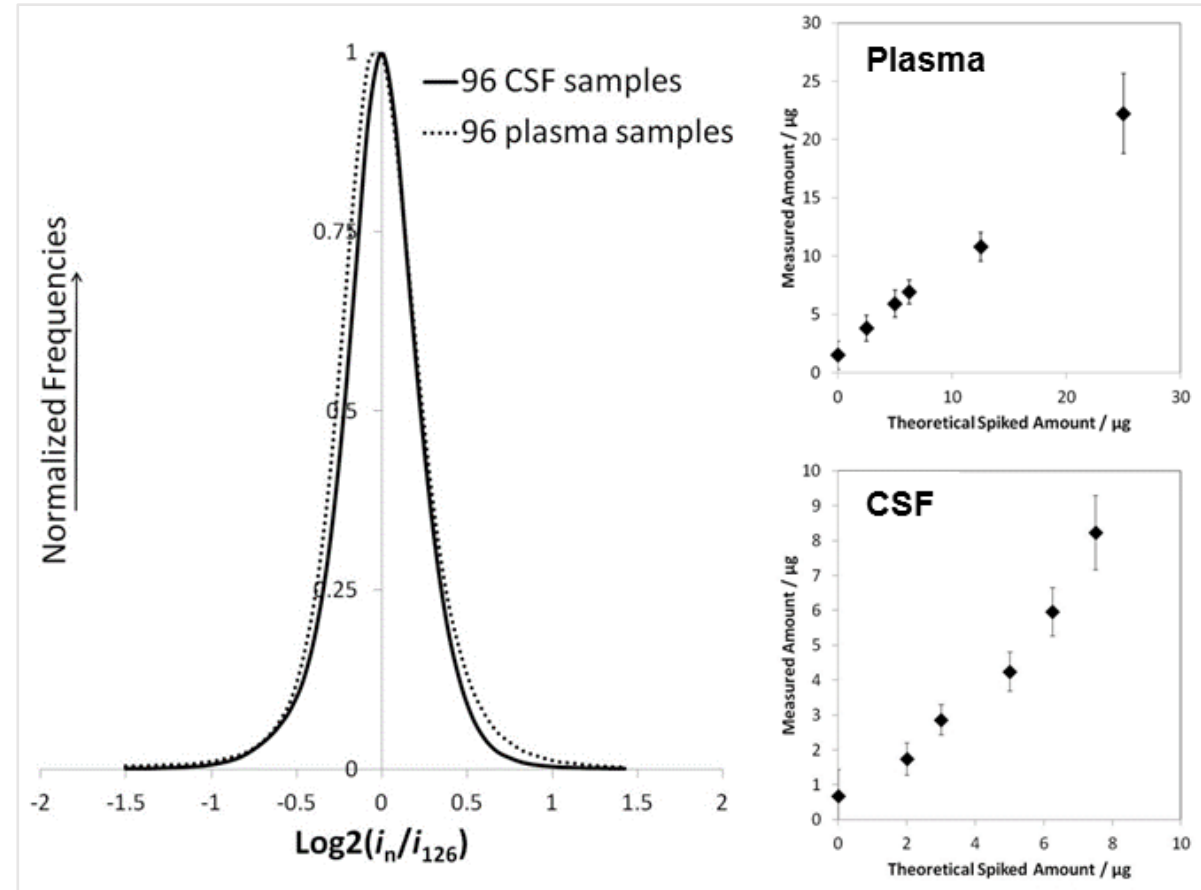
# Analysis of plasma and cerebrospinal fluid



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>



REVIEW



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

Loïc Dayon <sup>a,b</sup>, Ornella Cominetti <sup>a</sup> and Michael Affolter <sup>a</sup>

<sup>a</sup>Bioanalytics Department, Proteomics, Nestlé Institute of Food Safety & Analytical Sciences, Nestlé Research, Lausanne, Switzerland; <sup>b</sup>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.

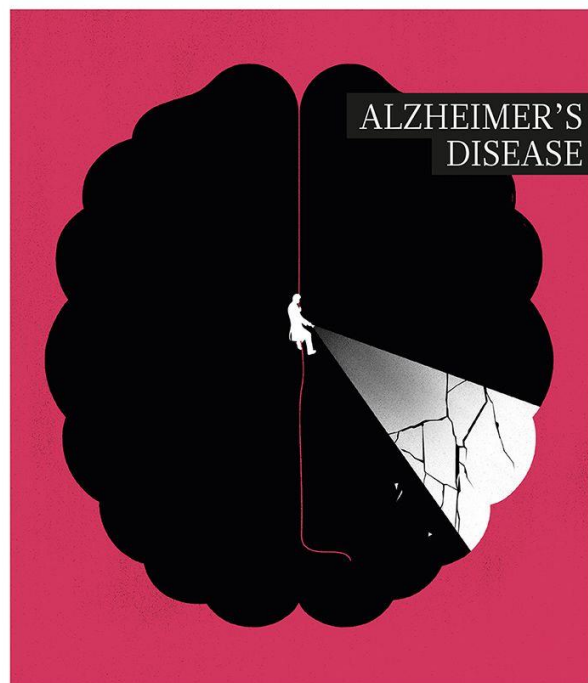
The identification of novel biomarkers with proven clinical utility classically relies on a complex process composed of discovery, qualification, verification, and validation steps (Figure 1). Proteomic biomarker discovery has provided numerous candidates, but only few of those have reached the validation 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.

# Alzheimer disease and biomarker needs

natureOUTLOOK



Produced with support from:



Produced with support of a grant from:



Exploring the depths of dementia

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 ( $A\beta$  load)



- CSF detection of  $A\beta$  peptide, tau and phosphorylated tau proteins

- ELISA
- Luminex



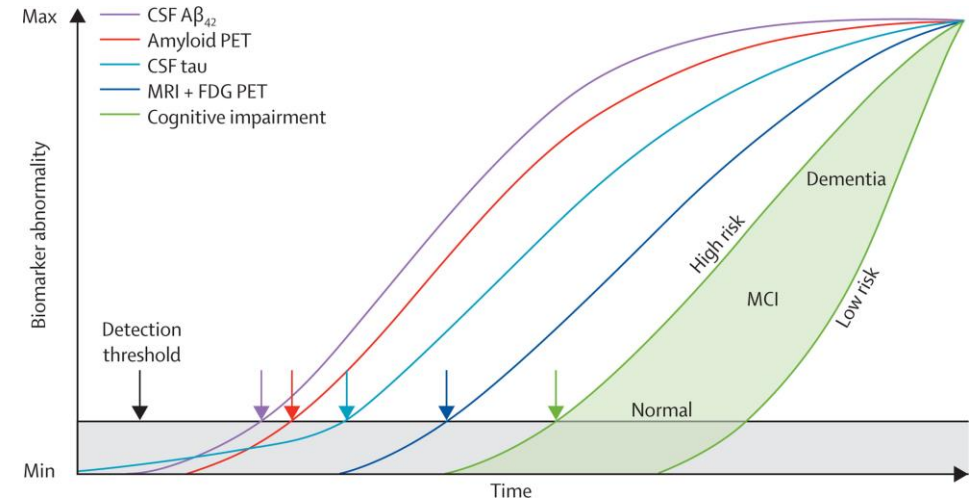
Nature | Outlook



Alzheimer's disease is getting easier to spot

Confirming a diagnosis of the condition used to be possible only after the patient's...

Elie Dolgin



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

# AD and Nutrition

- Still **no drug** can slow down the progression of AD
- **Preventive** 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**



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

Nature | Outlook

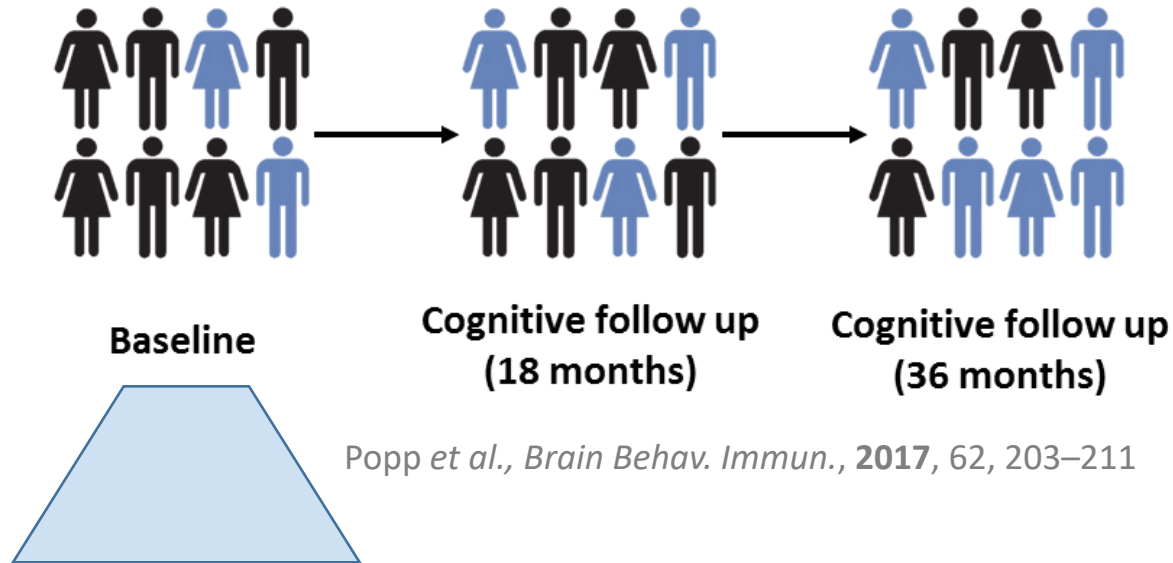


## How the evidence stacks up for preventing Alzheimer's disease

Scepticism towards the idea that lifestyle choices can reduce the risk of dementia is waning.

