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Allosteric Communication Pipelines in G-protein-coupled receptors

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Abstract

The binding of ligands to G-protein-coupled receptors (GPCRs) in the extracellular region transmits the signal to the intracellular region to initiate coupling to effector proteins. The mechanism of this allosteric communication remains largely unexplored. Knowledge of the residues involved in the pipeline of the allosteric communication from the extracellular to the intracellular region will provide means to (a) design ligands with bias in potency towards one signaling pathway over others, and (b) design allosteric modulators that show subtype selectivity in GPCRs. In this review we describe the current state of the computational methods that provide insights into the allosteric communication in GPCRs and elucidate how this information can be used to design allosteric modulators.

Keywords

GPCR; functional selectivity; allosteric communication; allosteric modulators; allosteric pipelines; allosteric binding sites

INTRODUCTION

Allosteric Communication in GPCRs

Membrane bound G-protein coupled receptors (GPCRs) communicate the agonist binding in the extracellular (EC) domain to the intracellular (IC) domain to initiate coupling to effector proteins. GPCRs are dynamic proteins and exist in an ensemble of functional states. Recent crystal structures show that the binding of both an agonist and the G protein is required to stabilize the “active state” and that there could be multiple active states of the receptor. Crystal structure of the active state of several class A GPCRs show significant conformational changes in the IC region of the transmembrane (TM) helices TM5, TM6 and TM7 of the receptor [1–4]. The largest change comes from the outward movement of the IC

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part of TM6 away from TM3. Crystal structures of some other class A GPCRs with only the agonist bound also show the outward movement of the IC region of TM6 but to a lesser extent than when bound to the G protein [5–7]. Biophysical experiments have shown that the binding of an agonist in the orthosteric site, leads to an increase in the affinity of the G-protein to the receptor [8]. Similarly, coupling of the G-protein to the receptor increases its binding affinity to the agonist [8]. Thus there is ample evidence that GPCRs are dynamic and exist in an ensemble of functional conformational states [9]. Ligand binding leads to stabilization of specific conformational states chosen from the ensemble of states [10,11].

In class A GPCRs, the distance between the orthosteric ligand binding site and the G-protein coupling site is about 30Å, implying that there is allosteric communication in propagating the activation signal from the orthosteric agonist binding site to the G protein coupling IC region of the receptor. This hypothesis is corroborated by the fact that mutations of residues distant from both the agonist binding site and the G protein interacting interface affect receptor activation [12•• and references therein]. The role of such mutations in their effect on the receptor activity, and the mechanism of allosteric communication are not well understood. Probing the allosteric mechanism of communication requires a confluence of experiments and computational methods. With the knowledge of the residues involved in allosteric communication, one could identify putative allosteric sites in the vicinity of these residues and use it to design allosteric modulators. In this review we describe the current state of the art in computational methods to delineate the residues involved in GPCR allosteric communication.

Allosteric communication in GPCRs mediates Ligand Bias

A single GPCR can mediate multiple signaling pathways depending on the ligand that activates the receptor. Agonist bound GPCRs couple to various intracellular effector proteins such as different heterotrimeric G proteins and β -arrestins that mediate different downstream signaling pathways. Ligands that cause differential signaling to various signaling pathways are called “biased ligands” and they show markedly improved therapeutic index (reduced side effects or off-target effects) as drugs [13–15]. Biased ligands have recently gained traction as effective therapeutics and there is growing literature on development of ligands biased towards β -arrestin mediated signaling pathways [15–18]. There are several factors that govern the biased signaling of an agonist-GPCR pair. The characteristics of the structural ensemble of the ligand-GPCR-effector complex are one of the key factors driving this phenomenon [16,17]. Allosteric communication between the orthosteric ligand binding site and the intracellular effector coupling site plays a significant role in regulating the conformational changes in the ligand-GPCR-effector complex. Biased agonists that show selectivity to specific signaling pathways do so by communicating their preference and effecting conformational changes in the GPCR, that result in coupling to specific G proteins or β -arrestins. Some GPCRs show promiscuity to coupling to different G proteins with varying affinities depending on the agonist that binds to the GPCR [19]. To explain this observation, we postulate that the residues involved in the allosteric communication could potentially modulate the varying degrees of affinity of the agonist-GPCR pair to various G-proteins or β -arrestins. Using this information one could potentially design biased ligands

that interact strongly with the residues involved the allosteric communication and thereby bias the efficacy of these ligands.

