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Reprogramming the Conformational Regulation of GPCR Signaling

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Signaling across biological membranes is critical to living cells and involves membrane-embedded receptors that transduce extracellular stimuli into cytoplasmic responses. G-protein coupled receptors (GPCRs) are one of the largest families of these receptors that serve this function and do so through long-range allosteric changes. Despite extensive genomic and functional studies on GPCRs, it was only in the past couple of years that new structural data have made accurately evaluating mechanistic and structural changes possible at the atomic level. My project is part of a general effort to uncover the sequence, structure, and energetic determinants governing GPCR signaling. I hypothesize that GPCR signaling is driven by conformational changes that allow them to toggle between functional states; computational designs that stabilize receptors in different states will allow us to reprogram their signaling properties and provide an atomic-level understanding of the underlying conformational regulation.

To address the relationship between protein stability and conformational plasticity in allosteric receptors, we have combined sequence bioinformatics techniques with the design mode of RosettaMembrane to probe the sequence space governing GPCR conformational stability. Sites predicted to be suboptimal for the stability of the receptor are targeted for design, and mutations that stabilize the receptor's inactive conformation are selected. The mutants are characterized via radioligand binding assays to measure apparent thermal stability. Our initial designs of the beta-1-adrenergic receptor (B1AR) have yielded mutants with increased apparent thermostability in comparison to a B1AR variant (M23) used in solving the B1AR structure (Warne et al. 2008). This method can be applied to receptors with unknown structure to facilitate structural determination. Additionally we are working to modulate and switch the functional states of GPCRs. Using multi-state design with RosettaMembrane, we have been able to design mutants of rhodopsin as well as dopamine D2 receptor (D2DR) with increased constitutive activity over their respective wild types. Furthermore, we are able to design mutants of D2DR that display increased active-state stability. With over eight hundred GPCRs in the human genome, many of which have been implicated in disease studies, it is apparent that there is a critical need for atomic-resolution structural and mechanistic understanding of their signaling. The bridging of computational molecular biophysics and molecular biology in the GPCR research field is rapidly developing and will contribute to the basic scientific knowledge of receptor signaling as well as provide a means to develop better treatment for a multitude of diseases.

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