



# Antibody-Independent, Deep-Dive Targeted Quantification of Proteins at 10 pg/mL Levels in Non-Depleted Human Serum/Plasma

**Song Nie, Tujin Shi, Thomas L. Fillmore, Yuqian Gao, Athena Schepmoes, Heather Brewer, Wei-Jun Qian, Karin Rodland, Richard Smith, and Tao Liu**

*Biological Sciences Division, Pacific Northwest National Laboratory*

**64th ASMS Conference on Mass Spectrometry and Allied Topics, San Antonio, TX, June 9, 2016**

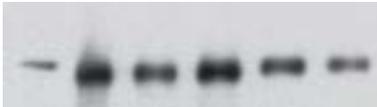


**Pacific Northwest**  
NATIONAL LABORATORY

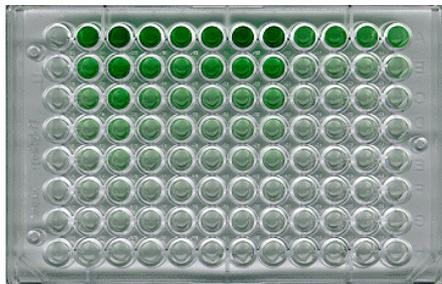
*Proudly Operated by Battelle Since 1965*

# Traditional affinity-based targeted quantification

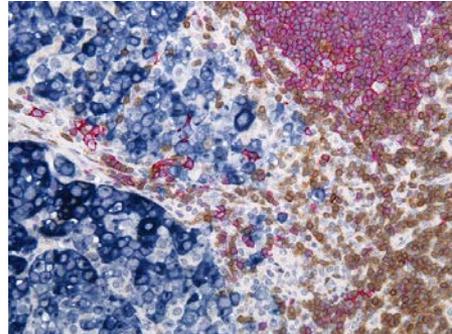
Western Blot



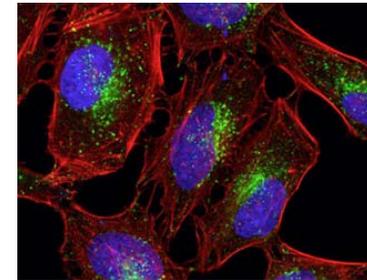
ELISA



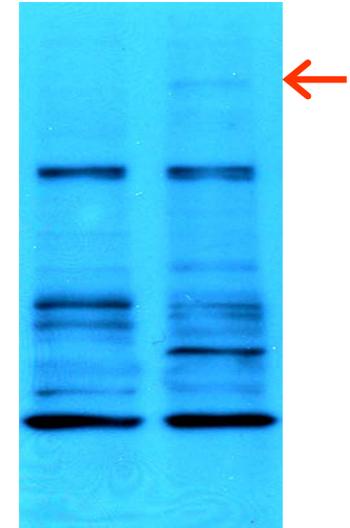
Immunohistochemistry



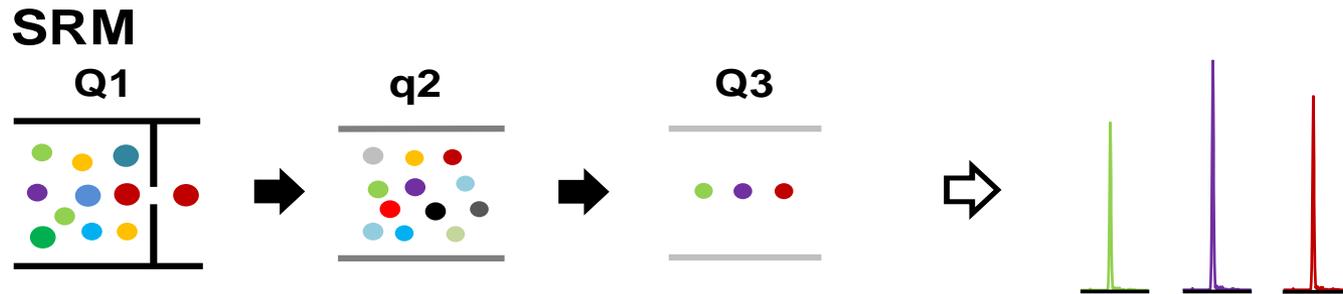
Immunofluorescence



- Low specificity
- long lead time
- Uncertain success rate



# Selected reaction monitoring (SRM/MRM)



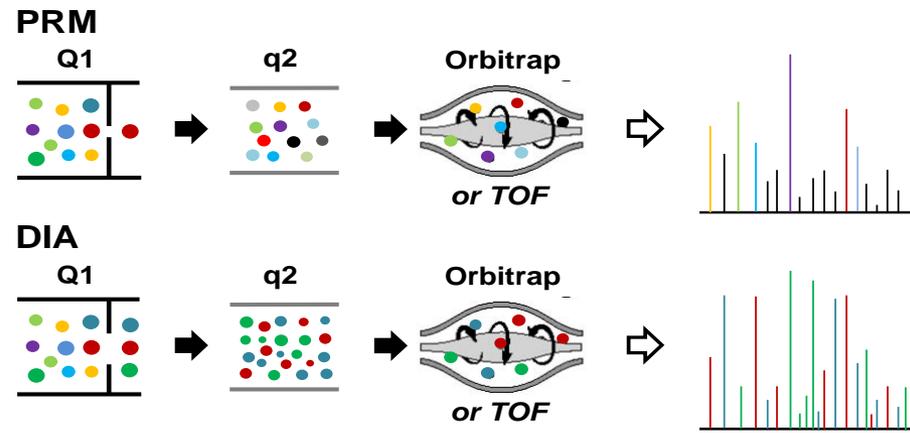
## Advantages:

- Ultra-high specificity
- Multiplexing capacity
- Relative/absolute quantification
- Protein isoform/variant/modification quantification

