

# Predicting ligand binding poses for low-resolution membrane protein models:

## Perspectives from multiscale simulations

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### Abstract

Membrane receptors constitute major targets for pharmaceutical intervention. Drug design efforts rely on the identification of ligand binding poses. However, the limited experimental structural information available may make this extremely challenging, especially when only low-resolution homology models are accessible. In these cases, the predictions may be improved by molecular dynamics simulation approaches. Here we review recent developments of multiscale, hybrid molecular mechanics/coarse-grained (MM/CG) methods applied to membrane proteins. In particular, we focus on our in-house MM/CG approach. It is especially tailored for G-protein coupled receptors, the largest membrane receptor family in humans. We show that our MM/CG approach is able to capture the atomistic details of the receptor/ligand binding interactions, while keeping the computational cost low by representing the protein frame and the membrane environment in a highly simplified manner. We close this review by discussing ongoing improvements and challenges of the current implementation of our MM/CG code.

## **Research highlights**

- Multiscale methods can provide insights into membrane proteins.
- Structural information on human G-protein coupled receptors is very limited.
- Docking becomes challenging when only low-resolution homology models are available.
- Our molecular mechanics/coarse grained approach improves ligand pose predictions.

## **Keywords**

G-protein coupled receptor; Chemosensory receptor; Bitter taste receptor; Homology modeling; Molecular docking; Molecular mechanics/coarse-grained simulations

## **Abbreviations**

MD, Molecular dynamics, MM/CG, molecular mechanics/coarse-grained, GPCR, G-protein coupled receptor, hGPCR, human GPCR, TAS2R, taste 2 receptor, OR, olfactory receptor, H-AdResS, Hamiltonian Adaptive Resolution Scheme

## 1. Introduction

Membrane proteins constitute 20% of the human genome [1, 2]. They are of utmost importance for pharmaceutical applications, as they are the target of about 60% of FDA approved drugs [3]. Thanks to increasingly reliable molecular docking programs [4], predictions of ligand binding poses to membrane proteins have tremendously improved over the last decade [5-8]. Nonetheless, while highly successful when experimental structural information is used, docking methods may have limited predictive power for low-resolution homology models [4, 9-13], i.e., models built with sequence identity of 35% or lower with their structural templates [2, 14-17]. In these cases, the modeled orientation of the side chains, an obvious key facet of ligand/receptor molecular recognition, may lack the sufficient accuracy to make structural predictions. In addition, most docking algorithms neglect the presence of explicit solvent [18], even though water molecules may be crucial to stabilize the ligand in a variety of membrane proteins, such as G-protein coupled receptors [19, 20]. Furthermore, the current molecular docking approaches provide docking scores or estimated binding free energies that may correlate with the experimentally measured  $EC_{50}$  or  $IC_{50}$  values, but do not correspond to the actual binding free energies.

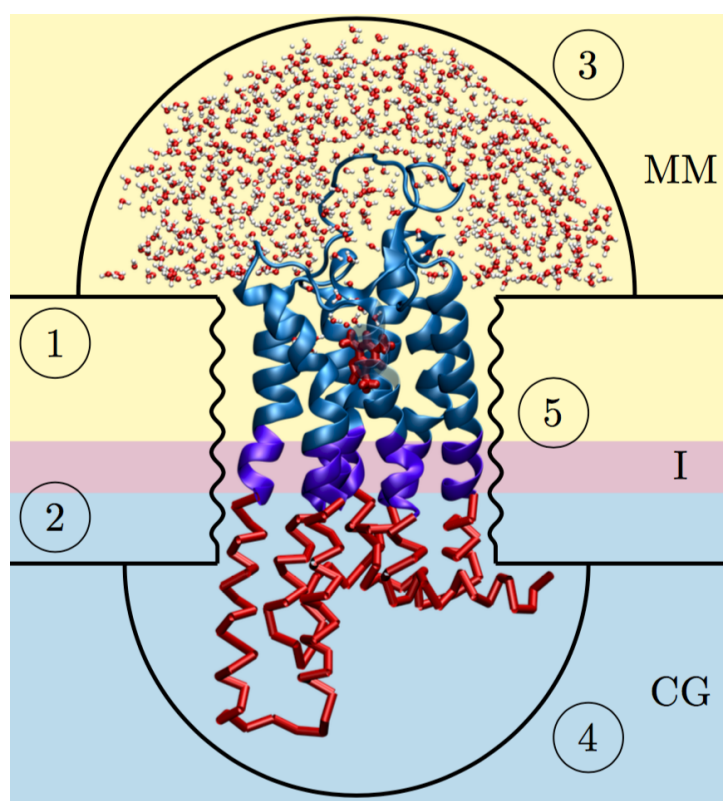
The predictions may be improved by combining docking with molecular dynamics (MD) simulations [21-23]. Unfortunately, these methods are still highly demanding in terms of resources, storage, and trajectory analysis, despite recent advances in hardware (such as the Anton massively parallel supercomputer [24, 25]) and software (such as the GPU-based algorithms [26-28] and MD-based enhanced sampling methods [29, 30]). Moreover, the sampling of the conformational space of the binding cavity might still be not long enough, especially when starting from low-resolution models. Thus, extensive validation by comparison with experimental data is needed [31, 32].

