Published in final edited form as:

Curr Opin Struct Biol. 2019 April ; 55: 121–128. doi:10.1016/j.sbi.2019.03.016.

Allostery in G Protein-Coupled Receptors Investigated by Molecular Dynamics Simulations

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Abstract

G-protein-coupled receptors (GPCRs) are allosteric signaling machines that trigger distinct functional responses depending on the particular conformational state they adopt upon binding. This so-called GPCR functional selectivity is prompted by ligands of different efficacy binding at orthosteric or allosteric sites on the receptor, as well as by interactions with intracellular protein partners or other receptor types. Molecular dynamics (MD) simulations can provide important mechanistic, thermodynamic, and kinetic insights into these interactions at a level of molecular detail that is necessary to rightly inform modern drug discovery. Here, we review the most recent MD contributions to understanding GPCR allostery, with an emphasis on their strengths and limitations.

Graphical Abstract

Introduction

The G protein-coupled receptor (GPCR) family encompasses several structurally-related membrane proteins with crucial roles in mediating a number of important cellular signaling processes. Located on the surface of a cell, GPCRs help transmit information from outside of the cell to the intracellular domain via a whole host of signaling paths. The modern view of GPCR function is one that incorporates the highly dynamic and allosteric nature of these

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proteins [1,2], whereby a pre-existing ensemble of active and inactive conformational states are sampled by the receptor with or without a ligand present. In the classical view of GPCR allostery, agonist binding at so-called “orthosteric” sites, which typically extend to the extracellular portion of the receptor, promotes allosteric coupling of the receptor to intracellular proteins, mostly G proteins and beta-arrestins, at a location several angstroms away from the ligand binding site. Thus, GPCRs function inherently as “allosteric machines”, wherein structural changes at one location – in this case the orthosteric ligand binding pocket – induces a change in structure at a distinct spatial region, thereby affecting its conformational dynamics and ability to couple to the intracellular proteins that promote cellular signaling. However, ligands of different efficacies, as well as intracellular binding partners, receptor dimerization, or even lipid molecules, will produce distinct population shifts in the natural GPCR thermal equilibrium by differentially stabilizing the multiple conformations sampled. This conformational selection is believed to form the basis for the receptor’s functional selectivity, also known as “biased agonism”, by promoting different cellular signaling responses.

Although more than 200 experimental high-resolution structures of GPCRs from more than 50 unique receptors have become available to date [2], these offer very limited conformational diversity. Not only do these structures represent stable conformations under specific experimental conditions, often lacking a natural lipid environment, but most of them correspond to inactive states of the receptor bound to orthosteric ligands. Yet, the ever-growing repertoire of active, inactive, and ligand-free GPCR experimental structures confirms the existence of different conformational states stabilized by ligands binding at different locations on the receptor, whether classical orthosteric or alternative, allosteric sites. Conformational states of GPCRs bound to allosteric modulators are particularly scarce, yet highly desirable given the expected cooperativity of these compounds with orthosteric agonists, and their potential for developing into selective drugs. The principles of conformational selection underlying the allosteric behavior of these molecules are particularly sought after in view of the ability of these compounds to either enhance or reduce the affinity/efficacy of endogenous or orthosteric ligands depending on whether the molecule acts as a positive allosteric modulator (PAM) or a negative allosteric modulator (NAM). Even more desirable is to understand how allostery is transmitted by interaction of the receptor with intracellular proteins or through dimerization/oligomerization with other receptors. However, current experimental structural information is limited to very few agonist-bound GPCR-G protein or arrestin complexes, to dimeric crystal structures that may or may not be physiologically relevant, and to specific experimental conditions (e.g., detergents, antibodies, mutations, fusion proteins, etc.) used to obtain such structures.

