

Crosstalk between phosphorylation and O-GlcNAcylation

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February 28, 2012

Cross Talk Between O-GlcNAcylation and Phosphorylation: Roles in Signaling, Transcription, and Chronic Disease

Gerald W. Hart,¹ Chad Slawson,¹
Genaro Ramirez-Correa,² and Olof Lagerlof¹

Annu. Rev. Biochem. 2011. 80:825–58

Site-specific interplay between O-GlcNAcylation and phosphorylation
in cellular regulation

Ping Hu, Shino Shimoji, Gerald W. Hart *

FEBS Letters 584 (2010) 2526–2538

VIEWPOINT

Glycosylation of Nucleocytoplasmic Proteins: Signal Transduction and O-GlcNAc

Lance Wells,* Keith Vosseller,* Gerald W. Hart†

23 MARCH 2001 VOL 291 SCIENCE

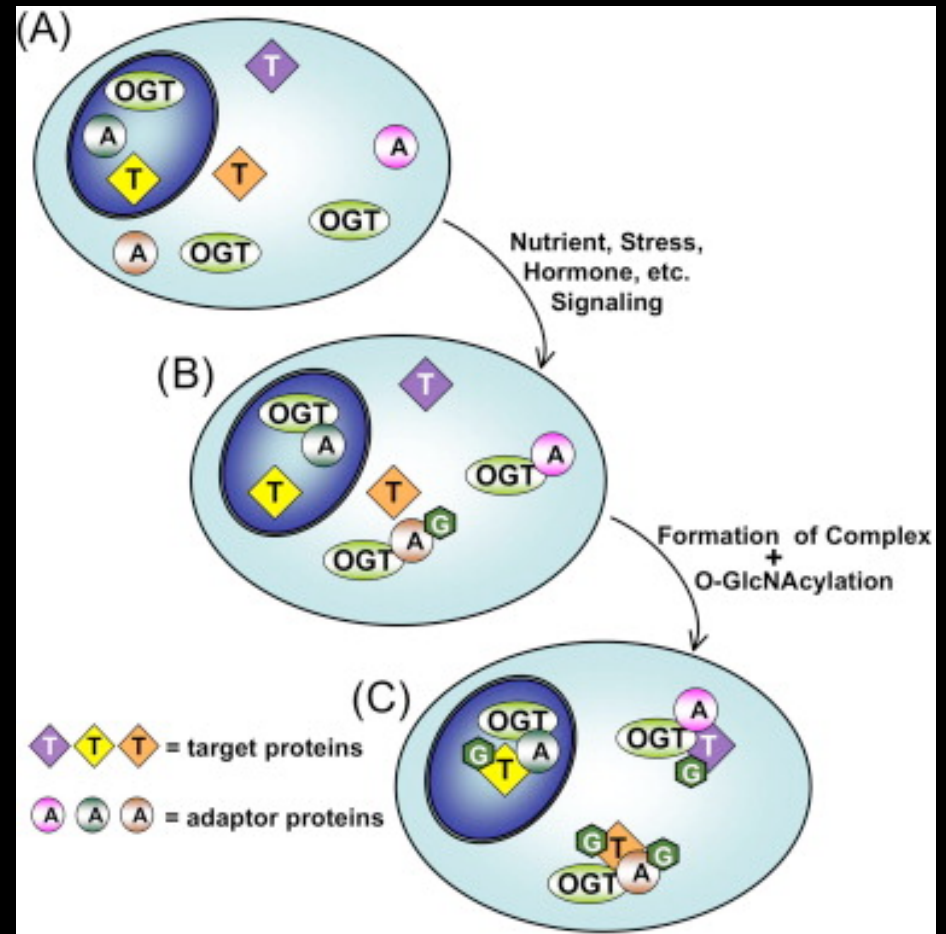
Phosphorylation VS. O-GlcNAcylation

Characteristic	Phosphorylation	O-GlcNAcylation
Amino acid	S, T and Y	S and T
Enzyme	518 kinases ¹	O-GlcNAc transferase (OGT)
Motif	Consensus motifs depends on kinases	No clear consensus motifs
Properties around sites	In and around disordered regions, surface residues, enriched in charged, hydrophilic (except for Y) and highly flexible residues ²	Close proximity to proline and valine, downstream tract of serines and absence of leucine and glutamine ³
Predictors	NetPhos, Scansite, DISPHOS, GPS, etc.	DictyOGlyc, NetOGlyc, OGlycPred, OGPET, YinOYang

