

# Development and Implementation of Parallel Reaction Monitoring Assays

Skyline User's Meeting June 31, 2015

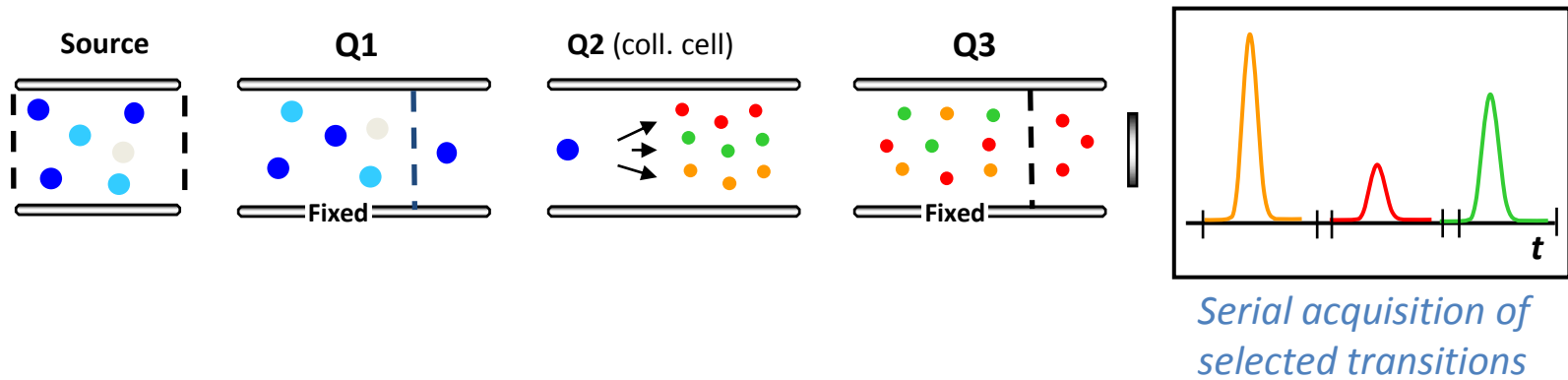
ASMS 2015 – St Louis, MO

***Bruno Doman, PhD***

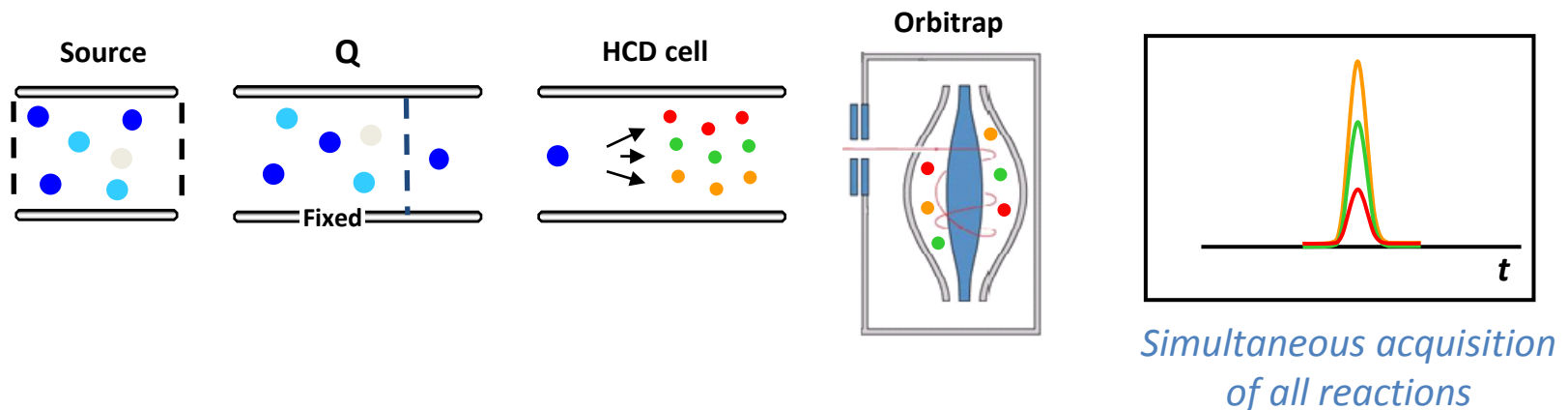
*Head Luxembourg Clinical Proteomics Center  
Invited Professor University of Luxembourg*

# Targeted Proteomics

- SRM experiments: triple quadrupole instrument



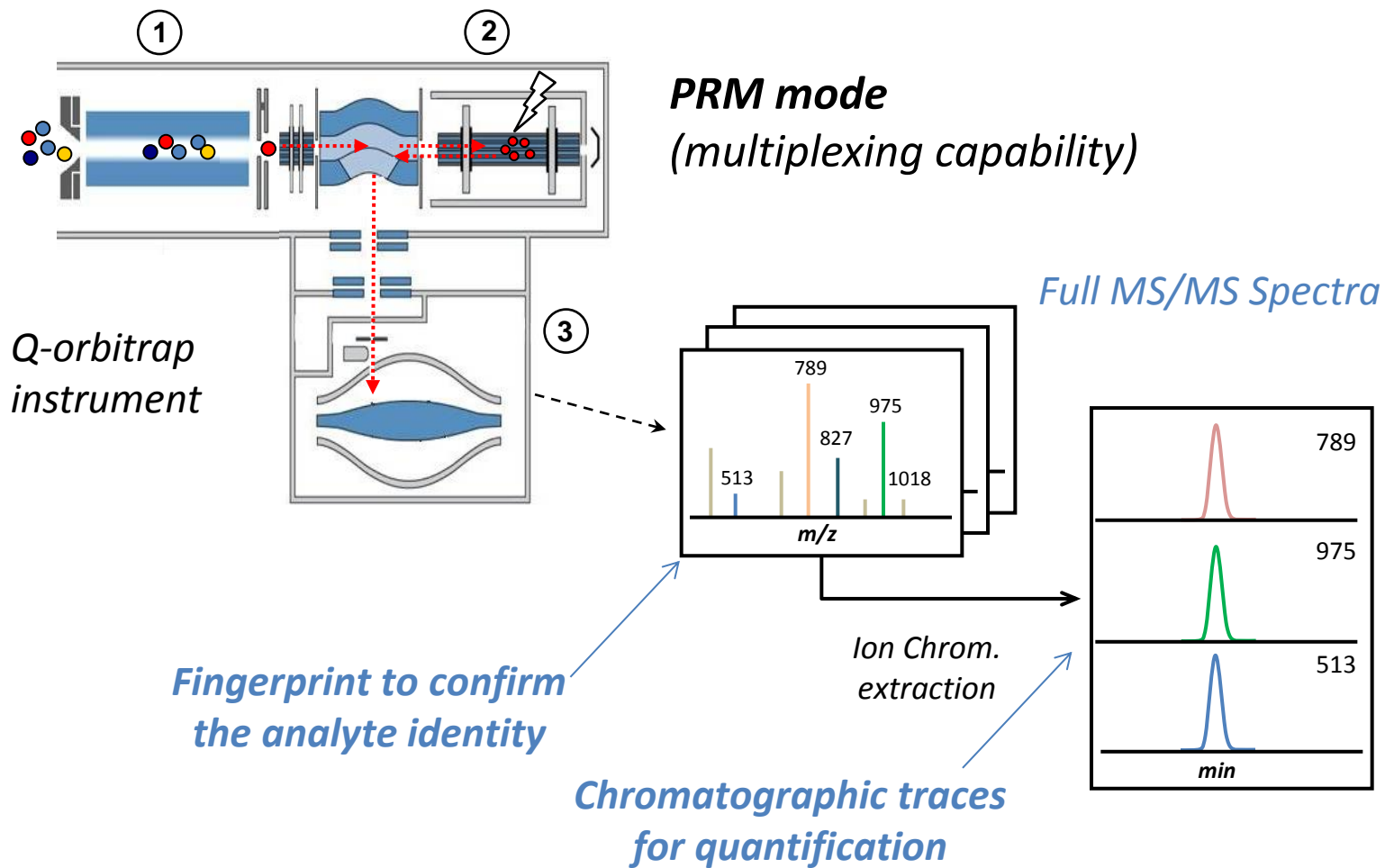
- PRM experiments: quadrupole orbitrap instrument