Molecular dynamics (MD) simulations have become an invaluable tool for investigating allosteric behavior in GPCRs [3]. This is because tracking the fluctuating atomic positions with high spatiotemporal resolution serves as a perfect complement to static snapshots of these proteins derived from experimental methods such as X-ray crystallography or electron microscopy. That MD simulations can now play this role is only possible due to the ever increasing methodological developments, at both the software and hardware level, that make them capable of simulating larger molecular systems for much longer timescales than were previously possible (now at least several microseconds using supercomputing clusters

commonly available at several academic institutions). In cases where the accessible timescale is still limited, careful use of enhanced sampling algorithms makes it possible to simulate even longer times. Furthermore, developments in Markov State Models (MSMs) and machine learning have also allowed to infer kinetic, thermodynamic, and mechanistic information from large MD datasets generated from simulating long-timescale biological processes [4]. Underpinning all the above developments are the atomistic or coarse-grained force fields used to model the energy interaction terms in the Hamiltonian that describe the biological system being simulated. Although these continue to be refined, they nonetheless represent an intrinsic approximation to all MD simulations. A schematic overview highlighting the available MD simulation strategies for studying complex biological systems is provided in Figure 1, along with their main limitation.

In what follows we summarize the most recent MD contributions and insights into the allosteric behavior of GPCRs, with a focus on ligand-induced receptor activation, as well as modulation by ligand or ion binding to allosteric sites. We conclude this overview with considerations of aspects of GPCR allostery that present a significant challenge for MD simulations due to the size of the systems and the timescale of the biological processes under study. Yet, some of these investigations have already started, and proven to nicely complement information derived from other biophysical methods such as, for instance, nuclear magnetic resonance (NMR) spectroscopy, single-molecule microscopy, as well as fluorescence, luminescence, electron paramagnetic resonance (EPR), or double electron-electron resonance (DEER) spectroscopy, towards a full understanding of GPCR signaling.

Binding and Action of Allosteric Modulators by MD

Although the number of high-resolution experimental GPCR structures has boomed over the last decade, it is important to keep in mind that most do not capture ligand/ion binding to GPCR allosteric sites nor the mechanisms of allosteric modulation. Using MD simulations, it is possible to help fill these gaps in several respects. First, placing allosteric modulators deep in the bulk, far from the ligand-GPCR complex, it is possible to track them along their binding pathways to their a priori unknown binding sites. Second, as the modulator relaxes in its allosteric site, it is possible, in principle, to extract information about the induced allostery across the receptor although this is not trivial, often requiring significant computational resources to achieve convergence of the simulation-derived observables.

One of the earliest studies of the mode of binding and action of GPCR allosteric modulators involved unbiased MD simulations on a number of PAMs and NAMs at the M2 muscarinic acetylcholine receptor (M2R) [5]. This study provided information about the binding pathway of these ligands, their binding modes, as well as the structural basis for their cooperation with orthosteric ligands, thus informing the rational design of allosteric modulators at M2R. Notably, all allosteric modulators in this study formed cation- π interactions with a number of aromatic residues located in the receptor extracellular vestibule, several angstroms away from the orthosteric binding site. It is interesting to note that in order to attain a proper description of these cation- π interactions, the parameters of the force-field had to be fine-tuned, which highlights the dependence of an MD-based result on the quality of the underlying force-field. Radioligand binding experiments on receptor

mutants confirmed simulation-based predictions of increased or decreased modulator affinities. Conformational changes of the orthosteric and allosteric binding sites, as well as electrostatic interactions between ligands in these sites, were suggested to contribute to positive and negative allosteric modulation of classical ligand binding, thus enabling the design of chemotypes with an altered allosteric effect.