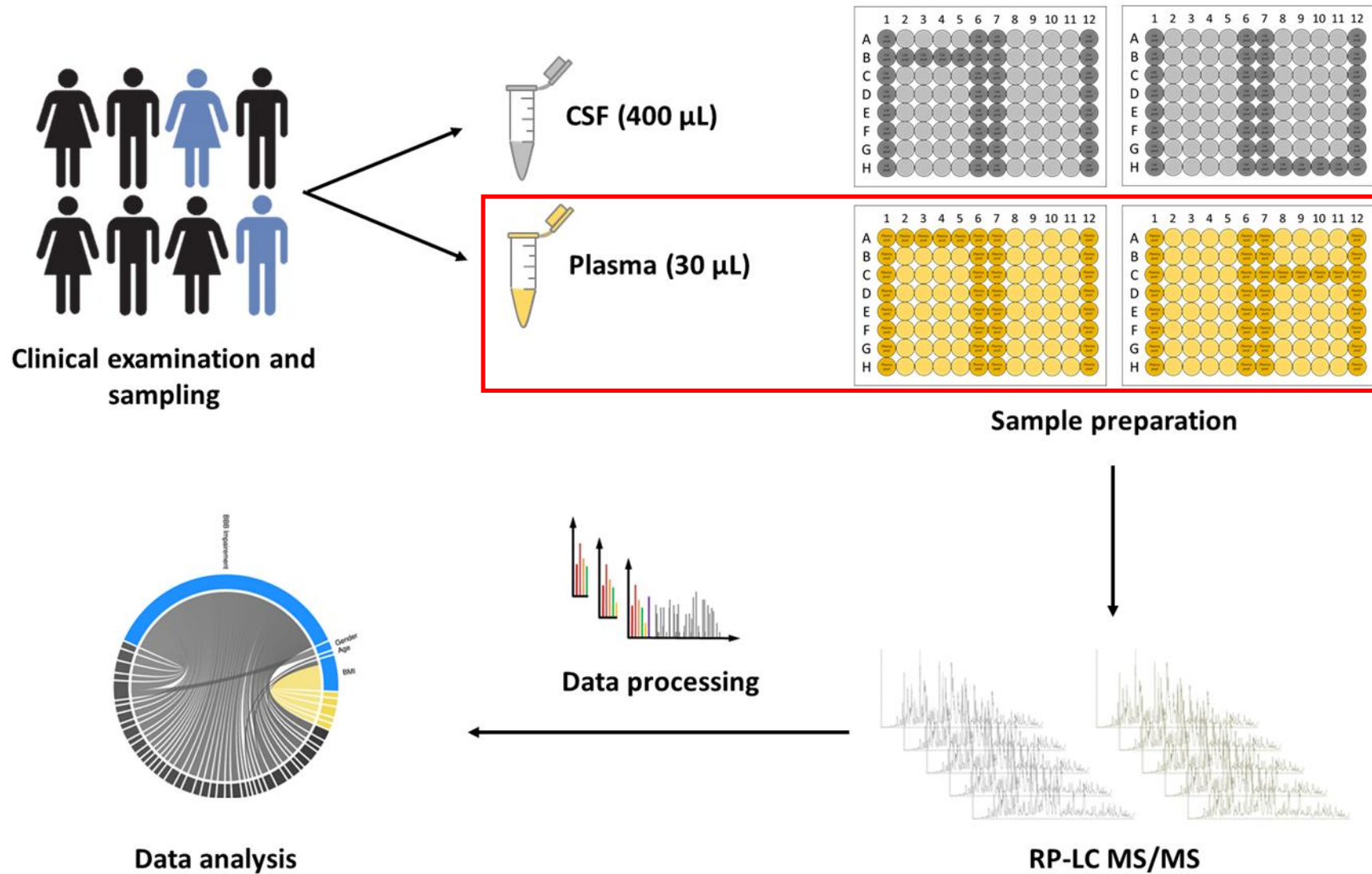
Emily Sohn

# An example of a study in brain health research



	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%)*

# Study design

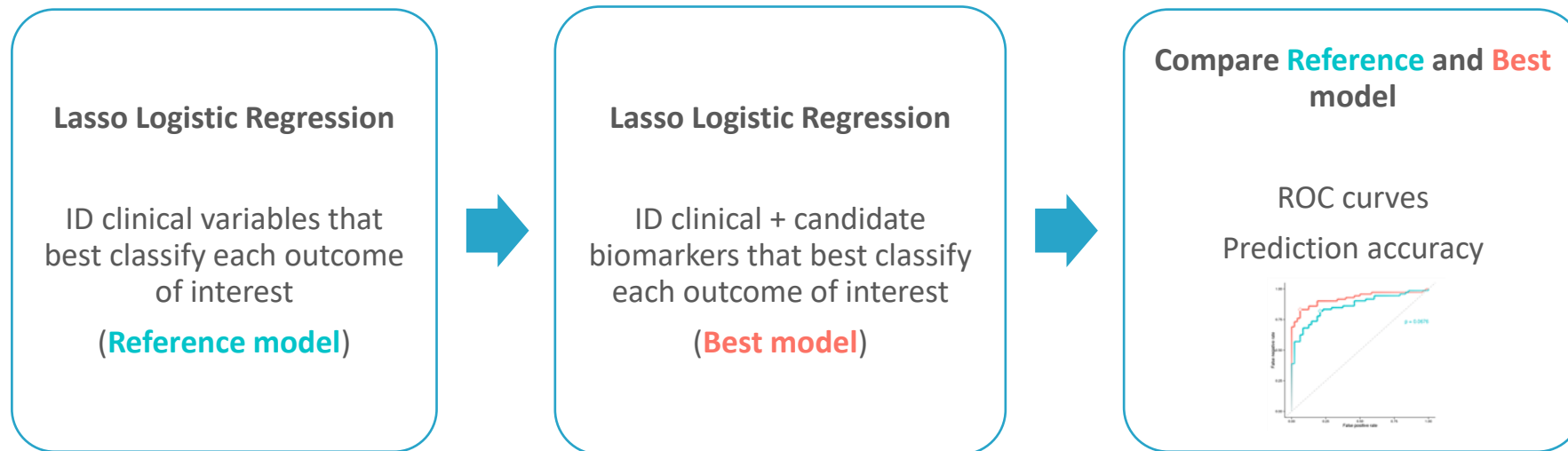


# Rationale and methodology

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

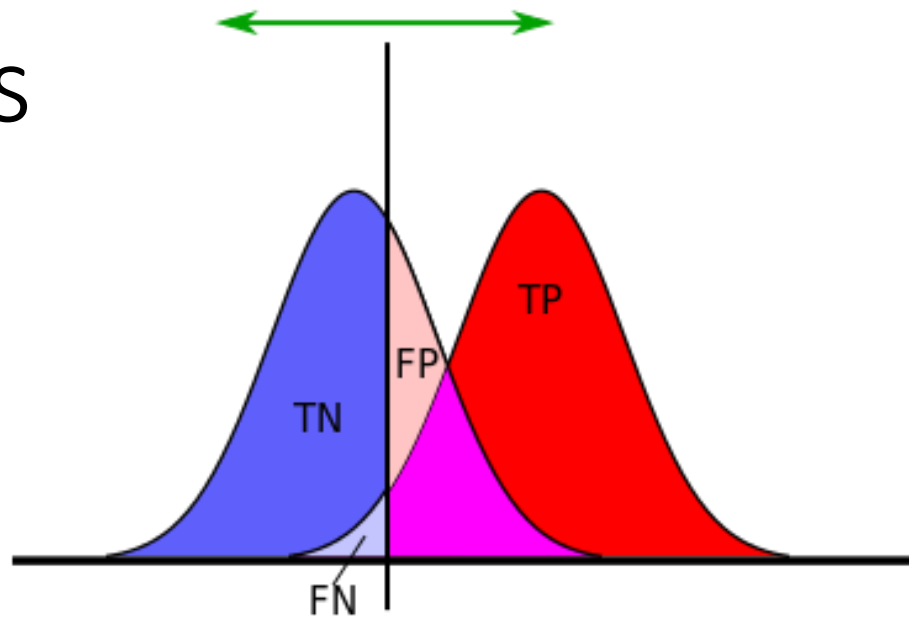
Endophenotype approach to define preclinical AD and cerebral amyloidosis based on well-accepted **CSF P-tau 181** and **A $\beta$ 1-42**

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



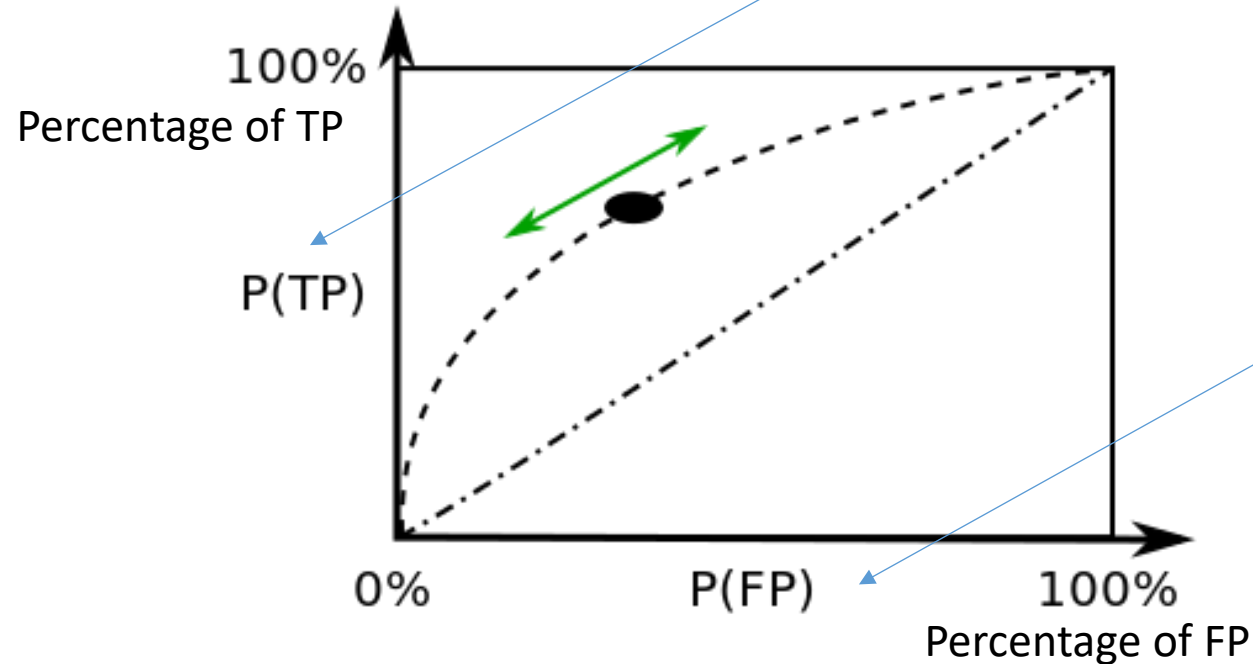
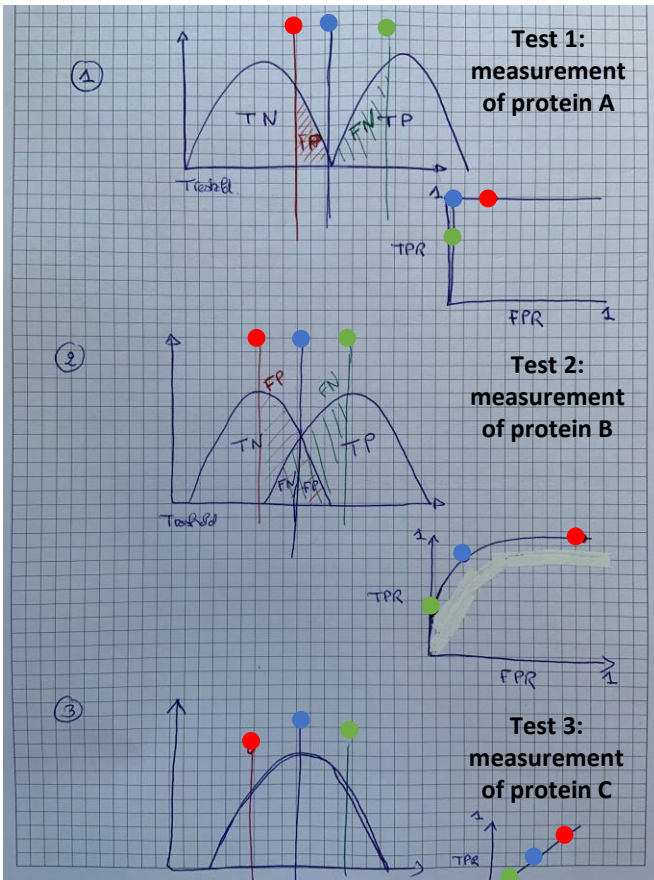
# ROC curves

Cases	Controls
TP	FP
FN	TN



$$\text{Sensitivity} = \frac{TP}{\text{Cases}} = \frac{TP}{TP+FN}$$

$$\text{Specificity} = \frac{TN}{\text{Controls}} = \frac{TN}{TN+FP}$$

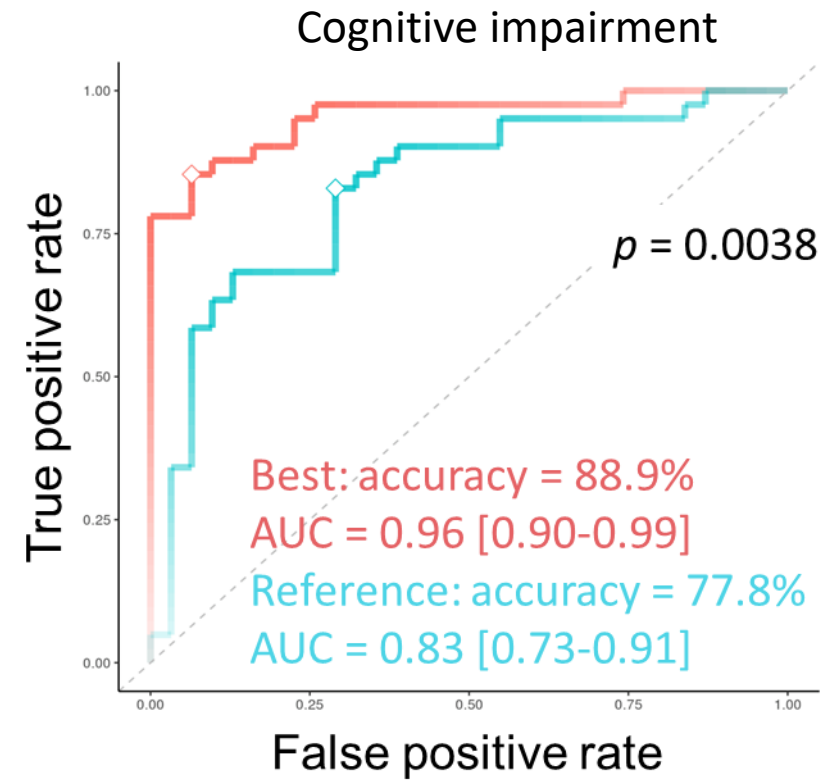
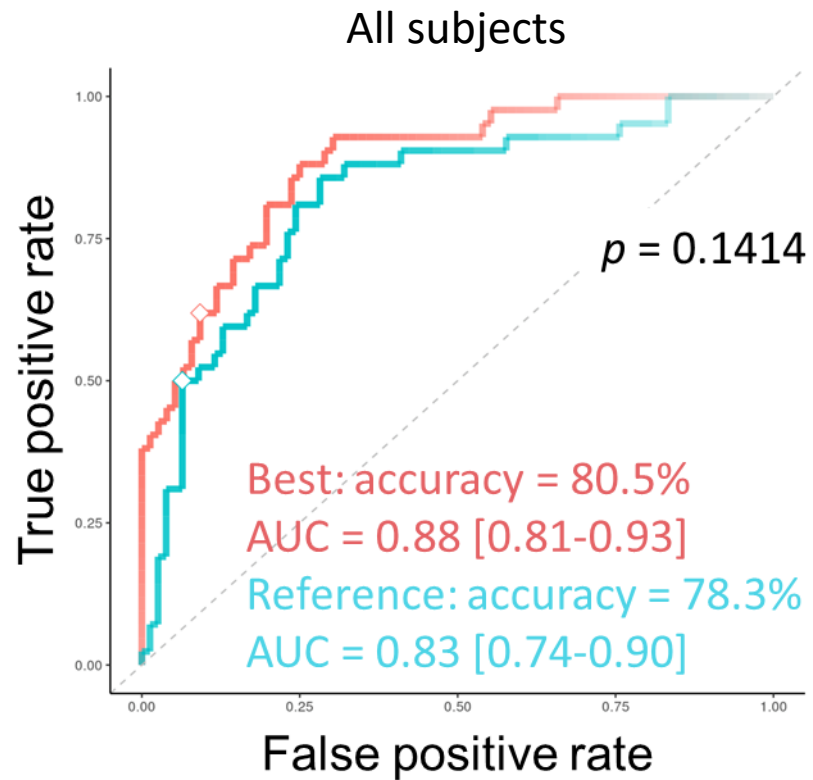


$$\text{FPR} = 1 - \text{specificity}$$

$$\text{FPR} = \frac{FP}{\text{Controls}} = \frac{FP}{TN+FP}$$

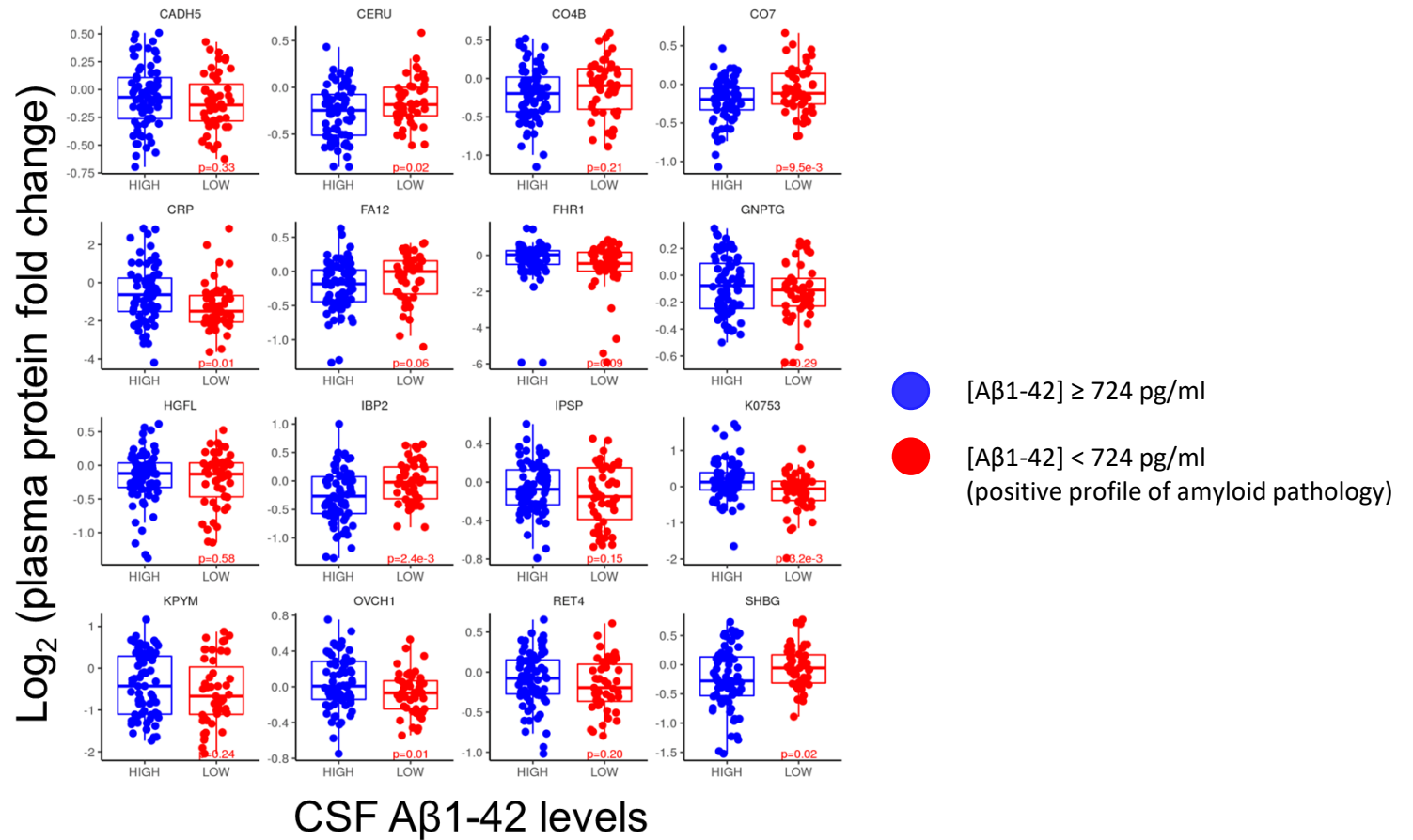
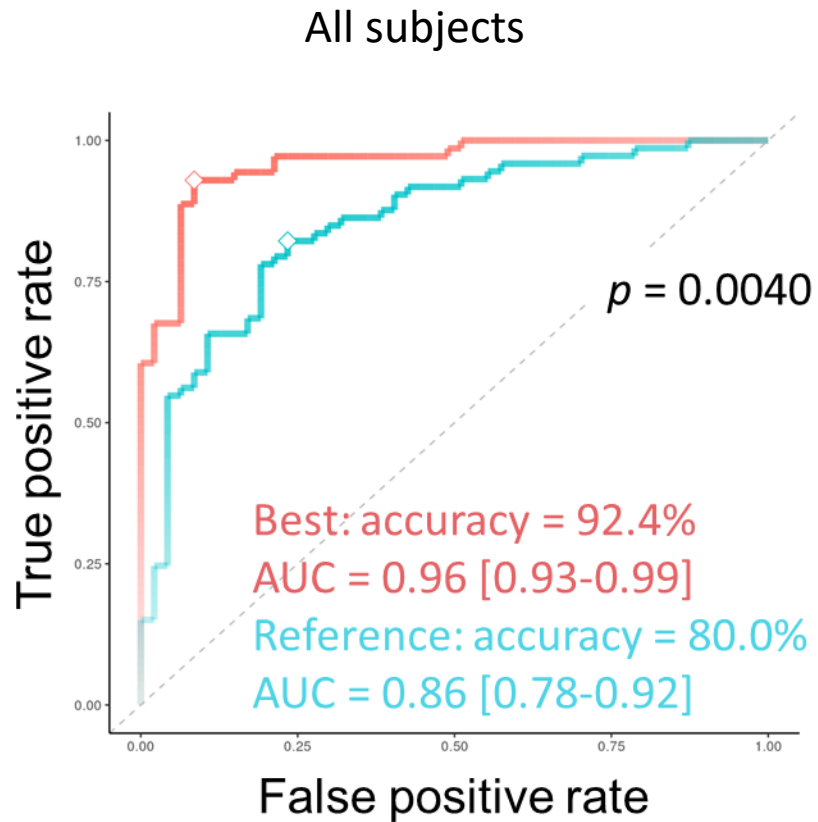
# Plasma proteomic profiles of AD

