CHAPTER

What can simulations tell us about GPCRs: integrating the scales

19

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CHAPTER OUTLINE

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Abstract

The functional dynamics of G protein-coupled receptors (GPCRs) encompasses multiple spatiotemporal scales, ranging from femtoseconds to seconds and Ångströms to micrometers. Computational approaches, often in close collaboration with experimental methods, have been invaluable in unraveling GPCR structure and dynamics at these various hierarchical levels. The binding of natural and synthetic ligands to the wild-type and naturally occurring variant receptors have been analyzed by several computational methods. The activation of receptors from the inactive to the active state has been investigated by atomistic simulations and ongoing work on several receptors will help uncover general and receptor-specific mechanisms. The interaction of GPCRs with complex membranes that contain phospholipids and cholesterol have been probed by coarse-grain methods and shown to directly influence receptor association. In this chapter, we discuss computational approaches that have been successful in analyzing each scale of GPCR dynamics. An overview of these approaches will allow a more judicious choice of the appropriate method. We hope that an appreciation of the power of current computational approaches will encourage more critical collaborations. A comprehensive integration of the different approaches over the entire spatiotemporal scales promises to unravel new facets of GPCR function.

INTRODUCTION

G protein-coupled receptors (GPCRs) are membrane receptors that are at the hub of several cellular signaling pathways (Pierce, Premont, & Lefkowitz, 2002; Rosenbaum, Rasmussen, & Kobilka, 2009). Figure 1 shows a schematic representation of a few representative GPCRs embedded in the cell membrane. These receptors

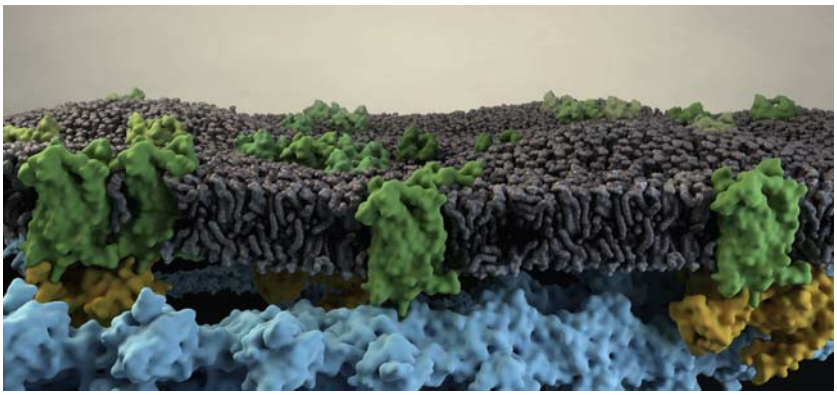

FIGURE 1

Schematic representation of G protein-coupled receptor (GPCR) organization highlighting the dynamic interplay between GPCR oligomers (green), membrane lipids (gray), actin (blue), and G-protein (yellow). For clarity, the complexity of the membrane is not highlighted in different colors. (See color plate)

were traditionally identified as being coupled to the heterotrimeric G-protein signaling pathway, but several G-protein-independent signaling pathways have been reported (Shukla, 2014). A large number of natural ligands and pharmaceutical drugs target these receptors, resulting in a myriad of signaling events (Lagerstrom & Schioth, 2008). An important hallmark of GPCRs is that they comprise of seven transmembrane helices connected by intracellular and extracellular loops. The ligand-binding site is situated between the transmembrane helices or at the extracellular loops. Although these ligands are receptor-specific, a high degree of cross-talk and off-target interactions have been observed within the family. Recent structural biological studies have been able to characterize both active and inactive states, and several apparent similarities have been observed between receptors (Cherezov et al., 2007; Huang, Chen, Zhang, & Huang, 2013; Liu et al., 2012; Rosenbaum et al., 2007). Interestingly, GPCR function has been shown to be modulated by the composition of the membrane bilayer (Chattopadhyay, 2014; Jafurulla & Chattopadhyay, 2013; Oates & Watts, 2011). Notably, cholesterol (Pucadyil & Chattopadhyay, 2004; Saxena & Chattopadhyay, 2012), phospholipids (Soubias & Gawrisch, 2012), and sphingolipids (Jafurulla & Chattopadhyay, 2015) have been shown to alter ligand binding and coupling of the signaling complex in a receptor-dependent fashion. Increasing evidence points toward the role of the underlying cytoskeleton in GPCR organization (Ganguly, Clayton, & Chattopadhyay, 2011). The question remains as to how the signaling diversity of GPCRs is maintained and regulated at the molecular level.

The functional dynamics of GPCRs can be considered to be hierarchical, occurring over several scales and levels. At one end of the range is the fast dynamics at the ligand-binding site, such as the femtosecond isomerization of retinal in rhodopsin (Schoenlein, Peteanu, Mathies, & Shank, 1991). Ligand binding results in pico-to-nanosecond timescale rearrangements at the active site (Henzler-Wildman & Kern, 2007). The activation of the receptor after ligand binding, i.e., the conformational changes from the inactive to the active state occur at the millisecond to second timescale (Manglik & Kobilka, 2014). At the other end, spatiotemporal organization of GPCRs occurs over micrometers in seconds or longer (Kasai & Kusumi, 2014). State-of-the-art experimental approaches have been able to probe several of these facets of GPCR biology. For instance, fluorescence-based microscopy and spectroscopic approaches have been able to capture GPCR-membrane interactions and GPCR association (Paila, Kombrabail, Krishnamoorthy, & Chattopadhyay, 2011; Saxena & Chattopadhyay, 2011). Crystallography and NMR methods have been able to identify conformational states and conformational dynamics (Cherezov et al., 2007; Liu et al., 2012; Park et al., 2012; Rosenbaum et al., 2007). Electron resonance spectroscopy is an emerging technique that has been able to probe facets of conformational dynamics (Manglik et al., 2015). However, the inherent resolution limitations of each of these experimental methods suggest that a more holistic approach with several complementary approaches is needed.