# PRM Analysis: Hallmarks

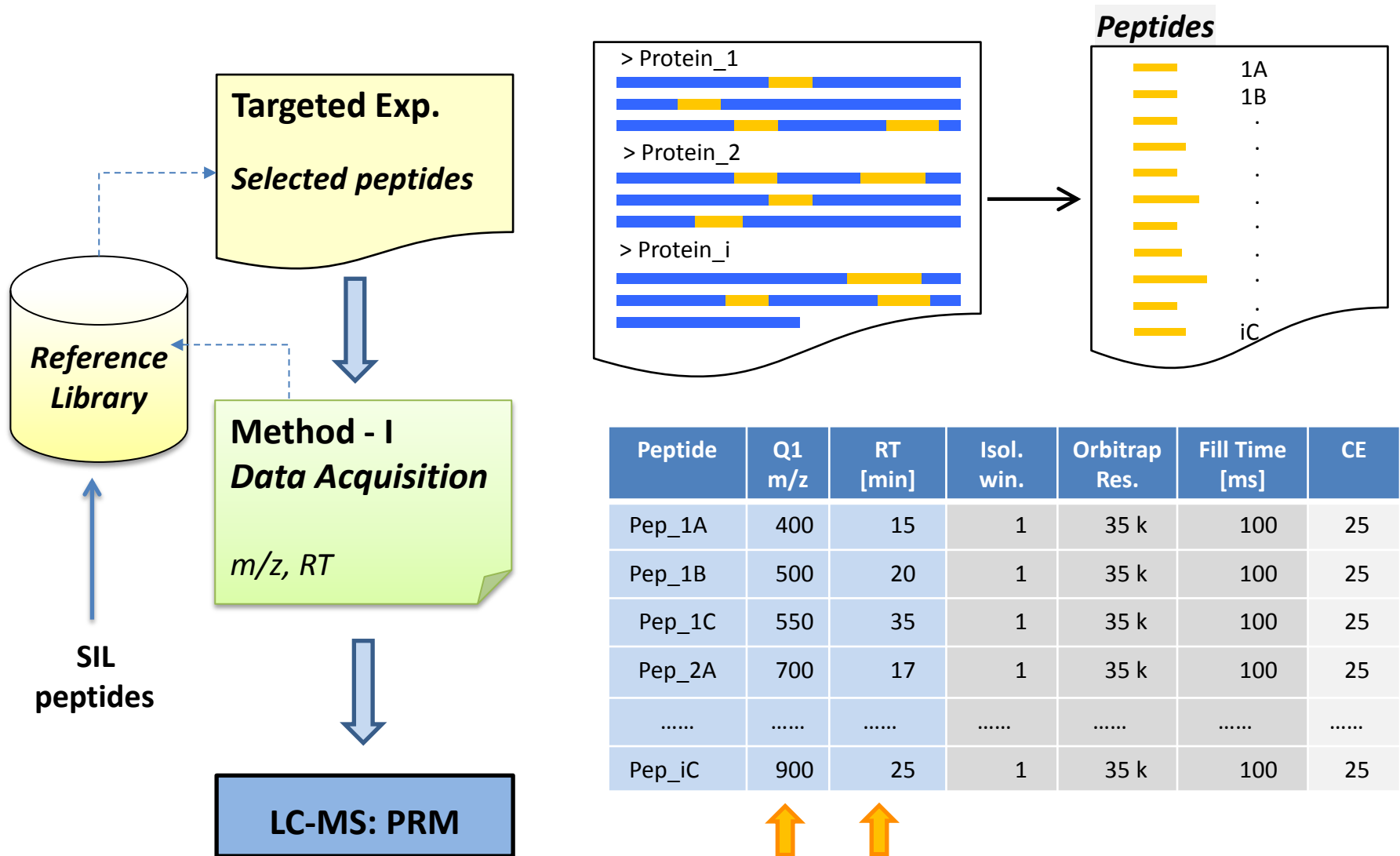
- The analysis is targeted
  - *i.e.* focused on a set of pre-defined peptides (/proteins).
  - Tools to select the peptides previously built for SRM can be used
- Full MS/MS spectra are systematically acquired
  - At each cycle (~ 2 sec) and store stored in the raw file.
- The data acquisition and the data analysis are decoupled,  
*Two distinct methods are created*
  - The Acquisition Method (Method-I)  
*Basic: Precursor ions  $m/z$  and predicted elution windows*
  - A Processing Method (Method-II)  
*Allows post-acquisition iterative data analysis (fragment ions)*

# Parallel Reaction Monitoring Experiment

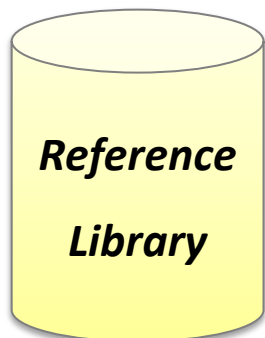


Gallien et al.; Mol. Cell. Proteomics, 2012

# Design of Acquisition Methods (PRM)



# Reference Libraries

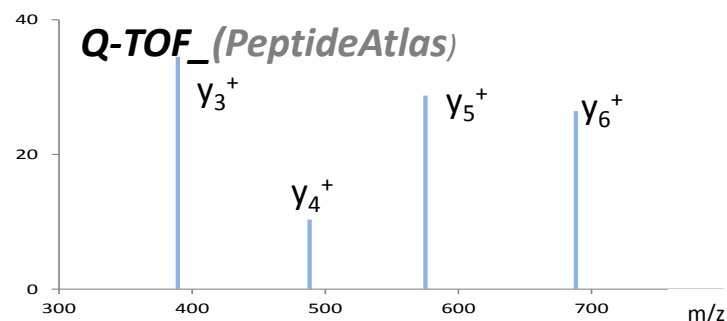
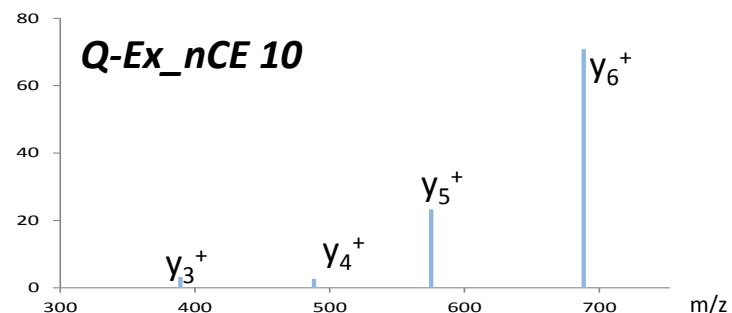
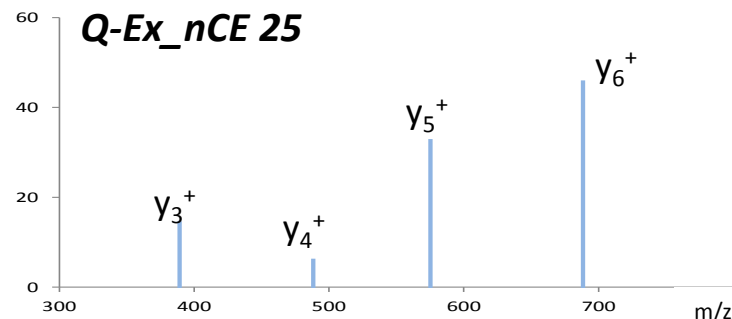


SQL  
(exported in mgf)

## Reference Library

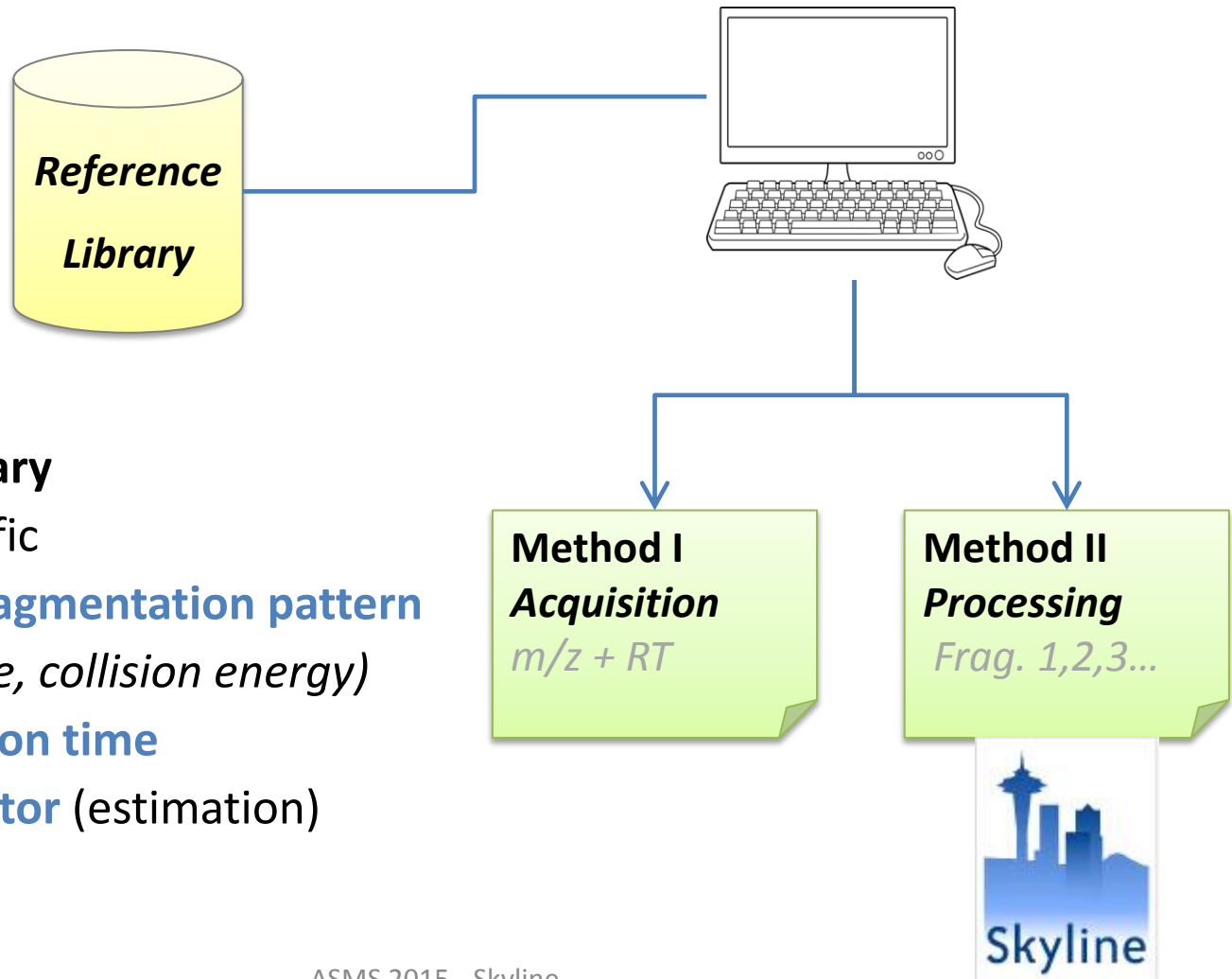
- Project specific (*SIL peptides*)
- MS/MS: Controlled fragmentation patterns (*m/z and rel. intensities*)  
(*gas pressure, collision energy*)
- LC: Defined **elution times**
- MS: Response **factor** (estimation)

