Proteomic analysis of biological fluids

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Biofluid proteomics: objectives and challenges

Serum
Plasma
Cerebrospinal fluid
Urine
Bile
Tears
Saliva
…

➢ Biological fluids are in close contact with tissue that may liberate protein components and their protein content may be affected by the disease

➢ discovery of new biomarkers for diagnosis and monitoring of therapy outcome

Key issues for biomarker identification using proteomic approaches:

• selection in the discovery phase of a **fluid in close proximity with the organ** affected by the disease, to increase the probability of finding a biomarker originating from pathological tissue

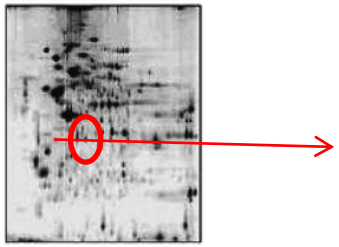
• **in-depth characterization of the fluid** using a proteomic method enabling the detection of a high number of species, even low-abundant ones

• analysis of a significant number of samples using **reproducible quantitative proteomic methods**, to yield statistically significant results

• a **validation phase** following the discovery phase, using a high-throughput method applicable on a larger cohort of patients

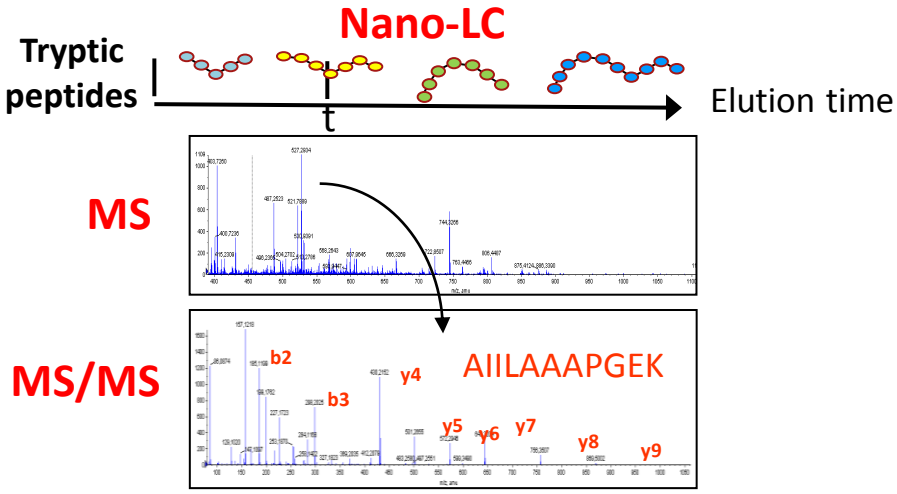
Analytical techniques for biofluids proteomics

- 2D gels
  - ✓Separation of each protein isoform on a 2D gel
  - ✓Spot detection/quantification performed by protein staining or fluorescence
  - ✓ A limited number of spots are then characterized by MS

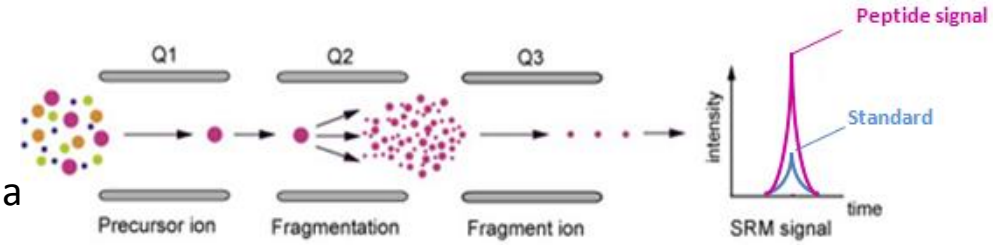


MS analysis
(protein identification)

- shotgun proteomics (nanoLC-MS/MS)
  - ✓ Trypsin digestion of protein mixtures
  - ✓Systematic sequençing by MS/MS -> protein identification
  - ✓ Quantification by analysis of MS signal



- MRM based quantitative assays
  - ✓ Trypsin digestion of protein mixtures
  - ✓ A few proteotypic peptides are specifically monitored as signature peptides for a panel of candidate proteins
  - ✓ Quantification by analysis of MS signal + interna peptide standards



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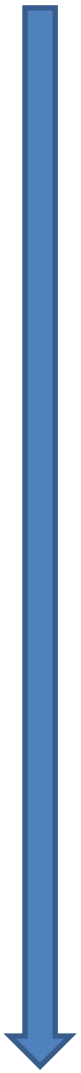

Dynamic range/ sensitivity

Quantitative analysis

Gel image analysis

Relative quantification of MS signal for >10 000 s peptide ions

Bioinformatics

Analysis of MS signal for a few peptide ions + internal standard

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Non-targeted Discovery

- MRM based quantitative assays
  - ✓ Trypsin digestion of protein mixtures
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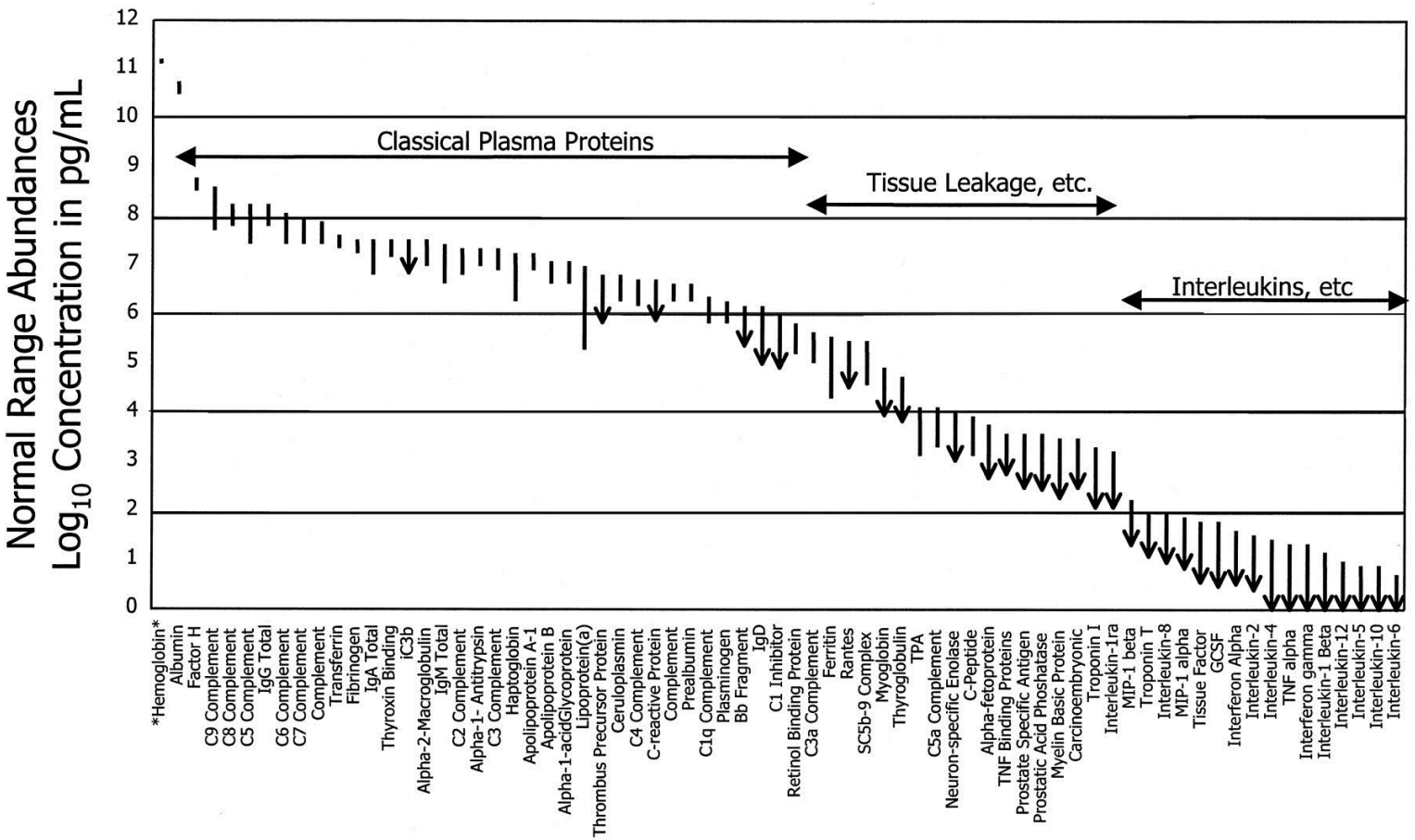
Targeted Validation

