Proteomics Standards Research Group (sPRG)

sPRG: yet another phosphoproteomics standard?

sPRG working group members

Antonius Koller (Chair)	Northeastern University
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David Hawke	UT MD Anderson Cancer Center
Anthony W. Herren	UC Davis
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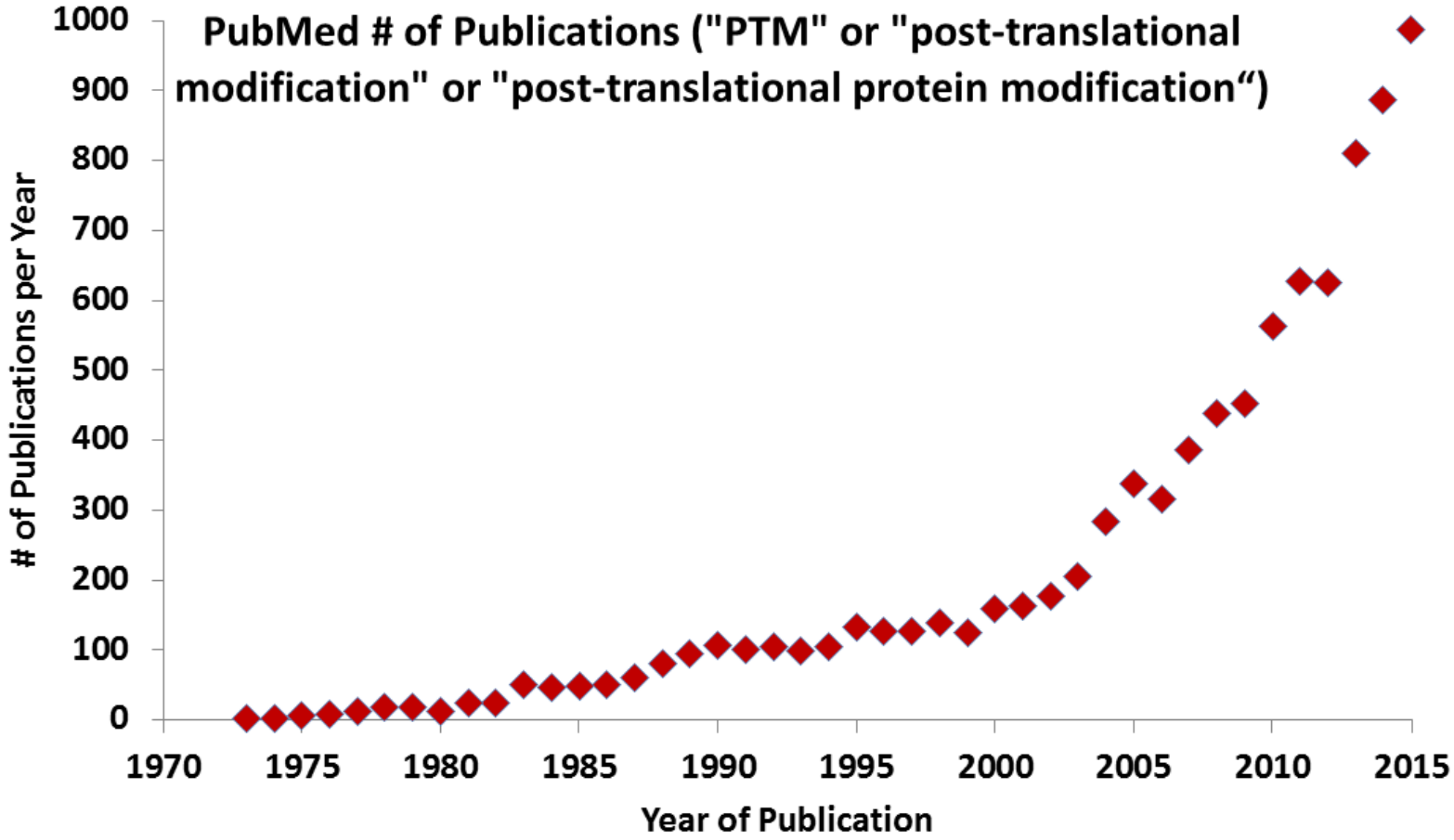
Brian C. Searle	University of Washington/Proteome Software Inc.
Allis Chien (EB Liaison)	Stanford University

Thank you!

Jenny Kim and Purvi Patel at Columbia University

PTMs continue to be a growing interest to proteomics



PRG 2003 phosphopeptide standard

- 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 phosphopeptide standard

- 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 phosphopeptide standard

- 6 digested proteins
- 23 synthetic phosphopeptides

Results:

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

sPRG 2012 phosphopeptide standard

- 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

Cross study comparison shows general improvement

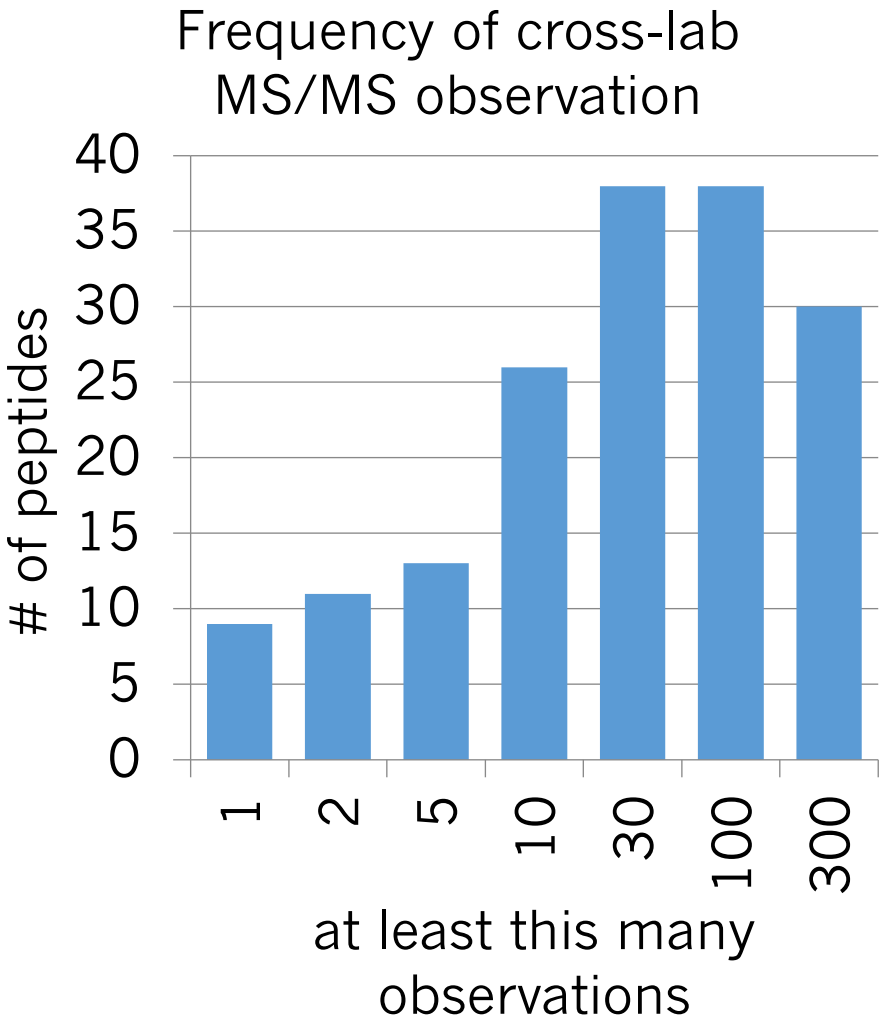
	PRG 2003	sPRG 2010	sPRG 2012
SVpSDYEGK	15%	40%	80%
THILLFLPKpSVSDYEGK	15%	62%	80%

