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Recent advances in computational studies of GPCR-G protein interactions

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Abstract

Protein-protein interactions are key in cellular signaling. G-protein-coupled receptors (GPCRs), the largest superfamily of human membrane proteins, are able to transduce extracellular signals (e.g., hormones and neurotransmitters) to intracellular proteins, in particular the G proteins. Since GPCRs serve as primary targets of ~1/3 of currently marketed drugs, it is important to understand mechanisms of GPCR signaling in order to design selective and potent drug molecules. This chapter focuses on recent advances in computational studies of the GPCR-G protein interactions using bioinformatics, protein-protein docking and molecular dynamics simulation approaches.

Keywords

Protein-Protein Interactions; GPCR-G Protein Interactions; Bioinformatics; Protein-Protein Docking; Molecular Dynamics

1. Introduction

Protein–protein interactions (PPIs) are central to many biological processes, including human immune responses and cellular signaling. PPIs have been targeted for developing small-molecule modulators as therapeutic drugs (Andreani & Guerois, 2014; Arkin & Wells, 2004). In particular, the interactions between G-protein-coupled receptors (GPCRs) and heterotrimeric guanine nucleotide-binding proteins (G proteins) are one of the most important cellular signaling events. Due to critical roles, GPCRs represent primary targets of ~1/3 of currently marketed drugs (Hopkins & Groom, 2002). The classical function of GPCRs is to transmit extracellular signals across the plasma membrane and activate intracellular proteins, e.g., the G proteins, which leads to further signaling of downstream effector proteins. The G protein (Moreira, 2014; Simon, Strathmann, & Gautam, 1991) consists of three structural subunits (Gα, Gβ and Gγ), for which 21, 6 and 12 different subtypes have been identified, respectively. There are ~700 unique heterotrimeric G proteins in the human genome. Moreover, GPCRs have ~800 different members in the superfamily. Interactions of GPCRs and the G proteins could thus involve hundreds of thousands of possibilities. However, GPCRs are known to selectively couple with the G proteins (Flock et al., 2017). It is important to understand the mechanism of GPCR-G protein coupling

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specificity, which will greatly facilitate effective drug design (Pardon et al., 2018; Weiss et al., 2013).

Recent breakthroughs in structural biology including X-ray crystallography and cryo-electron microscopy (cryo-EM) have enabled determination of more than ten GPCR-G protein complex structures (Carpenter, Nehmé, Warne, Leslie, & Tate, 2016; Chung et al., 2011; DeVree et al., 2016; Draper-Joyce et al., 2018; García-Nafría, Lee, Bai, Carpenter, & Tate, 2018; Huang et al., 2015; Liang et al., 2017; Rasmussen, Choi, et al., 2011; Ring et al., 2013; Scheerer et al., 2008). As summarized in Table 1, 17 GPCR structures are complexed with the G proteins or G protein mimics, including opsin coupled with the C-terminal peptide of the G_α subunit (Scheerer et al., 2008), the β_2 adrenergic receptor (β_2AR) with the G_s protein (Rasmussen, DeVree, et al., 2011) or the G-protein mimetic nanobody (Rasmussen, Choi, et al., 2011; Ring et al., 2013), rhodopsin coupled with arrestin (X. E. Zhou et al., 2017) or the G_i protein (Kang et al., 2018), the adenosine A_1 receptor (A_1AR) bound by the G_i protein (Draper-Joyce et al., 2018), the adenosine A_{2A} receptor (A_{2A}AR) bound by the "mini-G_s" (Carpenter et al., 2016) or G_s protein (García-Nafría, Lee, et al., 2018), the μ-opioid receptor (μOR) bound by the G-protein mimetic nanobody (Huang et al., 2015) or G_i protein (Koehl et al., 2018), the calcitonin receptor coupled with the G_s protein (Liang et al., 2017) and the serotonin 5-HT_{1B} receptor coupled with the G_o protein (García-Nafría, Nehmé, Edwards, & Tate, 2018). These structures provide important insights into active conformations of GPCRs and atomic GPCR-G protein interactions. However, the X-ray and cryo-EM structures are rather static images. It remains largely unknown how GPCRs dynamically recognize specific G proteins.