In-depth proteomic characterization: dynamic range

Dynamic range of protein concentrations spanning 12 orders of magnitude in plasma

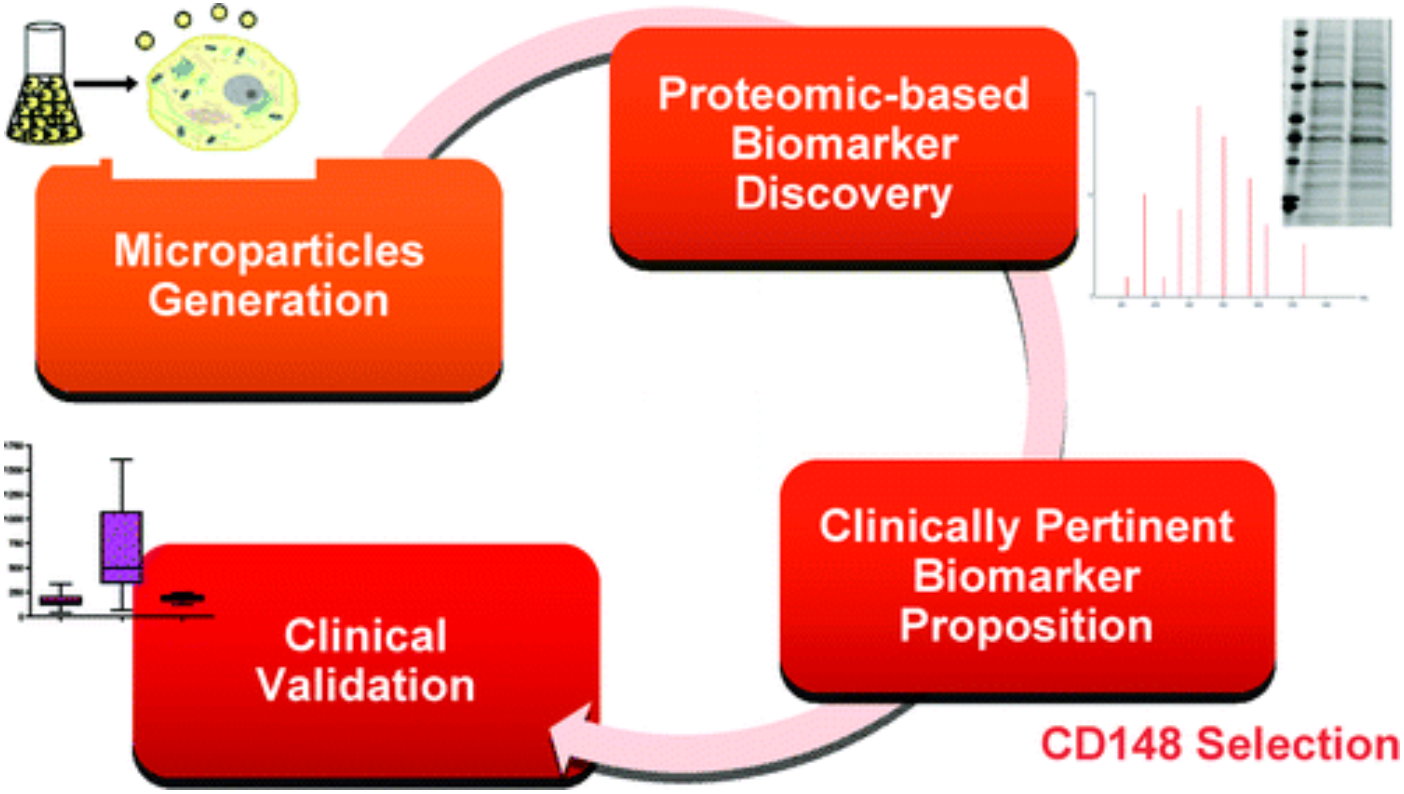


Anderson, N. L. (2002) Mol. Cell. Proteomics

How to deal with biofluids dynamic range?

