CHEMICAL APPROACHES TO STUDYING *O*-GLCNACYLATION OF PROTEINS

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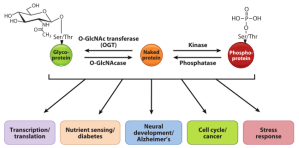
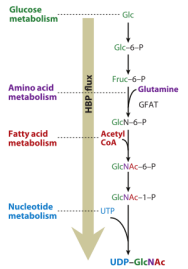
O-LINKED β-*N*-ACETYL GLUCOSAMINE MODIFICATIONS

O-GlcNAcylation Mediates Diverse Biological Functions

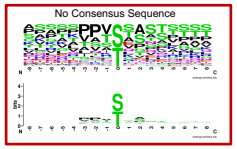
- *O*-GlcNAc is added to serine and threonine residues of intracellular proteins
- *O*-GlcNAc is a dynamic and inducible posttranslational modification (PTM)
- Found in all multicellular eukaryotes and essential for life
- *O*-GlcNAc is known to play an integral role in the regulation of many cellular processes in physiology and pathophysiology
- Exquisite interplay with other PTMs, such as phosphorylation

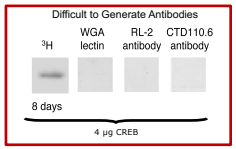




Challenges to Studying *O*-GlcNAc

- No consensus sequence for OGT
- Approaches rely on "snap shots" of *O*-GlcNAc
- Challenging to detect:
  - Substoichiometric
  - Labile
  - Found on low-abundance proteins

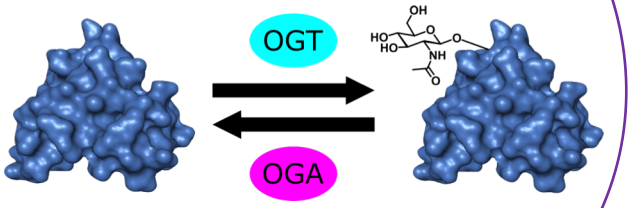




Because of these challenges, many methods for *O*-GlcNAc detection suffer from poor specificity and sensitivity

O-GLCNACYLATION: A DYNAMIC AND REVERSIBLE POST-TRANSLATIONAL MODIFICATION

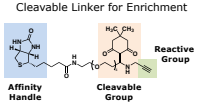
- *O*-GlcNAc marks are implemented by the enzyme OGT

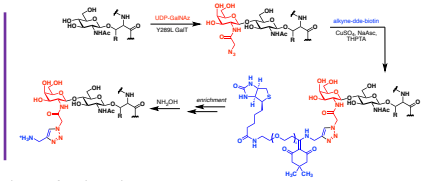


- *O*-GlcNAc marks are removed by the enzyme OGA

DEFINING THE *O*-GLCNACOME

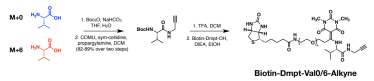
Chemoenzymatic Labeling to Detect *O*-GlcNAc and Enable Affinity Enrichment





- New chemical tag used to enrich sites of *O*-GlcNAcylation
- Molecules can be coupled to affinity tags with CuAAC and then purified by affinity pull-down
- Fluorescent tags may be coupled with CuAAC to enable visualization (in-gel fluorescence)
- Tags with high molecular weight may also be used for mobility shift assays
- Dde functional group provides a chemically cleavable handle to release tagged molecules

Moving Towards Quantitative *O*-GlcNAcomics for Disease-Related Studies

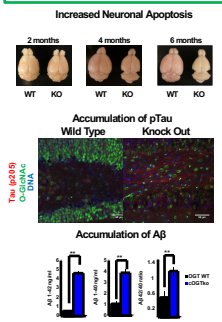


- Incorporation of an isotopically labelled amino acid into the linker enables quantitative, comparative *O*-GlcNAcomics

- Enables studies for comparison of physiological and pathological disease states

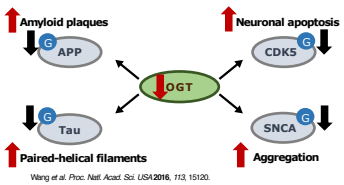
O-GLCNAC IN NEURODEGENERATIVE DISEASE AND CANCER

OGT Knock-out Results in Neurodegeneration



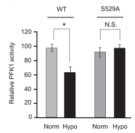
- Loss of OGT results in neuronal apoptosis
- Manifests as significant decrease in size of brain
- In AD, tau forms fibrils and is hyperphosphorylated
- Loss of OGT results in significant increases in phosphor-tau
- In AD, Aβ forms plaques in the brain
- Loss of OGT results in significant increases in Aβ

Molecular Mechanisms Contributing to Neurodegeneration



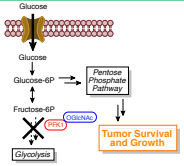
Wang *et al. Proc. Natl. Acad. Sci. USA* **2016**, *113*, 15120.

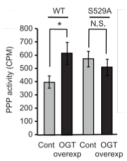
O-GlcNAc Regulates PFK1 Activity



- *O*-GlcNAcylation of PFK1, a key regulator of glycolysis, occurs in the binding site of its allosteric activator
- Modification of PFK1 inhibits the formation of active PFK1 tetramers

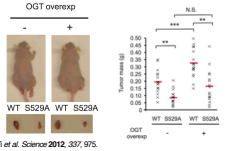
O-GlcNAcylation of PFK1 Regulates Glycolytic Flux





- Modification of PFK1 shifts carbon flux from glycolysis to pentose phosphate pathway (PPP)

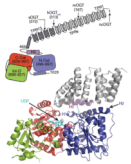
O-GlcNAcylation Promotes Tumor Growth



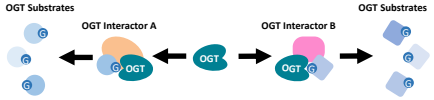
Yi *et al. Science* **2012**, *337*, 975.

DEFINING THE OGT INTERACTOME

How Does OGT Select Its Substrates?

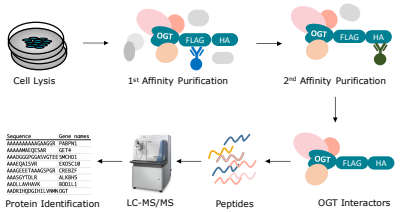


- Only one enzyme is responsible for the *O*-GlcNAcylation of thousands of substrates
- Activity of OGT is inducible towards specific substrates upon specific stimulation
- Studies demonstrate OGT is able to discriminate among substrates



- Interactors may influence substrate selectivity
- Interactor A may promote *O*-GlcNAcylation of substrate sub-set A
- Interactor B may promote *O*-GlcNAcylation of substrate sub-set B

Tandem Affinity Purification Identifies OGT Interactors



- Expression of dual-tagged OGT enables tandem affinity purification for pull-down of OGT interactors
- Mass spec analysis enables identification of OGT interactors

Identifying Functional Networks of OGT

- Biological networks in which OGT may play important roles can be identified by network analysis of OGT substrates and OGT interactors
- Importantly, lists of substrates and interactors are non-exclusive