NLLSVAYK



# Creation of the PRM Methods

- SIL peptides
- Discovery experiments



## Reference Library

- Project specific
- Controlled **fragmentation pattern**  
(*gas pressure, collision energy*)
- Defined **elution time**
- Response **factor** (estimation)

# Design of PRM Experiments

- **Acquisition Method** *{Decoupled from processing}*
  - Peptide specific parameters (*elution time, m/z precursor, coll. energy*)
  - Instrument parameters (*resolution, fill time*)
- **Data Processing** *{Paradigm shift}*
  - Confirmation of peptide identity (*fragmentation pattern*)
  - Quality control (*interferences ?*)
  - Quantification (*extraction of traces, integration*)

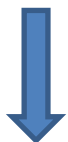


# PRM: Processing Method

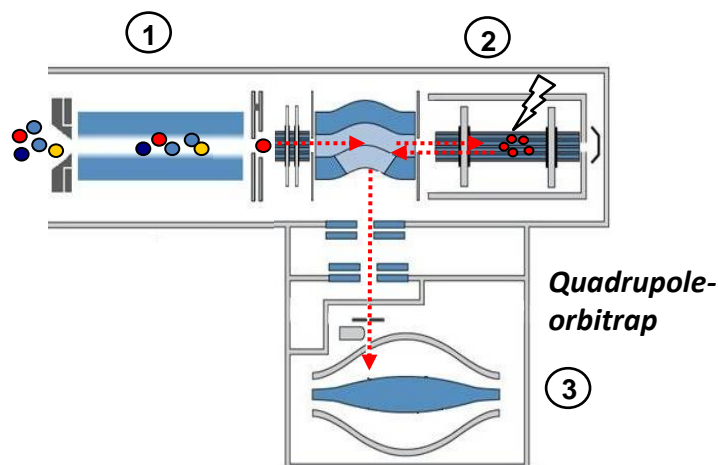
**Method - I**  
*Data Acquisition*



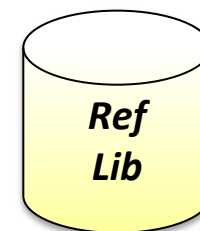