In addition to structural biology, experimental techniques including mutagenesis (Blin, Yun, & Wess, 1995; Burstein, Spalding, & Brann, 1998; Chen et al., 2010; Conklin, Farfel, Lustig, Julius, & Bourne, 1993; Erlenbach et al., 2001; Kostenis, Conklin, & Wess, 1997; Liu, Conklin, Blin, Yun, & Wess, 1995; Marin, Krishna, & Sakmar, 2001; Moro, Lameh, Hogger, & Sadee, 1993; Preininger et al., 2009; Schoneberg, Kostenis, Liu, Gudermann, & Wess, 1998; Slessareva & Graber, 2003; Valiquette, Parent, Loisel, & Bouvier, 1995; Wacker et al., 2008; Xiao et al., 1999), nuclear magnetic resonance (NMR) (Kim et al., 2013), hydrogen-deuterium exchange mass spectrometry (HDXMS) (Chung et al., 2011; Orban et al., 2012), and double electron-electron resonance spectroscopy (DEER) (Van Eps et al., 2018) have been utilized to investigate the GPCR-G protein interactions (Mahoney & Sunahara, 2016; Moreira, 2014; Preininger, Meiler, & Hamm, 2013). While the C-terminal α_5 helix in the G_α subunit has been suggested as the primary driver for specific receptor recognition (Blin et al., 1995), the G_α α_N helix and receptor intracellular loop (ICL) 2 and transmembrane (TM) helix 6 further contribute to the GPCR–G protein coupling specificity (Burstein et al., 1998; Chen et al., 2010; Neumann, Krause, Claus, & Paschke, 2005; Preininger et al., 2013; Timossi et al., 2002; Zhou, Yan, Yamamoto, & Tai, 1999). Furthermore, dynamic regions in the complex can be crucial for the coupling through allosteric conformational changes (Mahoney & Sunahara, 2016; Preininger et al., 2013). The precise conformation of active GPCRs also depends on chemical properties of the binding agonist. For example, agonist binding often leads to a change in the receptor conformation such as opening of the intracellular G protein binding pocket for coupling to the G proteins (Zocher, Fung, Kobilka, & Muller, 2012). The experimental studies have greatly advanced

our knowledge in the field, but the exact determinants of specific GPCR-G protein interactions remain unclear.

On the other hand, computational modeling has proven useful in studying PPIs (Janin et al., 2003; Shoemaker & Panchenko, 2007). Here, we review computational studies of GPCR-G protein interactions using various techniques, including bioinformatics, protein-protein docking and molecular dynamics (MD) simulation.

2. Bioinformatics of GPCR-G protein interactions

Significantly increasing information about the sequences, structures and signaling networks of GPCRs and the G proteins has become available in recent years. A number of bioinformatics and software tools as listed in Table 2 are useful for exploring GPCR-G protein interactions, including the protein data bank (PDB) (Berman et al., 2000), the GPCRdb (Munk et al., 2016), gpDB (Theodoropoulou, Bagos, Spyropoulos, & Hamodrakas, 2008) and human gpDB (Satagopam et al., 2010).

The GPCRdb is a widely used database for studying GPCRs (Munk et al., 2016). It contains valuable information about the structures, known mutations, homologues, ligands and phylogenetic relationships of GPCRs. Besides, the GPCRdb provides useful functions, such as generation of GPCR models and identification of ligand binding sites for virtual screening. Systematic analysis of data from the GPCRdb could deepen our understanding of GPCRs and the interactions with their G proteins. For example, Suku et al. performed systematic analysis of ligand binding pockets for GPCRs collected in the GPCRdb (Suku & Giorgetti, 2017). Ten residues including 3.32, 3.33, 3.36, 6.48, 6.51, 6.52, 6.55, 7.35, 7.39 and 7.43 (Ballesteros-Weinstein numbering of GPCRs (Ballesteros & Weinstein, 1995)) were identified to interact with ligands. In addition, these residues were found to be conserved and share a common evolutionary history. More recently, a bioinformatics approach has been applied to determine a selectivity barcode (patterns of amino acids) of GPCR-G protein coupling based on the data obtained from the GPCRdb (Flock et al., 2017). While universally conserved residues in the barcode allow GPCRs to bind and activate G protein in a similar manner, different receptors recognize unique positions of the G protein barcode through distinct residues. In summary, bioinformatics has become highly useful in extracting valuable information about GPCR-G protein interactions across the entire family of GPCRs and the G proteins.

