Proteomics Standards Research Group (sPRG)

sPRG: a Tale of Two Studies

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sPRG working group members

Toni Koller (Acting Chair)	**Columbia University**
Allis Chien (EB Liaison)	Stanford University
Christopher Colangelo	Primary Ion
David Hawke	UT MD Anderson Cancer Center
Alexander R. Ivanov	Northeastern University
Gordana Ivosev	Sciex
Paul Rudnick	Spectragen Informatics
Brian C. Searle	Proteome Software / U. of Washington
Scott A. Shaffer	U. of Massachusetts Medical School

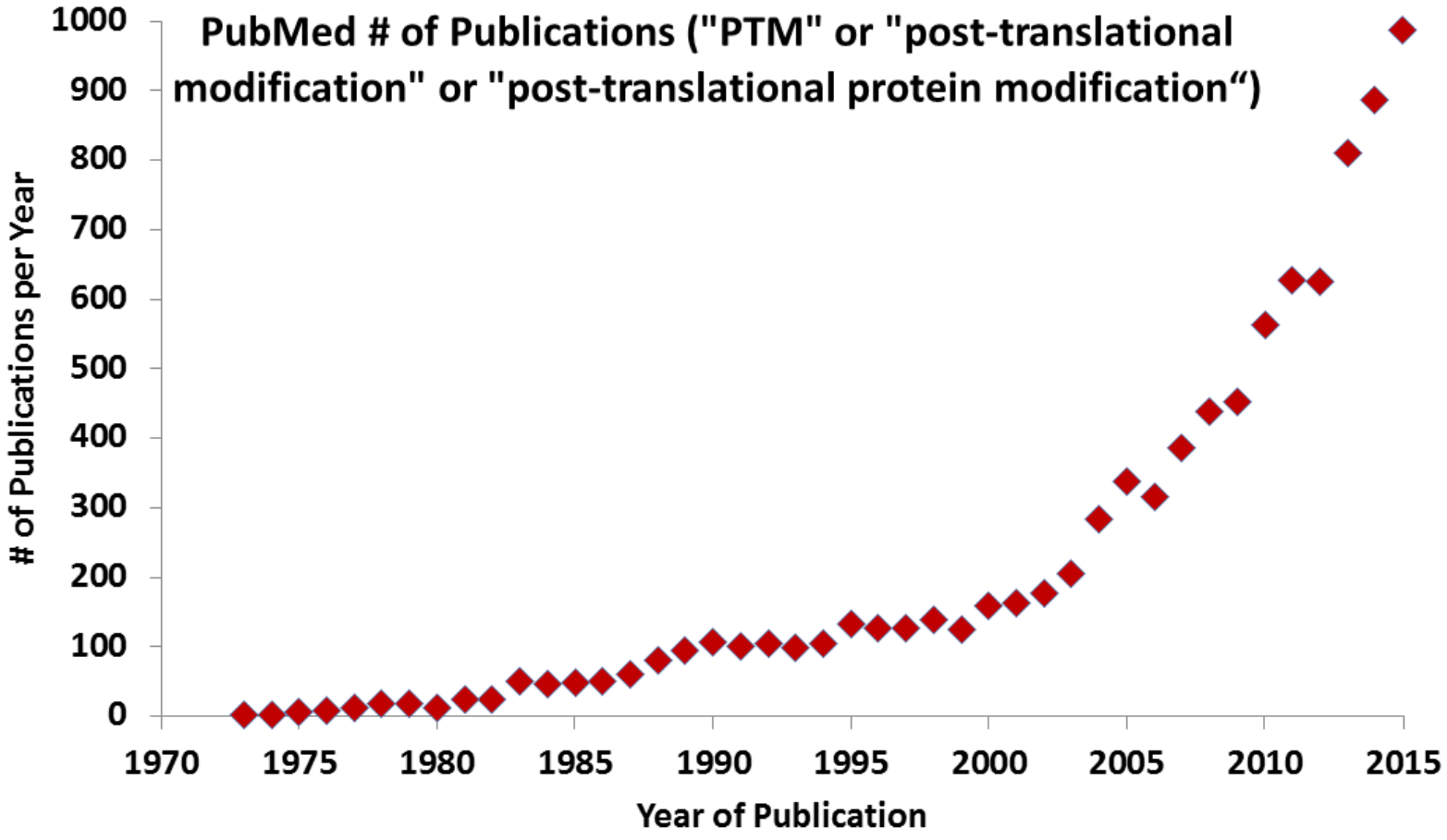
sPRG working group goals

- Revise interpretations of previous studies
- Prepare manuscripts
- Make ABRF standards available to the community

Proteomics Standards Research Group (sPRG)

Revisiting the sPRG 2012 PTM study

PTMs continue to be a growing interest to proteomics



PRG 2003

- 2 digested proteins
- 2 synthetic phosphopeptides

Results:

- 54 labs returned data sets
- 5 identified 1 phosphopeptide
- 5 identified the other
- 3 identified both
- **Massive over reporting**

sPRG 2007

- Mixture of 7 phosphorylated proteins

Results:

- 44 labs returned data sets
- 50 “known” sites of phosphorylation
- 27 sites identified by multiple labs
- 8 “bonus” sites identified by multiple labs
- Only 5 sites identified by ≥50% of labs
- **Over reporting? Interpretation hampered by unknowns**

sPRG 2010

- 6 digested proteins
- 23 synthetic phosphopeptides

Results:

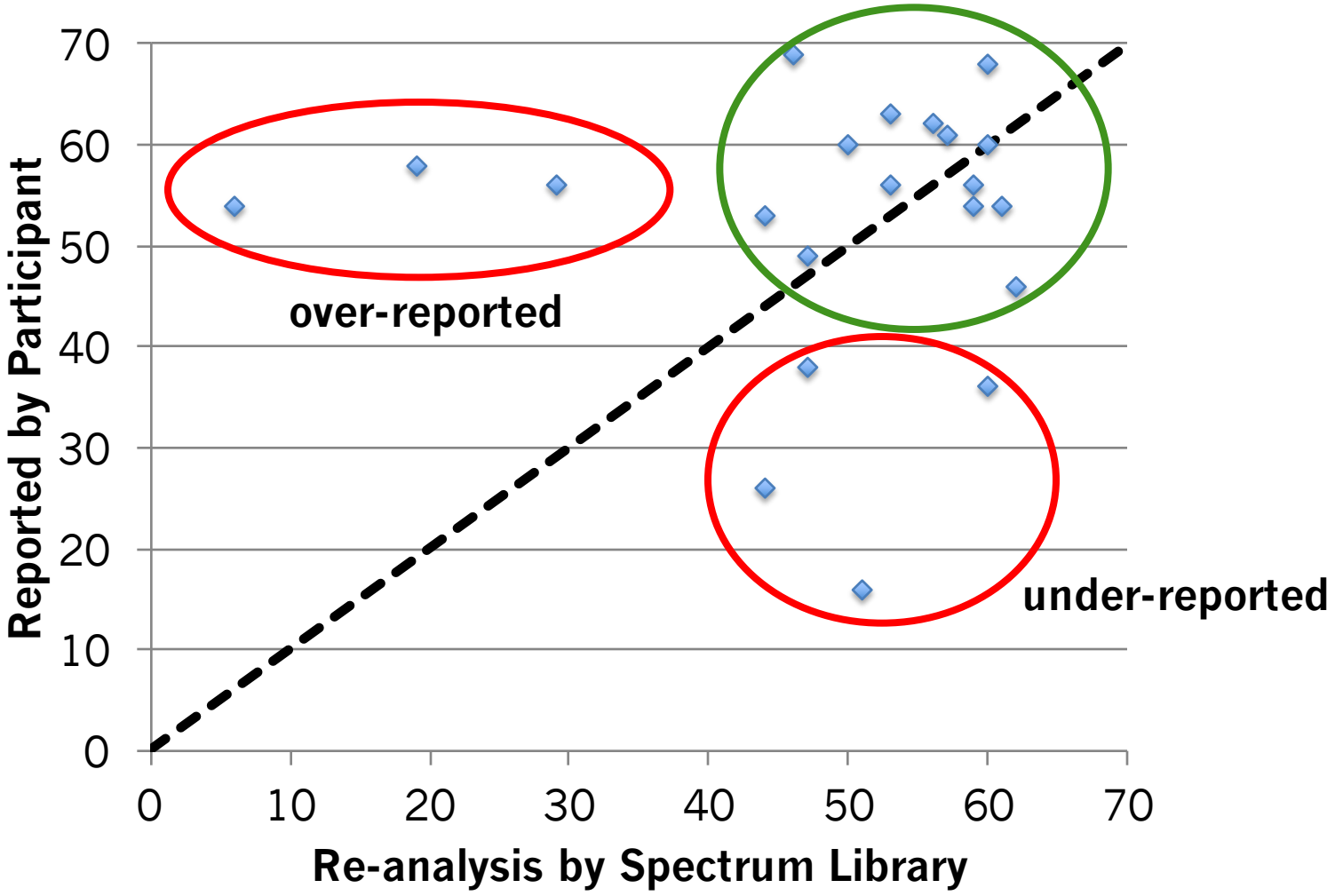
- 43 labs returned data sets
- 23 sites identified by multiple labs
- 16 sites identified by ≥50% of labs
- Multiply phosphorylated peptides still a challenge

sPRG 2012

- 6 digested proteins
- 45 synthetic phosphopeptides
  - (including positional isomers)
- 41 synthetic modified peptides
  - sulfated tyrosine
  - nitrosylated tyrosine
  - acetylated lysine
  - mono- di- and tri-methylated arginine/lysine
  - sym/asymmetric di-methylated arginine