**LC-MS: PRM**



**Method - II**  
*Data Processing*



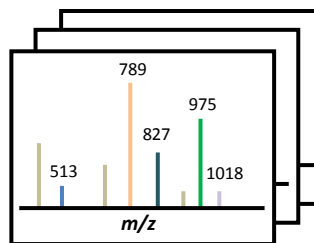
*Quadrupole-orbitrap*



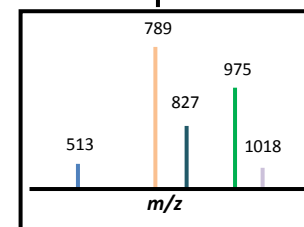
*Reference spectrum*

*Full MS/MS spectra*

*Identity confirmation*

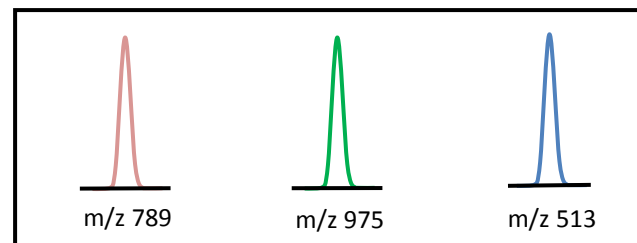


**Matching**

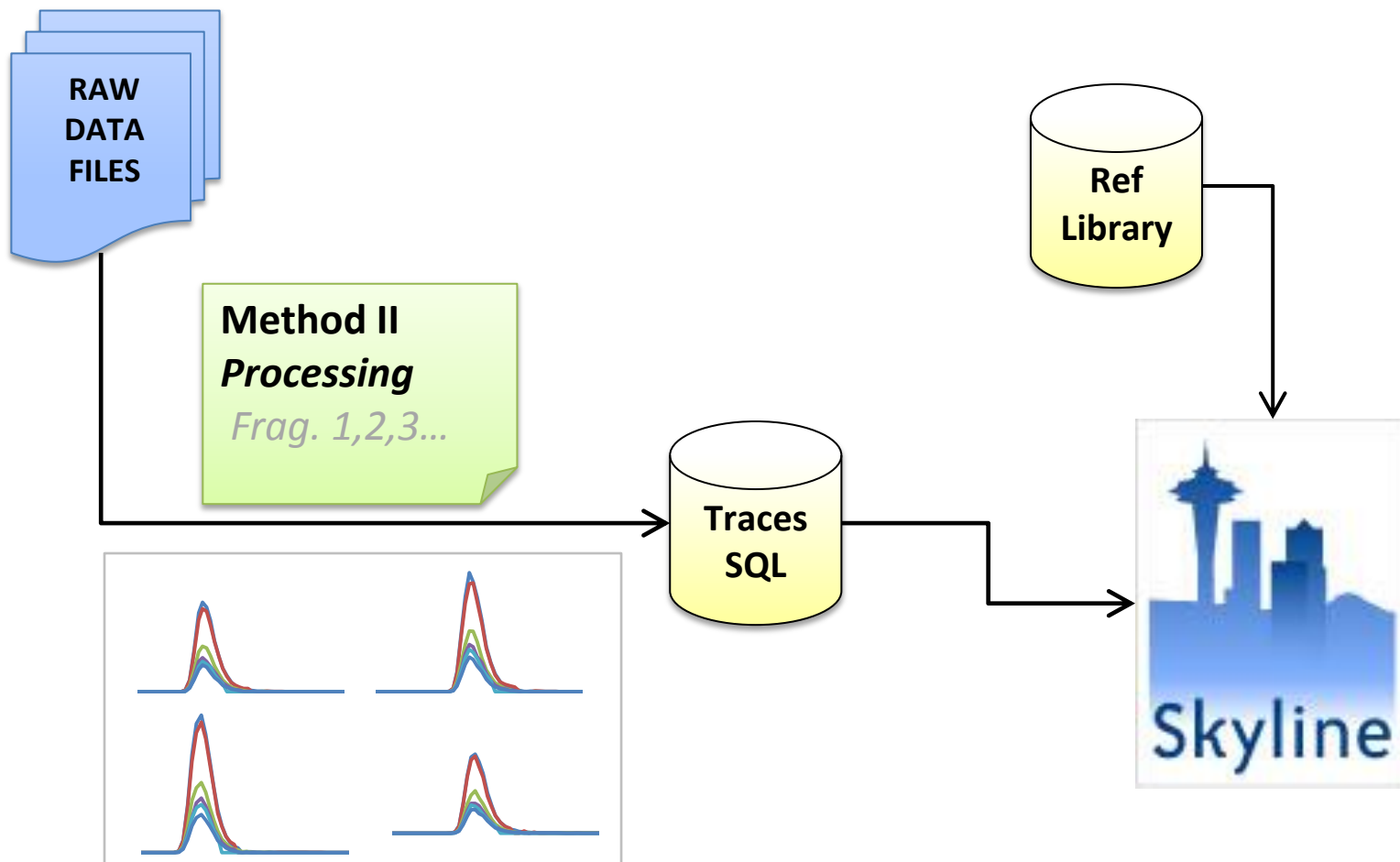


*Ion chrom. extraction*

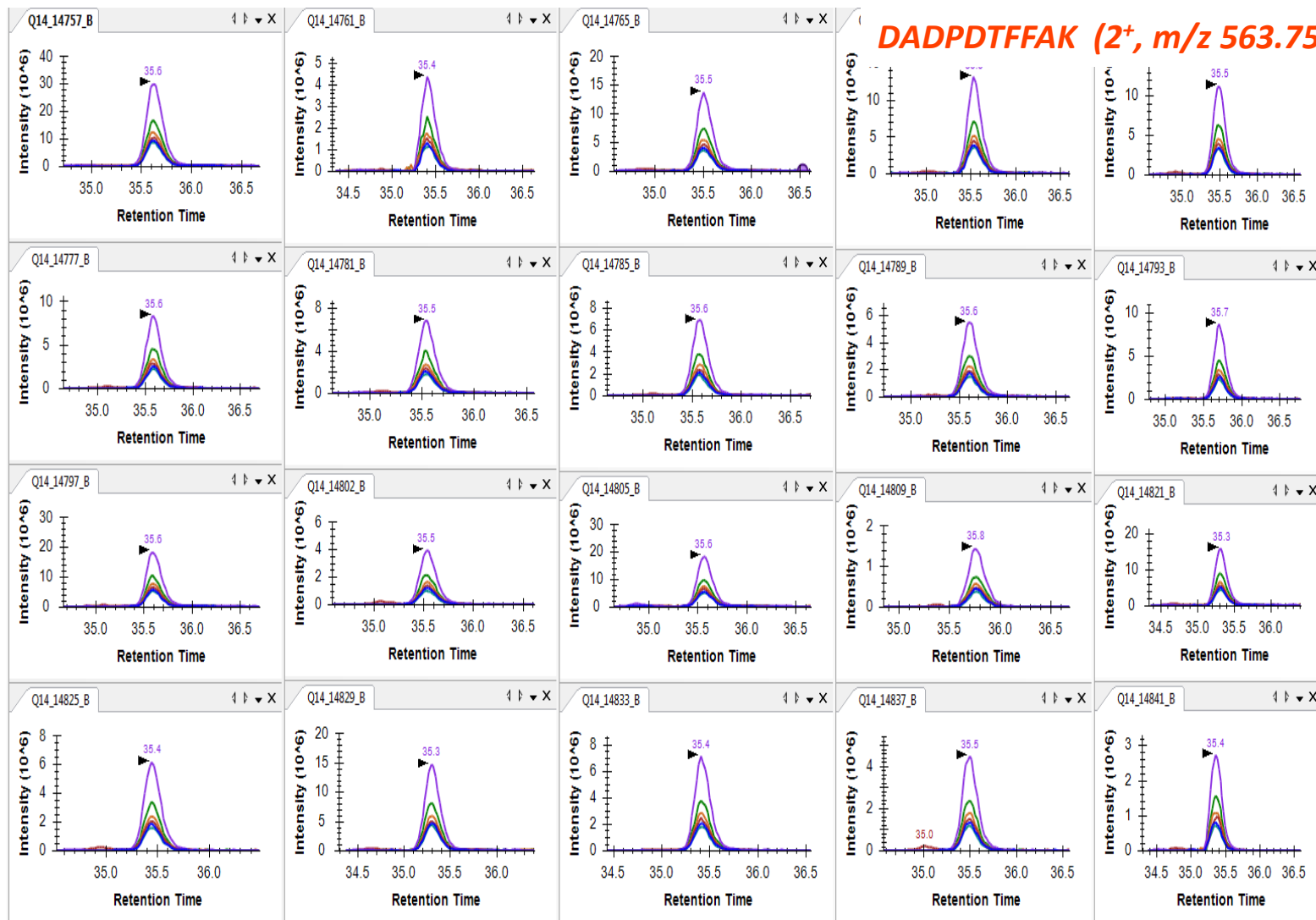
*Quantification*



# Proposed Data Analysis Workflow

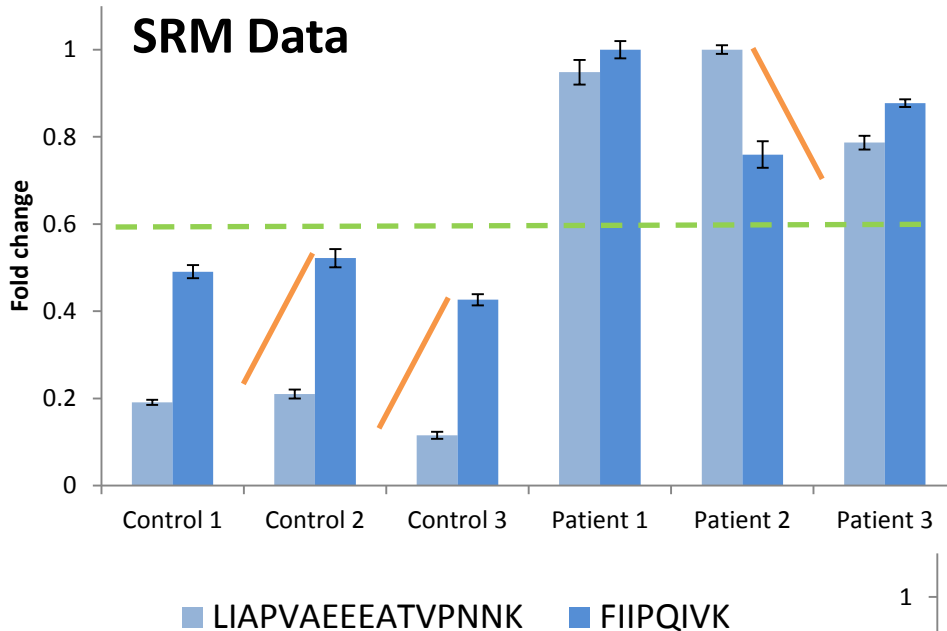


# Analysis of the Data Using Skyline



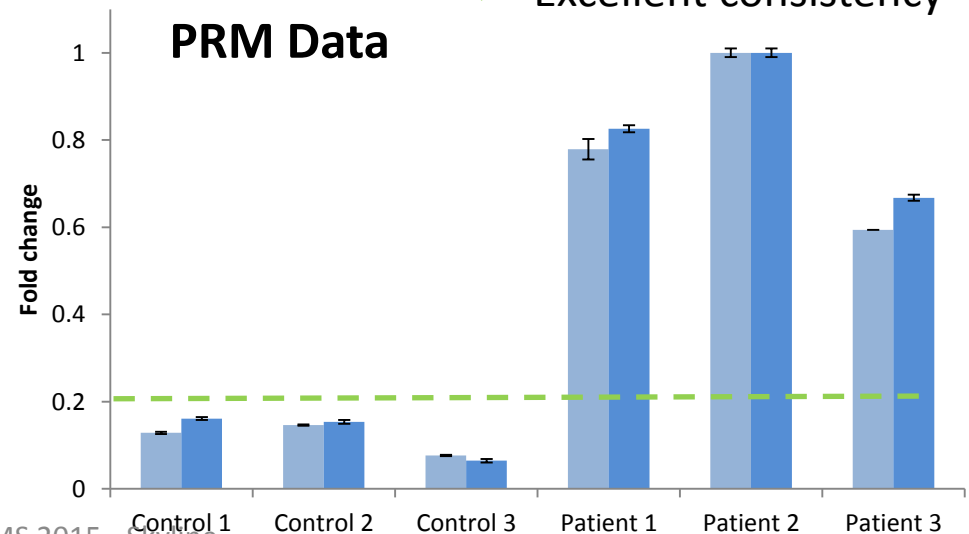
# Benefit #1: Enhanced Selectivity

✘ Poor correlation



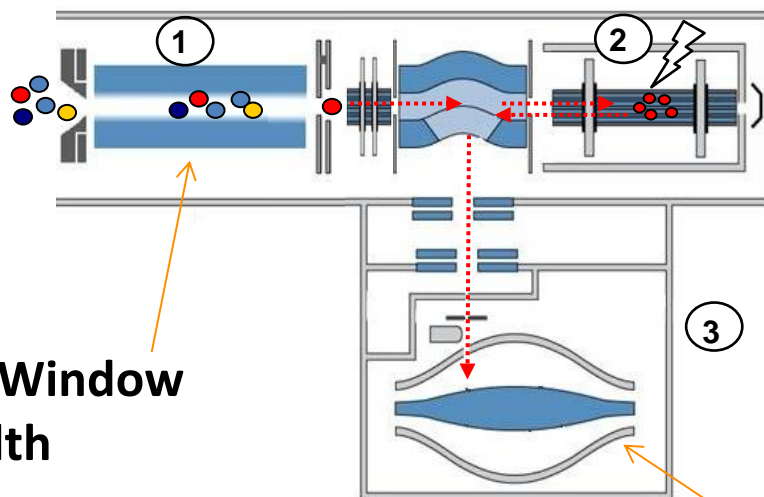
- Peptides **FIIPQIVK** and **LIAPVAEEEEATVPNNK**
- Surrogates: *L-lactate dehydrogenase*
- Plasma samples (*Alb+IgG depleted*)
- **Pilot study**: 3 controls and 3 disease