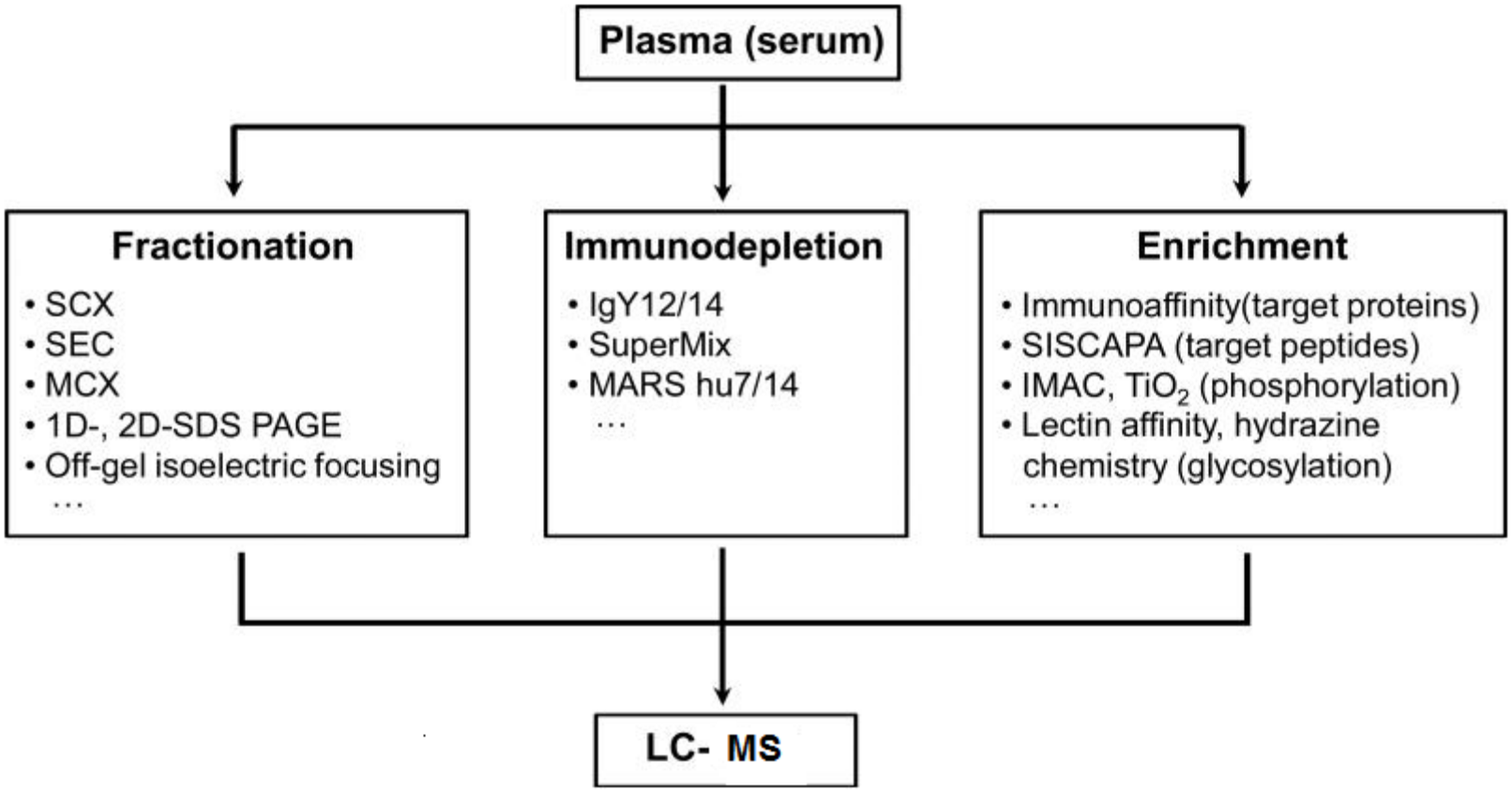
- Analysis of microparticles or exosomes

Miguet et al, J Proteome Res. 2009. Proteomic analysis of malignant B-cell derived microparticles reveals CD148 as a potentially useful antigenic biomarker for mantle cell lymphoma diagnosis.



How to deal with biofluids dynamic range?

➢ Prefractionation / depletion of highly abundant proteins



Shi et al, Proteomics 2012

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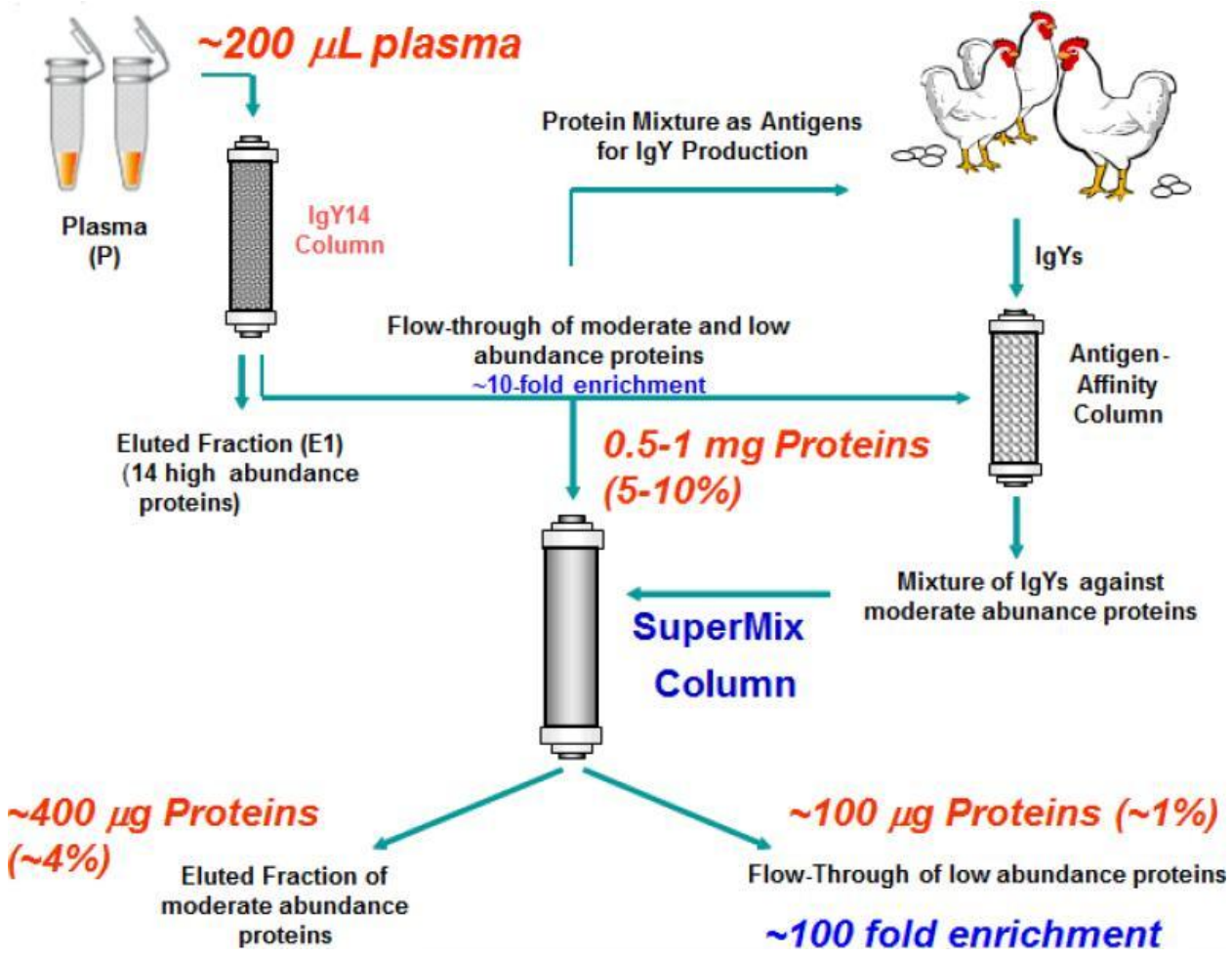
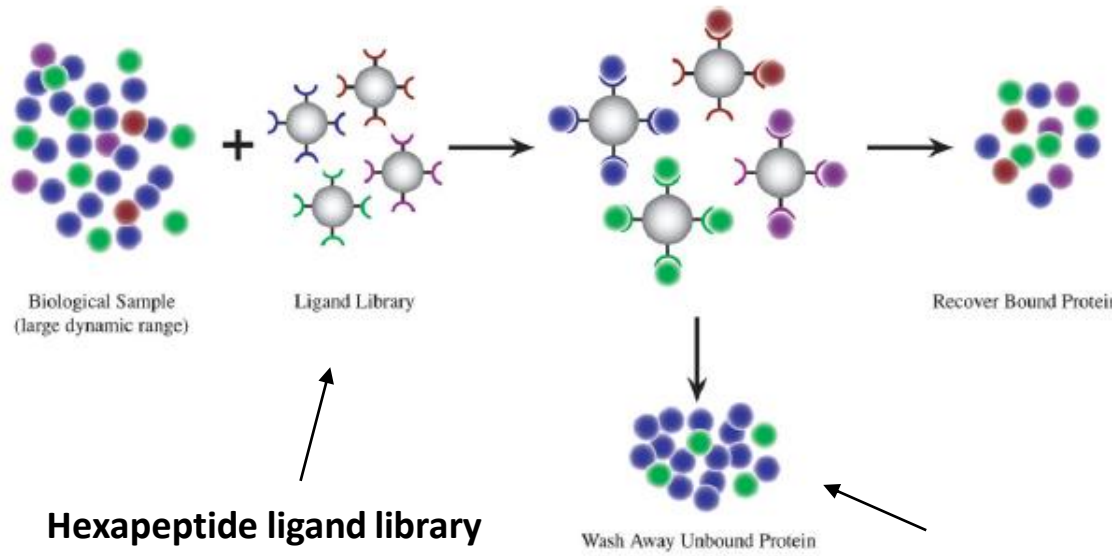