## Drawback:

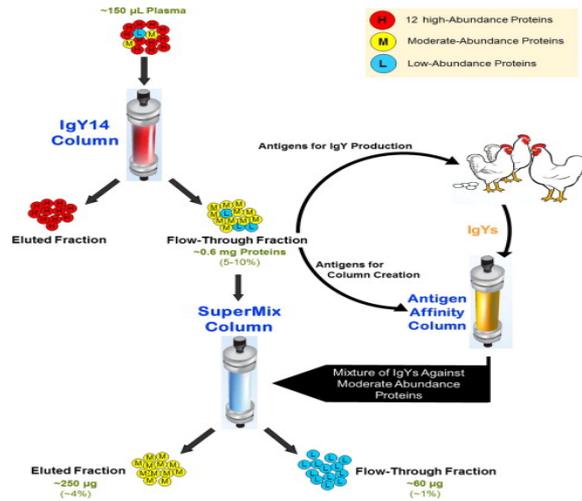
- LC-SRM sensitivity  
~100 -1000 ng/mL

## Other MS-based targeted proteomics

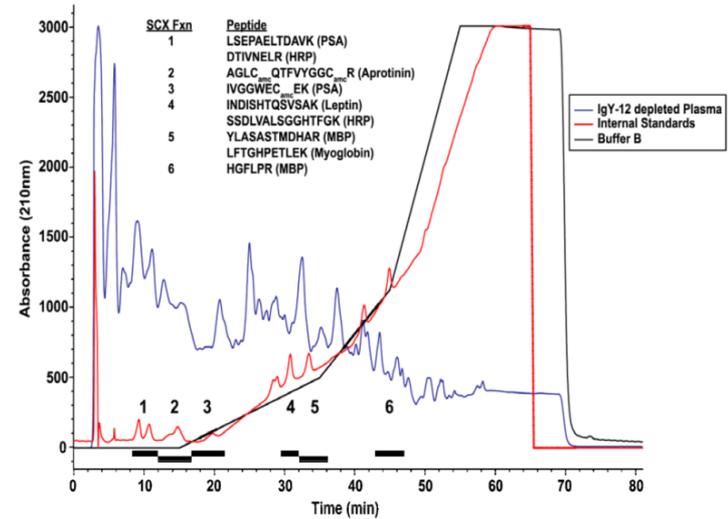


# Sample processing strategies enhancing the sensitivity of targeted quantification

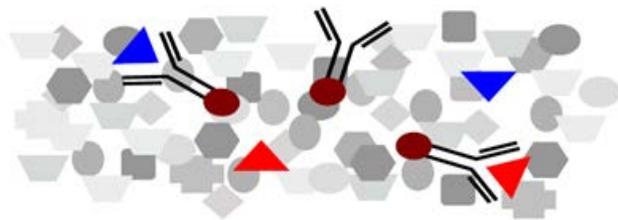
## Immunoaffinity depletion



## SCX



## SISCAPA



Anti-peptide antibody



Magnetic beads



Endogenous peptide



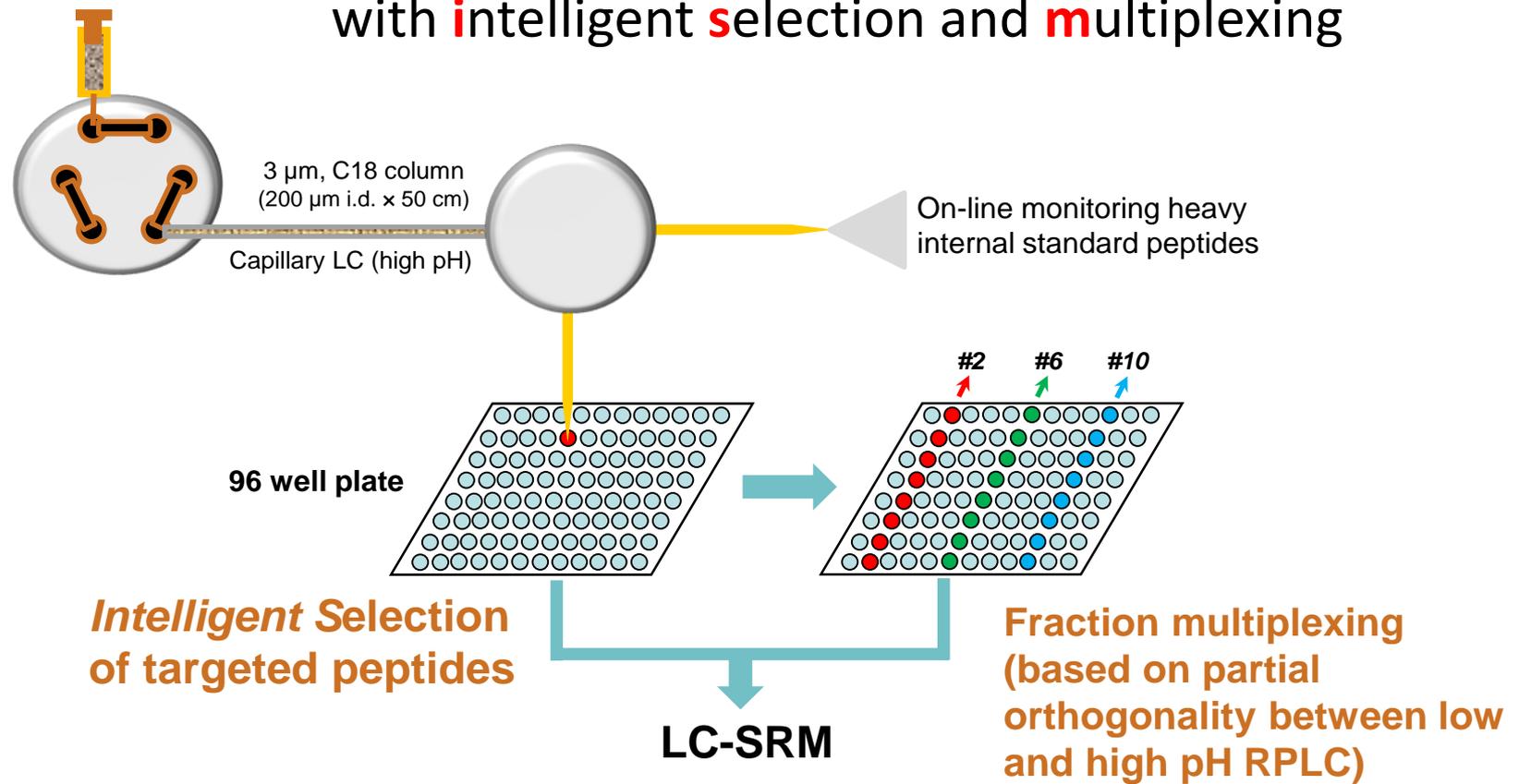
Spiked standard

~ 1 ng/mL  
~50-100 pg/mL (1 mL)

Qian et al., Mol. Cell. Proteomics. 2008, 7, 1963-1973.  
Keshishian et al., Mol. Cell. Proteomics. 2007, 6, 2212-2229.  
Anderson et al., Mol. Cell. Proteomics. 2009, 8, 995-1005.