- 30 data sets returned

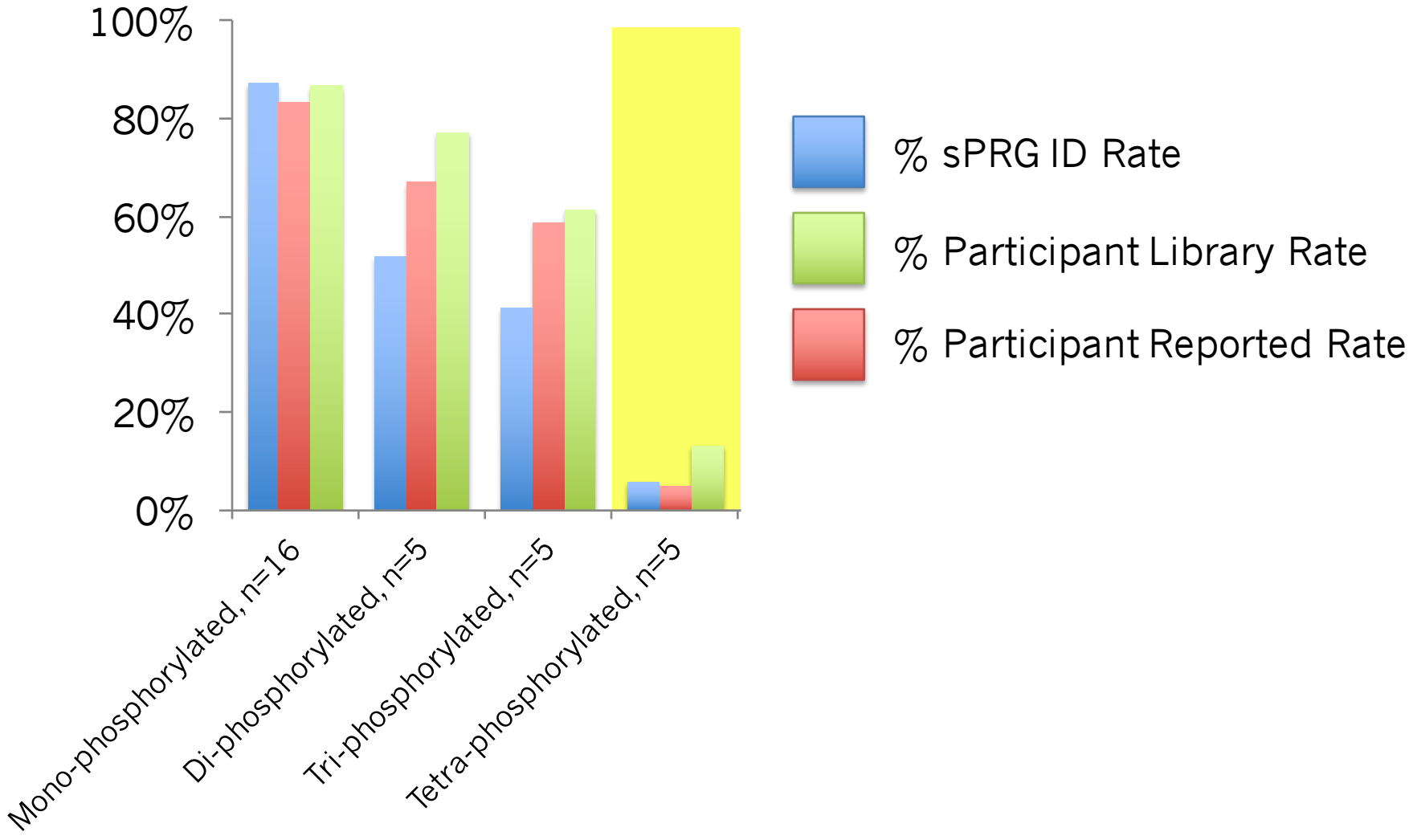
Over-reporting has been curbed somewhat



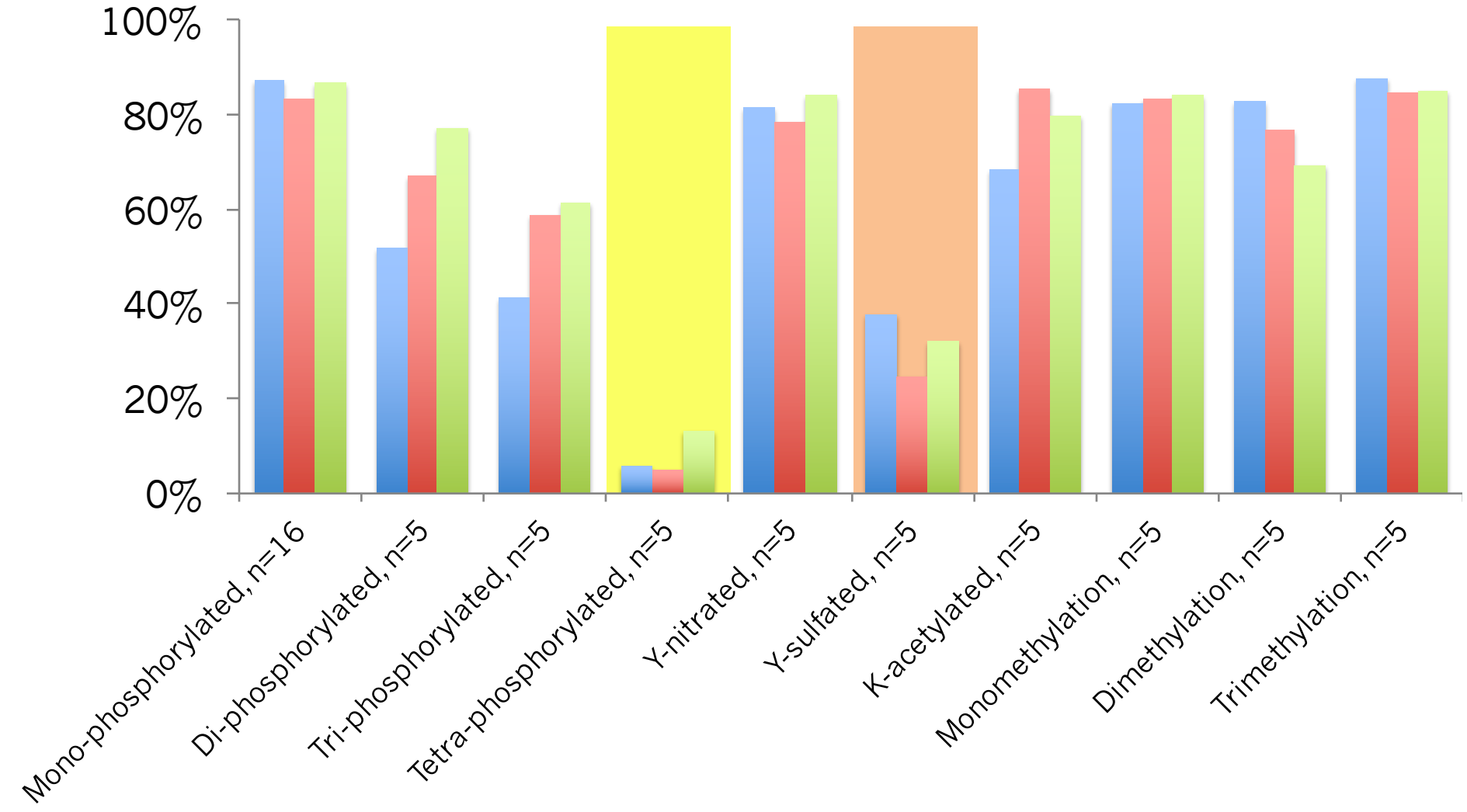
Cross study comparison shows general improvement

	PRG 2003	sPRG 2010	sPRG 2012
SVSpDYEGK	15%	40%	80%
THILLFLPKSpVSDYEGK	15%	62%	80%

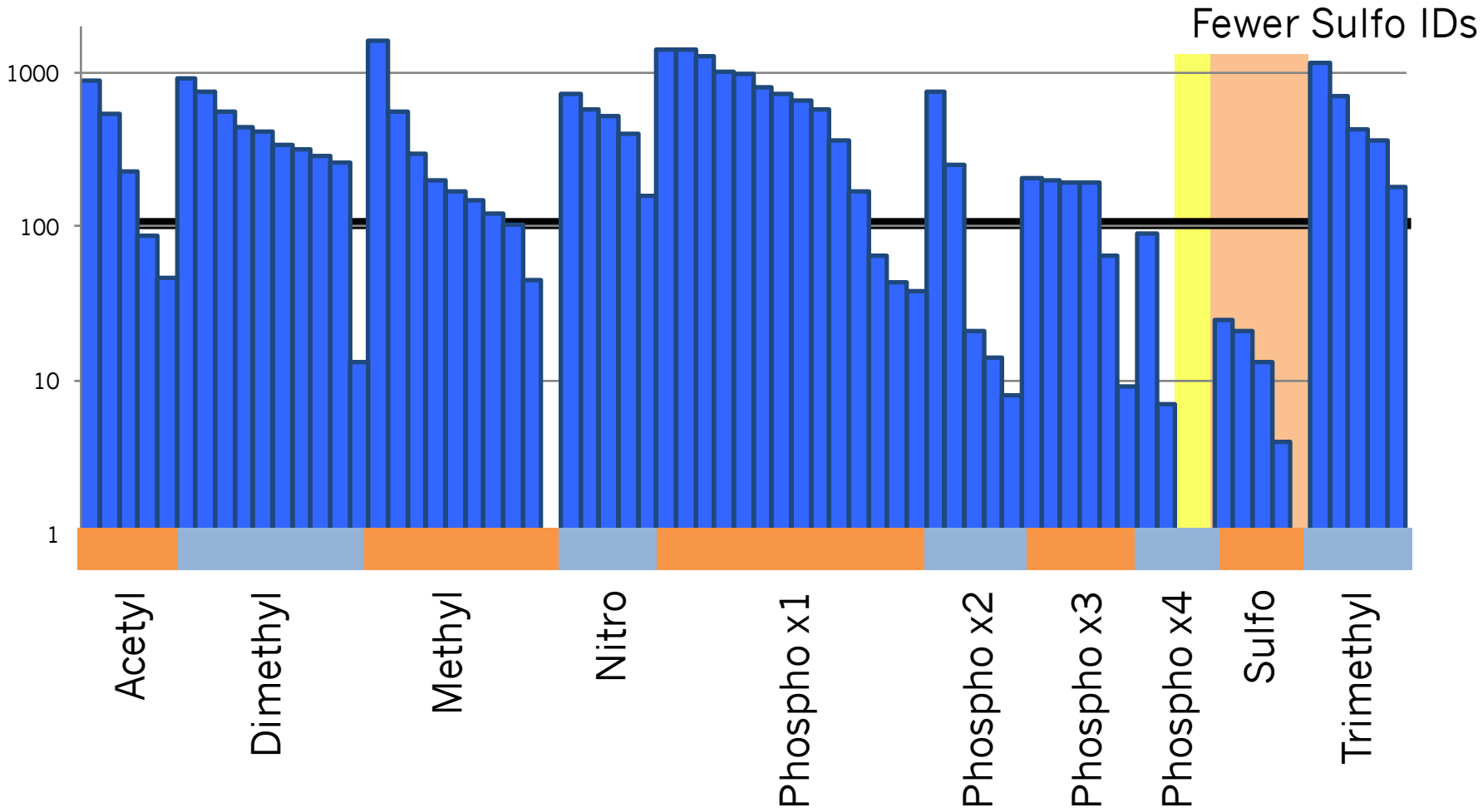
Still difficult to identify multiply phosphorylated peptides