3. Protein-protein docking on GPCR-G protein interactions

Since experimental structures of GPCR-G protein complexes are still very limited, protein–protein docking is an efficient computational approach to generate the complex models. It has been successfully applied to construct structures of GPCR-G protein complexes (Alexander et al., 2014; Pawlowski, Saraswathi, Motawea, Chotani, & Kloczkowski, 2014; Shim, Ahn, & Kendall, 2013), in addition to GPCR oligomers (Borroto-Escuela et al., 2018). Pawlowski et al. performed protein-protein docking to investigate the binding specificity between the human α2C-adrenoreceptor (ADRA2C) and the filamin-2 (FLN2) actin binding protein (Pawlowski et al., 2014). There was no experimental structure of the ADRA2C or

FLN2. Homology modeling was first performed to obtain their separate structures, which were used to build the complex structure with the HADDOCK server (de Vries, van Dijk, & Bonvin, 2010). Combining multiple sequence alignments and phylogenetic analysis, the authors found that electrostatic interactions between residues R454 and R456 in the ADRA2C and negatively charged residues in the FLN2 play an important role in the protein coupling. In order to investigate the mechanism of GDP release from the G protein, Alexander *et al.* utilized homology modeling, protein-protein docking and DEER experiments to construct a model of the active state of rhodopsin complexed with a heterotrimeric $G_{\alpha i\beta\gamma}$ protein (Alexander et al., 2014). With the template X-ray structure of the β_2AR-G_s complex, a homology model was first built using the Rosetta software. Then 1,000 independent protein-protein docking calculations were performed, resulting in a pool of 739 nonclashing models. Nine structures that could reproduce the DEER distances and signal shapes were identified. These structures suggested that the C terminus of the Gα α5 helix triggers conformational changes in the helical domain, which lead to GDP release. Based on the resulting models, energetic analysis was performed to identify residues that showed marked changes between the receptor-bound and free forms of the G protein. In another study by the same group, the important role of the α5 helix of Gα in the activation of the G protein was demonstrated through mutagenesis experiments (Kaya et al., 2014). Therefore, protein-protein docking has facilitated building structures and understanding protein interactions of the GPCR-G protein complexes. However, due to the flexible nature of GPCRs and the G proteins, insufficient accuracy of protein-protein docking has largely limited its applications in modeling GPCR-G protein complex (Kaczor, Selent, Sanz, & Pastor, 2013).

4. Molecular dynamics simulations of GPCR-G protein interactions

MD is a powerful computational technique for simulating biomolecular dynamics at an atomistic level (Karplus & McCammon, 2002). MD is able to provide dynamic information about the interactions between GPCRs and the G proteins, which is missing in static experimental structures and protein-protein docking. Thus, MD has been applied to investigate the GPCR-G protein interactions. Although there have been many MD applications on GPCRs or G proteins alone (Grossfield, 2011; Johnston & Filizola, 2011; Miao & McCammon, 2016a; Vanni & Rothlisberger, 2012; Yao et al., 2016), here we will focus on the GPCR-G protein interactions.

Since GPCRs are membrane proteins, their structural dynamics and function of GPCRs (including interactions with the G proteins) could be strongly affected by lipids (Yen et al., 2018). The orientation and position of GPCRs in the lipid membrane need to be carefully modelled. In this regard, the Orientations of Proteins in Membranes (OPM) database (Lomize et al., 2012) is useful in the modeling of membrane proteins, including GPCRs. In addition, CHARMM-GUI is an online webserver (http://www.charmm-gui.org/) (Jo et al., 2017; Jo, Kim, Iyer, & Im, 2008), which can be used to generate a simulation-ready system for a membrane-embedded protein and input files for various MD software packages, including AMBER, NAMD, GROMACS, and so on. It has significantly reduced the effort of system preparation for MD simulations.

Overall, MD simulations have greatly helped us understanding the GPCR-G protein interactions. However, due to limited timescales, direct MD simulations often suffer from insufficient sampling of the GPCR-G protein interactions. To overcome MD limitations, many enhanced sampling methods have been developed during the last several decades (Abrams & Bussi, 2014; Christen & van Gunsteren, 2007; Dellago & Bolhuis, 2009; Gao, Yang, Fan, & Shao, 2008; Liwo, Czaplewski, Ołdziej, & Scheraga, 2008; Miao & McCammon, 2016c; Spiwok, Sucur, & Hosek, 2015). Several enhanced MD methods have been successfully applied to study GPCR-G protein interactions, including umbrella sampling (Kästner, 2011; Rose et al., 2014; Torrie & Valleau, 1977), metadynamics (Alessandro & Francesco, 2008; Laio & Parrinello, 2002; Saleh, Ibrahim, & Clark, 2017; Saleh, Ibrahim, Saladino, Gervasio, & Clark, 2017; Saleh, Saladino, Gervasio, & Clark, 2017) and Gaussian accelerated molecular dynamics (GaMD) (Miao, Feher, & McCammon, 2015; Miao & McCammon, 2018; Pang, Miao, Wang, & McCammon, 2017). As summarized in Table 3, determinants of coupling selectivity between the GPCR and G protein (Kling, Lanig, Clark, & Gmeiner, 2013; Mnpotra et al., 2014; Rose et al., 2014; Shim et al., 2013), effects of different ligand binding on the stability of GPCR-G protein complexes (Bai, Zhang, Ban, Liu, & Yao, 2013; Feng, Hou, & Li, 2012; Goetz, Lanig, Gmeiner, & Clark, 2011; Miao & McCammon, 2016b; Saleh, Ibrahim, & Clark, 2017; Saleh, Saladino, et al., 2017; Shirvanyants, Ding, Tsao, Ramachandran, & Dokholyan, 2012), the G protein activation upon binding of a GPCR (Dror et al., 2015) and spontaneous binding of the G-protein mimetic nanobody to a GPCR (Miao & McCammon, 2018) have been investigated through MD simulations and will be discussed in the following.

