ABRF-sPRG 2017-2018: Development and Characterization of a Stable-Isotope Labeled Phosphopeptide Standard

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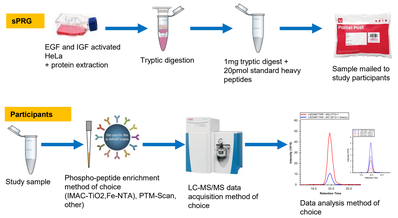
Introduction

The mission of the ABRF proteomics Standards Research Group (sPRG) is to design and develop standards and resources for mass-spectrometry-based proteomics experiments. Recent advances in methodology have made phosphopeptide analysis a tractable problem for core facilities. Here we report on the progress of a two-year sPRG study designed to target various issues encountered in phosphopeptide experiments. We have constructed a pool of over 150 heavy-labeled phosphopeptides that have previously been observed in mass spectrometry data sets. The specific peptides have been chosen to cover as many biologically interesting phosphosites as possible from seven different signaling pathways: AMPK signaling, death and apoptosis signaling, ErbB signaling, insulin/IGF-1 signaling, mTOR signaling, PI3K/AKT signaling, and stress (p38/SAPK/JNK) signaling. This standard should be helpful in a number of ways, including enabling phosphopeptide sample workflow development, as an internal enrichment and chromatography calibrant, and as a pre-built biological assay for a wide variety of signaling pathways. In this work we mixed the standard into an activated HeLa tryptic digest and distributed the mixture to over 60 ABRF member and non-member laboratories around the world. We asked participants to enrich phosphopeptides out of the HeLa background and report ratios of the heavy phosphopeptides to the endogenous levels. Here we report on our preliminary analysis of this cross-laboratory study.

Methods/Study Design



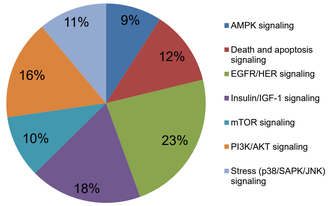
The sPRG prepared protein lysates from activated HeLa cells and digested with trypsin on S-Trap columns (Protifi). Study participants were sent 5pmol of pure heavy isotope phospho-peptide standard (Thermo) and 1mg of tryptic HeLa lysate spiked with 2pmol of heavy standard (Thermo). Participants were asked to enrich phospho-peptides from the spiked lysate, analyze with their method of choice, and anonymously report back light/heavy abundance ratios.

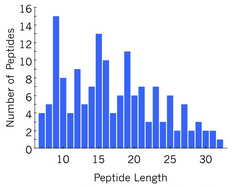
Generating a Synthetic Phosphopeptide Standard Year 1: 2016-2017

Site breakdown:
96 Serine
26 Threonine
36 Tyrosine

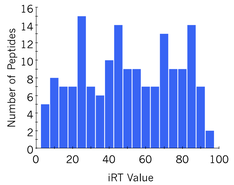
Occupancy breakdown:
143 single
6 double
1 triple

150 heavy isotope phosphopeptides from 89 proteins associated with known signaling pathways and commercially available antibodies

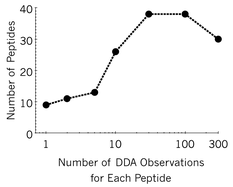




By necessity of synthesis and detection, many contain missed cleavages and are longer length



Balanced across chromatographic retention time for use as internal RT standard

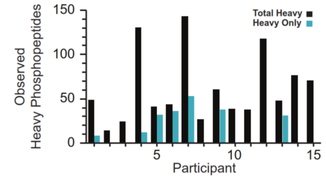


Phospho-peptides span wide dynamic range in previous DDA experiments (Phosphopedia database)

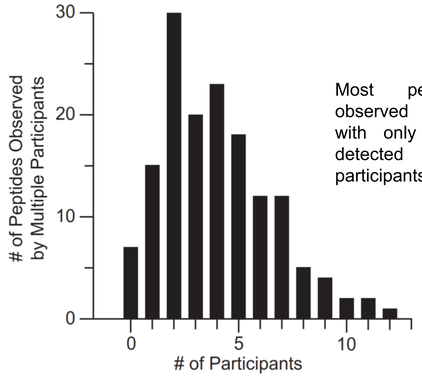
Peptide Type	Count	Percent
good signal	121	85.8%
low signal	6	4.3%
smear	9	6.4%
no signal	5	3.5%

Initial characterization of the pooled heavy isotope phospho-peptide standard using PRM gave clean fragmentation for 86% of peptides (2pmol injected on Thermo Fusion, QE, or QE-HF)

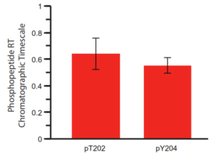
Phosphopeptide Detection in Spiked Lysates Year 2: 2017-2018



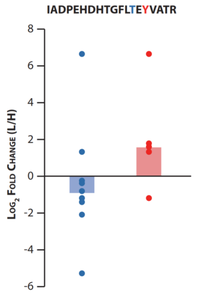
Phospho-peptide standard as observed by study participants (n=15) had a wide range of detection efficiencies with some reporting only the heavy spike-in and not the endogenous



Most peptides were observed at least once with only a few being detected by multiple participants

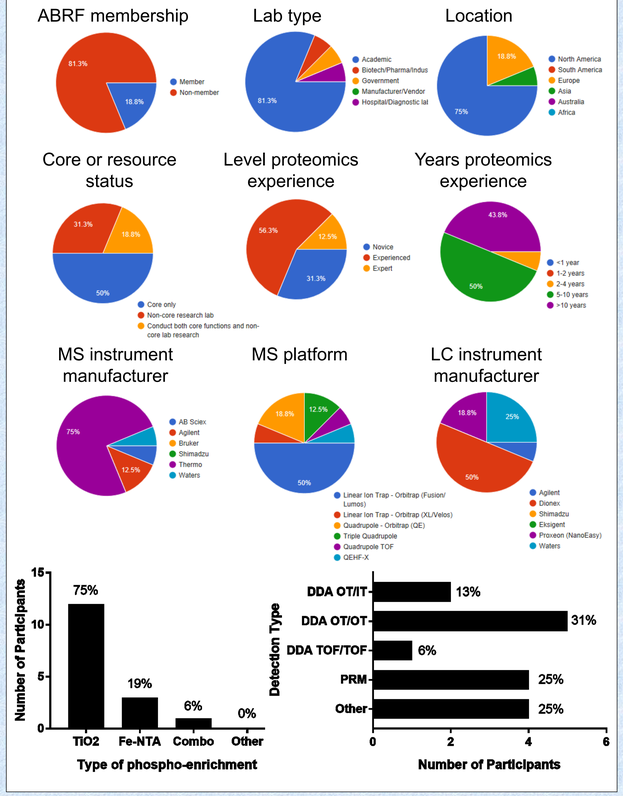


A MAPK3 phospho-isomer was differentially detected by participants (chromatographic RT normalized to longest reported RT)



Demographics, Experience, and Methods of Study Participants

N=16 participating labs



There's still time to submit data! Your participation is greatly appreciated.