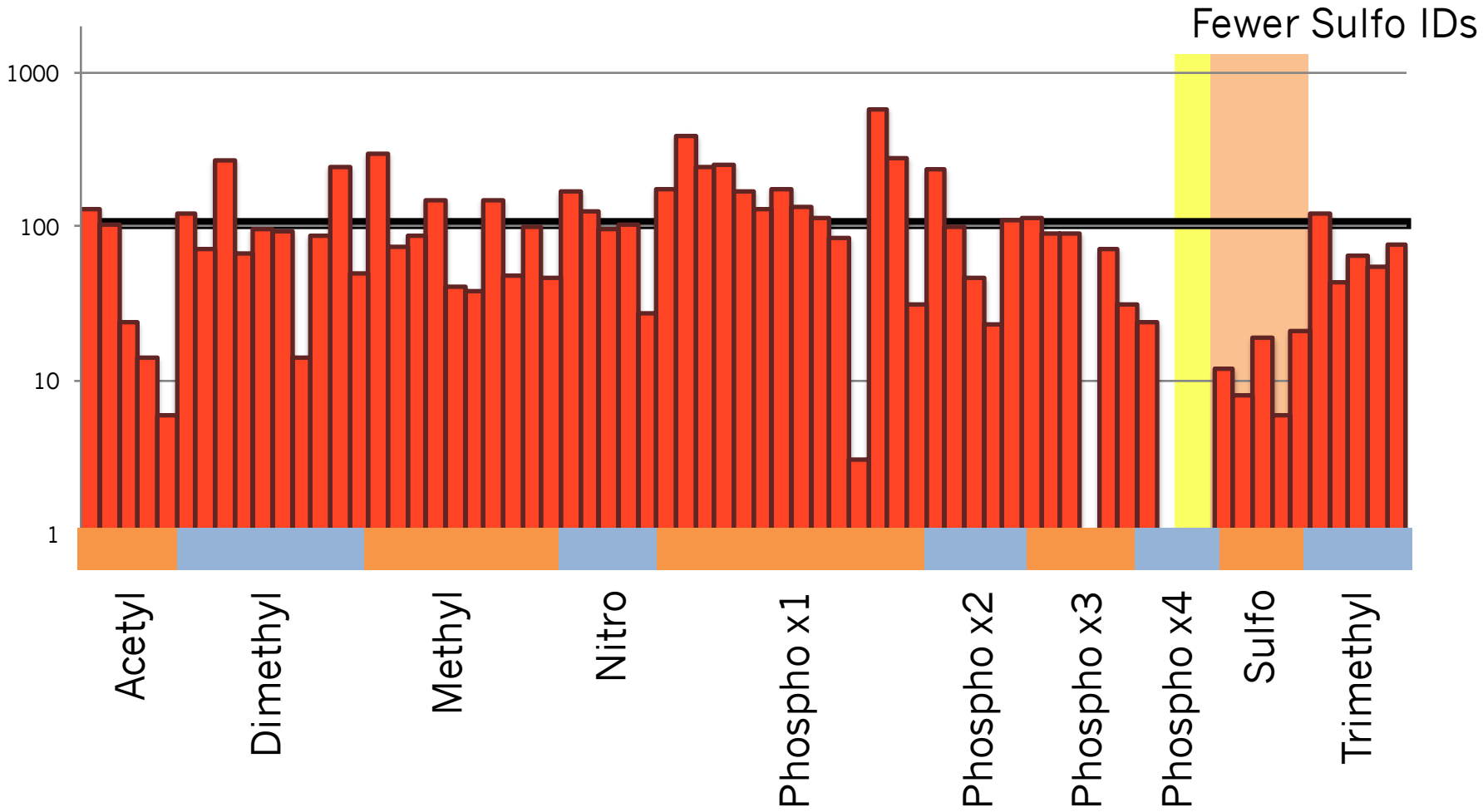
Analyses of other modifications are more successful