Computational approaches have emerged as important investigatory tools to complement and substantiate experimental findings, often at an improved resolution

(Henin, Baaden, & Taly, 2014; Ingolfsson et al., 2014; Stansfeld & Sansom, 2011). With increasing computational power and improvements in simulation techniques, computational studies are now able to probe the functionally relevant dynamics in several biological processes. In addition, simulations are able to unravel detailed molecular mechanisms and driving forces of these processes. Several computational approaches have been successful in analyzing the various hierarchical levels of GPCR dynamics described above (Grossfield, 2011; Johnston & Filizola, 2011; Sengupta & Chattopadhyay, 2015). The increasing number of approaches has been able to well reproduce the different hierarchies of GPCR dynamics, and also expanded the repertoire of appropriate models. In this chapter, we provide an overview of these approaches and the suitability and limitations of each of these methods.

1. MULTISCALE SIMULATIONS: ACCURACY, ADVANTAGES, AND LIMITATIONS

State-of-the-art simulation methods, including those that have been used to analyze the various facets of GPCR structure and function, encompass several length- and timescales. These methods include quantum chemical descriptors, classical models with an atomistic or coarse-grain representation, and phenomenological system-level models. Figure 2 depicts the various techniques and the length- and timescales

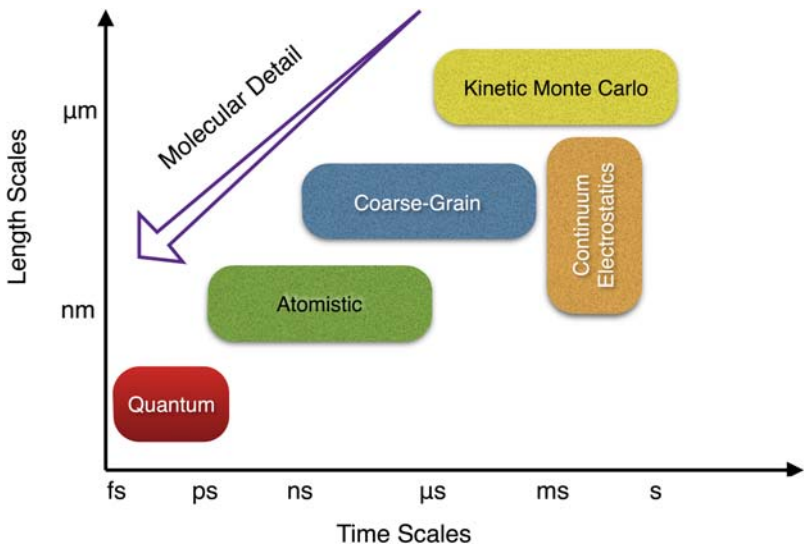

FIGURE 2

The time- and length scales currently sampled by different computational approaches. The molecular detail incorporated in the model increases as time- and length scales are decreased. For further details, see section "Multiscale simulations: Accuracy, advantages, and limitations."

they can currently sample. The molecular detail each model can represent has an inverse relation with the length- and timescales that can be represented with today's computational capabilities. This trade-off between the resolution of a model and the spatiotemporal scales it can sample, dictates the suitability of the model. Seemingly, a quantum chemical description of the system provides the most physicochemically accurate description of the model. However, we can only represent a few atoms with such a detailed description due to current computing capabilities. At the same time, reducing the degrees of freedom and describing the system under consideration with a less-chemically accurate model could still provide important mechanistic insights. After all, we do not need to include quantum mechanics to analyze the effect of earth's gravitation pull! Additionally, since the timescale of the dynamics that is under consideration dictates the suitability of a given computational model, we need to estimate the relevant time- and length scales of the different processes while choosing the relevant technique. In this chapter, we will highlight appropriate methods for each aspect of GPCR dynamics. We first briefly describe a few of these methods using the lipid bilayer membrane as an example. A schematic representation of the bilayer as described by different computational models, together with the assumptions that allow us to "step up" the time- and length scales is given in Figure 3. For a more rigorous mathematical treatment, we recommend the book *Simulating the physical world* (Berendsen, 2007).

1.1 QUANTUM MECHANICAL MODELS

To study biological processes that involve quantum effects, we need to study the system at a subatomic or quantum level. The fundamental formulation that describes quantum mechanical (QM) behavior is the Schrödinger equation and it is imperative to find solutions to it. A conundrum, however, is that the equation can only be accurately solved for a one-electron system and approximations are involved for all other systems. Ab-initio approaches such as Hartree−Fock methods are based on first principles involving the least approximations, which cause them to be computationally expensive. Semiemperical methods (such as MNDO, AM1, PM3) are parameterized based on experimental values and are much faster than ab-initio methods. Density functional theory is a mean field theory that maps the interacting electron problem to a noninteracting electron problem, in which energy is a functional of electron density. At the QM level, we represent the molecule in subatomic detail and calculate its molecular orbitals (MOs) from the electron potentials of each atom. The highest occupied MO (HOMO) of a lipid molecule is shown in Figure 3 (top). Due to the restrictions on the number of atoms that are feasible to include in the QM region, only a single lipid molecule or perhaps even just the head-group moiety can be represented. Often, QM methods are coupled with molecular mechanics (MM) to represent a part of the system with quantum descriptors and the remaining in an atomistic resolution, resulting in the QM/MM multiscale models.