Coarse-grained (CG) potential-based simulations [33-36] allow to sample the conformational space of biomolecules [37-50], including membrane proteins [51-54], more efficiently. Indeed, the computational cost of CG-based MD may be two to three orders of magnitude lower than that of all-atom (AA) force field-based MD simulations [42]. However, while CG-based MD provide information on large-scale phenomena, they obviously cannot describe the atomistic details of protein-ligand interactions. To address this issue, one may use hybrid multiscale methods that combine atomistic molecular mechanics (MM) with CG molecular dynamics [55-64].

In the case of membrane proteins, different MM/CG approaches have been applied [56, 57, 65-77]. They differ in (i) their partitioning of the system between the MM and CG regions; (ii) their MM and CG force fields; and (iii) the coupling scheme used to combine the MM and CG parts. In the approach developed by Voth and coworkers, the MM-CG and CG-CG interactions are determined using the so-called multiscale CG method [56, 57, 65]. It uses a force-matching procedure to determine an effective pairwise CG force field directly from the trajectory and force data generated by all-atom MD simulations. The resulting CG force field is used for the lipids and water molecules, whereas the AMBER all-atom force field is used for the protein. The same MM force field is used by Orsi and coworkers [66, 67]. However, they use different CG force fields for water (i.e., soft sticky dipole model [78]) and membrane (in which some of the lipid CG beads are ellipsoidal and thus are treated with the Gay-Berne potential [79], instead of the traditional Lennard-Jones potential [80, 81]). The MM-CG interactions are treated with the usual potential terms, but they are scaled by two constants parameterized on the basis of hydration free energy (for electrostatics) and transmembrane permeability (for the Lennard-Jones/Gay-Berne mixed potential) calculations. In contrast, electrostatic interactions are modeled explicitly in the ELBA CG force field used by Genheden and coworkers [68], and the MM-CG interactions are fine-tuned by introducing two empirical parameters scaling the non-bonded interactions between the MM protein (described with the AMBER force field) and the CG beads (representing the water and lipids). Alternatively, Marrink and coworkers couple the MM and CG levels of resolution by making use of massless virtual sites [69, 70], and describe the MM region (i.e., the protein) with the GROMOS united-atom force field [82-84], and the CG region (i.e., lipid and water molecules) with the MARTINI force field [85]. Additionally, this approach may include a dielectric screening of the MM-MM interactions due to the charged CG particles in order to improve electrostatics [70]. The same MM and CG force fields (GROMOS and MARTINI, respectively) are used by Han and coworkers in the PACE (Protein in Atomistic details coupled with Coarse-grained Environment) force field [71-73]. However, the MM-CG coupling in this approach is re-parameterized to reproduce thermodynamic experimental quantities.

