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

• Alexander Ivanov – *Northeastern University*

• Craig P. Dufresne – *Thermo Fisher Scientific*

• Scott A. Shaffer – *University of Massachusetts Medical School*
Mission of sPRG

- Design, Develop and Make ABRF standards available to the community
- Prepare Manuscript
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)
sPRG2017-2018

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

• Biologically relevant

• Useful for method development
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
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**

- Number of Peptides
- Peptide Length

**iRT Value**

- Number of Peptides
- iRT Value
# of DDA Observations for each peptide

![Graph showing the number of peptides vs. number of DDA observations for each peptide.](image)

- X-axis: Number of DDA Observations for Each Peptide
- Y-axis: Number of Peptides
Phosphopetide Characterization by DIA/PRM

<table>
<thead>
<tr>
<th>Peptide Type</th>
<th>Count</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>good signal</td>
<td>121</td>
<td>85.8%</td>
</tr>
<tr>
<td>low signal</td>
<td>6</td>
<td>4.3%</td>
</tr>
<tr>
<td>smear</td>
<td>9</td>
<td>6.4%</td>
</tr>
<tr>
<td>no signal</td>
<td>5</td>
<td>3.5%</td>
</tr>
</tbody>
</table>
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
  • TiO2 or IMAC enrichment
Your participation in this study would be greatly appreciated.
New members always welcome!!!!!!