P-tau 181/A $\beta$ 1-42 > 0.0779 (positive AD profile)



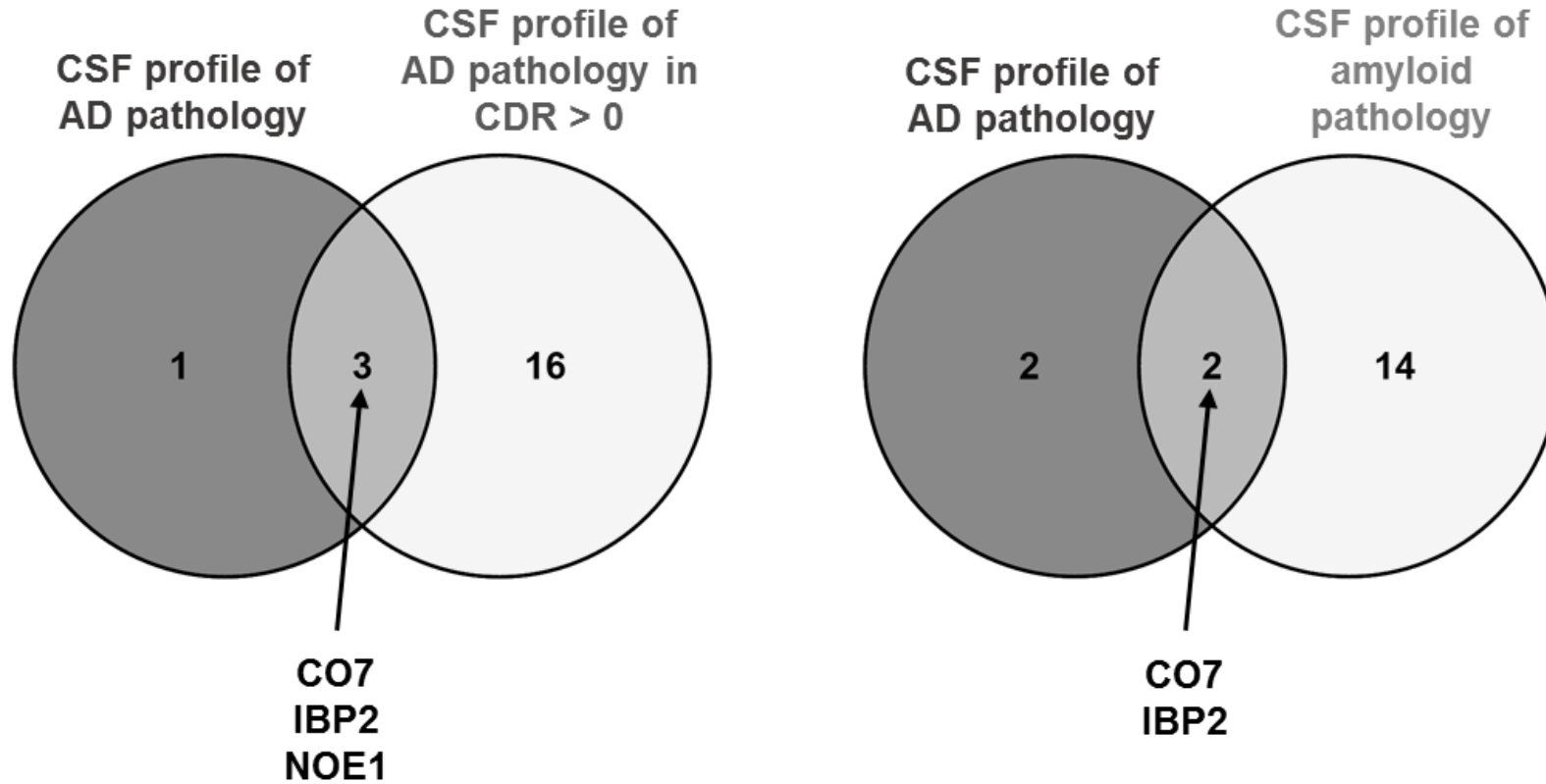
Dayon *et al.*, *J. Alzheimers Dis.*, **2017**, 60(4);1641–1652

# Plasma proteomic profiles of amyloid pathology



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

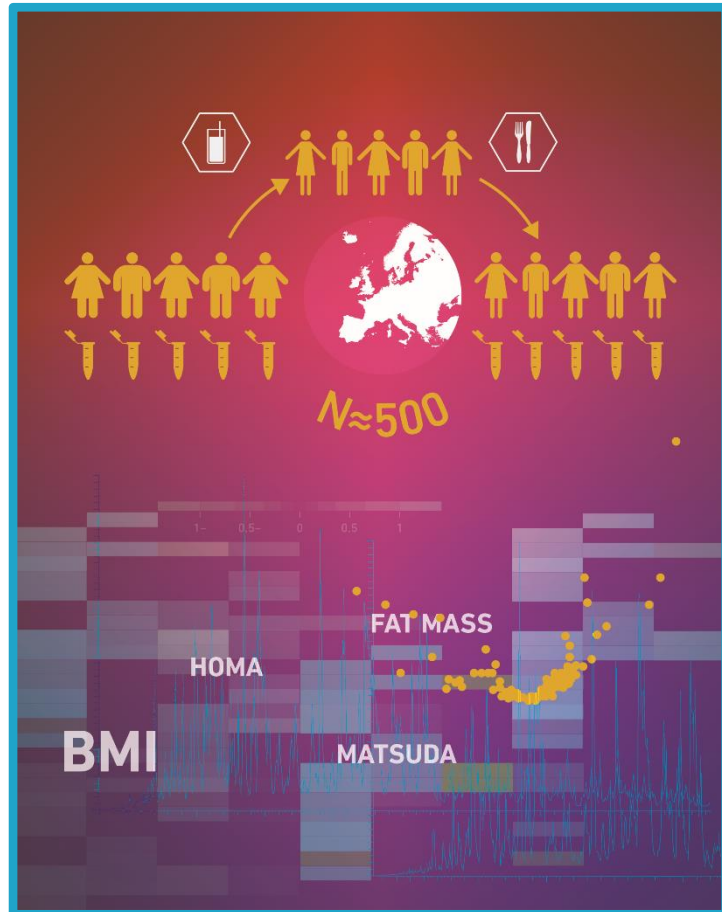
# Summary



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<sup>1,5\*\*</sup> , Ornella Cominetti<sup>1\*\*</sup>, Antonio Núñez Galindo<sup>1</sup>, Irina Irincheeva<sup>2</sup>, John Corthésy<sup>1</sup>, Arne Astrup<sup>3</sup>, Wim H.M. Saris<sup>4</sup>, Jörg Hager<sup>2</sup>, Martin Kussmann<sup>1\*</sup> and Loïc Dayon<sup>1</sup> 

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<sup>3</sup> Department of Nutrition, Exercise and Sports, Faculty of Science, University of Copenhagen, Copenhagen, Denmark

<sup>4</sup> Department of Human Biology, NUTRIM, School for Nutrition, Toxicology and Metabolism, Maastricht University Medical Centre, Maastricht, The Netherlands

<sup>5</sup> 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.

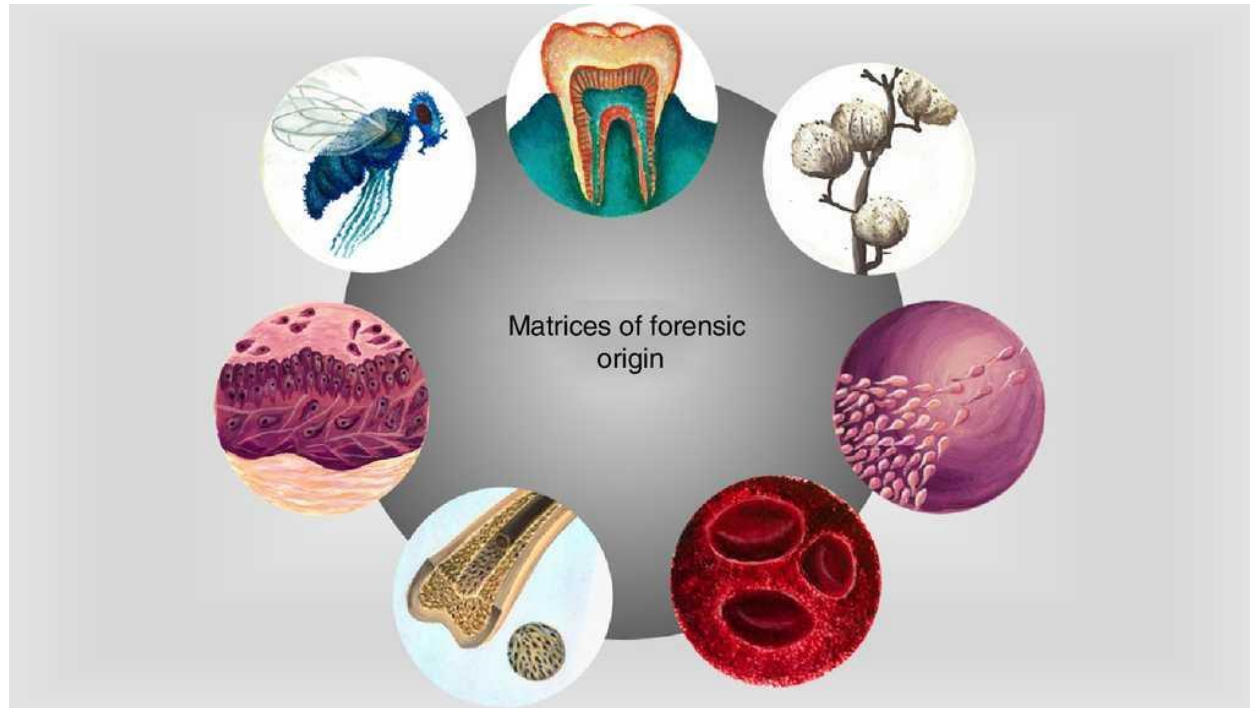
**Conclusion and clinical relevance:** MS-based proteomic analysis of a large cohort of non-diabetic overweight and obese individuals concomitantly identified known and novel proteins associated with weight loss and maintenance.

