

# UNRAVELING THE INTRAMOLECULAR DYNAMICS OF $\beta_2$ -ADRENERGIC RECEPTORS BY FRET-FCCS

Susobhan Choudhury<sup>1</sup>, Mike Friedrich<sup>1</sup>, Ashwin Balakrishnan<sup>1</sup>, Martin J. Lohse<sup>2</sup> and Katrin G. Heinze<sup>1</sup>

<sup>1</sup>Rudolf Virchow Center, Research Center for Experimental Biomedicine, University of Würzburg, Josef-Schneider-Str 2, 97080 Würzburg, Germany

<sup>2</sup>Max Delbrück Center for Molecular Medicine, Robert-Rössle-Str 10, 13125 Berlin, Germany

E-mail: susobhan.choudhury@uni-wuerzburg.de, katrin.heinze@uni-wuerzburg.de

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G-protein-coupled receptor (GPCR) mediated signal transduction is central to human physiology and disease intervention, yet the molecular mechanisms responsible for ligand-dependent signalling responses remain poorly understood. Understanding the molecular bases of their activation requires the characterization of the dynamic equilibrium between active and inactive states. Although inactive and active structures of GPCRs have been solved during the past few years, recent biophysical studies of various laboratories suggest that GPCRs operate beyond a two-state ‘on’-‘off’ model including several rapid (< ms) activation steps that together constitute the activation dynamics of GPCRs, and that ligands can influence these dynamics in distinct ways [1, 2]. In this juncture, a combined FRET-FCCS (Fluorescence Resonance Energy Transfer and Fluorescence Cross Correlation Spectroscopy) approach has been demonstrated to provide crucial information regarding such GPCR conformational fluctuations [3], however the studies so far are lacking live cell experiments. In the present study live cell FRET-FCCS was performed to access intramolecular dynamics of GPCRs. We generated two  $\beta_2$ -Adrenergic Receptor ( $\beta_2$ -AR) constructs, each conjugated to an EGFP in its intracellular loop 3 and a SNAP tag in its N-terminal and C-terminal, respectively. We labelled the SNAP tag with TMR-STAR probe (excitation: 555 nm, emission: 580 nm) to obtain the auto- and cross-correlation curves which reveal similar diffusion coefficient and colocalization of the two probes in the inactive state of  $\beta_2$ -AR. In addition, we observed FRET only in case of C-terminal construct while N-terminal SNAP tag does not exhibit FRET as expected. Several GPCRS-ligand interactions will help to further elucidate the dynamics and function of intermediate GPCR states and will help to quantify receptor activation in the transmembrane domain.

## References:

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