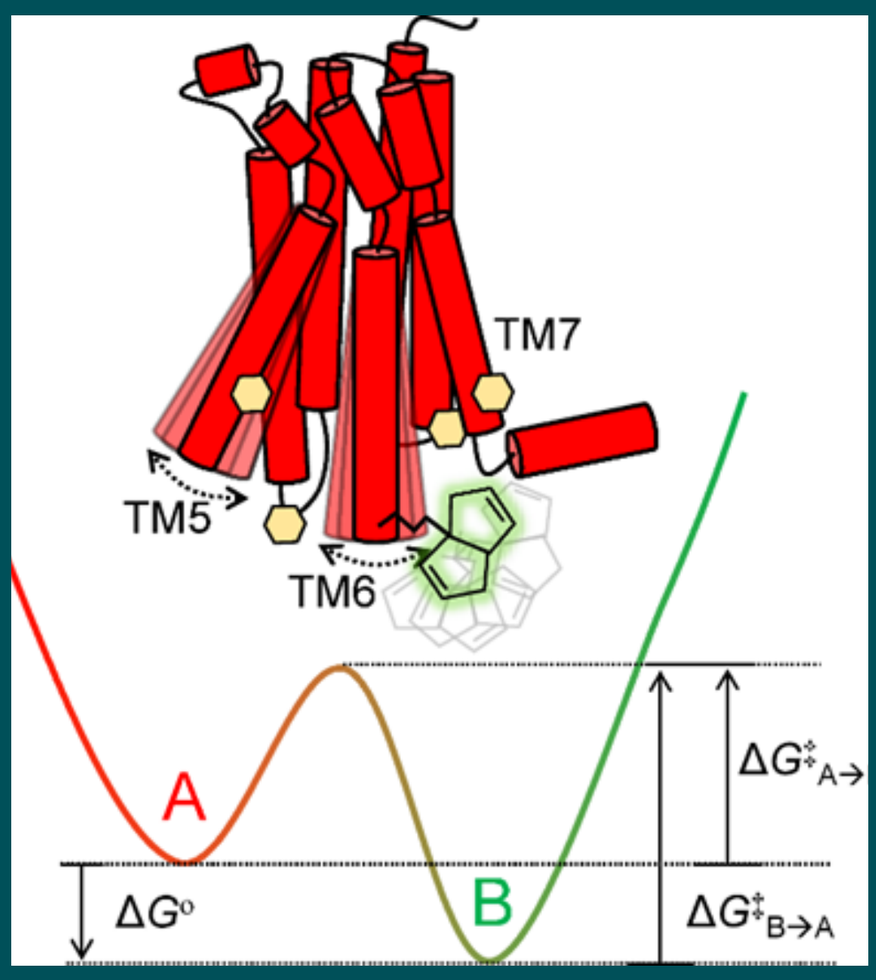
Chemical & Physical Sciences
UNIVERSITY OF TORONTO
MISSISSAUGA

COLLOQUIUM SEMINAR SERIES

MAPPING THE CONFORMATIONAL LANDSCAPE & SPATIAL ORGANIZATION OF G PROTEIN-COUPLED RECEPTORS USING SINGLE-MOLECULE FLUORESCENCE



G protein-coupled receptors (GPCRs) are the largest class of transmembrane proteins, making them an important target for therapeutics. Several aspects of GPCR signalling pathways, however, still remains elusive. In this thesis, two key aspects are addressed: the role ligands play in shaping the conformational landscape of GPCRs, and the oligomeric size of GPCRs during different stages of signalling. Here, a combination of ensemble and single-molecule fluorescence techniques were used to investigate the above, whereby the A2A adenosine receptor (A2AR), and the M2 muscarinic receptor (M2R) were used as model systems. Using single-molecule Förster Resonance Energy Transfer (smFRET), it was shown that A2AR exhibits a high degree of basal activity, and that the relative populations of inactive and active states is modulated upon binding of ligand. Furthermore, photoinduced electron transfer, monitored via Fluorescence Correlation Spectroscopy (PET-FCS), showed that ligands also effect the dynamics within and between several conformational states of A2AR. This work provides a quantitative approach to study how ligands modulate the conformational landscape of GPCRs. The oligomeric size of detergent-purified M2R and its attendant G protein (Gi1) were determined using single-molecule Photobleaching (smPB) analysis via a total internal reflection fluorescence (TIRF) microscope. The smPB analysis revealed that both M2R and Gi1 were oligomeric in nature prior to coupling, predominantly consisting of tetramers for M2R and hexamers for Gi1, regardless of ligand. In the coupled state (RG), it was found that a tetrameric M2R couples to tetrameric Gi1, whereby the binding of nucleotide (guanosine triphosphate) causes the disassembly of the RG complex, and a reduction in the oligomeric size of Gi1 from tetramers to dimers. The binding of an inverse antagonist also results in disassembly of the RG complex, and a reduction in the oligomeric size of M2R from tetramers to monomers or dimers. The work suggests that active M2R is tetrameric and solely capable of binding to tetrameric forms of Gi1, whereas the active form of Gi1 is dimeric and only activatable when coupled to M2R.

CPS AWARDS COLLOQUIUM

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Join us on Zoom at 3:10pm

https://utoronto.zoom.us/j/88646928603

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