ABRF 2017 Annual Meeting

A FORUM FOR ADVANCING TODAY'S CORE TECHNOLOGIES TO ENABLE TOMORROW'S INNOVATIONS

sPRG: Development and Characterization of a Stable-Isotope Labeled Phosphopeptide Standard

March 25-28, 2017

Town and Country Resort & Convention Center
San Diego, California

sPRG

Current sPRG Working Group Members

- Antonius Koller (Chair) - *Columbia University*
- Christopher Colangelo - *Primary Ion*
- David Hawke - *UT MD Anderson Cancer Center*
- Gordana Isovev - *Sciex*
- Brian C. Searle - *Proteome Software Inc.*
- Brett S Phinney - *Proteomics Core UC Davis Genome Center*
- Ryan Leib – *Stanford University*
- Bhavinkumar Patel – *Thermo Fisher*
- Allis Chien (EB Liaison) – *Stanford University*

sPRG

Past sPRG Members

- Alexander Ivanov – *Northeastern University*
- Craig P. Dufresne – *Thermo Fisher Scientific*
- Scott A. Shaffer – *University of Massachusetts Medical School*

sPRG

Mission of sPRG

- Design, Develop and Make ABRF standards available to the community
- Prepare Manuscript

sPRG

Short History of sPRG projects

- 2003: 2 digested proteins; 2 synthetic phosphopeptides
- 2007: mixture of 7 phosphorylated proteins
- 2010: 6 digested proteins; 23 synthetic phosphopeptides
- 2012: 6 digested proteins; 45 synthetic phosphopeptides; 41 synthetic modified peptides
  - http://spectragen-informatics.com/sprg
- 2015: 1000 stable-isotope labeled peptides
  - http://www.jpt.com/ SpikeMix ABRF (cross-species standard)

