Pacific Northwest NATIONAL LABORATORY

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Evaluation of SRM-, PRM- and DIA-based Targeted Quantification

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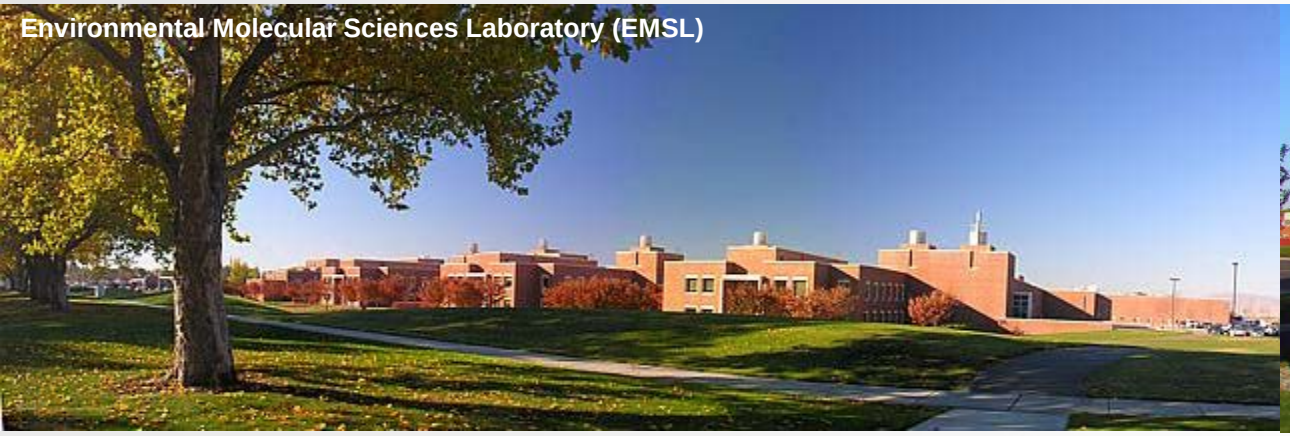


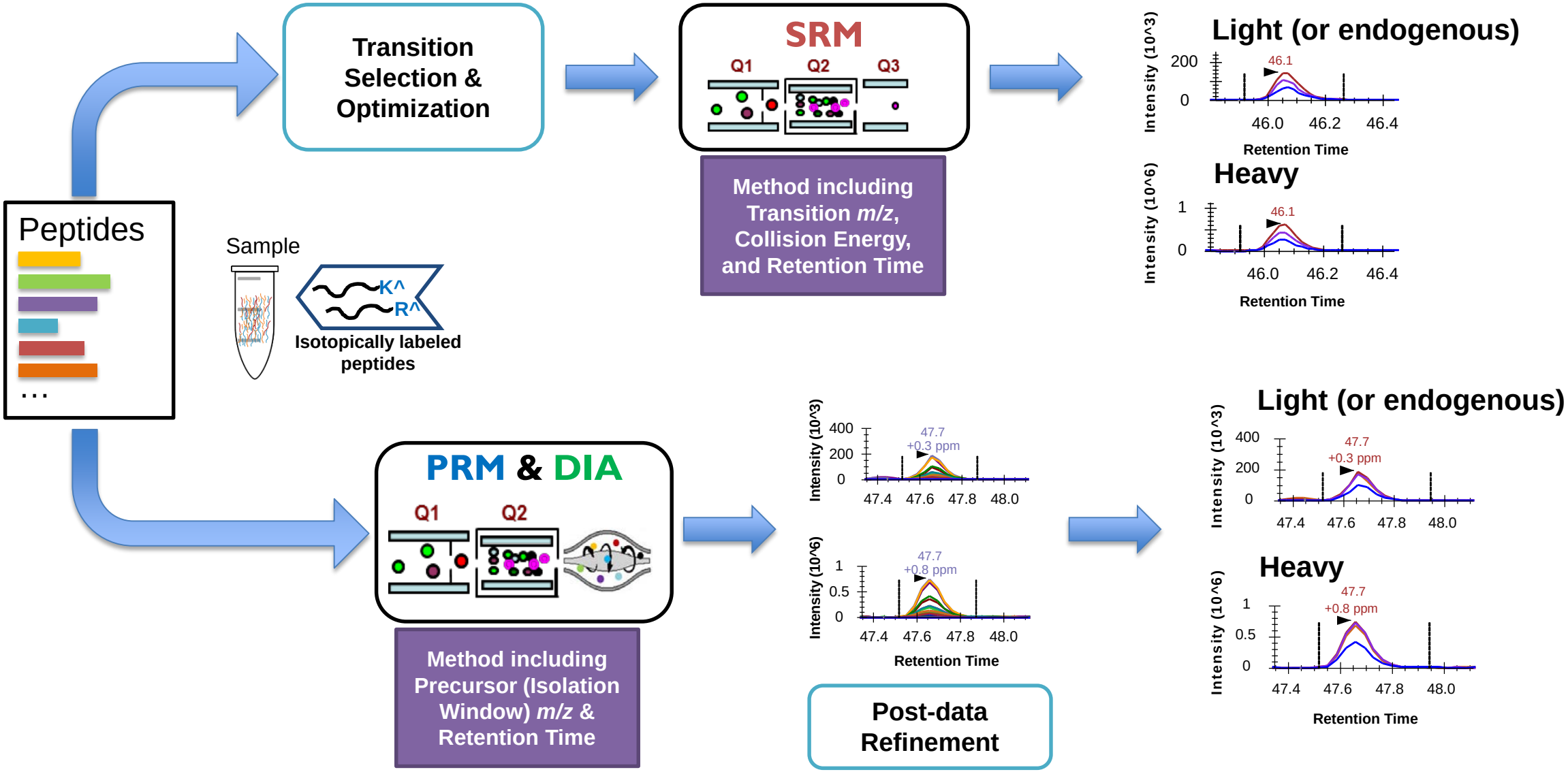
U.S. DEPARTMENT OF ENERGY

Outline

- Introduction
- Initial Evaluation of Targeted Proteomics Performance
- Large Scale Demonstration
  - Quantification of EGFR Pathway Proteins With/Without Fractionation
- Summary