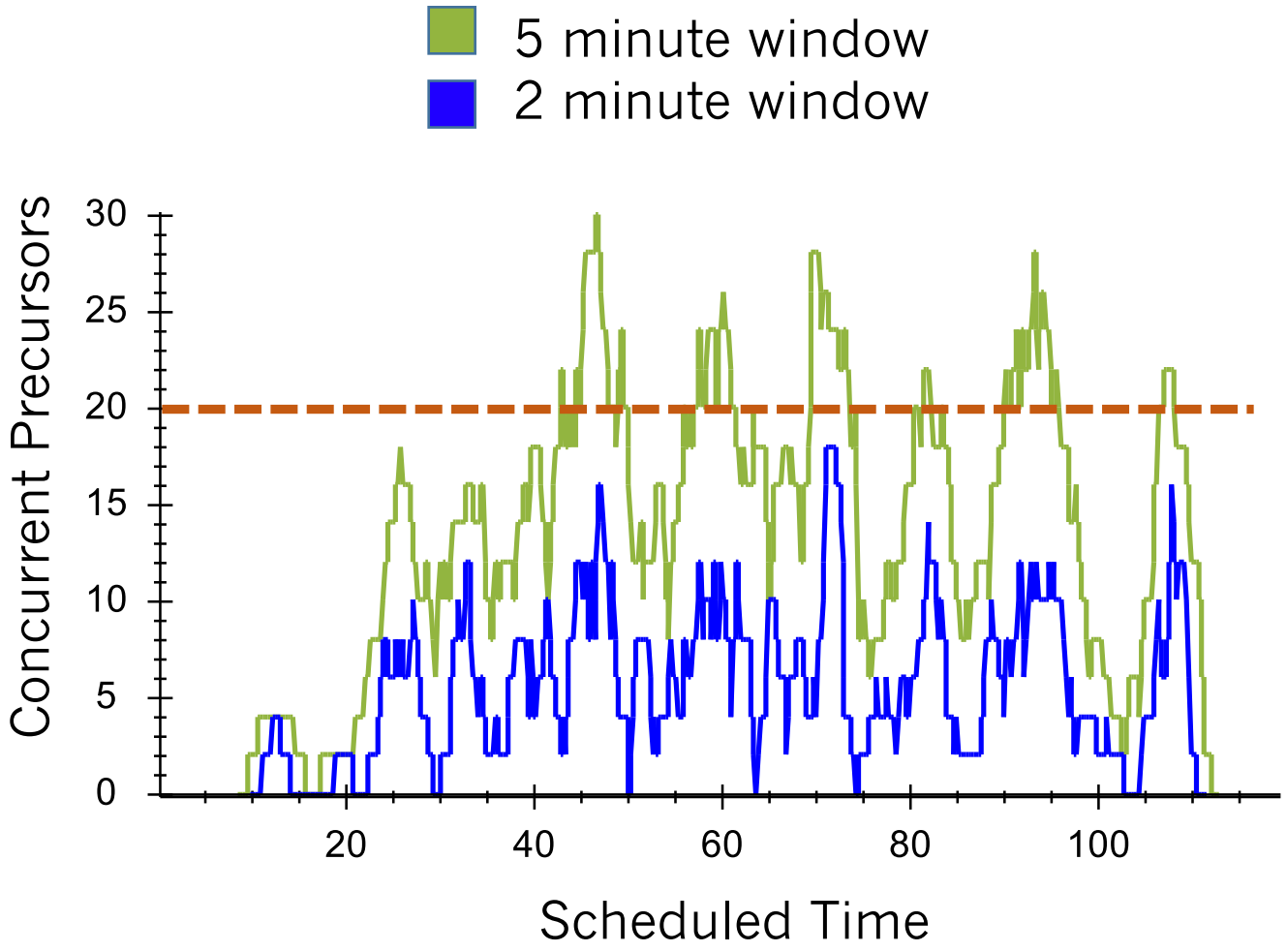
sPRG 2018 phosphopeptide standard

- 147 biologically interesting synthetic phosphopeptides

- Chosen to cover seven different signaling pathways:
  - AMPK signaling
  - death and apoptosis signaling
  - EGFR/HER signaling
  - insulin/IGF-1 signaling
  - mTOR signaling
  - PI3K/AKT signaling
  - stress (p38/SAPK/JNK) signaling

sPRG 2018 phosphopeptide standard



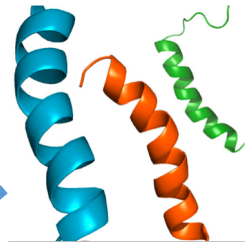


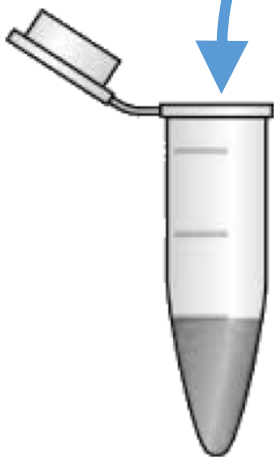
Lawrence et al, Nat Methods. 2016; 13, 431–434.

Our goal: to quantify endogenous phosphopeptides

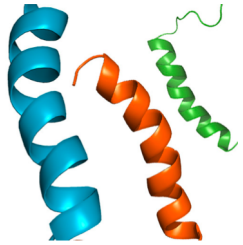
- 60 laboratories requesting samples received two vials:

~5 pmol of each standard peptide mixed

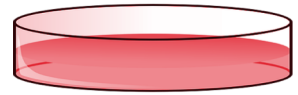


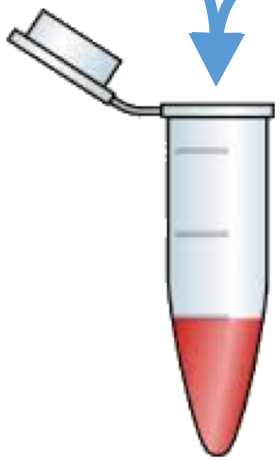


~2 pmol peptides



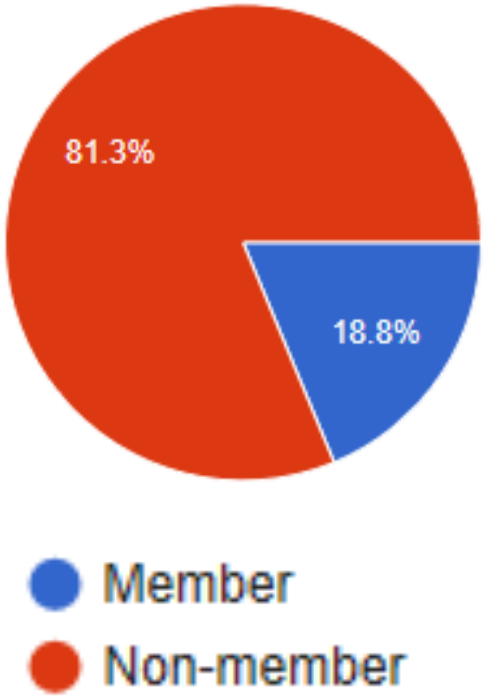
+ 1mg EGF and IGF activated HeLa cell lysate



