

Targeted proteomics assays for biomarker evaluation

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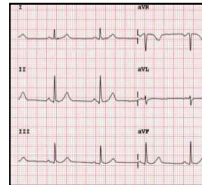
clinical
biomarkers

Biomarker workshop, Greece, September 2019

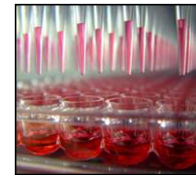
Introduction

About biomarkers...

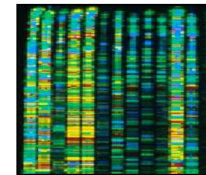
- **Definition** : Biomarkers are biological characteristics that are objectively measured and evaluated as indicators of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention.



ECG



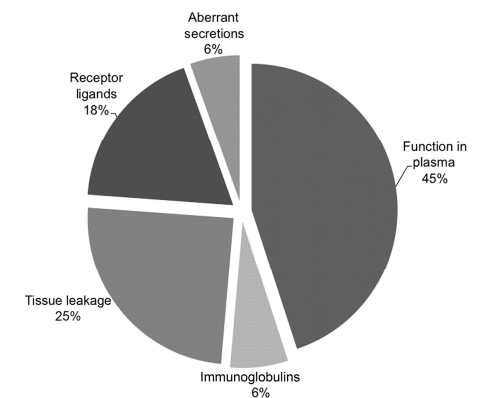
Proteins, metabolites



Genes

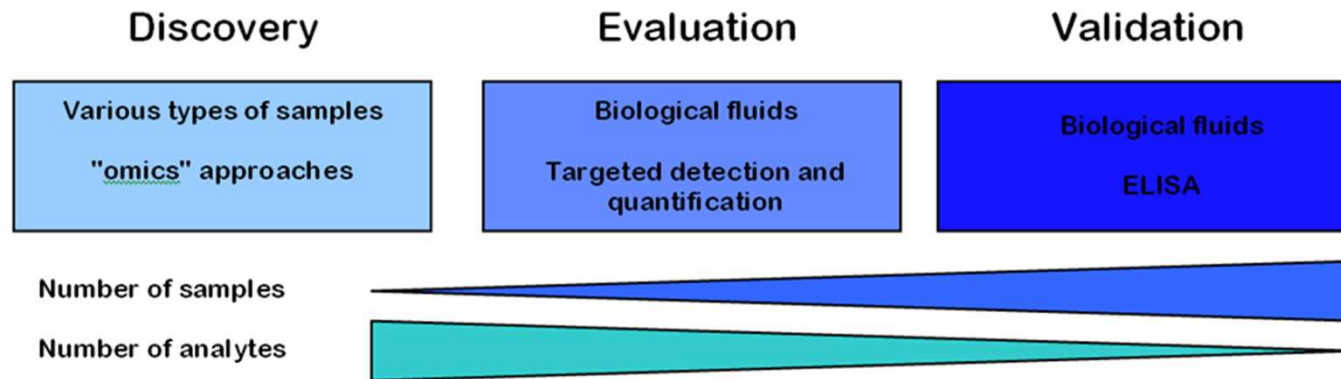
- To date, ≈ 200 plasma proteins are used as biomarkers in clinical biology for the diagnosis and monitoring of diseases, ≈ 110 are fully validated by health authorities (FDA, EMA)