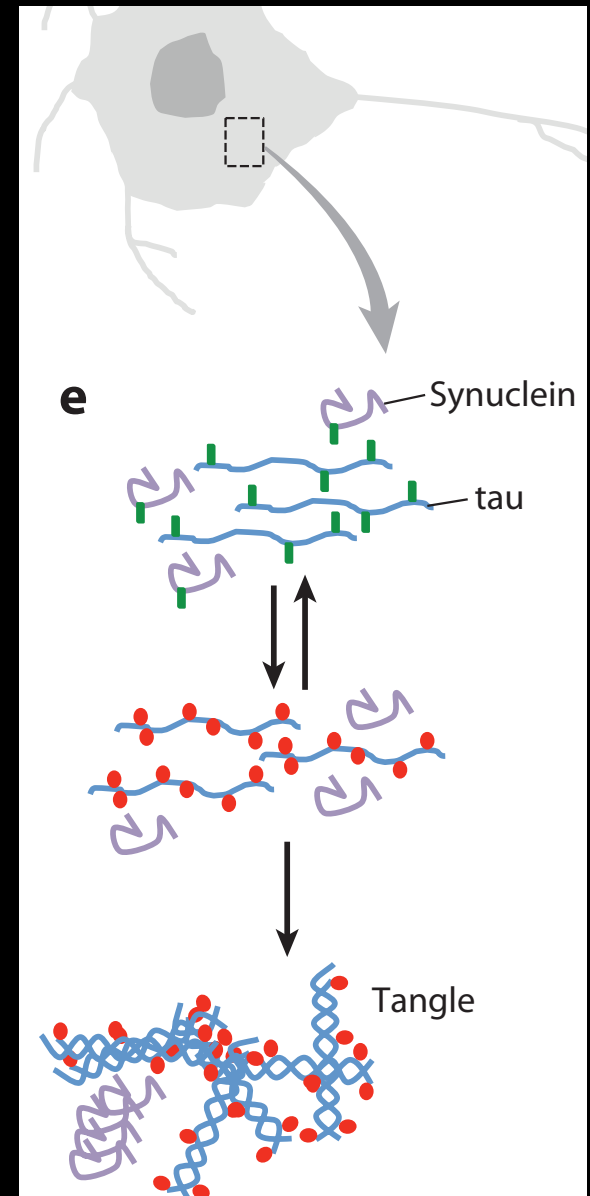
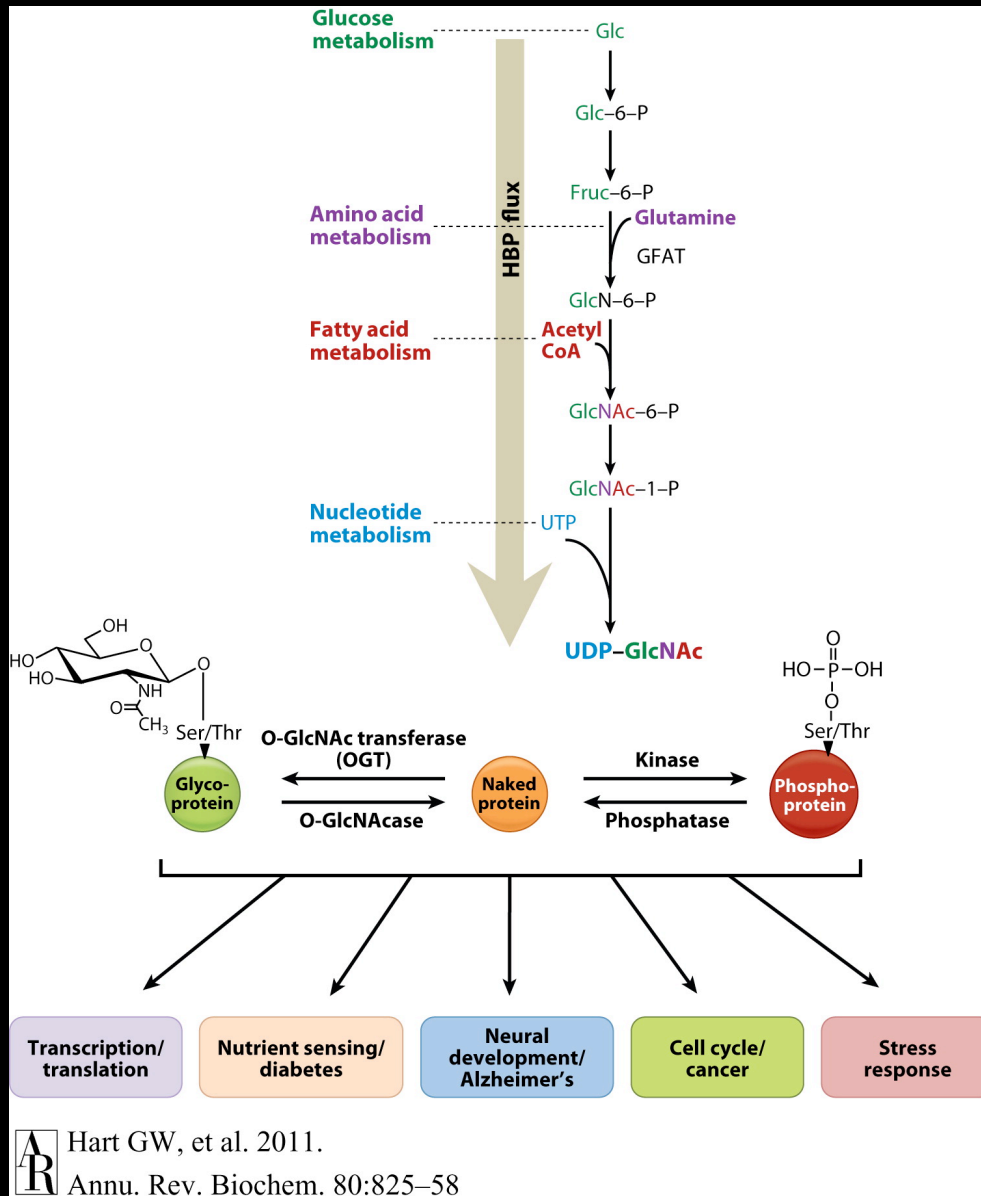
1. Manning et al. (2002). The protein kinase complement of the human genome.
2. Iakoucheva et al. (2004). The importance of intrinsic disorder for protein phosphorylation.
3. Gupta and Brunak (2002). Prediction of glycosylation across the human proteome and the correlation to protein function.