A more recent investigation by our lab used metadynamics as an enhanced sampling MD simulation approach to reduce the computational workload required to extract meaningful information from MD simulations for the binding of the PAM BMS-986187 to the δ -opioid receptor (DOR) in complex with the DOR-selective orthosteric agonist SNC-80 [6]. Metadynamics introduces a biasing potential in order to drive the system toward enhanced exploration of the available conformational space. In this study, it was determined that the DOR allosteric modulator visited multiple allosteric sites during the binding process, as well as two geometrically distinct, but energetically indistinguishable, binding poses during relaxation in the receptor extracellular vestibule. A few DOR residues forming stabilizing interactions with only one of the two predicted BMS-986187 poses were identified and validated by experimental testing, thus allowing us to evaluate the likelihood of the two poses *in vitro*. A critical issue in using metadynamics is the proper choice of typically up to two collective variables (CVs) upon which a biasing potential is deposited in order to force greater exploration of the conformational space. Care must be taken to make sure the CVs overlap with the slow modes of the system under study in order to ensure convergence of free energy profiles without being computationally prohibitive.

The binding of two PAMs, PD81723 and VCP171, to the Adenosine A₁ receptor (A1AR) has been the subject of recent MD simulations using another enhanced sampling method known as Gaussian-accelerated Molecular Dynamics (GaMD) [7]. The GaMD method introduces a ‘boost’ potential to speed-up sampling of the slow fluctuations while nonetheless being able of recovering the free energy landscape. Placing either of the two PAMs deep in the bulk and at least 20 Å from the GPCR complexed with the agonist NECA, the simulations captured transient interactions with different regions of the A1AR, as well as spontaneous PAM binding to a pocket in the extracellular loop 2 (ECL2), in agreement with mutagenesis experiments. Furthermore, the MD-predicted binding modes were corroborated by available experimental data. Upon comparing agonist motion within the orthosteric site for the agonist-GPCR complex with or without the PAMs in the simulation protocol, it was observed that the amount of conformational space within the canonical orthosteric site that the agonist would sample was dependent on the presence of the modulators. In particular, using the agonist structural RMSD, it was observed that the presence of the PAMs induced a more tightly bound agonist, implying a more stable complex. This was attributed to salt-bridge formation between the Glu172 and Lys265 residues in extracellular loops 2 and 3, respectively, upon PAM binding to its allosteric site.

Ion binding to GPCRs and consequent allostery propagated throughout the receptor have also been studied via MD simulations by our group and others. In early work using microsecond MD simulations, Selent et al. confirmed that Na⁺ ions entered the GPCR transmembrane region of the dopamine D₂ receptor (D2R) from the extracellular side and suggested transient binding of Na⁺ to the orthosteric site prior to “final” Na⁺ binding to the

allosteric site [8], which was stabilized by a hydrogen-bonding network. Notably, the presence of this type of network was later confirmed by a ultra-high resolution crystal structure of adenosine A_{2A} receptor [9] and the Na⁺ ion position in the highly conserved aqueous receptor pocket of δ -opioid and M2 muscarinic receptors was suggested to strongly respond to voltage changes [10]. Another important observation of the work by Selent et al. [8] based on enhanced sampling MD simulations is that Na⁺ binding to the allosteric site switches the torsional angle of Trp^{6.48} (superscript refers to the Ballesteros-Weinstein generic numbering scheme [11]) to the same value that has been seen in the inactive β 2 adrenergic crystal structure.

A joint experimental-computational work recently showed that Na⁺ not only modulates the affinity/efficacy of orthosteric ligands, but is also crucial for the action of a NAM, specifically SB269652, in modulating binding at the orthosteric site of the D₂R [12]. It was observed from these published simulations that the absence of Na⁺ bound to the crystallographic sodium binding site at Asp 80^{2.50} disrupts the binding mode of the NAM tetrahydroisoquinoline moiety at the orthosteric site, by weakening its salt-bridge interaction between the protonated tertiary amine and the Asp 114^{3.32} residue.