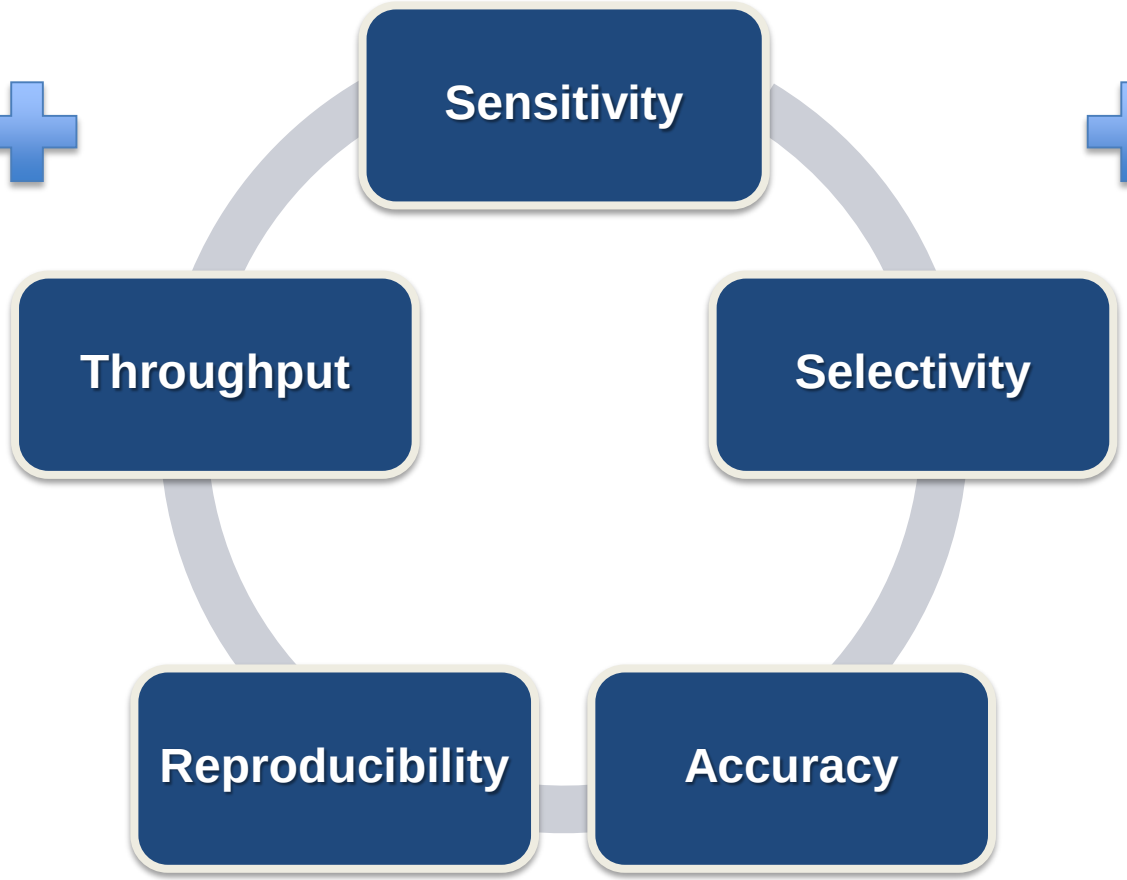
Targeted Proteomics Workflow

SRM: Selected Reaction Monitoring
PRM: Parallel Reaction Monitoring
DIA: Data Independent Acquisition



Aim: Evaluation of Targeted Quantification Performance

Same LC System + **PRISM Fractionation**



1. SRM and PRM: A.C. Peterson *et al*., *Mol Cell Proteomics,* 2012, 11, 1475.
2. SRM and PRM: S. Gallien *et al., J Proteomics,* 2013, 81, 148.
3. SRM and DIA: T. Schmidlin *et al*., *Proteomics,* 2016, 16, 2193.
4. SRM, PRM and DIA: T. Kockmann *et al*., *Proteomics, 2016,* 16, *2183*.
5. IS-PRM: S. Gallien *et al., Proteomics,* 2015, 14, 1630.
6. TOMAHAQ: B.K. Erickson et al., *Mol Cell,* 2017, 65, 361.

Experimental Design

1. Reverse Response Curve

Matrix: *Shewanella oneidensis* (bacteria)

