**Huntingtin Phosphorylation Analysis – 5th August 2016**

The peptide mass spectrometry data which has been accumulated from the 3 limited proteolysis/mass spectrometry/domain mapping experiments has now been further analysed to look for post-translational modifications of the huntingtin. In this first instance, just phosphorylation modifications were investigated.

**Methods:**

The following strategy was used to look for huntingtin phosphorylation motifs:

1. Collate all peptide data for the 3 mass spectrometry experiments. Sort peptides which contain phosphorylation motifs.
2. Conduct literature search for reported huntingtin phosphorylations. Assess huntingtin sequence for putative phosphorylations using prediction servers NetPhos 2.0 and 3.1 as well as data repository Phosphosite. Collate this data.
3. Conduct meta-analysis drawing all data together and assign scores for each detected phosphorylation on the basis of how many times it has been previously detected and/or predicted in the published literature as well as in how many experimental replicates it is seen.

**Sources and References:**

**Published Studies:**

<http://www.ncbi.nlm.nih.gov/pubmed/25799558>

<http://www.ncbi.nlm.nih.gov/pubmed/16782707>

**Prediction Servers and Repositories:**

Phosphosite

<http://www.phosphosite.org/homeAction.action>

NetPhos2.0

<http://www.cbs.dtu.dk/services/NetPhos-2.0/>

<http://www.ncbi.nlm.nih.gov/pubmed/10600390>

NetPhos3.1

<http://www.cbs.dtu.dk/services/NetPhos/>

<http://www.ncbi.nlm.nih.gov/pubmed/15174133>

**Results and Discussion:**

273 putative phosphorylations are predicted or detected and reported in the literature. For 81 of these experimental data has been published confirming the phosphorylation.

A summary of the data can be seen on the following page. The full analysis can be seen in the work book Phosphorylation\_PTM\_Huntingtin\_HEK293.xlsx.

 

Detailed in the tables above are 89 different phosphorylation sites which were detected in our in house mass spectrometry experiments of which 38 have been previously predicted and detected, 15 have been previously predicted but not detected and 36 had not been previously predicted or detected. The 38 phosphorylation sites which have been previously predicted and detected provide further supporting evidence that these sites could be phosphorylated in vivo.

Not all of the 89 phosphorylation sites detected overall are seen in every experimental replicate. For those which had not been detected previously a cut off of detection in at least 2 of the 3 experimental replicates was imposed. This left a short list of 6 predicted phosphorylation sites which we have experimentally detected for the first time as well as a further 7 phosphorylation sites which have not previously been predicted or described in the published literature. This short list is detailed in the table below.



All 6 of the previously predicted but not detected sites were predicted by both iterations of the NetPhos server with high confidence scores of >0.9. Of the 7 phosphorylation sites not previously described, none of these sites were predicted on the Phosphosite server. However they were all predicted, with varying degrees of confidence by the 2 iterations of NetPhos, version 3.1 assigning higher confidence scores in all cases than version 2.0.

The quality of the spectra for all 13 phosphorylations of this shortlist were analysed both my standard metrics (number of spectra, -10logP and Ascore) and visual inspection of the spectra. The spectra can be found in the accompanying document MS\_spectra\_analysis\_phosphorylations.pptx. For all 13 phosphorylation sites, high confidence spectra are observed.

**Next Steps:**

To begin to validate the physiological relevance of the putative phosphorylation sites, parallel analysis of huntingtin samples derived from a different cell line would be required or at the very least different preparations of the huntingtin protein from the same cell line (all of these experiments were conducted using protein derived from a single preparation of huntingtin from an over-expression system in HEK293 cells). Additionally, it must be considered that the over expression of huntingtin in the mammalian expression system could cause major changes in the equilibrium of cellular processes causing proliferative phosphorylation in a way which does not represent the physiological settings of huntingtin protein at all.