- Diagnosis
- Prognosis
- Therapeutic guidance and follow-up



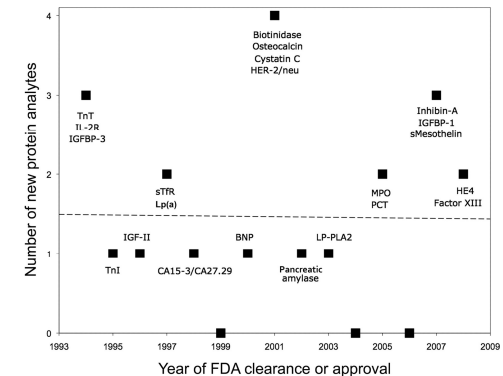
Source : Anderson, Clin Chem, 2010

The biomarker development pipeline



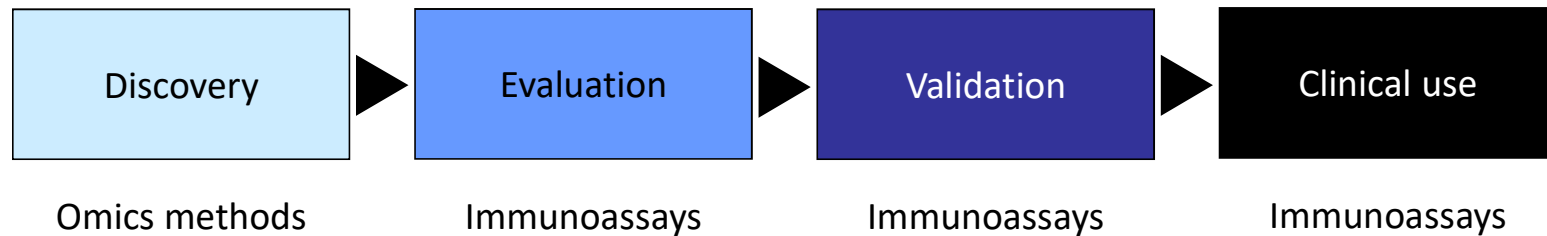
Adapted from Rifai *et al*, Nature Biotech, 2006

- The clinical implementation of a new biomarker depends on:
 - a proven medical value
 - a validated assay
 - socio-economical considerations
- A high attrition rate...



Source : Anderson, Clin Chem, 2010

Limitations in biomarker development



LIMITATION N°1

Availability of Abs & cost of immunoassay development

LIMITATION N°2

Detection of proteoforms
(Specificity issue)

[Cancer Biology & Therapy 8:12, 1083-1094; 15 June 2009]; ©2009 Landes Bioscience

Review

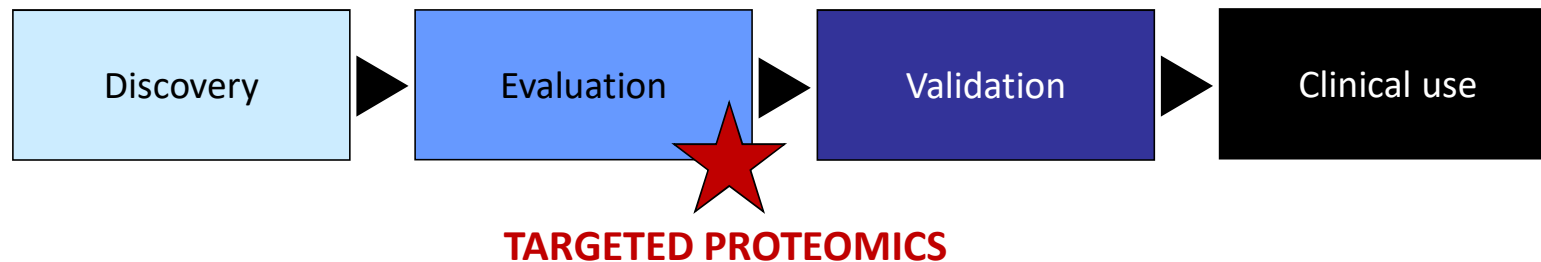
The evolving role of mass spectrometry in cancer biomarker discovery

Pei Wang, Jeffrey R. Whiteaker and Amanda G. Paulovich*

Fred Hutchinson Cancer Research Center; Seattle, WA USA

One current assay often employed is the ELISA, which is understandable since a well-functioning ELISA can be relatively high throughput and has extraordinary sensitivity for quantifying the target analyte. Unfortunately, ELISA development is costly (\$100 000–\$2 million per biomarker candidate) and associated with a long development lead time (>1 year) and a high failure rate [37,38], making it impractical to develop an ELISA for all putative biomarkers.

Targeted proteomics in biomarker development



KEY ASSETS

(comparison to immunoassays)

