Summary

G protein-coupled receptors (GPCRs) are a versatile family of eukaryotic membrane spanning proteins that initiate cellular signal transduction. Usually, external ligands of various types are binding to a GPCR and activating it. Thereby, the receptor can integrate external stimuli in a ligand-dependent manner such that multiple signal transduction pathways are activated with different efficacies. This phenomenon called ligand biased signaling is known for many receptors of the GPCR family. It remains to be clarified to what extend the receptor contributes to signaling bias, and which role the signaling network plays in its propagation and modification. As GPCRs are targets of many established drugs, the knowledge of biased signaling is promising to lead to the development of improved drugs.

In this study, I investigated the onset and propagation of biased signaling with theoretical and experimental approaches. A mathematical model was established based on the recent understanding that ligand binding stabilizes distinct receptor conformations and thereby introduces bias in signaling. The model suggests that ligand bias can occur due to conformation-dependent ligand binding efficacies and that modifiers acting on allosteric binding sites additionally influence the ratio of these receptor conformations. Further experimental investigations of the onset of ligand bias were conducted with a cell line stably expressing the human external calcium sensing receptor (CaSR), that is known for its multifarious ligand binding and signaling. Using a FRET sensor of the intracellular G protein $G_q$, we observed its direct activation upon CaSR stimulation with various ligands, a read-out at the very beginning of the signaling cascade. In our real-time measurements we found several ligands initiating $G_q$ activity that closely resembles downstream signaling read-outs that were described in literature about CaSR biased signaling. However, some ligands did not show bias in our assays in contradiction to published results of downstream read-outs. This indicates a modifying role of the signaling network downstream of the receptor. We conducted simultaneous measurements of $G_q$ activity and intracellular calcium release in order to assess the dynamic co-activation of these two signaling events. In these results, the $G_q$ activity and intracellular calcium mobilization show similar trends, but the precise co-variation of the signaling events depends on the ligand combinations used. These observations indicate that biased signaling results both from the receptor level and the downstream signaling network.

In summary, in this work advanced experimental tools and theoretical considerations were combined to investigate the onset and propagation of ligand biased signaling. This is a first step towards a deeper, network-level understanding of signal bias which might enable the targeted design and application of biasing drugs in future.