sPRG

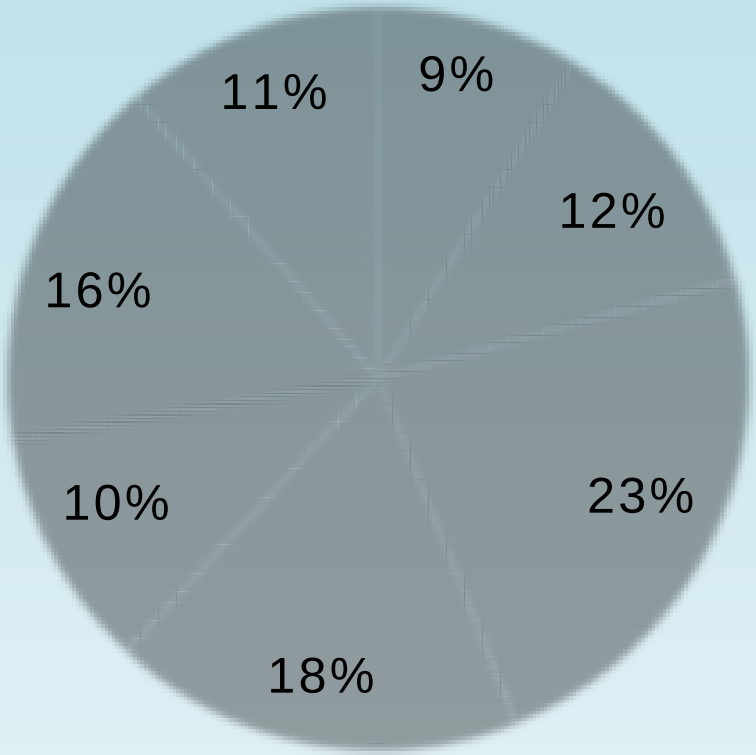
sPRG2017-2018

- **Development of 150 stable-isotope labeled phosphopeptides standard**

- Biologically relevant
- Useful for method development

sPRG

Signaling Pathways included



AMPK signaling

Death and apoptosis signaling

EGFR/ HER signaling

Insulin/ IGF-1 signaling

mTOR signaling

PI3K/ AKT signaling

Stress (p38/ SAPK/ JNK) signaling

sPRG

Phosphorylation site distribution

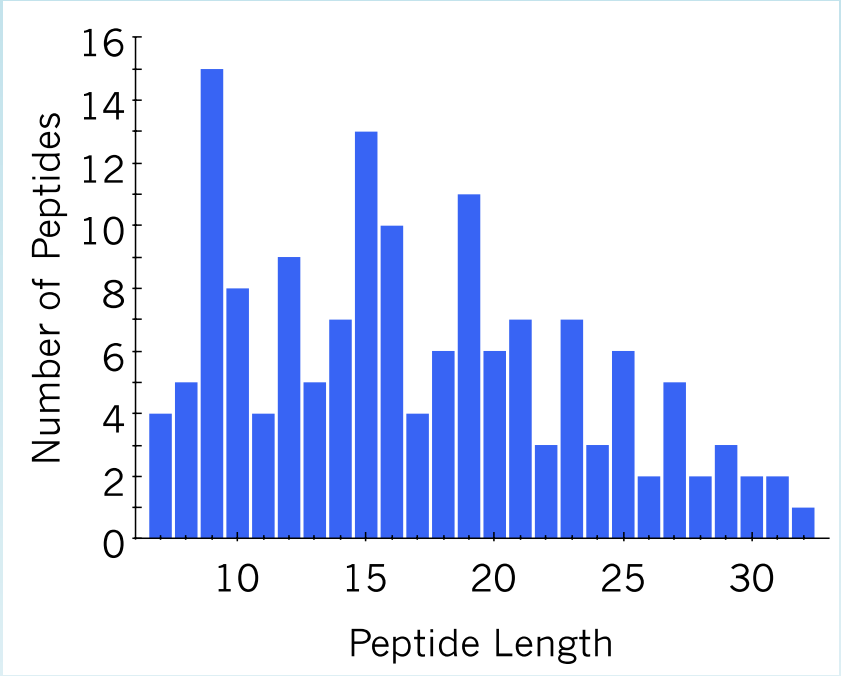
96 Serine phosphorylations
26 Threonine phosphorylations
36 Tyrosine phosphorylations

