MODIFICATION SPECIFIC PROTEOMICS; IDENTIFICATION AND QUANTITATION OF PHOSPHORYLATED PROTEINS AND SIALYLATED GLYCOPROTEINS DERIVED FROM PRIMARY BIOLOGICAL MATERIAL.

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Many proteins are post translationally modified and such modifications often determine the function, interaction and physio-chemical properties of these proteins. Many of the modifications involved in cellular regulation and signalling are reversible, where the shift between the addition and removal of the modifying group by specific enzymes provides a highly controlled regulation. For example protein phosphorylation, catalyzed by protein kinases and dephosphorylation catalyzed by protein phosphatases, is a central PTM in numerous cellular processes whereas glycosylation is involved in recognition, cell-cell interaction and binding of ligands and hormones to surface proteins. Common for most PTMs are the low stoichiometry of the modification, high heterogeneity (especially glycosylation) or pronounced changed in physiochemical properties which require specific methods in order to selectively and sensitively purify the given modified peptides/proteins from a highly complex sample before the modified peptide/protein is available for characterization. Therefore, a significant effort has been placed in developing new and better methods for the assessment of PTMs from various cellular systems. Presently, one of the largest challenges in modification specific proteomics is the development of robust methods for quantitative assessment of PTMs in proteins originating from primary cell material where often only low amount of protein can be extracted.

We have recently developed new and comprehensive strategies for the quantitative assessment of phosphopeptides and sialylated glycopeptides originating from primary cell materials employing titanium dioxide (TiO_2) chromatography, Sequential elution from IMAC (SIMAC) and hydrophilic interaction chromatography (HILIC). Examples will be given here of the study of depolarization dependent signalling in isolated nerve terminals, pro-inflammatory cytokine signalling in handpicked Islet of Langerhans and mice brain development.