Mechanistic insights into G protein association with a G protein-coupled receptor

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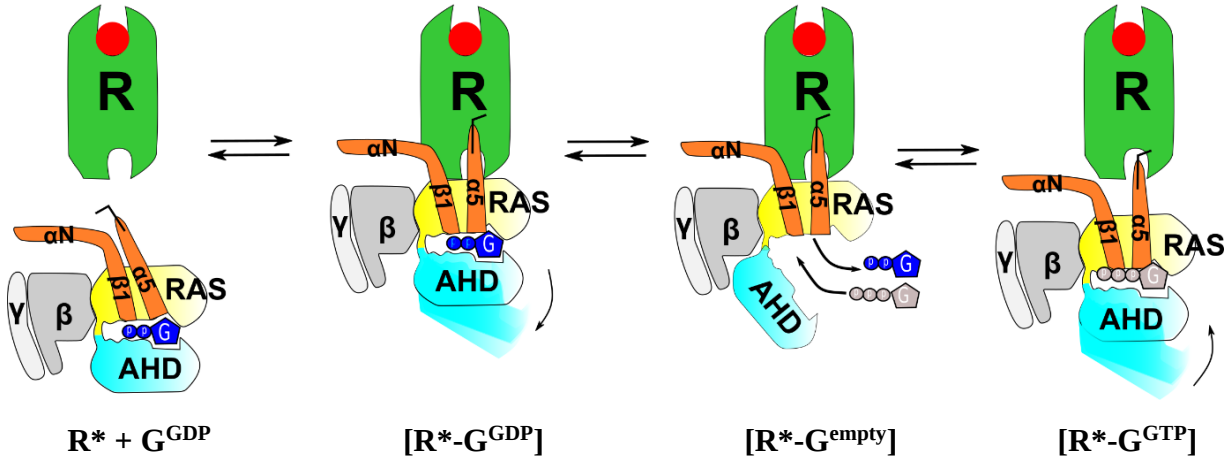

Heterotrimeric G proteins are activated by G protein-coupled receptors (GPCRs) mediating the exchange of guanine nucleotide in the Gα subunit. Using molecular dynamics (MD) simulations, we investigate atomistic details of the process of G protein association with a GPCR, describing the events that ultimately lead to ejection of GDP from its binding pocket in the Galpha subunit and formation of a nucleotide free R-G^{empty} complex. We simulated the first steps of this reaction sequence [1] for the β2AR-Gs signaling system, for which there is a wealth of biochemical, biophysical and structural data that can support further interpretation [2-4]. In classical all-atom µ-second (MD) simulations we observe association of membrane anchored Gs^{GDP} to the membrane embedded, agonist bound, β2-adrenoceptor (β2AR). We identify an extended binding interface of the receptor with the G protein compared to β2AR-Gs^{empty}, confirmed by site-directed mutagenesis and functional assays. Moreover, we observe major conformational changes at the nucleotide-binding pocket, significantly reducing the energy needed for GDP release. Our analysis sheds new light on the initial steps of receptor-mediated G protein activation and extends the limited view of nucleotide-free snapshots to include additional states and structural features responsible for signaling and G protein coupling specificity.

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