Expression and purification of membrane proteins in context of functionality

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Human membrane proteins, in particular G-protein-coupled receptors (GPCRs), are important drug targets. Precise characterization of ligands as well as crystallization requires sufficient amounts of pure and active protein to identify novel as well as more specific drugs.

In our projects, we compare different methods for expression, purification and stabilization of different membrane proteins. Notably, almost all proteins were expressed in their non-engineered, wild-type form, with different affinity tags like Rho1D4 or His tag added to the C- and N-termini. Bacterial and insect expression systems were employed.

In addition, an alternative affinity chromatography method was tested for its ability to enrich active protein based on interaction with a specific ligand. It is only reasonable to design such custom designed, target-specific affinity matrices for such relevant projects. Since free ligand has a higher affinity compared to the matrix-bound ligand, it is possible to elute the protein with the same ligand.

Biophysical measurements comparing ligand-binding properties of GPCRs purified from different expression hosts and by different affinity matrices will be presented, and the advantages and disadvantages of various stabilization methods, also in the light of subsequent membrane protein crystallizations, will be discussed.