143 singly phosphorylated
6 doubly phosphorylated
1 triply phosphorylated

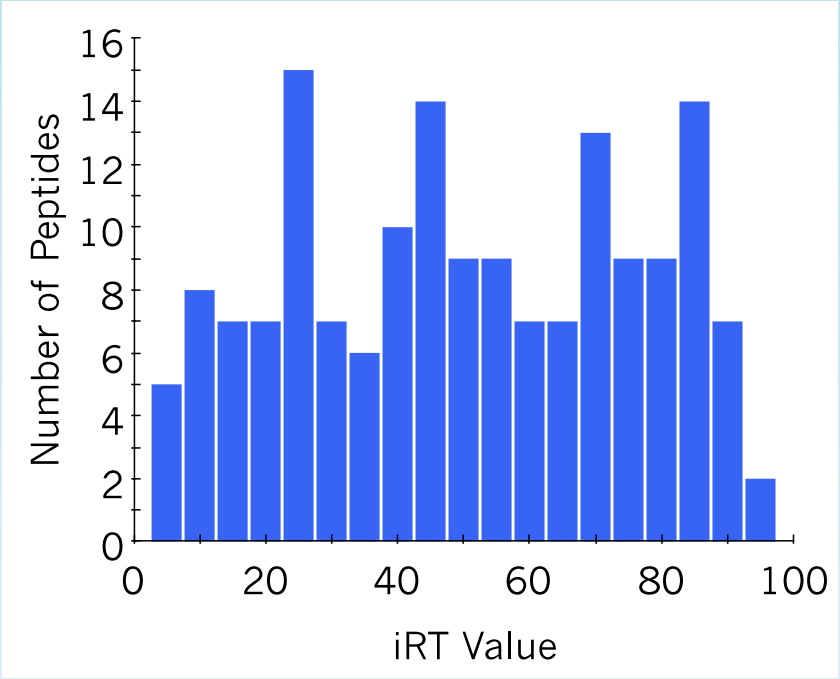
sPRG

Generating a Synthetic Phosphopeptide Standard

Peptide Length

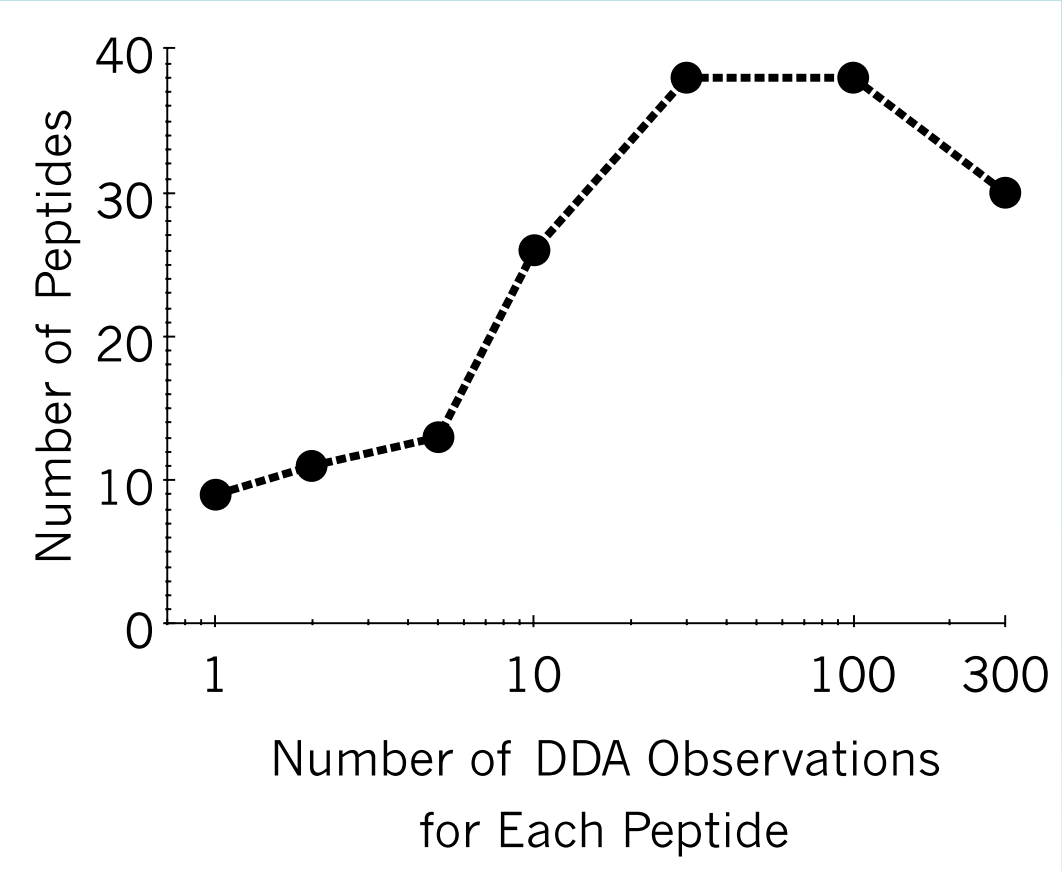


iRT Value



sPRG

of DDA Observations for each peptide



sPRG

Phosphopetide Characterization by DIA/PRM

Peptide Type	Count	Percent
good signal	121	85.8%
low signal	6	4.3%
smear	9	6.4%
no signal	5	3.5%

sPRG

Future Study

- In late summer/fall 2017, phosphopeptides will be sent out to willing participants.
  - Analysis of pure phosphopeptide standard
  - Analysis of phosphopeptide standard spiked into lysate
    - TiO_2 or IMAC enrichment

sPRG

Your participation in this study would be greatly appreciated.

sPRG

sPRG

New members always welcome!!!!!