23 Heavy Stock Concentration (fmol/uL)
0
0.0025
0.0125
0.025
0.125
0.25
2.5

- ✓ **Sensitivity**
- ✓ **Selectivity**
- ✓ **Accuracy**
- ✓ **Reproducibility**

SRM: TSQ Vantage; PRM&DIA: Q Exactive-HF
Technical triplicate

2. Large Scale Demonstration

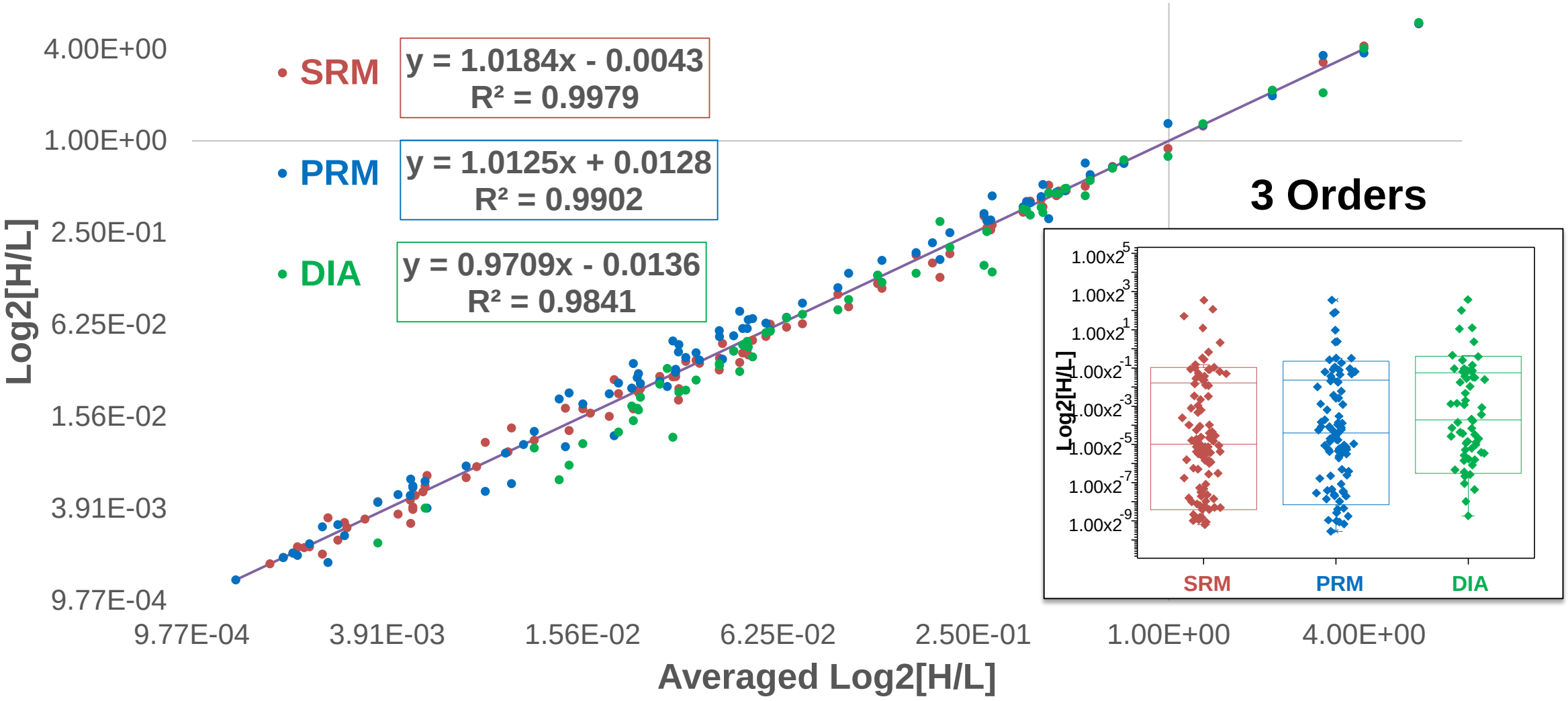
Targets: 133 EGFR Peptides

No Fractionation *vs.* PRISM Fractionation

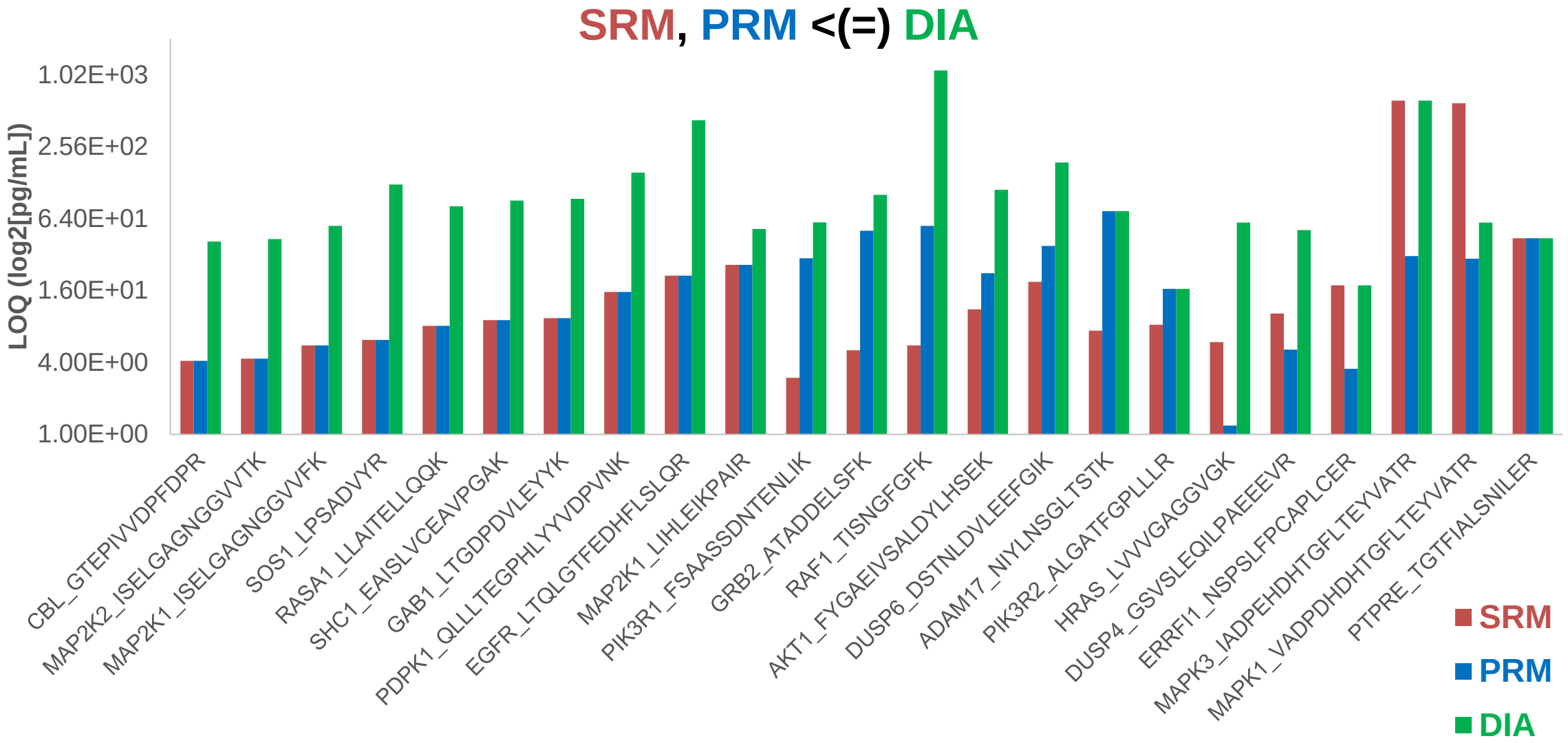
- ✓ **Throughput**
- ✓ **Sensitivity**

PRISM: Shi *et al., Proc Natl Acad Sci,* 2012, 109, 15395.

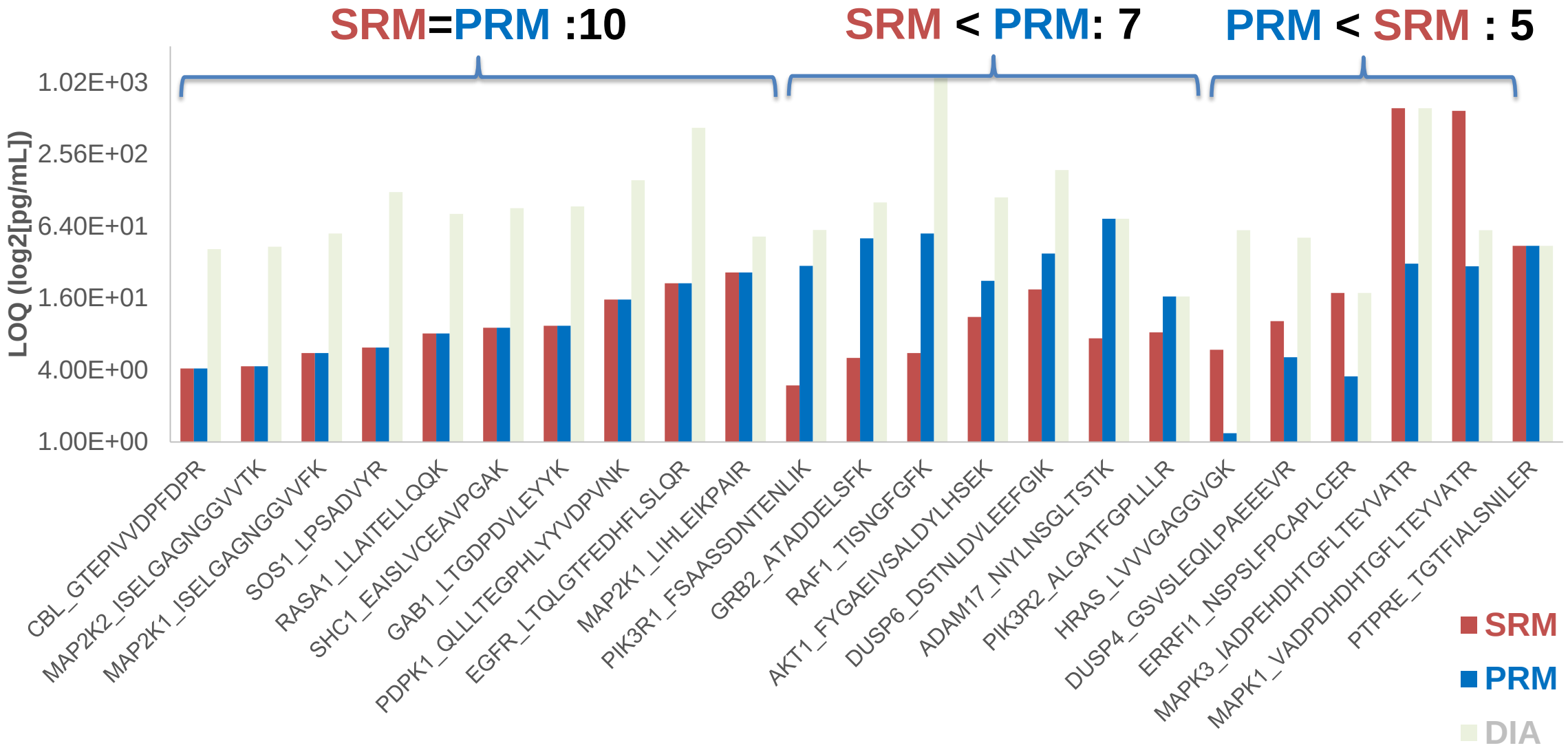
Linear Dynamic Range of Response: SRM ≥ PRM > DIA