Substrate specificities

- There are 518 known kinases in humans
- There is only one known gene for OGT in mammals and two in plants
- How does OGT modify more than 1000 different proteins?



Biological role of these PTMs

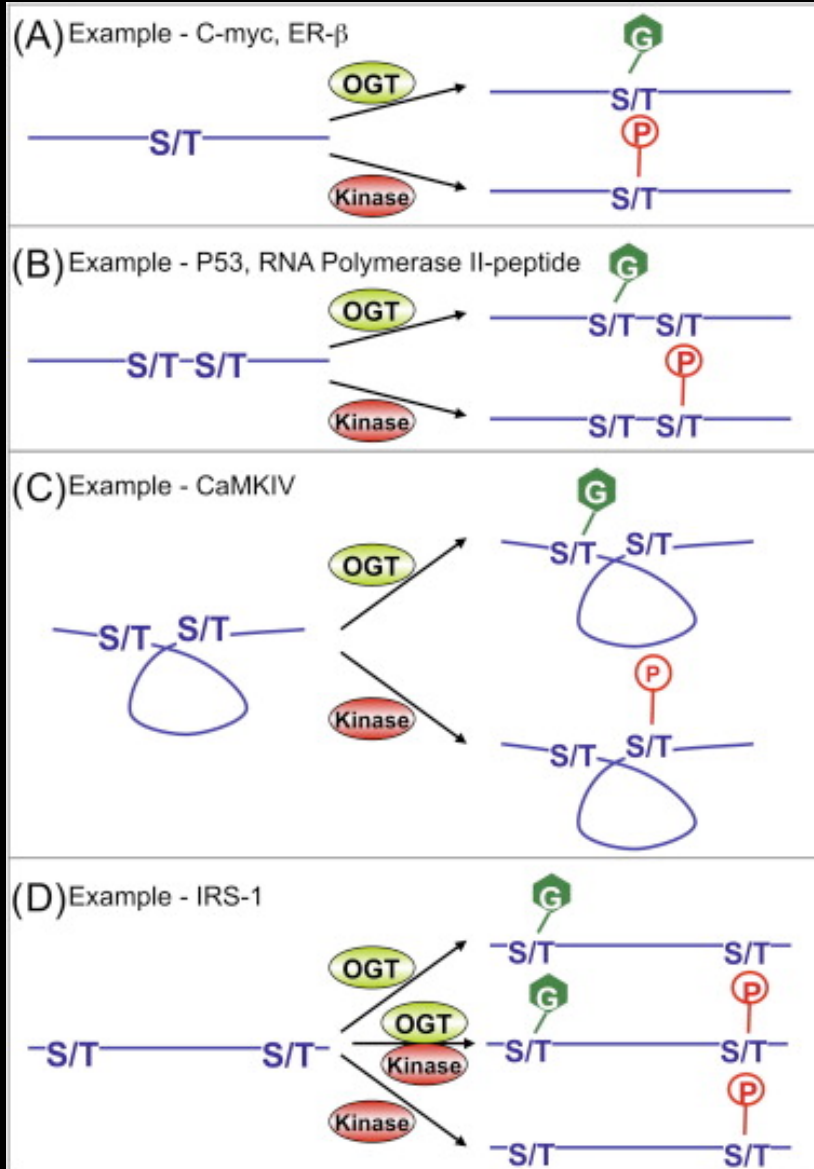


Case for crosstalk

- All known GlcNAcylated proteins are also phosphoproteins¹
- Same amino acids modified – “Thus far, virtually every O-GlcNAcylated protein can also be phosphorylated”
- O-GlcNAc half-life much shorter than polypeptide chain – dynamic like phosphorylation²
- Regulation of phosphorylation outside the kinase-phosphatase framework

