La Protéomique :

Etat de l’art et perspectives

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What is Proteomics?

"Proteomics includes not only the identification and quantification of proteins, but also the determination of their localization, modifications, interactions, activities, and, ultimately, their function."

S. Fields in *Science*, 2001.

Because proteomes are dynamic, proteomics great challenge is to measure accurately qualitative and quantitative changes of intracellular and extracellular protein content under different conditions to understand biological processes and define pathological states.

What can Proteomics do?

- **To provide lists of proteins and implement databases:**

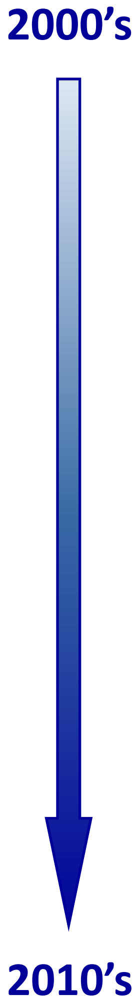
  Descriptive proteomics

- **To characterize and quantify proteins:**

  Functional proteomics

- **To decipher protein connections on a large scale:**

  Systems biology



2000’s

2010’s

How does MS-based Proteomics work?

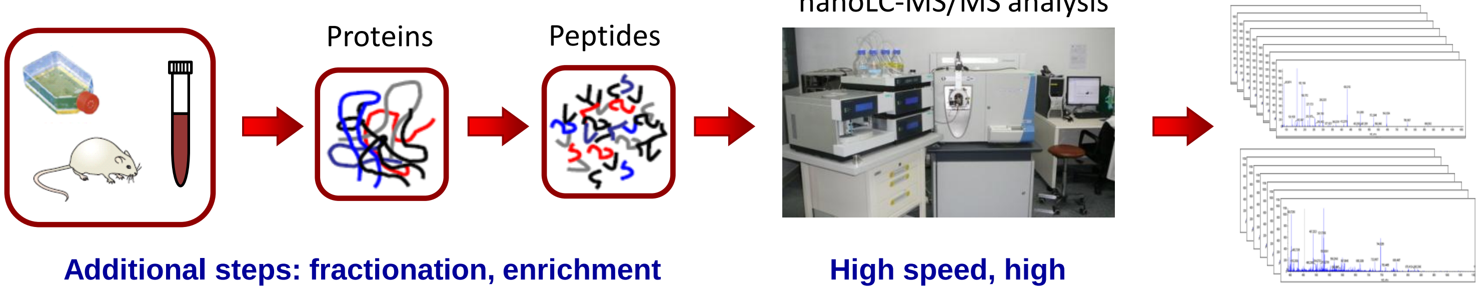
Sample preparation

Mass spectrometry analysis

Bioinformatic data analysis

Global proteomic approach for discovery studies:

in depth, unbiased, and quantitative proteome analysis



Identification

Quantification

Classification

Additional steps: fractionation, enrichment

High speed, high resolution acquisition

Reliable data processing using dedicated tools

Session 1, Lydie Lane

Session 3, Yves Vandenbrouck

- **Differential quantitative analysis of proteomes**
- **Biomarker discovery**

How does MS-based Proteomics work?

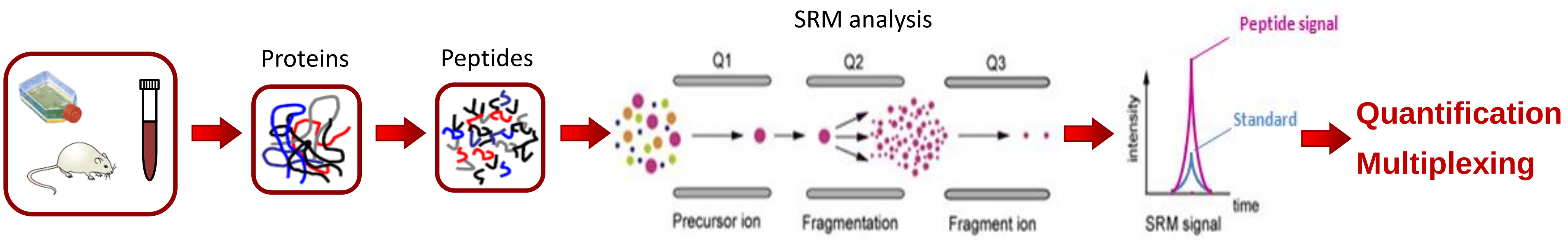
Sample preparation

Mass spectrometry analysis

Bioinformatic data analysis

Targeted proteomic approach for validation studies:

quantification of known proteins in many samples


Proteins

Peptides

SRM analysis

Quantification

Multiplexing

Minimal sample preparation

Selective, sensitive targeted acquisition

Reliable data processing using dedicated tools

- **Hypothesis driven studies: set of known proteins in specific pathways, …**
- **Biomarker validation**