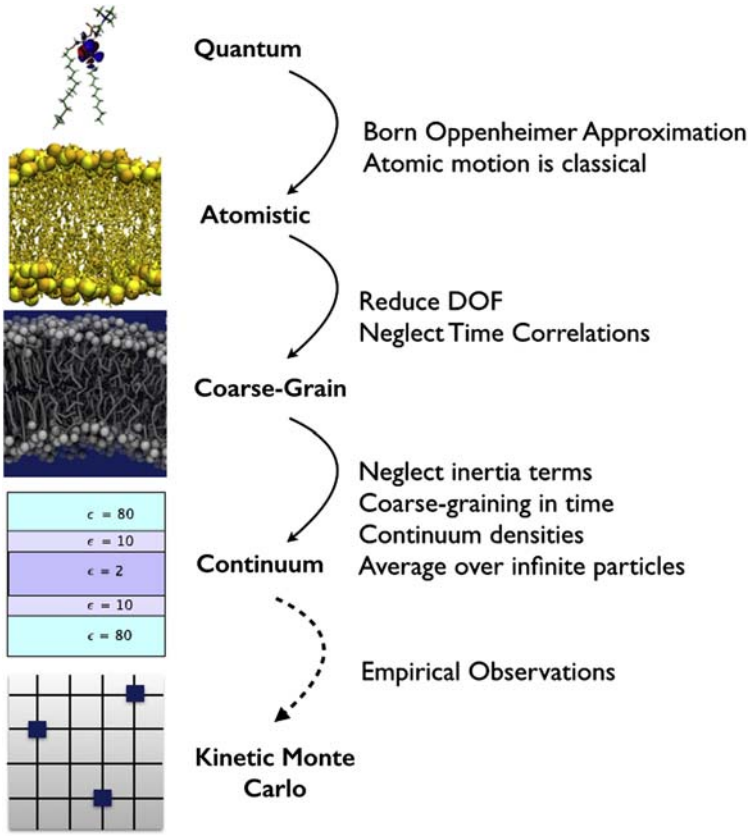

FIGURE 3

A schematic of a phospholipid bilayer as represented by different computational approaches and the underlying assumptions for each model. In the first panel, a single phospholipid is shown in the "ball and stick" representation, together with the HOMOs. In the second panel, a phospholipid bilayer is shown in atomistic detail. The acyl chains are shown as bonds in yellow (light gray in print versions) and the phosphorous and nitrogen as spheres in orange (gray in print versions) and yellow (light gray in print versions), respectively. In the coarse-grain representation of the bilayer, the acyl chains are shown in gray (black in print versions) and the head-group beads as white spheres. The water is not shown for clarity. The continuum membrane model is shown as slabs of varying dielectric depicting the water, head-group, and membrane core regions. In the bottom panel, the kinetic Monte Carlo membrane model is depicted as a grid. For further details see section "Multiscale simulations: Accuracy, advantages and limitations."

1.2 MECHANISTIC MODELS: ATOMISTIC AND COARSE-GRAIN REPRESENTATIONS

Under the Born–Oppenheimer approximation and the assumption that atomistic motion can be represented by classical Newtonian mechanics, we can model a molecule in its "atomistic representation." In this model, each atom is represented as a point particle, with its corresponding connectivity (Hug, 2013). A membrane patch comprising of several lipid molecules is shown in its atomistic representation in the second panel in Figure 3. For clarity, the atoms comprising the lipid head-group (phosphorus and nitrogen) are shown as spheres and the remaining atoms are represented only by the connecting bonds. Typically, membrane bilayers and vesicles comprising of hundreds of lipids (of dimension of several nanometers) are considered and GPCRs are embedded in them in a similar atomistic detail. The mathematical function to compute the energetics, along with the parameters describing the atomistic properties, such as mass and charge and connectivity such as bond length and angles is known as a force field. Typically, the force field comprises of the bonded terms (such as harmonic potential for bonds and angles) and nonbonded terms (van der Waals and coulombic interactions). Several force fields, such as CHARMM, GROMOS, and AMBER are commonly used to study GPCR dynamics (Monticelli & Tieleman, 2013). For further details on the methodology, we refer interested readers to related articles (Guixa-Gonzalez, Ramírez-Anguita, Kaczor, & Selent, 2012; Tai, Fowler, Mokrab, Stansfeld, & Sansom, 2008).

Mechanistic models with a coarse-grain representation have been gaining popularity and are well-suited for certain applications. In the coarse-grain model, we consider a set of atoms as a single bead and each molecule as a set of beads, reducing the degrees of freedom. We can think of this description as not representing each hydrogen, each carbon, or each nitrogen, but rather describing chemical groups such as an amine or alcohol. Currently, one of the most popular coarse-grain models is the MARTINI model (Marrink, Risselada, Yefimov, Tieleman, & de Vries, 2007; Monticelli et al., 2008; Yesylevskyy, Schäfer, Sengupta, & Marrink, 2010) that has been extensively used to analyze dynamic processes at the membrane. In the third panel of Figure 3, a lipid bilayer has been represented with the MARTINI force field, in which the lipid head-group is represented as beads (phosphate and choline moieties) and the lipid tails as bonds. The MARTINI model has been extended to proteins and the amino acid residues are represented by 1–5 beads, based on the size, flexibility, and physico-chemical properties. Similar to atomistic models, the parameters describing the bonded and nonbonded terms are included in the model. Other examples of coarse-grain models are the PRIMO model (Kar, Gopal, Cheng, Panahi, & Feig, 2014) that has been successful in analyzing protein folding and the model by Bereau and Deserno (Bereau & Deserno, 2009) that has been able to the partitioning of model peptides in bilayers.

Using these underlying models, we can perform both "unbiased simulations" or biased simulations with an additional bias (potential/force) (Pawar, Prasanna, & Sengupta, 2015). The unbiased methods sample the free energy landscape at

equilibrium, and are able to access the thermodynamically accessible energy landscape. The main limitation of these unbiased simulations is the phase space they sample and large barriers cannot be usually overcome. Extensive sampling is particularly required to probe rare events or multiple pathways. Biased simulations, such as umbrella sampling and force pulling, include an external bias and can be considered to be out of equilibrium studies. Careful analysis allows us to correctly estimate the underlying unbiased "true" energy landscape. However, it is possible that the external forces applied may bias the motion toward the target and drive the transition along unrealistic deformations.