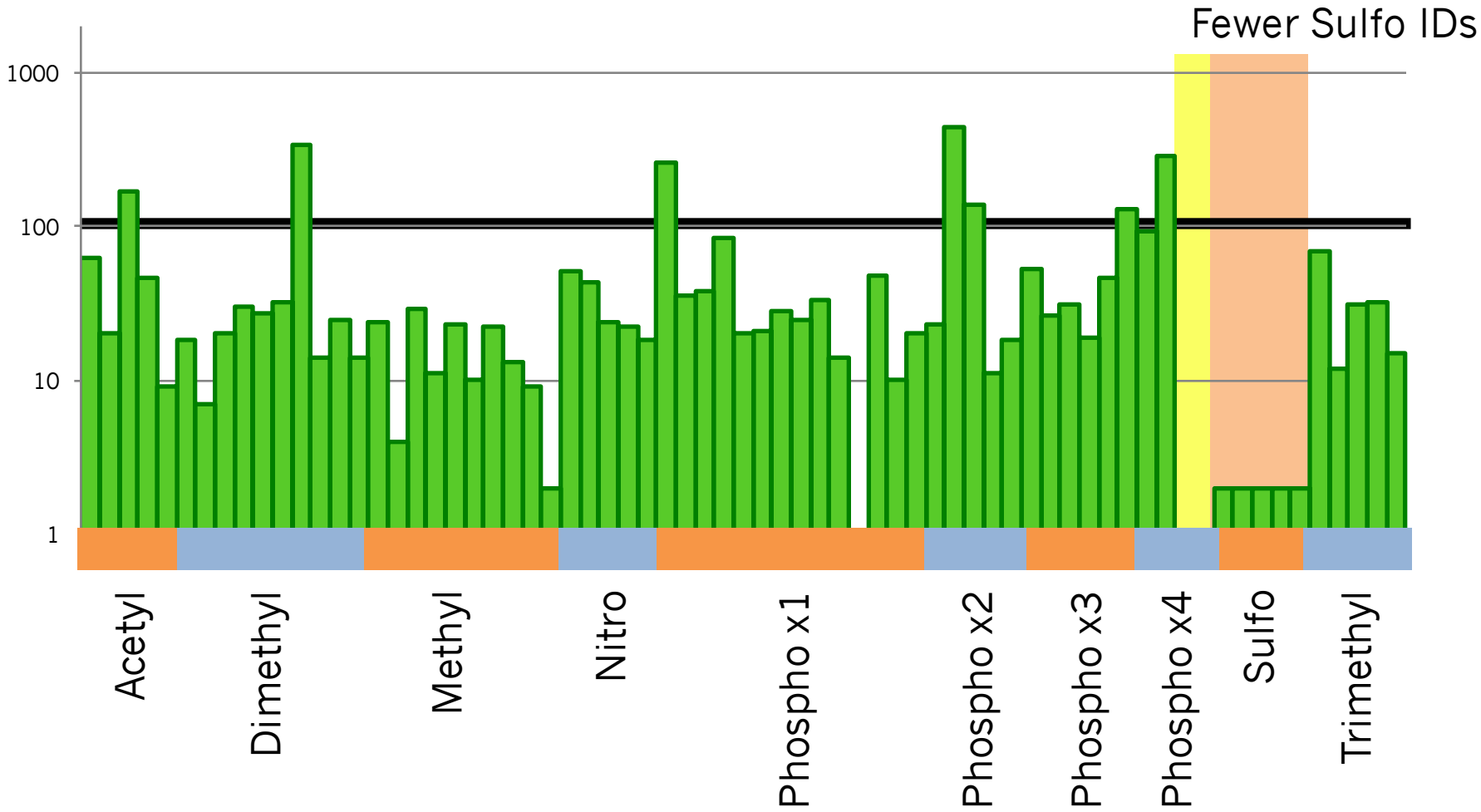
Confident CID identifications



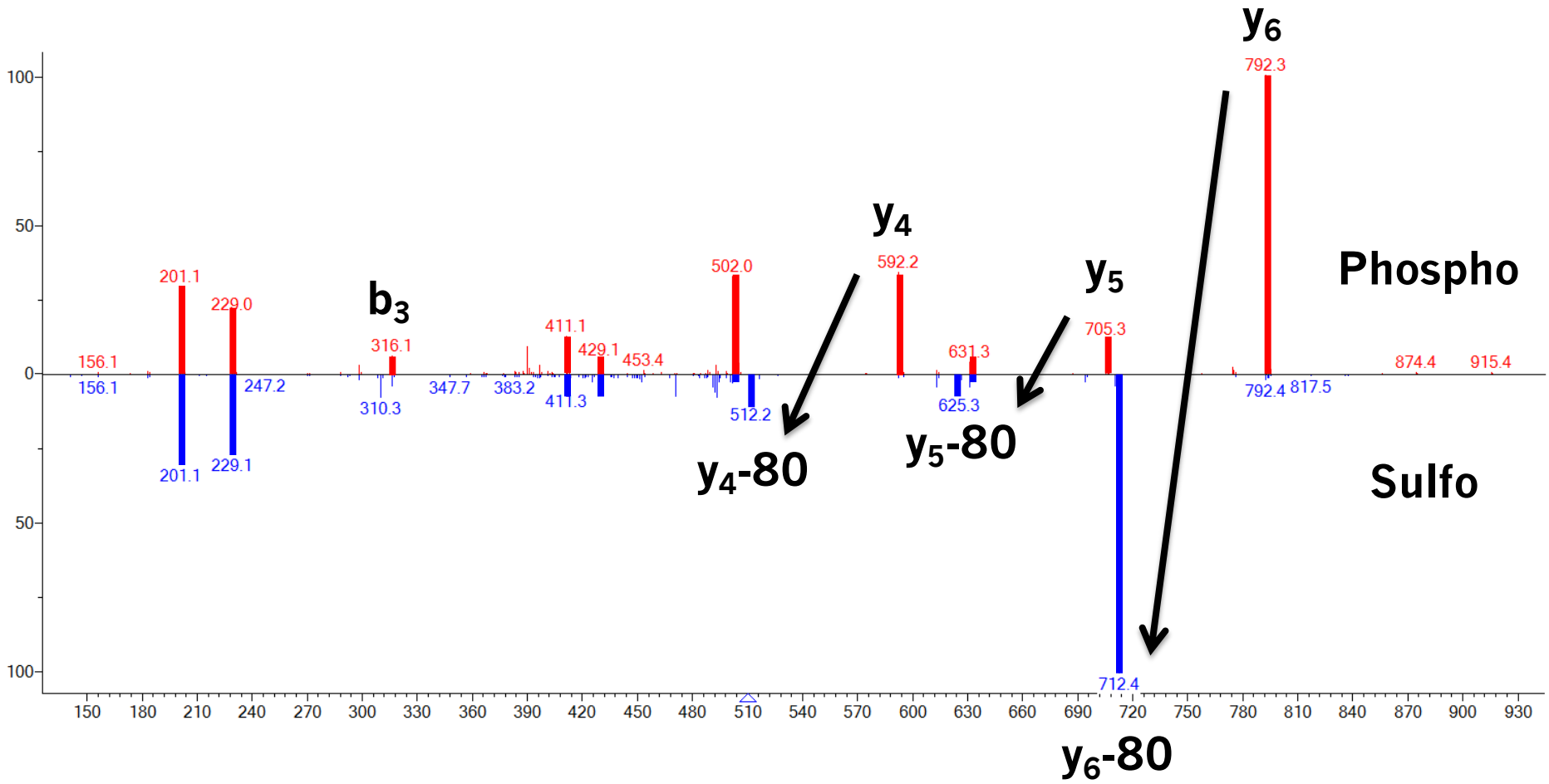
Confident HCD identifications



Confident Q-ToF identifications



DISL**S***DYK (Phospho/Sulfo)



Standard Availability

- Working with Thermo Fisher and Spectragen Informatics to distribute the sample with a new mass spectral library
- Revalidated the sample to confirm the make up
- Commercially available in limited quantities soon
- Sign up to be notified of its availability at http://spectragen-informatics.com/sprg

sPRG members involved in this study

Alexander R. Ivanov (Chair)	**Northeastern University**
Christopher Colangelo	Primary Ion
Craig Dufresne	Thermo Fisher Scientific
David Friedman	Vanderbilt University
Kathryn S. Lilley	University of Cambridge
Karl Mechtler	IMP Research Inst. of Molecular Pathology
Brett Phinney	University of California, Davis
Kristie Rose	Vanderbilt University
Paul Rudnick	Spectragen Informatics
Brian C. Searle	Proteome Software / U. of Washington
Scott A. Shaffer	U. of Massachusetts Medical School
Susan T. Weintraub	University of Texas HSC

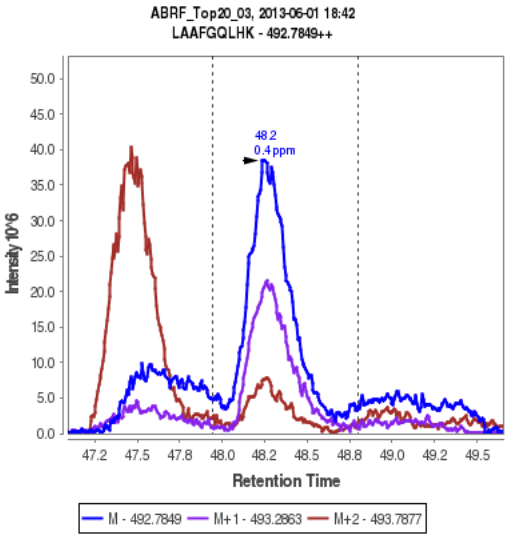
Proteomics Standards Research Group (sPRG)

Revisiting the sPRG 2014 “1000 Peptide” quantification study

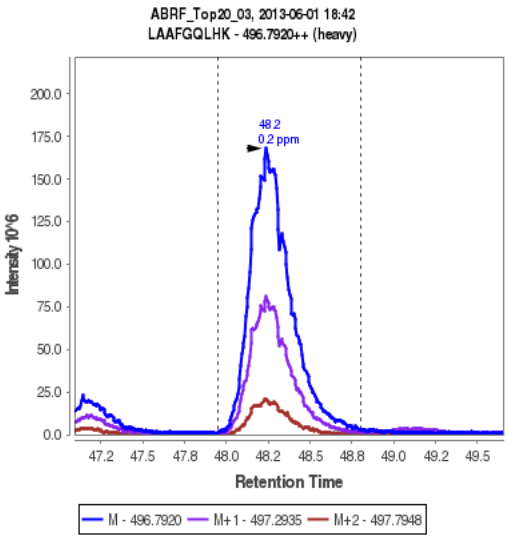
Relative Quantification with Stable Isotope Labeled Peptides

ILF3 (interleukin enhancer binding factor 3)