Mass spectrometry capabilities for global analyses

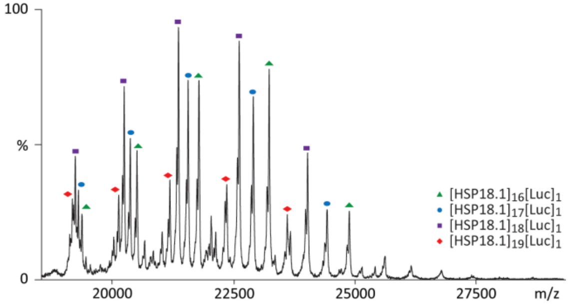
…

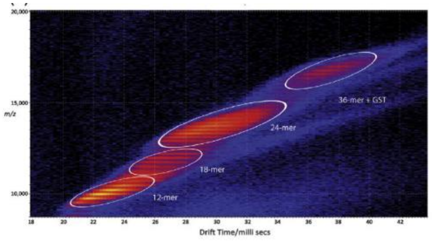
	Q-Star	LTQ-Orbitrap-XL	LTQ-Orbitrap-Velos
Year	2000	2005	2010
Resolution	10 000	60 000 – 100 000	60 000 – 100 000
Sequencing speed	4 MS/MS in 10 s	5 MS/MS in 1 s	20 MS/MS in 1 s
Sensitivity	4 fmol	0.5 fmol	0.5 fmol
Identified proteins	50	500	1500

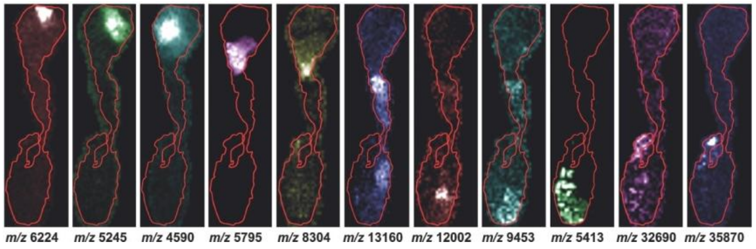
Mass spectrometry diversity

Mass spectrometry instrumentation is improving rapidly and constantly

Instrumentation	**Application**
Orbitrap	Global proteomics
Triple Quadrupole	Targeted proteomics
High resolution FT	Top down analysis of intact proteins
Ion mobility	Intact protein complexes analysis
MALDI TOF	Peptide/protein imaging
…	…





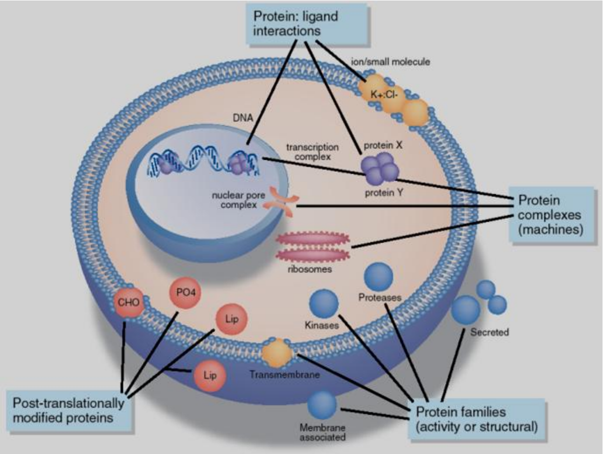


Session 1, Charles Pineau

Functional proteomics

Main objectives

- To understand molecular mechanisms
- To decipher protein interactions and networks
- To characterize cell signaling pathways
- To discover and validate biomarkers



Patterson, S.D. & Aebersold, R.H (2003)

Proteomic analysis

- Quantitative proteomics for relative protein abundance and dynamics
- Analysis of protein complexes including labile and transient partners
- Characterization of post-translational modifications
- Targeted proteomics

