

## RELATIVE QUANTITATION OF O-GLCNAC GLYCOSYLATION BY ETD USING TMT<sup>®</sup> ISOBARIC TAGS

VINER R., ZHANG T., SECOND T., ZABROUSKOV V.

*Thermo Fisher Scientific, San Jose, CA, USA*

Modifications of Ser/Thr residues in proteins by addition of a single O-linked N-acetylglucosamine (O-GlcNAc) plays an important role in cell regulation<sup>(1)</sup>. Understanding cellular mechanisms that regulate O-GlcNAc glycosylation has been challenging due to the difficulty in detection and quantitation of the modification. Mass spectrometry-based multiplex quantitative methods (including iTRAQ) have been successfully employed to measure relative phosphorylation with CID fragmentation. However, modifications such as O-GlcNAc are lost prior to fragmentation of the peptide backbone in conventional CID, preventing modification site localisation. This makes identification and quantitation of O-linked glycosylation sites impossible with traditional CID. To understand the regulation of O-GlcNAc glycosylation in cells will require the development new tools. In this study, we present a new strategy for relative quantitation of O-GlcNAc-containing peptides combining multiplexed tandem mass isobaric tag (TMT<sup>®</sup>) labelling and an alternative MS/MS fragmentation technique. Electron Transfer Dissociation (ETD). Experiments were conducted on a hybrid linear ion trap-orbitrap mass spectrometer equipped with ETD. Alpha crystalline (A and B chains) from different sources were chosen as a model protein because of its well known presence of O-GlcNAc sites and a low stoichiometry of glycosylation (<10%)<sup>(2)</sup>. The samples were digested, labelled with 6 plex TMT<sup>®</sup> tags (125, 128, 130 and 131 to produce 114, 116, 118 and 119 reporter ions, respectively, by ETD), and mixed in different ratios. ETD analysis of both labelled and unlabeled peptides pinpointed at least one O-GlcNAc-containing peptide per chain in addition to other PTMs (glycation, phosphorylation, acetylation). Reporter ion ratios measured by ETD had similar accuracy and precision as TMT<sup>®</sup> data obtained by HCD. Based on these results, we conclude that an ETD-based workflow can be used for simultaneous qualitative and quantitative proteomics of O-GlcNAc glycosylation.

### References:

1. Slawson, C., Hart, G.W. "Dynamic interplay between -GlcNAc and O-phosphate; the sweet side of protein regulation", *Curr.Opin.Struct.Biol*, 2003, 13:631-636
2. Khidekel, N. *et al* "Probing the dynamics of O-GlcNAc glycosylation in the brain using quantitative proteomics", *Nat ChemBiol*, 2007, 339-348