Allosteric GPCR Pharmacology

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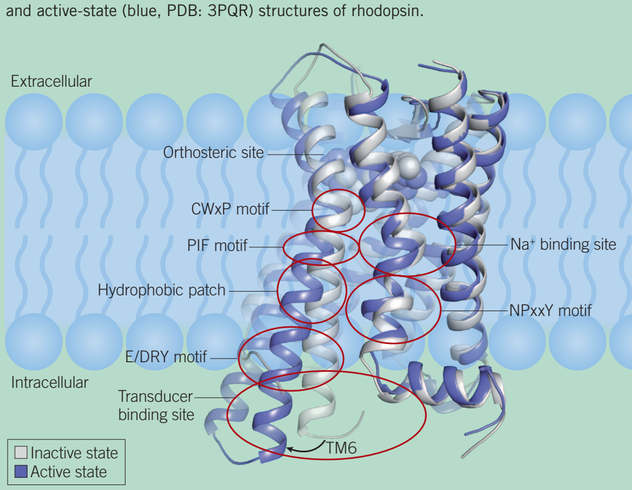
G protein-coupled receptors (GPCRs) are intrinsically allosteric proteins. They have evolved to transduce signals from one part of the protein to another through spatially distinct, but conformationally linked, binding sites. Endogenous activators bind to the cognate 'orthosteric' site of a GPCR, causing a conformational change in the GPCR that allows it to interact with intracellular transducers such as heterotrimeric G proteins. This is termed the 'allosteric transition'. This poster highlights some of the key insights into allosteric mechanisms of GPCR biology.

The Allosteric Nature of GPCRs

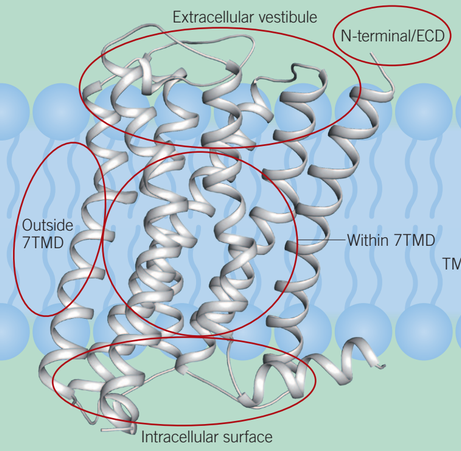
Microswitches Governing The Allosteric Transition (GPCR Activation)

The allosteric transition is governed by a set of relatively conserved conformational contractions in the extracellular domains, regulated by conserved microswitches and water molecules. These include a large outward movement of TM6 and a smaller inward movement of TM5 and TM7, governed by a series of conserved intermolecular interactions or 'microswitches', between orthosteric and transducer binding sites. Key microswitches in class A GPCRs include the CWxP motif, the PIF motif, an allosteric sodium-binding site, the NPxxY motif, and the E/DRY motif. The figure shows these common microswitches of the class A GPCR allosteric transition, mapped onto the inactive-state (white, PDB: 1F88) and active-state (blue, PDB: 3PQR) structures of rhodopsin.



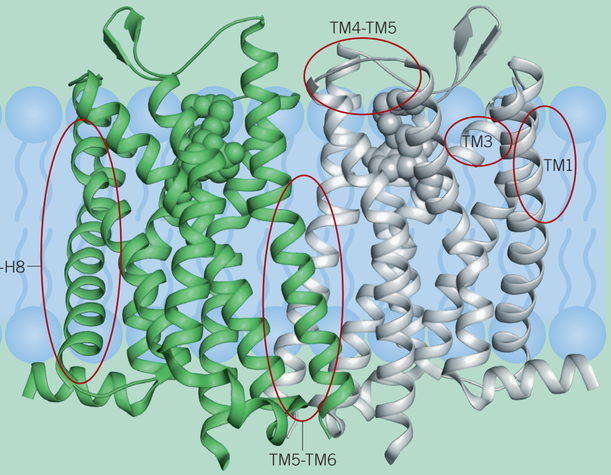
Allosteric Modulation

Indirect allosteric modulation describes the interactions between an orthosteric ligand and other conformationally-linked binding sites on the same receptor. 'Allosteric modulatory sites' can be grouped into five categories, depending on the location of the orthosteric site. The image below uses the example M_2 mAChR–iperoxo–LY2119620 structure (PDB: 4MQT).