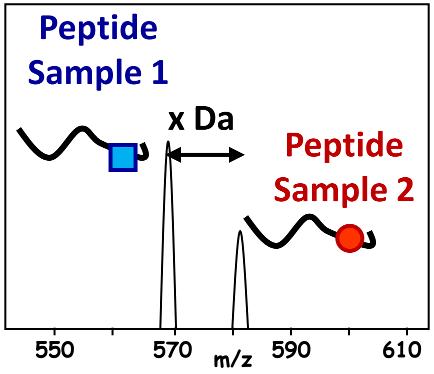
Quantitative proteomics strategies

Stable isotope labeling strategy

Sample 1 → Light isotope labeling ■

Sample 2 → Heavy isotope labeling ●

→ Mixed samples analyzed in one LC-MS/MS run →



Relative Quantification

Labeling methods: SILAC, ICAT, iTRAQ, …

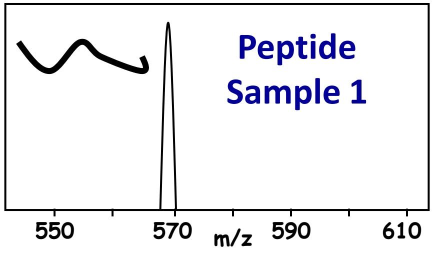
Label-free strategy

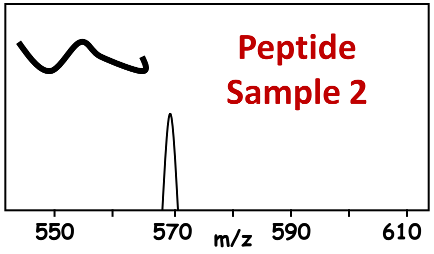
Sample 1 → LC-MS/MS analysis →

Sample 2 → LC-MS/MS analysis →

…

Sample n





→ Relative Quantification

MS peak intensities
MS/MS counting

Functional proteomics

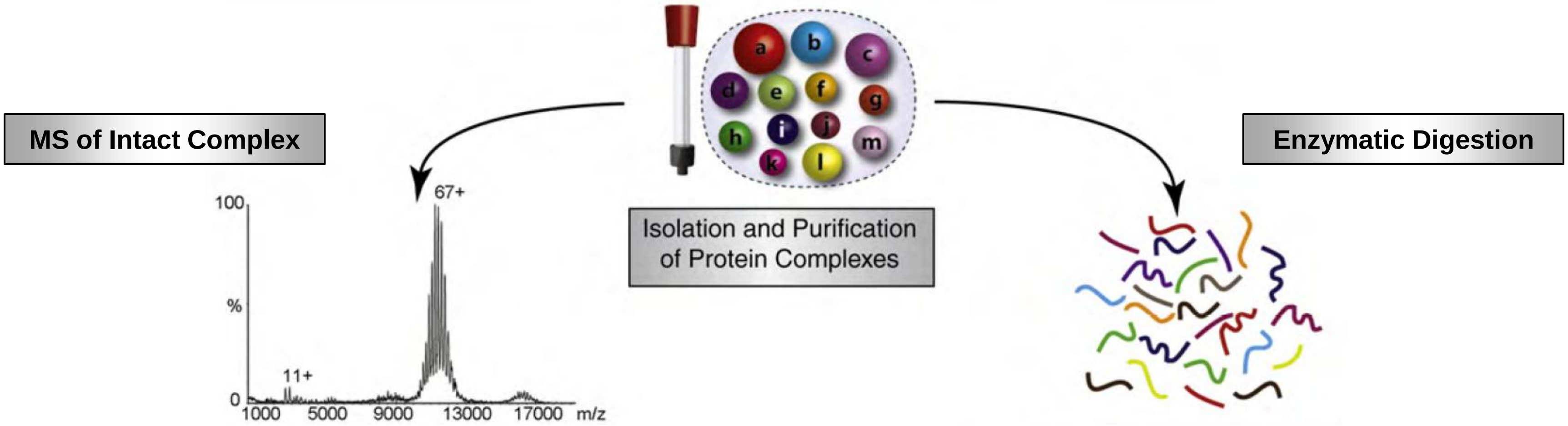
Technical challenges

- **Analysis of complex protein mixtures:** *fractionation, resolution, acquisition speed*
- **Differential quantitative analysis:** *repeatability, accuracy, bioinformatic tools*
- **Low-abundance proteins:** *enrichment, dynamic range, sensitivity*

Need for dedicated strategies for each biological objective in terms of sample preparation, mass spectrometry acquisition, and data analysis

Protein complexes

Protein complexes analysis by mass spectrometry



MS of Intact Complex

Isolation and Purification of Protein Complexes

Enzymatic Digestion

Structural biology approach

- Stoichiometry of subunits
- Assembly/2D architecture

Proteomic approach

- Protein subunit/partners identification
- Characterization of subunits (PTMs)
- Quantification and Dynamics
- Labile/transient partners identification

In-depth analysis of protein complexes:

Spatial organization and function

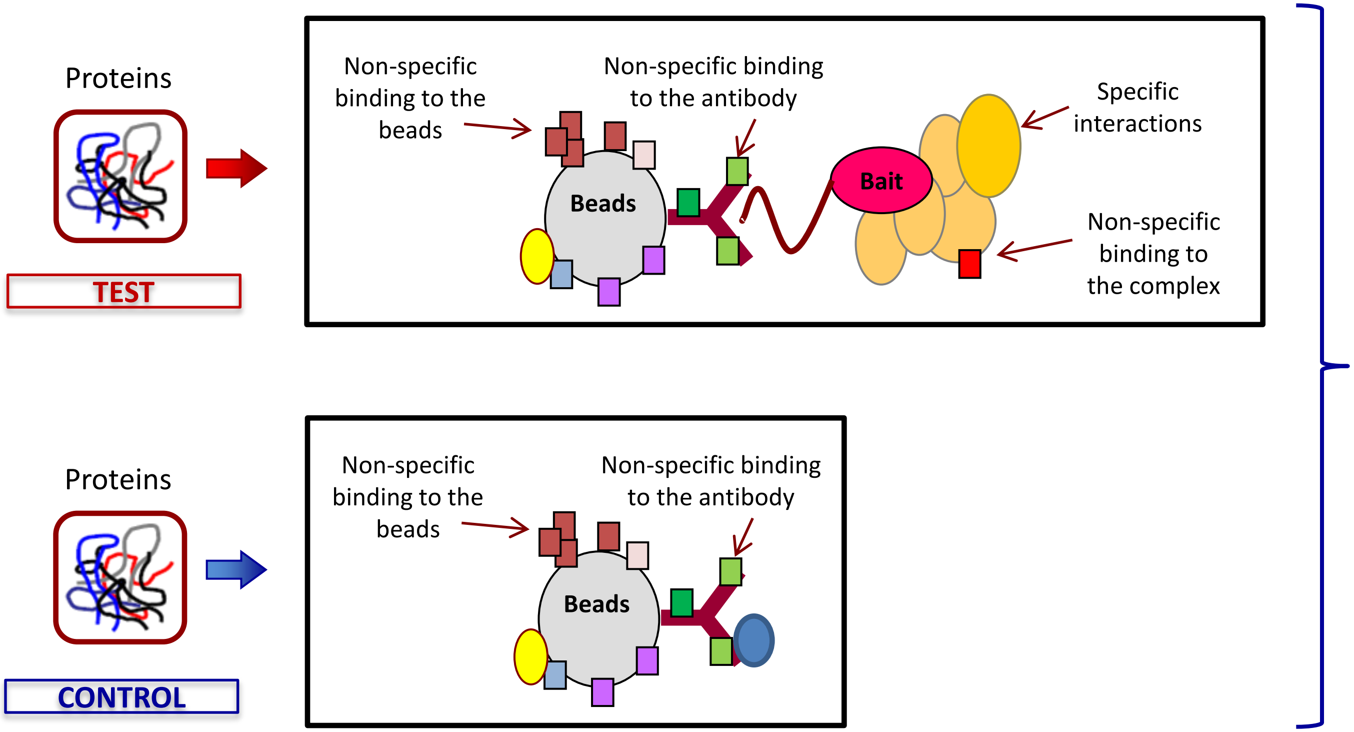
Challenges: Amount, stability, heterogeneity, contaminants