Shi et al, Methods 2012

How to deal with biofluids dynamic range?

➢ Reduction of sample dynamic range with ProteoMiner beads



LARGE DYNAMIC RANGE

REDUCED DYNAMIC RANGE

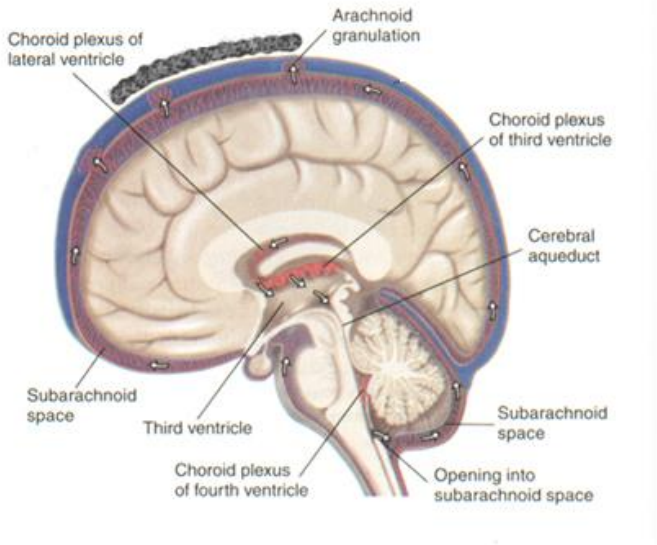
Hexapeptide ligand library
(~ 64 millions of different ligands)

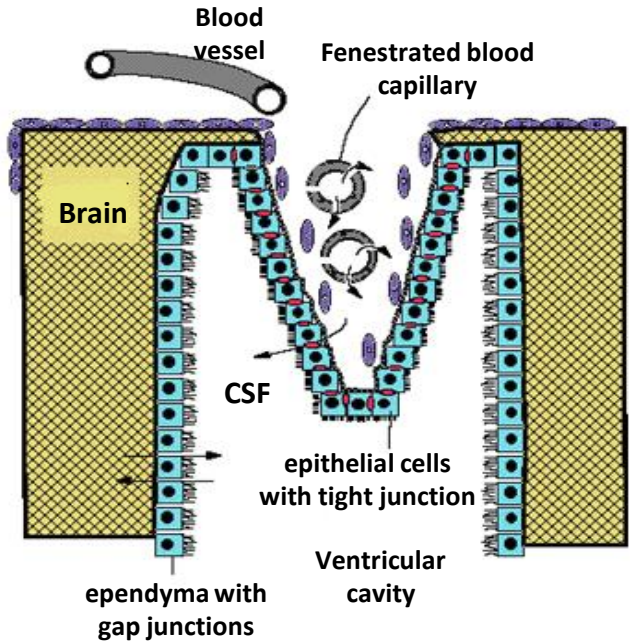
Flow-Through:
Highly abundant proteins

Roux-Dalvai et al. Extensive analysis of the cytoplasmic proteome of human erythrocytes using the peptide ligand library technology and advanced mass spectrometry. Mol Cell Proteomics 2008

Mouton-Barbosa et al, In-depth exploration of cerebrospinal fluid by combining peptide ligand library treatment and label-free protein quantification. Mol Cell Proteomics.2010