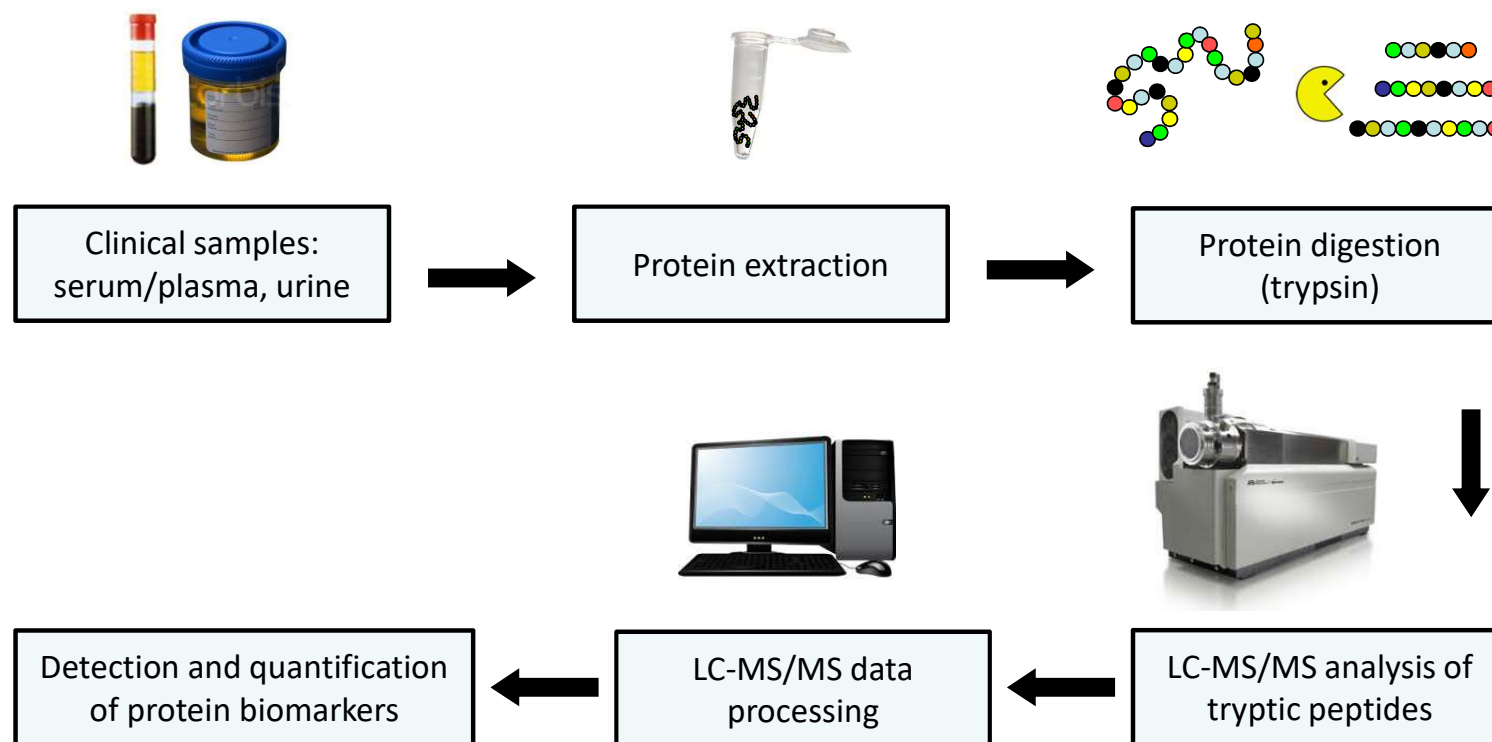
- Direct detection of analytes
- Excellent specificity
- Quantification
- Multiplexing capabilities

ISSUES TO SOLVE

- Dynamic range of biofluids → proteome coverage & low-abundant proteins
- Long, complex and manual processing of clinical samples
- Low throughput
- MS data analysis (expert processing and validation)

Development of targeted proteomics assays for biomarker evaluation

Classical proteomics workflow



Discovery proteomics vs targeted proteomics

Discovery analysis

Shotgun LC-MS/MS



MS1 → no selection

Fragmentation

MS2 → no selection (HR)



Targeted analyses

PRM



MS1 → ion selection

Fragmentation

MS2 → no selection (HR)