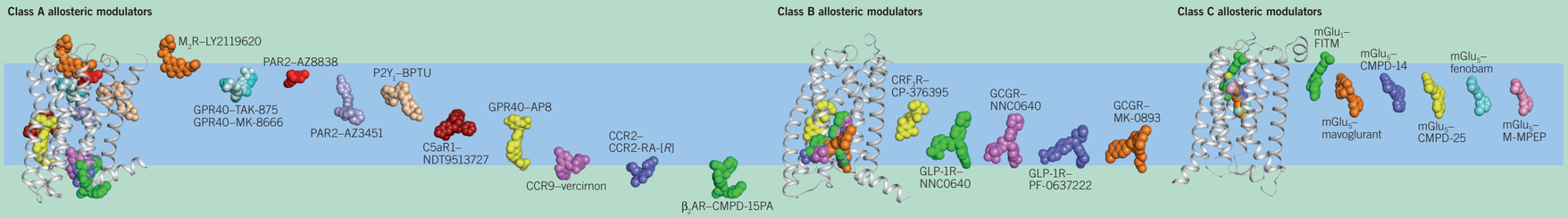
Impact of Oligomeric Architecture on Allosteric Signaling

GPCRs have been shown to oligomerize, adding an extra dimension to allosteric modulation, with studies highlighting the potential for allosteric interactions between binding sites on GPCRs within dimeric or higher-order oligomeric formations. Ovals in the diagram below, show the main interfaces that have been implicated in structural biology studies, mapped onto the dimeric structure of the μOR (PDB: 4DKL)[4].



Diversity in the Binding Sites of Synthetic Allosteric Modulators across GPCR Classes.

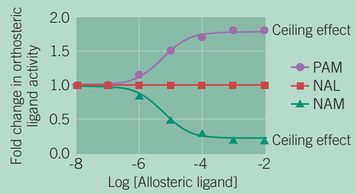
Chemical structures of allosteric modulators (colored spheres) mapped onto representative family members of class A (M_2 mAChR, PDB: 4MQT), class B (GCGR, PDB: 5EE7) and class C (mGlu5, PDB: 4OO9) GPCRs (PDB protein database; rcsb.org)[1].



Key Facets of GPCR Allostery

A. Positive allosteric modulators (PAMs), negative allosteric modulators (NAMs), and neutral allosteric ligands (NALs): Allosteric modulators are characterized through three different modes of behavior: PAMs NAMs or NAL[2]. Increasing concentrations of different allosteric modulators are titrated against a single concentration of an orthosteric ligand. The 'ceiling effect' is a key feature of allosteric drugs; beyond this concentration of allosteric drug the modulatory effect is saturated.

Allosteric modulation

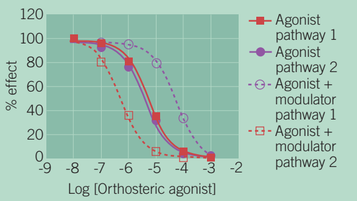


B. 'Probe dependence' is another unique pharmacological characteristic of allosteric modulators whereby the magnitude and direction of the allosteric effect can change depending on the orthosteric ligand[2].

C. Receptor subtype selectivity: Allosteric modulators can display selectivity in their ability to enhance the binding of a non-selective orthosteric ligand at one particular subtype relative to other related receptor subtypes.

D. Biased modulation: An allosteric ligand can promote more than one type of active state. This results in an agonist-modulator pair having different effects depending on the signaling pathway linked to each receptor conformation. In this example, the modulator increases the potency of an agonist for pathway 1 but decreases the potency of the agonist in pathway 2.