4.1. Determents of GPCR-G protein coupling specificity

MD simulations have been carried out to identify determinants of the GPCR-G protein coupling specificity. Kling et al. (Kling et al., 2013) reported microsecond MD simulations of ternary GPCR complexes, including the experimentally determined agonist-bound β_2AR-G_s and two homology models of the dopaminergic D_2 receptor (D2R) bound by the G_i protein. Important residues were located at the receptor intracellular end of the TM5 helix and the N-terminal region of the ICL3, which interacted with the α5 helix and α4/β6 loop in the Gα protein subunit.

The TM6 helix of GPCRs was identified as an important domain in determining the coupling selectivity between the receptors and G proteins (Kang et al., 2018; Rose et al., 2014; Shim et al., 2013; Van Eps et al., 2018). MD and umbrella sampling simulations were performed on the β_2AR bound by the C-terminal peptide of the Gα (GαCT) that was used as a surrogate of the G protein (Rose et al., 2014). The simulations suggested that distinct conformations of the β_2AR induced by binding of different G proteins co-existed in the G-protein free (*apo*) state of the receptor. Conformational heterogeneity of the TM6 emerged when the β_2AR was bound by the G_i or G_s protein. The important role of TM6 in the coupling selectivity was also demonstrated by Xu et al. (Kang et al., 2018) through structural biology and MD simulations. MD simulations were performed on four systems, including the G_i protein complexed with the rhodopsin and μOR and the G_s protein bound by the β_2AR and A_{2A}AR. Results showed that the outward movement of TM6 was less pronounced in the G_i-coupled than in the G_s-coupled receptors. This was consistent with

model of the rhodopsin-G_i protein complex, which was tested by MD simulation using the distance constraints from DEER experiments (Van Eps et al., 2018).

The G_α α5 helix of the G protein has been also shown to be important for specific GPCR-G protein coupling. Shim et al. combined MD simulation and mutagenesis experiments to identify critical regions for coupling of the CB1 receptor with the G_i protein (Shim et al., 2013). Guided by the X-ray structure of the β_2AR-G_s, a model was built for the CB1-G_i ternary complex. Through an 824 ns MD simulation, they found that tight interactions between the CB1 and the G_α α5 helix of the G_i protein were crucial for the receptor-G protein binding. Mnpotra et al. applied MD simulations to explore the interactions between the CB2 and G_i protein (Mnpotra et al., 2014). Results showed that the G_i protein could reorient to a different binding mode in comparison with orientation of the G_s protein in the β_2AR-G_s complex. During reorientation of the G_i protein, two major conformational changes occurred. First, the G_α α5 helix of the G_i protein tilted due to outward movement of the TM5 helix in CB2. Second, a 25° clockwise rotation of the G_i protein took place, leading to interaction of the receptor ICL2 with a hydrophobic pocket formed by residues Val34, Leu194, Phe196, Phe336, Thr340, Ile343 and Ile344 in the $G_{\alpha i}$. This structural model was highly consistent with the data obtained from cross-linking studies (Mnpotra et al., 2014).

In summary, the above MD studies have greatly advanced our knowledge of GPCR-G protein interactions. Several important structural motifs that contribute to the GPCR-G protein coupling specificity were identified, including the G_α α_5 helix of the G protein (Blin et al., 1995; Mnpotra et al., 2014; Shim et al., 2013) and the receptor ICL2/ICL3 and TM6 helix (Kang et al., 2018; Rose et al., 2014; Shim et al., 2013; Van Eps et al., 2018).