In the aforementioned methods, the protein is treated at the MM level, whereas the lipids are CG and the solvent can be CG or dual resolution. However, if protein models are extremely approximate (for instance, if the sequence identity with the templates is 35% or lower), one may question if it is reasonable to use atomistic descriptions of the lipid bilayer or even of the complete protein. Hence, in the MM/CG simulation approach developed in our group [74-76], we adopted a

dual-resolution strategy for the protein and an implicit representation for the membrane (Fig. 1). In particular, the binding site and the surrounding water molecules are treated using an atomistic force field (GROMOS), while the rest of the protein is described at the CG level by a Gō-like model [86]. The membrane is modeled implicitly by using wall potentials, instead of representing the lipids as CG beads. Therefore, this approach is particularly tailored to study ligand binding to membrane proteins, such as human G-protein coupled receptors (hGPCRs).<sup>1</sup> Here we first review the theoretical background of our MM/CG approach (Section 2), and then provide an overview of recent applications to the hGPCR membrane protein superfamily (Section 3). We close this review by outlining some of the improvements currently being implemented in our MM/CG scheme (Section 4).



**Fig. 1.** Simulation setup of our Molecular Mechanics/Coarse Grained (MM/CG) approach. The MM, I (interfacial), and CG regions are indicated with yellow, magenta, and blue backgrounds, respectively. Five walls around the membrane protein (here a human G-protein coupled receptor, hGPCR) are used to mimic the presence of a lipid bilayer and to keep the water in place: the planar walls (1, 2), the hemispheric walls (3, 4) and the membrane wall (5). The ligand is located in the center of the atomistic region and displayed in red sticks.

<sup>1</sup> A similar approach has been developed very recently by Feig and coworkers [77], in which the CHARMM36 [87] and the PRIMO [49, 50, 88] force fields are combined to describe the MM and CG parts of the protein, respectively, and the membrane is represented with the heterogeneous dielectric generalized Born (HDGB) model [89, 90]. However, so far this method has not been extensively tested for membrane proteins.

## 2. Theoretical background

The potential energy function in our MM/CG scheme reads:

$$V = E_{MM} + E_I + E_{I/MM} + E_{CG} + E_{CG/I} \quad (\text{Eq. 1})$$

where  $E_{MM}$ ,  $E_I$  and  $E_{CG}$  are the potential energies of the atomistic (MM) region, the interface (I) and the coarse-grained (CG) region, respectively.  $E_{I/MM}$  and  $E_{CG/I}$  describe the interaction energies between the interface and the MM region and between the interface and the CG region, respectively.  $E_{MM}$ ,  $E_I$  and  $E_{I/MM}$  have the same form as in the GROMOS96 force field [91], whereas  $E_{CG}$  and  $E_{CG/I}$  take the form of the Gō-like model [86], that is:

$$E_{CG} = \frac{1}{4} \sum_i k_b (|\vec{r}_i - \vec{r}_{i+1}|^2 - b_{ii+1}^2)^2 + \sum_{i>j} V_0 \{1 - \exp[-B_{ij}(|\vec{r}_i - \vec{r}_j| - b_{ij})]\}^2 \quad (\text{Eq. 2})$$

In equation (2), the first term describes the interaction between consecutive CG beads (Cα atoms), where  $k_b$  is the force constant and  $b_{ij}$  is the equilibrium distance corresponding to the native distance between CG atoms. Non-bonded interactions are taken into account in the second term using a Morse-type potential, where  $V_0 = 5.3 \text{ kJ} \cdot \text{mol}^{-1}$  is the well depth and  $B_{ij} = 5 + 6/b_{ij} \text{ nm}^{-1}$  is its modulating coefficient.  $E_{CG/I}$  takes the same form as equation (2), except that the summations run over the CG beads only in  $E_{CG}$ , whereas for  $E_{CG/I}$  they run over both the CG beads and the interface residues. Hence, the term  $E_{CG/I}$  ensures the integrity of the protein backbone by including bonded interactions between the CG atoms and the Cα atoms in the interface, as well as non-bonded interactions between CG atoms and the Cα and Cβ atoms at the interface. In addition, the thermal and viscous solvent effects acting on the system are mimicked by using the leap-frog stochastic dynamics with reference temperature  $T = 300 \text{ K}$  and time constant  $\tau = 0.4 \text{ ps}$  [92]. In the MM region, the solvent is treated in an explicit way using the SPC water model [93]. In particular, a “droplet” of water molecules is centered around the MM region (see Fig. 1).