LAAFGQLHK



LAAFGQLH**K***



light

heavy

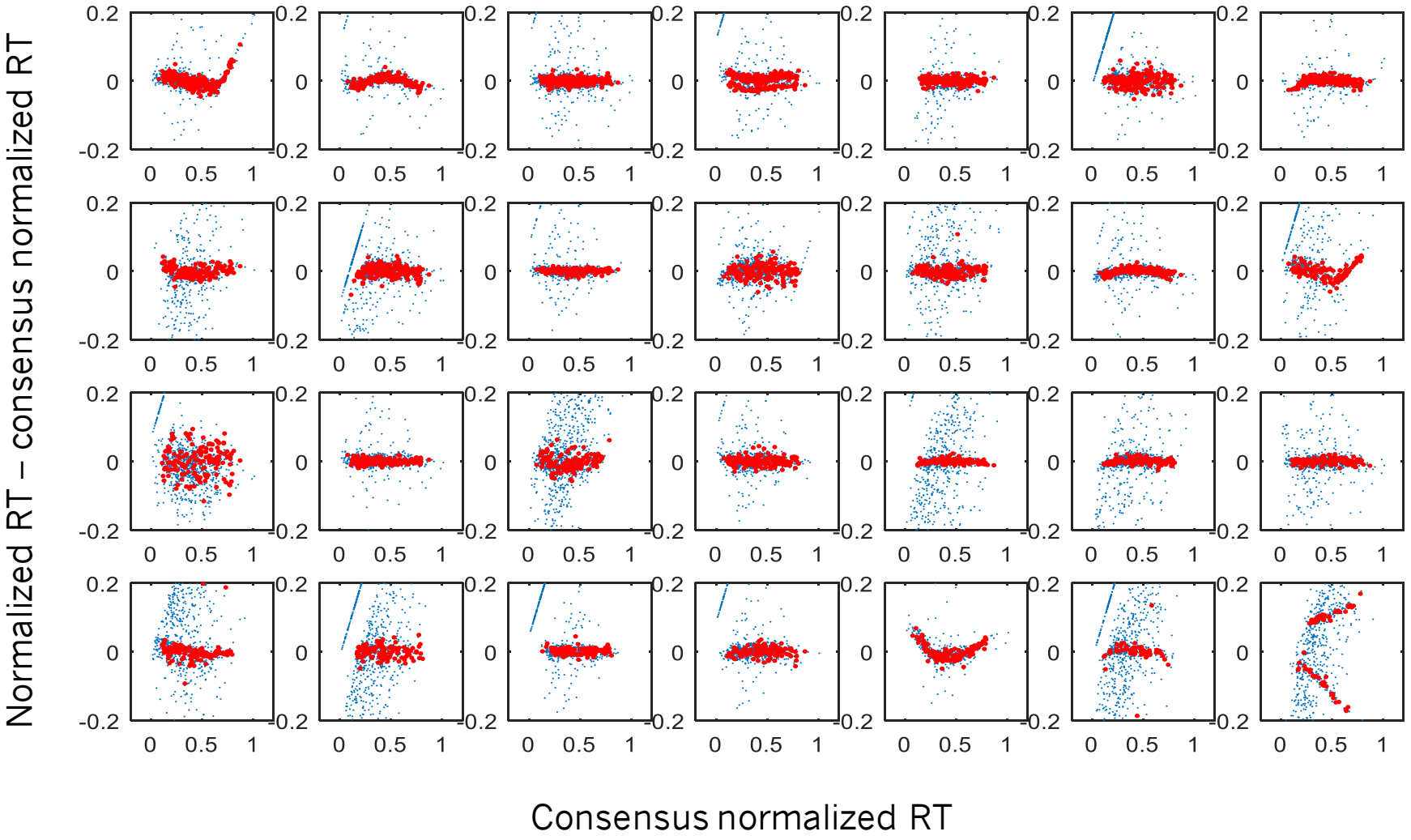
sPRG 2014

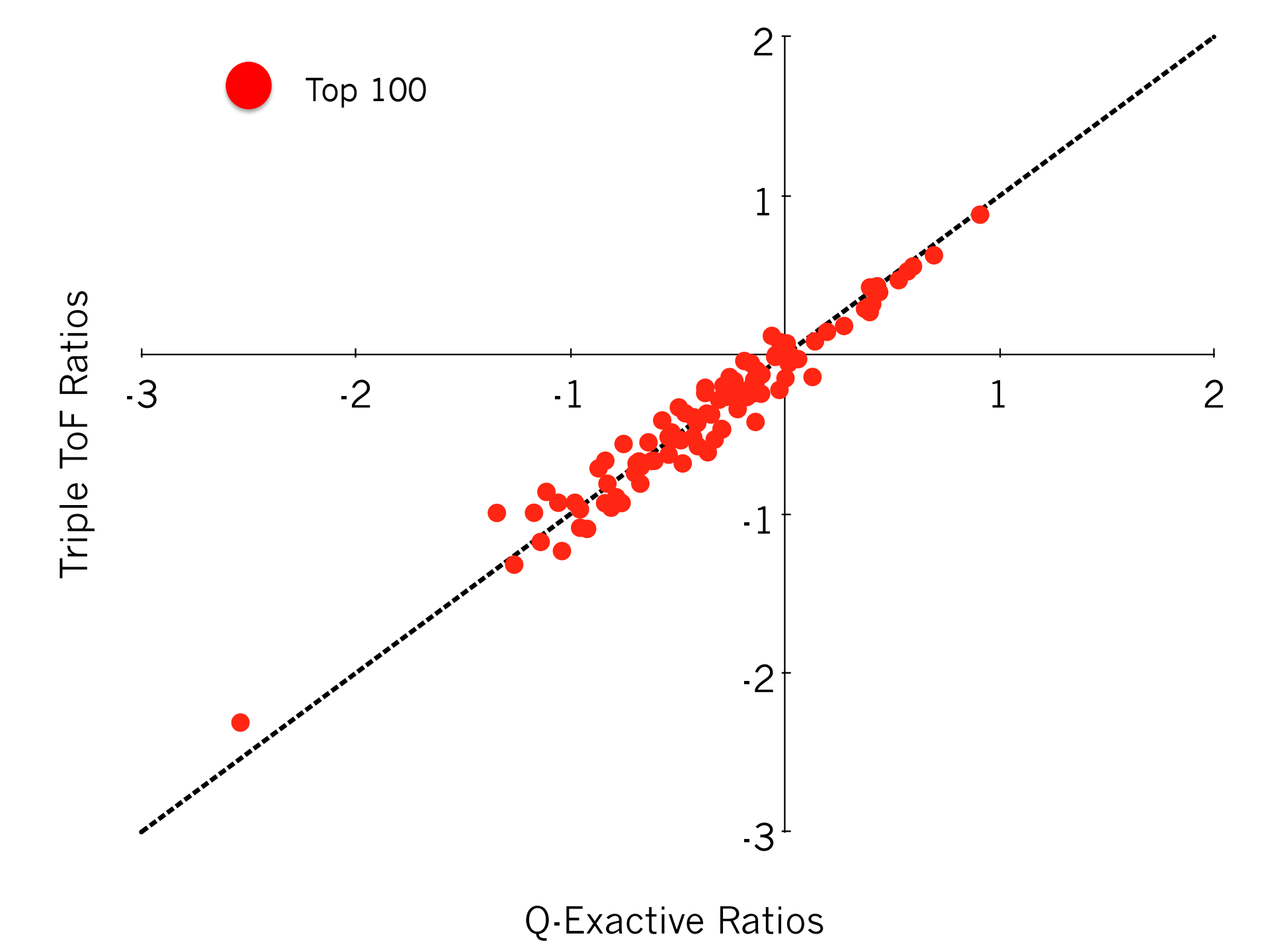
- 1000 tryptic peptides from 552 proteins synthesized by JPT
- Conserved across *Homo sapiens*, *Mus musculus* and *Rattus norvegicus*
- Chosen because of consistency of observation across three different labs
- stable isotope labeled at R and K

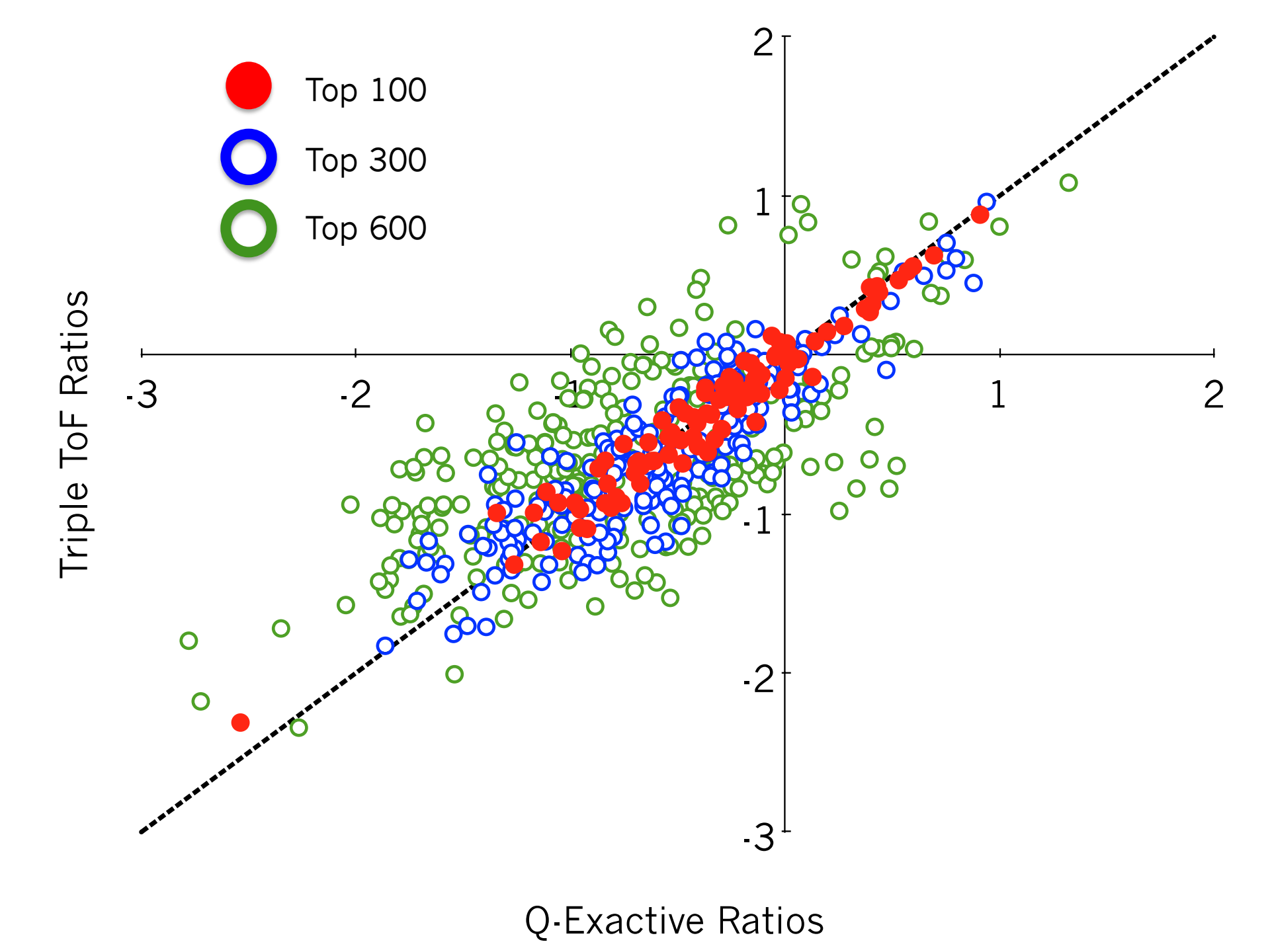
sPRG 2014

- Only light cleanup: we don't know the true abundance of the peptides
- When mixed with other samples: provides a relative standard to compare across platforms
- Initial study performed with HEK 293 matrix
- 49 labs returned data sets