1. Wang et al. (2008). Cross-talk between GlcNAcylation and phosphorylation: Site-specific phosphorylation dynamics in response to globally elevated O-GlcNAc.
2. Roquemore et al. (1996). Dynamic O-GlcNAcylation of the small heat shock protein alpha B-crystallin.

Crosstalk at different levels



Yin-Yang: serum starvation increases Thr GlcNAcylation and addition increases phosphorylation

Proximal: More common, in p53 it appears that there might not be enough space for both PTMs

Spatially proximal: Mutating Ser189 (O-GlcNAc site) increases phosphorylation at Thr200 & enzyme activity

Distal: Crosstalk between N-terminal phosphorylation sites and C-terminal O-GlcNAc sites regulates insulin signal

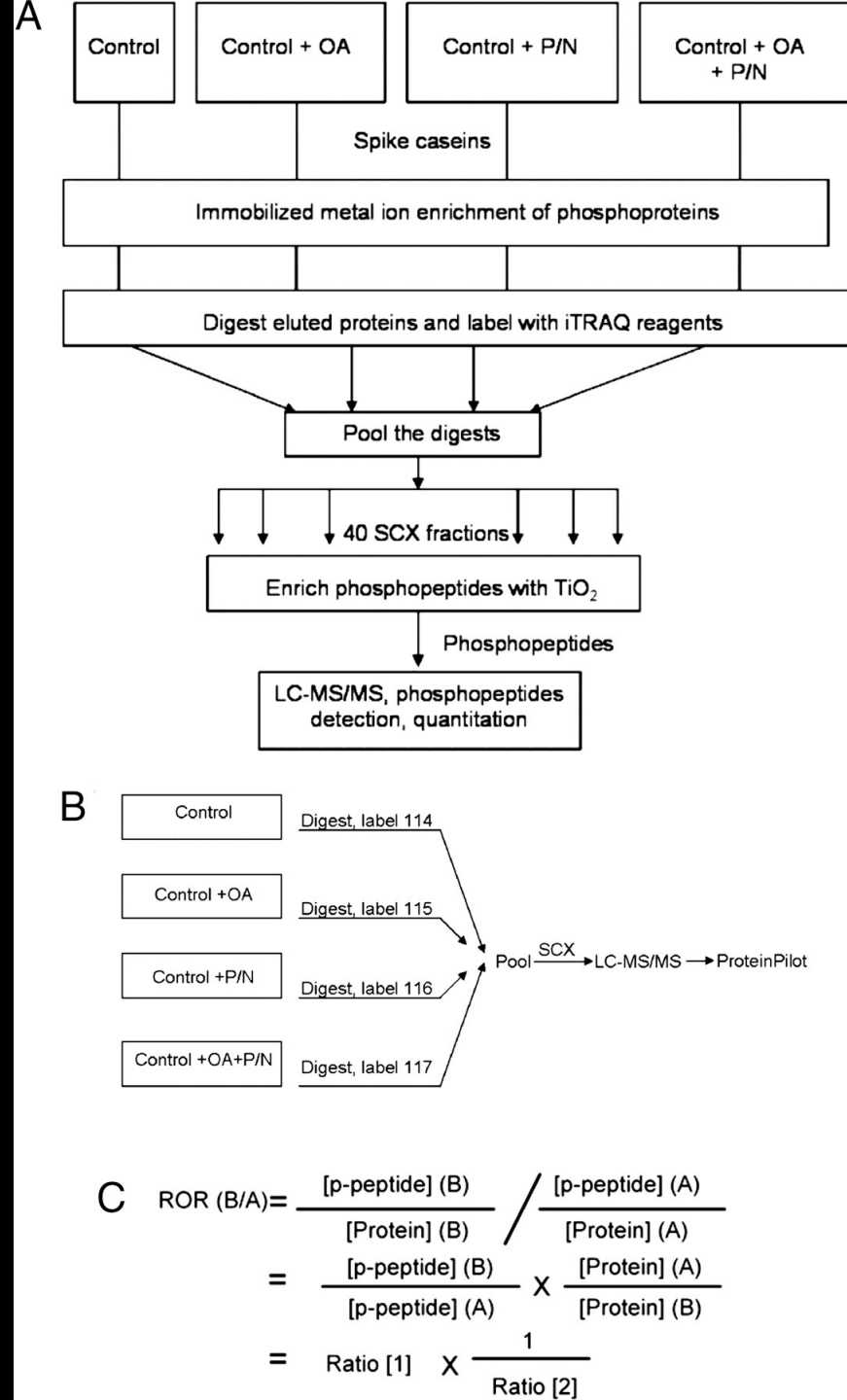
And growing evidence ...

