Researchers elucidate mechanisms behind protein selectivity in adenosine receptor

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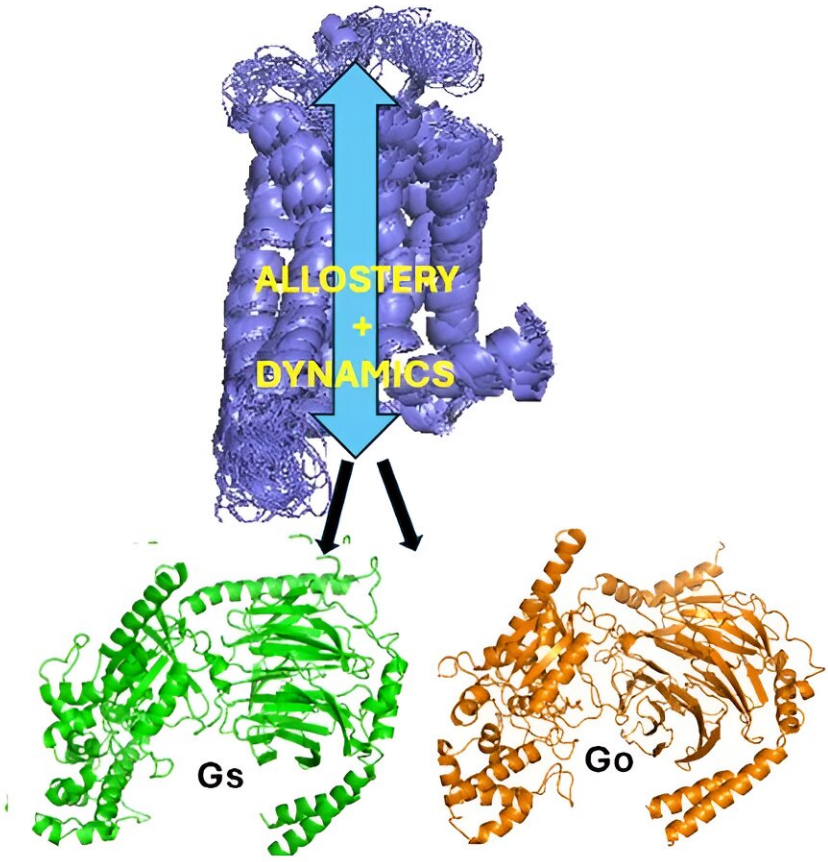


GPCR dynamics, shown in purple as the human A_{2A} receptor, and elegant modifications in activation pathways (allostery) indicated by the blue arrow, are critical for enabling GPCRs to bind to multiple G-proteins, shown in green and orange. Credit: *Nature Chemical Biology* (2024). DOI: 10.1038/s41589-024-01682-6

In a new study, a multinational research team led by Dr. Adnan Sljoka (RIKEN) and Prof. Akio Kitao (Tokyo Tech), in collaboration with Prof. Scott Prosser (University of Toronto), has carried out experimental and computational studies to elucidate the mechanisms behind G protein selectivity and efficacy in the human adenosine A_{2A} receptor ($A_{2A}R$).

$A_{2A}R$ is a member of major drug targets G protein-coupled receptor (GPCR) superfamily, which engages the G protein and initiates cell signaling, influencing heart health, inflammation, cancer, and brain diseases.

Scientists have made a breakthrough in understanding how $A_{2A}R$ can engage and activate multiple binding G-proteins and the mechanisms of this selective coupling. The work is published in *Nature Chemical Biology*.

The research team discovered that the hallmark coupling promiscuity in $A_{2A}R$ is a direct consequence of changes in activation conformations. Moreover, the long-range (allosteric) communication mechanisms elegantly control the sampling of specific conformers within a dynamic conformational ensemble.

This study offers profound insights into GPCRs selectivity and biased signaling. These findings are expected to have major implications in drug discovery and pave the way for novel GPCR-targeted therapeutic strategies in treating various human conditions, including cancer and neurogenerative disorders.

This research will also enable the design of more generalized computational and AI-driven studies, pushing the boundaries in GPCR activation mechanisms and next-generation pharmacology.