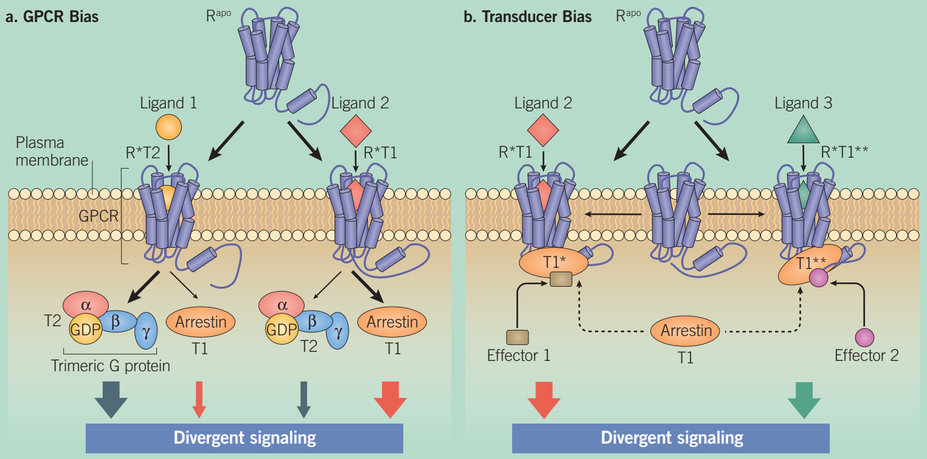
Biased modulation



Conformational Mechanisms of Biased Agonism

a. Different ligands (ligand 1 and 2) induce different conformations or active states within GPCRs (R*T1 and R*T2) promoting binding conditions for different transducer proteins such as G proteins and arrestins.

b. Two different agonists (ligands 2 and 3) promote a similar conformation or active state (R*), which binds transducer (T1), but bias arises through allosteric communication between the receptor, ligand and transducer, as the transducer undergoes conformational changes. Ligand 2 and ligand 3 stabilize two different transducer conformations or active states (T1*, T1**), which results in distinct downstream signaling events.



Therapeutic Applications of Allosteric Modulators

There are many potential advantages of allosteric modulators as therapeutic drugs in comparison to classic orthosteric ligands:

- The lower degree of amino acid conservation within allosteric sites relative to the orthosteric sites means there is greater potential for improved target selectivity, with fewer off-target effects.
- The effect of allosteric drugs is governed by the degree of cooperativity between orthosteric and allosteric sites, which permits greater control of on-target dose-related side effects. This potentially allows an allosteric medicine to be more efficacious at its GPCR target by opening up opportunities to exploit therapeutic windows that may have been too small to target with orthosteric medicines alone.
- 'Pure' PAMs or NAMs are quiescent in the absence of orthosteric endogenous agonist, exerting their effect only where and when the endogenous agonist is released. This means that tissue-specific conditions can be exploited where endogenous ligand levels in a given region are altered as a consequence of disease. For example, in a tissue with high endogenous hormone levels, a receptor will be differentially sensitive to the allosteric drug effects than the same receptor in other tissues in the body, reducing 'off-tissue' (on-target) effects.
- Allosteric ligands may cause endogenous agonists to exhibit biased signaling, providing another approach to selectively target desired pathways.

The recent expansion in structural knowledge of GPCR allosteric sites is being incorporated into drug-design studies. In addition to a number of GPCR allosteric modulators that are in clinical trials, there is increasing excitement about the potential for combining allosteric drugs with existing potent, but non-selective, orthosteric drugs to improve efficacy or to repurpose them[3,4].

ECD	extra cellular domain
GDP	guanosine diphosphate
GTP	guanosine triphosphate
NAL	neutral allosteric ligands
NAM	negative allosteric modulator
PAM	positive allosteric modulator
R^{apo}	absence of ligand or transducer
TMD	trans membrane domain

References:

1. **Burger** *et al* (2018) *J. Gen. Physiol.* **15** 1360–1372
2. **Christopoulos** *et al* (2014) *Pharmacol. Rev.* **66** 918–947
3. **May** *et al* (2007) *Ann. Rev. Pharmacol. Toxicol.* **47** 1–51
4. **Thal** *et al* (2018) *Nature* **559** 45–53

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- ML 382

$mGlu_1$ Receptors
- (*S*)-3,5-DHPG
- JNJ 16259685
- Bay 36-7620
- FTIDC, VU 0469650, FITM
- Ro 01-6128, Ro 67-7476, VU 0483605

$mGlu_2$ Receptors
- LY 379268
- LY 341495
- MNI 137
- LY 487379, CBiPES, BINA

$mGlu_3$ Receptors
- LY 379268
- LY 341495
- ML 337, LY 2389575

$mGlu_4$ Receptors
- Cinnabarinic acid
- VU 0155041, MPEP, VU 0364770
- LY 341495

$mGlu_5$ Receptors
- (*S*)-3,5-DHPG
- Fenobam
- CDPPB,VU 0360172, VU 0409551
- VU 0285683, ADX 10059, MFZ 10-7
- MPEP

$mGlu_7$ Receptor
- AMN 082
- (±)-ADX 71743, MMPIP
- LY 341495

$mGlu_8$ Receptor
- (*S*)-3,4-DCPG
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- AZ 12216052

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