Protein complexes analysis workflow

Sample preparation maintaining protein-protein interactions

Protein complex enrichment: Immuno-affinity purification

Quantitative MS analysis



T/C Ratio >> 1

Specific protein partners

T/C Ratio ≈ 1

Non-specific protein interactions

Session 3, Romain Roncagalli

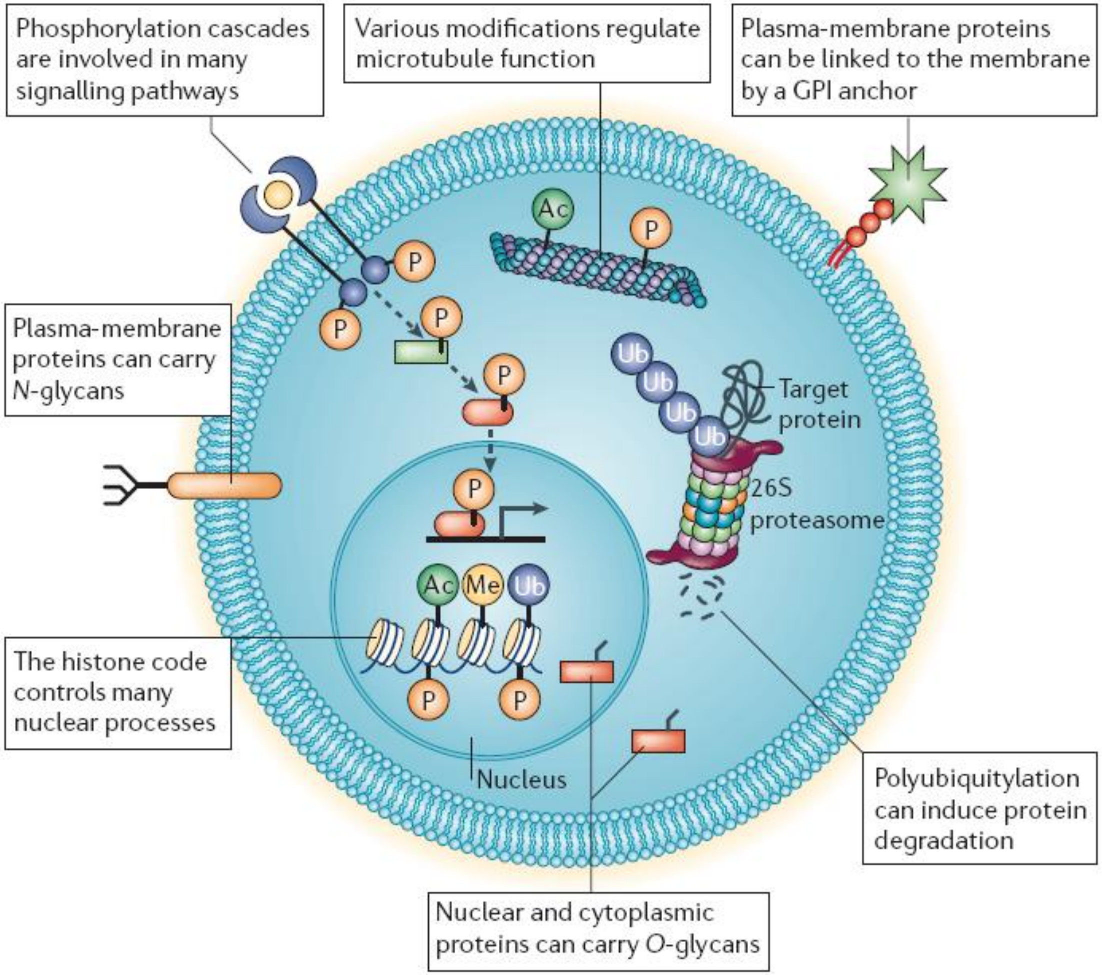
Protein complexes analysis

Perspectives and challenges:

- Quantitative MS analysis to study protein complexes dynamics
- Crosslinking combined to MS analysis
- Emerging new MS technologies (ion mobility MS)
- Combining complementary MS approaches
- Interaction networks on a large scale

Analysis of modified proteomes

Analysis of post-translational modifications



Phosphorylation cascades are involved in many signalling pathways

Various modifications regulate microtubule function

Plasma-membrane proteins can be linked to the membrane by a GPI anchor

Plasma-membrane proteins can carry *N*-glycans

The histone code controls many nuclear processes

Polyubiquitylation can induce protein degradation

Nuclear and cytoplasmic proteins can carry *O*-glycans

Jensen, *Nature Rev. Mol. Cell Biol*., 2006, 7, 391-403.