Background

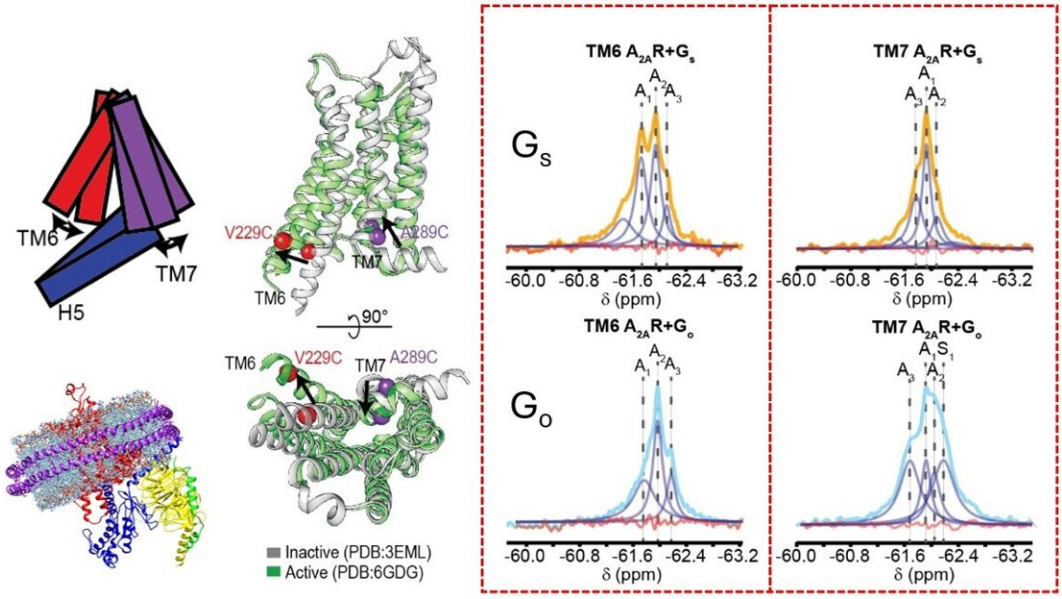
GPCRs are the largest receptor class, affecting almost every aspect of human physiology, with 35% of all approved drugs acting on GPCRs. They regulate sensory and neuronal signaling, as well as a myriad of processes associated with cell homeostasis, growth, and immune response.

GPCRs are primarily situated in the plasma membrane surrounding the cell, while the drug or ligand (such as hormones and neurotransmitters) that acts on the GPCR binds to an extracellular pocket. Activation is then communicated across the receptor, resulting in complexation with proteins on the cell interior.

Since the signal arrives at the cell exterior and initiates signaling pathways within the cell, this makes GPCRs particularly useful in drug discovery, as the drug in many cases need not enter the cell.

However, GPCRs activation is related to dynamic events, key intermediate states, and activation states that arise between the time a ligand binds and when the G protein is activated. Furthermore, many GPCRs are promiscuous as they selectively interact with different G proteins, each influencing a unique cellular response. In fact, G protein selectivity is one of the least understood aspects of GPCR biology.

Capturing the conformational dynamics of GPCRs, describing various functional states, and understanding allosteric mechanisms and their role in G protein selectivity, coupling promiscuity, activation and signaling mechanisms is a formidable challenge, making it difficult to predict or control GPCR behavior in drug development.



^{19}F-NMR spectra of $A_{2A}R$ (red box) identifies two activation states (orange peaks on left) used to couple to cognate (G_s) and one state (blue peak on left) to non-cognate (G_o) G proteins (TM6) while TM7 labeling reveals dynamic differences (right peaks) between G_s and G_o bound $A_{2A}R$. Dual TM6 activation states enable greater diversity of coupling partners while TM7 dynamics dictates coupling efficacy. Credit: *Nature Chemical Biology* (2024). DOI: 10.1038/s41589-024-01682-6

Overview of the research

Using experimental and computational techniques, including functional assays, Fluorine-nuclear magnetic resonance (^{19}F-NMR), mathematical rigidity theory, Molecular Dynamics Simulations and rigidity and geometry Monte Carlo simulations, the international research team discovered the mechanism behind GPCR-G protein selectivity.

The team focused their study on the human adenosine A_{2A} receptor ($A_{2A}R$). $A_{2A}R$ is a prototypical GPCR distributed in the nervous system, platelets, immune cells, lungs, heart, and vasculature, engaging several G proteins (notably, G_o) in addition to its cognate G_s protein.

$A_{2A}R$ drugs have been developed to address wound healing, vascular diseases, including atherosclerosis, restenosis, and platelet activation, as well as inflammation and cancer. However, pharmacological regimens are thought to act exclusively as antagonists or agonists to the $A_{2A}R$-G_s complex.

Thus, understanding the mechanism of G protein selectivity and efficacy in $A_{2A}R$, and the general bias and activation mechanisms in GPCRs, can yield new opportunities in pharmacology.

The researchers focused on studying key conformational states and dynamics of $A_{2A}R$ by complexing it with both cognate G_s and non-cognate G_o G-proteins with the same agonist ligand. ^{19}F NMR revealed several functional activation states of $A_{2A}R$ when it is coupled to G_s and G_o G-proteins.

