ITN - SBMPs
Structural Biology of Membrane Proteins

Postdoc project

GPCRs & their G proteins in native and reconstituted planar membranes: A generic platform to investigate receptor mediated signalling

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The project will focus on the monitoring of the activation of GPCRs and the subsequent interaction with their G proteins by single molecule imaging in order to quantify the details of these central signalling reactions. We will investigate two systems: (i) Recombinant expression of GPCRs and G proteins in heterologous cells and using the native cellular membranes for the optical measurements. (ii) Use of GPCRs expressed in vitro and reconstituted into artificial lipid membranes from the Frankfurt lab. We will establish the planar membrane system as a generic platform for investigating GPCR mediated cellular signalling. The project focuses on the following GPCRs: (i) Endothelin B receptor, which the Frankfurt group has already expressed without fluorescent probes; (ii) OR17-40, a prototypical human odorant receptor, which has been expressed recombinantly in HEK cells and investigated in live cells by the EPFL group. Odorant receptors are still very difficult to express efficiently in recombinant systems for yet unknown reasons. Therefore, the combination of in vitro expression and reconstitution in planar membranes will be of central importance, not only for elucidating molecular details of OR activation but also for the future design of artificial sensor systems based on native olfactory receptors.

The **group of H. Vogel** has long-standing experience with investigations of structural dynamics of membrane proteins in general and GPCRs in particular using different biophysical techniques. Of central importance in the present project are the novel techniques for site-selective labelling of membrane proteins, suitable for single molecule spectroscopy and fluorescence microscopy (Guignet 2004; Lill 2005; Meyer 2006a, b; Jacquier 2006; Prummer 2006).

The **group of F. Bernhard/V. Doetsch** has achieved a breakthrough in the expression of GPCRs in cell-free systems, suitable for functional investigations of these proteins by biophysical and structural approaches (Klammt 2005, 2006, 2007).

- Cell-free expression of wt and tagged GPCRs and their G proteins (delivered by F. Bernhard/V. Doetsch)

Endothelin B receptor and OR17-40 comprising polyhistidine sequences and fused to AGT or ACP tags as well as their G proteins, again with polyhistidine sequences will be expressed in cell free systems. Efficient expression protocols for the endothelin receptor and a fusion of this GPCR with G_{alpha} have already been established. Additional reaction conditions will be determined by robotic throughput screens. Different cell-free expression modes in presence of a variety of detergents or lipids will be analysed in order to result in highest protein quality. The structural and functional folding of the synthesized GPCRs will be evaluated by ligand binding assays or by a diverse number of complementary biophysical methods. Fusion

proteins of the olfactory GPCRs and their cognate G_{alpha} proteins will be constructed and cell-free produced for the characterization of signal transfer in planar membranes.

- Imaging GPCR-mediated signalling in planar membranes by single molecule microscopy (performed in H. Vogel's lab)

At EPFL, planar membranes comprising reconstituted GPCRs will be transferred to different solid supports, preferentially silicon chips, on one side covered by a 100 nm thick Al-oxide film, which comprises one or multiple (sub)micrometer-sized holes (Schmidt 2000; Danelon 2006). In this configuration, the chip functions as a zero-mode waveguide: light directed toward the chip generates a high-intensity evanescent field in the holes, suited for excitation of fluorophores in the planar membrane covering these holes. Fluorescence signals might arise from (i) fluorescent ligands bound to the GPCR, (ii) probes directly integrated in the receptor e.g. in form of GFP-fusion proteins, (iii) a reversible site-selective label of GPCR mutant proteins by polyhistidine sequences, or (iv) a labelled ACP-tag. The latter two techniques were pioneered by the EPFL group for imaging of membrane proteins, especially GPCRs (Guignet 2004, Meyer 2006 a, b; Jacquier 2006). The membrane on the chip will be freely accessible from both sides, and thus allow for introduction of G proteins (fluorescently labelled) from the cytoplasmic receptor side and fluorescent ligands from the extracellular receptor side, which would be able to activate the GPCR and in turn the G protein. We will use this generic chip platform to measure fluorescence signals arising simultaneously from the GPCR, its G protein and its activating ligand. This will yield information about the state of oligomerization of the signalling proteins involved and data to quantify the thermodynamics and kinetics of the molecular interactions of GPCR mediated signalling (Stanasila 2003; Lill 2005; Meyer 2006a; Perez 2006; Prummer 2006). The different GPCRs and their G proteins from the Frankfurt group will be equipped with orthogonal fluorescent probes and reconstituted into planar membrane systems. Single molecule fluorescence spectrosopy and microscopy will be used to investigate the monomer/oligomer state of the proteins as well as their mutual interactions during agonist-mediated activation of the receptors.

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