Engineering Heterotrimeric G Proteins to Facilitate Crystallisation of GPCRs in their Active Conformation

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G protein-coupled receptors (GPCRs) are integral membrane proteins that regulate cytoplasmic signalling, through heterotrimeric G proteins and β-arrestins. Structure determination of GPCRs in all activation states is vital to elucidate the precise mechanism of signal transduction, and to facilitate optimal drug design. However, due to their inherent instability, crystallisation of GPCR–G protein complexes has proved particularly challenging.

We used rational design mutagenesis to develop a minimal G protein, mini-G_s, which is composed of a single domain from the adenylate cyclase stimulating G protein (G_s). Mini-G_s induces similar pharmacological and structural changes in GPCRs as the heterotrimeric G protein, but eliminates many of the problems associated with crystallisation of these complexes, specifically their large size, conformational dynamics and instability in detergent.

We have determined the structure of the wild type human adenosine A_{2A} receptor ($A_{2A}R$) bound to mini-G_s at 3.4 Å resolution by X-ray crystallography. The structure revealed large, mutually induced conformational changes in both the receptor and G protein, and has provided unique insight into the mechanism of GPCR activation.