# PRISM-SRM

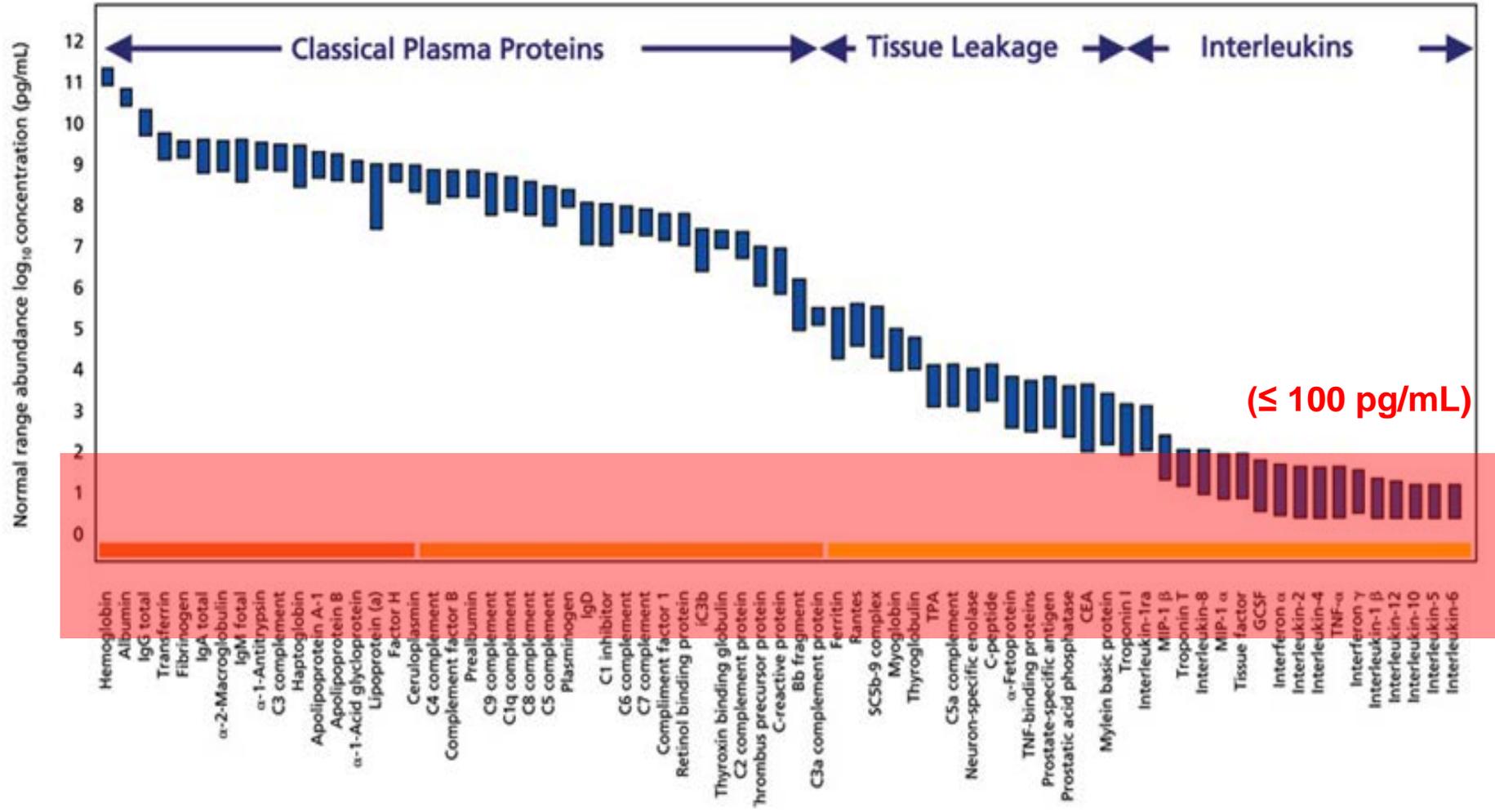
**PRISM**: high-**p**ressure high-**r**esolution separations with **i**ntelligent **s**election and **m**ultiplexing



# Sensitivity of current SRM platforms

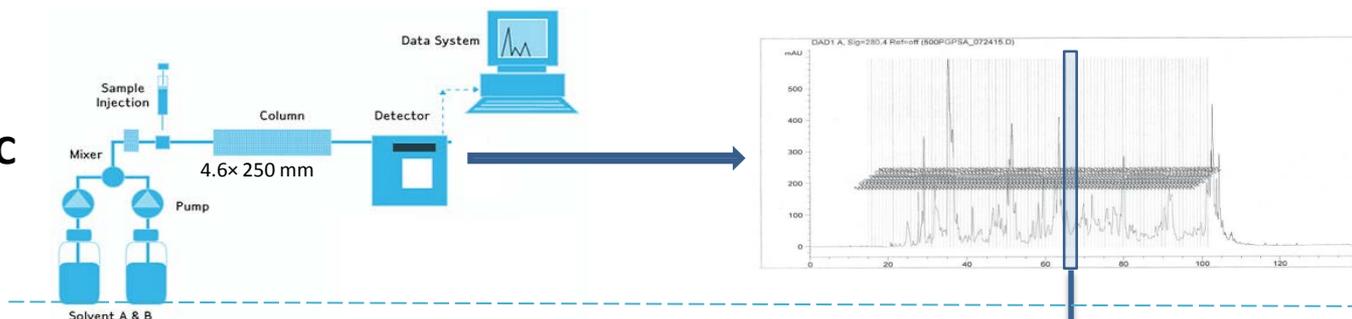
Platforms	LOQ	
	Plasma/serum	Mammalian cells
Conventional LC-SRM	~100-1000 ng/mL	
Long gradient LC-SRM (LG-SRM)	~10 ng/mL	
Ig Y14 depletion followed by SCX	~1 ng/mL	
High-pressure, high-resolution separations coupled with intelligent selection and multiplexing (PRISM)	~1 ng/mL	~100 copies/cell
		~1000 copies/cell (ERK phosphorylated forms)
SISCAPA	~1 ng/mL	
IgY14 depletion followed by PRISM (IgY14-PRISM)	~50-100 pg/mL	n/a
<b>New method (DD-SRM)</b>	<b>&lt; 10 pg/mL</b>	<b>1-10 copies/cell</b>