1.3 CONTINUUM ELECTROSTATIC MODELS

Continuum electrostatic models represent the membrane environment and the solvent particles implicitly, by representing the average electrostatic properties, such as varying polarity and screening. Commonly used methods for continuum representations of membranes are the Poisson−Boltzmann (PB) and Generalized Born methods (Ullmann & Bombarda, 2014). These models approximate the average influence of the environment on a solute. An example of a continuum model, in which the membrane is represented as zones of varying dielectric is shown in the fourth panel of Figure 3 (Sengupta, Meinhold, Langosch, Ullmann, & Smith, 2005). The proteins (or solutes) are usually represented in their atomistic representation, with an additional surface area (SA) term in a hybrid MM/PB-SA model. Such a model can then be used to determine equilibrium properties that are driven by membrane electrostatics, such as solute partitioning (Sengupta, Smith, & Ullmann, 2008). In a few cases, the protein can also be modeled with a continuum term (such as low dielectric region) to incorporate its average effect (Sengupta, Behera, Smith, & Ullmann, 2005).

1.4 PHENOMENOLOGICAL MODELS

At the other end of the scale, cellular-scale phenomenon has been probed by phenomenological models (Radhakrishnan, Halasz, Vlachos, & Edwards, 2010). The phenomenological models are based on experimental observations and provide an accurate description of the system at longer length and timescales. Although these models lack molecular details, they are able to analyze spatiotemporal dynamics that is not accessible by mechanistic models, even with enhanced sampling techniques. Phenomenological models can be broadly classified as stochastic or deterministic (Mayawala, Vlachos, & Edwards, 2006). Kinetic Monte Carlo methods and Gillespie models are the most commonly used stochastic models (Chatterjee & Vlachos, 2007; Gillespie, 2007). Deterministic models are often ODE- (ordinary differential equation) or PDE- (partial differential equation) based models that use a set of equations to model the reactions (association) and diffusion in the system (Athale & Deisboeck, 2006). Most stochastic models are particle based, which represent the system at a single-molecule resolution. Deterministic models are

continuum based that usually average over infinite particles to describe ensemble properties. Both sets of methods have been successfully used to describe membrane processes. For instance, in a kinetic Monte Carlo model studying lipid diffusion or confinement, each lipid molecule is represented as a point particle, and propagated during a simulation (see bottom panel of Figure 3). On the other hand, particle-based methods analyzing system-level properties of GPCRs do not account for the lipid bilayer explicitly. In such models, the effect of the membrane is described by the descriptors of the receptor itself. For instance, the lipid effects driving cluster formation can be modeled as long-range interactions between proteins, without explicitly accounting for the membrane (Duneau & Sturgis, 2012). Phenomenological models are being increasingly used to compare to single-molecule microscopy methods and help to unravel underlying mechanisms by detailed comparisons to experiments.

2. ANALYZING THE DYNAMICS OF GPCR—LIGAND INTERACTIONS

The structural characterization of GPCRs has opened up new avenues in our understanding of GPCR biology. Computational approaches build from the experimentally determined structures to model receptors of unknown structure using structure-based modeling approaches, such as homology modeling. Another important aspect of structural modeling is to predict the structural dynamics of the dynamic loop regions. These modeled structures can be further used to analyze GPCR—ligand interactions.

2.1 HOW DO WE UNRAVEL THE BIOLOGY OF SENSES THAT INVOLVE QUANTUM EFFECTS?

The photoisomerization of covalently bound 11-*cis*-retinal to all *trans*-retinal in rhodopsin and other members of the opsin family has been studied by various QM methods. An example of the electron density and the HOMO of retinal calculated by QM methods is shown in Figure 4. The formation of *trans*-retinal induces the formation of a sterically strained excited state rhodopsin that decays through a series of intermediates to form the active-state metarhodopsin II. Insights into the conformational cycle of rhodopsin are not possible unless the entire protein molecule is studied in conjunction with retinal. Since QM methods are computationally expensive, they are intractable for the entire receptor. A work-around is to use QM/MM methods in which the retinal, crystallographically resolved bound water molecules, and immediate residues (within 2 nm) are modeled using quantum mechanics while the remaining receptor is modeled using MM. ONIOM is a popular approach for QM/MM simulations, in which the system is divided into layers wherein a small region (such as the retinal and a few protein residues) is treated

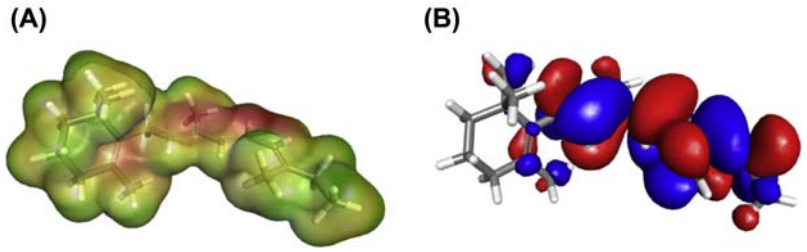

FIGURE 4

Schematic representation of (a) the electron density and (b) the highest occupied molecular orbital of retinal. The retinal molecule is shown as gray and white bonds.

with the most accurate ab-initio method and the remaining part of the system (the receptor and bilayer) is treated by an atomistic method. We suggest the review by Gascon et al. for detailed insights (Gascon, Sproviero, & Batista, 2006).