About Cerebro-Spinal Fluid…



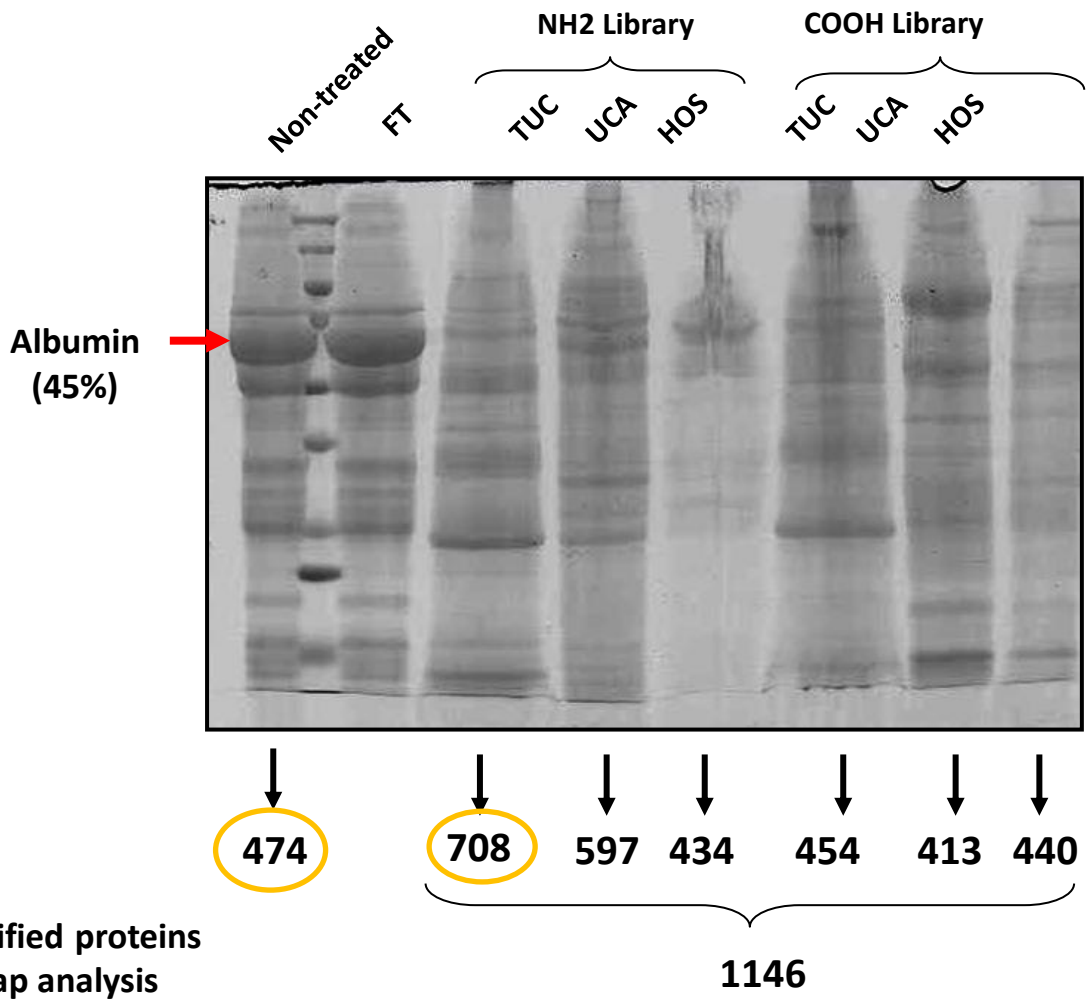


- Physical protection of the brain and metabolic function
- A small amount of CSF originates from the extracellular space of the brain
- potential biomarkers for neurological diseases

<u>Bottlenecks for proteomic studies on CSF</u>:

- Very large dynamic range of protein concentrations : 10^{10}, albumin = 45% of total protein
- Low protein concentration : 0.40 mg/ml (200x less than serum)
- Low available volume : Lumbar puncture = 1 to 2 mL

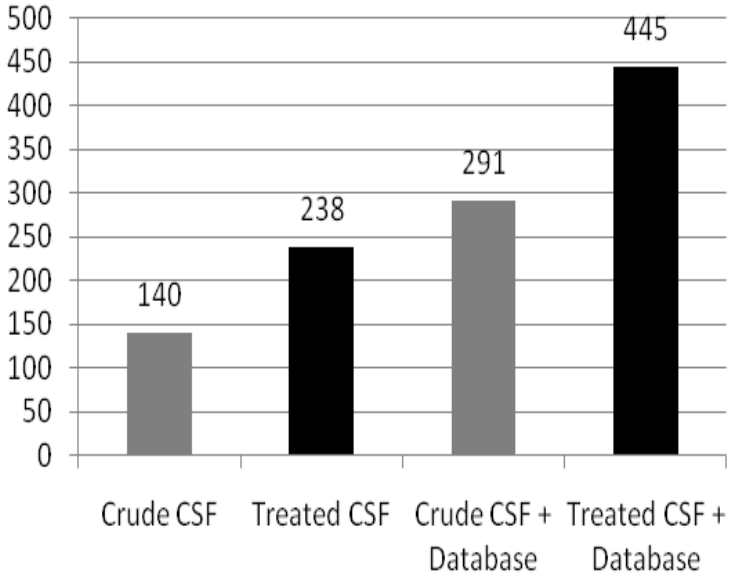
ProteoMiner treatment of CSF



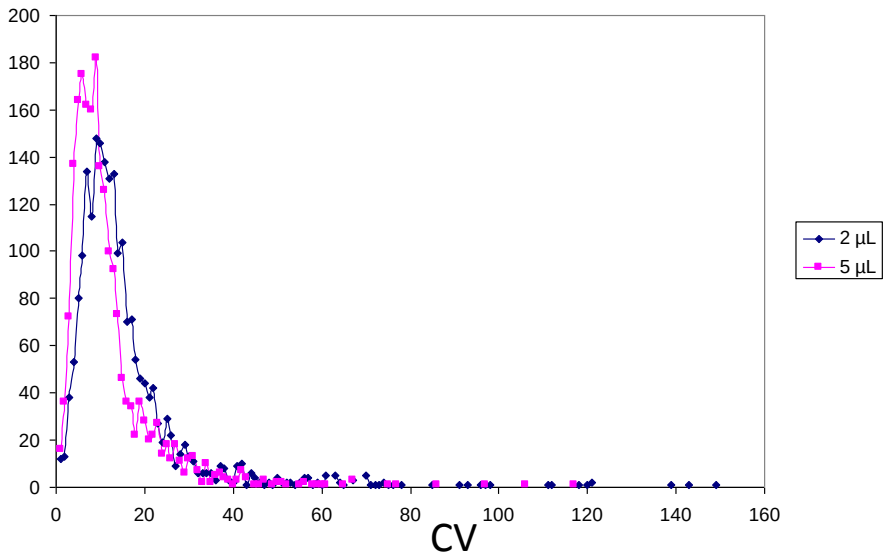
Number of identified proteins
by LTQ-Orbitrap analysis

Label-free quantification of ProteoMiner-treated CSF using MFPaQ and an identification database

Number of quantified proteins after single run analysis



Distribution of Coefficients of Variation for peptides intensities (4 replicates) after equalization on 5µL or 2µL beads