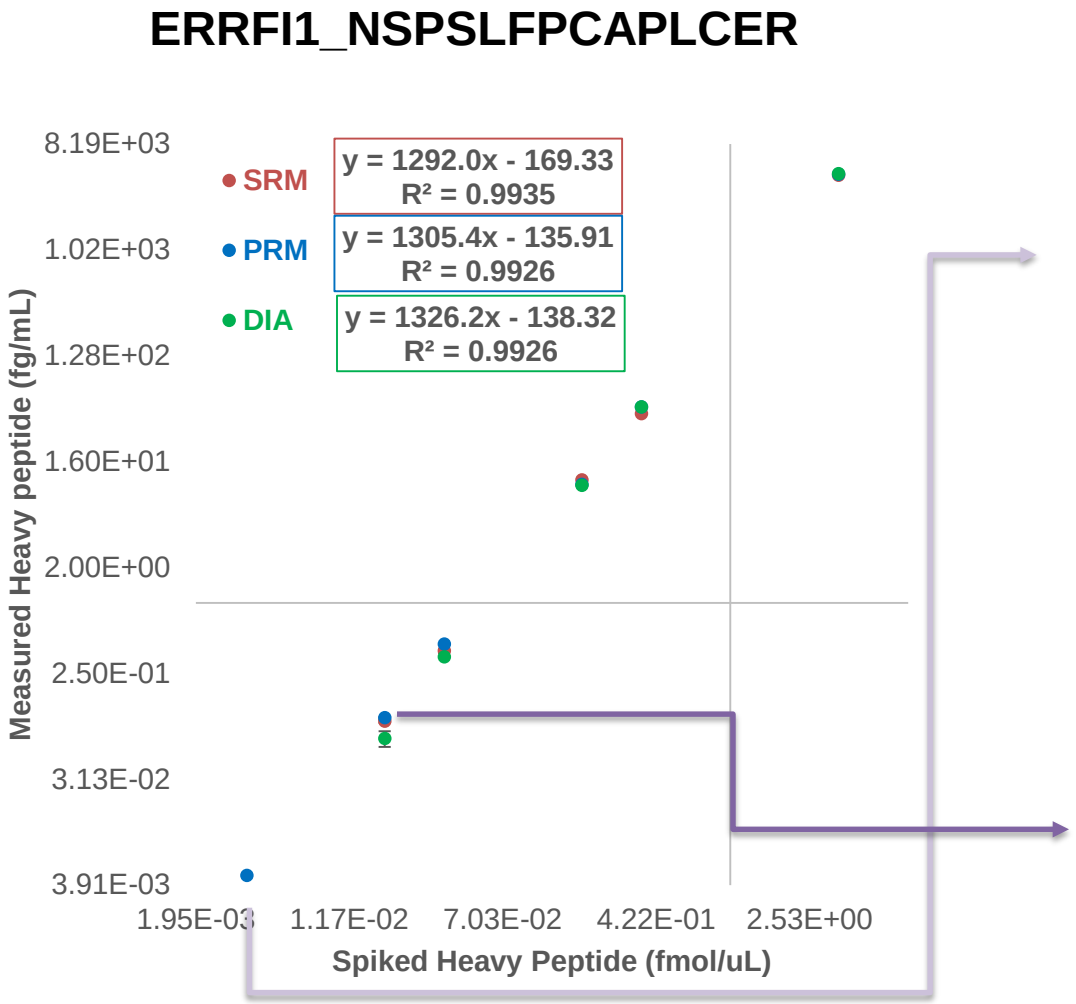
LOQ Values: SRM ≤ PRM < DIA

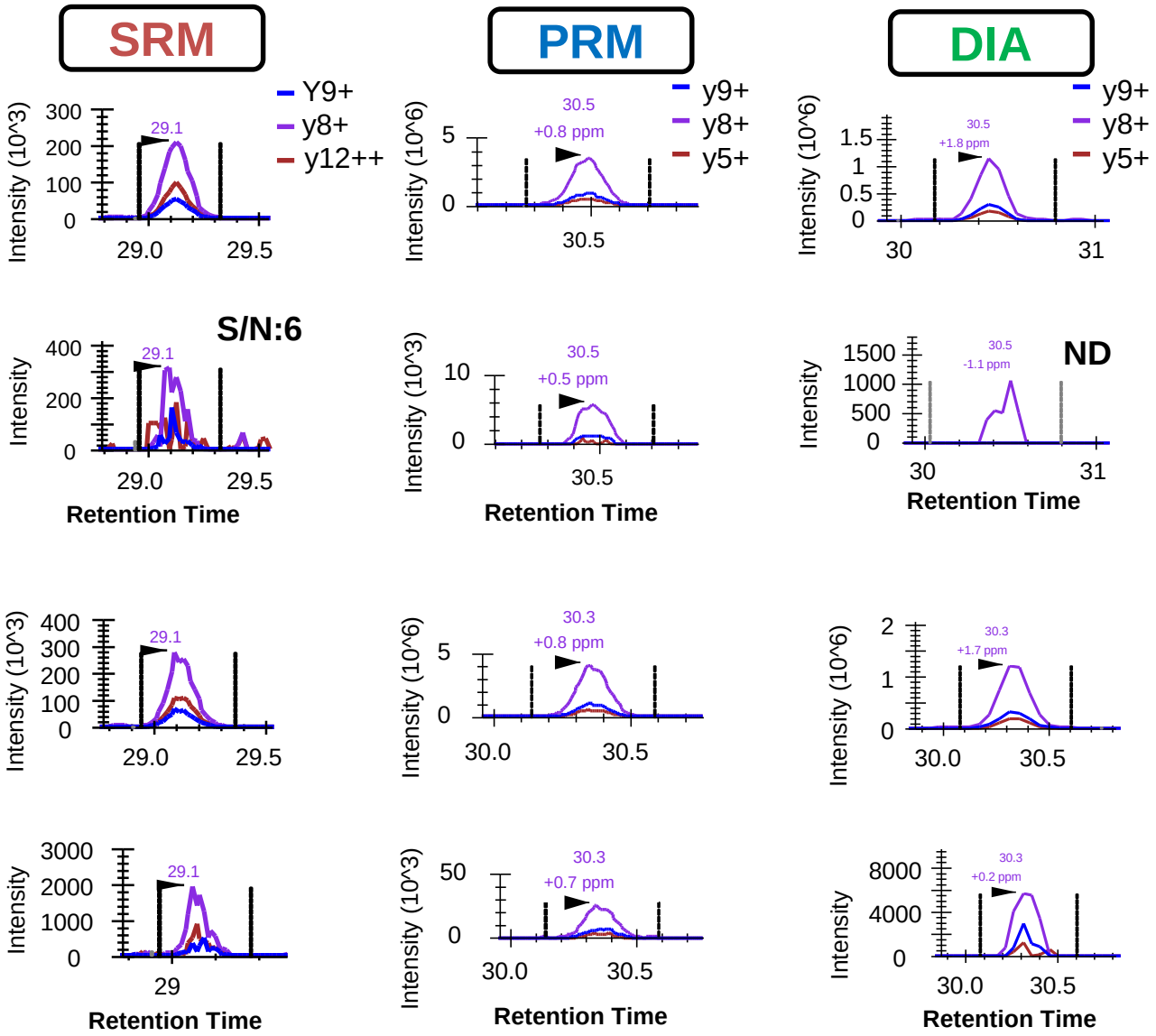


LOQ Values: SRM ≤ PRM < DIA



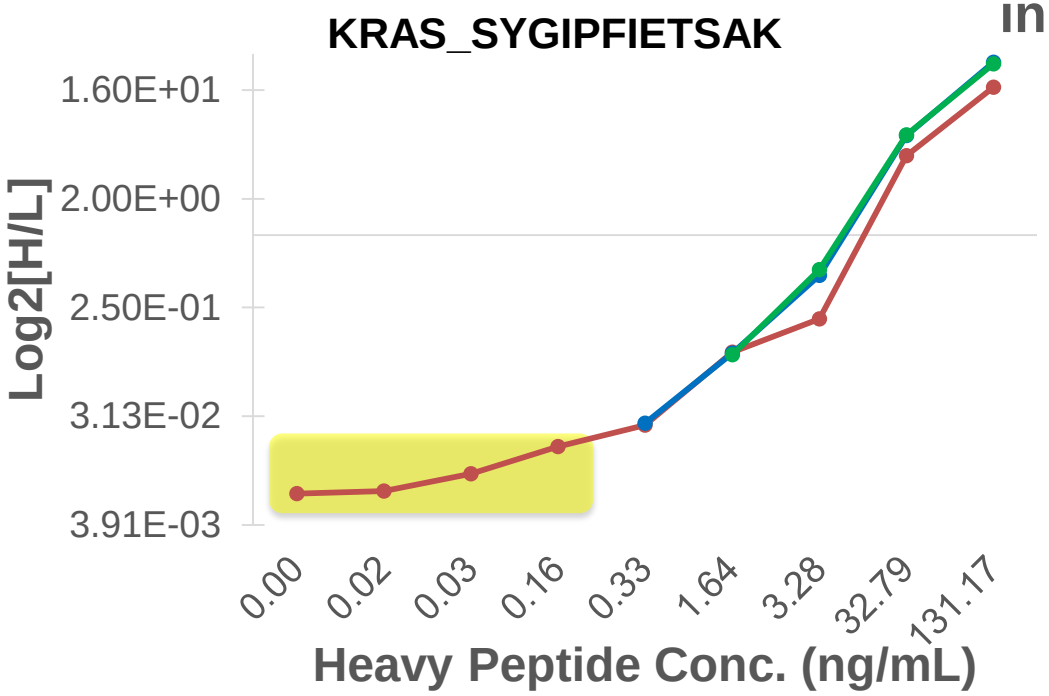
PRM: Selectivity → Sensitivity

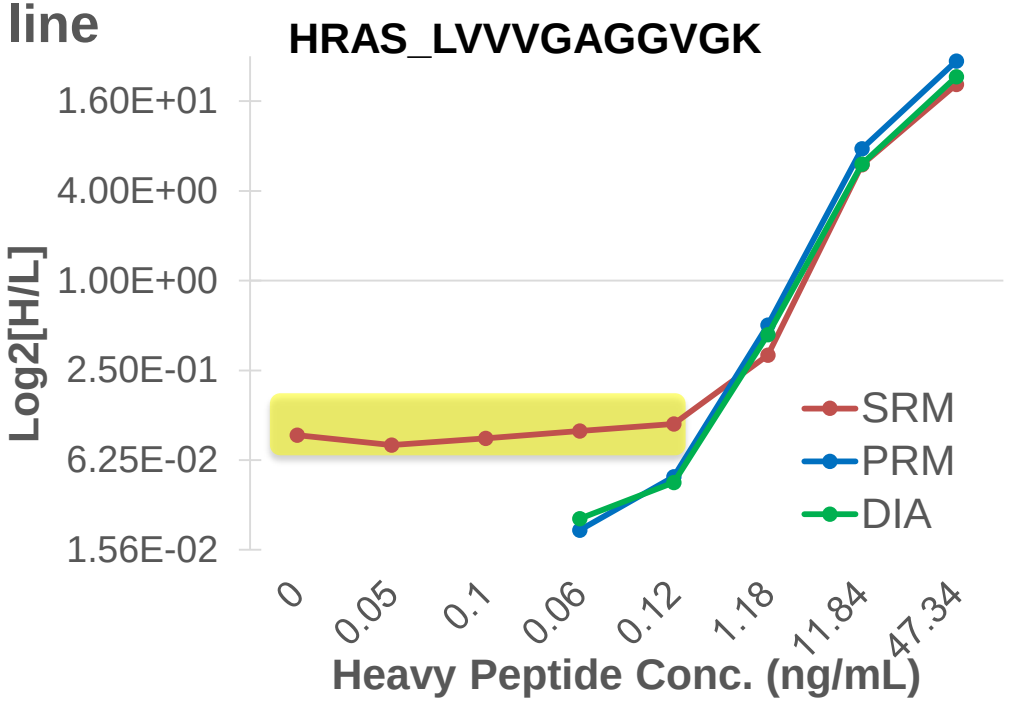




PRM: Selectivity → Quantification Accuracy