In order to tailor this approach for membrane proteins, the membrane is included in an implicit way, and the water droplet is hemispherical, so it does not protrude inside the hydrophobic core of the membrane (see Fig. 1). The representation of the implicit membrane and the maintenance of the water droplet surrounding the ligand binding cavity is realized in practice by introducing five walls into the system (Fig. 1) [76] described by five corresponding “wall” functions ( $\Phi_i, i = 1 \dots 5$ ). A level-set approach [94] is used to indicate whether  $\vec{r}$  is outside of the protein/membrane region ( $\Phi(\vec{r})_i < 0$ ), at the exact position of the wall ( $\Phi(\vec{r})_i = 0$ ) or inside the protein/membrane ( $\Phi(\vec{r})_i > 0$ ). The first two walls ( $i = 1, 2$ ) are planar walls that coincide with the height of the heads of the membrane lipids and are represented by

$$\Phi_i(\vec{r}) = \|\vec{r}_z - \vec{r}_{iz}\| \quad \text{for } i = 1, 2. \quad (\text{Eq. 3})$$

In addition, two hemispheric walls ( $i = 3, 4$ ) are used to cap the extracellular and cytoplasmic ends of the protein, which are described by the functions

$$\Phi_i(\vec{r}) = r_i - \|\vec{r} - \vec{\chi}\| \quad \text{for } i = 3, 4, \quad (\text{Eq. 4})$$

which are defined only outside the membrane region. The center  $\vec{\chi}$  of each hemisphere is located at the height of the phospholipid headgroups, above and under the center of mass of the protein. The radius  $r_i$  of each hemisphere is defined such that the protein can not reach the wall during the simulation. The fifth wall implicitly represents the hydrophobic region of the membrane and is defined by

$$\Phi_5(\vec{r}) = \vec{r}_p - \min_j \|\vec{r} - \vec{c}_j\|, \quad (\text{Eq. 5})$$

where the distance between the point  $\vec{r}$  and the closest initial position of C $\alpha$  atoms  $\vec{c}_j$  is computed, and  $r_p$  is the distance between the C $\alpha$  atoms and the wall is set to 2.0 Å by default. Additionally, a smoothing technique [76] is applied to avoid discontinuities in the wall. Boundary potentials are added to the MM/CG potential energy function as functions of the distance of an atom to the closest wall

$$d(\vec{r}) = \min_{i=1\dots 5} (\|\Phi_i(\vec{r})\|), \quad (\text{Eq. 6})$$

so that:

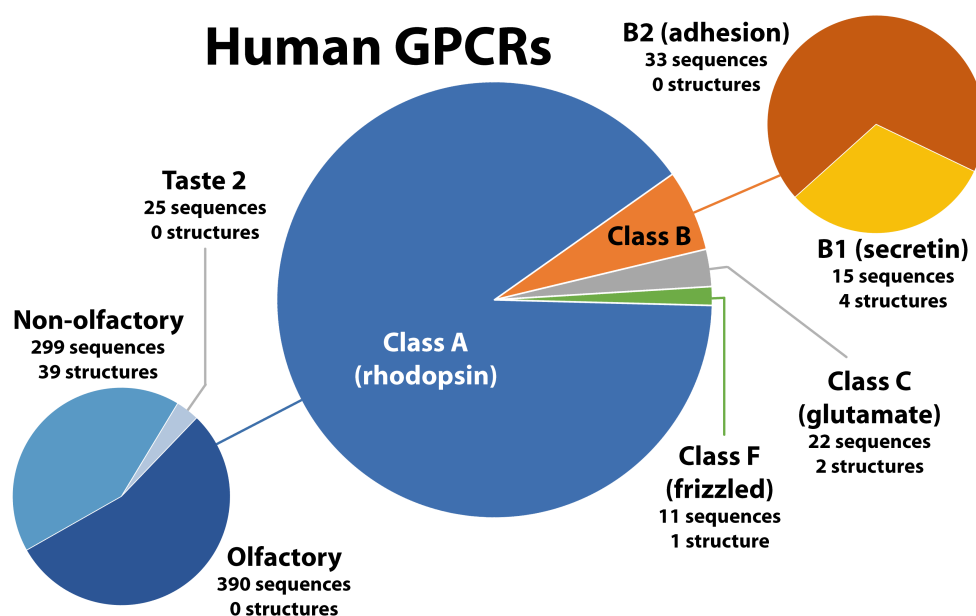
$$V(d) = \begin{cases} \frac{1}{d} & \text{for } d = \|\Phi_i(\vec{r})\| \text{ with } i = 1, 2, 3, 4 \\ 4\varepsilon \left[ \left(\frac{\sigma}{d}\right)^2 - \frac{\sigma}{d} \right] & \text{for } d = \|\Phi_5(\vec{r})\|. \end{cases} \quad (\text{Eq. 7})$$

