Contribution ID : **120** Type : **Oral Presentation**

# Decoding GPCR Activation: Minute-Scale Simulations Reveal a Novel Pseudo-Active State of the Adenosine A2A Receptor

G protein-coupled receptors (GPCRs) represent membrane proteins of significant pharmacological importance, being the target of more than one-third of commercially available drugs. 1-5 These receptors are activated by orthosteric ligands, undergoing substantial conformational changes that facilitate the coupling with diverse effector proteins.6-7 To achieve precise control over the pharmacological response of drugs, it is crucial to illuminate the poorly understood aspects of gpcr activation. In this study, we elucidate the complete activation mechanism of the adenosine A2A receptor (A2AR), a class A GPCR, utilizing minute timescale molecular dynamics and free energy calculations. We thoroughly explore the conformational landscape of A2AR in its basal apo form and under various ligated conditions, revealing insights into ligand intrinsic activity and the receptor's lowest energy functional states. Notably, we identify a novel pseudo-active state (pAs) of the A2AR apo form stabilized by specific interactions involving "microswitch" residues, including the salt bridge between the class A conserved residues R5.66 and E6.30. In this "pAs" state, A2AR can couple to the Gs protein upon rearrangement of the intracellular end of TM6, providing unique structural insights into receptor functioning. This discovery paves the way for future signaling studies and offers a promising framework for drug design strategies aimed at developing A2AR-biased ligands. Our simulation protocol is generalizable and can be applied to study the activation mechanism of any GPCRs and to predict the intrinsic activity of ligands based on their effect on the receptor conformational dynamics, resulting in a valuable tool for investigations on GPCR activation and drug design.

**Primary author(s) :** D'AMORE, VINCENZO MARIA (Dipartimento di Farmacia Università di Napoli Federico II); Dr. CONFLITTI, PAOLO (Università della Svizzera Italiana); MARINELLI, LUCIANA; Prof. LIMONGELLI, VITTORIO (Università della Svizzera Italiana)

**Presenter(s) :** D'AMORE, VINCENZO MARIA (Dipartimento di Farmacia Università di Napoli Federico II)