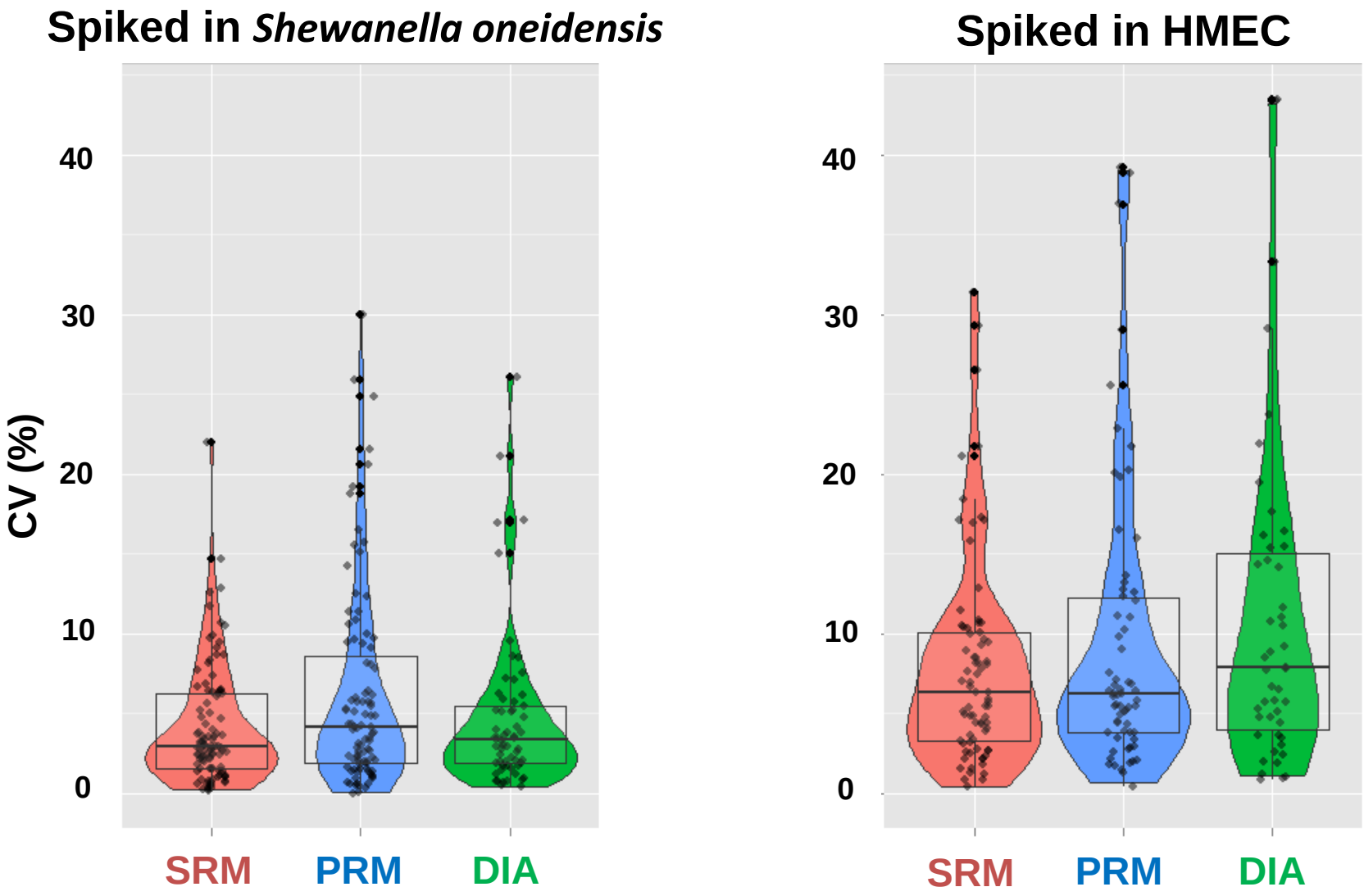
in HMEC cell line



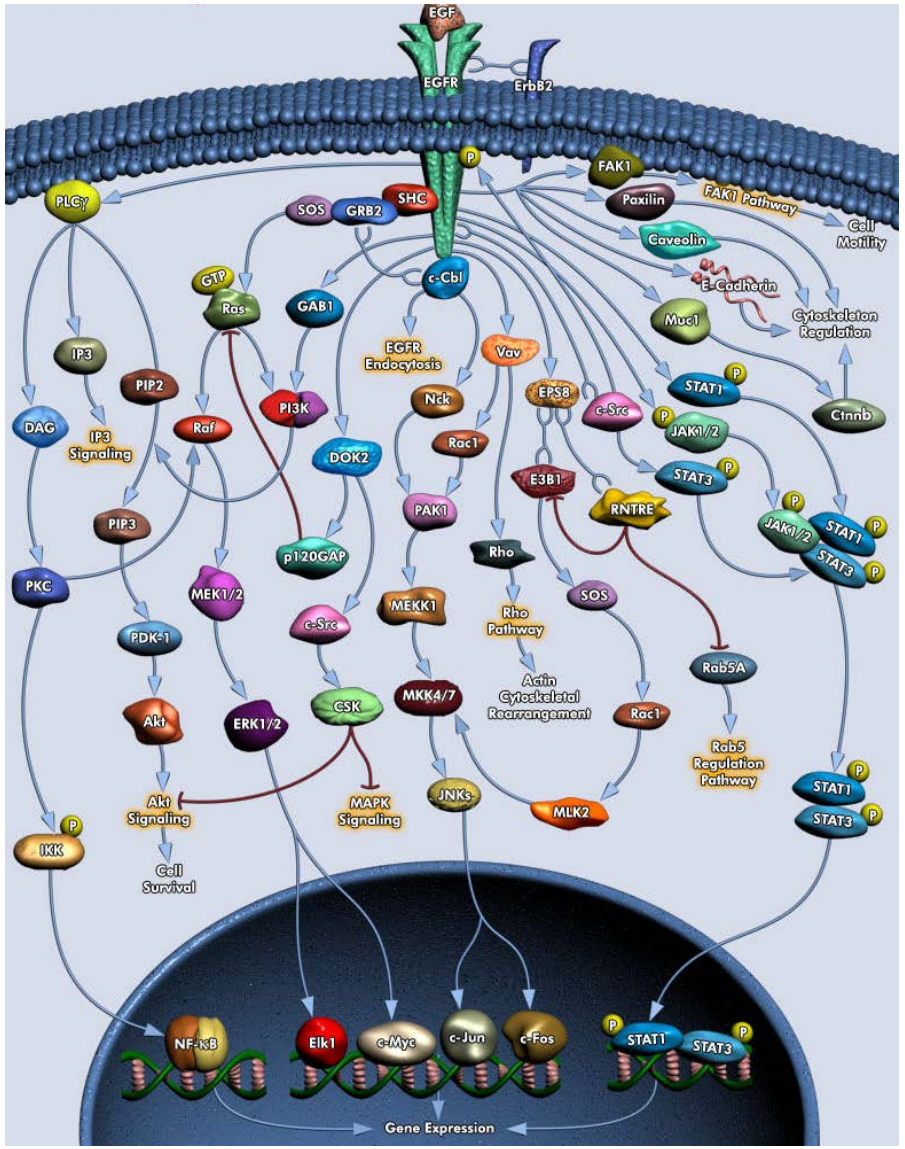


- Potential matrix interference in SRM
  - Low mass resolution and accuracy afforded by QqQ mass spectrometer
- High accuracy for PRM
  - High resolution and tight mass tolerance applied for data acquisition and extraction

