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GERMAN CANADIAN
COMPLEX MEMBRANE PROTEINS
IN CELLULAR DEVELOPMENT AND DISEASE

# P7

## Rational reconstitution of membrane proteins (Sandro Keller)

One of the main bottlenecks in membrane-protein research is their functional reconstitution into artificial lipid bilayers. We have developed new approaches towards overcoming this limitation by establishing high-sensitivity isothermal titration calorimetry (ITC) as a powerful method for monitoring the reconstitution of membrane proteins into lipid vesicles and bicelles in a non-invasive and fully automated manner. So far, this has been exemplified for a prokaryotic ion channel as a robust and well-characterised model protein, which was functionally reconstituted at unprecedentedly high protein densities in a proof-of-principle project. We now aim to extend this approach to eukaryotic proteins of high physiological and pharmacological relevance, such as ion channels and intramembrane proteases. These proteins will first be produced by heterologous expression and purified by chromatographic methods in sufficient quantities and purities. Next, we will reconstitute them into liposomes and bicelles of various compositions and monitor these processes with the aid of ITC in order to achieve high protein/lipid ratios. The resulting proteoliposomes are used for protein transfer into planar lipid membranes, which enable a qualitative corroboration of ion-flux specificity and a quantitative assessment of the yield of functionally reconstituted protein. Finally, proteoliposomes and bicelles with high protein/lipid ratios shall be employed for, respectively, 2D and 3D crystallisation trials aiming at providing the starting material for structure elucidation by electron microscopy and X-ray diffraction in collaborating groups.