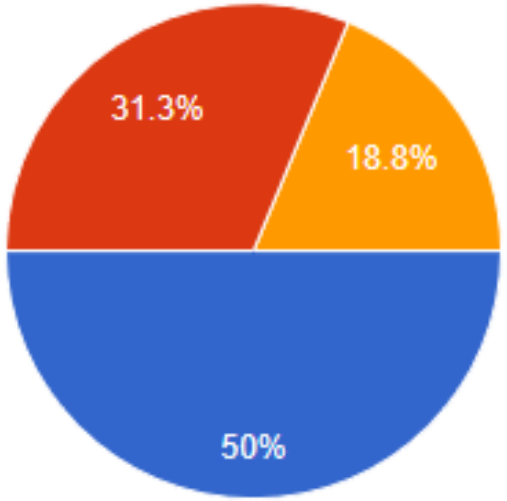


Participant demographics

ABRF membership: 19%

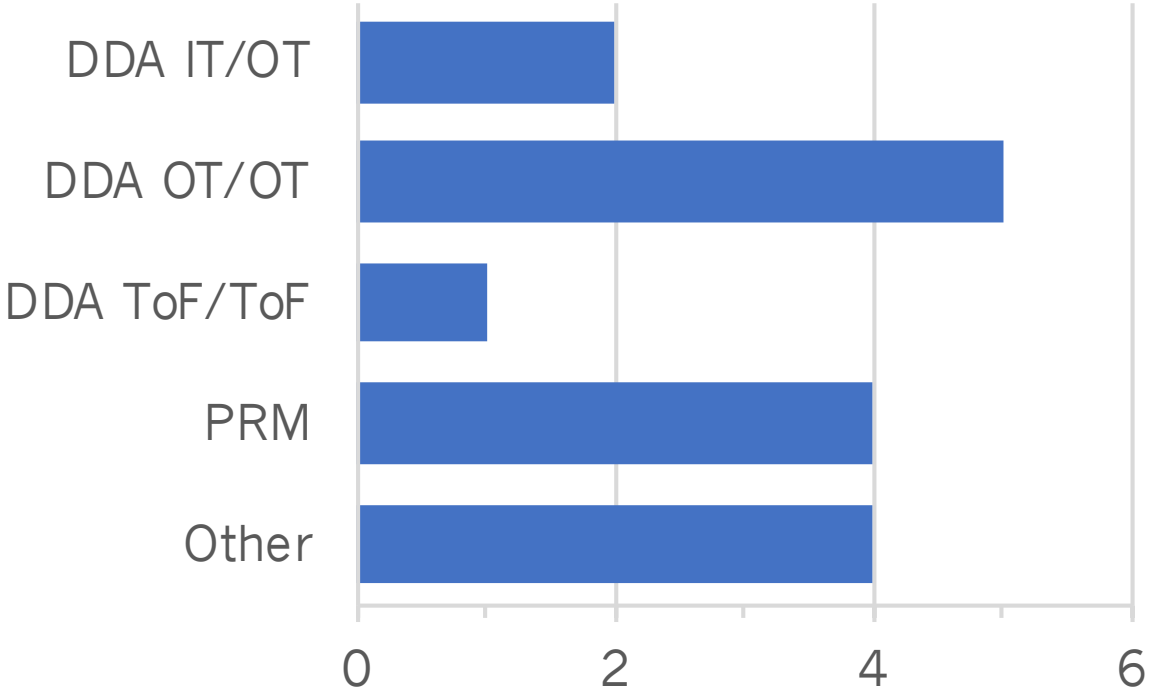


Core status: 69%

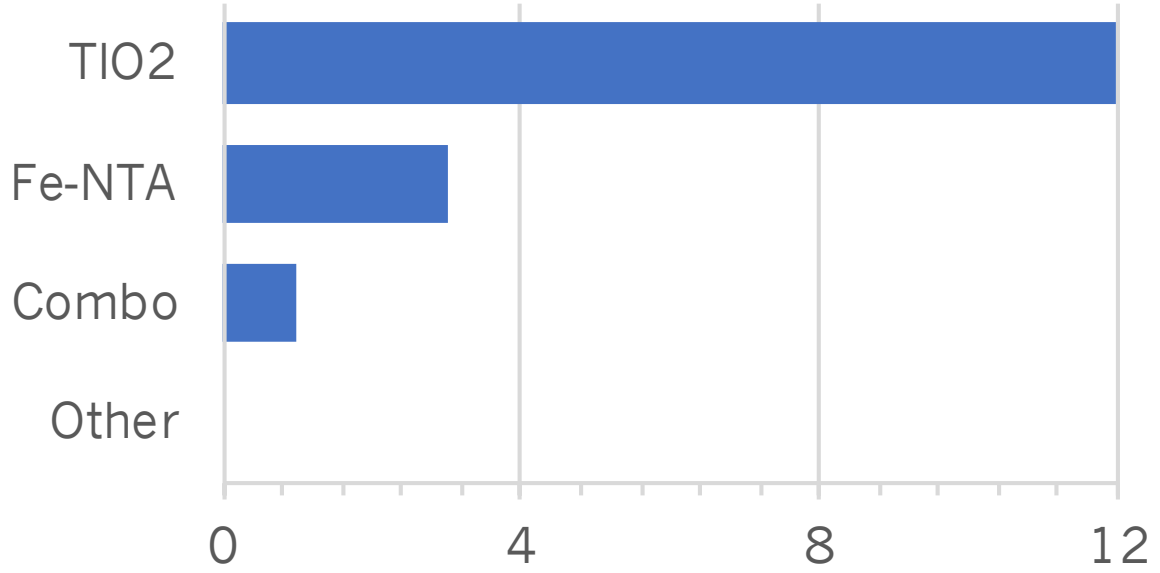


Participant methods

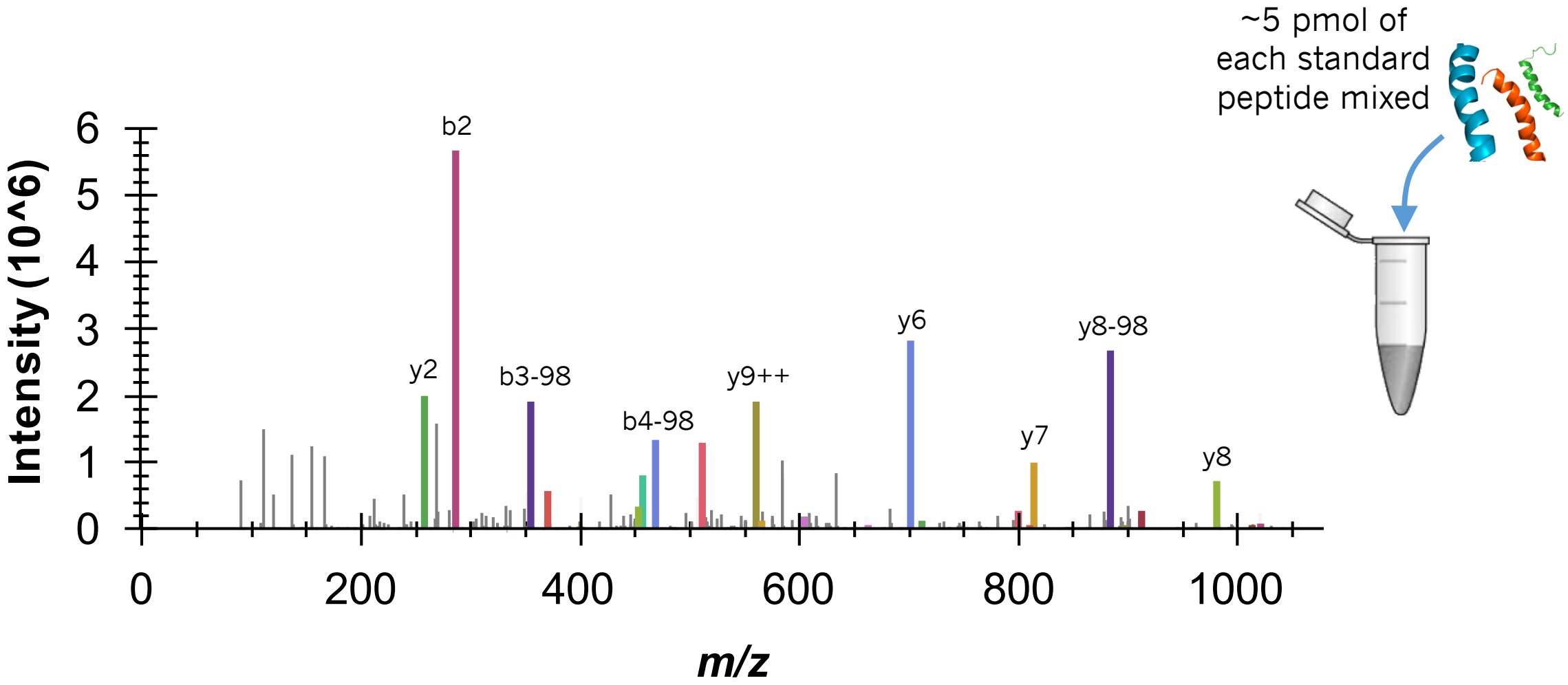
DDA=50%, PRM/SRM/DIA=50%