4.2. Effects of ligand binding on GPCR-G protein interactions

GPCR signaling occurs via ternary complexes formed under cooperative binding between the receptor, ligand and an intracellular binding partner (IBP). Ligand binding could lead to a conformational change of the receptor (e.g., opening of the intracellular pocket) for coupling to the G protein (DeVree et al., 2016). Conversely, binding of the G protein in the intracellular binding site could allosterically influence ligand binding in the receptor orthosteric site. DeVree et al. demonstrated that binding of the G protein in the β_2AR could allosterically close the receptor extracellular ligand-binding pocket (DeVree et al., 2016). The allosteric interaction between the orthosteric site and the G protein binding pocket is thus involved in the GPCR-G protein interactions. MD simulations were performed on the agonist-β_2AR-$G_{\alpha s}$ complex system (Feng et al., 2012). Interaction between the β_2AR and G_s protein was found to be stable when the complex was bound by the Nb35 nanobody. Without Nb35, the agonist could trigger conformational changes of β_2AR from the extracellular to the intracellular domains. The importance of nanobody in stabilizing the GPCR-agonist-IBP ternary complex was also demonstrated using GaMD in the simulations of the M2 muscarinic receptor (Miao & McCammon, 2016b). The intracellular domain of TM6 could remain in its active state when the receptor was bound with the Nb9–8 nanobody, while removal of Nb9–8 led to inward movement of the TM6 and deactivation of the M2 receptor. These simulation findings were consistent with experimental data obtained from NMR (Nygaard et al., 2013) and DEER studies (Manglik et al., 2015), which indicated

that binding of a G protein or G protein mimic is required to stabilize the active conformational state of GPCRs in addition to agonist binding. Metadynamics simulations were also performed to investigate the similarities and differences between β_2AR-agonists bound by different IBPs, including the G_s protein and the G protein mimetic nanobody (Saleh, Ibrahim, & Clark, 2017). Important intermediate states were identified for the GPCR upon binding of different agonists and IBPs.

Goetz et al. applied MD simulations to determine the effects of different agonist and inverse agonist binding on stability of the β_2AR complexed with the C terminus of the Gαs subunit ($G_{\alpha s}$CT) (Goetz et al., 2011). The simulations showed that the ligand-binding pocket conformation and interaction between the $G_{\alpha s}$CT and β_2AR were different upon binding of the isoprenaline agonist and carazolol inverse agonist. Isoprenaline induced an inward movement of the TM5 in the orthosteric binding site of the β_2AR, whereas carazolol blocked rearrangement of the extracellular domains of the receptor. Moreover, the β_2AR and $G_{\alpha s}$CT formed stable interaction in the presence of isoprenaline, while the complex was destabilized by binding of carazolol. In another study, MD simulations of the β_2AR complexed with the entire G_s protein were performed by Bai et al. to investigate the binding effects of three different ligands (e.g. agonist BI-67107, inverse agonist ICI 118,551 and antagonist alprenolol) (Bai et al., 2013). Their results suggested that BI-67107 formed three more stable hydrogen bonds with the receptor (residues Ser203$^{5.42}$, Ser207$^{5.46}$ and Asn293$^{6.55}$) than ICI 118,551. Thus, BI-67107 was able to stabilize the β_2AR in the active state. Binding of the ICI 118,551 inverse agonist could change β_2AR from the active to the inactive state, as well as inducing dissociation of the G_α and $G_{\beta\gamma}$ subunits.

More recently, Saleh et al. applied metadynamics simulations to investigate structural dynamics and free energy profiles of the β_2AR-arrestin and β_2AR-G_s complexes, in the absence or presence of different ligands (Saleh, Saladino, et al., 2017). The ligands included the full G_s/arrestin agonist isoprenaline, the G_s/arrestin unselective antagonist alprenolol, the G_s inverse agonist/arrestin antagonist ICI 118,551 and the G_s inverse agonist/arrestin partial agonist carvedilol. The simulations suggested that agonists and partial agonists increased the binding affinity of the G protein or arrestin to the β_2AR. Antagonists left the binding affinity largely unaffected or decreased it slightly. Inverse agonists decreased it significantly. An extended ternary complex model was then proposed, in which the ligand bias towards either the G-protein or arrestin pathway is regulated by cooperative binding of the receptor, ligand and IBP. The free energy changes could be used to characterize the ligand signaling bias, which was suggested to be a promising approach for rational design of GPCR biased agonists.

