



# HUPO HPP MS Resource Pillar Phosphopeptide Challenge Report

**Susan Weintraub, Michael Hoopmann  
Magnus Palmblad and Ulrike Kusebauch**



# MS Resource Pillar Phosphopeptide Challenge

A list of 94 pSTY-peptides (human sequences) was assembled from resources at the ISB (89 non-phosphorylated).

All peptides have been frequently observed in multiple experiments, as reported in 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

Varied 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

Phosphopeptides and their non-phosphorylated counterparts were synthesized by SynPeptide Shanghai and provided **free-of-charge** to participants.

For some peptides there is more than one phosphorylated form.

Phosphorylated peptides were mixed with their non-phosphorylated counterparts at varied concentrations.

The same peptide mixture was provided in a matrix consisting of 6  $\mu\text{g}$  of trypsin-digested yeast lysate.

Resuspension in 100  $\mu\text{L}$  results in concentrations of 3 - 30  $\text{fmol}/\mu\text{L}$  of synthetic peptides.

# **MS Resource Pillar Phosphopeptide Challenge**

**The goal for the study is to provide the best method(s) to:**

- 1. Identify the peptide sequences in the vial and determine the number and location of phosphorylation sites on each peptide.**
- 2. Determine the relative abundance of phosphorylation at each modified site by comparison with its non-phosphorylated counterpart.**
- 3. Enrich for phosphorylated peptides from the sample containing the yeast background matrix and re-analyze by MS.**

# MS Resource Pillar Phosphopeptide Challenge

Participants were provided information about the 89 peptide sequences.

*Carbamidomethyl-cysteine indicated by C[160]						
**SSR, sequence-specific retention / relative hydrophobicity (Krokhin OV, Anal Chem. 2006, 78(22):7785-95)						
Sequence*	SSR**	MW (Da)	Length	Expected charge	Proteins	UniProt Accession Number
TAQVPSPPR	10.8	951.5138	9	2	1	Q9UKV3
AQTPPGPSLSGSK	11.1	1225.6302	13	2	1	Q9UQ35
VGGSDEEASGIPSR	12.5	1359.6266	14	2	1	Q9NQ55
NVHSEDFENR	12.6	1245.5374	10	3	1	Q9H2P0
ATVTPSPVK	13.3	898.5124	9	2	1	Q9H1E3
LGAGEGGEASVSPEK	13.3	1386.6626	15	2	3	Q13428,Q13428-4,Q13428-6
YNLDASEEEDSNK	13.4	1512.6216	13	2	2	O95218,O95218-2
SLDSDESEDEEDDYQQK	13.9	2030.7712	17	2	1	Q13442
GDVTAEEAAGASPAK	14.3	1372.6470	15	2	1	P49006
AAVLSDESEDEEK	14.4	1291.5779	12	2	2	Q96ST2,Q96ST2-3
ATENDIYNFFSPLNPVR	42.4	1995.9690	17	2	1	P31943
SLSQSFENLLDEPAYGLIQK	45.0	2251.1372	20	2	1	Q8TC07-2