Notably, free energy profiles and kinetics of Na⁺ binding derived from MD simulations of 18 different GPCRs and Markov state models suggested a conserved mechanism of Na⁺ binding for GPCRs [13]. In a very recent contribution by our group [14], we used a multi-ensemble Markov model framework combining equilibrium and non-equilibrium atomistic MD simulations to estimate the energy differences and required timescales of Na⁺ translocation in inactive and active μ -opioid receptor (MOR) crystal structures. Not only did these simulations provide kinetic estimates of Na⁺ translocation, but they also presented evidence for a continuous ion pathway through the MOR active, but not inactive, conformation, as well as testable hypotheses of molecular determinants and correlated motions that might affect MOR signaling estimates, alongside estimates of Na⁺-induced changes to agonist binding that were validated by radioligand measurements. In another very recent joint computational-experimental work, MD simulations provided a structural context to evidence from ¹⁹F NMR spectra that the divalent Ca²⁺ and Mg²⁺ cations shift the equilibrium toward active states of A2AR whereas Na⁺ stabilizes inactive- or partial-agonist-induced states of this receptor [15]. Specifically, analysis of several μ s-long unbiased MD simulations of different conformational states of A2AR in the presence of a high concentration of monovalent and divalent cations using rigidity-transmission allostery theory revealed that divalent cations bring the extracellular ends of TM5 and TM6 in closer proximity via coordination with residues on these helices, forcing an allosteric opening for G-protein binding at the intercellular domain. An important point to keep in mind regarding MD simulations of cations is that force fields tend to provide a somewhat poor description of cationic species, especially of divalent cations.

Mechanisms, Thermodynamics, and Kinetics of Ligand-Induced Receptor Modulation by MD

MD simulations have made relevant contributions to our current understanding of GPCR activation mechanisms, thermodynamics, and kinetics. In work immediately following the publication of one of the earliest active GPCR crystal structures, that of a β 2-adrenergic receptor (B2AR) bound to a G-protein-mimetic nanobody in addition to a potent agonist, long unbiased MD simulations revealed spontaneous deactivation of the receptor in the absence of the intracellular binding partner, despite the presence of a bound agonist [16]. Furthermore, an intermediate metastable state with a somewhat longer residence time than the active state was found to connect the active and inactive conformations along the deactivation pathway, with helix 7 approaching its inactive conformation but helices 3,5,6 fluctuating around their active state conformations. An important contribution from this MD simulation study was the atomic-level mechanistic evidence for the loose allosteric coupling between the extracellular drug-binding site and the intracellular G-protein binding site, with each region maintaining the ability to interconvert between active and inactive conformations. Moreover, the receptor had a larger propensity to acquire an inactive conformation in the absence of a bound intracellular binding partner notwithstanding the agonist likely ability to increase the fraction of active receptors. Despite the long MD simulations in this study, sampling by standard MD identified only one intermediate region along the B2AR activation pathway, in contrast to our earlier suggestions from enhanced sampling approaches that GPCRs sample at least two different intermediate regions in addition to states similar to the available active and inactive crystallographic structures along the activation pathway, as we demonstrated for rhodopsin [17], B2AR [18], and most recently, MOR [19,20]. Notably, the presence of several different kinetic states along multiple B2AR activation paths, and their differential modulation by ligands of different efficacy, was also observed from processing a massive amount (two milliseconds) of MD-based data afforded on the rather exclusive Google's Exacycle cloud-computing platform [21].

In our most recent work aimed at elucidating dynamic and kinetic elements of MOR functional selectivity [19], we used an adaptive sampling MD simulation protocol [22] in order to access conformational states inaccessible via brute-force MD. Here, the effect of both morphine – a classic opioid agonist – and TRV-130, an agonist biased towards G-protein recruitment and signaling, on the activation process were investigated. With the use of a MSM along the slowest dynamical modes of the agonist-GPCR systems, 14 kinetically distinct macrostates making up 4 conformationally distinct regions were determined [19]. These regions corresponded to two different intermediate regions in addition to those containing active and inactive crystal-like states of the receptor. In line with the ensemble picture of GPCR activation supported by DEER and NMR measurements (e.g., see [23]), even in the presence of agonists, the inactive GPCR conformational region was dominant, with 50% of the simulation for the morphine-bound system and 58% for the TRV-130-bound receptor [19] sampling the inactive state region [19]. As shown for B2AR by DEER and NMR measurements [23], our simulations sampled inactive conformations with either a broken or formed hydrogen bond between the side chains of R165^{3.50} and T279^{6.34}, which