4.3. Activation of the G protein upon binding of GPCRs

The G proteins are molecular switches that turn on intracellular signaling cascades in response to the activation of GPCRs by extracellular stimuli. Their switching function depends on the ability of the G_α subunit to cycle between an inactive GDP-bound state and an active GTP-bound state. Thus, mechanisms about how the GPCR catalyzes GDP release on cognate G protein and how the G protein transits between its different states are significantly important in understanding the signal transduction within the GPCR-G protein

complex. Both experimental and computational techniques have been utilized to address the above questions (Duc, Kim, & Chung, 2015; Mahoney & Sunahara, 2016; Nguyen Minh, Hee Ryung, & Ka Young, 2017). Extensive MD simulations performed by Dror *et al.* demonstrated that separation of the Ras and helical domains was necessary but not sufficient for GDP release from the G protein (Dror et al., 2015). Conformational changes in the G_α α5 helix was concomitant with opening of the helical domain. The repositioned α5 helix weakened binding of the GDP, facilitating its release from the G_s protein. These predictions were validated by the DEER spectroscopic experiments. Conformational changes in the G_α α5 helix and opening of the helical domain in the β_2AR-G_s complex were also observed in computational modeling by Pachov et al. using a Kino-Geometric Sampling (KGS) method (Pachov et al., 2016). This study demonstrated that interactions between the αN helix of the G_s protein and the receptor ICL2 facilitate nucleotide exchange by weakening a salt bridge between the P-loop and switch 1 in the G_s protein. Despite these advances, we still lack a detailed understanding of the mechanisms of the G protein binding to GPCRs, the G protein catalyzed hydrolysis of GTP to GDP and allosteric modulation of nucleotide binding in the G proteins by GPCRs (Duc et al., 2015; Mahoney & Sunahara, 2016; Nguyen Minh et al., 2017).

4.4. Mechanism of GPCR-G protein binding

MD simulations of protein-protein binding are challenge, due to the limited simulation timescales while slowly evolving protein dynamics. Nevertheless, remarkable advances in supercomputing have enabled the D.E Shaw research group to successfully simulate binding of five different protein-protein systems through exceptionally long-timescale MD simulations (Pan et al., 2018). Hundreds-of-microseconds conventional MD simulations captured spontaneous protein-protein binding events. Furthermore, repeated protein association and dissociation were observed in enhanced MD simulations using a “tempered binding” approach (Pan et al., 2018).

In the context of GPCR-G protein interactions, powerful enhanced MD simulations successfully captured spontaneous binding of a G-protein mimetic nanobody to a muscarinic GPCR using the GaMD method (Miao & McCammon, 2018). With X-ray structure of the agonist-nanobody-M2 receptor complex, the agonist and nanobody were initially displaced to >20 Å far away from the active M2 receptor. Five 4.5 μs independent GaMD simulations were performed. Although the agonist could not reach the X-ray binding pose in the receptor orthosteric binding pocket, the nanobody could successfully bind to the receptor G-protein coupling site in one GaMD simulation with a minimum RMSD of 2.48 Å in the nanobody core domain compared with the X-ray structure. The GaMD simulations showed significant conformational changes in both the orthosteric ligand-binding pocket and intracellular domains of the M2 receptor upon nanobody binding. Binding of the nanobody switched the orthosteric pocket from the “open” to “closed” conformation and led to activation of the M2 receptor with an increase in the intracellular TM3–TM6 distance. Moreover, two important low-energy intermediate conformational states were identified during binding of the G-protein mimetic nanobody. The nanobody formed transient electrostatic, hydrogen bonding and hydrophobic interactions with the receptor through the binding process. The flexible receptor ICLs played a key role in the recognition and binding of the nanobody (Miao &

McCammon, 2018). Therefore, GaMD simulations provided important insights into the mechanism of the G-protein mimic binding to a GPCR.

5. Discussions and Outlook

Interactions with the intracellular G proteins represent a canonical signaling pathway of GPCRs, key membrane proteins that serve as primary targets of ~1/3 currently marketed drugs. Structural determination of GPCR-G protein complex has exploded in very recent years, due to breakthroughs in X-ray crystallography and cryo-EM (Table 1). Extensive research studies have been focused on GPCR-G protein interactions using various experimental techniques (e.g. mutagenesis, NMR, HDXMS and DEER) in addition to the structural biology. These studies have greatly facilitated our understanding of GPCR-G protein interactions. However, the experimental techniques often suffer from limited spatial and temporal resolutions, as well as high cost. In this regard, computational modeling has proven useful and efficient in studies of GPCR-G protein interactions. Complementary experimental and computational techniques have been combined in numerous studies in order to obtain a more detailed picture of GPCR-G protein interactions. Here, we have focused on reviewing recent studies of GPCR-G protein interactions with computational approaches, including bioinformatics, protein-protein docking and MD simulations.