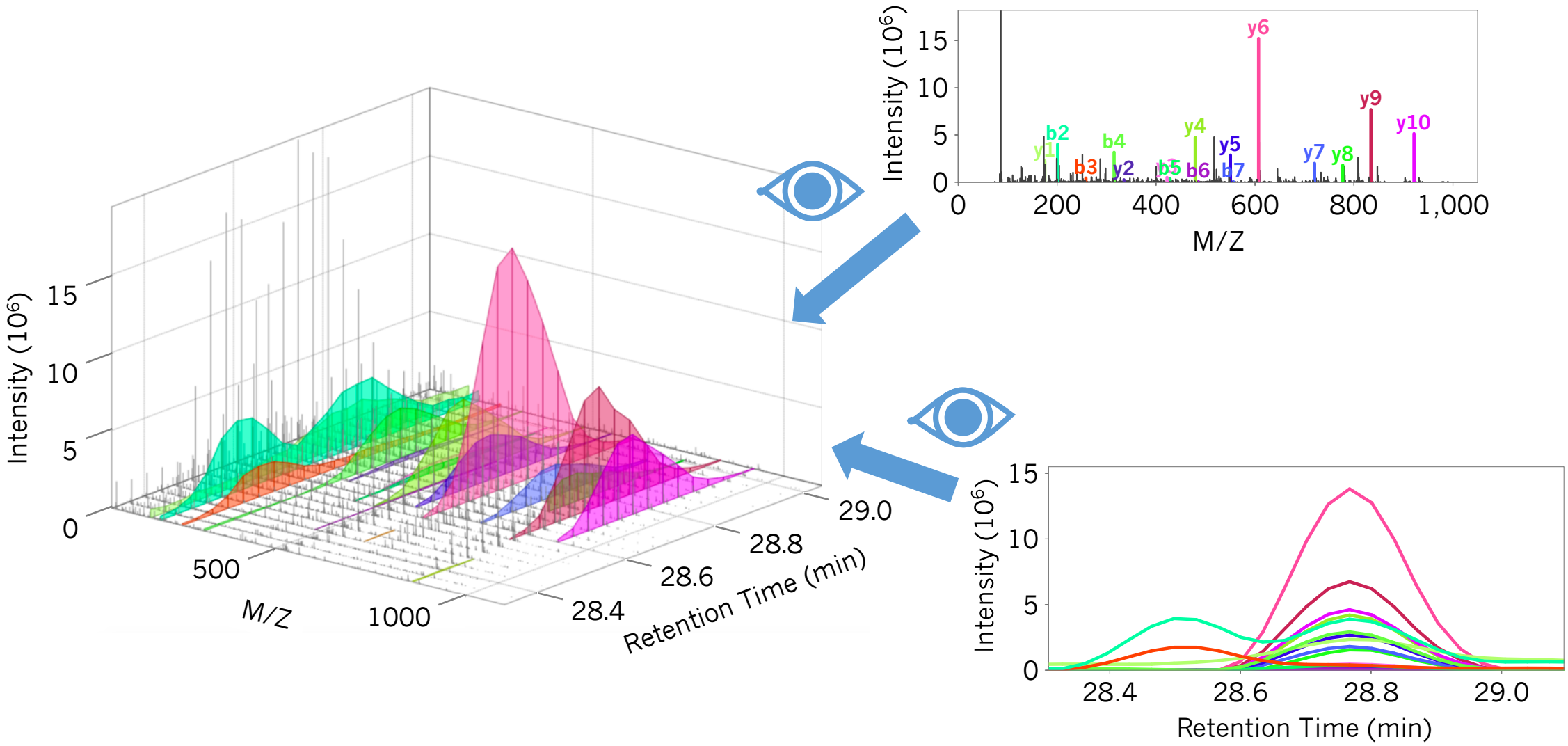
TIO_2=75%, Other=25%



Standard alone enables spectrum library building

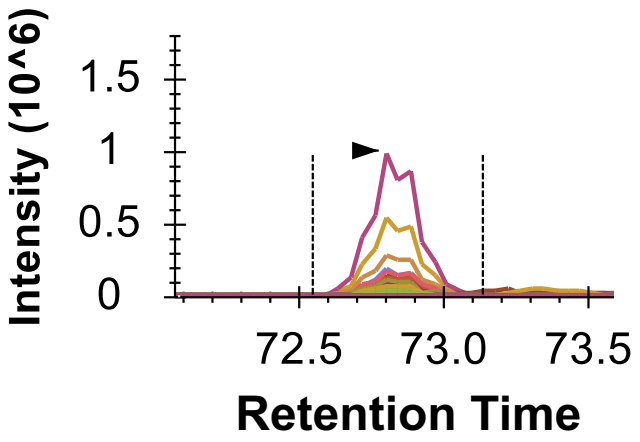


Multiple views of MS/MS data

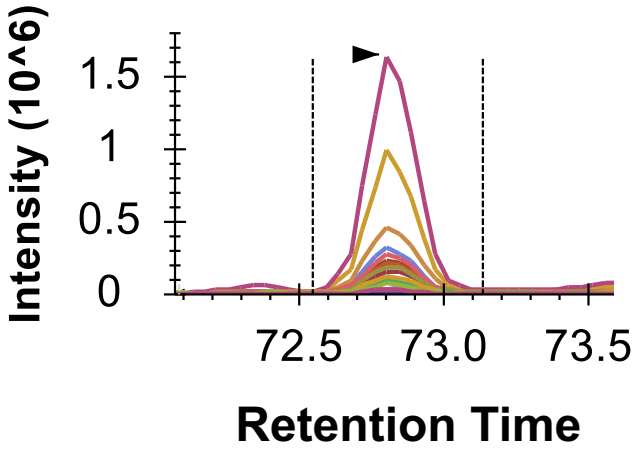


Using the standard to quantify phosphopeptides

Light (endogenous)

