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(G*) Tracking Diffusion and Oligomerization of M2 Receptors in Live Cells

Tuesday, 7 June 2022 12:00 (15 minutes)

Important insights about the signaling mechanisms of G Protein Coupled Receptors (GPCRs) can be learned from their supramolecular assembly. Recent studies in our lab have shown that the M2 muscarinic receptor (M2R), as well as its cognate G protein (Gi), can be purified as oligomers, yet the size and the dynamics of these oligomers, as well as their function are not fully understood in vivo. We used single-molecule fluorescence techniques, such as single-particle tracking (SPT) and single-molecule photobleaching (smPB) to identify the oligomers of M2R in live HEK293 cells. The receptors have been expressed with a HaloTag at their extracellular interface, allowing for labelling with fluorophores with HaloTag ligand (HTL), such as JF549 HTL. The movement of M2 receptors in the cell membrane is spatially and temporally heterogeneous, transitioning between normal and anomalous diffusion regimes. As controls, SPT measurements were performed on pure monomeric (CD86) and dimeric (CD28) membrane proteins. Intensity traces of immobile, single receptor complexes in the membrane of fixed cells was analyzed using in-house smPB code based on change-point and Bayesian algorithms. The results show a distribution of multiple stepwise decreases and indicate that the M2R mediated signaling proceeds, at least in part, via oligomers of receptors and G proteins.

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