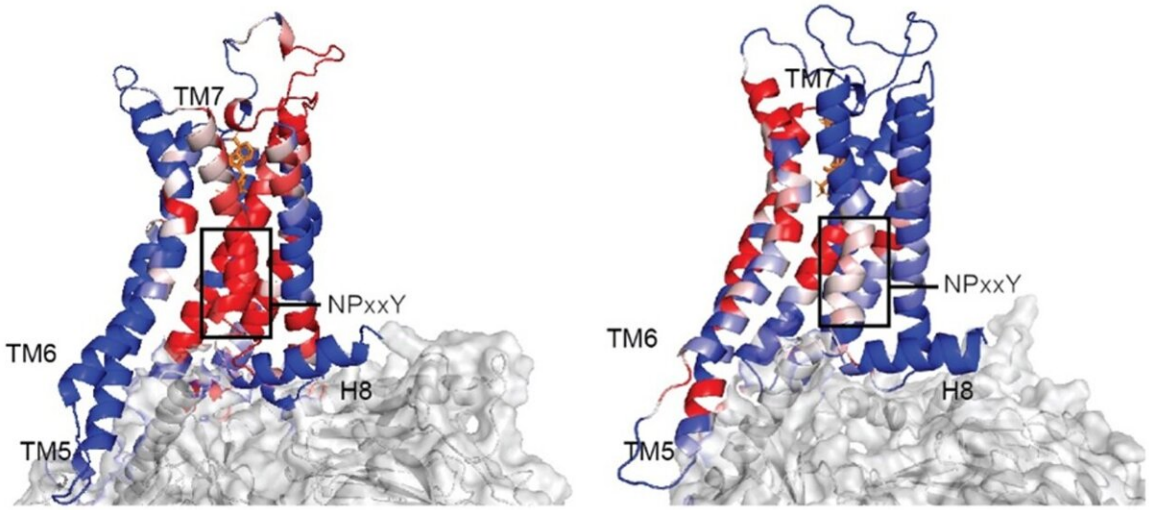
When $A_{2A}R$ is engages with its preferred G_s partner, the receptor adapts long-lived and highly populated activation states. However, when coupled to G_o, these activation conformational states are significantly less engaged and populated. In fact, one of the activation states was mainly observed when the receptor was engaged with G_s but not G_o.

Molecular dynamics simulations and dPaCS-MD/MSM calculation, performed at Dr. Kitao lab, indicate that G_s forms more interactions with $A_{2A}R$ and has stronger binding affinity compared to G_o. Additional Monte Carlo simulations performed by Dr. Tucs confirmed when $A_{2A}R$ engages G_s or G_o G-proteins, there are major changes in the receptor's dynamics, which dictate the populations and interconversions between

activation states identified through NMR. This led to the hypothesis that allostery (long range communication) might be at play.

To probe allostery in the receptor, the researchers utilized rigidity theory techniques developed by Dr. Sljoka. Rigidity theory analysis validated the presence of multiple adaptive allosteric networks which were distinct when the receptor engages with G_s and G_o. The allosteric mechanisms directly control the dynamic differences and transitions between distinct functional states, playing a major role in G protein selectivity and G-protein-receptor coupling.

The ability of GPCRs to connect with multiple proteins through distinct activation states could lead to better treatments, highlighting the complex yet fascinating ways our body's cells communicate and respond to signals. The findings in this study offer important insights into GPCRs selectivity, allostery, partial agonism and biased signaling, with major implications in drug discovery.



Computational analysis based on modeling and mathematical rigidity theory identifies activation (allosteric) network pathway (red) propagating through the $A_{2A}R$ GPCR and the differences in the pathways in the $A_{2A}R$-G_s (left) and $A_{2A}R$-G_o (right) complexes. These pathways play a major role in the control of GPRC activation, the control in activation states, and G protein selectivity. Credit: *Nature Chemical Biology* (2024). DOI: 10.1038/s41589-024-01682-6

Future developments

While the current study provides an unprecedented mechanistic understanding of coupling and promiscuity in $A_{2A}R$, future studies will no doubt focus on trying to generalize to other GPCRs and incorporation of advanced AI models.

These efforts have significant implications for designing safer and more selective therapeutics targeting GPCRs and will deepen our overall understanding of cellular signaling mechanisms.

More information: Louis-Philippe Picard et al, Balancing G protein selectivity and efficacy in the adenosine A2A receptor, *Nature Chemical Biology* (2024). DOI: 10.1038/s41589-024-01682-6

Provided by Tokyo Institute of Technology

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