Retention times are extremely consistent across labs and platforms



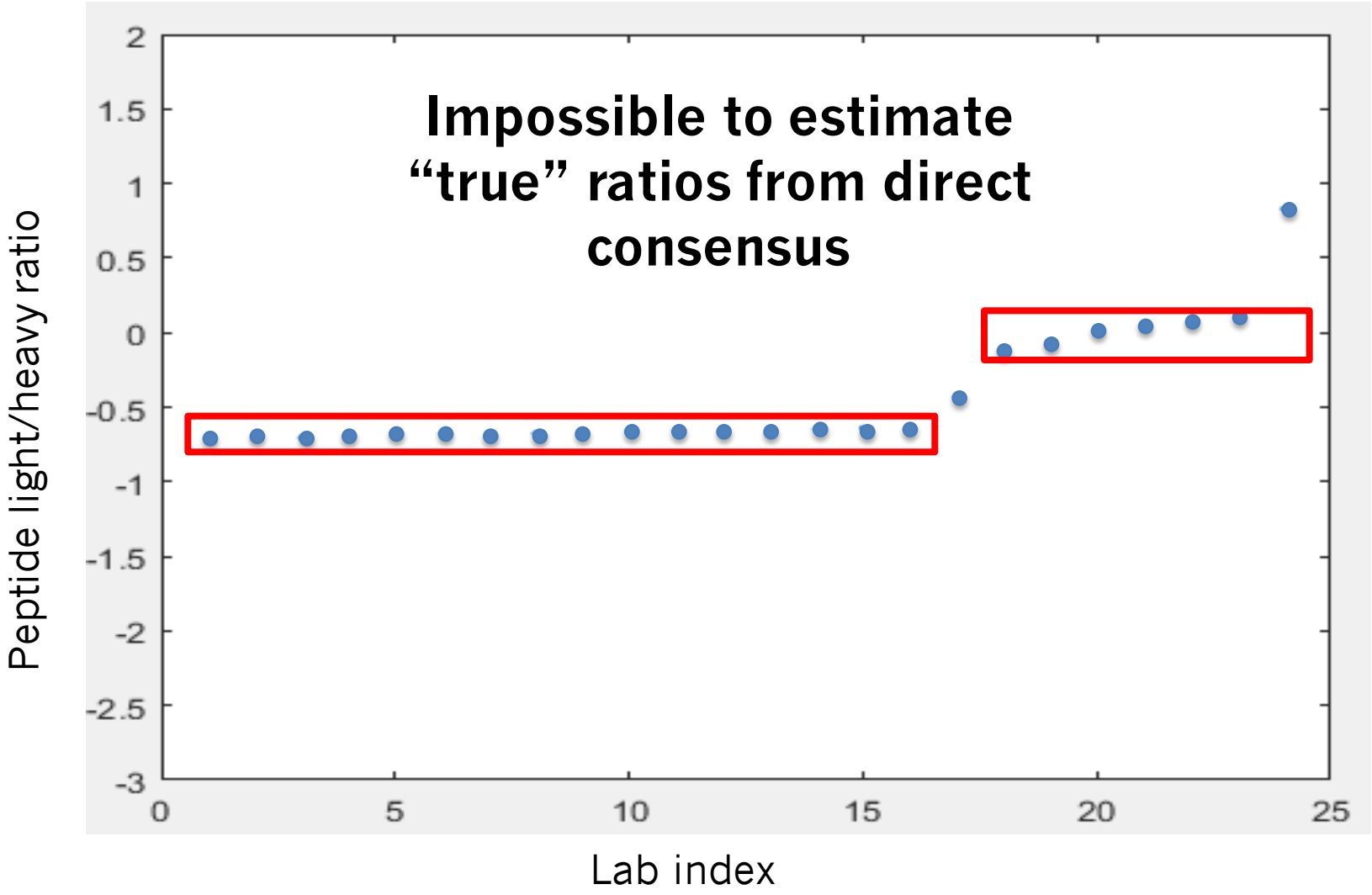

Top 100
Triple ToF Ratios
Q-Exactive Ratios
2
1
-1
-2
-3
-3
-2
-1
1
2


Top 100
Top 300
Top 600
Triple ToF Ratios
Q-Exactive Ratios
2
1
-1
-2
-3
-3
-2
-1
1
2

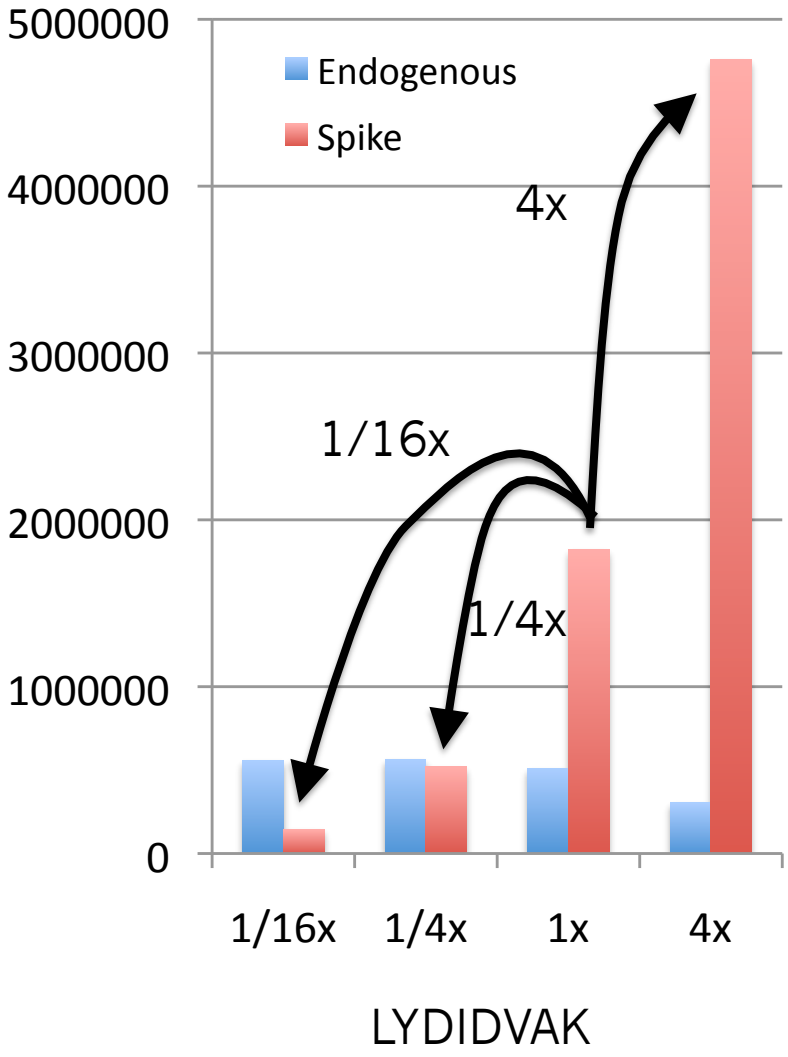
Much more quantitative variability than we expected!

- Worked to assign a better “true” ratio to improve alignment
- Worked to understand where the variability was coming from

What is the “true” peptide ratio?