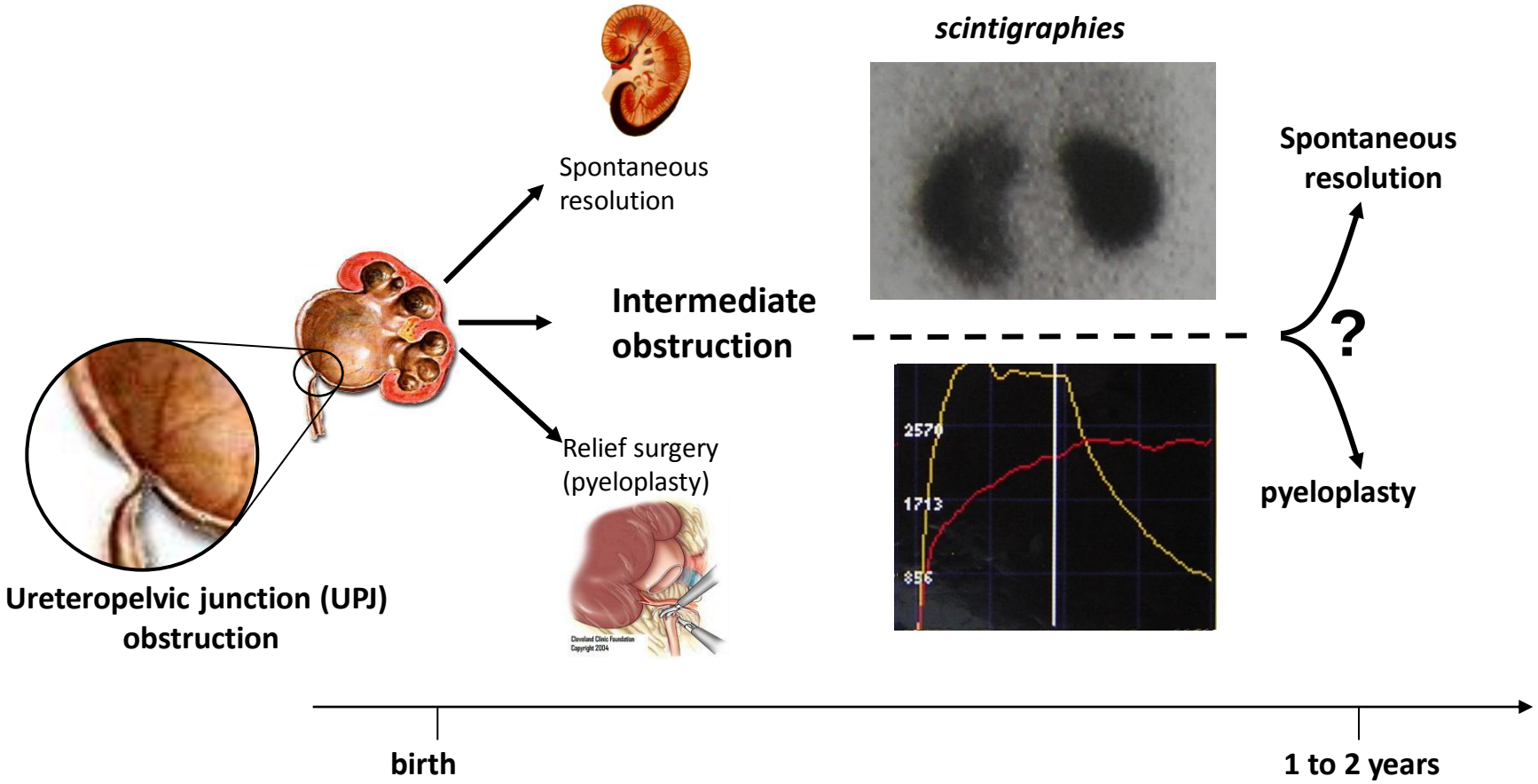


- Reduction of sample dynamic range through sample treatment (Proteominer beads)
- Use of a label-free quantitative approach, easy to implement on biological fluids (MFPaQ software)
- Increase the number of quantified proteins through the use of a protein identification database

PROTELL: Biomarkers identification in CNS relapse of diffuse large B cell lymphomas
Coordinator: Catherine Thieblemont, hôpital Saint-Louis, Paris

Discovery of candidate urinary biomarkers of congenital unilateral ureteropelvic junction (UPJ) obstruction

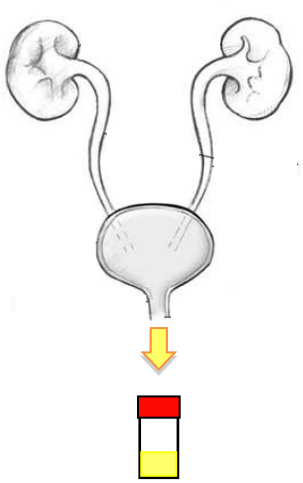
(Chrystelle Lacroix, collaboration équipe Joost Schanstra, INSERM U858, Toulouse)



- Identify early urinary biomarkers indicative for surgery
- Better understand the pathophysiology of UPJ obstruction