✔ Excellent consistency





# Selectivity of PRM Measurements



**Selection Window  
Width**

[Typically: 1.0 (2.0)  $m/z$ ]

High selectivity: 0.7 (0.4)  $m/z$ ]

(High performance Quad)

**High Resolution (OT)**

[35,000 – 140,000]

# Targeted Experiments (PRM)

## Classical Quant. Experiment

- **Precise quantification**  
*(biomarkers)*
- **Internal standards**  
*(calibrated amount)*
- **Limited number of analytes**

## Screening Experiment

- **Detection of peptides**  
*in complex matrix*
- **Large scale**  
*(hundred of candidates)*
- **Multiplexing capability**

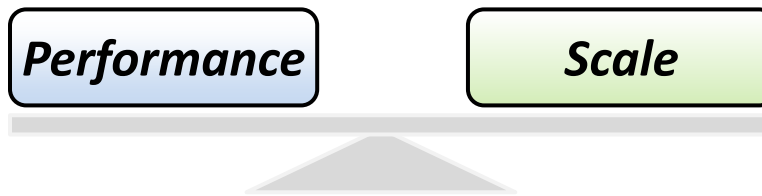
*Selectivity + Sensitivity*

*Nb Peptides*

**Performance**

**Scale**

# Targeted Experiments (PRM)



Parameters	Q-Exactive HF	
Resolving power	60 k	15 k
Transient time [ms]	128	32
Fill time [ms]	< 120	< 30
Nb peptides / cycle	6 ~ 10	60

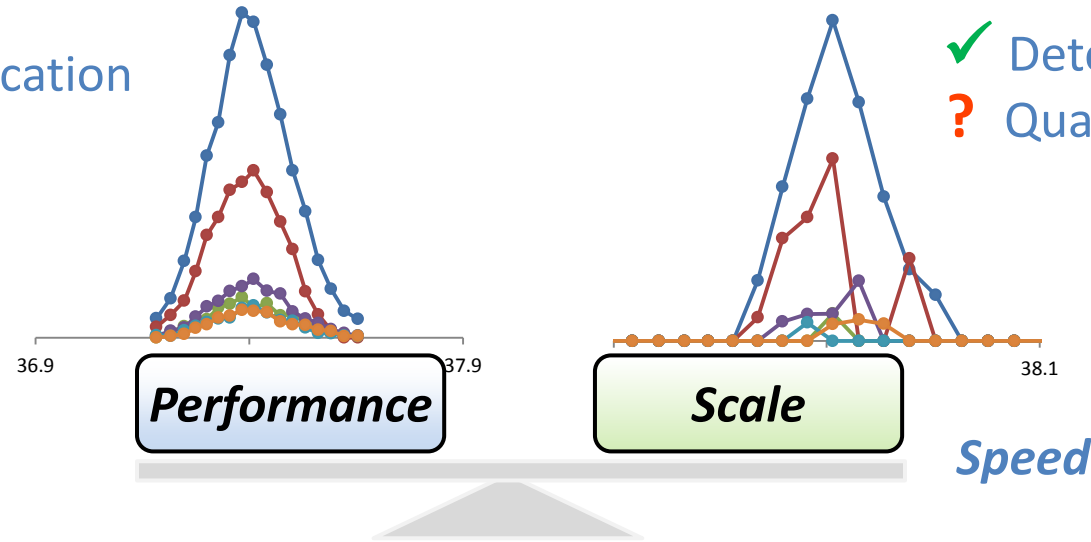
(\*) Cycle time: 2 s;