Received: October 27, 2016

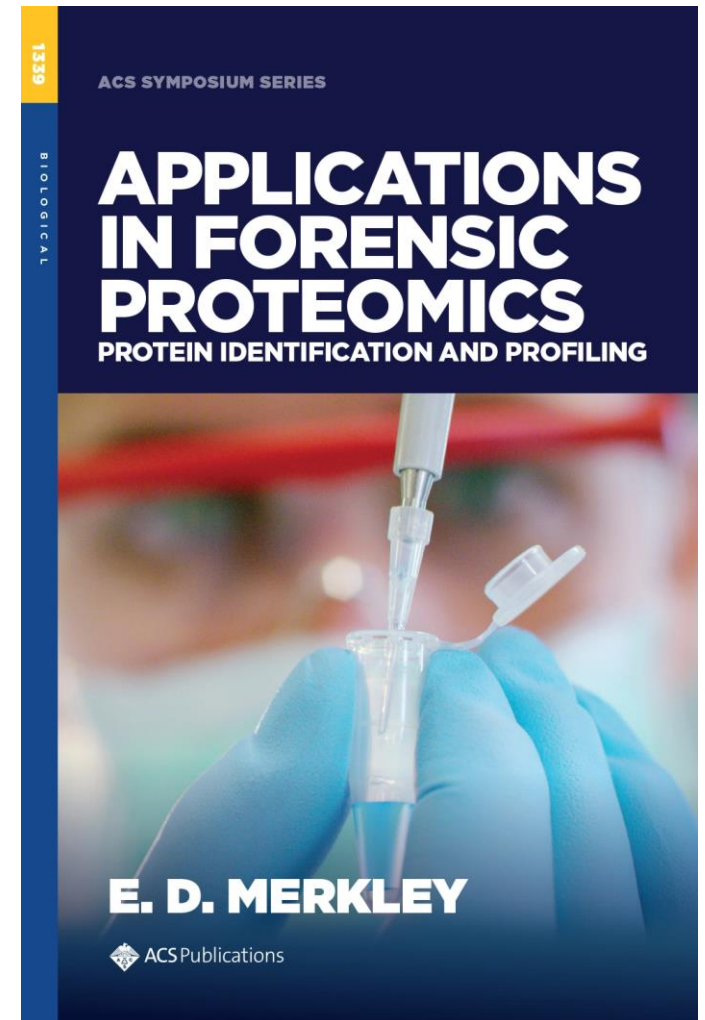
Revised: March 1, 2017

Accepted: March 27, 2017

## 5.3. Forensics

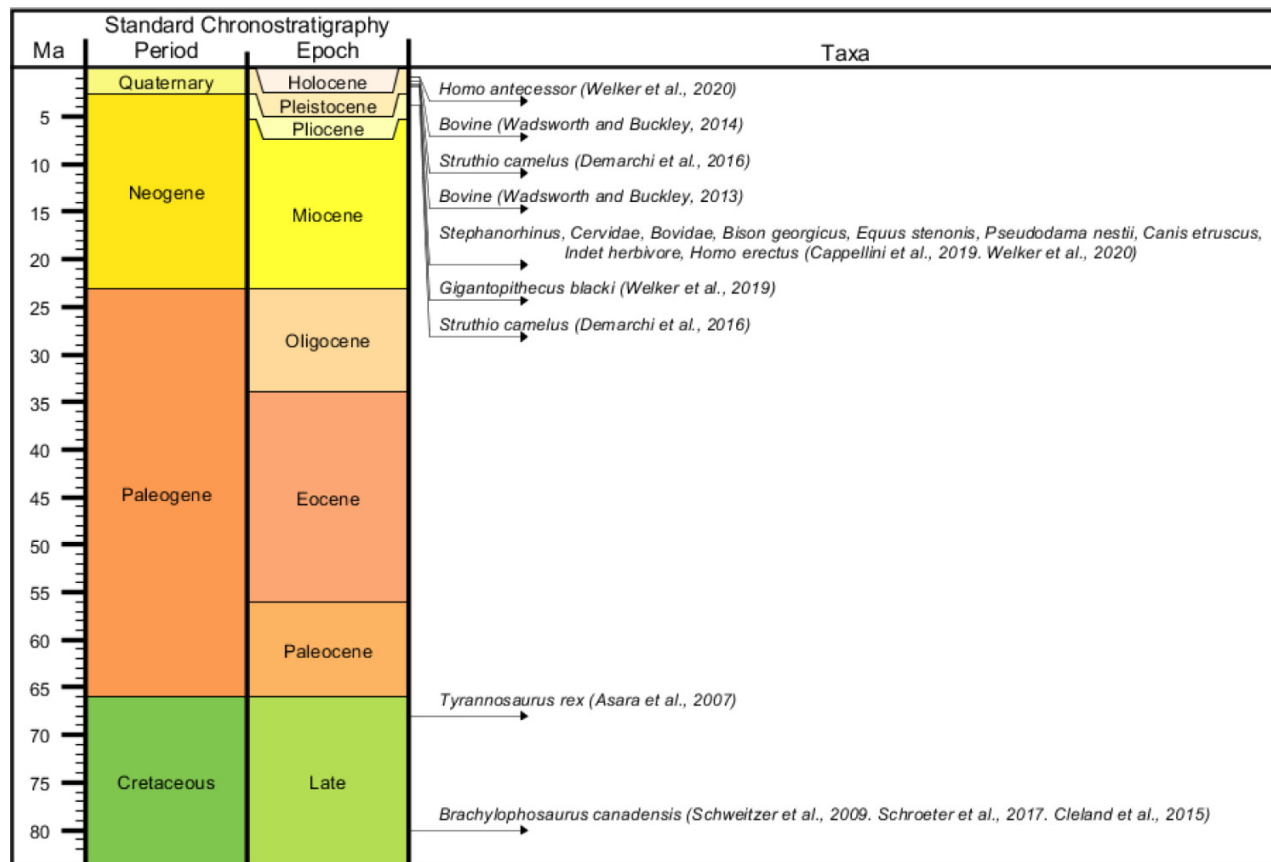


Spanish Journal of Legal Medicine. 2019;45:114–22



DOI: 10.1021/bk-2019-1339

# Proteins are more stable than DNA



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

ACS  
central  
science

In collaboration with C&EN

## Proteomics Offers New Clues for Forensic Investigations

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

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Article Recommendations

Carolyn Wilke

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



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

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

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

© 2021 American Chemical Society 1595

https://doi.org/10.1021/acscentsci.1c01232  
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**

# 5.4. Targeted mass spectrometry-based approaches



METHOD OF THE YEAR

NEWS FEATURE | SPECIAL FEATURE

## Targeted proteomics

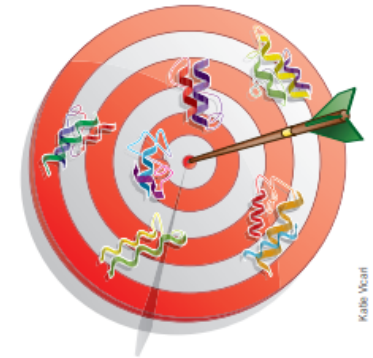
Analysis of a preselected group of proteins delivers more precise, quantitative, sensitive data to more biologists. Vivien Marx reports.

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



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

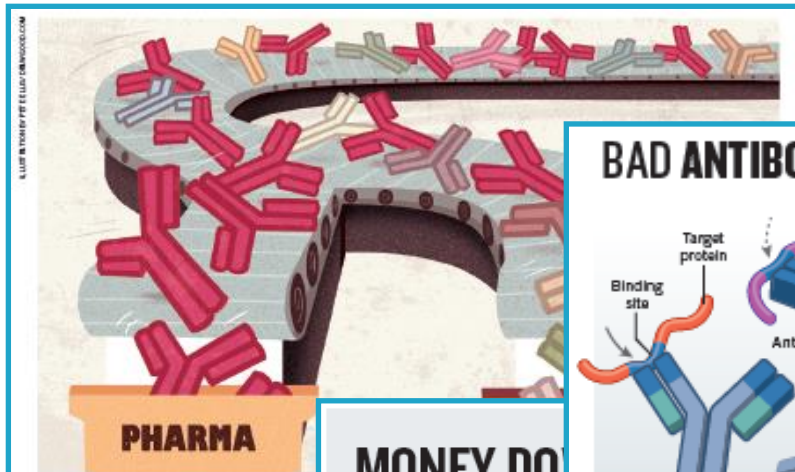
are not inherently the same,” says Ruedi Aebersold, from the Institute of Molecular Systems Biology at the Swiss Federal Institute of Technology in Zurich. Neither person is necessarily wrong: the contradiction stems from their measurement of different subsets of the whole proteome,

© 2013 Nature America, Inc. All rights reserved.



Ruedi Aebersold hopes many laboratories will adopt targeted proteomics.

# Why targeted MS?



PHARMA

Standard used

To save millions of dollars and do reagents must be defined by the say Andrew Bradbury,

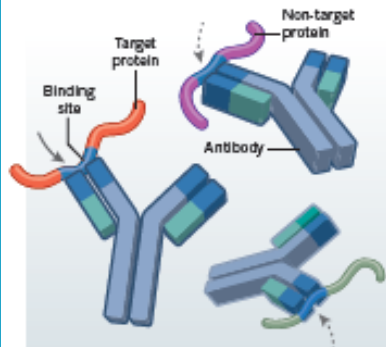
Central to reproducibility in biomedical research is being able to use reagents that are identical to those described in publications. Alarming, there are serious flaws in the reliability of antibodies, the most widely used class of protein-binding reagent.  
In the body, antibodies help to fight pathogens. In the lab, biologists have long used them to track proteins of interest because they

MONEY DO

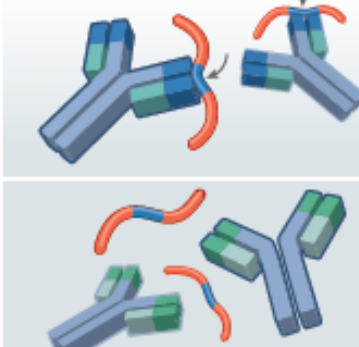
Global spending protein-binding reagents \$1.6 BILLION

## BAD ANTIBODIES

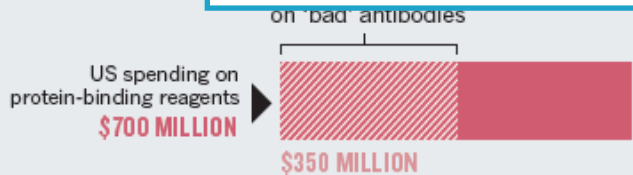
The most common problems with antibodies and how to avoid them.



**CROSS-REACTIVITY**  
Problem: An antibody is supposed to recognize only its target protein, but sometimes binds to others, depending on the proteins present in a sample.  
Solution: An antibody should be tested for off-target binding using positive and negative controls.



**VARIABILITY**  
Problem: Separate batches of antibody can perform differently. This happens most often when the antibody is produced from a new set of animals.  
Solution: Researchers should confirm lot numbers and characterization data with vendors.



Nearly half of all money wasted on 'bad' antibodies worldwide is spent in the United States.

All costs estimates assume that 50% of antibodies are validated and that researchers buy 'bad' antibodies as often as they buy 'good' ones.

NEWS FEATURE

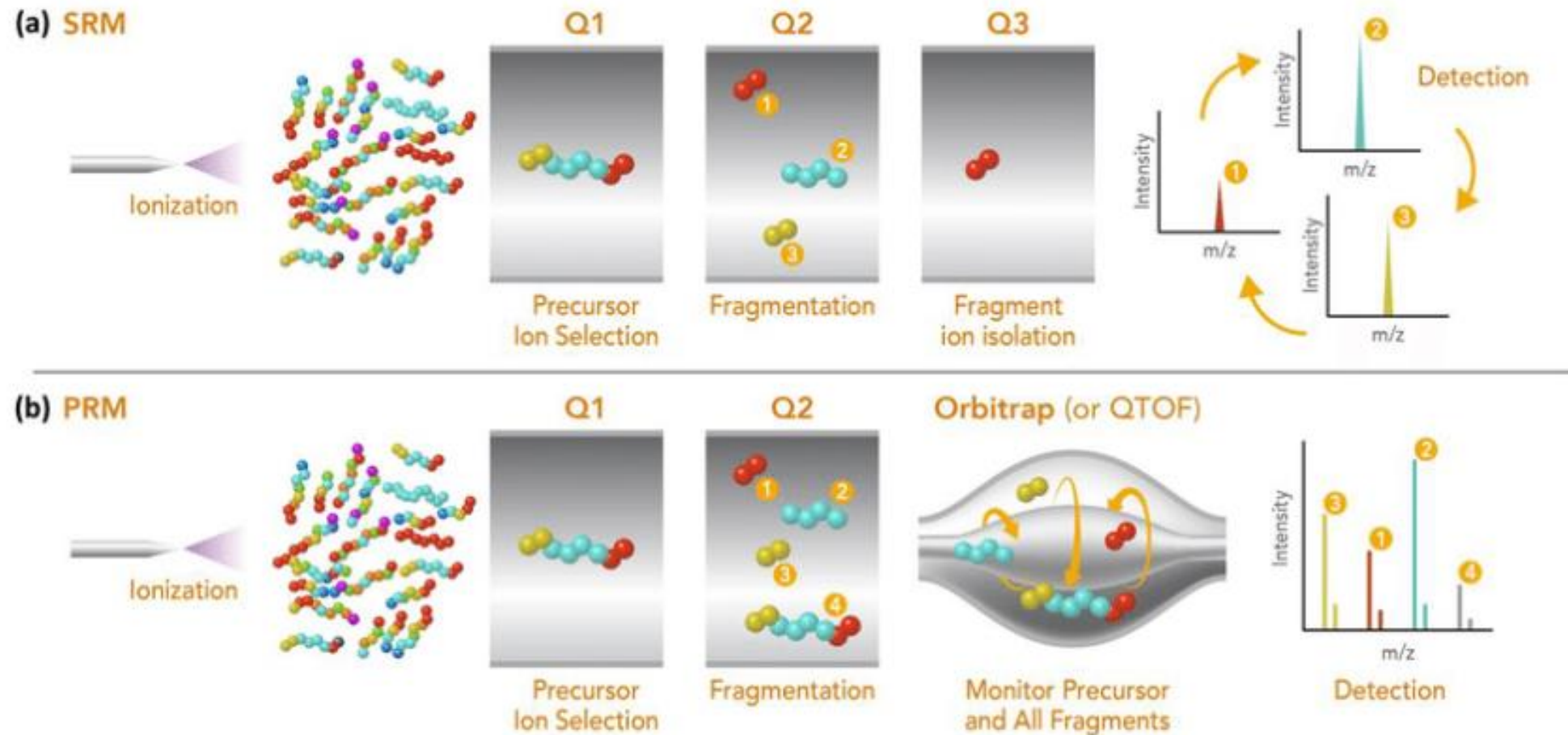
# BLAME IT ON THE ANTIBODIES

Antibodies are the workhorses of biological experiments, but they are littering the field with false findings. A few evangelists are pushing for change.