- Activation of protein kinase A and C decreases global O-GlcNAcylation and inhibition increases it
- Inhibition of GSK-3 increases O-GlcNAcylation at least 10 proteins (mostly cytoskeletal) & decreases O-GlcNAcylation on 19 proteins (mostly RNA-binding)
- O-GlcNAc found to regulate several kinases and OGT regulated by phosphorylation
- In hippocampal neuronal synapses elevation of O-GlcNAc increases phosphorylation of synapsin I/II at specific sites
- Yin-Yang model – reciprocal occupancy of phosphate and O-GlcNAc moieties at Ser/Thr

Large-scale study

- 711 phosphopeptides quantified
- ~48% of phosphosites not cycled
- Of the remaining, increased O-GlcNAcylation resulted in lower phosphorylation at 280 sites and increased phosphorylation at 148 sites
- Multiple causes attributed

Wang et al. (2008). Cross-talk between GlcNAcylation and phosphorylation: Site-specific phosphorylation dynamics in response to globally elevated O-GlcNAc.



Bioinformatics in this area

- Most work has focused on Yin-Yang sites
- No work on sequence and conservation analysis of these sites
- Only one predictor so far – YinOYang¹
- An ensemble of nine neural network classifiers trained on local sequence features and surface accessibility around O-GlcNAc site
- Runs this predictor and then runs NetPhos – those sites with high scores for both, predicted as Yin-Yang sites

1. Gupta and Brunak (2002). Prediction of glycosylation across the human proteome and the correlation to protein function.

An alternative approach

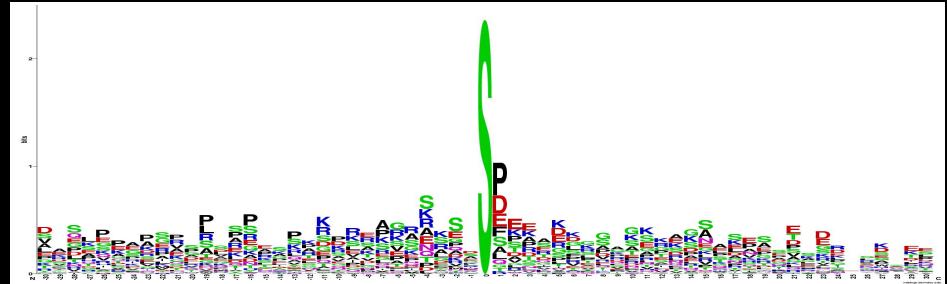
- Train on known Yin-Yang sites as positives and any sites not known to be occupied by both PTMs as negatives
- Tried as class project by Chao and group
- Used dataset from Wang et al.
- If the phosphorylation level dropped significantly after P/N treatment for a specific site, the site was a Yin Yang site