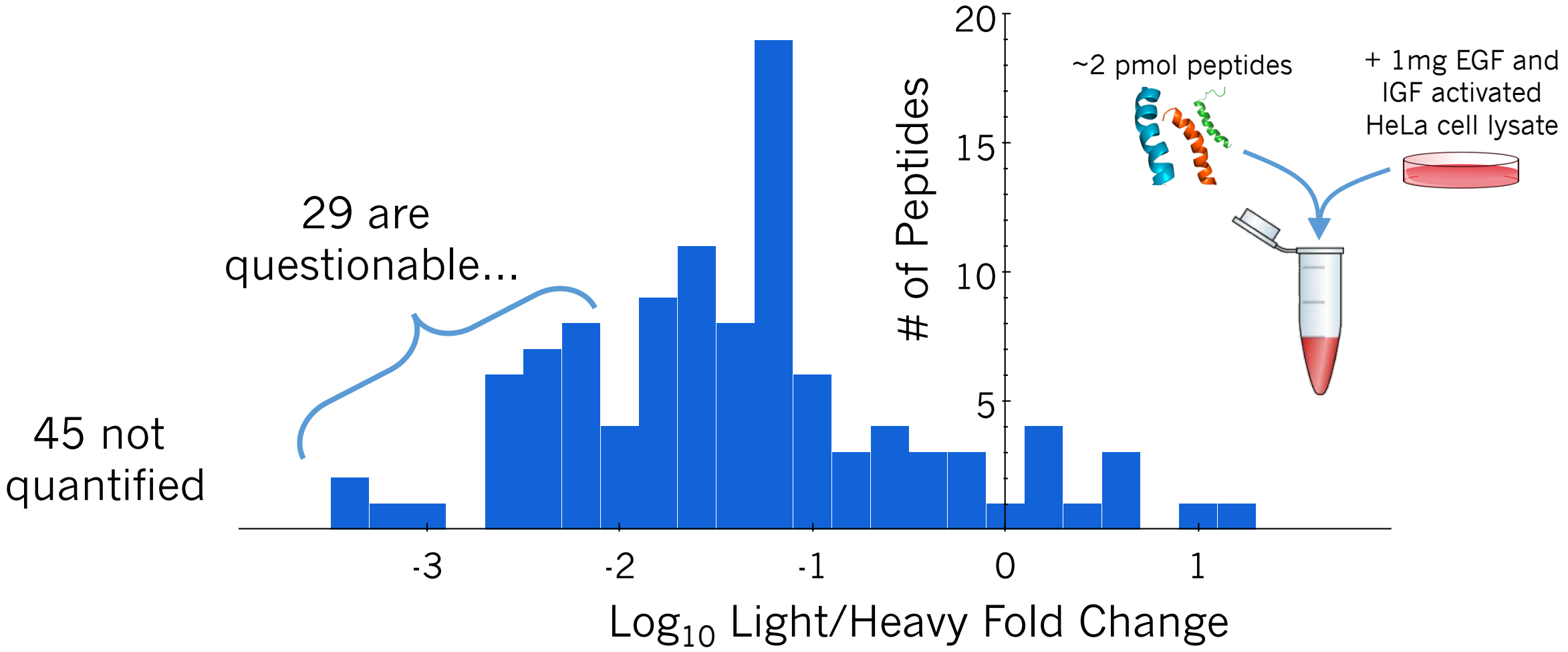


Heavy (standard)



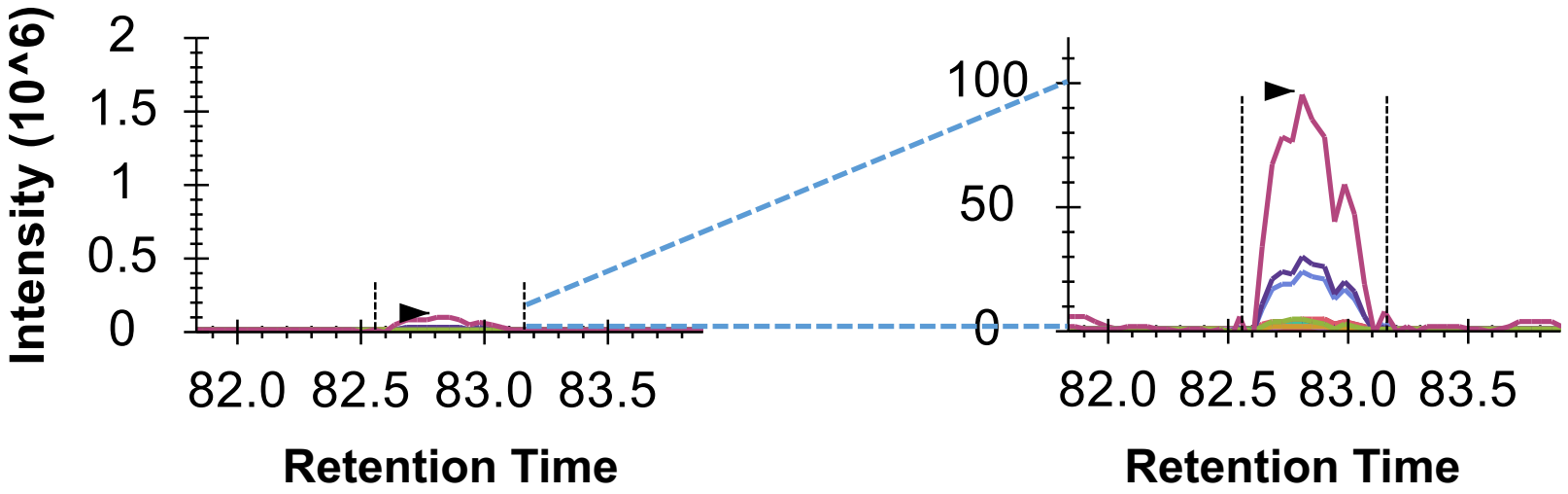
1:1.7 fold change

Many of our biologically interesting peptides are measurable *in vivo*

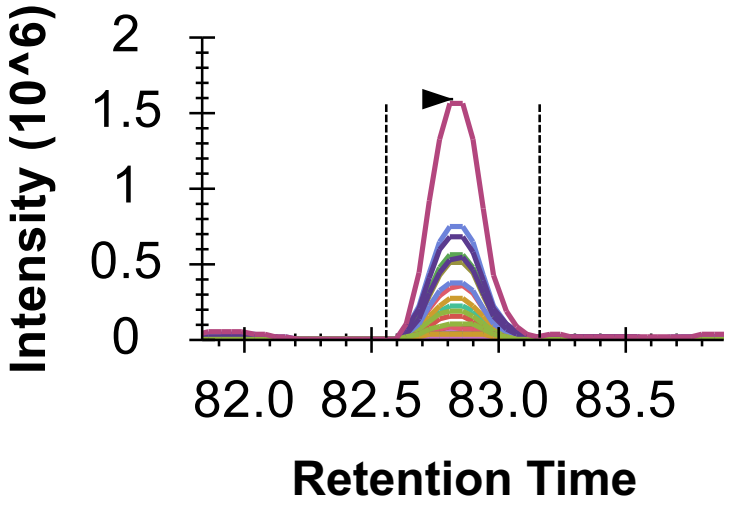


Many peptides are challenging without standards

Light (endogenous)

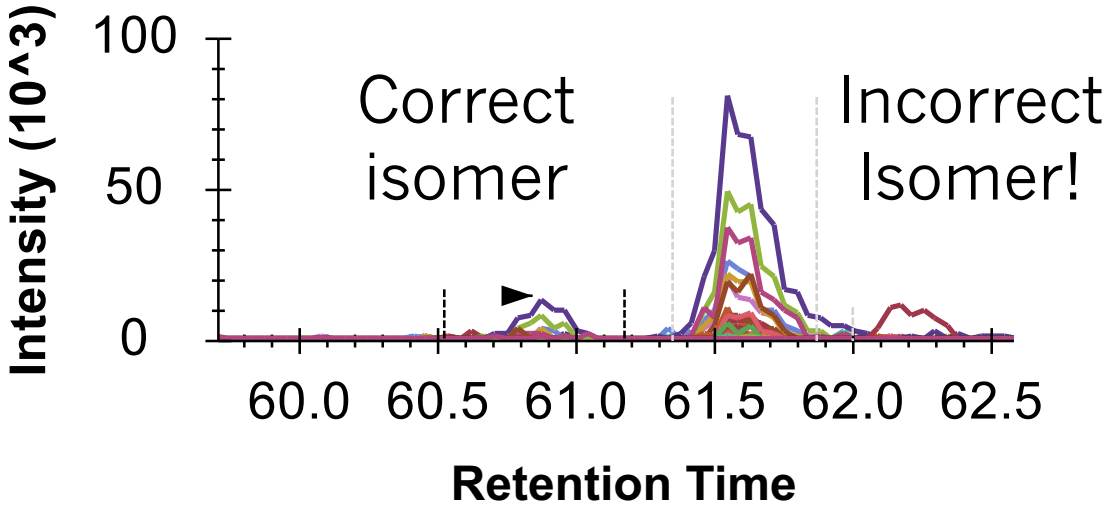


Heavy (standard)