SRM



MS1 → ion selection

Fragmentation

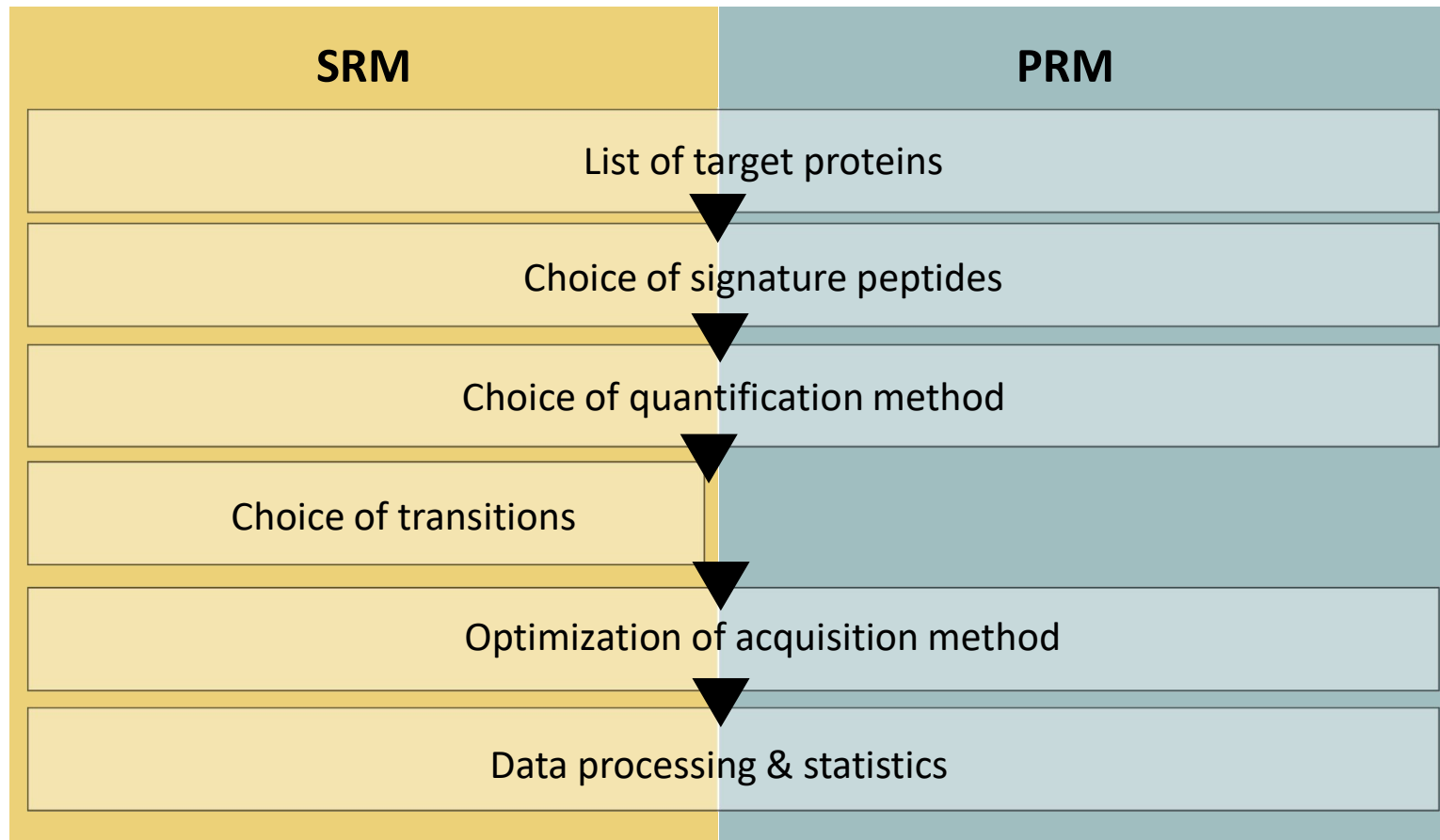
MS2 → ion selection



Proteome coverage

Sensitivity

SRM and PRM workflows



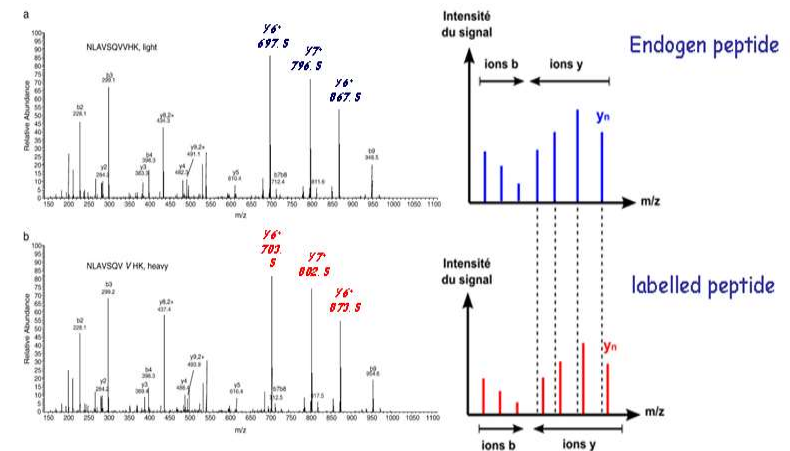
Choice of signature peptides (and transitions)



