

## Single Molecule Analysis of GPCR Dimer Formation in Living Cells

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The group of GPCRs with more than 800 members is one of the largest gene families and its members are key controllers for many different signaling systems in virtually every eukaryotic tissue (~ 150 different GPCR types in every cell). GPCRs connect extracellular stimuli with intracellular responses which in turn regulate various processes ranging from homeostasis over inflammatory and immune responses to neuronal communication. An aspect in GPCR signaling that is poorly understood is the occurrence and the biological meaning of changes of protomer assembly upon receptor stimulation. Whereas members of the GPCR subfamily C form obligatory oligomers, the situation for the majority of GPCRs remains under debate. In particular for class A (~ 90% of all GPCRs) it has been frequently postulated that they exist in a dynamic equilibrium between mono- and oligomers which may be shifted upon ligand treatment.

Many experimental techniques that aim at detecting protein interactions require stable association of the proteins and therefore are not able to effectively and quantitatively resolve transient interactions. Beyond that, high expression levels might introduce artifacts in proximity-based assays like FRET or cross-linking. We have overcome this obstacle by directly imaging fluorescence labeled GPCRs in real-time at the single molecule level with a high signal-to-noise ratio. Currently we apply our approach to study oligomerization events of clinically relevant class A members.