ONE MAN'S NOISE IS ANOTHER'S SIGNAL – QUANTIFYING GPCR DIFFUSION DYNAMICS USING FLUORESCENCE CORRELATION SPECTROSCOPY

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As G protein-coupled receptors (GPCRs) are among the most important drug targets, great efforts are being undertaken to understand their activation dynamics. Such activation dynamics have been indicated to be more complex than a simple 'on/off' switching [1,2]. Several methods suited for live-cell experiments cover the millisecond time scale and have been applied to understand the dynamics of GPCRs in this regime [1,3]. Faster dynamics in the microsecond or nanosecond range are thought to be highly relevant [1,2,4], however, less readily accessible. Thus, we aim to understand such fast dynamics of β_2 adrenergic receptors in live cells using Fluorescence Correlation Spectroscopy (FCS)-based approaches complemented by time-resolved anisotropy measurements. Our FCS experiments revealed two translational diffusion times in the lower and upper millisecond timescales, respectively. This observation is in line with recent single-particle tracking data that revealed the coexistence of different motility states in GPCRs ranging from trapping to superdiffusive motion [5]. Time-resolved anisotropy data however gave a single, rapid rotational diffusion coefficient. Like the FCS results, the anisotropy-derived rotational diffusion constant was comparable for three different GPCR constructs including a bifunctional conjugation of the GFP label. Our results present robust, unexpectedly fast GPCR dynamics that are not explained by the label's freedom of movement alone and underline the complexity of GPCR dynamics.

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