INVESTIGATION OF CONFORMATIONAL DYMAMICS OF A G-PROTEIN COUPLED RECEPTOR USING SINGLE-MOLECULE FLUORESCENCE MICROSCOPY

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G-protein coupled receptors (GPCRs) orchestrate critical processes in human body and are targets for 30% out of all FDA-approved drugs. GPCR function is determined by conformational dynamics of the receptor, but only for less than 1% of GPCRs any model of conformational landscape was suggested. For those few GPCRs studied from this point of view, the data presented in literature are contradictory.

To get information about number of states and kinetic rates of interstate transitions we use single-molecule fluorescence microscopy and PIFE (protein-induced fluorescence enhancement) effect. In the experiment based on PIFE, conformational changes in protein induce changes of local environment of the dye. Being exposed to different environments the dye exhibits different spectroscopic properties and therefore can serve as a reporter of conformational changes.

We expressed and purified 6 mutant variants of GPCR and achieved site-specific labeling of the receptor in membranes with environment-sensitive fluorescent dyes with different labeling positions. To test the dye sensitivity in the selected positions we analyzed changes of fluorescence spectra and lifetimes upon ligand binding on ensemble level. Finally, we performed burst-wise measurements of fluorescence lifetime, anisotropy and intensity from single freely diffusing molecules using a confocal microscope.

To pave the way for tracking of slow sub-second conformational changes in receptor, we immobilized it on glass surface in lipid nanodiscs. We tested different variants of nanodiscs and liposomes containing biotinylated lipids or nanodiscs with peptide-tags with affinity to streptavidin. In case of nanodiscs we showed that conventional strategies of immobilization are prone to unspecific binding to the PEG-coated glass surface. We used microscale thermophoresis to test interaction between streptavidin and nanodiscs or liposomes used for protein immobilization.

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