

Sweet insight in proteomics: Experimental strategies for deciphering the glycome

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Glycosylation is so far the most abundant form of post-translational modifications (PTMs) and plays important roles in development, immunity, cancer, and infectious diseases, it is also particular interest for Biotechs, as far as many of recombinant therapeutics are glycoproteins. Analyzing and understanding the glycosylation changes associated with various pathological processes provides a way to the development of new therapeutic and diagnostic means, through the discovery of new “glycomarkers” and “glyco-targets”. Just as with the other efforts contributing to systems biology, the fields of glycoproteomics and glycomics are strongly dependent on the current and future advances in analytical methodologies and instrumentation.

Mass spectrometry (MS) is routinely employed as a key methodology in the structural elucidation of glycoconjugates. Although mass spectrometers using single mass analyzers (mainly MALDI-TOF) can now readily and routinely assist acquisition of compositional data on glycans in terms of isobaric monosaccharides, we must keep in mind that the structural analysis of glycans requires determination of branching, linkage position, monomer anomericity, glycosylation site occupancy (microheterogeneity). For that reason, in addressing the analytical needs for a complete structural investigation of a complex glycoprotein, a combination of several analytical methods and also several MS instrumental approaches will be required. **Glycoproteomics** will share many analytical approaches with the mainstream of proteomics such as “top down,” “bottom up” or “shotgun” approaches. Quite different will be **Glycomics** which necessitate a quantitative removal of glycans from the polypeptide backbone prior to their separate analysis using different MS ionization techniques and/or mass analyzers displaying glycomic profiles (Glycomaps) for a given glycoprotein, biological fluid, cell or tissue.

Glycoproteomics as part of a differential proteomic approach will also generally address to low abundant proteomes consisting mainly of membrane-associated glycoproteins. Their analysis will therefore be strongly dependent from solubilisation, selective enrichment and analytical separations (e.g.: lectins, affinity -tag separation ...) as well as from other *in vitro* or *in vivo* labelling strategies.

Through different examples, all these aspects will be emphasized in comparison to the more traditional methodologies used for years in structural glycoanalysis.