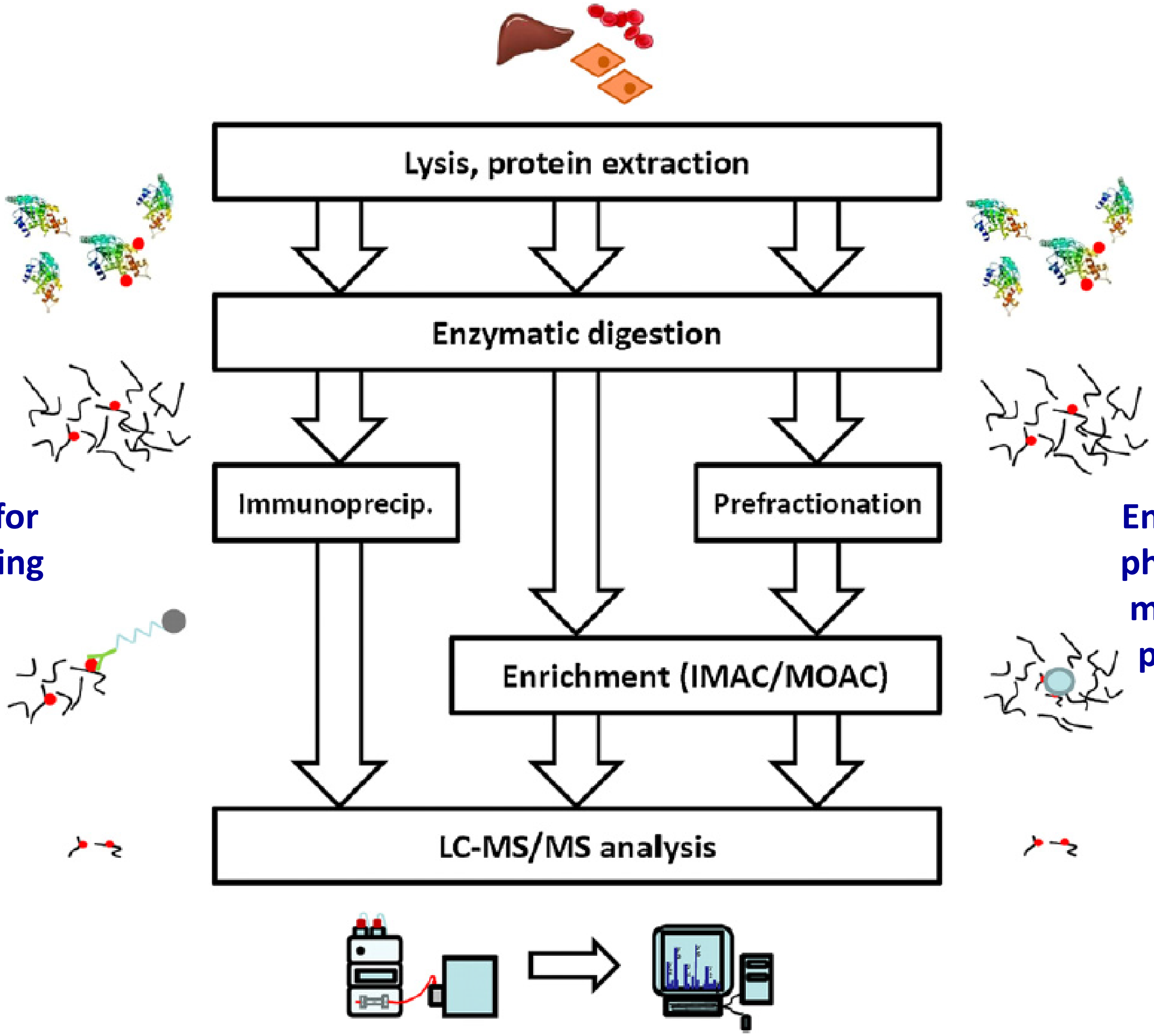
Analysis of post-translational modifications

Technical challenges

- **Low amount of modified proteins:** *enrichment, sensitivity*
- **Transient state of the modification:** *inhibitors to freeze the system when available*
- **Stability of the modification:** *appropriate buffers and pH, MS analysis conditions*
- **Localization of the modification:** *sequence coverage and approriate MS/MS*

Need to adapt analytical strategies to the modification of interest

Workflow for the analysis of phosphoproteomes



Enrichment for pTyr-containing peptides

Enrichment for all phosphopeptides: mainly pSer- and pThr-containing peptides

Leitner et al., Analytica Chimica Acta, 2011.