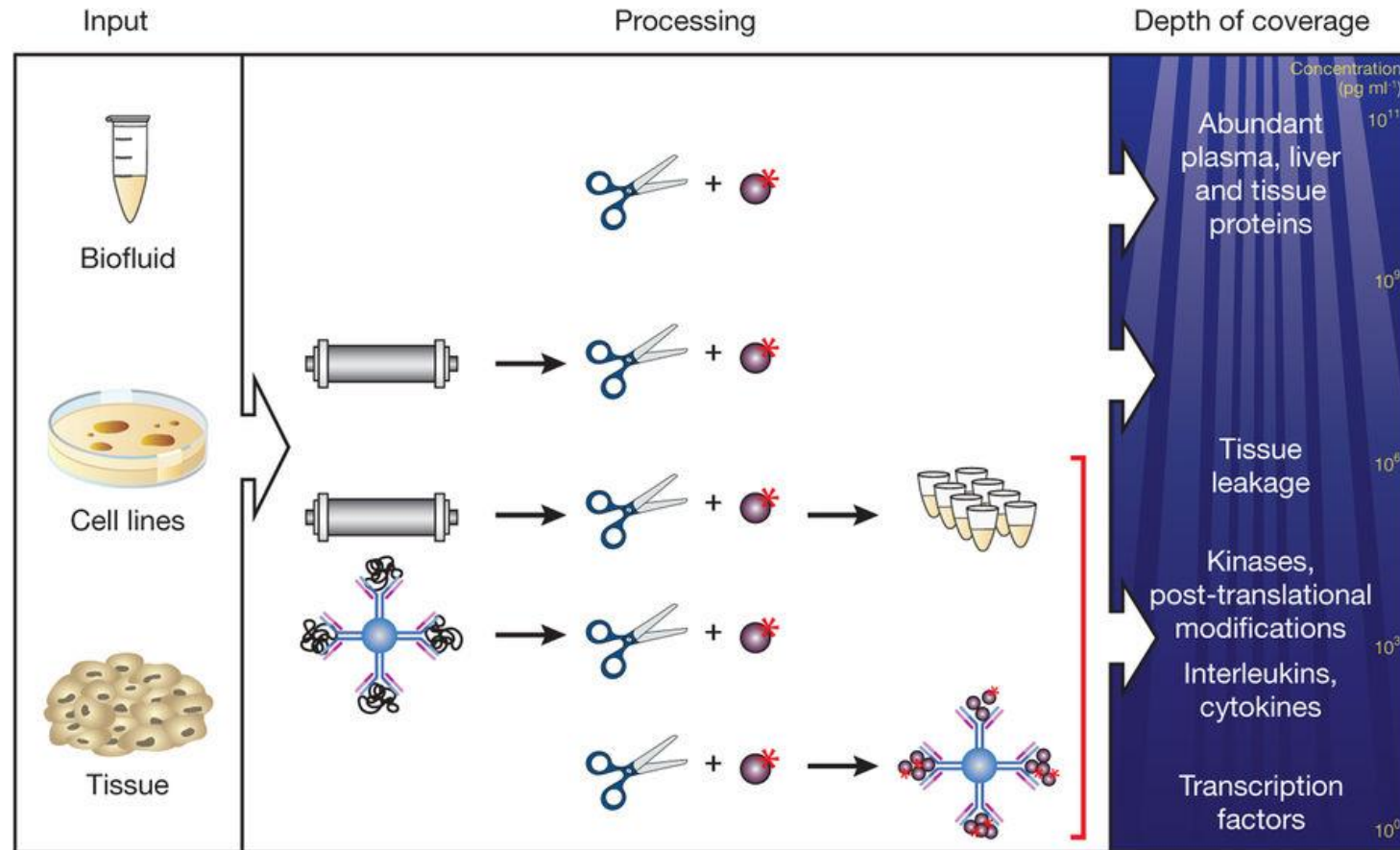
BY MONYA BAKER



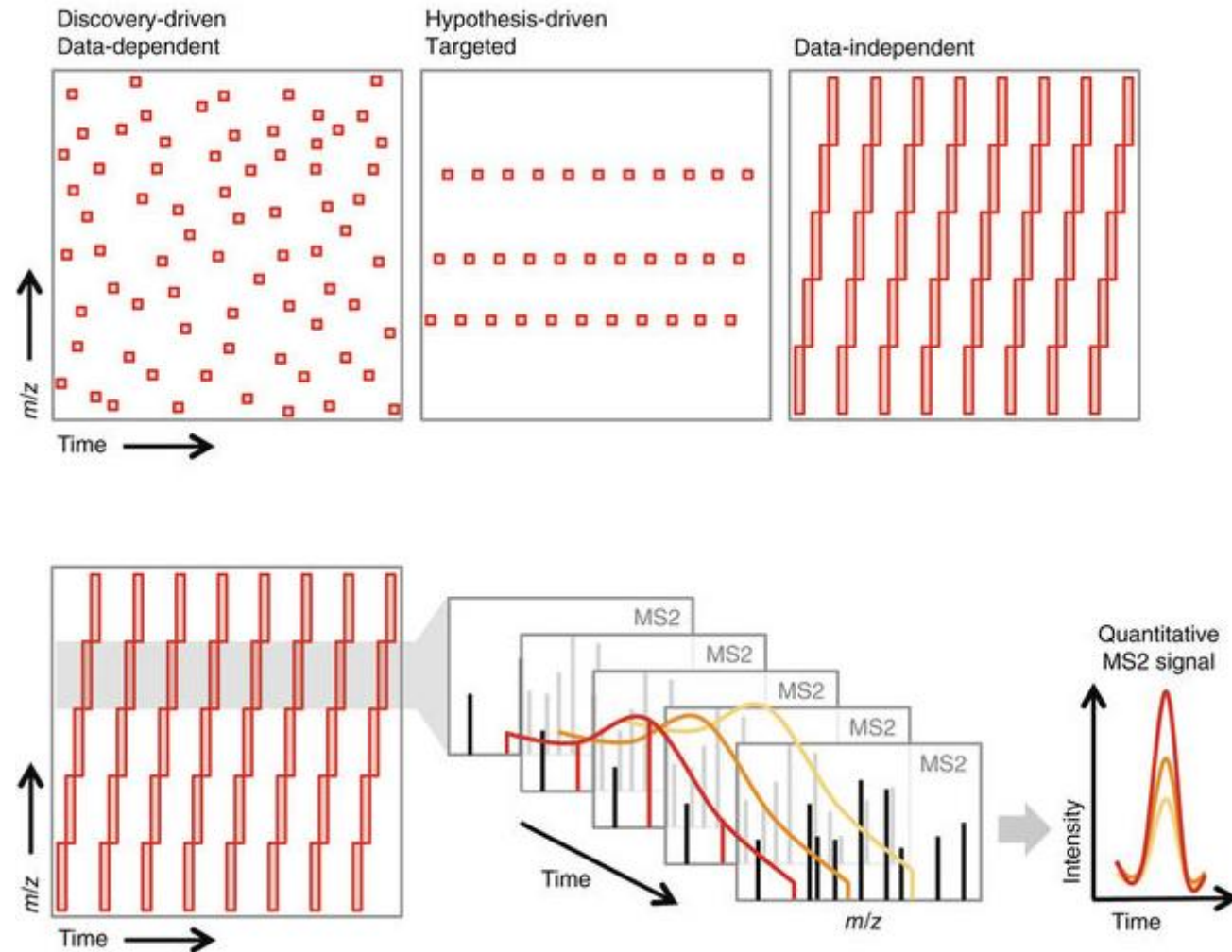
# Selected reaction monitoring (SRM) and parallel reaction monitoring (PRM)



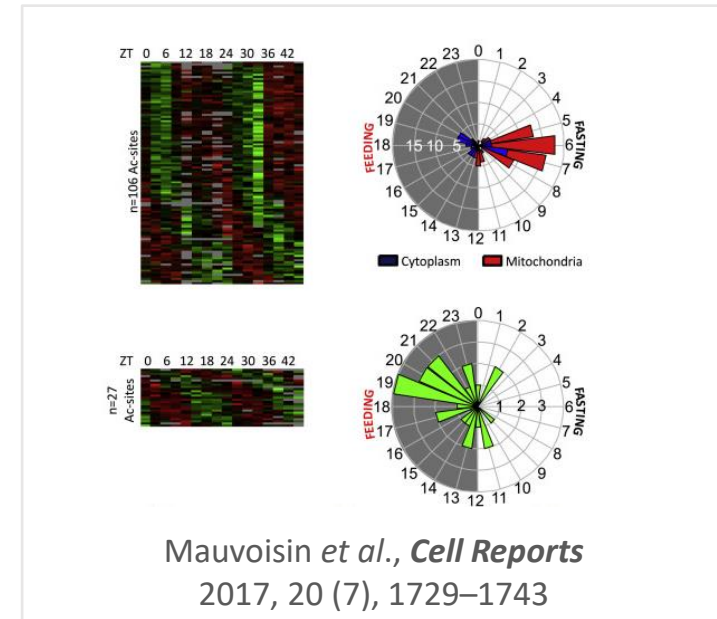
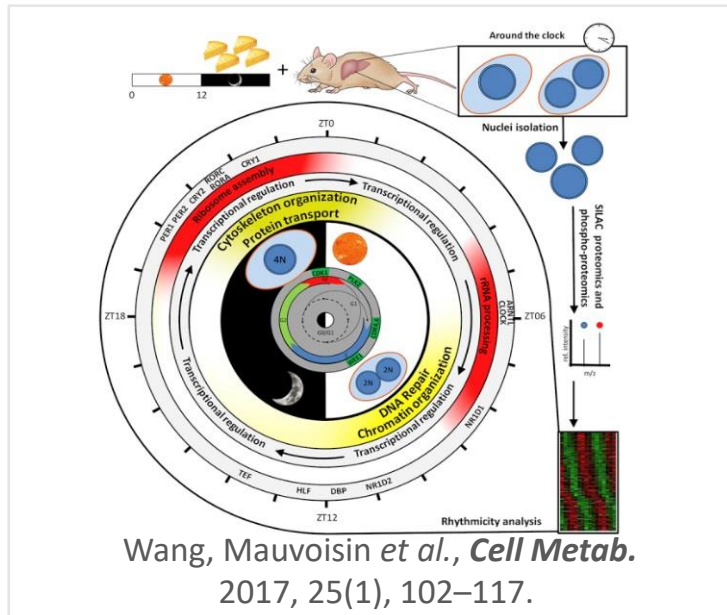
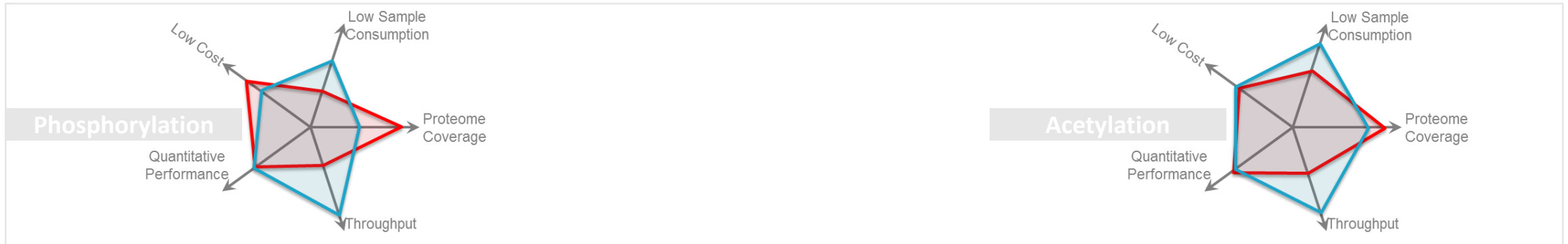
# Enrichment strategies to increase sensitivity and specificity of analyte detection in SID-MRM-MS



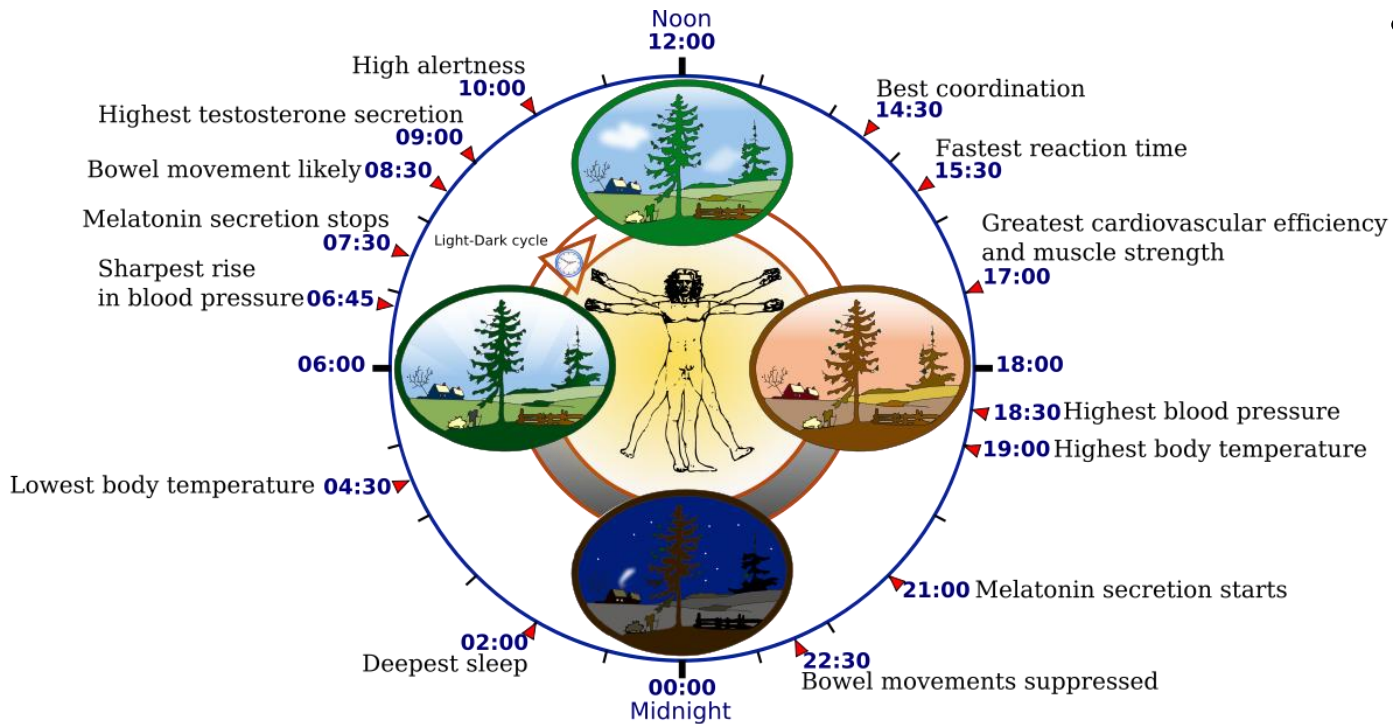
# Untargeted MS approaches with targeted data analysis



# Targeted sample preparation with untargeted MS approaches



# 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.

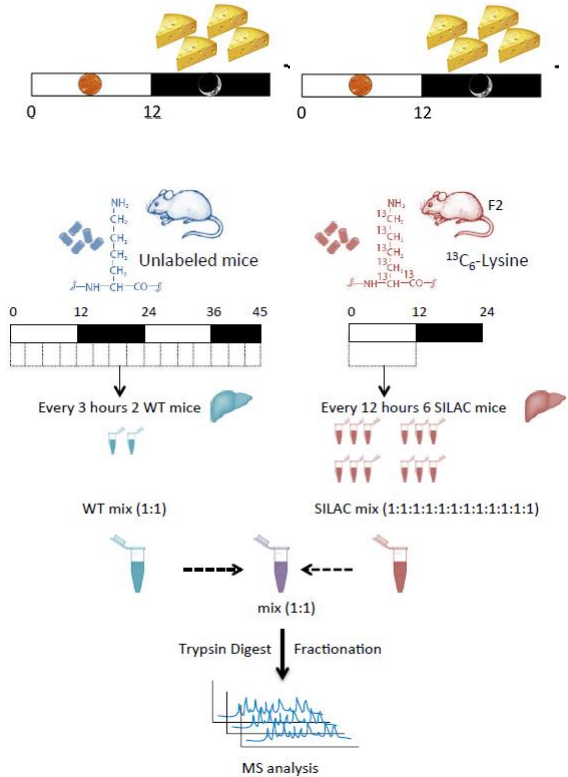
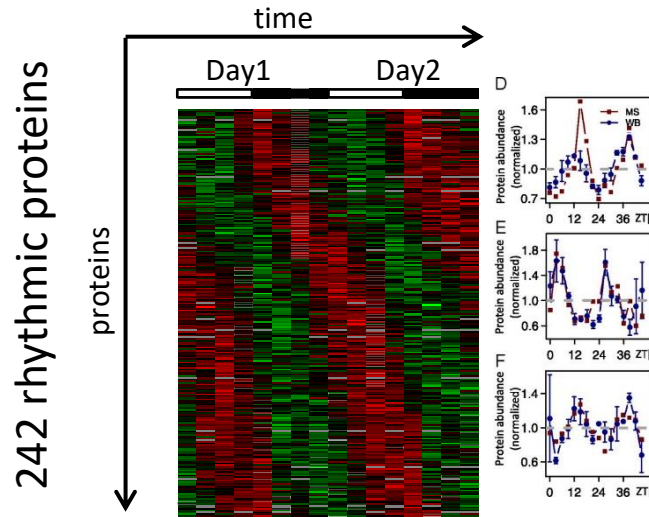
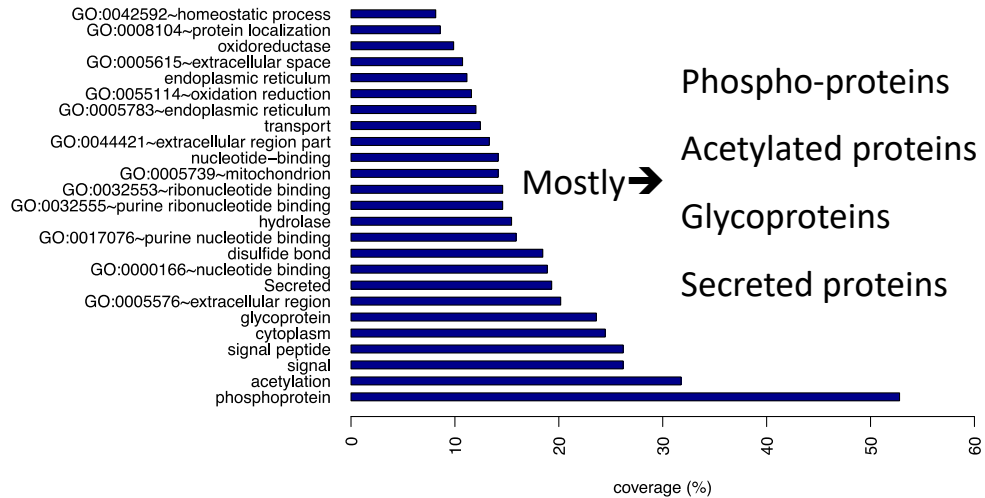
# Full circadian proteome