With dramatically increasing information that is collected about GPCR-G protein interactions, bioinformatics has been applied to determine major determinants of coupling selectivity between GPCRs and the G proteins (Flock et al., 2017). Bioinformatics is useful in providing an overview of protein-protein interactions for the entire family of GPCRs and the G proteins. On the other hand, docking and MD simulation are able to generate a more detailed picture of target GPCR-G protein interactions of interest. There are several advantages for protein-protein docking. It is highly efficient. The docking software tools and webservers are mostly user friendly. Docking calculations are usually fast without the need of expensive computational resources. They are able to generate computational models of protein complex, e.g. GPCR-G protein complex structures. These models could provide valuable information about overall conformations of the GPCR-G protein complexes. However, applications of protein-protein docking in modeling GPCR-G protein interactions are still limited due to low accuracy. Limited capability to account for protein flexibility and inaccuracy of docking scores often require the use of protein-protein docking in combination with experiments (Alexander et al., 2014) and/or MD simulations for further validation (Shim et al., 2013).

Applications of MD simulations in molecular biology and drug discovery have dramatically increased in recent years especially in the research field of GPCRs (Hollingsworth & Dror, 2018; Latorraca, Venkatakrishnan, & Dror, 2017; Miao & McCammon, 2016a). Remarkable developments in both the computing hardware (e.g. the Anton specialized super computer and fast GPUs) and software tools have enabled long-timescale MD simulations. MD simulations have been performed over microseconds to milliseconds. The MD simulations have provided important insights into the dynamic mechanism of GPCR-G protein interactions at an atomistic level (Latorraca et al., 2017). Nevertheless, binding of intracellular G proteins to GPCRs is challenging for conventional MD simulations. In this

regard, enhanced MD simulations are useful to help address the challenge. Notably, enhanced simulations using the GaMD method captured spontaneous binding of the G-protein mimetic nanobody to a GPCR (Miao & McCammon, 2018). Pan et al. simulated both binding and unbinding of five different protein-protein systems using a “tempered binding” approach (Pan et al., 2018), although the GPCR-G protein was not included in their simulated systems. Therefore, innovations in both computing hardware and enhanced sampling methods have opened a new era in MD simulations of protein-protein binding. Continued developments are expected to enable simulations of binding processes between GPCRs and the cognate G proteins in the near future. Such studies will potentially reveal mechanisms of the GPCR-G protein interactions and the cooperative activation of GPCRs and G proteins at an atomistic level.

In summary, bioinformatics, protein-protein docking and MD simulation have proven useful for exploring the GPCR-G protein interactions. Combination of computational and experimental modeling and complementary experiments will help us to obtain a detailed understanding of the GPCR-G protein interactions and GPCR signaling mechanism. This will greatly facilitate more effective computer-aided drug design of GPCRs (Huang et al., 2015; Korczynska et al., 2018; Miao et al., 2016; Miao & McCammon, 2016a).

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Table 1.

Structures of GPCRs complexed with the G proteins or G protein mimics

GPCR	G protein or mimetic nanobody	PDB ID (resolution)	Method	Reference
Opsin	C-terminal peptide of G_α transducin	3DQB (3.2Å)	X-ray	(Scheerer et al., 2008)
β_2AR	G_s and nanobody Nb35	3SN6 (3.2Å)	X-ray	(Rasmussen, DeVree, et al., 2011)
β_2AR	Nanobody Nb80	3P0G (3.5Å)	X-ray	(Rasmussen, Choi, et al., 2011)
M2	Nanobody Nb9–8	4MQS (3.5Å)	X-ray	(Kruse et al., 2013)
β_2AR	Nanobody Nb6B9	4LDE (2.7Å), 4LDL (3.1Å), 4LDO (3.2Å)	X-ray	(Ring et al., 2013)
μOR	Nanobody Nb39	5C1M (2.1Å)	X-ray	(Huang et al., 2015)
A_{2A}AR	Mini-G_s	5G53 (3.4Å)	X-ray	(Carpenter et al., 2016)
GLP-1R	G_s	5VAI (4.1Å)	Cryo-EM	(Zhang et al., 2017)
Calcitonin receptor	G_s	5UZ7 (4.1Å)	Cryo-EM	(Liang et al., 2017)
Rhodopsin	Arrestin	5W0P (3.0Å)	X-ray	(Zhou et al., 2017)
GLP1	G_s	6B3J (3.3Å)	Cryo-EM	(Liang et al., 2018)
KOR	Nanobody Nb39	6B73 (3.1Å)	X-ray	(Che et al., 2018)
μOR	G_i	6DDE (3.5Å), 6DDF (3.5Å)	Cryo-EM	(Koehl et al., 2018)
A_1AR	G_i	6D9H (3.6Å)	Cryo-EM	(Draper-Joyce et al., 2018)
A_{2A}AR	G_s and nanobody Nb35	6GDG (4.1Å)	Cryo-EM	(García-Nafría, Lee, et al., 2018)
Rhodopsin	G_i	6CMO (4.5Å)	Cryo-EM	(Kang et al., 2018)
5-HT_{1B}	G_o	6G79 (3.8Å)	Cryo-EM	(García-Nafría, Nehmé, et al., 2018)

Table 2.

