CellPress

Modeling G protein-coupled receptors in complex with biased agonists

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The biological response to the activation of G protein-coupled receptors (GPCRs) typically originates from the simultaneous modulation of various signaling pathways that lead to distinct biological consequences. Hence, 'biased agonists' (i.e., compounds that selectively activate one of the pathways while blocking the others) are highly sought-after molecules to provide fine-tuned pharmacological interventions. This review describes strategies that can be deployed to model the conformation of GPCRs in complex with ligands endowed with specific signaling profiles useful for the generation of hypotheses on the structural requirements for the activation of different signaling pathways or for rational computer-aided ligand discovery campaigns. In particular, it focuses on strategies potentially applicable to model the global or local conformational states of GPCRs stabilized by specific ligands.

Introduction: GPCRs and biased signaling

GPCRs are integral membrane proteins that function as cellular receivers for stimuli that, in most cases, are given by extracellular molecules known as receptor agonists [1,2]. These can be endogenous compounds, for instance neurotransmitters or hormones, but also exogenous natural or artificial compounds, for instance odorants or drugs.

On binding to the receptors, agonists trigger the activation of receptor-mediated signaling pathways that initiate with the interaction of intracellular signaling proteins with the domains of the receptor exposed to the cytosol. Other ligands, known as antagonists, impede the agonist-mediated activation of the receptor. Finally, ligands known as inverse agonists, besides interfering with agonists as do antagonists, suppress the constitutive activity of the receptor [3,4].

Several studies pioneered by Robert Lefkowitz demonstrated that the biological response to the activation of GPCRs typically originates from the simultaneous modulation of various signaling pathways [1–3,5–9]. Some of these are mediated by the activation of G proteins, whereas others are modulated by proteins known as arrestins. Because the activation of different pathways leads to distinct biological consequences, there is significant interest in the identification of 'biased agonists' of GPCRs, which are agonists that selectively activate only one signaling response. As a result, biased agonists have been identified for several GPCRs [2], some examples of which include the β-adrenoceptors [8], the parathyroid hormone receptor [10], a nicotinic acid receptor (GPR109A) [11], the angiotensin receptors [12–18], the opioid receptors [19–21], and the dopamine receptors [22].

The molecular bases of biased agonisms rest on the fact that GPCRs are essentially allosteric systems: the binding of agonists to a first site stabilizes a conformation of the receptor that promotes the activation of specific intracellular signaling partners that bind to a second site. In particular, the receptors are formed by a single polypeptide chain that spans the membrane seven times, with seven α-helical transmembrane domains connected by three intracellular and three extracellular loops [23]. The structure folds to form a membrane-embedded helical bundle, with the N terminus of the chain in the extracellular milieu and the C terminus in the cytosol. For most GPCRs, the binding of agonists occurs in a cavity formed within the helical bundle and exposed to the extracellular milieu [23]. Conversely, intracellular partners bind to regions of the receptor exposed to the cytosol [24,25]. As research studies have shown, GPCRs can assume a range of conformations stabilized by different ligands and associated with different signaling states; some conformations are signaling silent, whereas others trigger the activation of signaling cascades [4,9,26–30]. Specifically, some conformations prevent the interaction of the receptor with all of its signaling partners. Conversely, other conformations promote the association with one or more intracellular signaling proteins, some of which require prior phosphorylation of specific amino acid residues of the receptor exposed to the cytosol [25]. The fact that biased agonists have been found for several systems suggests that the receptor conformations associated with the activation of different pathways are distinct; hence, one conformation can be silent with respect to a given pathway but lead to the activation of another pathway [9].

Thanks to several technical and methodological advancements, the field of GPCR structural biology is currently in full blossom and is yielding a steady output of structures solved at atomic resolution [31–36]. Each experimental structure reflects a specific conformational state of the receptor stabilized by the ligand and all of the other conditions employed for the structural determination. Hence, to gain insights into the conformational

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Keywords: flexible docking; global conformational changes; local conformational changes; molecular dynamics; normal mode analysis; virtual screening.

0165-6147/
 http://dx.doi.org/10.1016/j.tips.2014.04.004

requirements for the activation of the various signaling pathways, efforts are being made toward the solution of structures of GPCRs in complex with ligands endowed with different signaling profiles [37–39]. In particular, there is significant interest in the solution of GPCR structures in complex with biased agonists through X-ray crystallography [37,38] and toward the generation of NMR-supported hypotheses on the conformations linked to the various signaling states [39]. One of the challenges related to the characterization of the molecular mechanisms that underlie biased agonism is that some of the structures solved in complex with biased agonists, although showing receptor–ligand interactions not found with non-biased ligands, captured the receptor in its inactive conformation and do not show the conformational changes responsible for biased signaling [37].