# The “dynamic range” challenge of complex samples

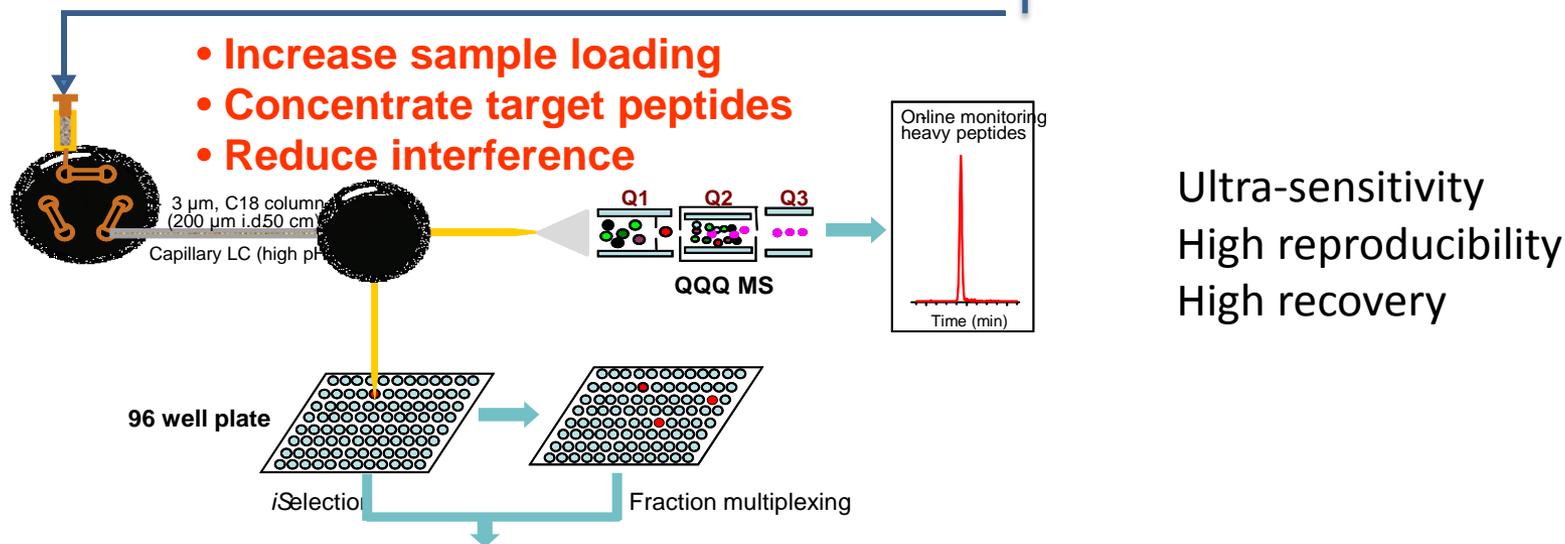


# The Deep Dive SRM concept (DD-SRM)

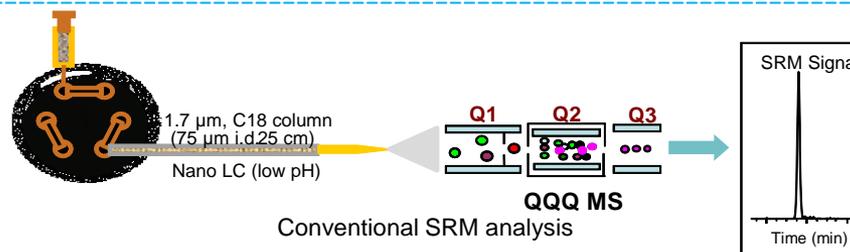
Low pH RPLC



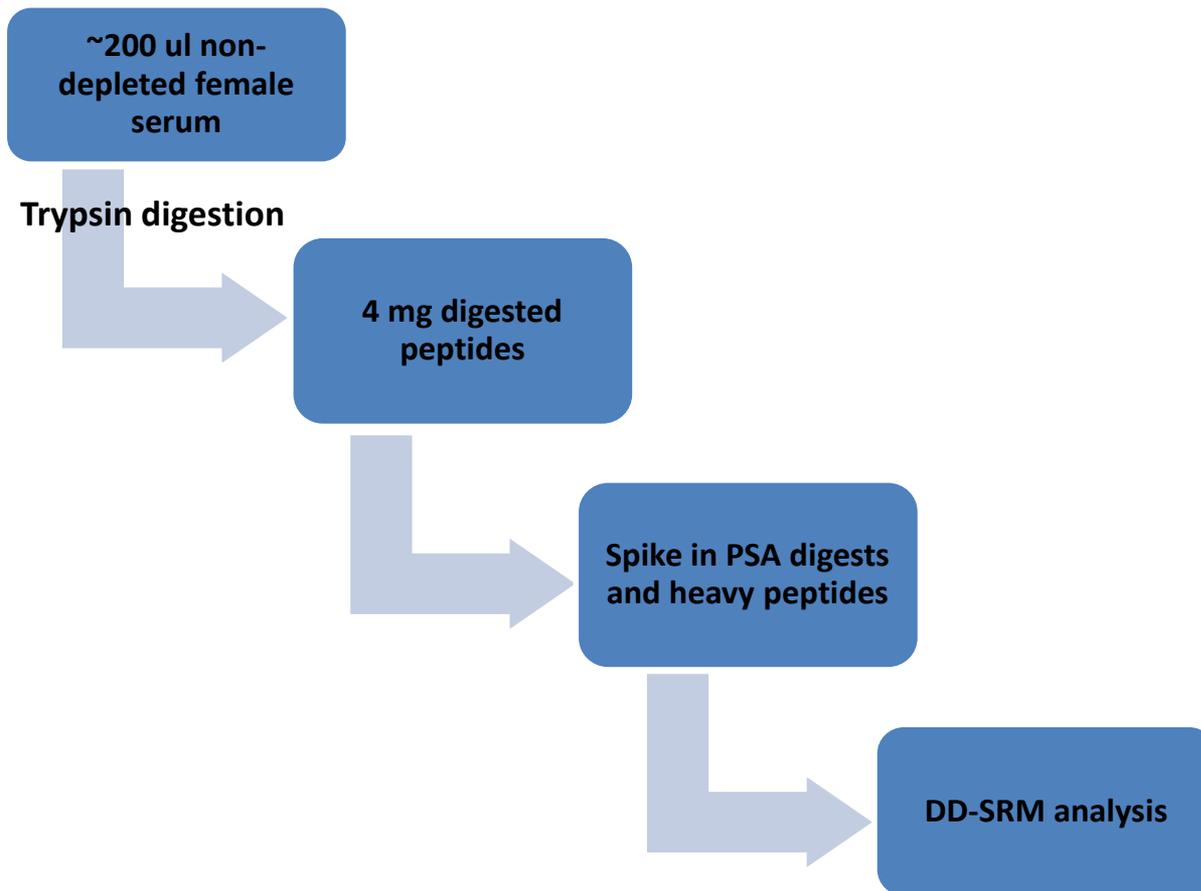
High pH RPLC



Low pH RPLC

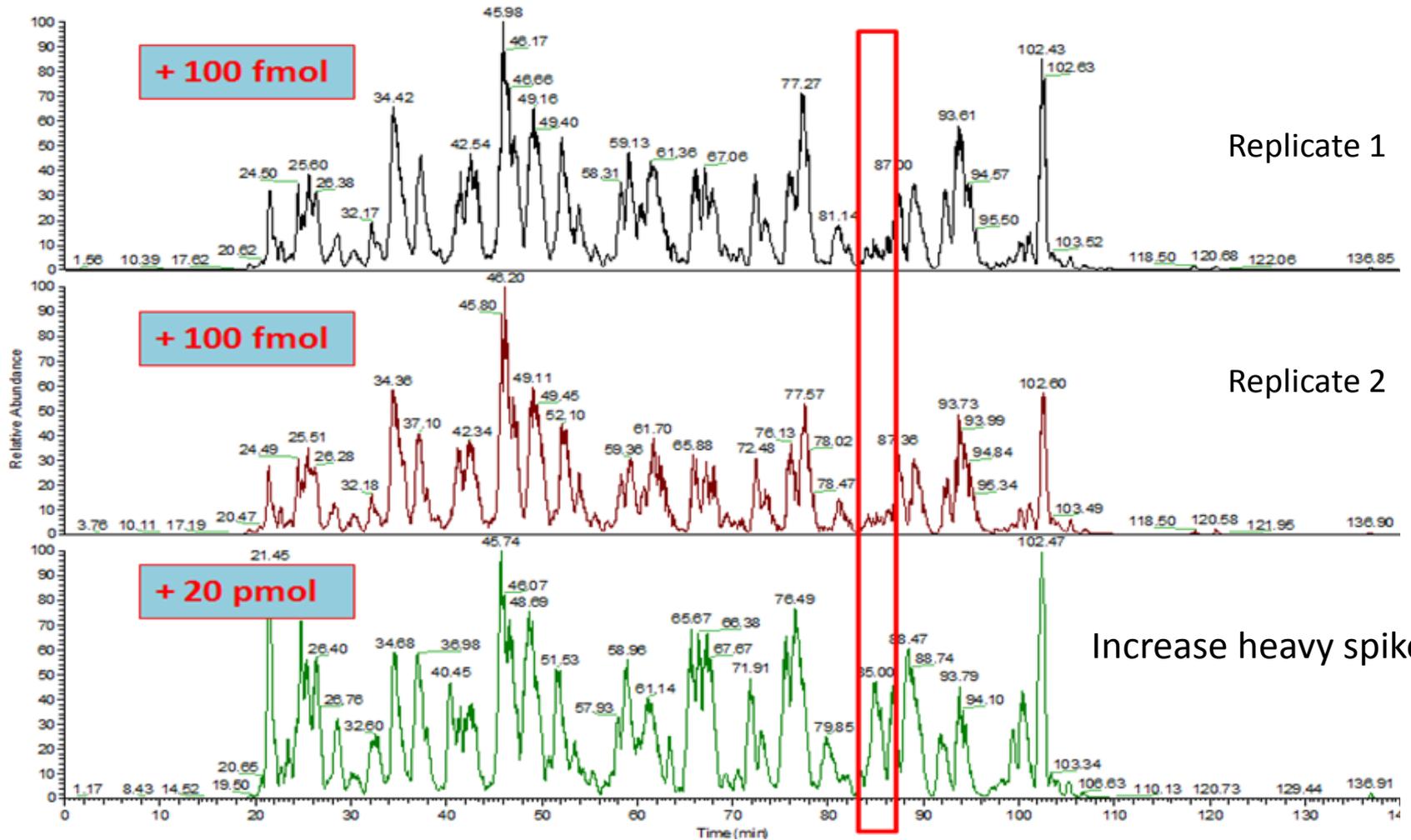


# Proof of concept: PSA spike-in experiments

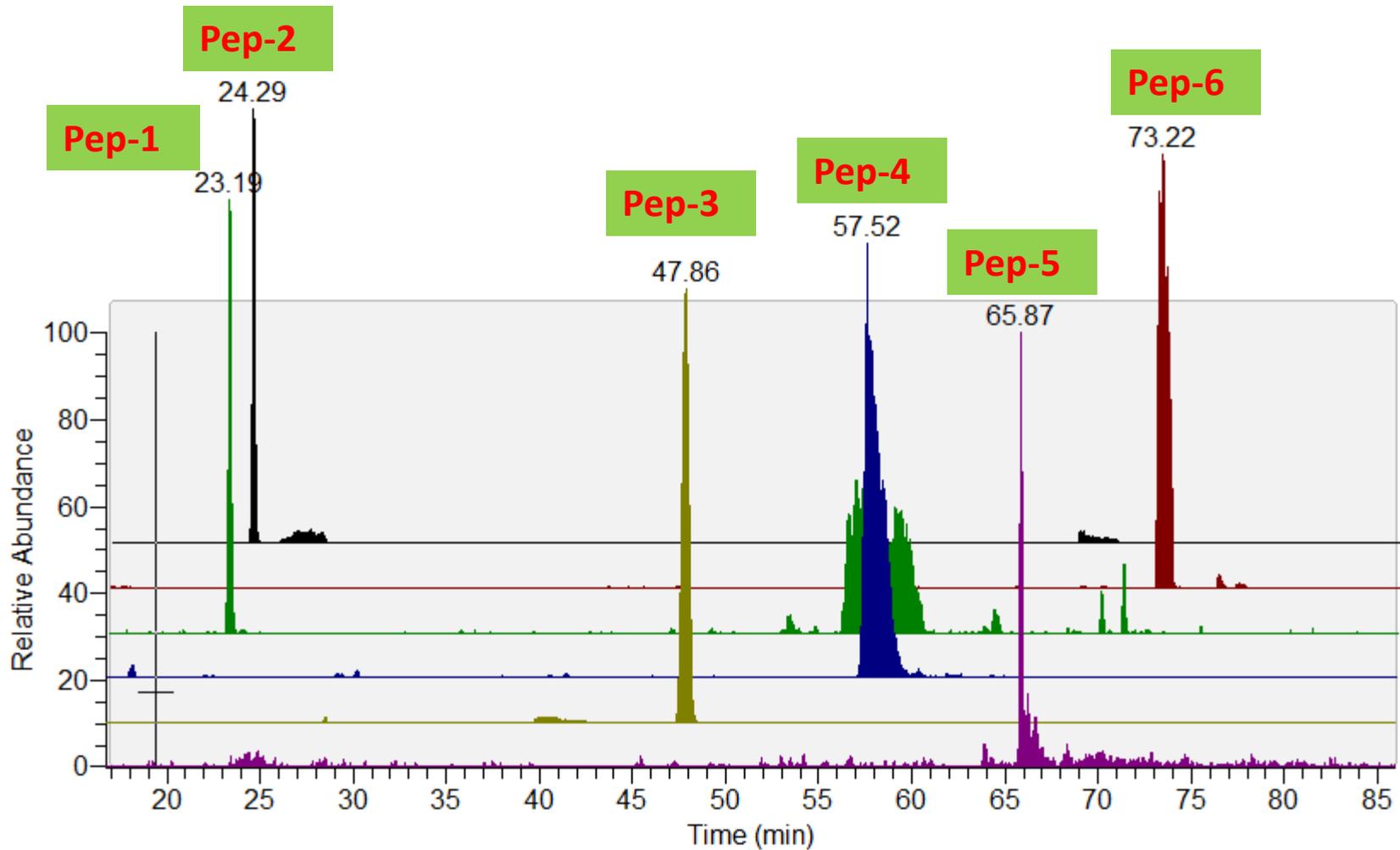


