SINGLE-MOLECULE FRET MEASUREMENTS OF THE CONFORMATIONAL DYNAMICS IN ADENOSINE RECEPTOR A_{2A}

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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. The multi-state conformational behavior of GPCRs delineates their complex pharmacology and, therefore, challenges modern drug design. The adenosine receptor A_{2A} is a G-protein coupled receptor (GPCR) that regulates the cardiovascular tonus and promotes healing of inflammation-induced injuries. A_{2A} is a promising target for drugs against insomnia, chronic pain, depression, Parkinson's disease, and cancer. Here we applied single-molecule FRET (smFRET) to investigate the conformational dynamics of the A_{2A} in freely diffusing lipid nanodiscs without immobilization.

We combined fluorescence intensity, lifetime, and anisotropy information to measure smFRET between two dyes attached to genetically introduced cysteines in the A_{2A}. We observed that FRET efficiency in the double-labeled A_{2A} increases upon agonist binding. Sub-millisecond dynamics was revealed by several complimentary burst-wise fluorescence analysis approaches: E vs TauD plot, FRET-2CDE, BVA, and fFCS.

We propose a dynamic model of A_{2A} activation that involves a slow (>2 ms) exchange between the active-like and inactive-like conformations in both apo and antagonist-bound A_{2A}, explaining the receptor's constitutive activity. For the agonist-bound A_{2A}, we detected faster (390±80 μs) ligand efficacy-dependent dynamics.

The general strategy developed in our work can be extended to study the effects of various modulators (ligands, ions, lipids, etc.), membrane-mimicking systems (micelles, lipid nanodiscs, liposomes, etc.) and genetic modifications on the activity of A_{2A} and, in perspective, other GPCRs.

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