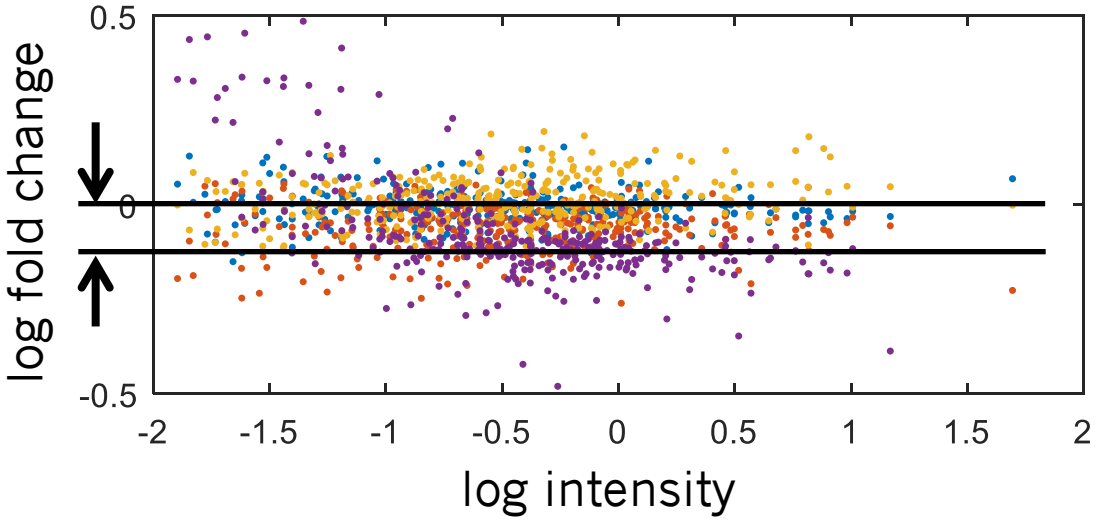


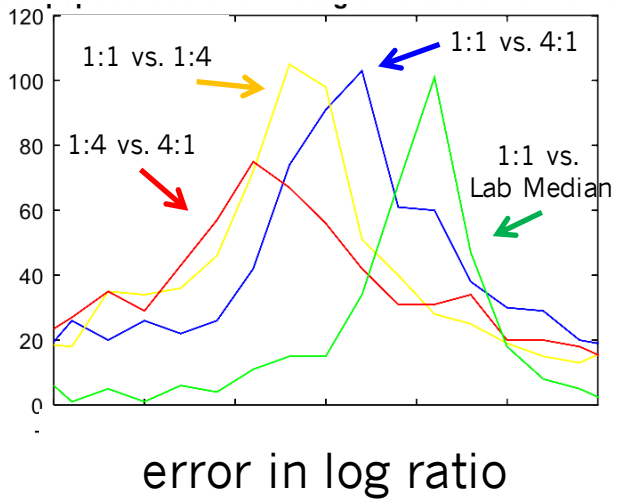
Constructing an accurate “true” ratio



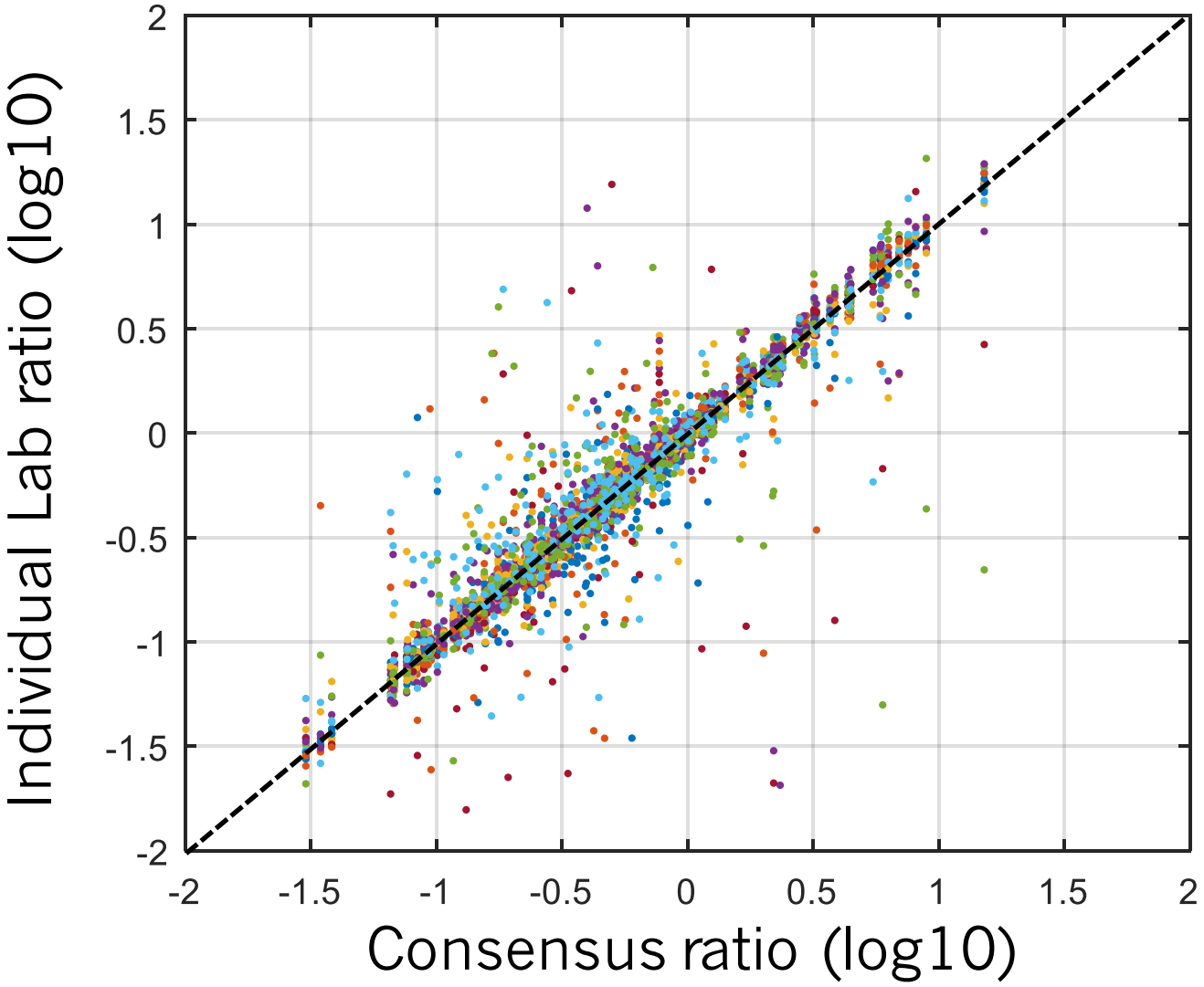
- Only 1x was given to participants
- Triplicate analysis of all mixtures
- Two very different instruments / configurations

Assigning a “true” ratio from all the dilution mixtures

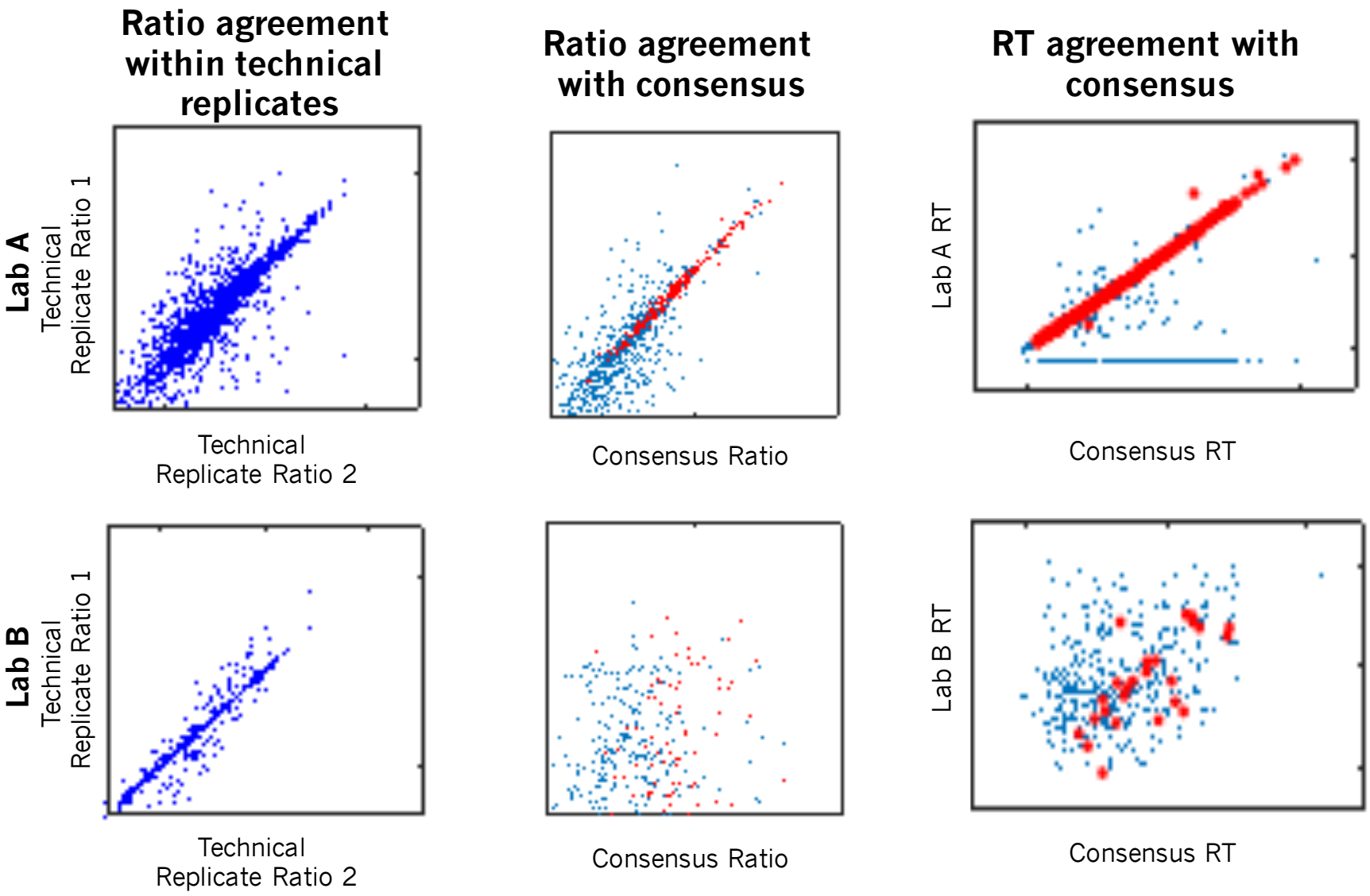




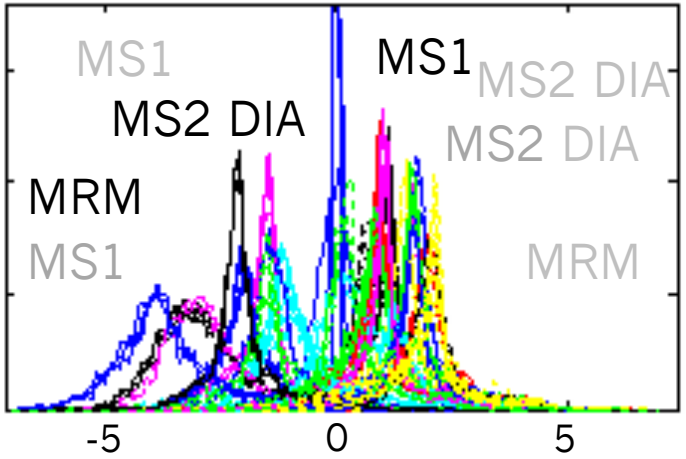
357 peptides in good agreement across most labs



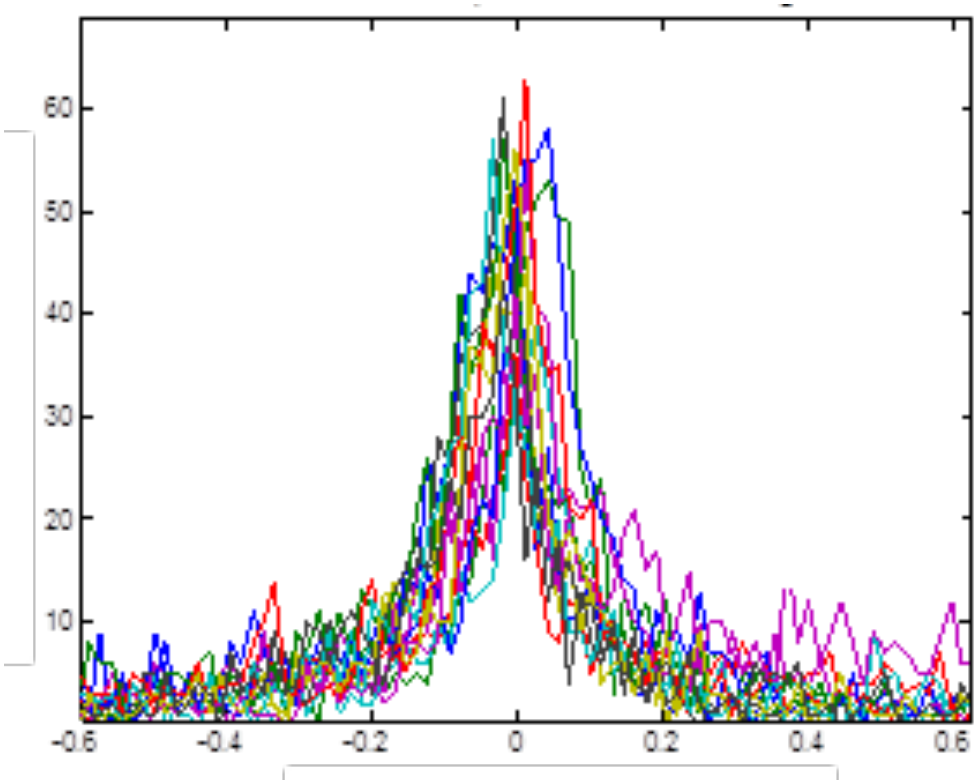
Replicate accuracy doesn't necessarily imply "true" accuracy