in opioid receptors replaces the classical R^{3.50}-D/E^{6.30} salt bridge that stabilizes the inactive conformation of TM6 in a number of inactive GPCR crystal structures. However, ~96% of the sampled inactive MOR conformations bound to either morphine or TRV-130 contained a broken R165^{3.50}-T279^{6.34} hydrogen bond. Notably, our study provided evidence for a different dynamic behavior of MOR induced by morphine or TRV-130, and revealed kinetically distinct, and potentially relevant druggable states that may be difficult to obtain experimentally, yet potentially useful for the purpose of drug discovery. Another interesting result of our study was that several activation paths were found to be available to the agonist-GPCR complex, with the majority of them traversing only one of the two identified intermediate regions (the same also identified in earlier studies [16]), in addition to various different active-like and inactive-like states. Notably, despite the heterogeneity of deactivation paths in both systems, only in the TRV-130-bound MOR system were the majority of these paths traversing a specific active-like state in the transition between MOR active and inactive crystallographic states (see Figure 2 for an illustration of the structural differences between inactive, active, and this crucial TRV-130-stabilized active-like conformation of MOR). In contrast, the most probable deactivation pathways of the morphine-bound MOR system featured either a direct transition to the inactive-like crystallographic state of MOR or one that involved a state of the MOR active region [19]. Finally, an important contribution of this work was the calculation of the rates for interconverting between the macrostates, which fell naturally from MSMs, and which suggested that the receptor conformational changes that precede interactions with intracellular partners at the cytoplasmic side are not the rate-limiting step in receptor activation.

In another very recent joint experimental-computational study [24], insights were drawn from MD simulations regarding the selectivity of the antagonist AF-DX384 towards the M2 muscarinic receptor. In particular, the MD simulations looked into the structural basis for selectivity of the M2 receptor over M3 despite their orthosteric site residues being highly conserved. The reason was suggested to be a contraction of the binding site due to the formation of several close contacts formed by the ligand with TM2 and TM3. In addition, the MD simulations probed the thermostability and increased binding affinity of the antagonist towards the S110R mutant of M2 receptor, whose crystal structure was obtained in this work. Based on free energy perturbations theory, the binding free energy calculated for the mutant complexed with AF-DX384 was 6 kcal/mol higher, confirming the enhanced affinity for the mutant. This stabilization of the antagonist in complex with M2 was attributed to residues Y80^{2.61}, W99^{3.28}, L100^{3.29}, and Y104^{3.33} from TM2 and TM3 making closer interactions with the AF-DX384 [24].

Notwithstanding the important insights that can be derived from these types of studies, hundreds of μ s simulations on specialized computational resources are still required to achieve free-energy convergence in GPCRs. A recent methodological extension from our group aims to make the accurate computation of both free energies as well as kinetic rate constants for the GPCR activation process much more affordable than current approaches [20]. Using the metadynamics-based enhanced sampling approach to drive forward the exploration of the conformational landscape, together with maximum caliber to achieve the best available kinetic model given available information on the system under study, we

showed that these estimates were in good agreement to those using the more expensive approach described above [19]. In particular, the maximum caliber approach makes it more tractable to compute the transition matrix in MSMs that estimates the rates for interconverting between different kinetic states [20].