PSA (pg/mL)
0
10
25
50
100
500
1000
10000
100000

# Challenge: locate target fraction in the 1<sup>st</sup> dimension LC separation



# Use of “internal marker” peptides from albumin

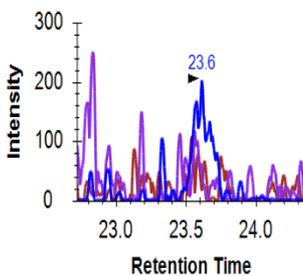


**6 endogenous albumin marker peptides RT distribution in one run.**

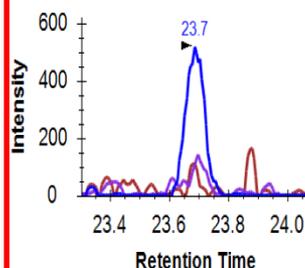
# XICs of PSA peptide

## LSEPAELTDAVK (PSA)

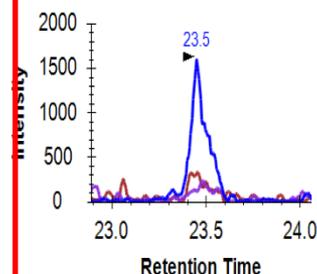
0 pg/mL



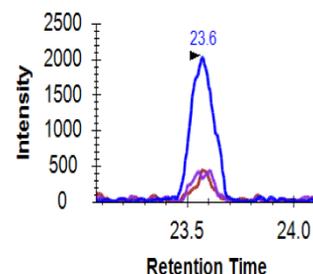
10 pg/mL



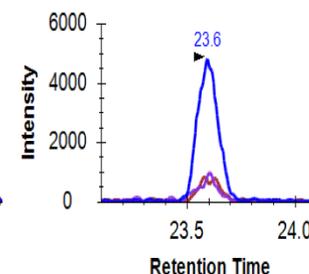
25 pg/mL



50 pg/mL

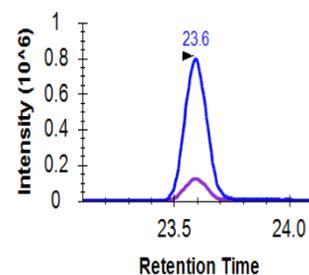
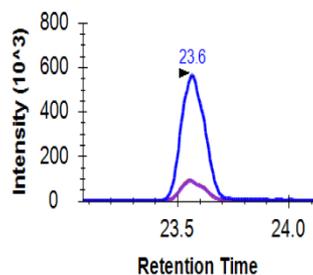
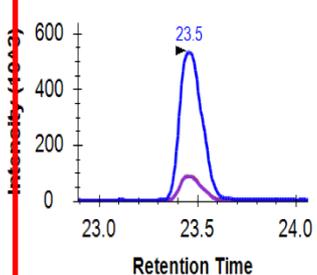
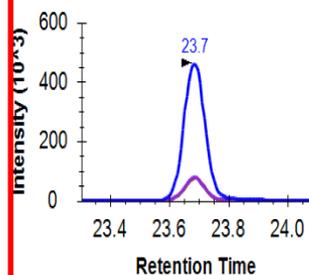
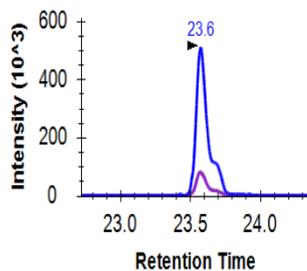