Adding standard to sample allows comparison to other labs/platforms



Ratio of Lab X to Lab Y



Ratio of Lab X/Std to Lab Y/Std

Re-characterized standard mixed with in HeLa

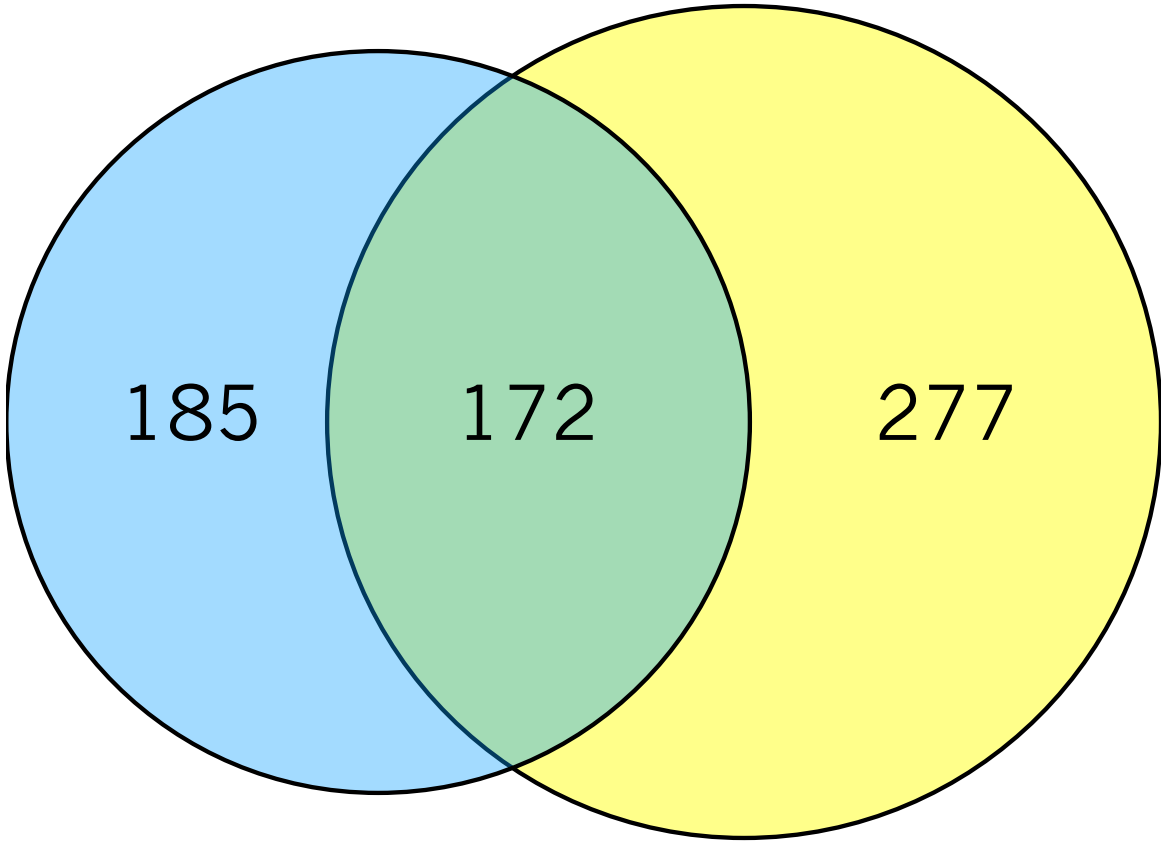
- Logically, if you can compare very disparate platforms, you should be able to compare cell lines

- We ran acquisitions on 3x different instrument platforms

Different 300 peptides in HeLa?!

357 consensus
HEK peptide ratios

449 confidently IDed
HeLa peptide ratios



What does that mean? Take homes

- Matrix complexity has a huge effect on which peptides are visible
- “1000 peptide” standard doesn’t mean 1000 peptides are quantitative in your sample
- 1000 peptides sounds like overkill but it guarantees some peptides are quantitative
- Of the 1000 peptides, we believe approximately 1/3rd are quantitative in any given cell line

What can you use this sample for if you don't have multiple platforms?

- Costs ~ 50¢ per sample (50 fmol)
- Cheap quantitative standard (if it overlaps with your peptides of interest)
- Loading standard
- iRT alignment standard for improving identification rates

jpt
Innovative Peptide Solutions

Proteomics & Enzyme Profiling

SpikeMix™ ABRF (cross-species standard)
100pmol - $ 1049.00
10pmol - $ 545.00
1pmol - $ 164.00

sPRG members involved in this study

Christopher Colangelo (Chair)	**Primary Ion**
Craig Dufresne	Thermo Fisher Scientific
David Hawke	UT MD Anderson Cancer Center
Gordana Ivosev	Sciex
Toni Koller	Columbia University
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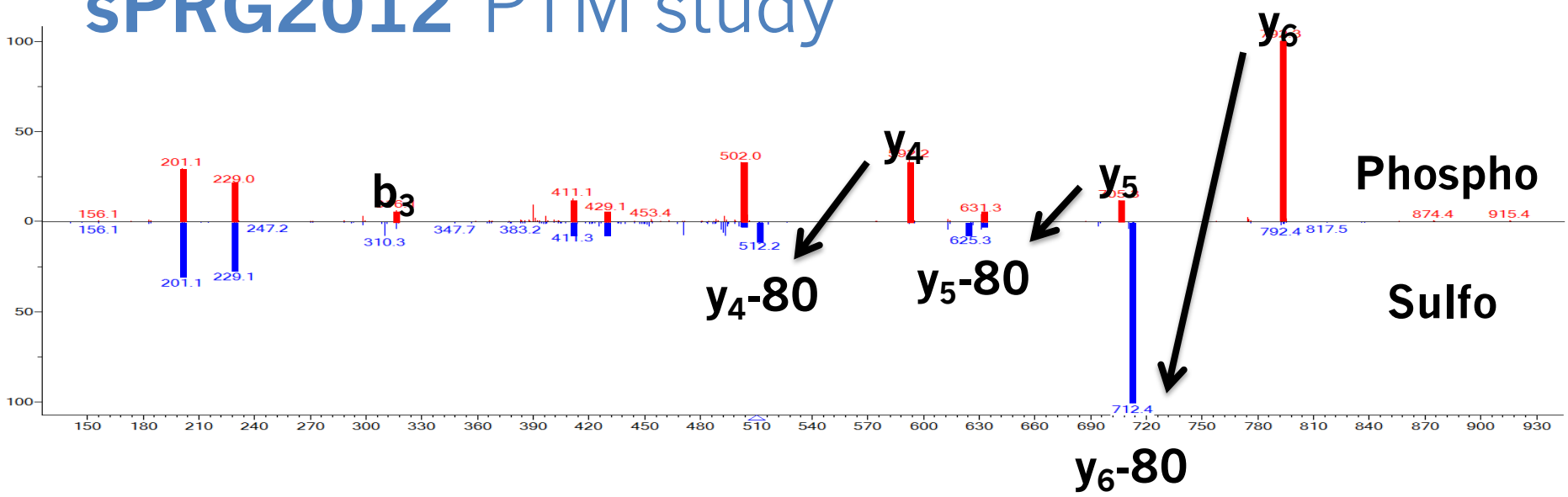
Kristie Rose	Vanderbilt University
Paul Rudnick	Spectragen Informatics
Brian C. Searle	Proteome Software / U. of Washington
Scott A. Shaffer	U. of Massachusetts Medical School
and:	
Brendan MacLean	U. of Washington
Vagisha Sharma	U. of Washington

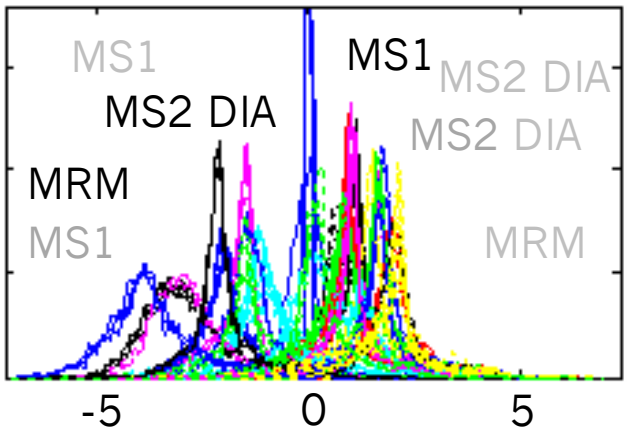
sPRG 2017?

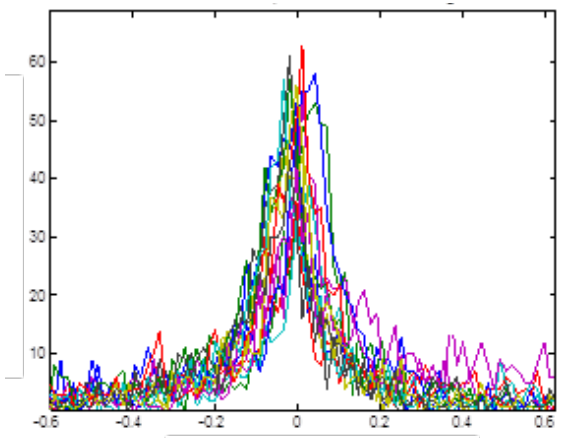
We have several new study ideas,
but need new members!

sPRG2012 PTM study



sPRG2014 1000 peptide study





Ratio of Lab X/Std to Lab Y/Std