## 2.1

# Novel Lectin-based Enrichment of *O*-GlcNAc Peptides: Application to *O*-GlcNAc Proteomics of Post-synaptic Density Fraction From Mouse Brain

**K. Vosseller[1], J. Trinidad[2], C. Specht[3], A. Thalhammer[3], A. Lynn[2], J. Snedecor[2], K. Medzihradszky[2], R. Schoepfer[3], and A. Burlingame[2]**

**[1]Drexel University College of Medicine, Philadelphia, PA; [2]University of California, San Francisco, CA; [3]University College, London, UK**

*O*-GlcNAc is a widespread dynamic carbohydrate modification of cytosolic and nuclear proteins which may act analogously to phosphorylation in regulating protein function. However, knowledge of specific *O*-GlcNAc regulatory roles is limited, in part due to difficulties in mapping sites of modification. *O*-GlcNAc site-mapping strategies involving enzymatic tagging or chemical derivatization have yielded only a small number of *O*-GlcNAc attachment sites, perhaps due to methodological drawbacks including low sensitivity and lack of specificity. We report a strategy for effective direct enrichment and identification of *in vivo* natively modified *O*-GlcNAc peptides, involving unique lectin (Wheat Germ Agglutinin) based isocratic HPLC chromatography coupled with mass spectrometry. The approach is validated through isolation of a synthetic *O*-GlcNAc modified peptide from a complex mixture. Enrichment of a low abundance well characterized *O*-GlcNAc modified peptide from a tryptic digest of the model *O*-GlcNAc modified protein alpha-crystallin is demonstrated. Finally, the application of this strategy for proteomic scale *in vivo* site-mapping is shown through enrichment of greater than 100 *O*-GlcNAc modified peptides from a post-synaptic density (PSD) fraction from mouse brain. The PSD is critical in synaptic transmission, and a variety of signaling and cytoskeletal proteins concentrated in this fraction are shown here to be *O*-GlcNAc modified. The pre-synaptic active zone vesicle associated protein Bassoon was found to be *O*-GlcNAc modified at 14 distinct sites. Often, due to the lability of *O*-GlcNAc during CID, MS/MS of natively modified peptides did not allow for specific site assignment. Beta-elimination/Michael addition derivatization of WGA enriched *O*-GlcNAc modified peptides facilitated mapping of specific modified residues in some cases. Additionally, the value of electron capture dissociation (ECD) as a strategy to preserve the labile *O*-GlcNAc modification during fragmentation and facilitate site-mapping of natively modified peptides is demonstrated.

## 2.2

# Chemical Approaches to Understanding Protein Glycosylation in the Brain

**L. Hsieh-Wilson[1], N. Khidekel[1], N. Lamarre-Vincent[1], S. Ficarro[2], and E. Peters[2]**

**[1]California Institute of Technology, Division of Chemistry and Chemical Engineering, Pasadena, CA; [2] The Genomics Institute of the Novartis Research Foundation, San Diego, CA**

The covalent modification of intracellular proteins by *O*-linked $\beta$-*N*-acetylglucosamine (*O*-GlcNAc) is emerging as a crucial regulatory posttranslational modification akin to phosphorylation. Numerous studies point to the significance of *O*-GlcNAc in cellular processes such as nutrient sensing, protein degradation and gene expression. Despite its importance, the breadth and functional roles of *O*-GlcNAc are only beginning to be elucidated. Advances in our understanding will require the development of new strategies for the detection and study of *O*-GlcNAc-modified proteins *in vivo*. We will discuss methods to study the modification in cells, as well as functional implications of *O*-GlcNAc for gene regulation and information storage. One method exploits an engineered galactosyltransferase enzyme to selectively label *O*-GlcNAc proteins with a ketone-biotin tag. The tag permits enrichment of low-abundance *O*-GlcNAc species from complex mixtures and enables localization of the modification to short amino acid sequences.

Using this approach, we discovered twenty-three new *O*-GlcNAc glycosylated proteins from the mammalian brain, including regulatory proteins associated with gene expression, neuronal signaling, and synaptic plasticity. The functional diversity represented by this set of proteins suggests an expanded role for *O*-GlcNAc in regulating neuronal functions. Moreover, the chemoenzymatic strategy described here should prove valuable for quantifying dynamic changes in glycosylation and facilitate studies of the physiological significance of *O*-GlcNAc across the proteome.

Downloaded from https://www.mcponline.org by guest on October 27, 2020



© 2005 by The American Society for Biochemistry and Molecular Biology, Inc.
**This paper is available on line at http://www.mcponline.org**

## 2.3
## MS Imaging in Clinical Proteomics

D. Hochstrasser[1], D. Walther[2], M. Quadroni[3], S. Catherinet[2], M. Tuloup[2], P. Binz[2], J. Burgess[1], A. Vaezzadeh[1], C. Zimmermann[1], G. Corthals[1], J. Sanchez[1], and R. Appel[2]

[1]Clinical Pathology and Pharmacy Section, Geneva University and University Hospital, Geneva, Switzerland; [2]Swiss Institute of Bioinformatics, Centre Medical Universitaire, Geneva, Switzerland; [3]Institut de Biochimie, Lausanne, Switzerland

Proteome complexity conditions and guides the future trends in clinical proteomics. We have now reached a stage where industries and laboratories develop and integrate simplified and somewhat automated large-scale approaches to fractionate samples, to compare them and to for example unravel new biomarkers. Mass spectrometry (MS) progressively plays a pivotal role. Since recently mass spectrometers are also being used as molecular imaging device. However, central and critical elements emerge that hold back rapid progress: these are the display, interpretation, scoring and evaluation of data. In particular, efficiently assessing the quality and reproducibility of the vast amount of data is difficult. Most current proteomics experiments perform qualitative and semi-quantitative profiling of proteins in complex mixtures using high-resolution separations followed by MS analysis. More and more MS is used after the separation steps to scan peptide data at a high sampling rate producing highly correlated data. Exploiting the correlations that exist within MS datasets allows for the extraction of information that is not obtainable otherwise. Thus innovative software is being developed to access the full range of information contained in those data. The vast possibilities to discover more and better information offered by proteome imaging have so far been unsuspected. As MS analyses are probabilistic in nature, for adequate data interpretation, stringent and rigorous methods, as well as software tools to display the original data, should scrutinize experimental results & data comparisons, and outcomes should be accompanied by statistic, positive and negative predictive values. With better and more rigorous data acquisition and display, comparison and interpretation, clinical proteomics will establish itself as a major component of biomarker discovery, target selection and validation, quality control and toxicology evaluation during drug development and drug discovery and possibly in routine clinical diagnostic procedures.

Downloaded from https://www.mcponline.org by guest on October 27, 2020