100 pg/mL



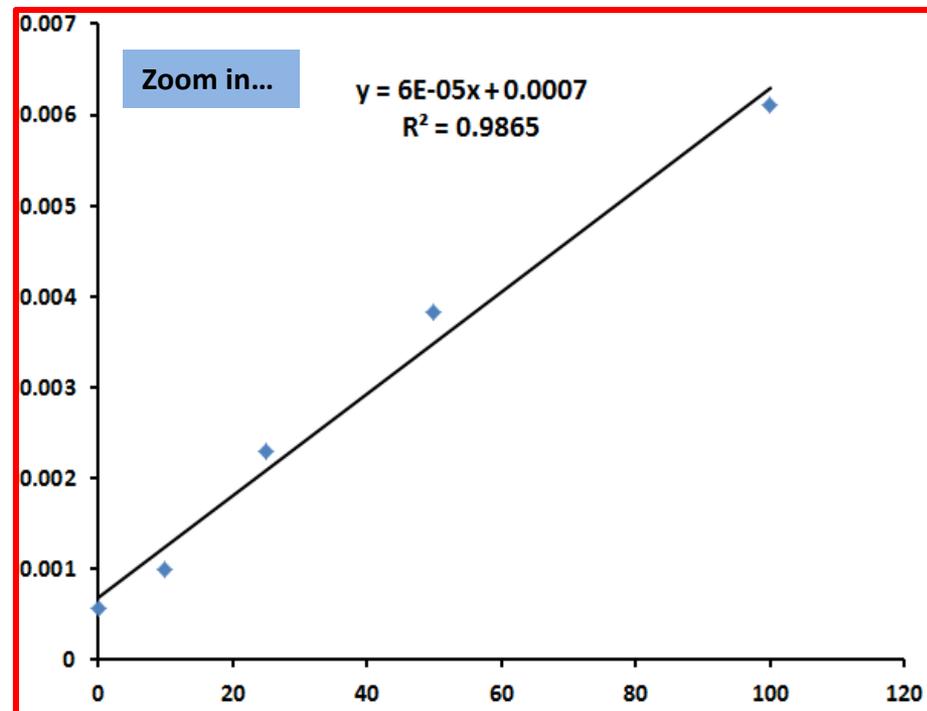
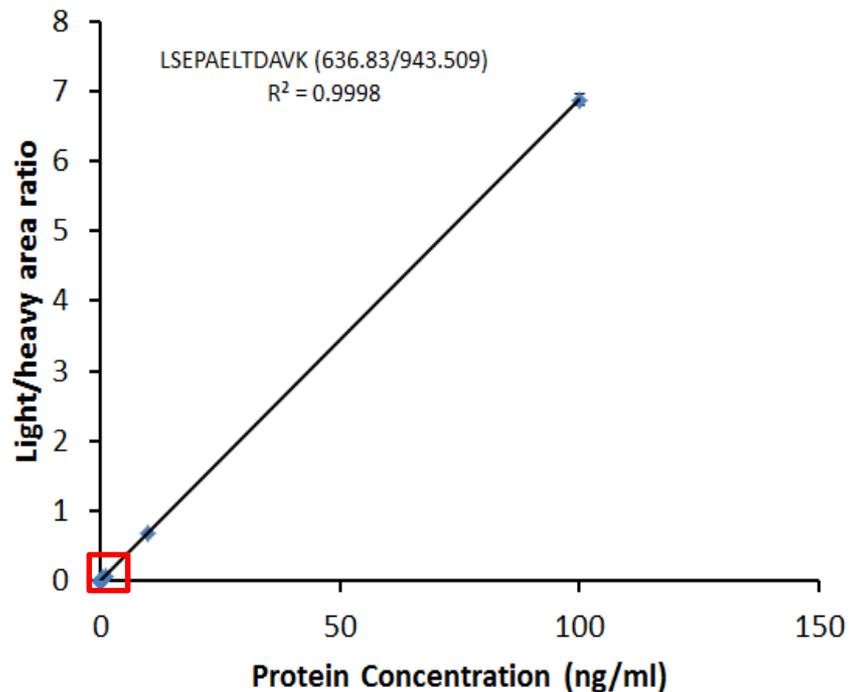
Light

## LSEPAELTDAV(13C6,15N2)K (Internal Standard)



Heavy

# Calibration curve for PSA peptide



An average CV below 5%

Concentration (ng/mL)	0	0.01	0.025	0.05	0.1 <sup>a</sup>	0.5	1	10	100
Light/heavy	N.D.	$1.00 \times 10^{-3}$	$2.30 \times 10^{-3}$	$3.83 \times 10^{-3}$	$6.10 \times 10^{-3}$	$3.52 \times 10^{-2}$	$7.15 \times 10^{-2}$	0.687	6.87
CV(%)	N.A.	10.0	4.35	5.43	2.84	2.3	3.96	3.92	1.21

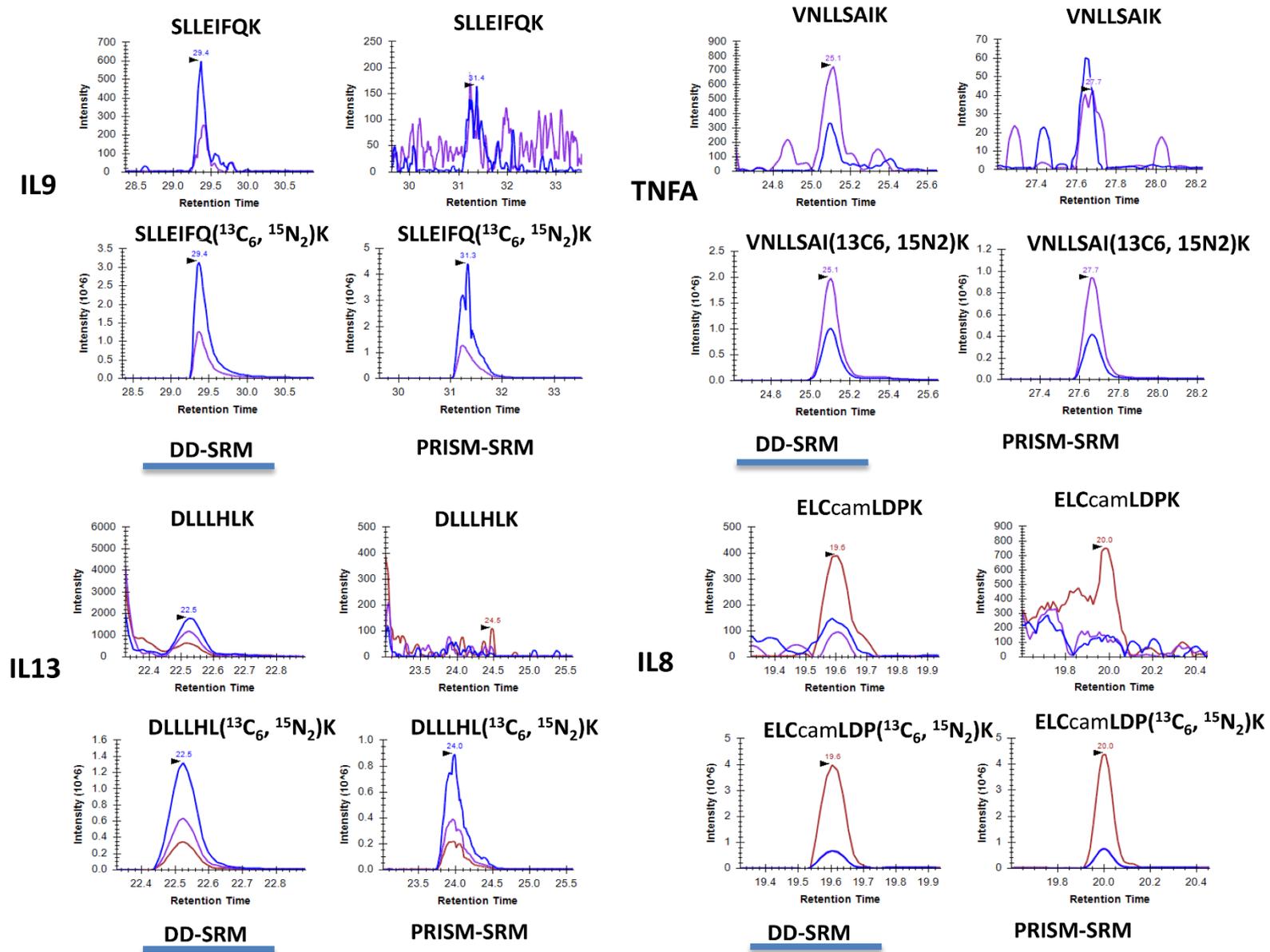
<sup>a</sup> Three processing replicates followed by three SRM technical replicates

