



## MS Resource Pillar Committee

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# **MS Resource Pillar of the Human Proteome Project (HPP)**

**A list of 96 peptides was assembled from resources at the ISB.**

## **Considerations for selection**

Hydrophobicity/HPLC retention time

Length

Expected charge state

Tryptic C-terminus

Number of phosphorylations

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

**All peptides have been frequently observed in multiple experiments.**

**From experience gained in the Human SRMAtlas project, it was expected that the sequences would be suitable for peptide synthesis.**

## Examples of Synthesized Phosphopeptides

Sequence	No. of Sites	SSR	MW	Length	Expected charge
ADEP <sup>ss</sup> EEsDLEIDK	3	20.4	1902.61	15	2
VVEAVNsDsDSEFGIPK	3	28.2	2031.75	17	2
sPVFsDEDEsDLDFDISK	3	35.6	2154.74	17	2
ATVt <sup>s</sup> PsPVK	2	13.3	1058.45	9	2
SLDsDEsEDEEDDYQQK	2	13.9	2190.7	17	2
AAVLSDsEDEEK	2	14.4	1451.51	12	2
SGAQASSt <sup>s</sup> PLsPTR	2	15.2	1518.61	14	2
AGGPTt <sup>s</sup> PLsPTR	2	15.6	1313.54	12	2
AGGPA <sup>t</sup> PLsPTR	2	16.1	1283.53	12	2
AGGPA <sup>t</sup> PLSP <sup>t</sup> R	2	16.1	1283.53	12	2
AQtPPGPSLSGSK	1	11.1	1305.6	13	2
VGGsDEEASGIPSR	1	12.5	1439.59	14	2
NVHsEDFENR	1	12.6	1325.5	10	3
LGAGEGGEASVsPEK	1	13.3	1466.63	15	2
SIsLGDSEGPIVATLAQPLR	1	40.0	2103.06	20	2
sIsLGDSEGPIVATLAQPLR	1	40.0	2103.06	20	2

# MS Resource Pillar of the Human Proteome Project (HPP)

The committee partnered with SynPeptide Shanghai to produce the complex set of human phosphopeptides and their unphosphorylated counterparts.

Singly- and multi-phosphorylated (Ser, Thr or Tyr)

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

The peptide mixtures can be used for:

- Method development

- Verification for phosphopeptide enrichment

- Sequence analysis by mass spectrometry

- Bioinformatic processing

## Stage 1 (A)

Interested members of the HUPO community will obtain a set of the peptides and apply their own methods and bioinformatic analysis to fully characterize the peptides as a neat mixture and in a tryptic digest background.

Results reports and details of methods will be sent to the MS Resource Pillar committee.

# **MS Resource Pillar of the Human Proteome Project (HPP)**

## **Stage 1 (B)**

“Experimentalist” members of the MS Resource Pillar will analyze the samples by multiple MS approaches.

Various methods for enrichment will also be utilized.

Data files from the committee will be sent to the “Informatics” members for assessment of different strategies for data processing, phosphosite localization and site probability determination.

## **Expected outcomes**

A variety of 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 raw files and meta data will be deposited in PRIDE.

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

# **MS Resource Pillar of the Human Proteome Project (HPP)**

**A huge “Thank You” to**

**Rob Moritz**

**Ulli Kusebauch**

**Mike Hoopmann**

**Sarah Li**

# MS Resource Pillar of the Human Proteome Project (HPP)

**Peptide sets and information/instructions  
can be obtained at the  
SynPeptide Shanghai booth (#48)**