2.2 CAN WE PREDICT HOW NATURAL LIGANDS AND DRUGS BIND GPCRs?

Docking studies coupled with atomistic molecular dynamics simulations have emerged as an important method to analyze the binding of ligands at the active site (Tautermann, Seeliger, & Kriegl, 2015). A community-wide GPCR-DOCK challenge is conducted to assess the progress in docking of small molecule to GPCRs. The latest challenge demonstrated that correct prediction of extracellular loops and activated structures of GPCRs were the main bottlenecks for accurate prediction of allosteric ligand binding (Kufareva et al., 2014). High-throughput docking studies can be used to screen molecules that could potentially bind to a given GPCR (Tikhonova et al., 2008), or even orphan GPCRs with unknown ligands (Tabata, Baba, Shiraishi, Ito, & Fujita, 2007).

The accuracy of current docking approaches is high, especially for small rigid molecules. Commonly used docking suites, such as GLIDE-XP (Friesner et al., 2006) and AutoDock, (Morris, Huey, & Olson, 2008) are able to reproduce ligand binding to a crystal structure, i.e., the docked pose has a low deviation from the orientation of the crystallographically resolved ligand. A comparison of the binding orientation of carazalol observed in the crystal structure and in docking studies (using the structure that was co-crystallized with it) is shown in Figure 5 and exhibits a good fit. One of the main drawbacks of commonly used docking tools is that only a single receptor conformation is used, and flexibility of both the ligand and the receptor are often ignored. Consequently, docking of ligands other than that with which it was cocrystallized could exhibit large deviations. Further, water molecules often mediate the ligand binding and are usually ignored in these docking studies. To overcome these limitations, one may consider several conformational states of the receptor, such as those generated by atomistic molecular dynamics simulations. Multiple

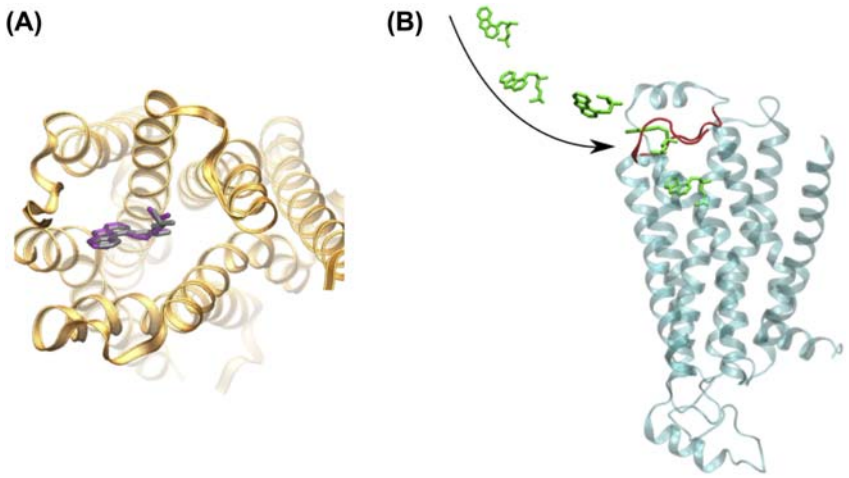

FIGURE 5

The binding of carazalol to β_2-adrenergic receptor. (a) comparison of the binding pose as observed from crystallographic studies (purple (gray in print version)) and docking studies (gray (light gray in print version)). The figure is based on data from Shahane et al. (2014) (b) A schematic representation of a putative binding pathway of the antagonist, carazol to β_2-adrenergic receptor. The receptor is shown in ribbon representation as translucent helices. The residues corresponding to the ligand entry site, vestibule 1, is highlighted in red (grey loop in print version). The carazalol molecule is shown in green (solid grey in print version).

homology models, distinguished by the environment in which they reside have been used by us to differentiate between docked posses of agonists and antagonists (Paila, Tiwari, Sengupta, & Chattopadhyay, 2011).

2.3 IS IT POSSIBLE TO CLASSIFY LIGANDS BY THEIR BINDING FREE ENERGY?

Analyzing the relative strengths of ligands, to screen for potential agonists and antagonists is an important aspect of GPCR–ligand interactions (Gohlke & Klebe, 2002). However, the "docking score" obtained from the above-mentioned docking studies that is used to distinguish between different docked posses only includes the enthalpic contribution to ligand binding. To analyze the binding free energy of the ligands, one needs to consider the receptor and the surrounding membrane. Solvation-free energies can be calculated by MM/PBSA approaches using a continuum membrane model (Waszkowycz, 2008). The main limitation of such a model is the absence of the receptor and ligand dynamics. Free-energy calculations can provide the binding free energy of the ligand using a series of molecular dynamic simulations, in which "alchemic modifications" of the ligand are performed (Henin et al., 2006). Current research is tuned toward improving these estimates.

2.4 IDENTIFYING THE PATHWAYS OF LIGAND ENTRY AND EXIT

An important strength of computational methods is to analyze the pathways of association. Dror et al. (2009) analyzed the entry of ligands into β_2-adrenergic receptor and its subsequent binding at the binding site using microsecond timescale unbiased atomistic molecular dynamic simulations. They proposed two main entry points of the ligand with vestibule 1 (highlighted in red in Figure 5) being the most important entry site. Although the study established the use of long timescale atomistic molecular dynamics to analyze ligand entry pathways, these timescales remain out of reach for most computational studies today, especially to analyze multiple ligand-specific pathways. To increase the sampling of the process, ligand exit pathways have been studied using enhanced sampling methods (Gonzalez, Perez-Acle, Pardo, & Deupi, 2011; Wang & Duan, 2009). Importantly, the same vestibules described from the unbiased simulations have been identified in the enhanced-sampling studies. Although, unbiased simulations can improve the sampling of the pathways and enhance the dynamics along different pathways, one has to be cautious since the extra force added in the unbiased simulations may drive the system along nonphysical pathways. These studies pave the way to study ligand binding/unbinding in other GPCRs, but further studies are required to test the generality of these methods.