Examples of phosphoproteomes studies

Combination of quantitative approaches and phosphoproteome analyses to study signaling pathways on a global scale

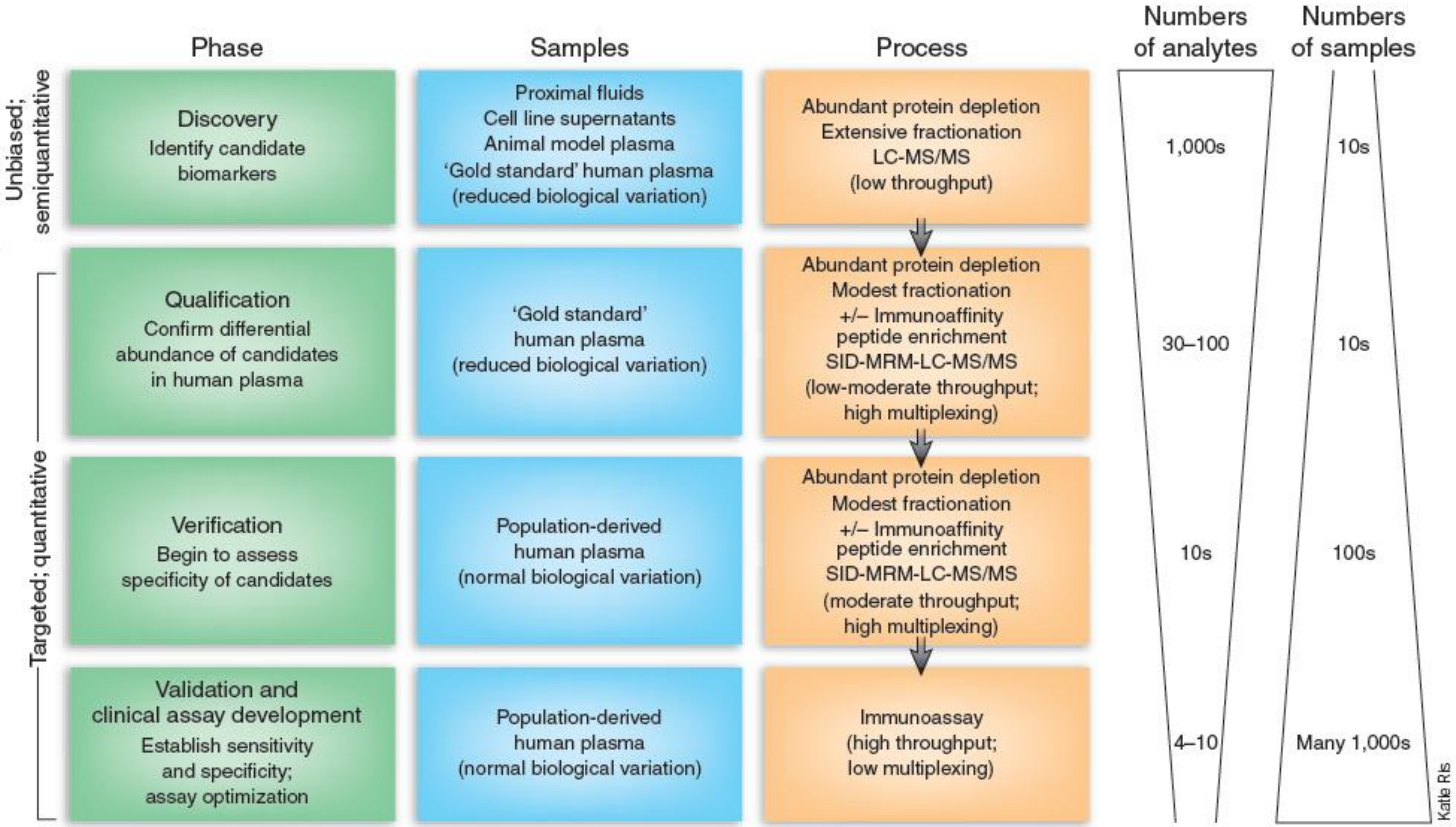
Starting material	Objective	Method	Total # of phospho sites	Reference
Human Jurkat T cells (100 million cells)	TCR signaling	SILAC TiO_2 and pY enrichments	10 665 (696 regulated) from multiple runs	Mayya et al., Science Signaling, 2009
Human B cells (630 million cell nuclei)	DNA damage response	SILAC + time points TiO_2 enrichment	7 043 (594 regulated)	Bennetzen et al., Mol Cell Proteomics, 2010
Human embryonic stem cells (50 million cells)	Differentiation	SILAC + time points TiO_2 enrichment	15 004 from multiple runs 10 066 (4 504 regulated) 11 104 (3 380 regulated)	Rigbolt et al., Science Signaling, 2011
Mouse liver (10 mg proteins)	Insulin signaling	Spike-in SILAC (Hepa1-6 cells) TiO_2 enrichment	14 857 (1 000 regulated)	Monetti et al., Nature Methods, 2011

Remaining challenges:

Phosphorylation sites localization, stoichiometry, interplay with other PTMs

Biomarkers

Process flow for the development of biomarkers



	Phase	Samples	Process	Numbers of analytes	Numbers of samples
Unbiased; semiquantitative	**Discovery** Identify candidate biomarkers	Proximal fluids Cell line supernatants Animal model plasma 'Gold standard' human plasma (reduced biological variation)	Abundant protein depletion Extensive fractionation LC-MS/MS (low throughput)	1,000s	10s
Targeted; quantitative	**Qualification** Confirm differential abundance of candidates in human plasma	'Gold standard' human plasma (reduced biological variation)	Abundant protein depletion Modest fractionation +/– Immunoaffinity peptide enrichment SID-MRM-LC-MS/MS (low-moderate throughput; high multiplexing)	30–100	10s
	Verification Begin to assess specificity of candidates	Population-derived human plasma (normal biological variation)	Abundant protein depletion Modest fractionation +/– Immunoaffinity peptide enrichment SID-MRM-LC-MS/MS (moderate throughput; high multiplexing)	10s	100s
	Validation and clinical assay development Establish sensitivity and specificity; assay optimization	Population-derived human plasma (normal biological variation)	Immunoassay (high throughput; low multiplexing)	4–10	Many 1,000s