# Extremely low-abundance endogenous cytokines using DD-SRM in non-depleted serum

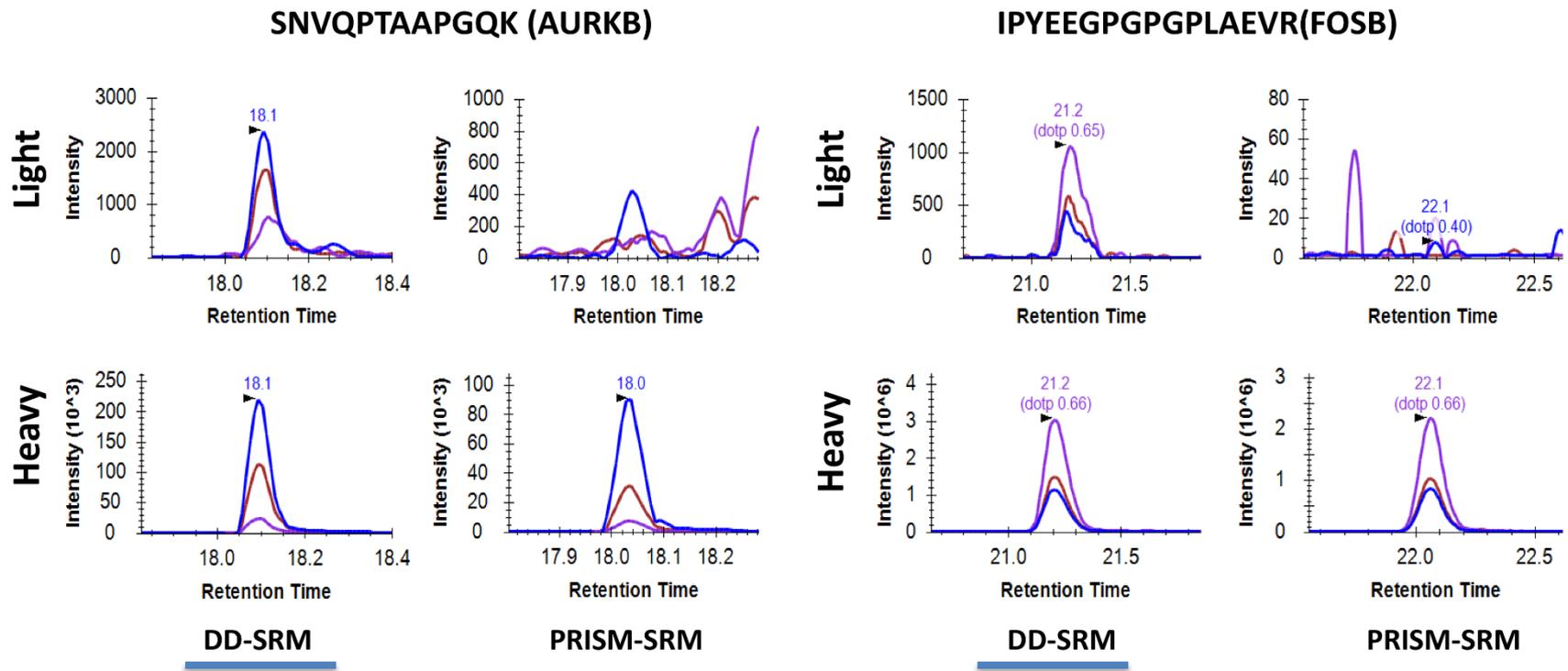
Protein	Serum concentration(pg/mL)*
IL8_HUMAN	7.26
IL9_HUMAN	17.0
IL13_HUMAN	7.23
TNFA_HUMAN	21.9

\* The concentrations are from other reports, measured by ELISA.

# Detection of four cytokines by DD-SRM in non-depleted serum from healthy individuals



# Detection of low-abundance cellular proteins by DD-SRM in an ovarian tumor tissue



Tissue sample loading amount: 1 mg  
~20 copies/cell

# Summary

- A new targeted quantification strategy was developed based on multi-dimensional reversed-phase LC separation, enabling quantification of proteins at <10 pg/mL level in serum/plasma sample, without the need for antibody or immunoaffinity depletion.
- Throughput is limited at present for applications with large numbers of targets or samples: several samples/day.
- A approach is applicable to other MS analyses, e.g., DIA,PRM.
- This method provides a means to quantify extremely low abundance protein in complex biological samples, such as cytokines in serum, transcription factors in cells and tissues.

# Acknowledgements



**The Integrative Omics Group at PNNL**



U24-CA-160019



National Institute of  
General Medical Sciences  
*Basic Discoveries for Better Health*

BTRR: P41GM103493