The Relevance of GPCR Allosteric Communication in Drug Design

The orthosteric ligand binding sites across members of a GPCR subfamily are typically highly conserved. This poses a challenge to develop ligands that selectively bind and activate specific receptor subtypes. Selective ligands are desirable since they are (a) ideal chemical tools for studying the *in vivo* function of the receptors and, (b) can be developed as drugs with minimal off-target effects. Since targeting the orthosteric ligand binding sites often does not yield subtype selective ligands, a better strategy is to identify and target alternate binding cavities in the GPCR, with low sequence similarity among the subtypes. Such binding sites are known as allosteric binding sites since they affect the potency and/or efficacy of the orthosteric ligand at a distance without competing directly for binding. An excellent review of the role of GPCR allostery in ligand bias and subtype selectivity is presented in reference 20 [20••].

“Allosteric Modulators” are molecules that occupy allosteric binding sites and can act as an agonist by themselves, as well as increase or decrease the binding and/or potency of the orthosteric ligand [21, 22•]. Identification of putative allosteric modulator binding sites in GPCRs would substantially aid and speed up the discovery or the design of allosteric modulators. This remains a challenge given that there are only a few GPCR crystal structures with allosteric modulators bound [22•, 23•]. Analysis of the ligand binding site and cholesterol binding sites in the crystal structures of various class A, class B and class C GPCRs show that agonists, antagonists, and allosteric modulators bind in different regions of the GPCR structure as shown in Figure 1. The crystal structures [22•–34] shown in Figure 1, reveal that ligands can bind in the EC region, in the lower half of the TM region (Figure 1A) and also in the extrahelical region of the receptor [24••] between the TM helices as shown in the surface representation in Figures 1B and C. The various binding sites shown in Figure 1, could be targeted as potential allosteric modulator binding sites [22•, 23•, 27•] for identifying allosteric modulators in other GPCRs. However, an understanding of how these potential binding sites communicate with the G protein coupling site, and the residues involved in the communication would add immense value to harnessing these sites for design of allosteric modulators. Computational methods play a crucial role in identifying residues involved in allosteric communication. In the following sections we review the methods available to date and discuss the significance of the results to our understanding of allosteric mechanisms in GPCRs.

Methods to Study Allosteric Communication in GPCRs

NMR, DEER and fluorescent spectroscopic studies on GPCR conformational dynamics, have shown concerted conformational changes between residues in the ligand binding site and those in the G protein binding site [35, 36•]. The concerted conformational changes are allosteric in nature and occur in micro to millisecond time scale.

Computational methods used for mapping networks of residues involved in allostery in proteins, range from bioinformatics [37••, 38] to elastic network models [39, 40], to analysis

of contact maps [41], force distribution [42] and correlated residue motions [43•• – 46]. A comprehensive account of the computational methods to study protein allostery has been reviewed [47]. Here we discuss only the computational methods that have been applied to GPCR allostery extensively. The Evolutionary Trace (ET) bioinformatics based method analyzes the amino acid sequence alignment among class A GPCRs and provides an ET rank for each residue. The ET rank of a residue is high if a particular residue is mutated at the point of where major branches in the evolutionary tree diverge and the residue is more conserved within each of the minor branches [48]. A cluster of residues with top ET rank within a representative sub family signifies residue “community” of functional importance. The ET analysis (ETA) has been applied to redesign receptor mutations that could mimic ligand specificities and also mutations that switch the signaling bias [49••, 50•]. More recently Sung et al. incorporated mutual information (MI) of the co-variation of distal residue pairs in the ETA, to identify residue pairs in dopamine D2 receptor that are involved in allosteric communication [51]. Sung et al. used ETA to predict a network of residues involved in allosteric communication in the dopamine receptor D2, shown as yellow spheres in Figure 2A. The ETA cannot distinguish functional residues from the ones that confer structural stability. It does not take into account that the dynamics of conserved residues contributing differentially to allosteric communication.

Another computational method used for identifying residues involved in allosteric communication is derived from molecular dynamics (MD) trajectories. This method involves calculating correlated movements between pairs of residues in Cartesian coordinates from the MD simulations [44]. The result of such analysis gives network of residues involved in allostery in proteins. However, the use of Cartesian coordinates in calculating the correlated movements of residue pairs, introduces noise in the correlation due to high frequency motions [43••, 45]. This leads to spurious correlations and hence errors in the prediction of allosteric residues. Additionally, this method does not map the continuous pathway of residues involved in allosteric communication from the ligand binding site to the effector binding region of the GPCR.