# **MS Resource Pillar Phosphopeptide Challenge**

**Complete submission included the following:**

**Method details (sample handling, analysis, enrichment, data processing)**

**Results template with site localization and relative quantification**

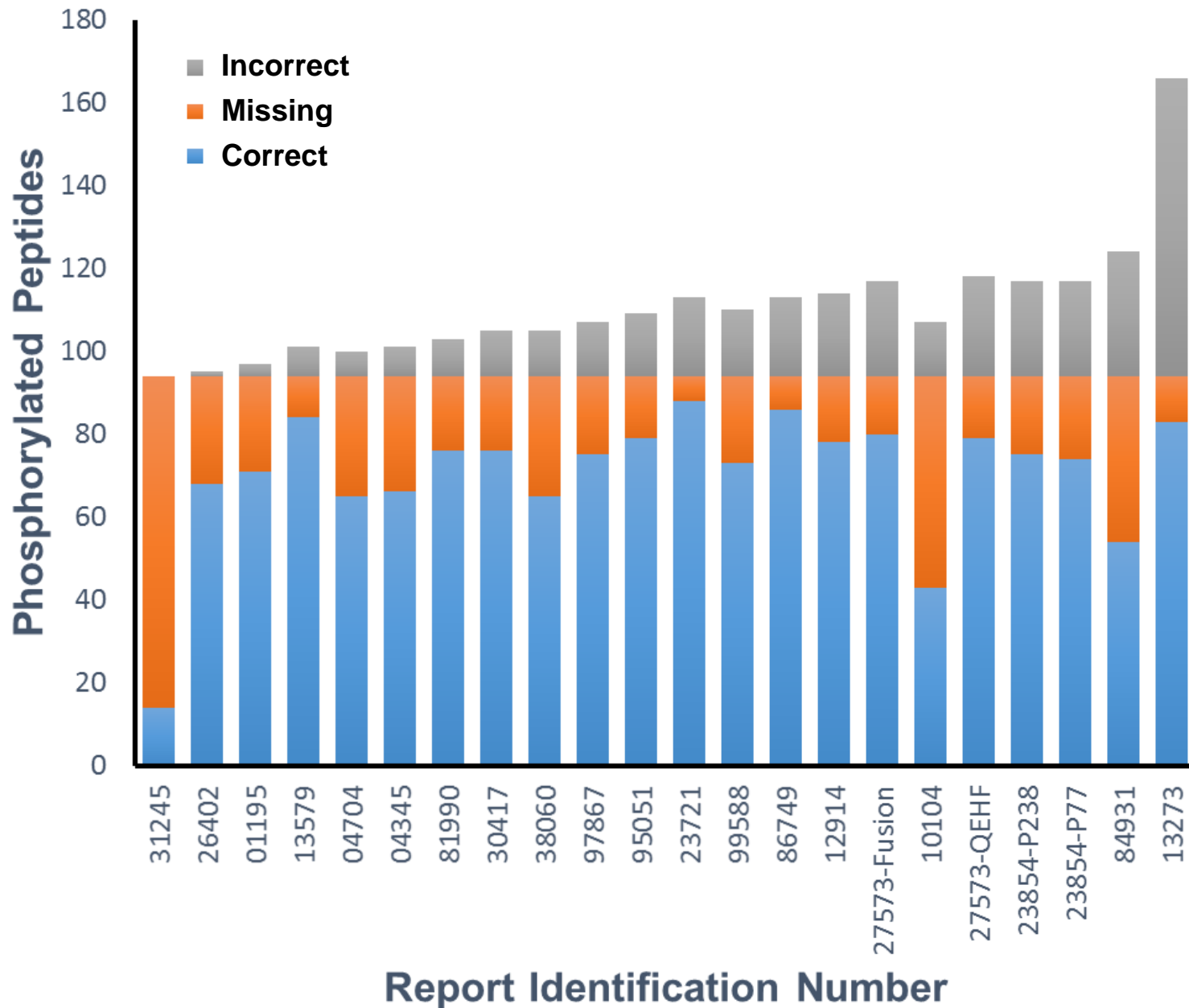
**Raw data files**

# MS Resource Pillar Phosphopeptide Challenge - Participants

<b>Country</b>	<b>Participants</b>
<b>Australia</b>	<b>2</b>
<b>Canada</b>	<b>1</b>
<b>France</b>	<b>2</b>
<b>Germany</b>	<b>1</b>
<b>Italy</b>	<b>1</b>
<b>Korea</b>	<b>1</b>
<b>The Netherlands</b>	<b>3</b>
<b>Russia</b>	<b>1</b>
<b>South Africa</b>	<b>1</b>
<b>Spain</b>	<b>3</b>
<b>Sweden</b>	<b>1</b>
<b>United States</b>	<b>4</b>
<b><i>Total</i></b>	<b><i>21</i></b>



# Identification Summary



# What's Next?

**Present current results at Bioinformatics Hub (Wednesday AM) to obtain ideas and assistance for further examination of the data**

**Compile details about phosphopeptide enrichment (Mike Hoopmann and Ulli Kusebauch)**

**Evaluate efficiency of enrichment strategies (Mike Hoopmann and Ulli Kusebauch)**

**Convert files for viewing/in-depth interrogation at the PSM level in MassIVE (Mike Hoopmann and Nuno Bandeira)**

**Continue recalibration of precursor ions (Magnus Palmblad)**

**Evaluate data to see if reliable trends can be discerned to formulate best practice recommendations**

**Complete manuscript on methods used/best practices**

**New initiative?????**

**A big “Thank You” to**

**SynPeptide** 

**ReSyn Biosciences** 

**Robert Moritz**

**Dave Campbell**

**Nuno Bandeira**

**Sarah Li**

*and*

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