Essential dynamics to understand the transitional pathways of GPCRs in different lipid environments

Our structural understanding of GPCR activation has increased over the last few years owing to the increased number of crystallographic structures of receptors in a series of conformational states. However, the environment in which the receptors have been crystallised does not reflect the contribution the lipid environment has on transitional pathways between active and inactive states. Membrane lipids are not passive bystanders and exhibit a number of properties such as hydrophobic thickness, lateral pressure and membrane curvature profiles that have recently been shown experimentally to have a regulatory role on GPCR activation (1). Furthermore, despite the wealth of receptor structures the transitional pathway between active and inactive states is poorly understood even in the absence of the lipid environment.

To address this we have used molecular dynamics simulations of the well studied β_2AR. Inactive and active β_2AR structures were inserted into pre-equilibrated DOPC, DOPE and DOPG bilayers. Each of the 6 systems was subsequently simulated for 200ns and essential dynamics was applied to obtain insights into the inactive to active transition for each of the 3 lipid environments.

Visual inspection of the transitional pathway between inactive and active states for each of the 3 lipid environments revealed a similar pattern of backbone and side chain movements however the degree of the conformational change as measured by root mean square deviation to the active structure was DOPE > DOPC > DOPG with the latter simulation giving the smallest RMSD compared to the crystal structure of the β_2AR active state. In addition, we have also used the essential dynamics simulations as a series of conformational snapshots for umbrella sampling to quantify the free energy difference between the active and inactive states for the 3 lipid environments. The transitional pathway for each lipid group was separated into 40 evenly spaced snapshots from the complete transition. Each snapshot was subsequently simulated for 50ns (totalling 120 simulations for this study). Interestingly, the order of the free energy changes were consistent with the order of activation seen experimentally for the β_2AR with 3.32 ± 1.2 kcal/mol, 26.1 ± 2.9 kcal/mol and 37 ± 3.2 kcal/mol for the inactive to active transitions in the DOPG, DOPC and DOPE lipid environments, respectively.

We are currently using *in silico* mutagenesis and density profiles in order to determine if there are any “binding” sites for lipids on the external face of GPCRs.

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(1) Dawaliby R *et al* (2016). *Nat. Chem. Biol* **12**:35–39.