Module 7: The O-GlcNAc modification

Faculty Leader: *Natasha Zachara Ph.D.*

Background:

As we discussed in our lecture, the O-GlcNAc modification can be challenging to detect. In part this is a reflection of the β-O-glycosidic bond, which is both acid and base labile and ionized easily during many forms of mass spectrometry. Moreover, the addition of N-acetylglucosamine doesn't alter the pI of proteins and rarely alters the molecular weight of proteins. Complicating analysis further, UDP-GlcNAc is used for the synthesis of many types of glycans so metabolic labeling and manipulation of the hexosamine biosynthetic pathway can result in changes to other glycoconjugates. Finally, while there are only two enzymes for the addition and removal of O-GlcNAc, only the O-GlcNAcase has been successfully and specifically inhibited *in vivo*.

Possible techniques:

There are numerous techniques for the detection, enrichment, and analysis of O-GlcNAc. We have chosen to focus on the following techniques:

A) O-GlcNAc transferase (OGT) assays;

B) O-GlcNAcase (OGA) assays;

C) Western blotting with antibodies and lectins, and

D) Labeling with Galactosyltransferase.

The latter two techniques were covered in Metabolic Engineering/Glycan Labeling, Release & Detection.

Samples

Today we are OGT and OGA isolated from different tissues. OGT will be assayed against a variety of peptide substrates, whereas we will use different inhibitors to assess the levels of activity of HexA/B (lysosomal hexosaminidases) and Hex C (the O-GlcNAcase).

OGT and OGA Assays

OGT and OGA are both inhibited by detergent (>0.1 (v/v)% NP-40). To address this challenge, we typically desalt lysates using Zeba spin desalting plates or use a detergent free lysis. The latter is being used today. To ensure we don't impact the OGA assay, lysates will be made in the absence of TMG and PUGNAc.