



# HPP MS Resource Pillar Phosphopeptide Challenge

**Sue Weintraub**

**HPP Investigator Meeting, 17th HUPO World Congress, Orlando, FL**

**September 30, 2018**



## **MS Resource Pillar Committee**

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Sue Weintraub  
Gilberto Domont  
Daniel Figeys  
Michael Hoopmann  
Peter Hovatovich  
Don Hunt  
Alexander Ivanov  
Oliver Kohlbacher  
Ulrike Kusebauch

Henry Lam  
Alexey Nesvizhskii  
Eunok Paek  
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Stephen Pennington  
Andrea Urbani  
Juan Antonio Vizcaino  
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# MS Resource Pillar Phosphopeptide Challenge

Develop a sample containing a complex set of human phosphopeptides and their unphosphorylated counterparts to be used for:

- Method development
- Phosphopeptide enrichment
- Sequence analysis by mass spectrometry
- Bioinformatic processing



Partnered with SynPeptide Shanghai

SynPeptide is providing the peptides **free of charge** for this project.

# Peptide Selection

- A list of 96 pSTY-peptides was assembled from resources at the ISB (89 stripped)
- All peptides have been frequently observed in multiple experiments (PeptideAtlas)
- From experience gained in the Human SRMAtlas project, it was expected that the sequences would be suitable for peptide synthesis
- **Considerations for selection**
  - Hydrophobicity/HPLC retention time: SSR 10-45
  - Length: 8-20
  - Expected charge state: 2-4
  - Tryptic C-terminus
  - Number of phosphorylated sites: 1-3, Ser, Thr, Tyr
  - Level of difficulty for computational site localization
- **Exclusions**
  - Met-containing peptides (prone to oxidation)
  - Peptides with *N*-terminal amino acids that are likely to cyclize

# MS Resource Pillar Phosphopeptide Challenge

## Stage 1

Interested members of the HUPO community

- Received a set of the peptides at the HUPO Congress 2017 in Dublin
- Were asked to apply their own methods and bioinformatic analysis
- Fully characterize the peptides as a neat mixture and in a tryptic digest background

## Expected outcomes

Multiple purification schemes, analytical protocols and data processing strategies will be evaluated.

The results will be collated by the MS Resource Pillar committee members who will determine the approach(es) that provide the highest coverage of phosphopeptides with accurate site localization.

The outcomes will be reported to HUPO members by various mechanisms.

# What was provided to participants?

- Phosphorylated peptides (purified!), mixed with their non-phosphorylated counterparts, for some peptides there is more than one phosphorylated form
- All peptides contained human sequences, various concentrations.
- Same peptides in a background matrix consisting of 6  $\mu\text{g}$  of trypsin-digested yeast lysate
- Resuspension in 100  $\mu\text{L}$  will result in synthetic peptide concentrations of 3  $\text{fmol}/\mu\text{L}$  – 30  $\text{fmol}/\mu\text{L}$ .
- Result template with 89 stripped sequences (SSR etc.), but not the number of pSTY peptides
- No other guidelines, choice of own methods and software, no database provided.
- Data submission anonymous (5-digit code), MassIVE Repository

# We asked participants for:

- Identification and site localization of pSTY-peptides in neat mixture and after enrichment from yeast background
- Relative quantification in neat mixture by comparison with its non-phosphorylated counterpart
- Method details
- Raw data
- Return result template

Please enter a "1" under the sequence location of each phosphorylated residue per peptide.

If multiple phosphopeptide forms are detected, please copy the sequence to another line and indicate the modification sites.

Sequence (Carbamidomethyl-cysteine indicated by C[160])	Sequence location																				"NEAT" vial Rel. quant.	Detected after enrichment? from "Yeast" (Y/N)
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20		
TAQVPSPPR																						
AQTTPGPSLSGSK																						
VGGSDEEASGIPSR																						
NVHSEDFENR																						
ATVTPSPVK																						
LGAGEGGEASVSPEK																						



# Participation

- Samples were obtained at the HUPO Congress 2017 Dublin.
- 90 participants from 26 countries collected samples.
- 18 returned data sets - 20% return! (status: August 2018)