In the absence of the experimental structure of a GPCR in the conformational state stabilized by a given ligand, a structure of the same receptor solved in a different conformation has the potential to serve as a starting point for computational experiments intended to model the conformational state of interest. In particular, efforts to model the conformation of a GPCR in complex with a specific ligand can have two distinct scopes: a broader one, intended to forecast the global conformational state of the receptor–ligand complex [40–45]; or a more circumscribed one, intended to forecast the local conformational state of the ligand-binding cavity (Figure 1) [46–61].

In this review, I discuss ways in which experimental structures of GPCRs cocrystallized with non-biased ligands could be used as platforms to generate models of the receptors in complex with biased agonists. I begin with an illustration of the published strategies that attempt to model the overall structural features of GPCRs in complex with biased agonists [40,41]. I then provide an overview of the strategy that my research group has employed to: (i) model the local conformation of the ligand-binding cavity of GPCRs stabilized by specific compounds [46]; and (ii) use such models to steer virtual screening campaigns toward the identification of ligands with desired signaling profiles [62]. We reported the application of such a procedure to the generation of models that well discriminate between agonists and blockers; although theoretically conceivable, its application to the construction of models that can distinguish between agonists endowed with different signaling profiles (e.g., agonists biased toward the exclusive activation of a single signaling pathway) remains to be demonstrated. I conclude with a view of the possible evolution of the field. The case studies that we discuss are all related to the β_2-adrenoceptor (β_2AR), a prototypical GPCR naturally activated by epinephrine and targeted by drugs for various indications including lung diseases [63] and hypertension [64].

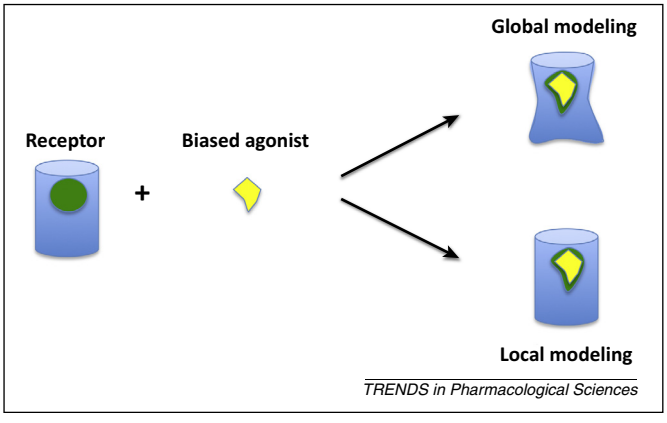

Figure 1. Modeling the global and local conformational state of G protein-coupled receptors (GPCRs) in complex with biased agonists. Given the experimental structure of a GPCR and a model of a given biased agonist, different modeling strategies can be applied to the generation of hypotheses on the global conformation of the resulting complex or its local conformation with respect to the ligand-binding cavity.

Modeling the overall conformation of GPCRs in complex with biased ligands

Broad modeling endeavors aspire to forecast the global conformational state of the receptor in complex with ligands endowed with different signaling profiles. Reaching this goal would provide powerful mechanistic insights into the requirements for the activation of the different signaling pathways. Although modeling global conformational states is challenging, the results of several studies indicate that the field is moving closer to its attainment [40–45]. In the following paragraphs we describe two case studies that illustrate two modeling strategies that have been applied or are potentially applicable to the global conformation of GPCRs in complex with biased ligands [40,41].

The first is a modeling report of the effect of ligands endowed with different signaling profiles in the stabilization of different conformations of the β_2AR, described in 2013 by Tikhonova and coworkers [40]. Specifically, the authors investigated how receptors bound to ligands with different signaling profiles transition from the active to the inactive state through a modeling approach based on accelerated molecular dynamics. This is a modification of conventional molecular dynamics that aims at achieving a more thorough conformational sampling by 'boosting' dihedral potentials, thus allowing rotations of the dihedral angles defined by atomic bonds in the backbone and the side chains of proteins to overcome energetic barriers. The authors subjected to the computational study models of the receptor in complex with salbutamol, a non-biased agonist also known as albuterol that is widely used for the treatment of asthma, and the biased agonist N-cyclopentylbutanepherine, an agonist that is more efficacious toward the β-arrestin than the G_s-mediated signaling pathway [5]. Both models were constructed on the basis of the activated crystal structure of the receptor solved in complex with a nanobody [65]. To study the transition of the receptor from the activated to the inactive state, the authors conducted the molecular dynamics simulations in the absence of the nanobody, which is fundamental for the stabilization of the activated conformation [65]. Hence, they observed that, in the course of the simulations, the receptor moved toward an inactive-like conformation in presence of both the non-biased and the biased agonist, as well as in the absence of a ligand. However, interestingly, they also observed that the simulations conducted with the two different ligands caused distinct patterns of motion to the seventh transmembrane domain (TM7) of the receptor. Because it drives the receptor toward its inactive conformation, the