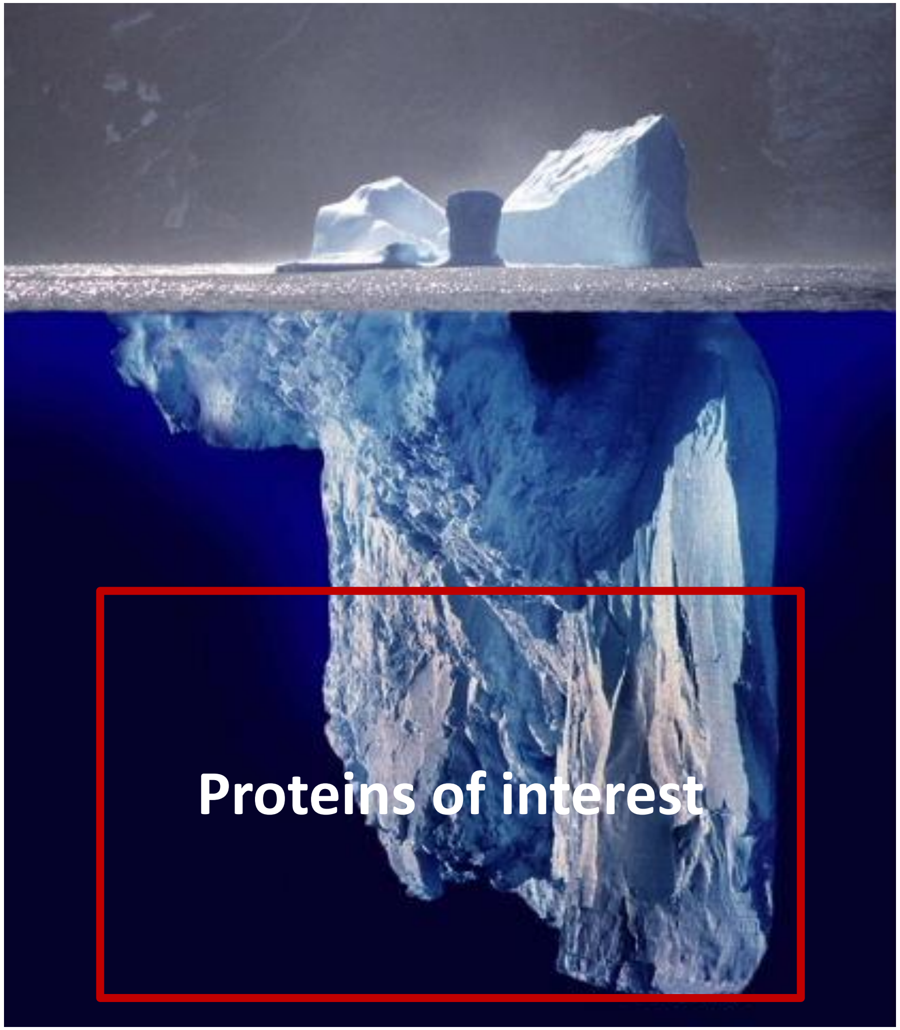
Katie Ris

Rifai et al., Nature Biotechnol., 2006.

Proteomic analysis of biological fluids

Technical challenges:
- ► Protein concentration dynamic range
- ► Few proteins are highly abundant