# Targeted Experiments (PRM)

✓ Quantification

✓ Detection  
? Quantification

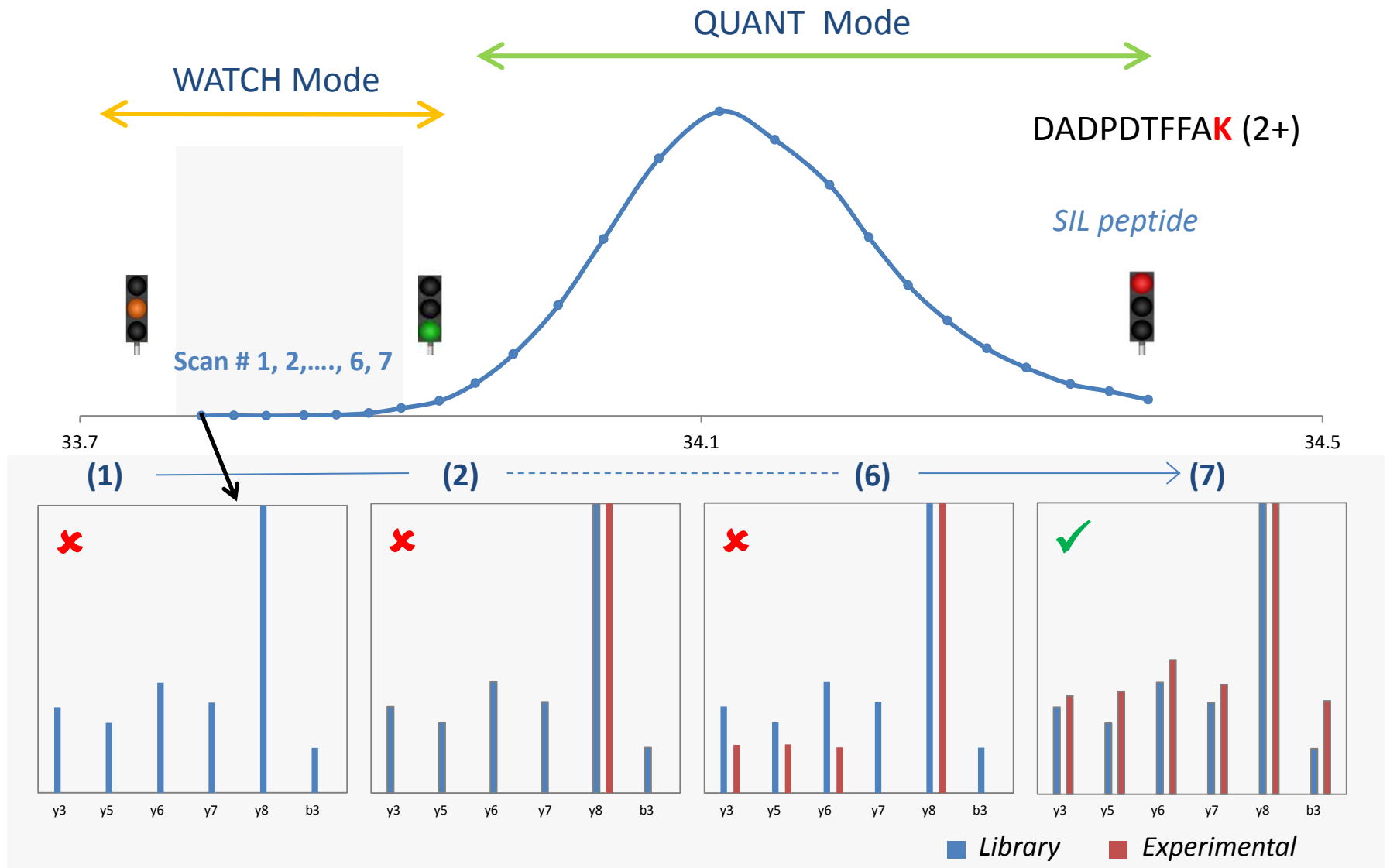


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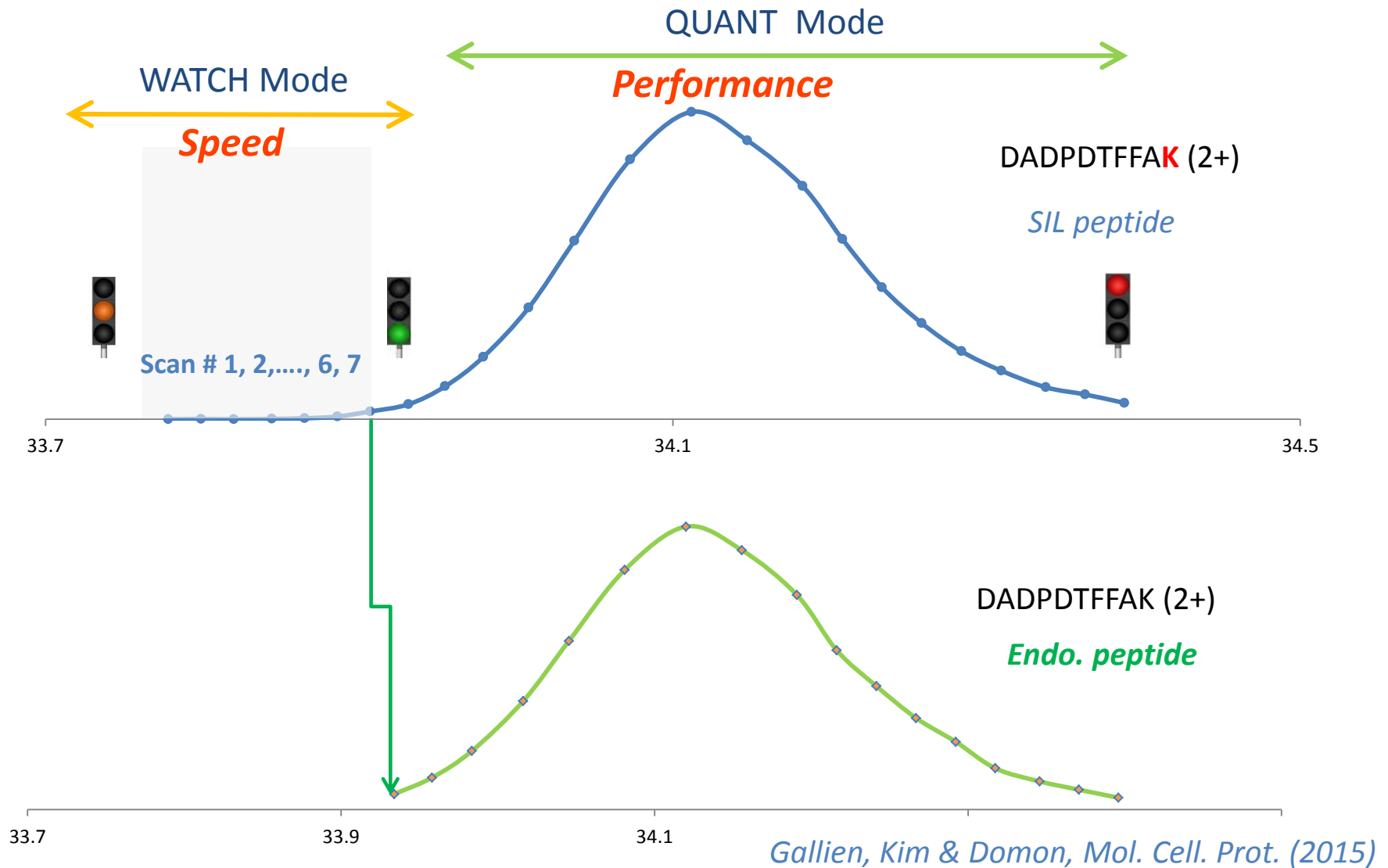
(\*) Cycle time: 2 s;

*Can we marry both worlds ?*

# IS Triggered-PRM: WATCH Mode (IS-PRM)



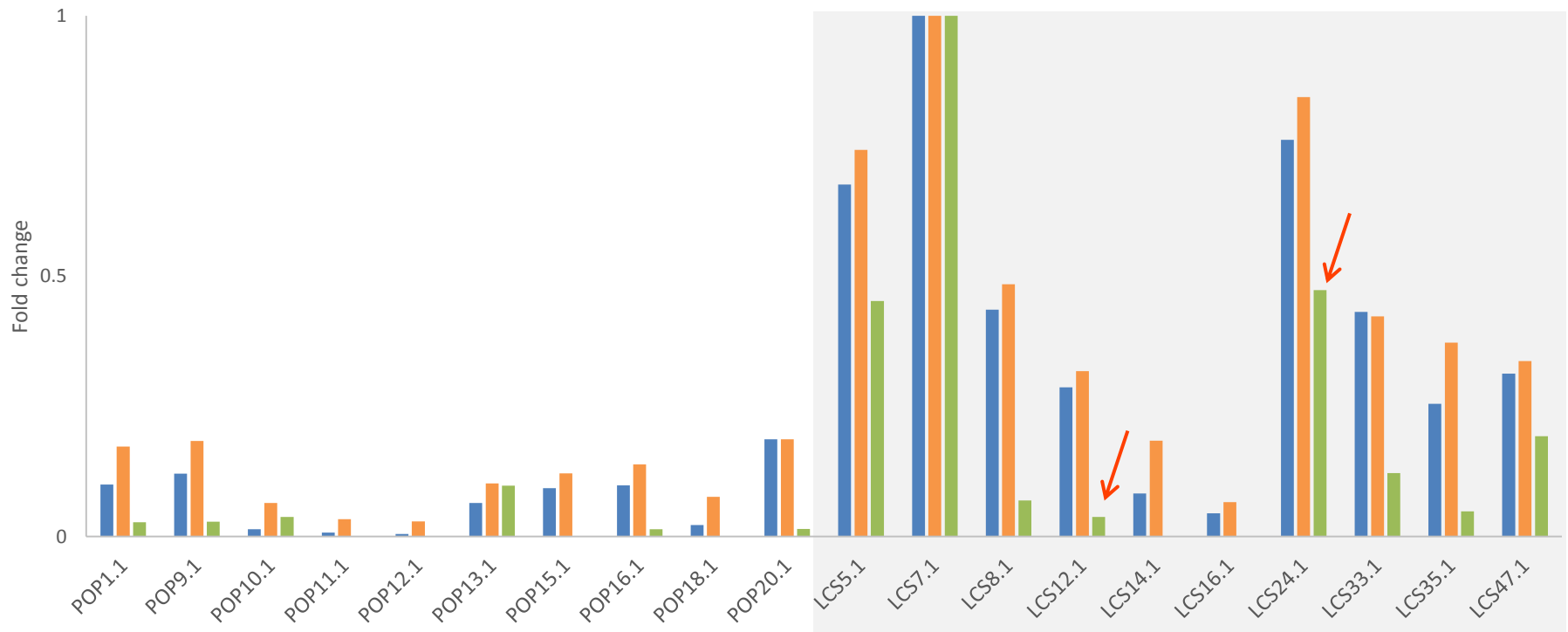
# IS Triggered-PRM: QUANT Mode (IS-PRM)