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

5827 proteins identified

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

Coverage of measured proteins



**Circadian clock-dependent and -independent rhythmic proteomes implement distinct diurnal functions in mouse liver**

Daniel Mauvoisin<sup>a,b,1</sup>, Jingkui Wang<sup>c,1</sup>, Céline Jouffe<sup>a,b</sup>, Eva Martin<sup>a,b</sup>, Florian Atger<sup>a,b</sup>, Patrice Waridel<sup>d</sup>, Manfredo Quadroni<sup>d</sup>, Frédéric Gachon<sup>a,b,1,2</sup>, and Felix Naef<sup>c,1,2</sup>

<sup>a</sup>Department of Pharmacology and Toxicology, University of Lausanne, CH-1005 Lausanne, Switzerland; <sup>b</sup>Diabetes and Circadian Rhythms Department, Nestlé Institute of Health Sciences, CH-1015 Lausanne, Switzerland; <sup>c</sup>The Institute of Bioengineering, School of Life Sciences, Ecole Polytechnique Fédérale de Lausanne and Swiss Institute of Bioinformatics, Lausanne CH-1015, Switzerland; and <sup>d</sup>Protein Analysis Facility, University of Lausanne, CH-1015 Lausanne, Switzerland

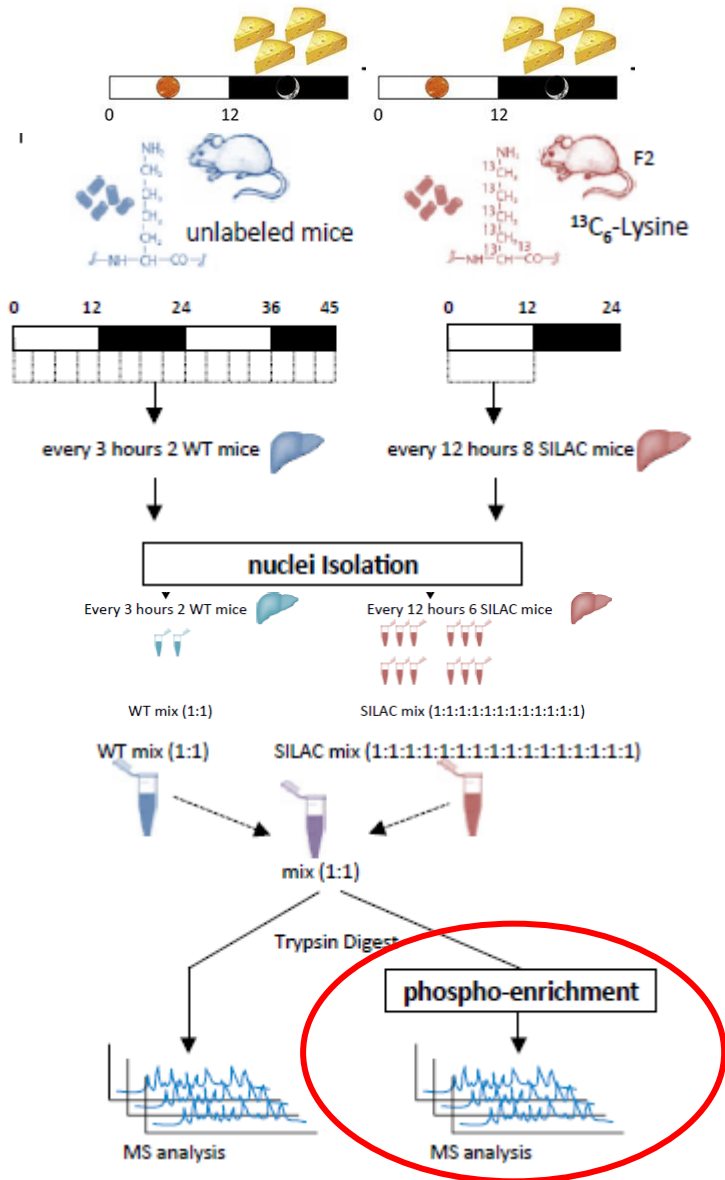
DOI: 10.1073/pnas.1314066111

# Circadian phosphoproteome

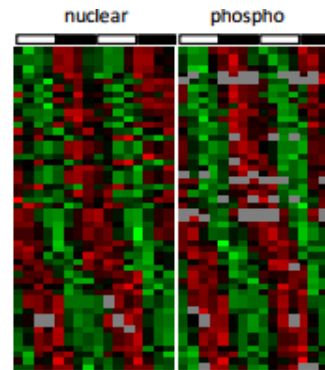
Almost 5000 phosphopeptides identified

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

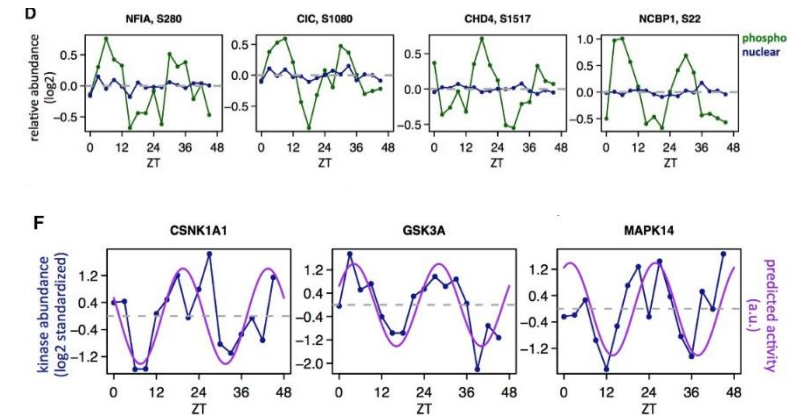
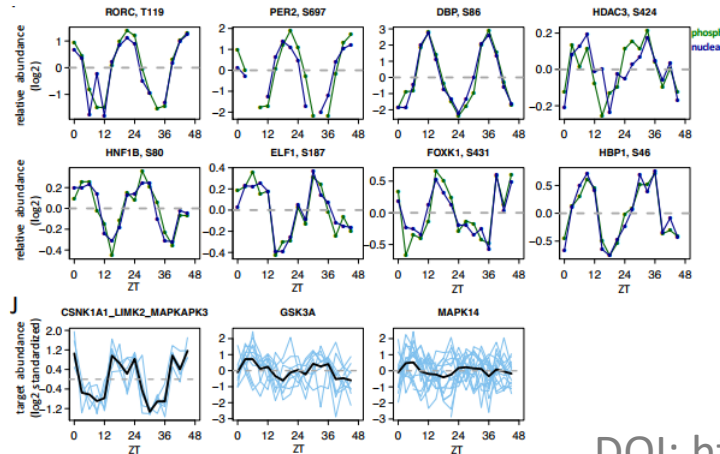
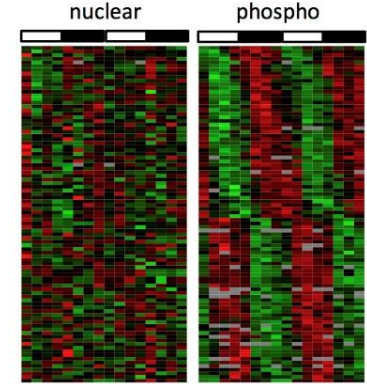
113 phosphoproteins presented rhythmicity



Corresponding rhythmicity



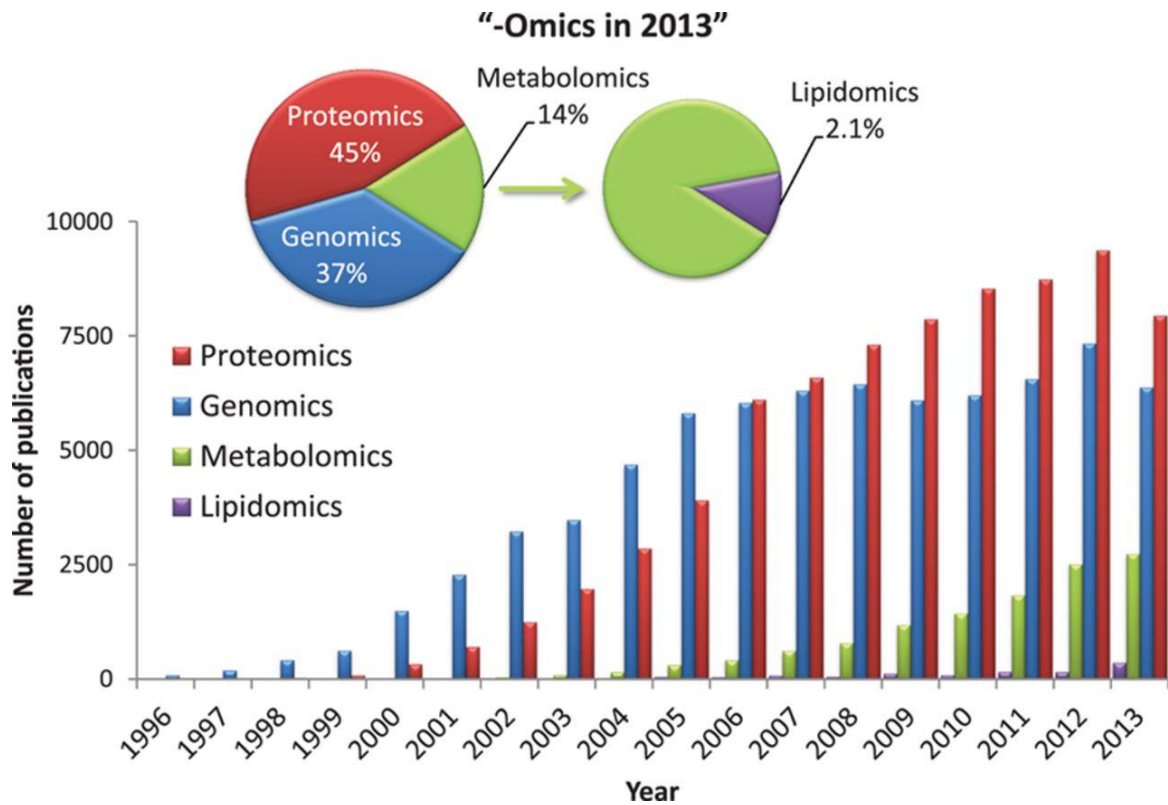
Non Corresponding rhythmicity



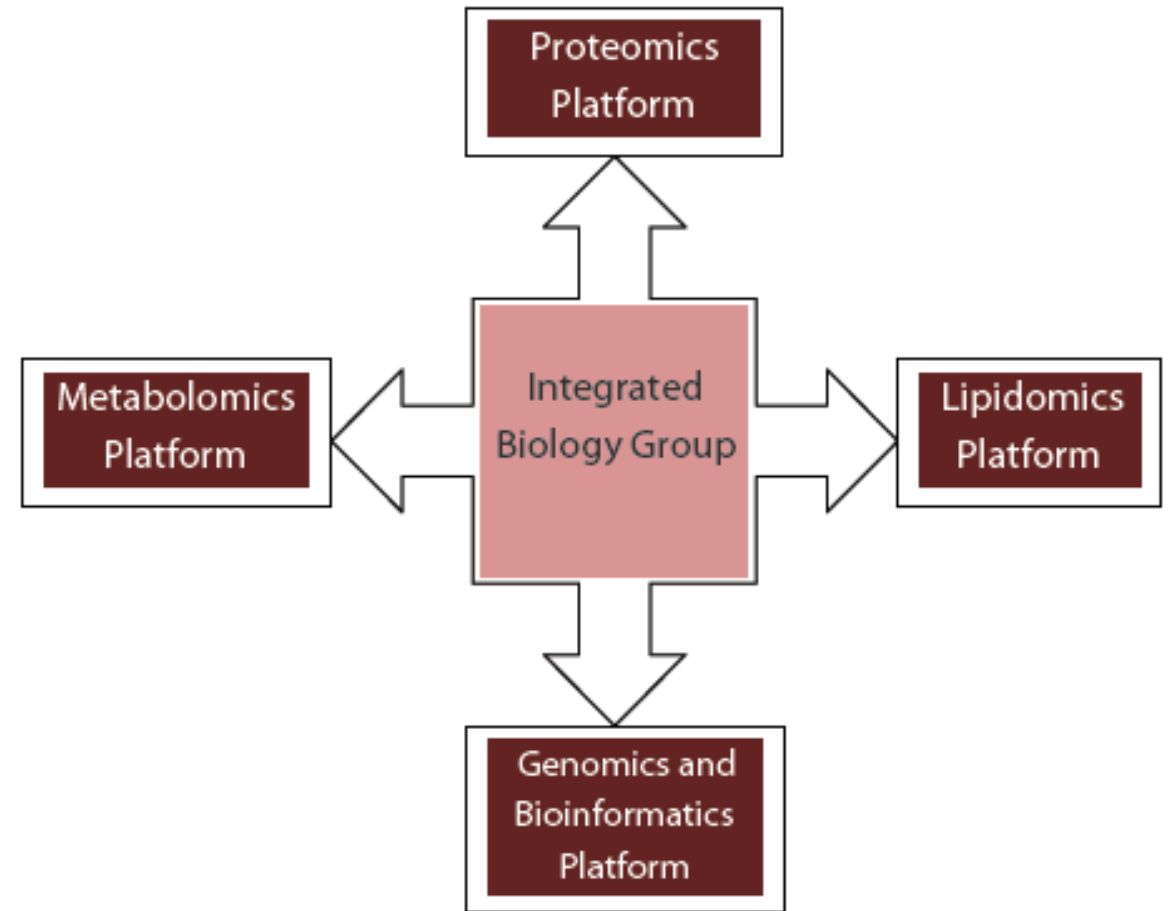
DOI: <https://doi.org/10.1016/j.cmet.2016.10.003>