Normalized Ratio
1 : 0.5 : 0.59 : 0.79
1 : 1 : 0.73 : 0.89
1 : 0.95 : 1.1 : 1.13
1 : 0.9 : 0.61 : 0.85
1 : 0.95 : 0.64 : 0.85
1 : 1.27 : 0.89 : 1.18
1 : 2.59 : 1.07 : 1.65
1 : 0.81 : 0.73 : 0.77
1 : 0.81 : 0.65 : 1.04
1 : 1.49 : 0.82 : 1.13
1 : 1.14 : 0.59 : 1.22
1 : 1.88 : 1.56 : 1.11
1 : 2.77 : 0.69 : 1.6
1 : 2.19 : 1.94 : 3.63
1 : 2.2 : 1.94 : 3.85
1 : 1.24 : 0.18 : 1.37
1 : 1.36 : 0.75 : 1.47
1 : 2.95 : 0.73 : 1.68
1 : 1.38 : 1.13 : 1.2
1 : 1.38 : 1.11 : 1.19
1 : 2.53 : 0.51 : 2.69
1 : 1.54 : 1.1 : 1.62
1 : 1.14 : 1.15 : 1.65
1 : 0.86 : 1.75 : 1.57
1 : 0.9 : 1.15 : 1.05
1 : 1.16 : 0.71 : 0.98
1 : 0.76 : 0.97 : 1.02
1 : 1.69 : 1.04 : 1.21
1 : 1.14 : 0.6 : 1.01
1 : 0.4 : 0.84 : 1.19
1 : 2.23 : 1.38 : 2.19

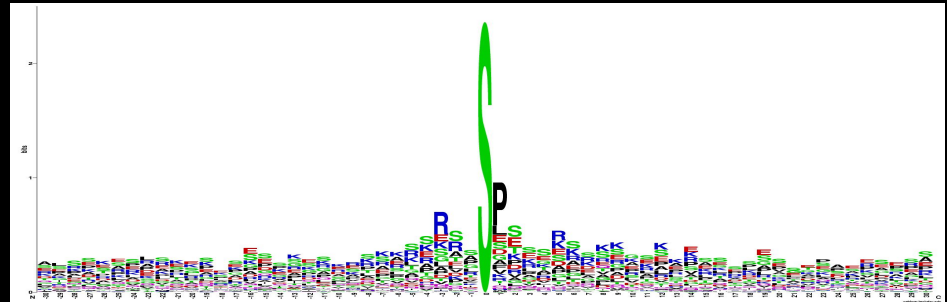
$$\begin{aligned}
 \text{C} \quad \text{ROR (B/A)} &= \frac{[\text{p-peptide}] (B)}{[\text{Protein}] (B)} \bigg/ \frac{[\text{p-peptide}] (A)}{[\text{Protein}] (A)} \\
 &= \frac{[\text{p-peptide}] (B)}{[\text{p-peptide}] (A)} \times \frac{[\text{Protein}] (A)}{[\text{Protein}] (B)} \\
 &= \text{Ratio [1]} \times \frac{1}{\text{Ratio [2]}}
 \end{aligned}$$

