DECIPHERING FAST DIFFUSION DYNAMICS OF β_2-ADRENERGIC RECEPTORS WITH TIME RESOLVED FLUORESCENCE

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G-protein coupled receptors (GPCRs) are the largest superfamily of membrane proteins targeted by pharmaceutical drugs as they act as gatekeepers in the signal transduction of various neurotransmitters in our body, hence making their function an important aspect to understand. Many structural and biophysical studies have been carried out so far, but it has been challenging to study basal and activation dynamics of GPCRs in live cells, particularly across a large time regime. In our study, we uncover fast rotational and translational diffusion constants that β_2-Adrenergic Receptor (β_2-AR) on live cells possess from nanoseconds to seconds using advanced time-resolved fluorescence techniques including Fluorescence Correlation Spectroscopy, Time Resolved Anisotropy and polarisation resolved FCS with full correlation from picoseconds to seconds. In the case of translational diffusion constants, we show the presence of two constants, a slow diffusion constant (~0.1 $\mu m^2 s^{-1}$) stemming from receptors on the plasma membrane and a fast one (~10 $\mu m^2 s^{-1}$) originating from membrane bound receptors in close proximity to the plasma membrane. With the rotational correlation times, we observe a fast component (~50 ns) as reported before [1] and a slower one in the µs range which turns out to be the missing piece proving that β_2-AR fits the Saffman-Delbrück model. Ligands effect these dynamics, however our data do not show any evidence for cluster formation upon ligand binding. Our findings suggest that probing a wide time regime of nanoseconds to seconds is essential to eventually get a complete picture of GPCR dynamics in the future.

References:

1. Spille, J. H., A. Zürn, C. Hoffmann, M. J. Lohse, and G. S. Harms. Rotational diffusion of the alpha(2a) adrenergic receptor revealed by FlAsH labeling in living cells. Biophys J 100(4):1139-1148 (2011).