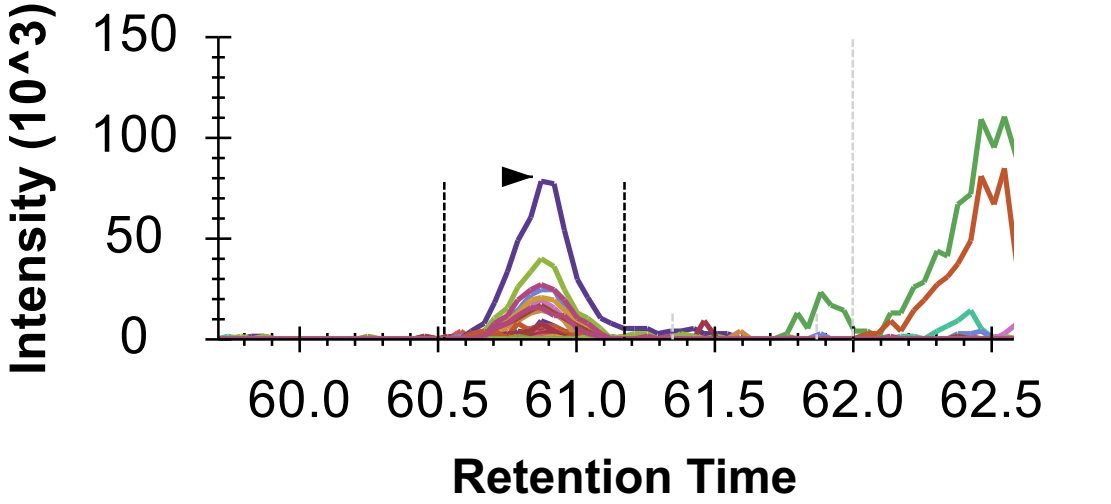


The standard helps determine positional isomers

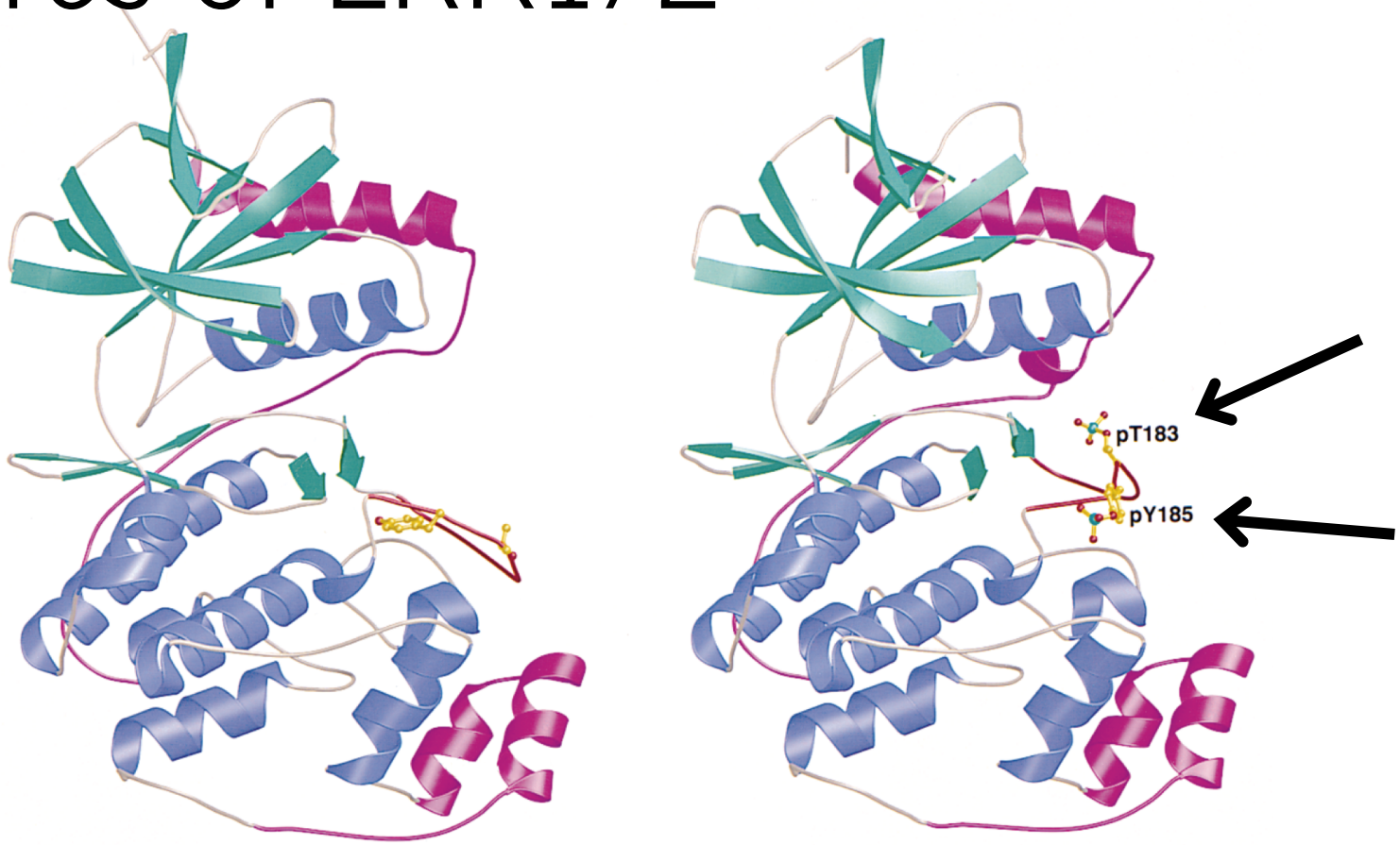
Light (endogenous)



Heavy (standard)