Protein concentration dynamic range:
10^{10} - 10^{12}



Mass spectrometry dynamic range:
10^{3} - 10^{4}

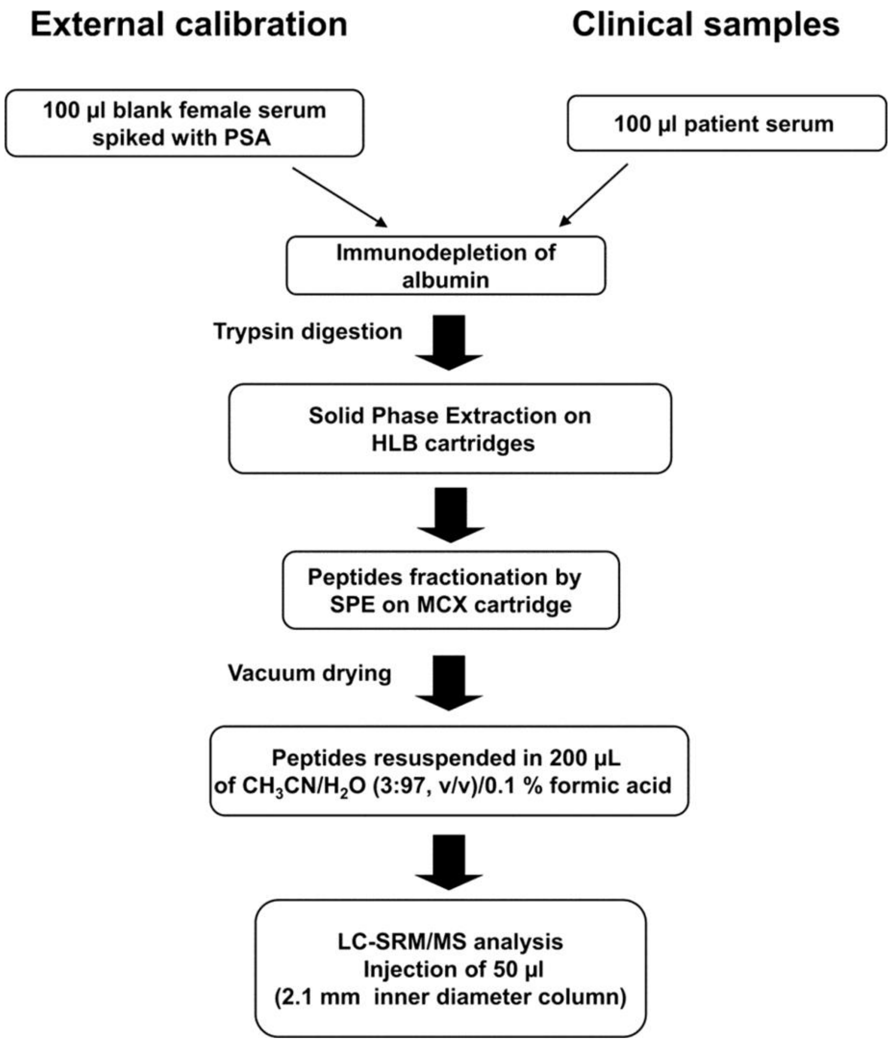
Equalization

Depletion

Session 3,
Anne Gonzalez de Peredo

SRM analysis of PSA biomarker in patients serum

Experimental workflow



External calibration

Clinical samples

100 µl blank female serum spiked with PSA

100 µl patient serum

Immunodepletion of albumin

Trypsin digestion

Solid Phase Extraction on HLB cartridges

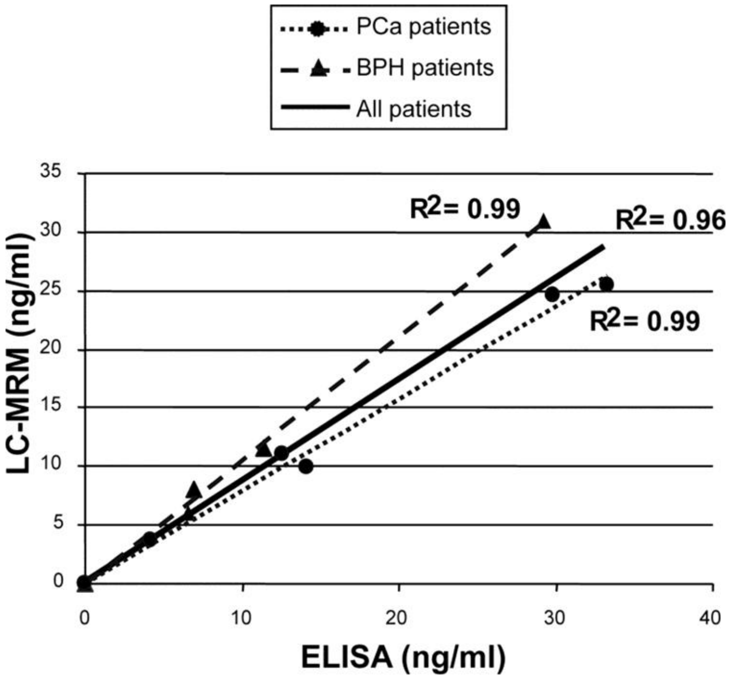
Peptides fractionation by SPE on MCX cartridge

Vacuum drying

Peptides resuspended in 200 µL of CH_3CN/H_2O (3:97, v/v)/0.1 % formic acid

LC-SRM/MS analysis
Injection of 50 µl
(2.1 mm inner diameter column)

SRM and ELISA correlation for PSA quantification

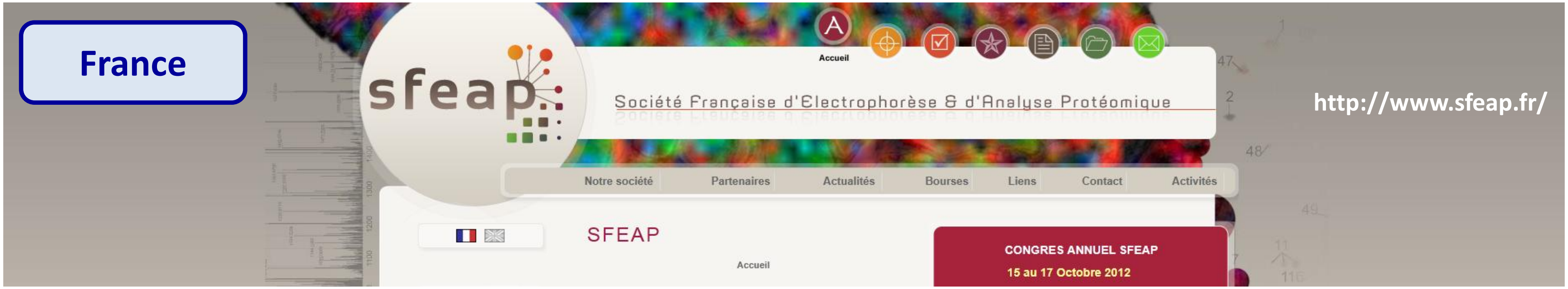


LOQ: low ng/ml range

Fortin et al., Mol Cell Proteomics, 2009.

Proteomics community

France



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Europe

Eupa european proteomics association

http://www.eupa.org/

International

HUPO

Human Proteome Organisation

Fostering international proteomic initiatives to better understand human disease

http://www.hupo.org/

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