No consensus sequence

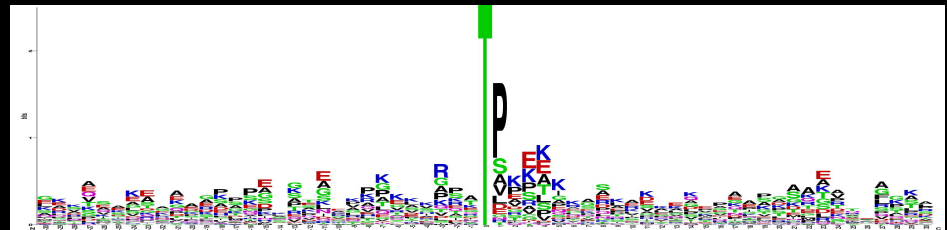
Pos Ser



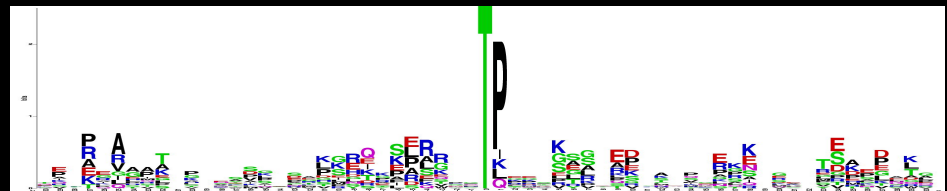
Neg Ser



Pos Thr

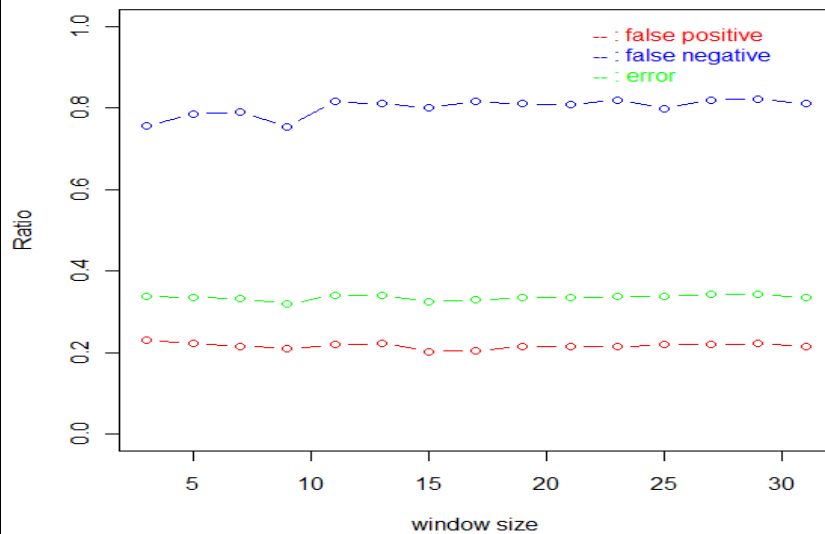


Neg Thr



Profile model and ANN

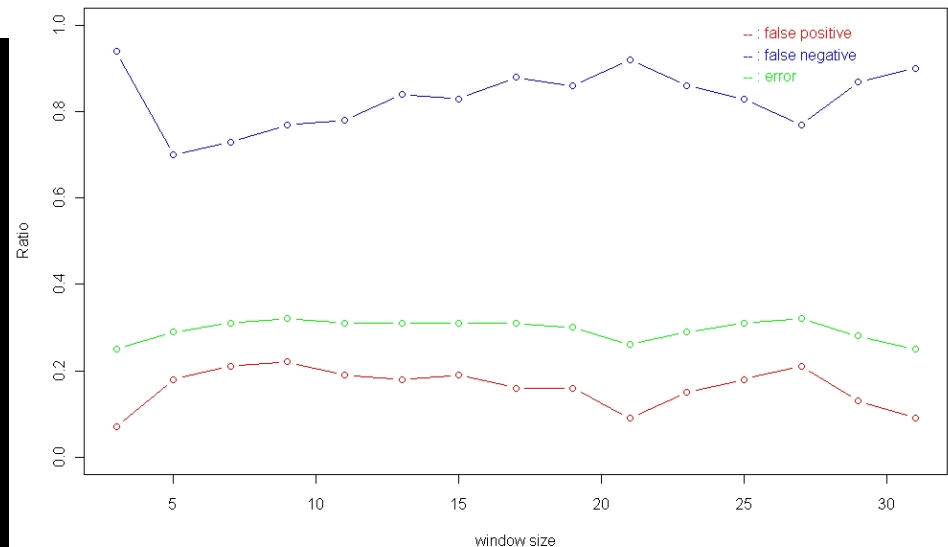
Profile model



- Window centered around site
- 4-fold cross validation is used to evaluate the model

- Feed-forward ANN with sigmoidal nodes and one hidden layer
- The input layer has n binary input groups for window length n

Result



Discussion

- Training with Yin-Yang data makes sense as opposed to the YinOYang approach
 - But training data quantity and quality?
 - ANN with two outputs for phosphorylation and O-GlcNAcylation – (0, 0), (0, 1), (1, 0) and (1,1)?
- What about the idea that “virtually every phosphorylation site can be O-GlcNAcylated”?
- Other features such as intrinsic disorder?
- Computational prediction and analysis of crosstalk other than Yin-Yang sites?

Thanks for listening