Reproducibility among Three Techniques



Large Scale Demonstration: Quantification of EGFR Pathway Proteins



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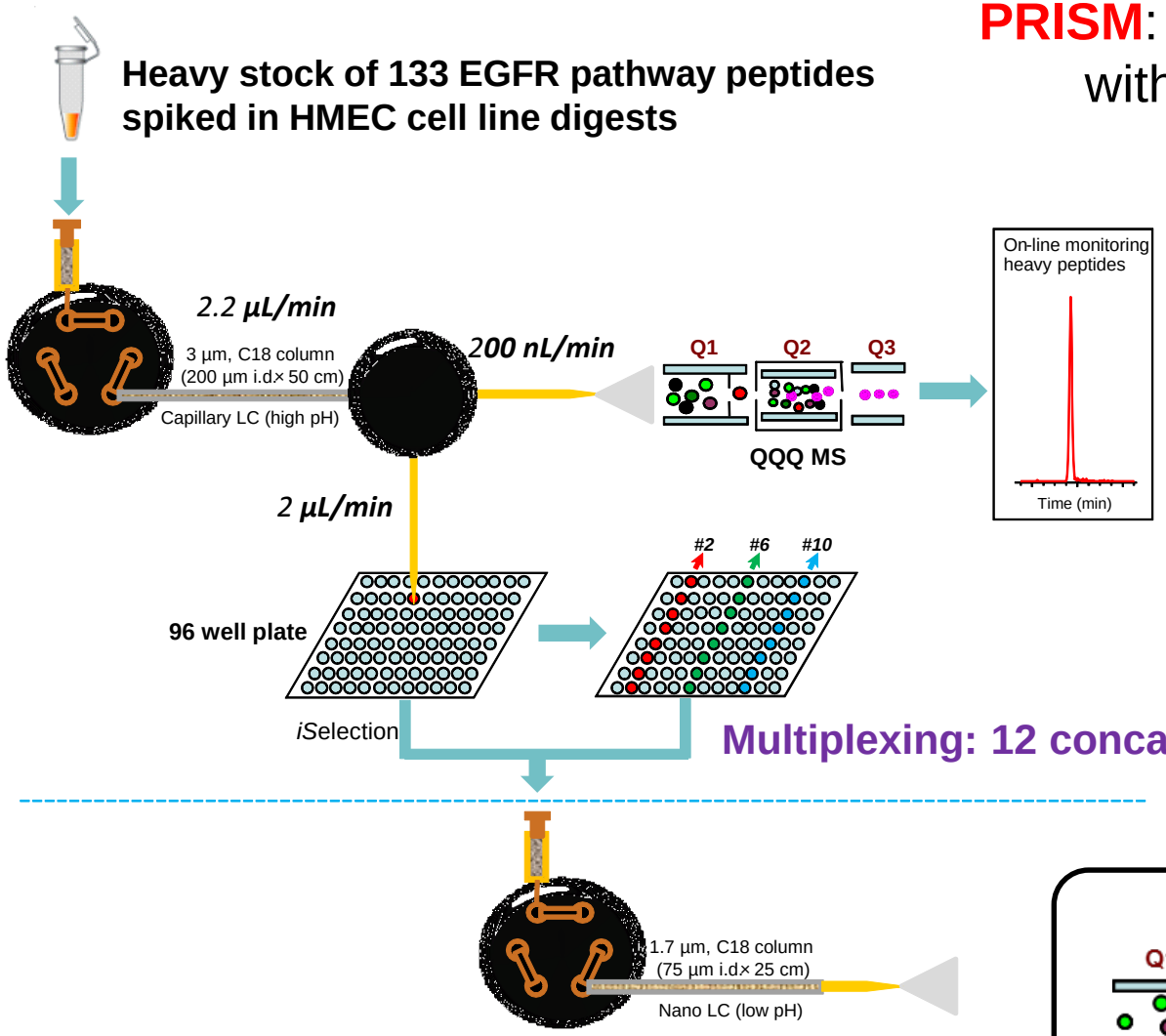
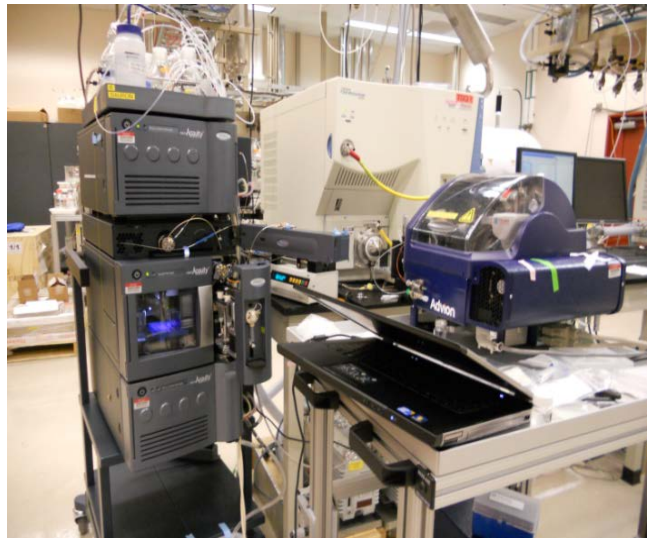
- EGFR pathway
  - One of the most important pathways
  - One of the most well-studied signaling networks
  - Underlying diseases (dysregulation of signaling networks)
  - Key players (low abundance regulatory proteins) and their PTMs
- 133 peptides/ 32 proteins (including 6 phosphopeptides)
- Quantification with and without PRISM fractionation
- Sample
  - PRISM: 25 ug of peptides
  - No PRISM: 0.5 ug of peptides

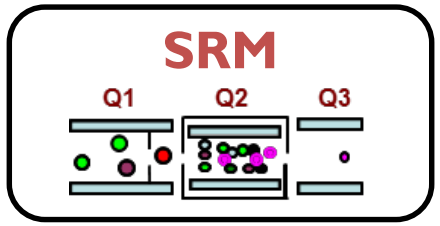
Oda *et al.*, *Mol Syst Biol* 2005, 1:2005.0010.
Shi *et al.*, *Sci Signal* 2016, 9(436):rs6.