2.5 MOLECULAR BASIS OF POPULATION-WIDE VARIATIONS IN DRUG RESPONSE

The naturally occurring variants of GPCRs have warranted a detailed study due to their importance as therapeutic targets. Several nonsynonymous single nucleotide polymorphisms (nsSNPs) have been identified and linked to disease susceptibility and varied drug response. One of the best characterized nsSNPs in the β_2-adrenergic receptor is the Arg16Gly variant in the N-terminal of the receptor that has been linked to a differential response to albuterol, a common asthma drug (Green, Turki, Innis, & Liggett, 1994). In order to study the molecular mechanisms that could be responsible for these interindividual differences, we would need to compare the dynamics of the wild-type and variant receptors. Atomistic molecular dynamic simulations have been able to successfully reproduce receptor dynamics, both the local and global conformational dynamics and could be used to distinguish between the variants.

We have recently performed microsecond timescale atomistic simulations of the Arg16Gly variant to analyze these conformational differences around the mutant residue and the long-distance effects in the variant receptor (Shahane, Parsania, Sengupta, & Joshi, 2014). Our simulations predicted that the N-terminal loop of the Arg variant is more dynamic than the Gly variant, leading to its differential orientation with respect to the receptor. Interestingly, the position and dynamics of the N-terminal region was observed to affect ligand binding-site

accessibility and structure. The study was one of the first to analyze the functionally relevant conformational dynamics of naturally occurring nsSNPs of GPCRs, and further work analyzing polymorphisms in other GPCRs is anticipated. Understanding the molecular mechanisms responsible for differences in drug binding among individuals remains an emerging field and could provide important clues on the functional single nucleotide polymorphisms identified in the recent large-scale sequencing initiatives.

3. CAN CURRENT SIMULATION TECHNIQUES ALLOW US TO STUDY GPCR ACTIVATION?

The majority of studies on GPCR dynamics have focused on either the inactive or the active state based on the conformations resolved crystallographically. A typical starting conformation of such a study, using as an example the β_2-adrenergic receptor in the inactive state (PDB: 2RH1 (Cherezov et al., 2007)) embedded in a lipid bilayer, is shown in Figure 6. Characteristics of the inactive state, such as the ionic lock (salt bridge between transmembrane helices III and VI) have been investigated using atomistic molecular dynamics simulations. Similarly, simulations of the active

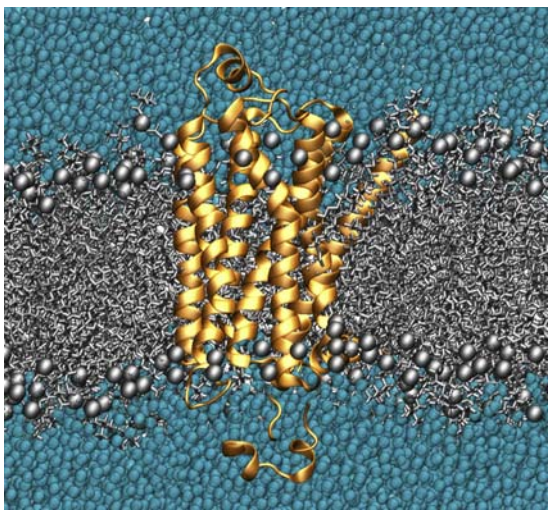

FIGURE 6

A schematic representation of the β_2-adrenergic receptor (inactive state, PDB ID: 2RH1) embedded in a POPC bilayer. The receptor is shown in the ribbon representation in metallic yellow, the lipid bilayer in gray, and the water in cyan. (See color plate)

state, although limited, have identified key interactions with the ligand and bound G-protein. Interestingly, atomistic MD simulations were able to predict the instability of the active state of the β_2-adrenergic receptor in the absence of a coupled G-protein or mimic (Rosenbaum et al., 2011).

3.1 THE INACTIVE TO ACTIVE STATE TRANSITIONS

An important success of computational studies has been to analyze receptor activation, starting from the inactive state of the receptor to its active state (Dror et al., 2011; Nygaard et al., 2013). Atomistic molecular dynamic simulations were performed totaling to hundreds of microseconds in order to analyze these transitions. A large conformational diversity in inactive, active, and intermediate states has been observed, in line with previous hypothesis and more recent NMR studies (Nygaard et al., 2013). Despite the success of atomistic molecular dynamic simulations in sampling receptor activation, the number of studies have been restricted due to limited computational resources.

An interesting study demonstrated that it is possible to use Google's Exacycle cloud-computing platform to simulate shorter trajectories with a total length of several milliseconds (Kohlhoff et al., 2014). Enhanced sampling techniques have been used to analyze receptor activation and have provided key insights into the molecular mechanisms of activations (Miao, Nichols, Gasper, Metzger, & McCammon, 2013; Singh, Ahalawat, & Murarka, 2015). Additionally, a combination of biased and unbiased simulations were used to investigate the effect of water dynamics on the activation (Yuan, Filipek, Palczewski, & Vogel, 2014). These methods appear to be good alternatives to millisecond timescale unbiased simulations.

3.2 HOW CAN WE ANALYZE THE FORMATION OF THE SIGNALING COMPLEX?

Understanding the coupling of GPCRs to their signaling partners remains one of the open questions in GPCR biology. The active state structure of the β_2-adrenergic receptor bound to a G-protein paved the way for simulations of the activated complex. Investigating the pathway of association of the signaling complex promises to reveal further insights. Although atomistic simulations would be needed to accurately study the conformational changes associated with receptor activation and subsequent coupling, we can envision that a coarse-grain model could perhaps be able to reproduce this coupling. Another aspect of this complex formation is the specificity of the G-protein coupling, as well as the non-G-protein partners. Protein–protein docking has not yet proven to accurately reproduce this coupling. Due to the length of the simulations, we believe we cannot yet use atomistic simulations as a scoring function to predict the binding partners of GPCRs. The coupling of the signaling complex remains one of the aspects of GPCR biology that are yet to be studied by computational methods.