# Screening of Biomarkers

Example : *PGK1 Phosphoglycerate kinase 1*

PRM



■ YAEVTR

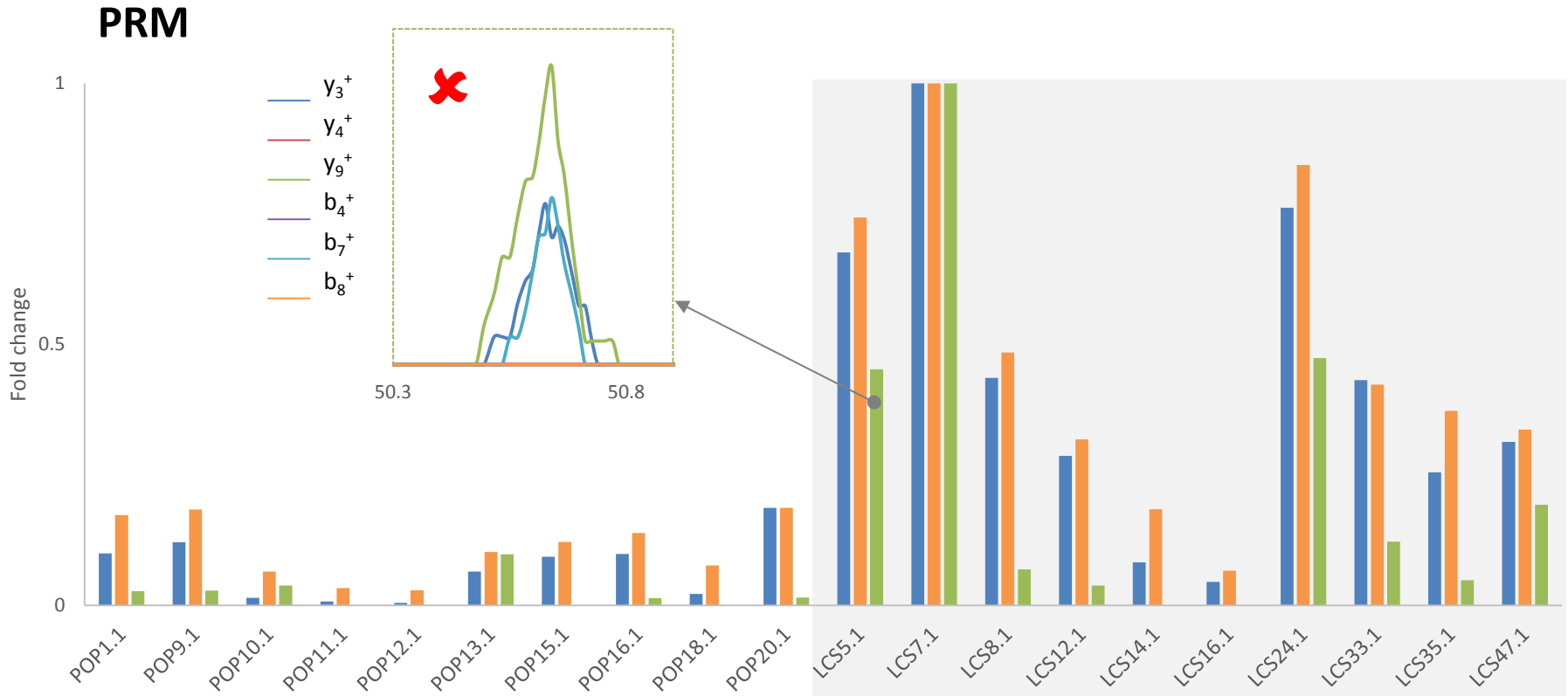
■ ALMDEVVK

■ VLPGVDALSNI

Poor Consistency

# Screening of Biomarkers

Example : *PGK1 Phosphoglycerate kinase 1*



■ YAEVTR

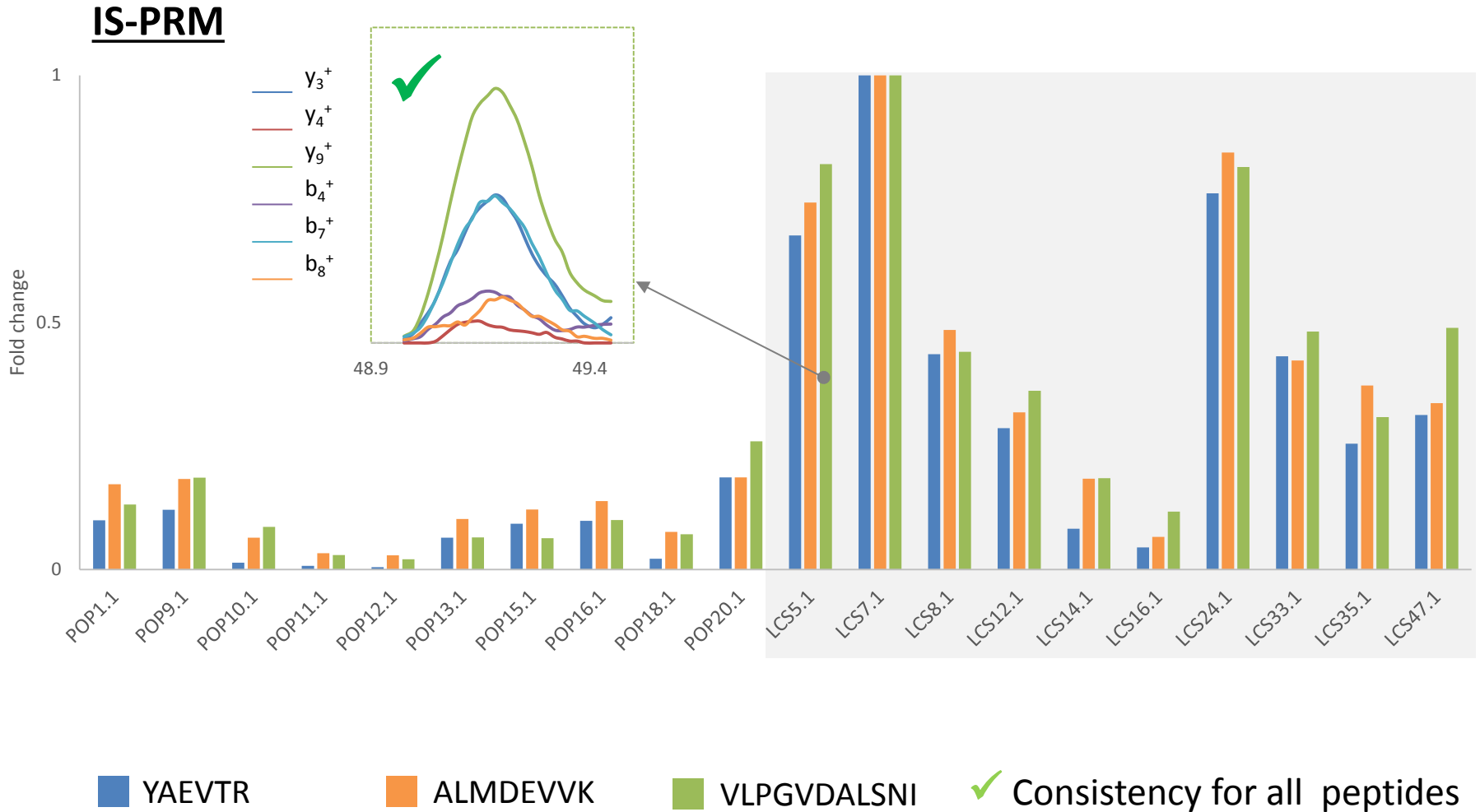
■ ALMDEVVK

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Poor Consistency

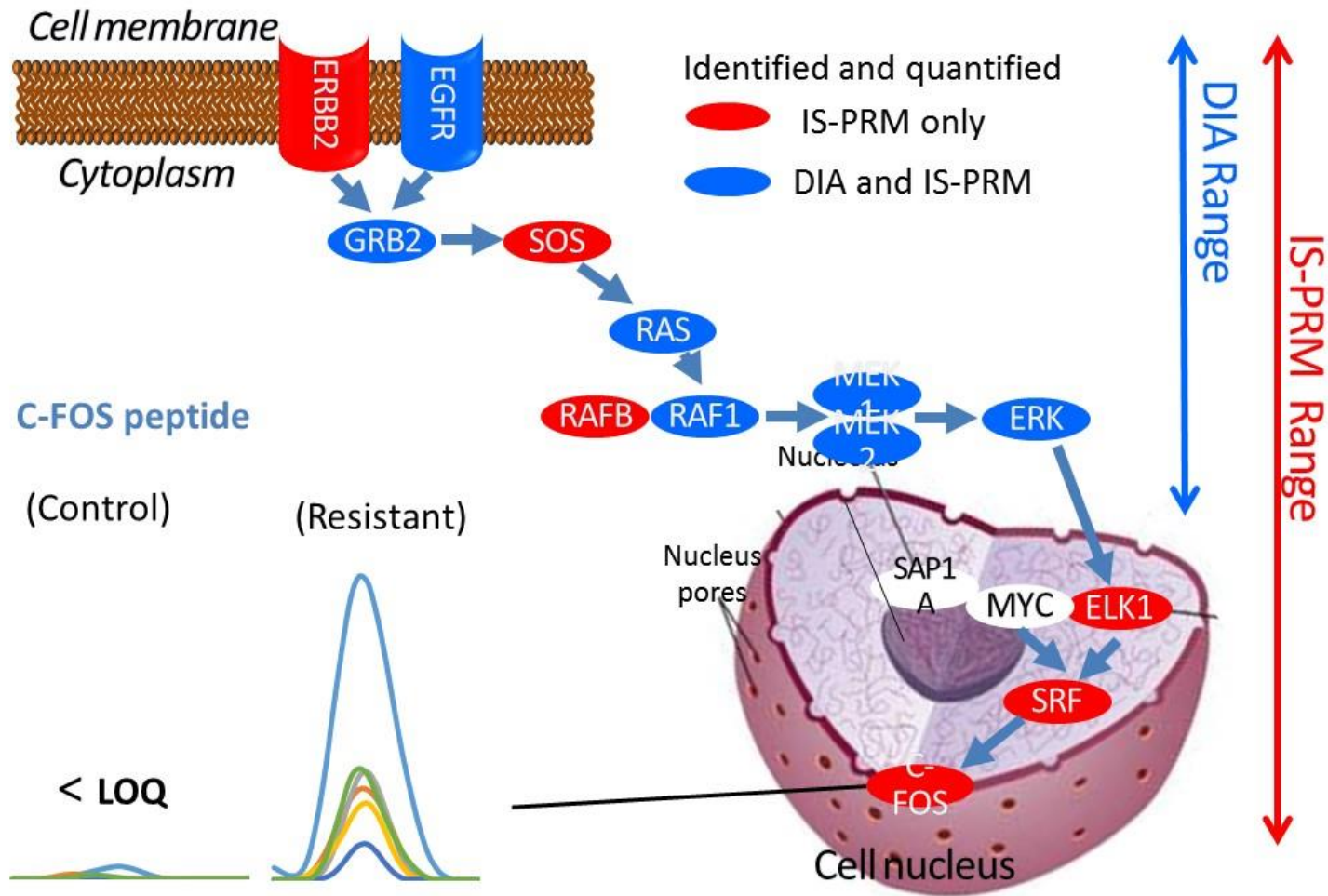
# Screening of Biomarkers:

Example : PGK1 Phosphoglycerate kinase 1



# Analysis of MAPK Signaling Pathway

Poster  
ThOP # 342

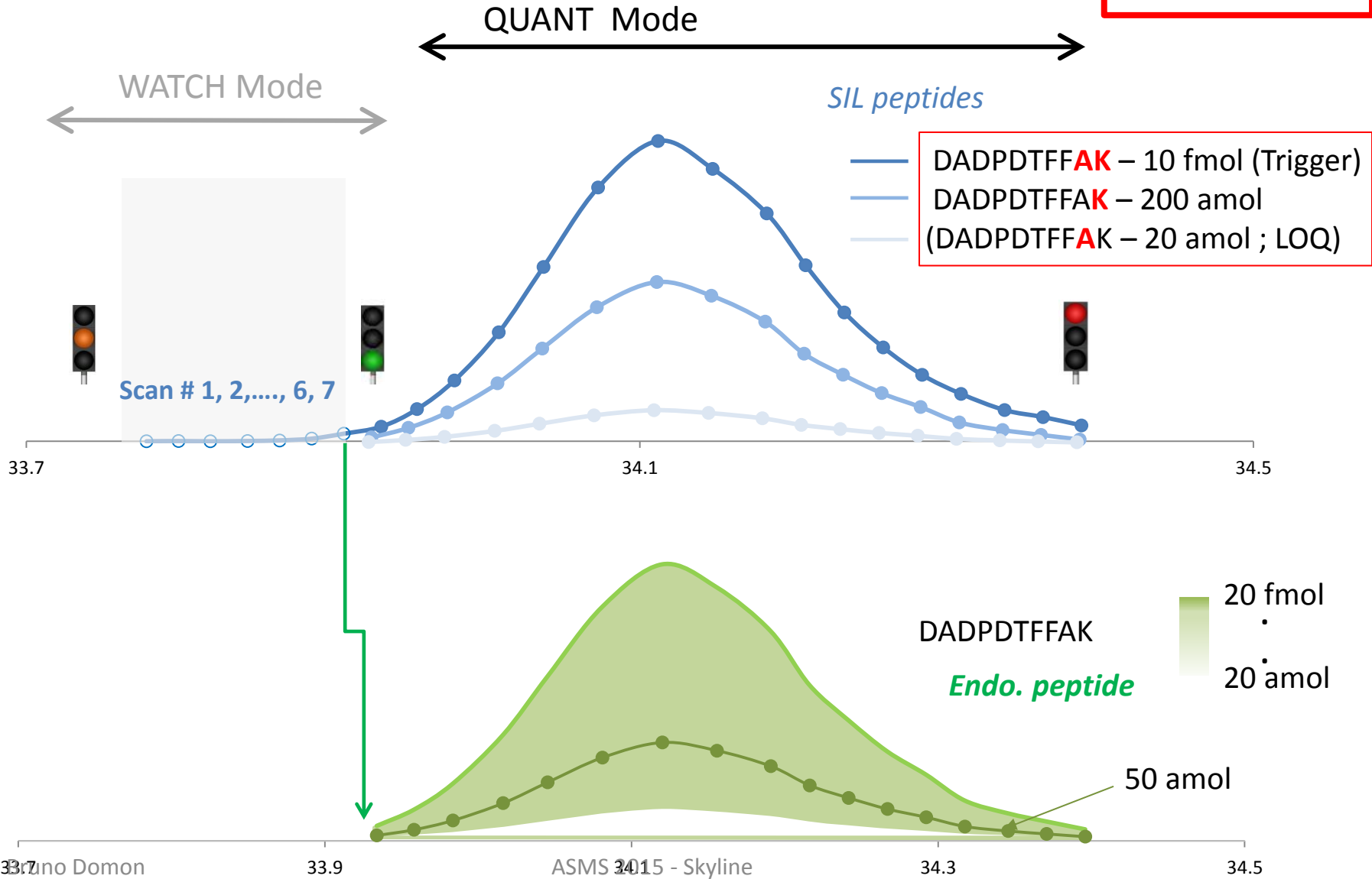


**Application of IS-PRM**

# IS-PRM Vers. 2.0

S. Gallien et al.

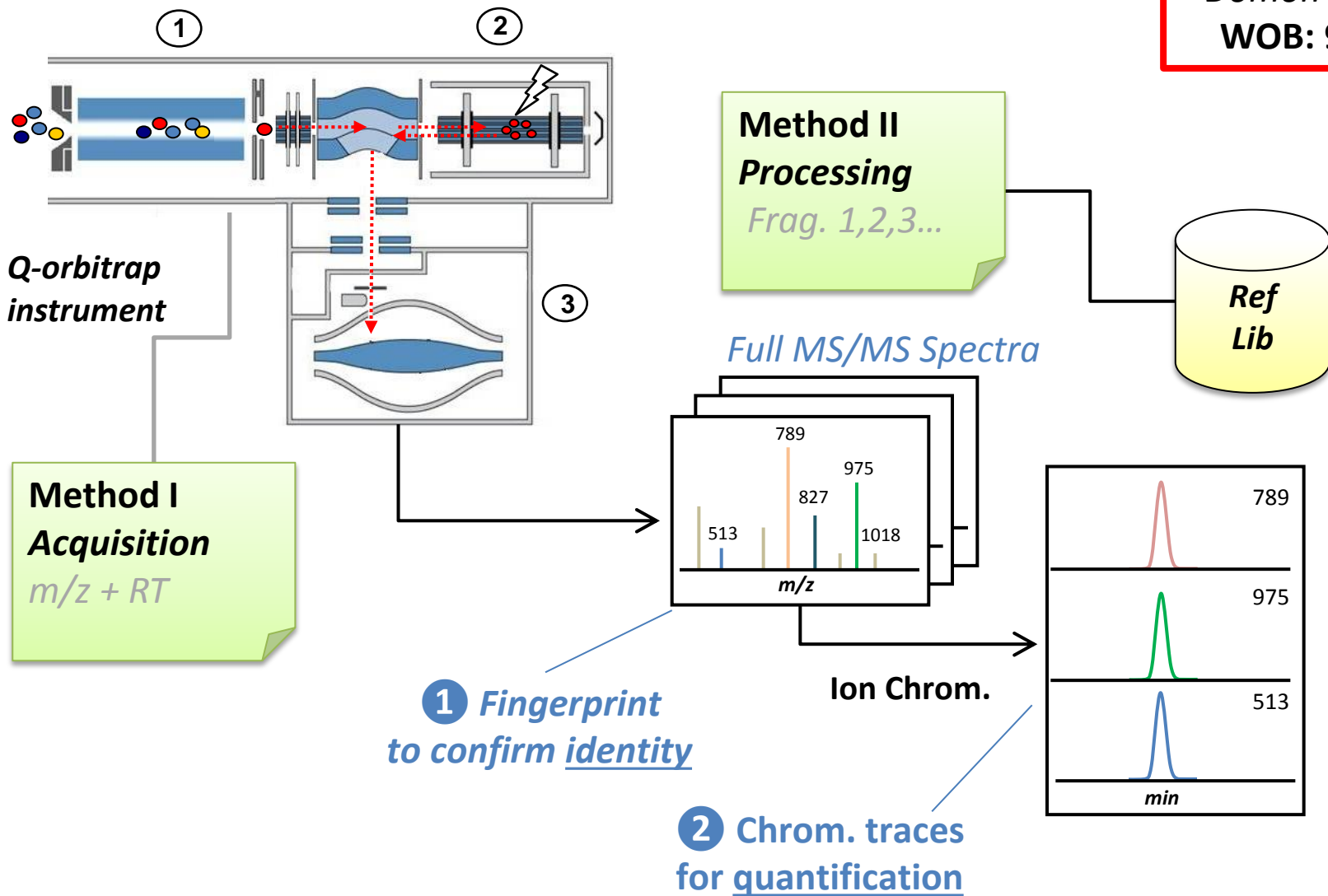
ThOG: 9:10





# PRM has Shaped a New Paradigm

Domon et al.  
WOB: 9:30



# Conclusion

- **Quantification in PRM mode**

- PRM represents an alternative to SRM
- Large-scale experiments are possible using *internal standard triggering*

- **Increased analytical precision**

- Better selectivity (high resolution)
- High sensitivity (trapping device)
- Increased confidence in assignments  
(*accurate mass, fragmentation pattern*)

- **Simple workflow**

- Fast and simple design of acquisition methods
- Straightforward, iterative data analysis using *Skyline*.

# Acknowledgements

- Sebastien Gallien, LCP
- Sang-Yoon Kim, LCP
- Adele Bourmaud, LCP
- Daniel Ayoub, LCP
- Antoine Lesur, LCP

## ***Thermo Scientific***

- Catharina Crone, Thermo
- Tabiwang Array, Thermo
- Andreas Kuehn, Thermo
- Michael Blank, Thermo
- Andreas Huhmer, Thermo

## ***Skyline***

- Brendan MacLean

## **Funding:**

- Fonds National de la Recherche, (FNR, PEARL Grant) Luxembourg
- Ministry of Research and Higher Education (MESR)