Courtesy of A. Núñez Galindo

# 5.5. Other biological applications of mass spectrometry

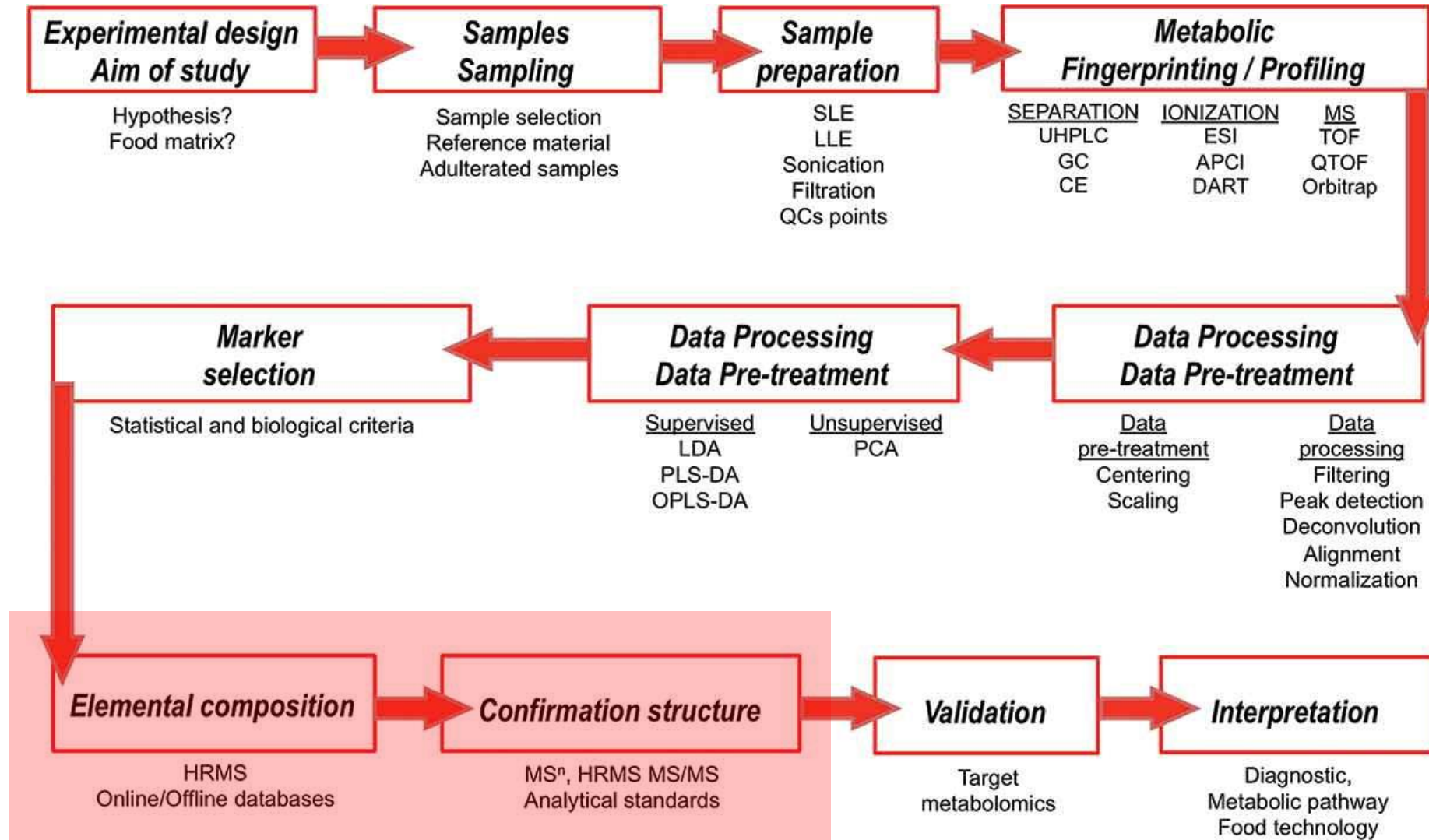


<https://doi.org/10.1161/CIRCGENETICS.114.000550>



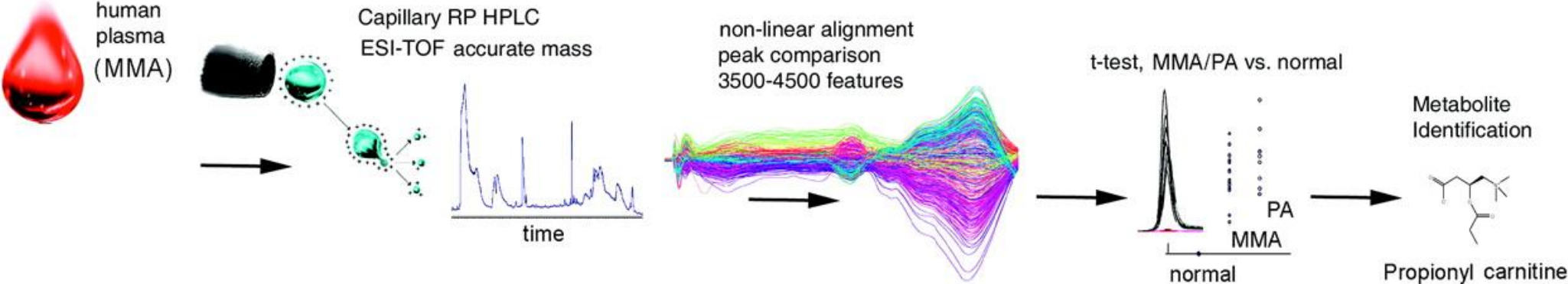
DOI:10.4172/jcsb.1000082

# Mass spectrometry-based metabolomics (1)

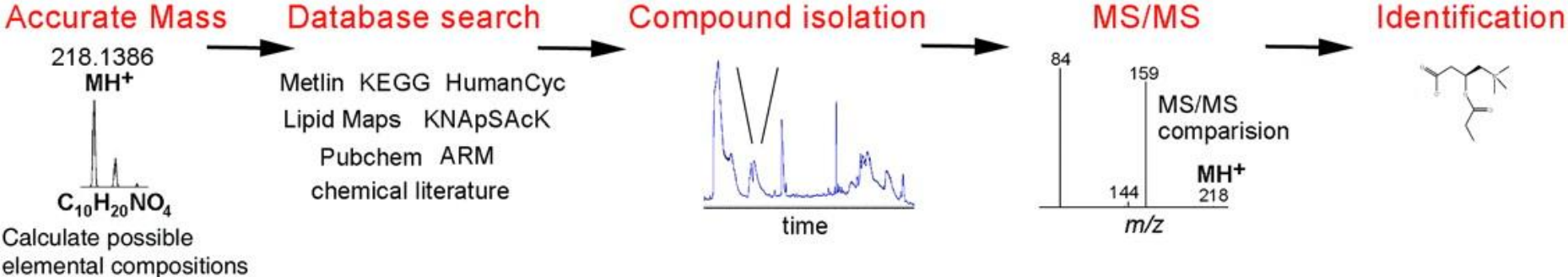


# Mass spectrometry-based metabolomics (2)

## A. Finding Metabolomic Differences (untargeted)

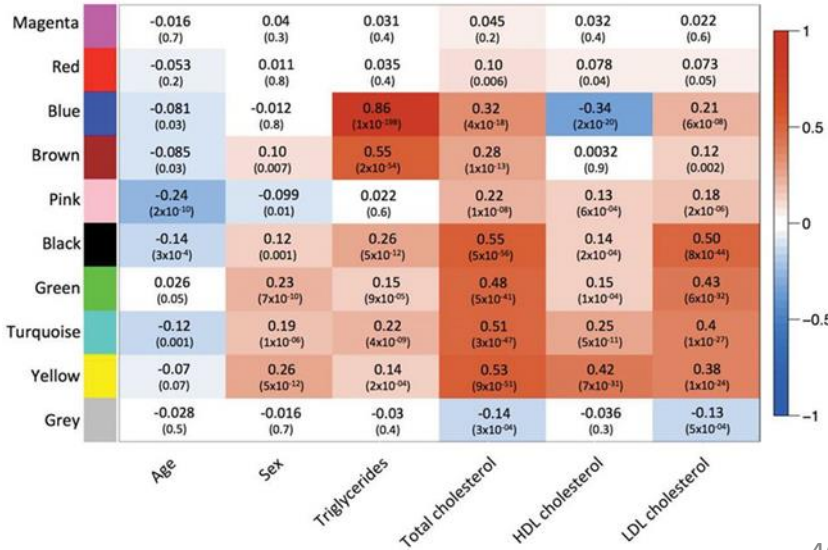
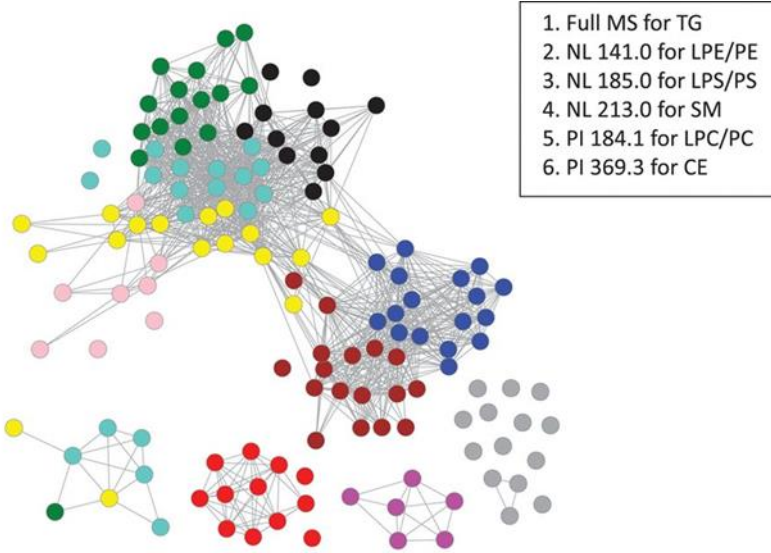
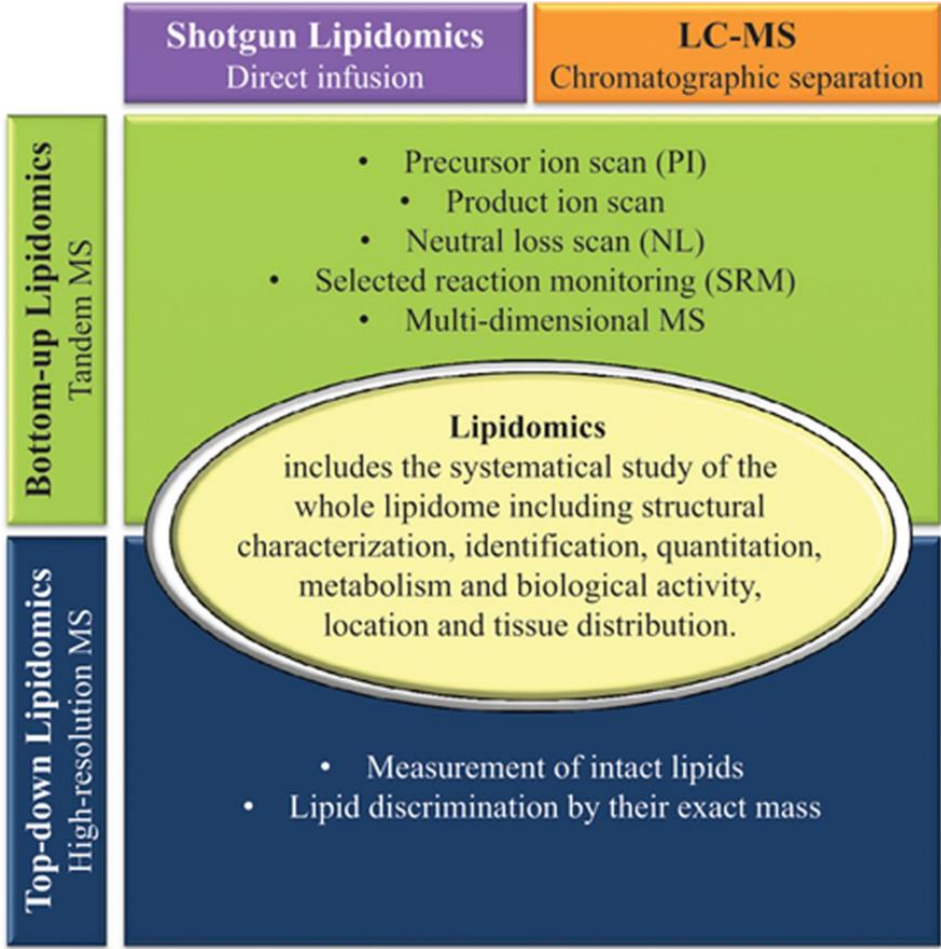


## B. Identification of Unknown Metabolites (targeted)



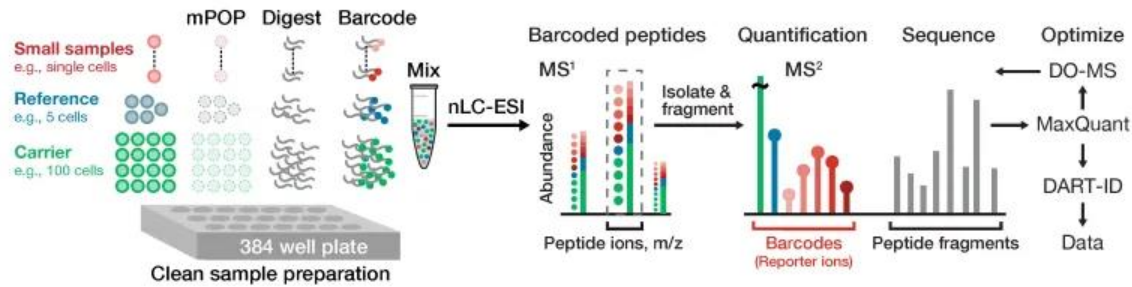


# Mass spectrometry-based lipidomics (2)

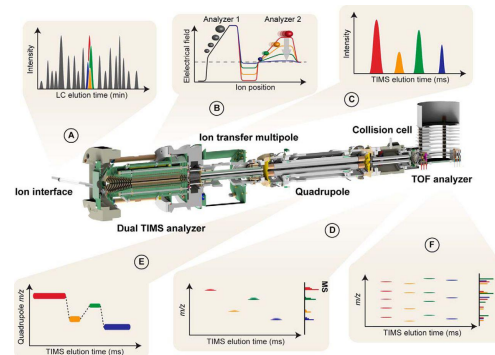


# 5.6. Advanced innovations (single-cells, 4D proteomics, multi-omics) and emerging technologies

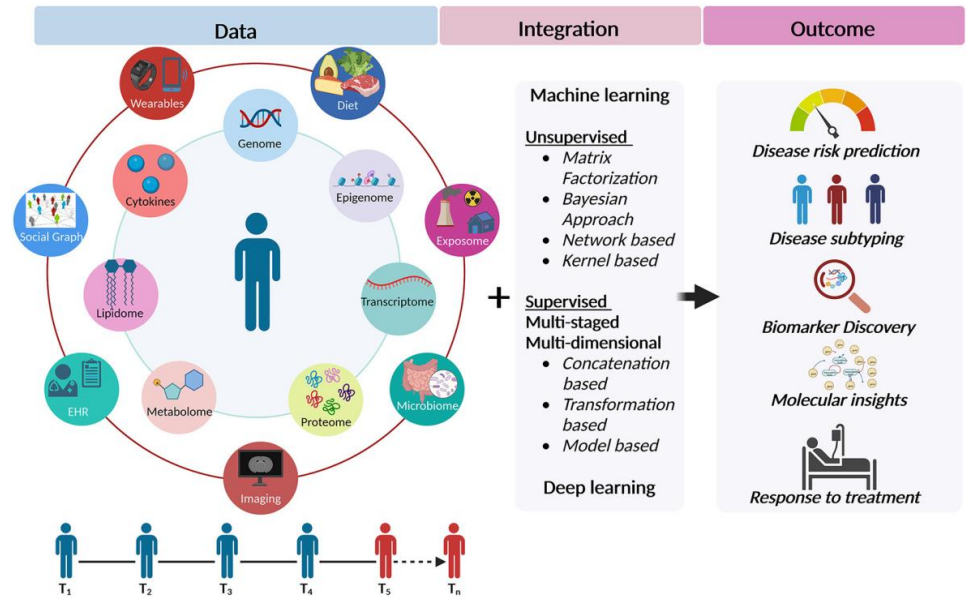
## Single-Cell Proteomics by Mass Spectrometry (SCoPE2)