- Easily detected using liquid-chromatography-mass spectrometry
- Specific for the protein target (isoform) → BLAST
- 6-32 amino acids in length (+2, +3 charge state); mass range QqQ (400–1600 m/z)
- Generate 'intense' y product ions (+1 charge state) → used to established SRM transitions
- No more than 40% hydrophobic residues
- No arginine or lysine doublets (RR, KK, RK, KR)
- No missed cleavage
- No PTM or chemical modification
- Transitions per peptide: minimum 3

Useful tools

-ESP predictor (Fusaro V. et al., *Nature Biotechnology*, 2009)
<http://www.broadinstitute.org/cancer/software/genepattern/espselector>
 -PeptideSieve (Mallick P. et al., *Nature Biotechnology*, 2007)
<http://tools.proteomecenter.org/wiki/index.php?title=Software%3APeptideSieve>
 -PepFly (Sanders W. et al., *BMC Bioinformatics*, 2007)
<http://www.mybiosoftware.com/pepfly-peptide-flyability-prediction.html>



Source : Dupuis et al, *J Proteomics* 2009

Choice of quantification standard(s)

- **PSAQ Protein Standard Absolute Quantification**

☞ Brun *et al*, Mol Cell Proteomics, 2007

- **PrEST Protein Epitope Signature Tag**

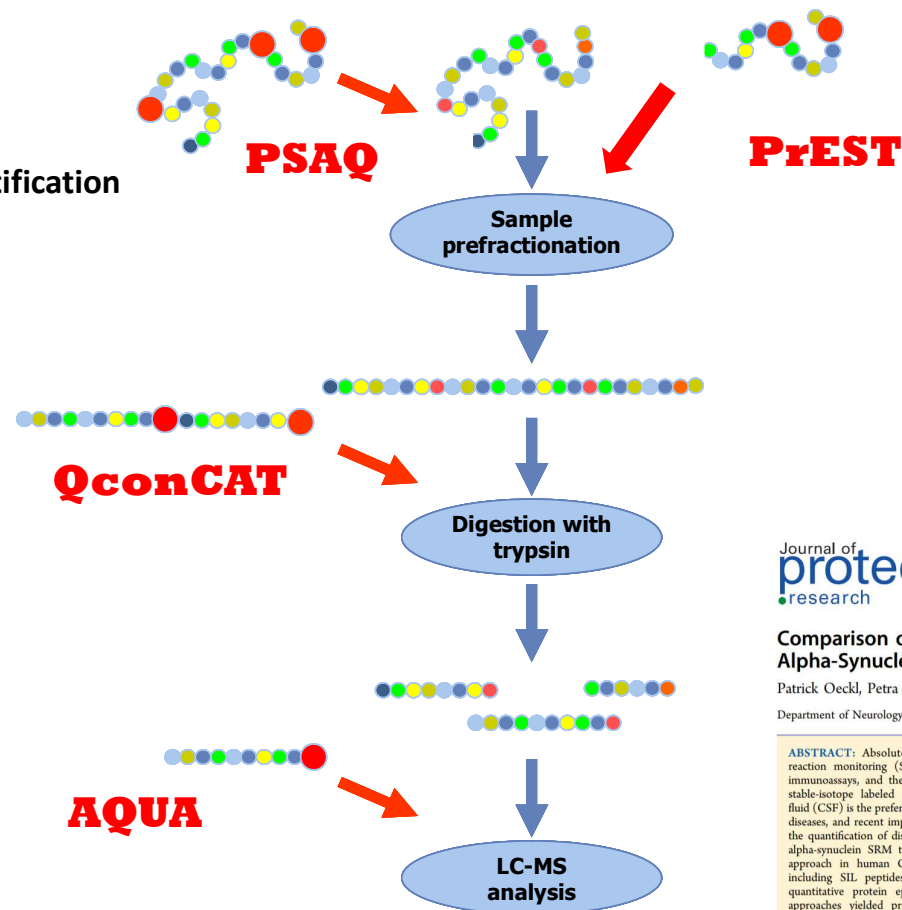
☞ Zeiler *et al*, Mol Cell Proteomics, 2012