Databases and software tools for modeling GPCR-G protein interactions

Database/ Software	Description	Reference and website
PDB	A database contains biological macromolecular structures determined by experiments.	(Berman et al., 2000) https://www.rcsb.org/
GPCRdb	A database contains structures, diagrams and web tools of GPCRs.	(Munk et al., 2016) http://gpcrdb.org/
gpPDB	A database contains information about GPCRs, effectors of GPCRs and their known interactions.	(Theodoropoulou et al., 2008) http://bioinformatics.biol.uoa.gr/gpDB/
Human gpDB	A database contains information about 713 human GPCRs, 36 human G-proteins and 99 human effectors.	(Satagopam et al., 2010) http://bioinformatics.biol.uoa.gr/human_gpdb/
OMP	A database provides information about structural classification of membrane proteins, topology, spatial positions in the lipid bilayer, and intracellular localization.	(Lomize, Pogozheva, Joo, Mosberg, & Lomize, 2012) https://opm.phar.umich.edu/
CHARMM-GUI	A web-based graphical user interface that helps preparation of biomolecular systems (including GPCRs and the G proteins) for molecular dynamics simulations.	(Jo et al., 2017) http://www.charmm-gui.org/

Table 3.

A summary of MD simulation studies on GPCR-G protein interactions.

System	Method	Major findings	Reference
β_2AR-G_αCT	MD	Interactions between the β_2AR and G_αCT are ligand dependent.	(Goetz et al., 2011)
β_2AR-G_s	MD	Nanobody plays an important role in stabilizing the β_2AR-G_s complex.	(Feng et al., 2012)
β_2AR-G_s	MD	Binding of different ligands affects stability of the β_2AR-G_s complex.	(Bai et al., 2013)
β_2AR-G_s D_2R-G_i	MD	Receptor ICL3 and the α5-helix of Gα play an important role in GPCR-G protein coupling.	(Kling et al., 2013)
CB1-G_i	MD, Ala mutation	The Gα α5 helix of the G protein plays an important role in the CB1-Gi coupling.	(Shim et al., 2013)
CB2-G_i	MD, cross-linking	The ICL2 in CB2 and the Gα α5 helix of the G protein play an important role in the CB2-G_i coupling.	(Mnpotra et al., 2014)
β_2AR-G_i/G_s	MD	The TM6 helix in the β_2AR plays an important role in binding selectivity of G_i and G_s proteins.	(Rose et al., 2014)
β_2AR-G_s	MD, DEER spectroscopy	Separation of the Ras and helical domain of the Gα subunit is necessary but not sufficient for rapid nucleotide release.	(Dror et al., 2015)
β_2AR-G_s	Kino-Geometric Sampling	Interaction between the αN helix of the G protein and the receptor ICL2 is important for nucleotide release.	(Pachov, Fonseca, Arnol, Bernauer, & van den Bedem, 2016)
M2-nanobody	GaMD	Nanobody is important in stabilizing the active conformational state of the M2 receptor.	(Miao & McCammon, 2016b)
β_2AR-G_s M2R-nanobody μOR-G_s/ nanobody	Metadynamic	The binding of intracellular binding partners alters agonist binding modes.	(Saleh, Ibrahim, & Clark, 2017)
β_2AR-G_s β_2AR-arrestin	Metadynamics	The structure and dynamics of GPCR-G protein complexes depend strongly on the nature of small-molecule ligands.	(Saleh, Saladino, et al., 2017)
Rhodopsin–G_i	MD, DEER	A model of rhodopsin-G_i is presented.	(Van Eps et al., 2018)
M2-nanobody	GaMD	GaMD captured spontaneously binding the G protein mimic nanobody to a muscarinic GPCR.	(Miao & McCammon, 2018)
Rhodopsin-arrestin	MD, Fluorescence spectroscopy	GPCRs could stimulate arrestin through interactions mediated by the receptor phosphorylated cytoplasmic tail (RP tail) only, the receptor core only, or both the receptor core and RP tail.	(Latorraca et al., 2018)