Remaining Challenges in Studying GPCR Allostery by MD

One of the important open questions regarding GPCR allosteric modulation is the extent to which intracellular partners, oligomerization, or even the lipid solvent can affect the dynamic behavior of the receptor, thus dictating its conformational selection and consequent functional selectivity. This is challenging from an MD perspective due to the size of the systems, the long timescales of the biological process under study, and related methodological difficulties/limitations. Yet, a few studies have started to investigate the effect of intracellular partners or oligomerization on the receptor dynamic behavior, and the power of these early MD applications stems from their integration with experimental biophysical methods. For instance, using coarse-grained representations of inactive or active crystallographic states of MOR, we recently studied the dimerization process of this receptor using MD simulations and MSMs to reveal that the dimeric MOR structures tended to be low-population, transient states [25]. An important conclusion of this work, which was corroborated by FRET experiments, was that extrapolating to physiological GPCR concentrations would make the dimeric structures negligible. Although we considered both active and inactive structures of MOR, the transition between them was prevented by the elastic network required by the coarse-grained force field we used. Another limitation – inherent to all coarse-grained representations – is the loss of spatial resolution in these simulations.

A different aspect of GPCR allostery that presents a significant challenge for all-atom, standard MD simulations is the study of the dynamic coupling of GPCRs with G proteins or β -arrestins, which is of course how the GPCR transmits information to the cellular environment. A recent investigation from Latorraca et al., however, used unbiased MD simulations to probe the mechanism behind arrestin activation [26]. In particular, these simulations of rhodopsin-mediated activation of arrestin-1 provided atomic-level evidence for the importance of both the transmembrane receptor core as well phosphorylated cytoplasmic receptor tail in arrestin activation. Initiating MD simulations with arrestin-1 bound to either the rhodopsin core or tail, it was observed that the rotation angle of the C-domain relative to the N-domain – one of the main metrics differentiating active from inactive arrestin state -- remained much closer to its active state value. Concurrent experiments from Eichel et al. [27] found that β -arrestin-2 could accumulate in clathrin-coated structures within HEK 293 cells in the presence of either active β 1-adrenergic receptor or B2AR, but without co-accumulation of the GPCRs, and that the β -arrestins were activated through transient binding to the GPCR core. Unbiased MD simulations from Eichel et al. [27] revealed that salt bridge formation between residues K78 and E314 stabilizes the inactive state of β -arrestin 2, making it possible that the GPCR core induces arrestin activation through destabilizing their interaction. Although MD simulations were used to study the GPCR mediated activation process of arrestin, the timescale issue (i.e. simulating long enough times) prevented simulating the actual binding process, which is

likely to be the rate determining step in the process of arrestin activation and likely to contain important insights.

In a recent publication [28], using site-directed spin labeling and the EPR spectroscopic method, structural constraints for both the Gi protein and active-state rhodopsin were determined in order to arrive to a model for the rhodopsin-Gi protein complex. This model provided the first structure for a GPCR-G protein complex not based on Gs based proteins, with the aim of illuminating possible structural features behind GPCR selection of specific G proteins for coupling. Very short unbiased MD simulations from the final model structure showed that the complex -- as well as their binding pose -- were stable, lending further support to the model in the absence of solved experimental structures. In particular, a comparison with the B2AR-Gs complex showed that the binding mode for the model rhodopsin-Gi complex was different, with the TM5-TM6 loop in rhodopsin contacting the β 6-sheet of the Gi protein, which was absent in the B2ARGs complex [28]. Very recent μ s-long unbiased MD simulations of the angiotensin II type 1 receptor (AT1R) gave further support to the significant conformational heterogeneity of the inactive state of AT1R demonstrated by a series of DEER experiments designed to map the conformational distribution of AT1R either in the absence or presence of different biased ligands [29]. One of the interesting conclusions of this study is that β -arrestin can recognize a larger repertoire of conformations, from open to occluded at the intracellular side, whereas the G protein can only bind open conformations.

