

Exploring cell surface proteomes - Specific identification of *Drosophila* plasma membrane glycoproteins via mass spectrometry

Ralph Schiess¹, Bernd Wollscheid², Julian Watts² and Ruedi Aebersold^{1,2}

(1) Institute for Molecular Systems Biology, ETH Zurich, 8093 Zurich, Switzerland

(2) Institute for Systems Biology, 1441 North 34th Street, Seattle, WA 98103-8904, USA

* correspondence: <schuess@imsb.biol.ethz.ch>

Membrane proteins are responsible for most membrane functions, serving as specific receptors, enzymes and transport proteins. The proteins within the plasma membrane therefore give each cell type its characteristic functional properties. We have developed a methodology that allows us to specifically identify a subset of *Drosophila* plasma membrane cell surface proteins via Mass Spectrometry (MS). Glycoproteins on the surface of intact, living cells are chemically modified and coupled to a biotin-containing linker molecule that does not damage the cells nor penetrates the cells. The tagged cells are then disrupted and a fraction enriched in membrane proteins is isolated from the lysate via ultracentrifugation. Upon digestion of the isolated membrane proteins, the tagged glycopeptides are affinity-purified and optionally labeled with stable isotope labeling techniques for subsequent relative quantitation. *N*-linked glycopeptides are specifically released and analyzed via electrospray ionization ion trap tandem mass spectrometry (LC MS/MS).

The technology allows for the specific identification of proteins which were present on the cell surface of a given cell at the time of labeling. Cell surface proteins in *Drosophila* cells can therefore be identified in a discovery-driven approach. This methodology has the potential for the discovery of biomarkers that might differentiate cancer cells from control cells. The potential detection of new differentiation markers could also permit the classification of cell populations in more detailed developmental stages or, for example, could indicate lineage commitment of stem cells. Furthermore, the identification of *N*-glycosites allows the determination of the topology of the identified glycoprotein within the plasma membrane, since the glycosylated and labeled peptides are only expected to be present on the extracellular part of the transmembrane proteins.