4. THE DYNAMIC INTERPLAY BETWEEN THE MEMBRANE AND RECEPTORS

In the previous examples, we have focused on a single receptor monomer and its mechanism of action. However, it is becoming clear that these receptors do not function in isolation (Chakraborty & Chattopadhyay, 2014; Chattopadhyay, 2014). The interaction of these receptors with their complex membrane environment and other receptors is being increasingly reported by experimental methods. Recent computational endeavors have been able to provide detailed mechanisms of some of these phenomenon (see Figure 7). Computational models that can accurately describe these complex interactions promise to unravel aspects of GPCR biology that are inaccessible to current experimental approaches.

4.1 EMERGING CONCEPTS IN MEMBRANE LIPID INTERACTIONS PREDICTED BY SIMULATIONS

In order to study the interaction of membrane lipids with GPCRs, we need to represent both the membrane and the receptor with a model that can correctly predict the interaction of the protein and the lipid moieties. Initial developments in the field were necessarily divergent and earlier studies were not able to capture details of this interaction, especially for cholesterol. For instance, atomistic simulations had been predicted that cholesterol is mainly excluded from the receptor surface of rhodopsin (Grossfield, Feller, & Pitman, 2006), although recent data suggest the presence of high cholesterol density sites (Horn, Kao, & Grossfield, 2014). Recent atomistic molecular dynamic simulations have pointed to cholesterol-interaction sites in several GPCRs (Sengupta & Chattopadhyay, 2015), including adenosine$_{2A}$ receptor (Lee & Lyman, 2012) and β_2-adrenergic receptors (Cang et al., 2013). One of the cholesterol-interaction sites predicted from simulations have been associated with cholesterol densities in crystallographic studies (Lee & Lyman, 2012). Atomistic molecular dynamics simulations are as a result able to provide a good prediction of the sites of the receptor that could interact with cholesterol, but the relative strengths of binding remain unclear.

Coarse-grain simulations using the MARTINI force field provide a good alternative method to study GPCR–cholesterol interactions sampling over several tens of microseconds (Sengupta & Chattopadhyay, 2012). At these timescales, the cholesterol binding–unbinding events appear to be well sampled. However, in the absence on multiple association–dissociation events, the relative energetics calculated are only qualitative. Several of the cholesterol interactions sites identified in coarse-grain studies of β_2-adrenergic receptor correspond directly to those identified from atomistic simulations (Prasanna, Chattopadhyay, & Sengupta, 2014). It is encouraging to see that irrespective of the models used, the predicted interaction sites match well. The main advantage of using coarse-grain simulations is that we can access longer timescales and consider complex membrane compositions.

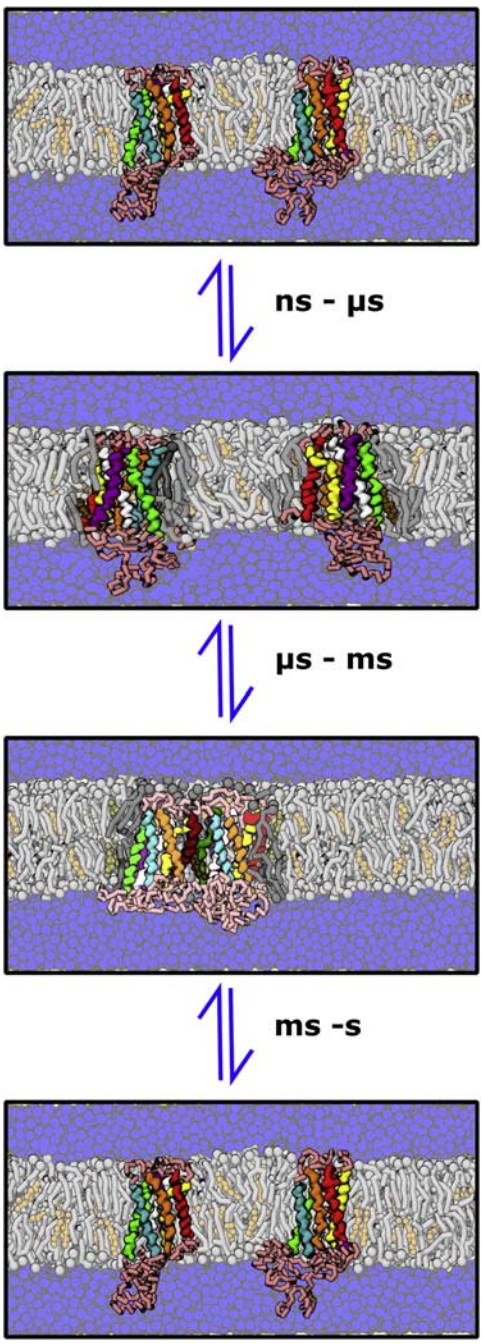

FIGURE 7

A schematic representation of the important interactions of G protein-coupled receptors (GPCRs) with the membrane and its role in GPCR association. The timescales of these

Additionally, since coarse-grain simulations are able to sample unbinding events, we can analyze the relative timescales of these events. Two distinct timescales of association have been identified: a fast nanosecond and a slow microsecond timescale dynamics, that are in line with subsequent NMR studies (Gater et al., 2014). With increasing computational power, coarse-grain approaches will be able to quantitatively analyze cholesterol association energetics.