It is also known that the solvent medium, through a possible role on GPCR conformational dynamics, will likely be influential to the GPCR activation processes. Yet, most MD simulations of GPCR-lipid interactions published to date use very simple, often coarse-grained, lipid compositions of the cell membrane, and very few investigate the effect of different lipid environments on the receptor allosteric behavior, perhaps due to the large computational resources required to address these types of questions as well as the questions regarding the reliability of their force-fields [30]. A recent unbiased all-atom MD-based investigation, however, looked into the effect of cholesterol embedded in the lipid bilayer on the serotonin 5-hydroxytryptamine 2A receptor (5-HT2A) [31]. Here, a correlation was found between the presence of cholesterol and the structural flexibility of the system. Furthermore, projection of the conformational dynamics onto the subspace of the two principal modes of the system revealed a tendency for a cholesterol-rich system to sample a much greater conformational variation in contrast to a cholesterol-depleted system. A decrease of 10 % in the number of hydrogen bonds formed between 5-HT2A and the first lipid bilayer shell with the inclusion of cholesterol was observed to be a major cause for their different conformational dynamics. In another very recent, but coarse-grained MD simulation study, A2AR in one of three different conformational states was placed in a lipid bilayer containing 10 different lipids in order to approach the behavior of GPCRs *in vivo* [32]. Three of the 10 lipids -- namely cholesterol, GM3 and PIP2 -- were found to interact with A2AR regardless of its conformational state, although their relative affinities for the receptor was found to be highly dependent on receptor conformation. In particular, it was found that PIP2 bound tightly in a space between the TM6 and TM7 and thereby stabilized the outward movement of TM6 accompanying receptor activation and consequent G protein activation. This was corroborated by a one-dimensional free energy profile for the

dissociation of a mini-Gs protein from the receptor, which was ~10 kcal/mol smaller in the absence of PIP2. Interestingly, the presence of cholesterol has been implicated in the GPCR dimerization process as well [33]. In general, the studies of lipid interaction with GPCRs involve coarse-grained MD simulations in order to reach the μ s timescale and are therefore dependent on the quality of the available coarse-grained force fields. We refer the interested readers to a recent review devoted solely to lipid-GPCR studies via coarse-grained simulations [30].

Conclusions

The mechanistic, thermodynamic, and kinetic information that emerges from MD simulations has made it an invaluable complementary tool to the more static picture that emerges from the majority of experimental structural biology approaches. With the continuing developments in methodology, MD simulations have now been successfully deployed to study a whole host of biological systems including GPCRs, with particular emphasis on understanding allostery, and its role in receptor activation and functional selectivity. Here we have summarized recent MD simulation studies of allostery in GPCRs. We expect that the number of contributions of MD to GPCR structure, function, and dynamics will continue to increase, and provide unique testable hypotheses to complement information from experimental biophysical methods.

Acknowledgments

The Filizola lab is funded by National Institutes of Health grants DA045473, DA026434, DA038882, MH107053, and DA034049 for work on GPCRs. Computations are run on resources available through the Scientific Computing Facility at Mount Sinai and the Extreme Science and Engineering Discovery Environment under MCB080077, which is supported by National Science Foundation grant number ACI-1053575.

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- of special interest
- of outstanding interest

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Figure 1. A schematic diagram depicting three standard approaches to using MD simulations. The text in green font is used to highlight the usefulness of each approach whereas the text in red font is used to highlight one of the major limitations associated with each approach. Notice that the classical force fields used to approximate interaction terms between particles is an inherent limitation to all approaches, whether at the all-atom or coarse-grained level.

Figure 2. Structural differences emerging from the dynamic behavior of MOR bound to a classical opioid agonist (morphine) or a G protein-biased ligand (TRV-130) during receptor activation.

While one of the most probable deactivation pathways of the morphine-bound MOR system involves a direct transition from the active-like crystallographic MOR state (blue cartoon) to the inactive-like crystallographic state of MOR (gray cartoon), the majority of activation paths in the system bound to TRV-130 traverse a specific active-like state (salmon cartoon). Notice major rearrangements in TM5, TM6 and helix 8.