We have recently developed a computational method “*Allosteer*” that uses long time scale MD simulation trajectories to analyze the correlated movement among residue pairs in torsional angles [12••], instead of Cartesian coordinates. Use of torsional angles eliminates the high frequency noise and improves the predictions of residues involved in allosteric communication [43••]. Higher the correlated movement between pairs of residues more involved is the residue pair in the allosteric communication. *Allosteer* also calculates the continuous pipeline of residues involved in mediating the allosteric communication from the ligand binding EC region to the effector coupling IC region in GPCRs, shown as blue and red pipelines in Figure 2B. The residues located in the allosteric communication pipeline are termed as “allosteric hubs”. The results from *Allosteer* show [12••]: 1) that the agonist bound receptor conformational state has weaker allosteric communication from the ligand binding site to the G protein coupling site than the antagonist bound inactive state, 2) mutation of the allosteric hub residues increase or decrease the activity of the receptor towards G protein coupling, and 3) void space around the allosteric hub residues can be used as putative allosteric binding sites to design/identify allosteric modulators. It should be noted

that *Allosteer* does not take into account the allosteric communication that could be actively mediated by water or other factors such as lipids in the environment.

Residues mediating allosteric communication modulate receptor activity

Comparison of the allosteric communication pipelines calculated using *Allosteer* on β 1- and β 2-adrenergic receptors, dopamine D3 receptor (D₃DR), histamine receptor 1 (H₁R), M₂ and M₃ muscarinic acetylcholine receptors (M₂R, M₃R), protease activated receptor 1 (PAR1) and A_{2A} adenosine receptor (A_{2A}R), showed several common pipelines that communicate between the EC and the IC domains present in all the receptors. Figure 2B shows the two most common top scoring allosteric pipelines as red and blue patches, present in all these receptors. The red allosteric pipeline starts at the interface residues located between the ECL2 (extracellular loop 2) and EC end of TM2, traverses through the orthosteric ligand binding pocket and ends at the IC end of TM5 and TM6, where the G-protein couples to the receptor. The blue allosteric communication pipeline passes through TM7 and terminates in helix 8. Since the red allosteric communication pipeline shown in Figure 2B is the strongest in the inactive state (stronger the allosteric communication, less dynamic is the receptor), it could play a role in stabilizing TM5 and TM6 in their inactive conformation. The allosteric hub residues that are common to all the eight receptors are shown as spheres in Figure 2B. The residues 2.39, 3.50, 5.51, 5.62 and 6.37 (Ballesteros-Weinstein GPCR numbering scheme [52] used here) are common allosteric hubs in all the eight class A receptors including the peptide receptor PAR1. The first number in the Ballesteros-Weinstein GPCR numbering scheme refers to the TM helix in which the residue is present. The second number marks the position of the residue with respect to the most conserved residue in that TM helix that is numbered as 50. Experimentally identified constitutively active and uncoupling mutations (that are far away from ligand binding site or G-protein coupling site) and those that overlap with the allosteric hubs from MD analysis, are shown as green and red spheres respectively in Figure 2C. This is evidence that the allosteric hub residues modulate the activity of the receptor and explains the hitherto unexplained role of the constitutively active mutants (CAM) and uncoupling mutations (UCM). CAMs are mutant receptors with higher basal activity compared to their wild type and UCMs are mutants with reduced basal activity compared to their wild type receptors. The allosteric hubs identified by both ETA and *Allosteer* are circled in orange in Figure 2C. The allosteric hub residues thus identified can be mutated to modulate the efficacy of an agonist for one signaling pathway over the other, thus altering the bias factor of ligands. It is noted that there are more constitutively active and uncoupling mutants predicted from the MD analysis that were not identified by ETA method. Thus a combination of ETA and analysis of allosteric hubs using MD simulations make a powerful toolset for prediction of residues involved in allosteric communication.