Structures of ERK1/2

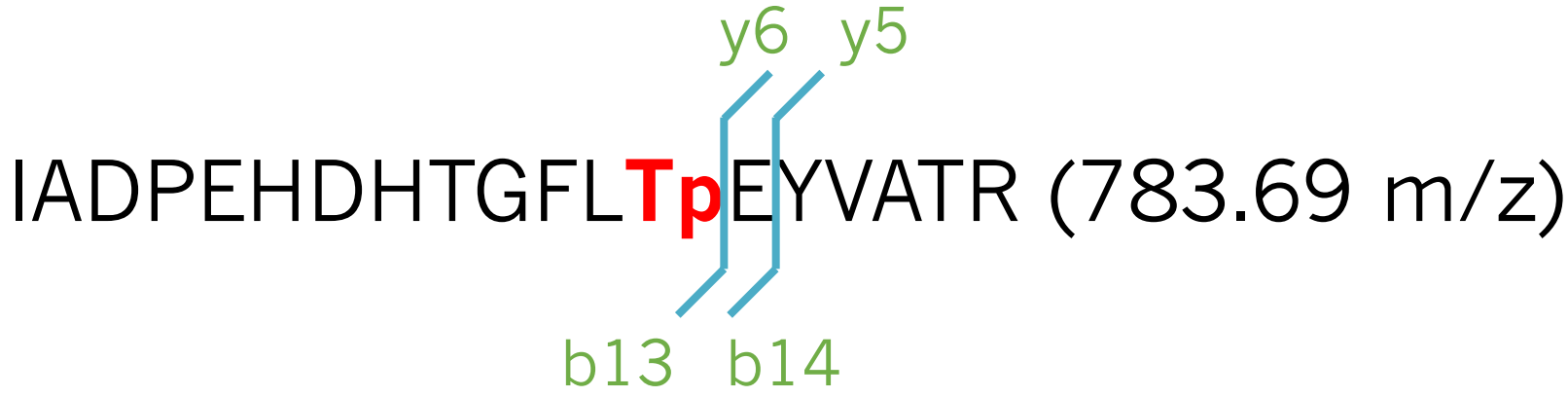


Inactive form

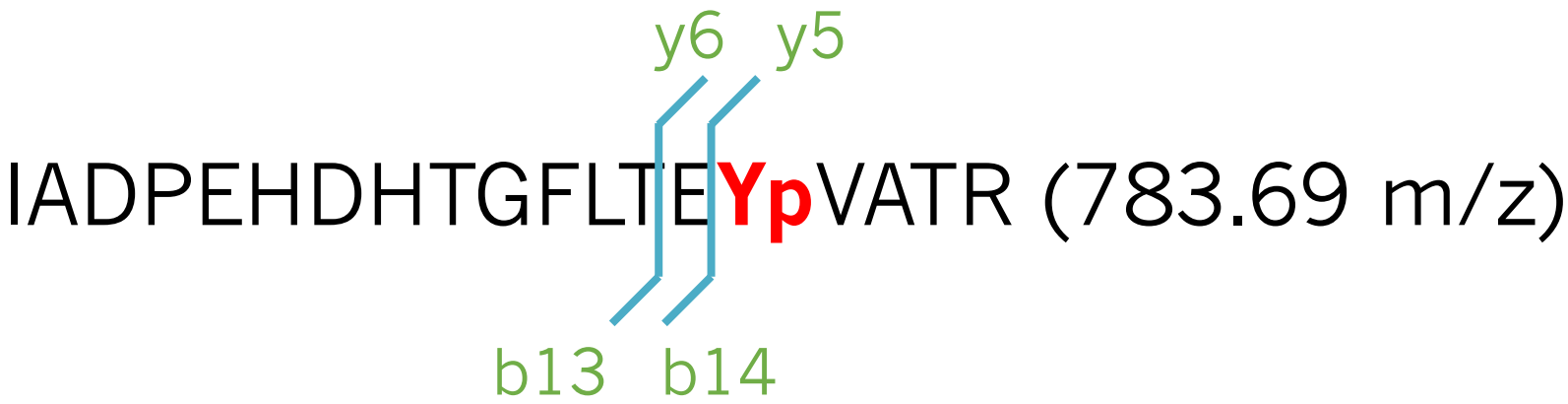
Active form

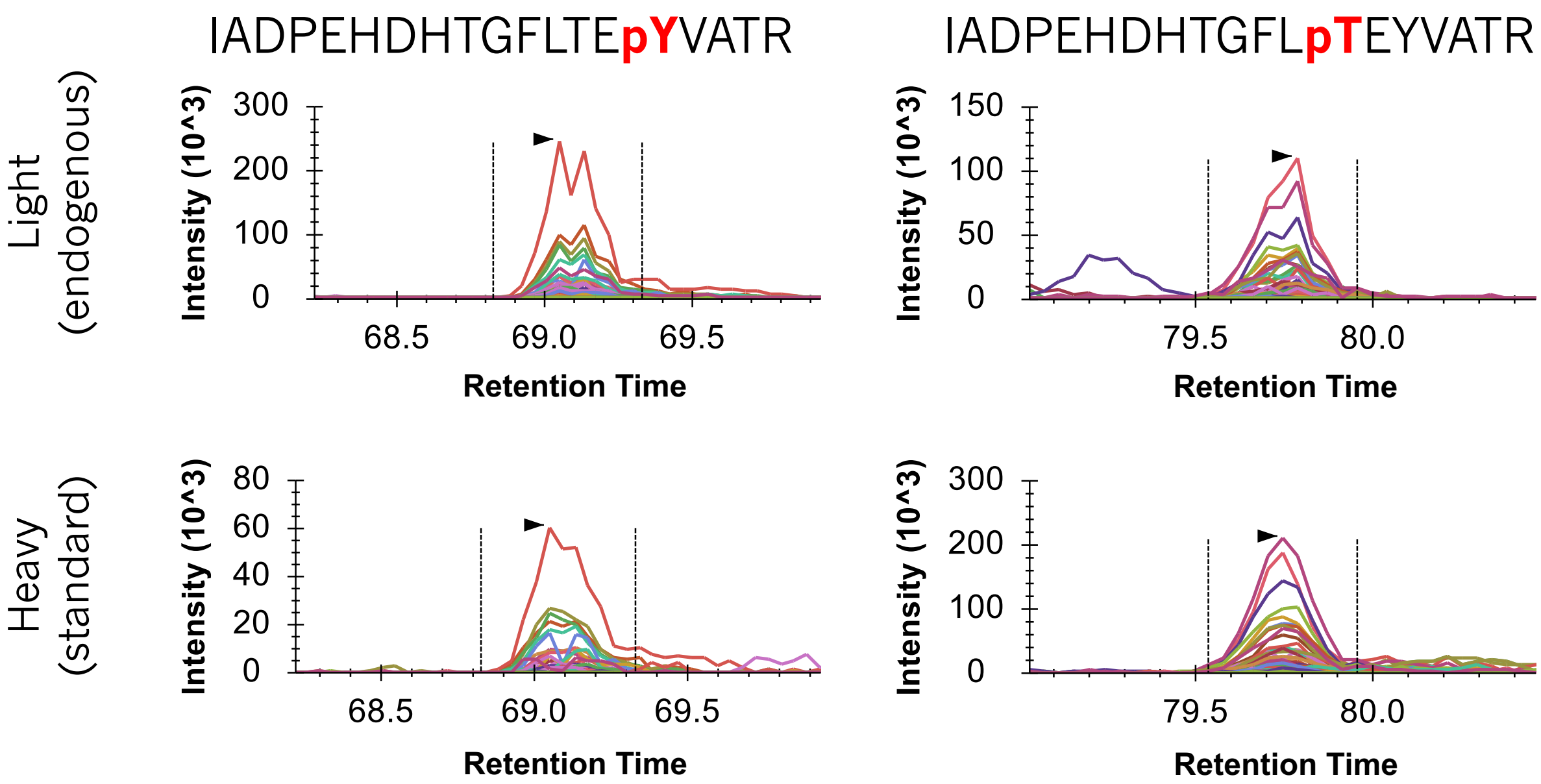
B. J. Canagarajah et al., 1997, *Cell* **90:**859

Differentiating two phosphosites in ERK1



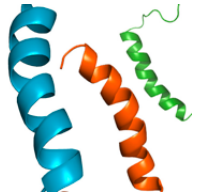
versus




IADPEHDHTGFLTEpYVATR
IADPEHDHTGFLpTEYVATR
Light (endogenous)
Heavy (standard)
Intensity (10^3)
Retention Time
300
200
100
0
150
50
80
60
40
20
68.5
69.0
69.5
79.5
80.0

How do we derive an answer key?