- **QconCAT concatemer**

☞ Beynon *et al*, Nat Methods, 2005

- **AQUA peptides**

☞ Gerber *et al*, PNAS, 2003

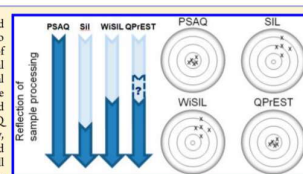


Comparison of Internal Standard Approaches for SRM Analysis of Alpha-Synuclein in Cerebrospinal Fluid

Patrick Oeckl, Petra Steinacker, and Markus Otto*

Department of Neurology, Ulm University Hospital, D-89081 Ulm, Germany

ABSTRACT: Absolute protein quantification by selected reaction monitoring (SRM, also MRM) is an alternative to immunoassays, and the gold standard here is the addition of stable-isotope labeled (SIL) proteins (PSAQ). Cerebrospinal fluid (CSF) is the preferred source of biomarkers for neurological diseases, and recent improvements in mass spectrometry enable the quantification of disease-relevant proteins in CSF. We used alpha-synuclein SRM to investigate alternatives to the PSAQ approach in human CSF regarding precision and accuracy, including SIL peptides, winged SIL (WISIL) peptides, and quantitative protein epitope signature tags (QPrESTs). All approaches yielded precise results in CSF with CV values



Optimization of acquisition method



SRM

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 www.molecularsystemsbiology.com



REVIEW

Selected reaction monitoring for quantitative proteomics: a tutorial

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of analytes. Proteomic studies are commonly performed using a shotgun approach, in which the sample proteins are enzymatically degraded to peptides, which are then analysed by mass spectrometry (MS). Thereby, a subset of the peptides present in the sample is automatically and in part stochastically selected by the mass spectrometer in a process referred to as data-dependent precursor selection. Systems biology requires accurate quantification of a specified set of peptides/proteins across multiple samples derived from cells in differentially perturbed states (Ideker *et al.*, 2001). This stringent requirement is driven by the long-term goal of systems biology to generate mathematical models that simulate the system and make specific predictions about its

Sensitivity

Dwell time:
 Has to be as long as possible

Between 5ms and 100ms

Accuracy

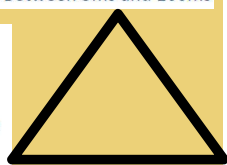
Cycle time:
 Has to be as short as possible
 Conditions the number of points measured per chromatographic peak

Between 1.5s and 3s

Multiplexing

Number of transitions:
 Proteins, peptides, L/H...

3 to 6 transitions per peptide
 4 to 5 peptides per protein
 ~1000 transitions per run



PRM

2146

DOI 10.1002/jpmic.201500543

Proteomics 2016, 16, 2146–2159

REVIEW

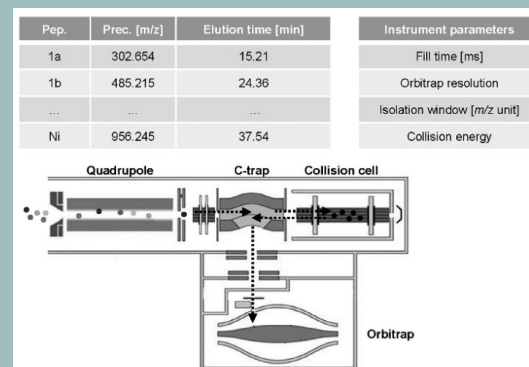
Parallel reaction monitoring using quadrupole-Orbitrap mass spectrometer: Principle and applications

Adele Bourmaud^{1,2}, Sebastien Gallien¹ and Bruno Domon^{1,2}

¹ Luxembourg Clinical Proteomics Center, Luxembourg Institute of Health (LIH), Strassen, Luxembourg
² Doctoral School in Systems and Molecular Biomedicine, University of Luxembourg, Esch-sur-Alzette, Luxembourg

Targeted mass spectrometry-based approaches are nowadays widely used for quantitative proteomics studies and more recently have been implemented on high resolution/accurate mass (HRAM) instruments resulting in a considerable performance improvement. More specifically, the parallel reaction monitoring technique (PRM) performed on quadrupole-Orbitrap mass spectrometers, leveraging the high resolution and trapping capabilities of the instrument, offers a clear advantage over the conventional selected reaction monitoring (SRM) measurements

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Data processing and statistics



Skyline Targeted Mass Spec Environment ☐ Skyline

Skyline Targeted Mass Spec Environment



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