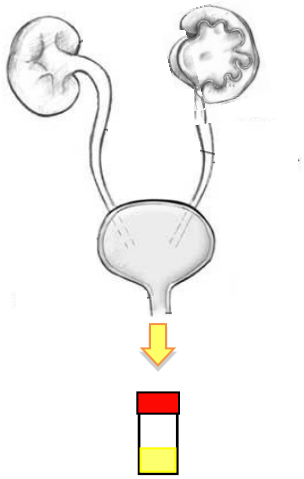
Samples (n=5/group) for discovery

Healthy



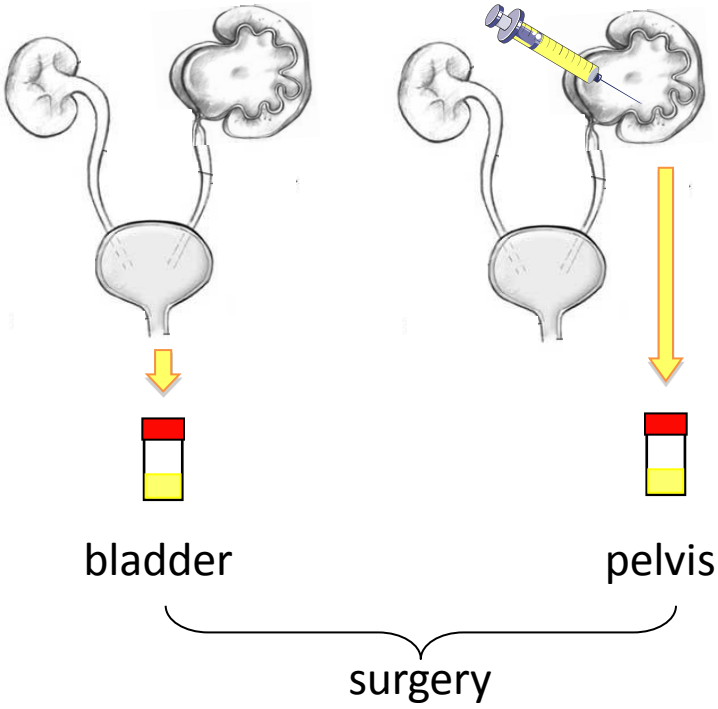
healthy

Mild obstruction
No surgery



spontaneous resolution

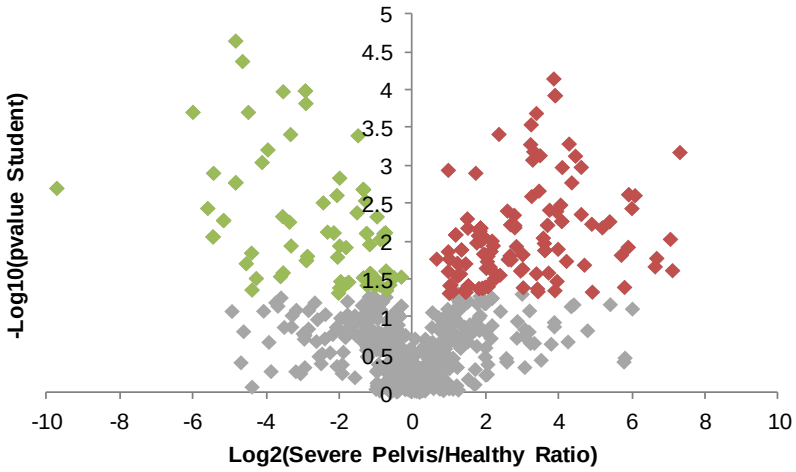
Severe obstruction
Need surgery

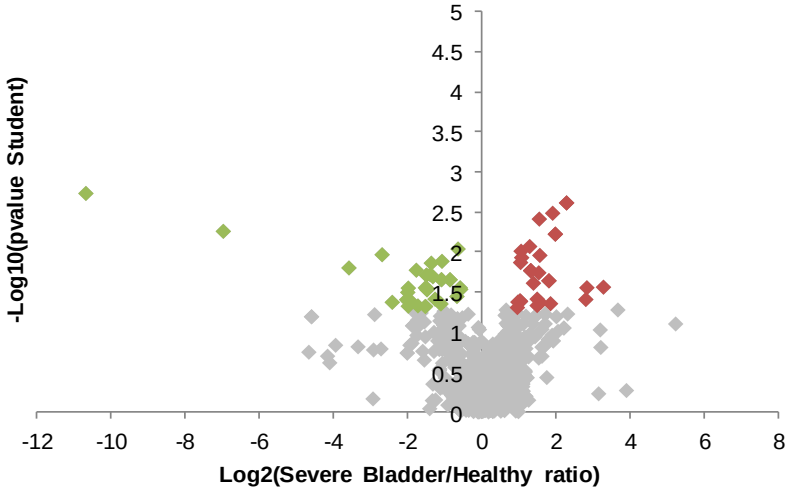


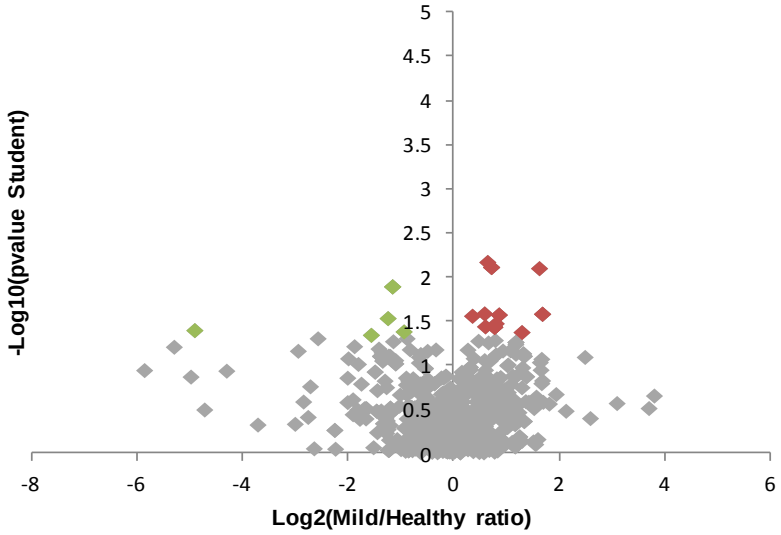
- Sample processing by FASP
- nanoLC-MS/MS analysis on a LTQ-Orbitrap Velos,2h gradients
- Quantitative analysis with MFPaQ

Label-free quantitative analysis of urine samples using MFPaQ

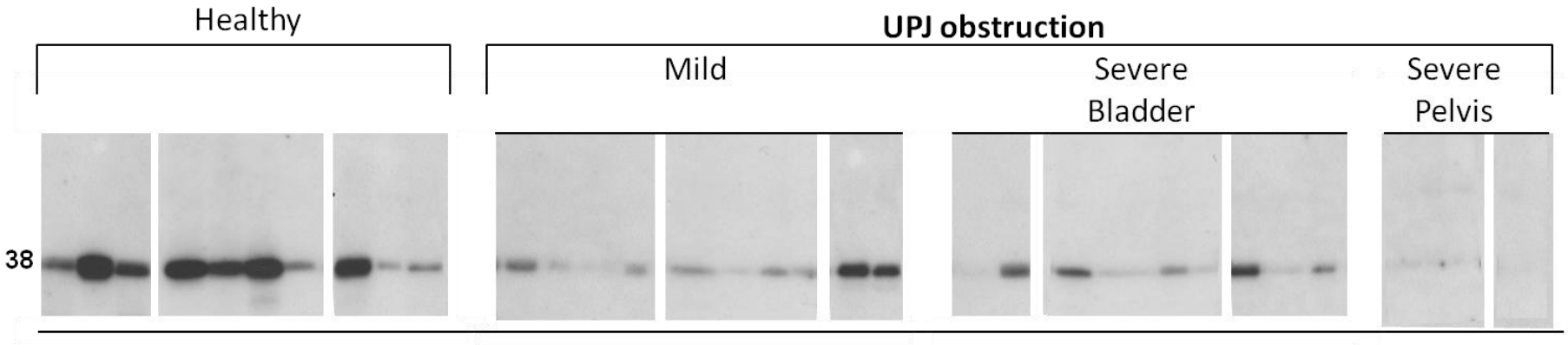
- *Around 800 urinary proteins quantified*
- *Volcano plot showing t test p-values versus protein ratio*