***Big thank you to everyone who participated and returned results!!!***



# Preliminary Results

## What was returned by participants?

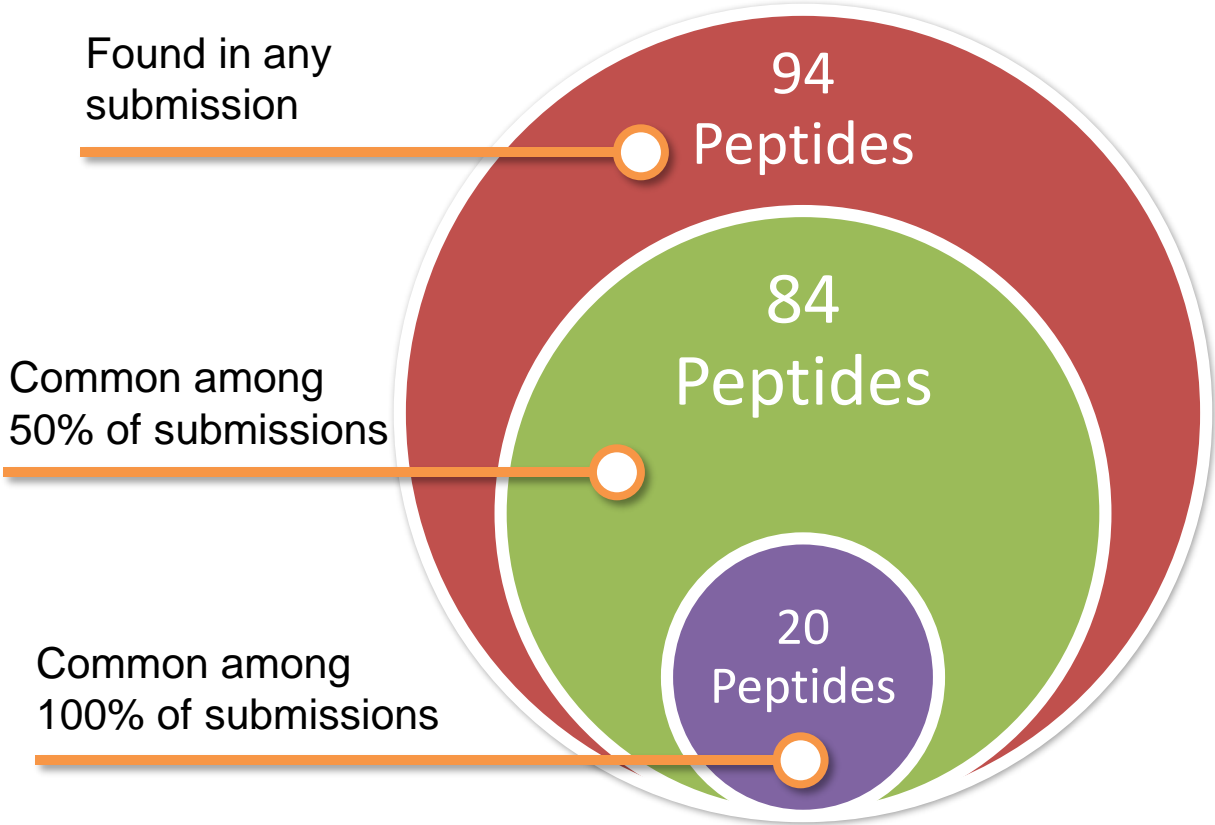
- Some groups provided excellent details about methods – Thank you!
- For several data sets, methods were missing or reported methods were incomplete (e.g., no information about db, method for site localization not specified, results provided but no methods, or methods provided but results template not used).
- Some groups reported combined results from multiple strategies (e.g., DDA and PRM analysis).
- Some groups used two different instruments or methods and reported two data sets.

**Broad range of methods (enrichment and MS) and software tools used!**

# Distribution of Correct Phosphopeptide Identifications

## 18 Total Analyses

Average of 73 phosphopeptides correctly identified per submission



# Next Steps

- Presentation of preliminary results at the Bioinformatics Hub (Special Interest Topic #1, Monday 9.30 am) and on HPP Post-Congress Day
- Extend data submission deadline until December 15, 2018
- Detailed analysis of data.
- Plan stage 2, based on outcomes, discussions at the Bioinformatics Hub and communications with MS Resource Pillar committee members.

**A big “Thank You” to**

**SynPeptide**   
High Quality Peptide

**ReSyn Biosciences**



**Robert Moritz**

**Dave Campbell**

**Nuno Bandeira**

**Sarah Li**

*and*

**All participants who provided their data sets!**