QUANTIFYING DIFFUSION DYNAMICS OF β_2-ADRENERGIC RECEPTOR USING TIME RESOLVED FLUORESCENCE SPECTROSCOPY

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Membrane proteins act as gatekeepers and transducers to a cell, converting chemical signals from ligands to downstream effects. The majority of receptors belong to the superfamily of G-protein-coupled receptors (GPCRs), responsible for mediating complex cell responses to e.g. hormones and neurotransmitters. Thus, they are prominent drug targets, in turn making it important to understand how they function. A major focus in the study of GPCR function is the receptor´s activation by ligands. A plethora of structural and biophysical studies has been performed to understand GPCR activation dynamics [1]. Particularly, in the case of adrenergic receptors, recent studies have hypothesised that activation involves multiple intermediate steps occurring on millisecond and microsecond timescales [2, 3]. Time-resolved fluorescence spectroscopic methods such as Fluorescence Correlation Spectroscopy (FCS) and time-resolved anisotropy are useful in resolving such fast dynamics. In this work, we are using FCS and time-resolved anisotropy to uncover fast dynamics of β_2-Adrenergic Receptors (β_2-AR) in live cells. We achieve this by conjugating β_2-AR to different fluorescent tags such as fluorescent protein (EGFP) and organic dyes (conjugated using SNAP tag and unnatural amino acid (UAA) chemistry). These tags vary in their size and photophysical properties, thereby helping us differentiate the dynamics of each fluorophore in a live cell from the dynamics originating from the receptor itself. Our results show the presence of at least two different translational diffusion components and one rotational diffusion component: A fast translational diffusion component originating from β_2-AR diffusing in the cytosol and a slow one stemming from the β_2-AR on the plasma membrane. Meanwhile, the observed rotational diffusion coefficient is in line with that observed in α_{2A}-AR but unlike e.g. rhodopsin [4]. Our aim now is to further investigate micro- and nanosecond dynamics using continuous wave (cw)-FCS and perform measurements in the presence of agonists and antagonists, in essence gaining knowledge about dynamic effects imparted by different fluorophores and the fast dynamics of the protein in tandem.

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