~2 pmol peptides



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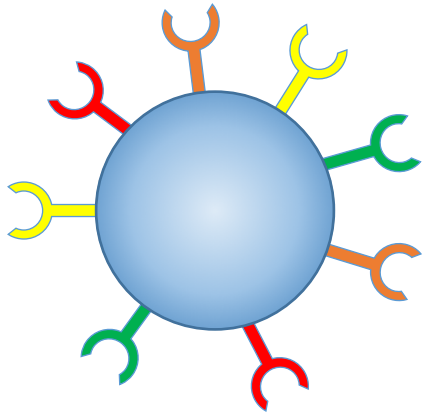
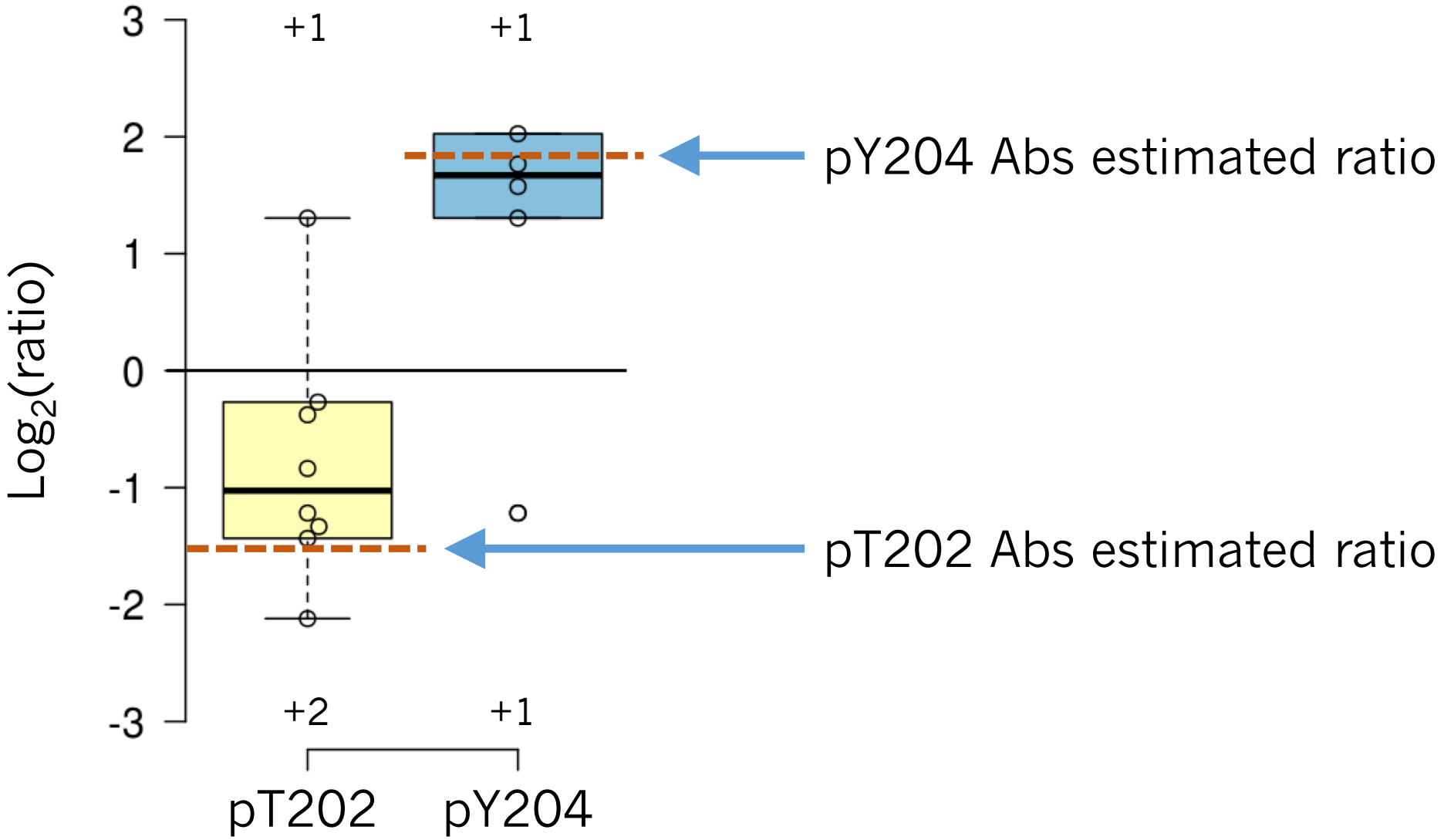


Site specific peptide antibodies
using PTMScan (CST)

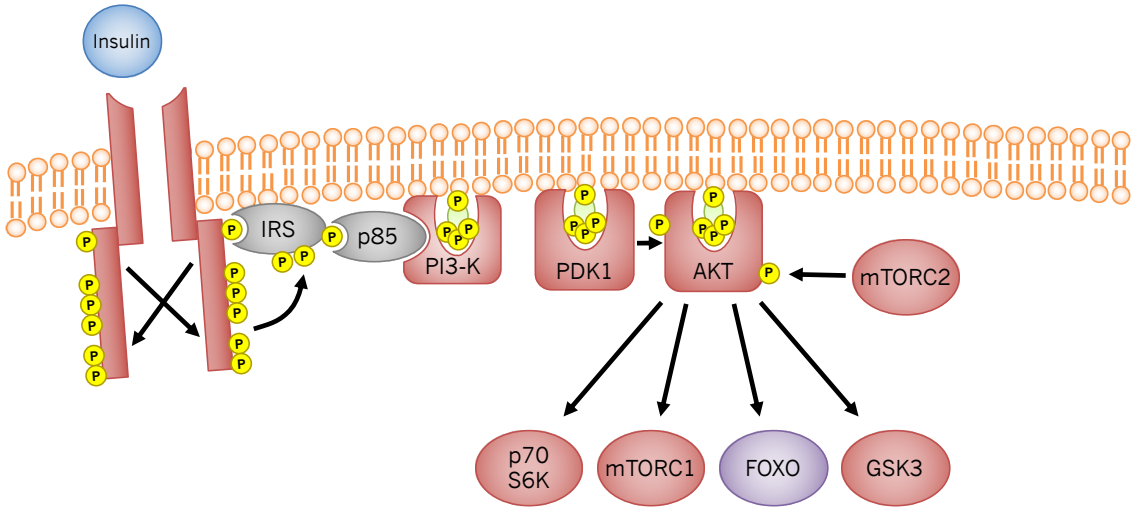
QQQ SRM analysis

How did participants do?



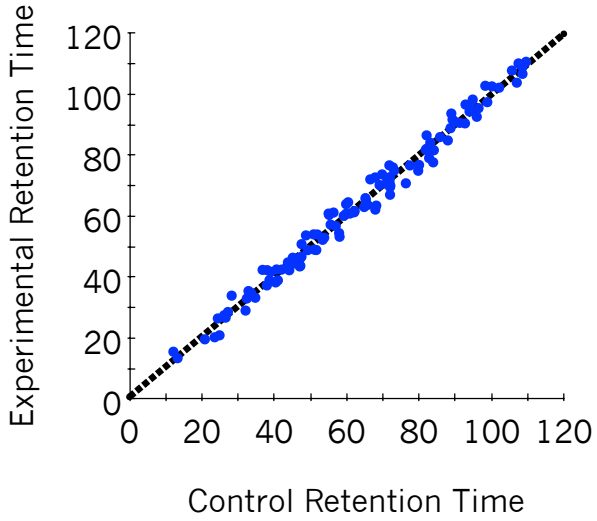
What is this standard good for?

- As a pre-built biological assay



- As an enrichment normalizer/benchmark

- As a phosphopeptide-based retention time standard (e.g. PRTC, iRT)



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sPRG 2019?

Only 15 of 60 participants returned results...
We need more measurements returned!

New participants and new study ideas?

Thanks again!

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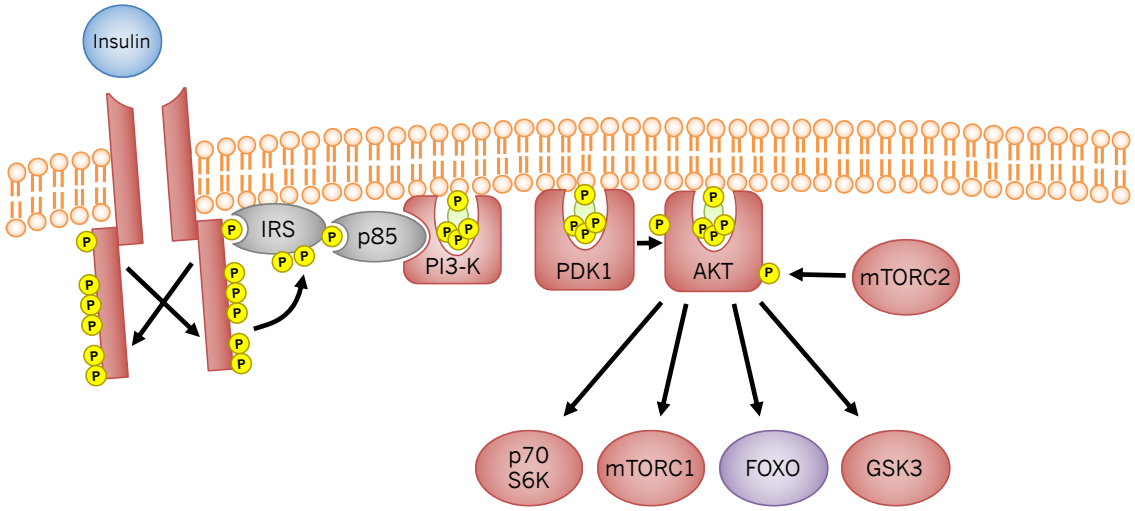
Jenny Kim and Purvi Patel at Columbia University

Questions? **Poster Board Number: 201**

- As a pre-built biological assay



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