Predicting Allosteric binding sites

Allosteric binding sites are much sought after for identification of allosteric modulators for GPCRs. GPCR crystal structures with allosteric modulators, and information from pharmacological studies show possible allosteric modulator binding sites [22•, 23•, 26, 27•, 53]. However, in cases where there is no known allosteric binding site, we require methods to map putative allosteric binding sites. Void space located distant from the orthosteric sites,

found in the GPCR crystal structures or identified by MD simulations has been designated as potential allosteric binding sites [54]. However, any putative void space in the GPCR structures mapped from MD simulations may not have an allosteric effect on the ligand bound in the orthosteric site. The allosteric site(s) should have functional communication with the orthosteric site. Combining the putative ligand binding sites in GPCR structures with the predicted allosteric pipelines and residues involved in the pipelines, one could authenticate which of the putative small molecule binding sites are predicted to have allosteric communication with the orthosteric site. This concept is illustrated in Figure 3. The allosteric hubs identified in GPCRs using MD simulations [12••], are shown as cyan spheres in Figure 3. The ligand binding sites from multiple GPCR crystal structures are highlighted as transparent spheres in Figure 3. Please note that the ligand binding sites shown in this figure includes class B and class C GPCRs in addition to class A GPCRs. These positions have been included to show all possible allosteric ligand binding sites in GPCRs, as gleaned from crystal structures. It is seen that the position of the allosteric hubs overlay with the potential ligand binding sites in GPCRs. Thus the void space in the receptor near allosteric hubs is a more reliable predictor of potential allosteric binding sites that can be used to design allosteric modulators.

Conclusions

Allosteric communication is a central mechanism by which GPCRs communicate the ligand binding information in the EC region to the IC region where the GPCR couples specifically to the G proteins or the β -arrestins. Understanding the mechanism of the allosteric communication and delineating the residues involved will aid (a) the design of biased ligands towards specific signaling pathway over others, (b) design allosteric modulators that show subtype selectivity and (c) design mutant GPCRs that show specificity to a ligand of choice. Computational methods play an integral role in delineating the allosteric communication mechanism. The combination of sequence alignment based ETA and structure based molecular dynamics methods provide a robust and reliable predictor of functional residues in the GPCR structures and potential allosteric binding sites.

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Abbreviations

DEER	Double Electron Energy resonance
NMR	Nuclear Magnetic Resonance
GPCR	G-protein-coupled receptor
ETA	Evolutionary Trace Analysis
MD	Molecular Dynamics

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Highlights

- GPCRs modulate functional selectivity to effector proteins via allosteric mechanism.
- Finding allosteric binding sites is critical to design of subtype selective ligands.
- Advanced computational methods identify residues involved in allosteric communication.
- Combination of computational methods provides new allosteric sites to target in GPCRs.

Figure 1.

Positions of ligands from crystal structures of GPCRs overlaid on the crystal structure conformation of the inactive state β_2 -adrenergic receptor; the color codes are: blue – ligands binding to the extracellular loops and N terminus, green – ligands binding to the extracellular part of the transmembrane domain; magenta – ligands binding to the intracellular part of the transmembrane domain or near the G protein interface, yellow – cholesterol; (A) β_2 -adrenergic receptor shown in cartoon representation with all ligands overlaid; (B and C) surface representation of β_2 -adrenergic receptor showing the ligand binding extra helical sites. We have shown ligands from the following crystal structures: pdb

IDs: 4Z35, 5CGC, 4OO9, 4OR2, 4K5Y, 4ZJ8, 4XEE, 2RH1, 3PWH, 4RWS, 5EE7, 3VW7, 4MQT, 4XNV and 4PHU.

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Figure 2.

(A) Functional residues in the dopamine D2 receptor identified using ETA and displayed on the inactive state crystal structure of β_2 -adrenergic receptor (pdb id: 2RH1); (B) allosteric hub residues that are common among eight class A GPCRs identified using *Allosteer*; (C) allosteric hubs from *Allosteer* that are also experimentally shown to be CAMs or UCMs are highlighted in green and red respectively; allosteric hubs that are common with those reported by ETA are shown with orange circles; all residues are displayed in the inactive state crystal structure of β_2 -adrenergic receptor.

Figure 3.

Common allosteric hubs as cyan spheres overlaid on the inactive state crystal structure of β 2-adrenergic receptor (pdb id: 2RH1); the ligand binding sites from multiple GPCR crystal structures are highlighted as transparent spheres; extracellular ligand binding sites are colored green, intracellular sites are colored magenta; the two major allosteric pipelines are also shown as reference. The various ligand binding sites inside the TM region of GPCR crystal structures overlap with the void space in the vicinity of allosteric hub residues.