REVEALING FAST DYNAMICS OF G-PROTEIN-COUPLED RECEPTORS WITH FLUORESCENCE CORRELATION SPECTROSCOPY

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G-Protein-Coupled Receptors (GPCRs) are the largest class of membrane-bound receptors transducing different stimuli. Such stimulation leads to a GPCR conformational change and activates downstream G-proteins. Structures of both the active and the inactive conformation have recently been solved, and a lot of effort has been made to study the dynamics of GPCR activation. Based on biophysical studies it appears that a simple 'on/off' model is not sufficient to describe the GPCR activation mechanism [1,2,3]. Even if most 'live-cell' applications can capture GPCR activation times in the millisecond range [1,4] GPCR activation dynamics may also occur within the microsecond range and beyond [1,2,5]. To capture fast components of GPCR activation fluorescence fluctuation methods such as Fluorescence Correlation Spectroscopy (FCS) are an excellent way to proceed as FCS operates over a wide range of time scales that span the microsecond to the upper millisecond range. Here, we demonstrate live-cell FCS experiments in a home-built setup to investigate GPCR dynamics. Our results reveal that the receptors show two distinct diffusion times, while the fast component would be hardly detectable by commonly used imaging techniques. Qualitatively, the finding seems to be robust as observed in three different fluorescent β_2-adrenergic receptor constructs produced with various labeling positions and strategies. We believe, such fast dynamics are crucial to understand GPCR activation dynamics. Quantitatively, we detect a significant mobility bias dependent on the labels' freedom of movement. These findings give valuable insights for the development and choice of suitable fluorescent GPCR constructs and may help to make fast single molecule fluorescent approaches even more reliable.

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