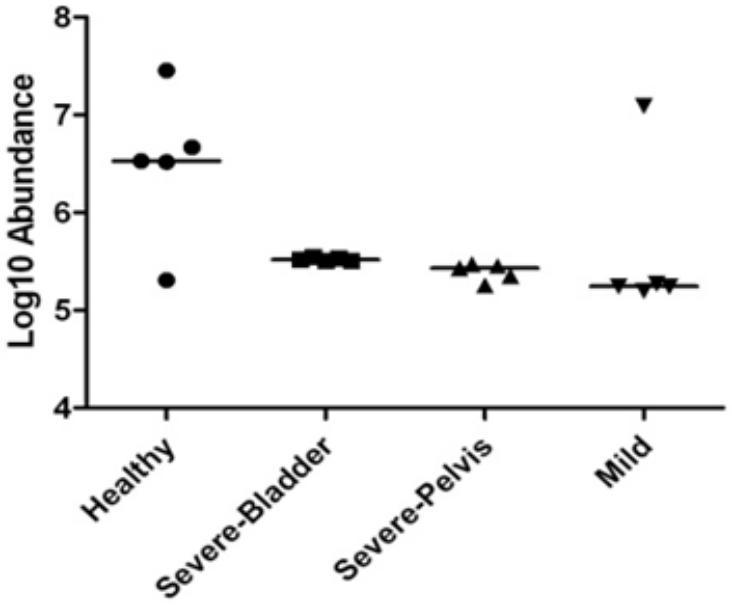




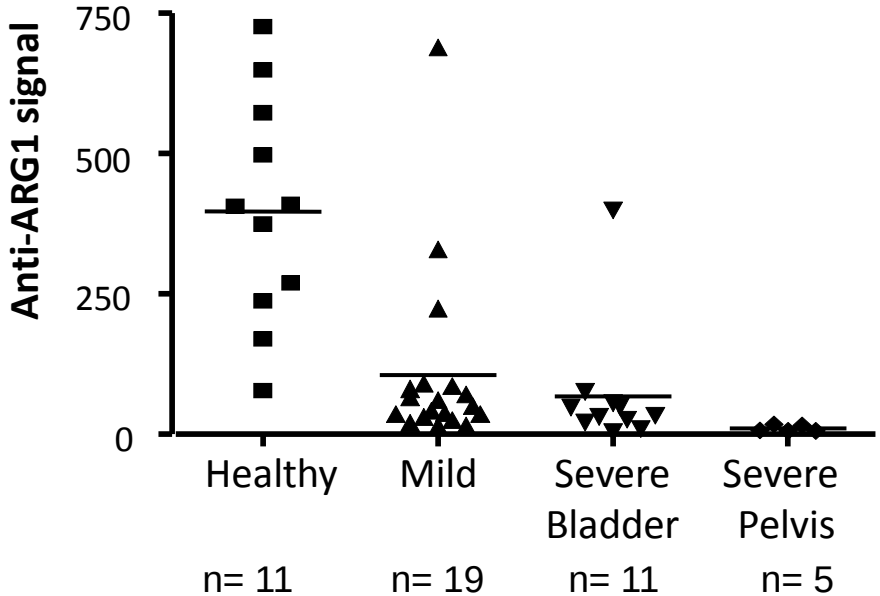
ARG1 immunodetection in urinary samples



LC-MS data



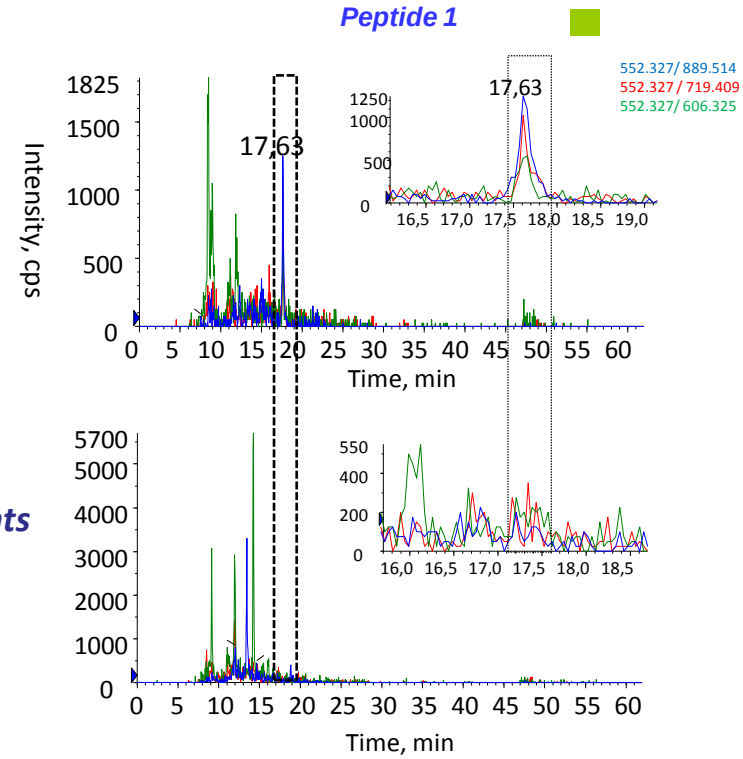
Western-blot data

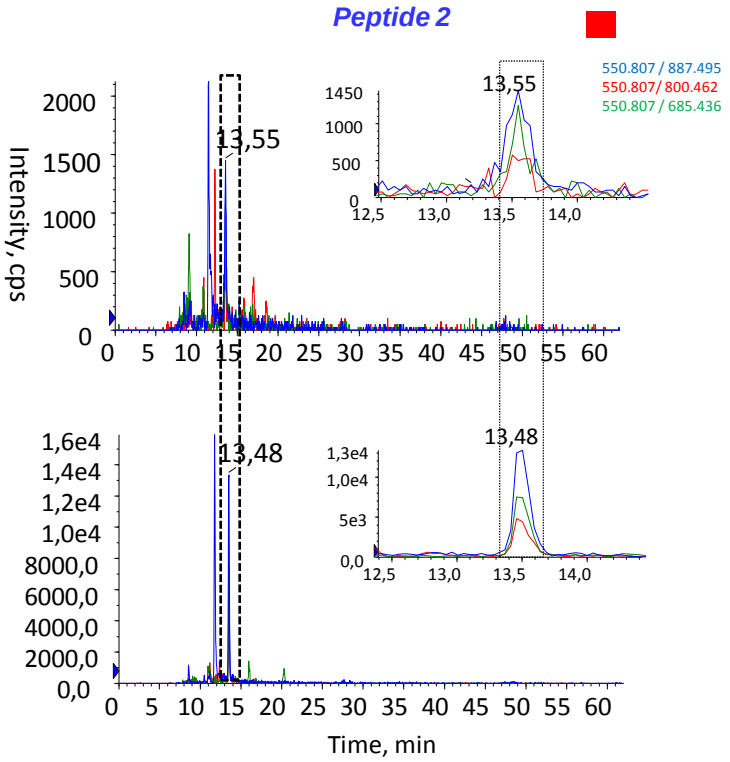


Validation by MRM

Healthy sample

Pool of patients





Confirmed expression where Western blot failed

Perspectives :

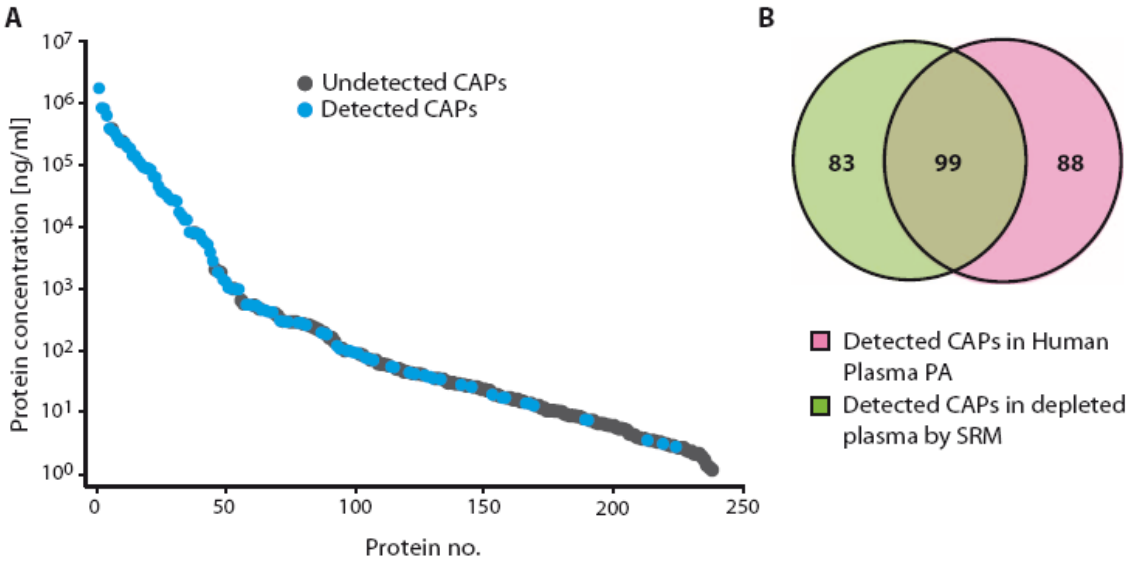
- ***Detection on the smallest volume of urine to increase patient number during validation***
  - ***Optimisation of the sample preparation***
  - ***Relative quantification of the proteins for validation on a cohort of 20 new samples per group***

Monitoring biomarkers by MRM in large patient cohorts

Hüttenhain et al, Reproducible quantification of cancer-associated proteins in body fluids using targeted proteomics. Sci Transl Med. 2012 Jul

➢Generation of a library of MRM assays for more than 1000 proposed candidate biomarker proteins, previously associated with cancer

➢ Detectability in biofluids: 182 proteins detected in depleted plasma and 408 in urine



➢ Monitoring of 34 biomarkers candidates across 83 patient plasma samples

Remerciements

Proteomics and Mass Spectrometry of Biomolecules

Florence Dalvai
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Bernard Monsarrat