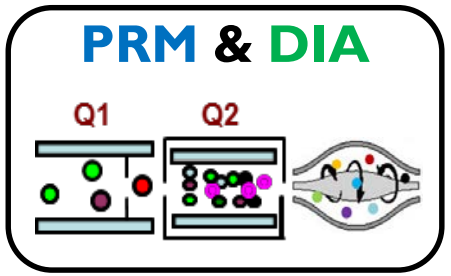
PRISM

PRISM: high-**p**ressure high-**r**esolution separations with **i**ntelligent **s**election and **m**ultiplexing



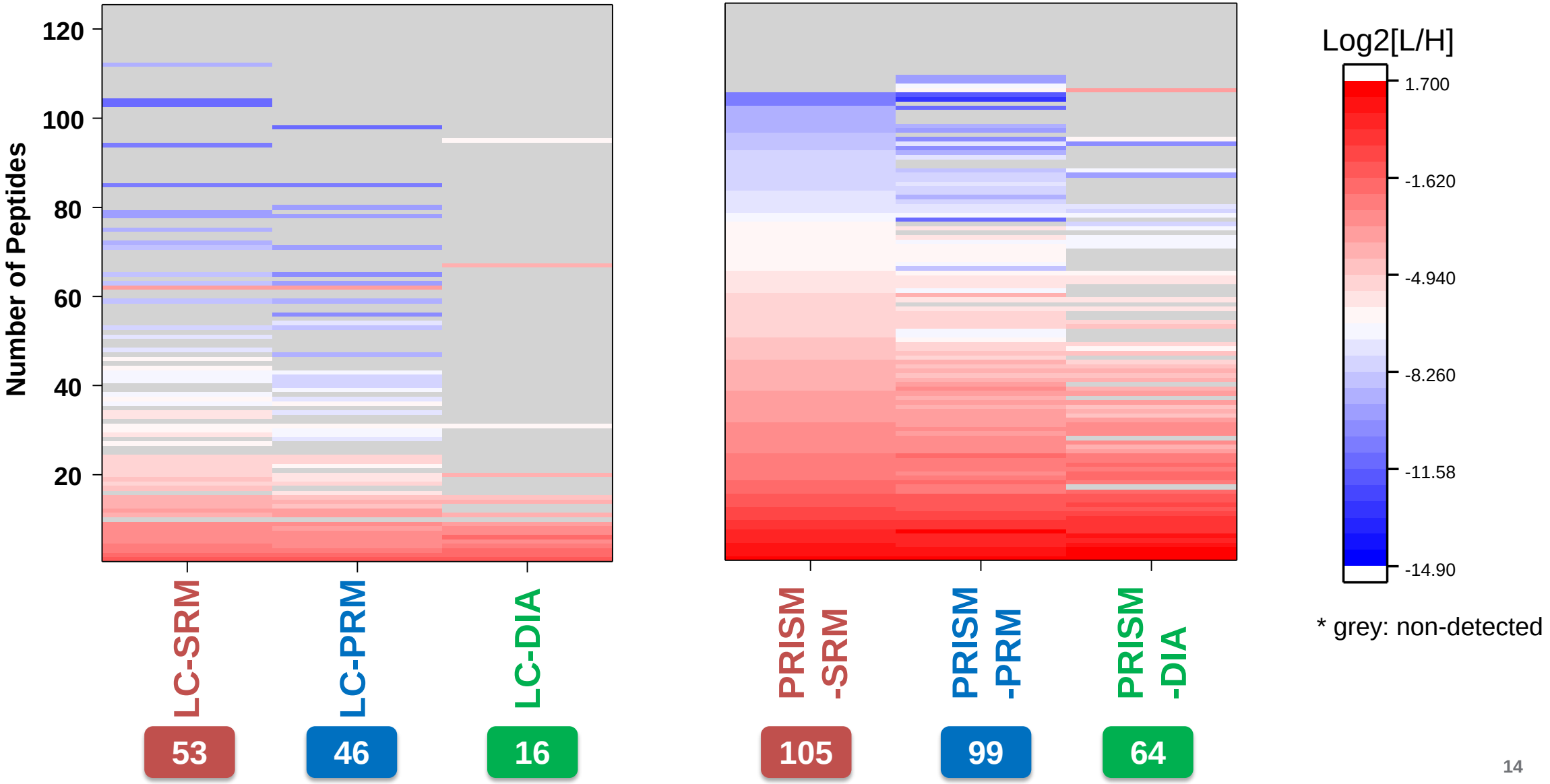




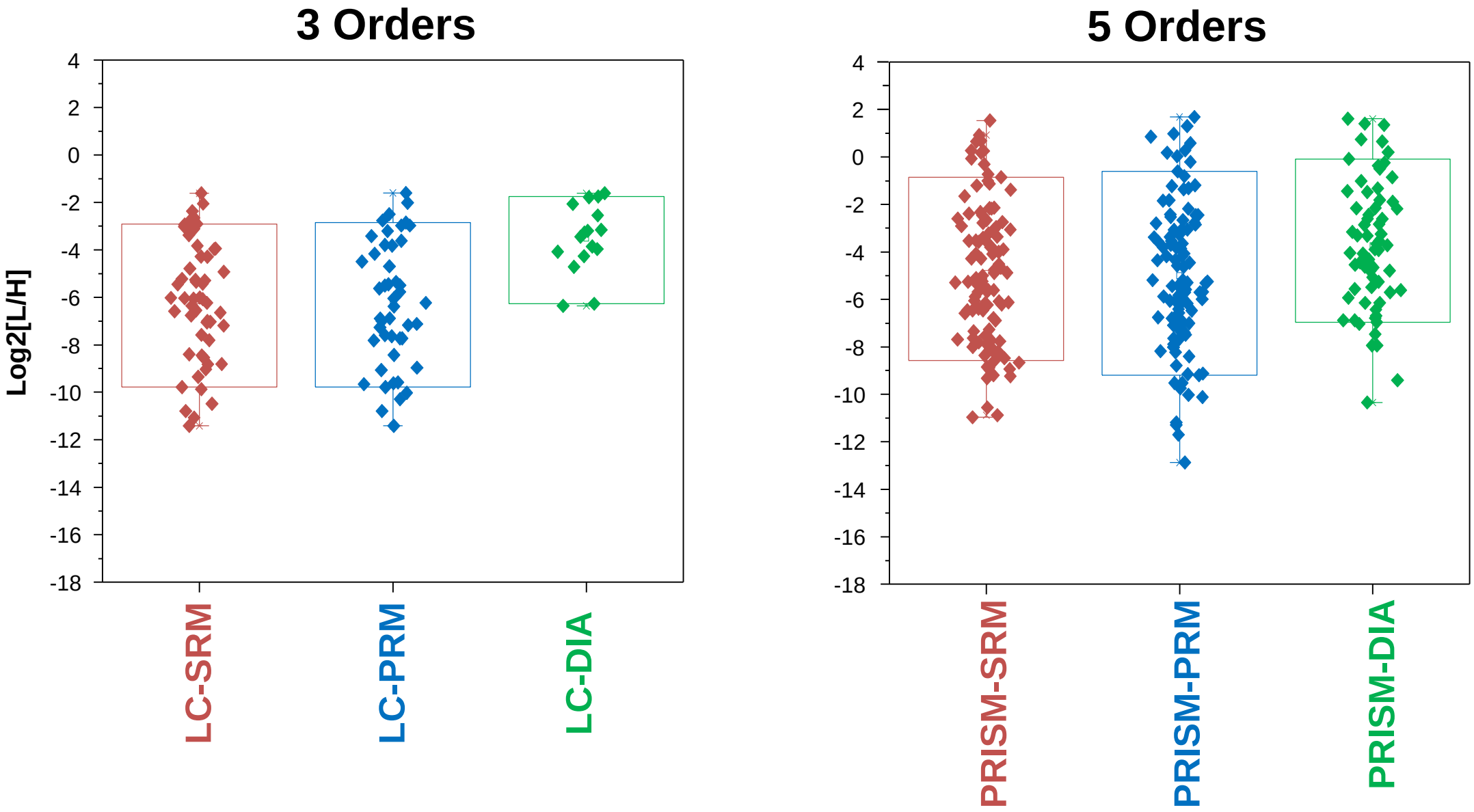


Shi *et al.*, *Proc Natl Acad Sci,* 2012, 109, 15395.