Coarse-grain simulation studies have been successful in identifying phospholipid-binding sites on the receptor (Prasanna, Chattopadhyay, & Sengupta, 2015). Although the dynamics of phospholipids is slower than cholesterol in the membrane bilayer, simulations totaling to hundreds of microseconds have been able to identify sites with high occupancy of phospholipids. The most important site identified is at the groove of transmembrane helices I and VII. Importantly, the same site was seen to correspond to a "stably bound" lipid molecule in atomistic simulations (Patra et al., 2015). However, due to the limited timescale of the atomistic simulations, the dynamics and diffusion of several other lipid molecules was confined, and comparison to coarse-grain simulations helped to identify its significance.

The effect of the membrane composition on GPCR structural dynamics is less clear. Structural differences in the kink regions of transmembrane helices has been reported to cholesterol dependent (Khelashvili, Grossfield, Feller, Pitman, & Weinstein, 2009), but were later suggested to be force-field dependent (Lee, Patel, & Lyman, 2013). A recent work pointed to an increased flexibility of the *trans*-membrane helices of the serotonin$_{1A}$ receptor in the absence of cholesterol (Patra et al., 2015), but longer timescales and a more comprehensive sampling is required to analyze the detailed conformational dynamics. Long timescale atomistic simulations and detailed analysis on the structural dynamics will be required to probe membrane effects in GPCRs.

4.2 WHAT METHODS CAN WE USE TO STUDY GPCR ASSOCIATION?

Rhodopsin oligomerization was analyzed using the MARTINI coarse-grain force field and demonstrated to be suitable for probing receptor association (Periole, Huber, Marrink, & Sakmar, 2007). Multiple receptors were considered and microsecond timescale simulations were performed. During the course of the simulation, the receptors interacted with each other and main interacting surfaces

processes are given with the arrows. Coarse-grain simulations are able to capture the nanosecond to microsecond cholesterol and lipid interactions with GPCRS, as well as the microsecond to millisecond GPCR association. The dissociation of GPCRs at the millisecond to second timescales have not been yet reported by coarse-grain simulations. The backbone of the receptor is shown in different colors corresponding to the transmembrane helices, the lipid bilayer in gray (white in print versions), the cholesterol in yellow (light gray in print versions), and the water in blue (gray in print versions).

The figure is based on data from Prasanna et al. (2014).

could be identified. Interestingly, the sites with increased protein−protein contact corresponded to the sites with increased membrane perturbations around the receptor monomer. The study paved the way for further work and several studies used similar starting conformations to analyze GPCR homoassociation (Horn et al., 2014; Mondal et al., 2013). The main limitation of these simulations was that a high protein−lipid ratios was considered that could result in a possible bias of the final oligomer states.

Cholesterol-dependent dimer interfaces of the β_2-adrenergic receptor have been probed by us, using the same force field at lower protein−lipid ratios (Prasanna et al., 2014). In the study, a larger initial interreceptor distance was considered and the proteins diffused freely, interacting with each other at a microsecond timescale. The dimer structures were identified from the simulations by calculating contact maps of the interacting interfaces. The cholesterol-dependent dimer interfaces were correlated to specific cholesterol association sites, with a reduced nonspecific effect of the membrane. The results suggested that a few dimer interfaces of the β_2-adrenergic receptor are of similar energetics, which are tuned by the membrane under varying compositions. The study demonstrated the importance of sampling protein−lipid interactions in addition to the protein−protein contacts. The work opens up further questions about cholesterol and membrane effects in GPCR association, and detailed simulations on related receptors is needed to extract general effects.

5. GPCR ORGANIZATION IN THE CELL MEMBRANE

The spatiotemporal organization of GPCRs in cells occurs over large time- and length scales. Recent super resolution microscopy has revealed a receptor-dependent organization ranging from millisecond association/dissociation of dimers, to larger oligomers that are stable up to seconds (Kasai & Kusumi, 2014). Importantly, association appears to be ligand-independent, but it has been suggested that different oligomeric species could have varying ligand-binding affinities. It is therefore important to analyze the spatiotemporal dynamics of GPCRs, in order to address the underlying mechanisms of association.

Both stochastic and deterministic models have been used to probe facets of GPCR organization. Using a combined stochastic and deterministic model, Fallahi-Sichani and Linderman (2009) showed that microdomains can both amplify or attenuate signals in ligand-dependent GPCR association. However, recent experimental studies on several GPCRs suggest that the dimerization of these GPCRs is independent of ligand binding (Kasai & Kusumi, 2014) and it is not clear whether micro-domain dependence will be supported by a ligand-independent model. On the other hand, a classical free-diffusion model of GPCRs was shown to be consistent with the experimental kinetic data, only if the complexes are modeled to be weaker than suggested experimentally (Schöneberg, Heck, Hofmann, & Noe, 2014). We have recently proposed a kinetic Monte Carlo model and tested it

to analyze association of model peptides (Pawar, Deshpande, et al., 2015). The parameters of the model, such as dimer lifetime and on-rates were estimated from coarse-grain simulations, and shown to match in vitro estimates. The organization at seconds timescale was then explored from the emergent properties of the kinetic Monte Carlo model by calculating the resultant dimer population. Such a model could prove to be useful to analyze the organization of GPCRs with parameters measured from experiments and estimated from atomistic and coarse-grain simulations. A comprehensive ligand-independent modeling of membrane receptors, in particular GPCRs, that could explain the recent microscopic data is still missing and paves the way for future modeling efforts.

CONCLUSIONS

Computational methods have proven to be indispensable to study various facets of GPCR biology. As such, there are no "one size fits all" models and a judicious choice of the appropriate methodology is recommended. A critical analysis of the advantages and limitations of each model is needed to decide the appropriateness and relevance of the model used, and make further improvements in analyzing all aspects of GPCR dynamics. Additionally, a better understanding of the computational methods will help improve the decision making for an appropriate choice of model. A close iterative collaboration with experiments and computational approaches promises to uncover key aspects of receptor function and organization.

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