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

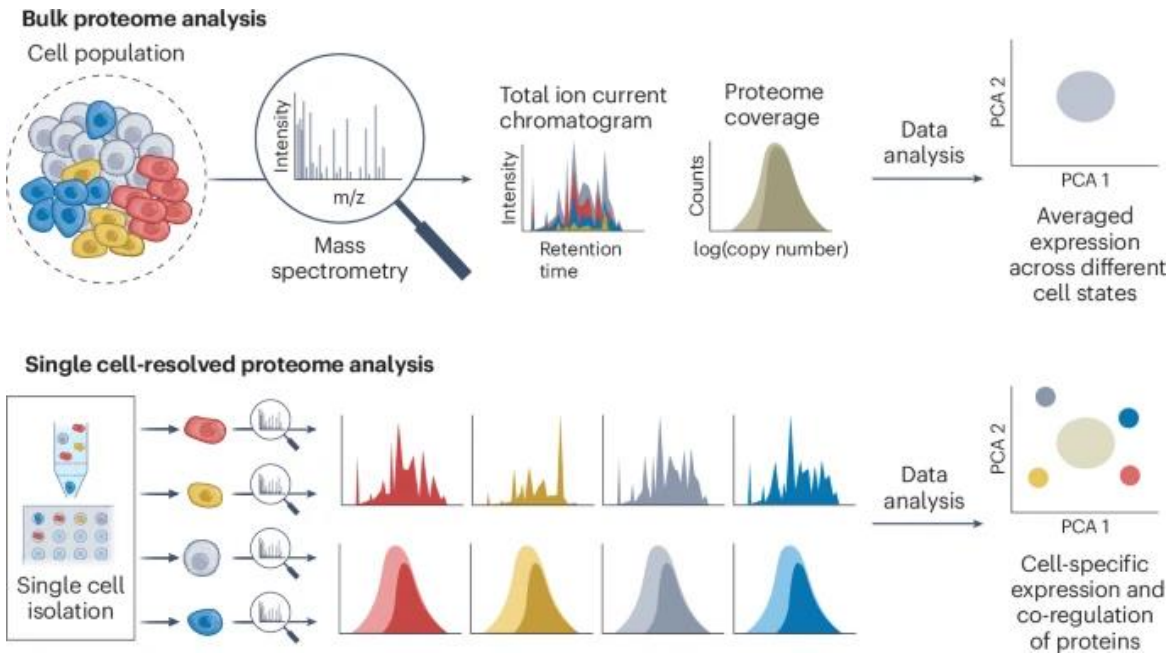


Molecular & Cellular Proteomics 2018 172534-2545DOI: (10.1074/mcp.TIR118.000900)



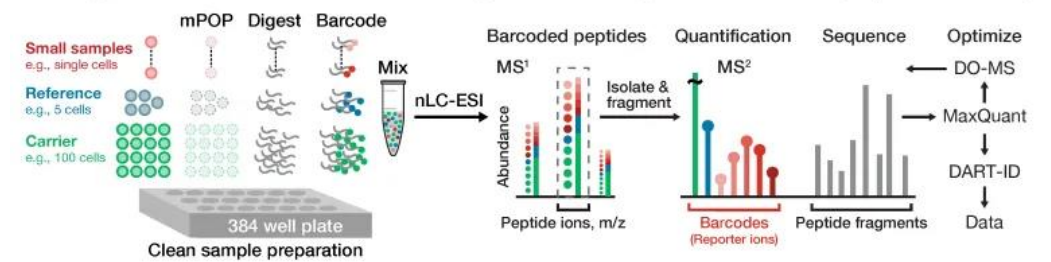
<https://doi.org/10.1016/j.mcpro.2023.100561>

# Single-cell proteomics



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

## Single-Cell Proteomics by Mass Spectrometry (SCoPE2)

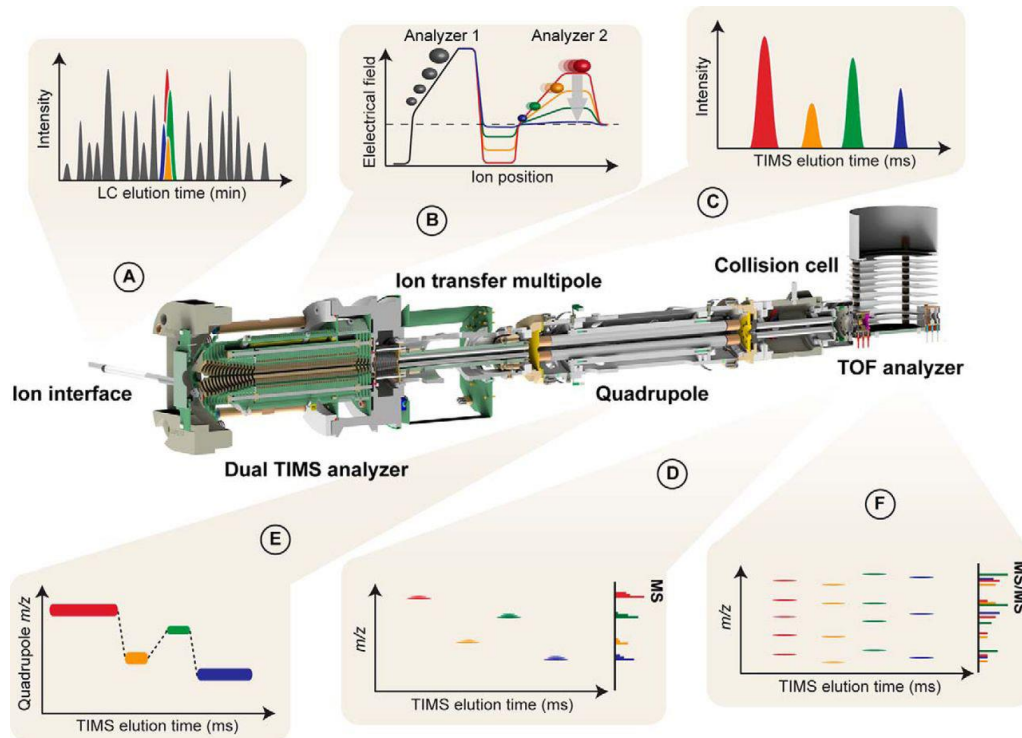


<https://web.northeastern.edu/slavovlab/blog/single-cell-proteomics/>

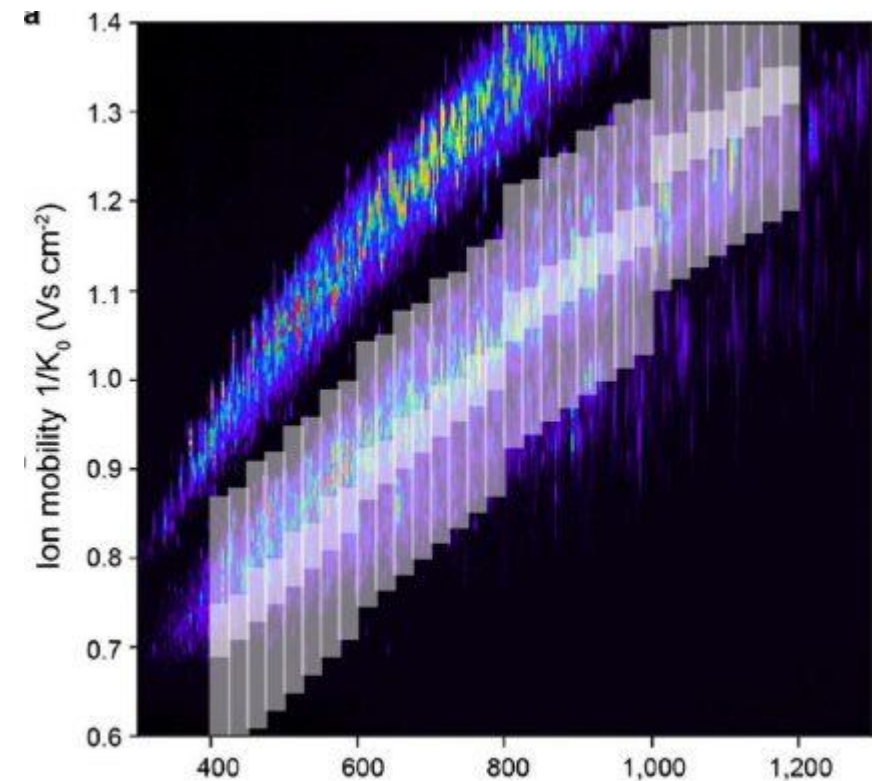
- Sensitivity challenge
- Sample preparation
- Use of isobaric labelling

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

- 4D proteomics

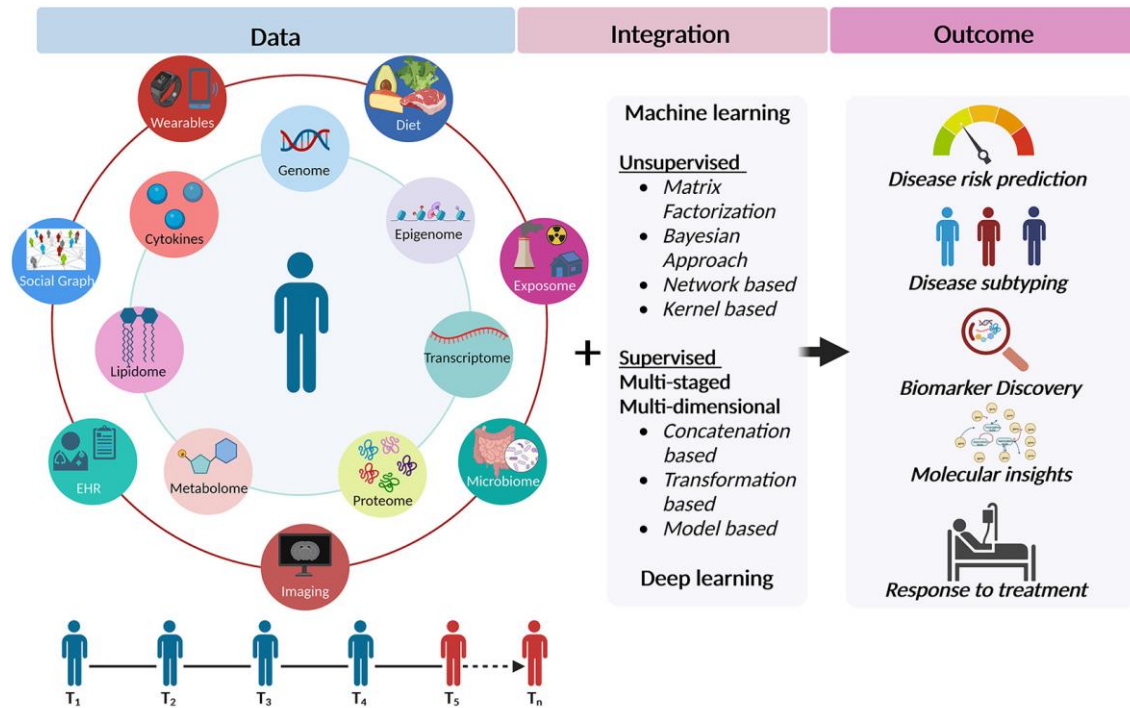


Molecular & Cellular Proteomics 2018 172534-2545 DOI: (10.1074/mcp.TIR118.000900)



<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>



## REVIEW

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

Ornella Cominetti <sup>1</sup> and Loïc Dayon <sup>1,2</sup>

<sup>1</sup>Proteomics, Nestlé Institute of Food Safety & Analytical Sciences, Nestlé Research, Lausanne, Switzerland; <sup>2</sup>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>

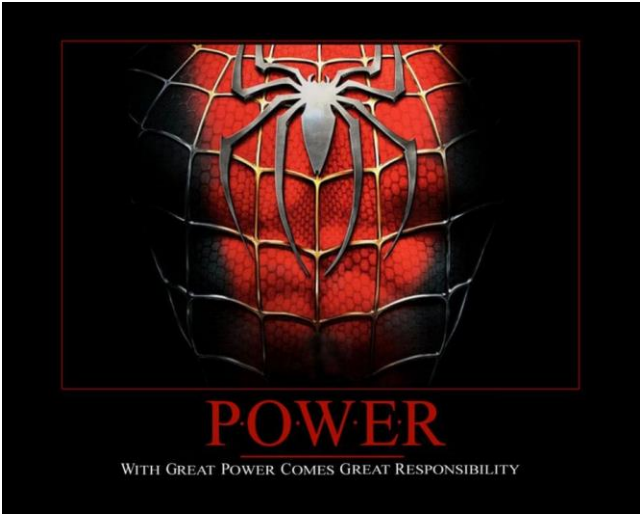
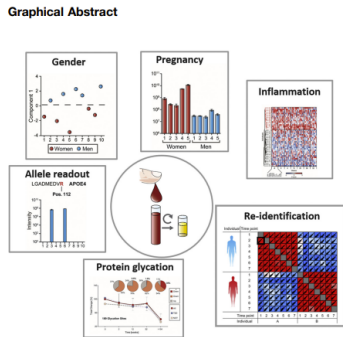
# 5.7. Opportunities, limitations and ethical consideration

## 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

**Correspondence**  
geyer@biochem.mpg.de;  
mmann@biochem.mpg.de

**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.



This Photo by Unknown Author is licensed under [CC BY-NC-ND](https://creativecommons.org/licenses/by-nc-nd/4.0/)

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

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

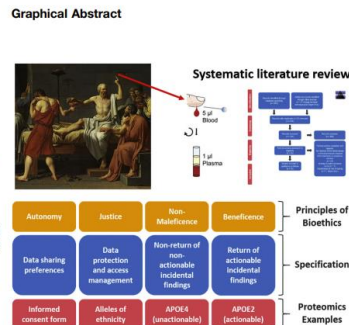
MCP PERSPECTIVE

## Ethical Principles, Constraints, and Opportunities in Clinical Proteomics

**Authors**  
Sebastian Porsdam Mann, Peter V. Treit, Philipp E. Geyer, Gilbert S. Omenn, and Matthias Mann

**Correspondence**  
sebastian.porsdammann@philosophy.ox.ac.uk; mmann@biochem.mpg.de

**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.



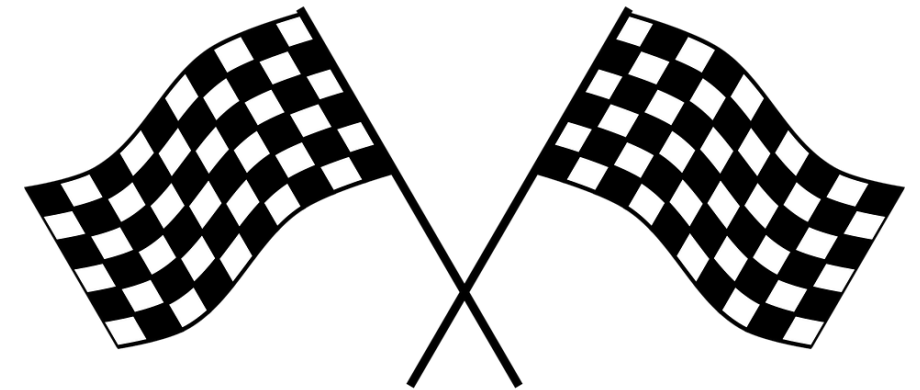
<https://doi.org/10.1016/j.mcpro.2021.100046>

# 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?