Quantification of EGFR Pathway Proteins by LC *vs.* PRISM-S/PRM and -DIA

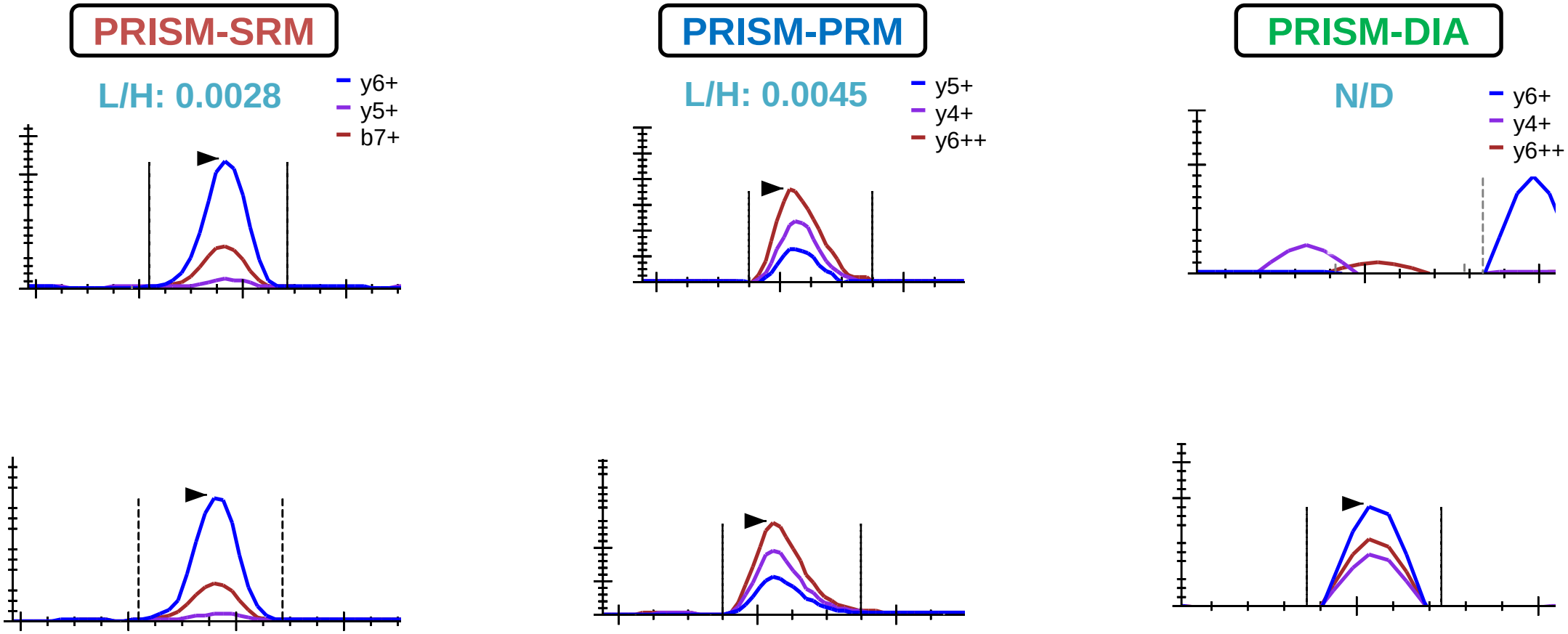


Dynamic Range of Quantification: PRM ≥ SRM >DIA

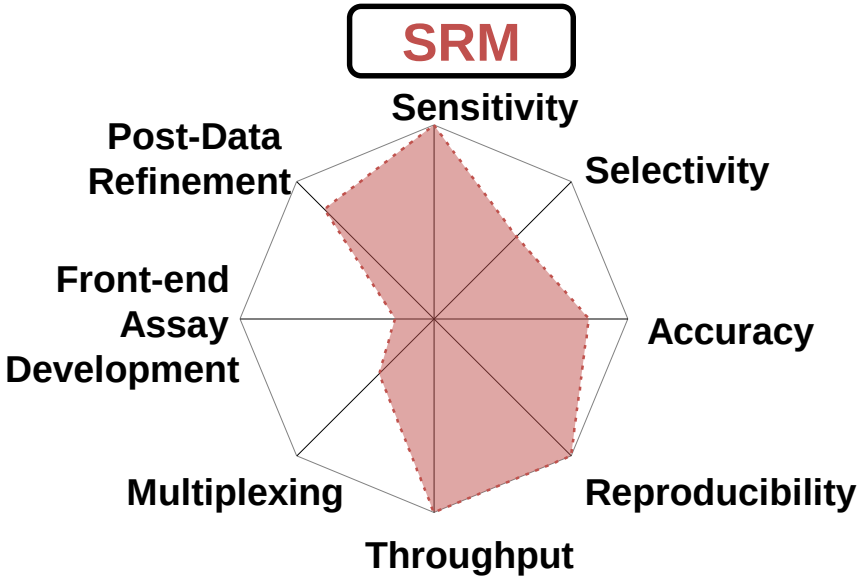


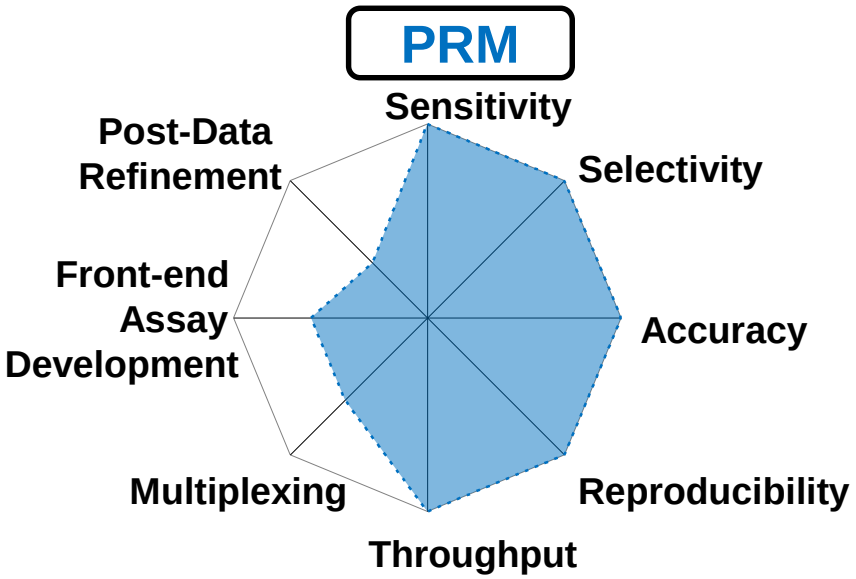
XICs of Very Low Abundant Peptide Quantified by PRISM

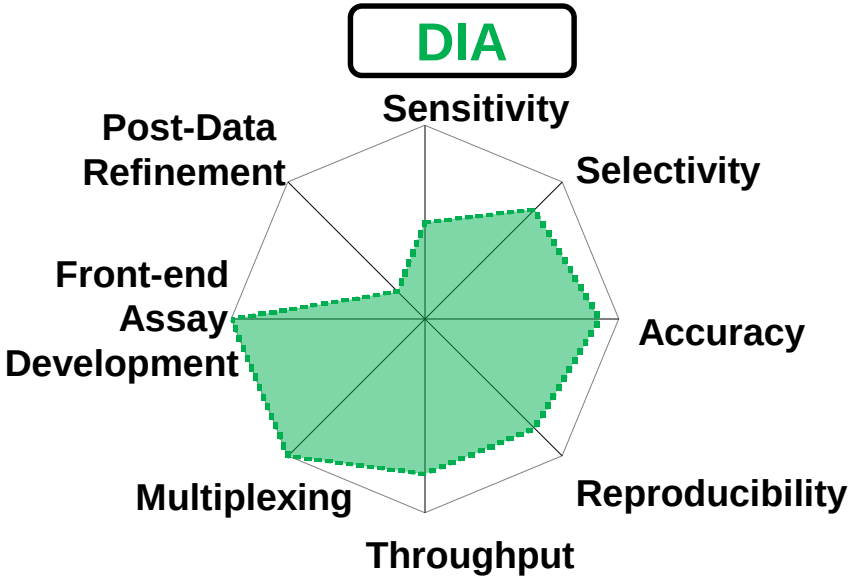
SPRY4_LQHPLTILPIDQVK (copies/cell: ~900)



Summary







- Sensitivity: SRM ≥ PRM > DIA
- Selectivity: PRM > DIA > SRM
- Accuracy: PRM > DIA > SRM
- Reproducibility: SRM ≥ PRM > DIA
- Throughput: SRM ≥ PRM > DIA

- In combination with the front-end fractionation, overall quantification performance was improved for all three techniques.

Acknowledgements

The Integrative Omics Group at PNNL

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CLINICAL PROTEOMIC TUMOR ANALYSIS CONSORTIUM

Early Detection Research Network
Biomarkers: the key to early detection