The potential  $V$  is purely repulsive for walls 1 to 4, whereas for wall 5 it is a softened Lennard-Jones-type potential, where  $\varepsilon$  is the depth of the potential well and  $\sigma$  is the finite distance at which the potential is zero, so that the minimum of the potential is at  $d = 2\sigma = r_p$ . Water, C $\alpha$  atoms of both MM and CG regions, as well as atoms belonging to aromatic residues Phe, Trp and Tyr (the so-called “anchor residues”), are influenced by these potentials. The membrane wall potential (Eq. 7,  $i=5$ ) constrains the shape of the protein while maintaining a good degree of flexibility. Nonetheless, we would like to note here that this model neither allows distinguishing between different types of bilayers nor includes electrostatics (see also Section 5). The cut-off distance of the potentials is set to 15 Å for the repelling walls ( $i = 1$  to 4), and to  $1.5r_p$  for the membrane wall ( $i = 5$ ). The first value is chosen such that a water molecule cannot pass through this distance during one time step, while the second value guarantees that the force does not affect the MM

region. The force is shifted so that it is continuous at the cut-off distance and set to a finite value ( $1000 \text{ kJ mol}^{-1} \text{ nm}^{-1}$ ) near the wall in order to prevent too large forces acting on the system. The current version of the MM/CG code described here is implemented within the GROMACS 4.5 code [95-99].

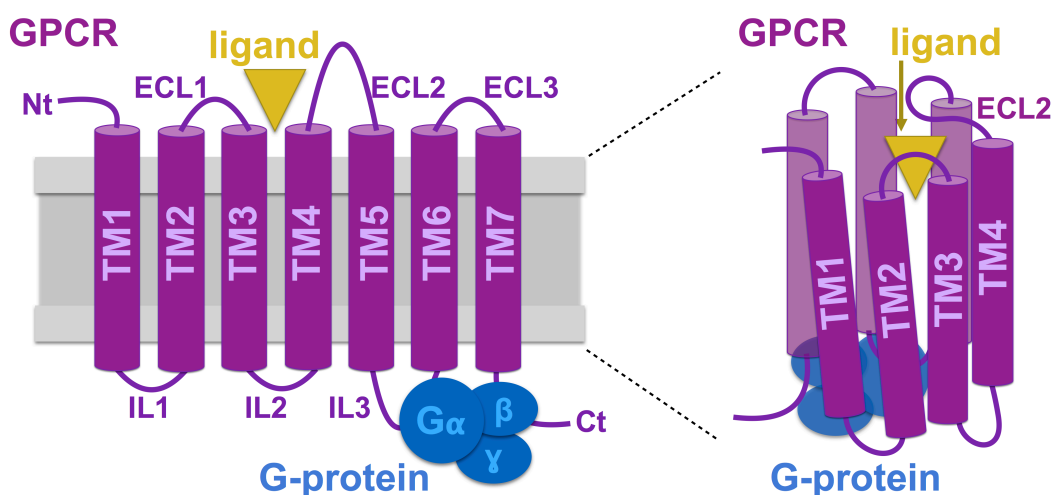
### 3. Applications to human G-protein coupled receptors

The largest membrane-bound receptor family expressed in humans is that of G-protein coupled receptors (hGPCRs). These proteins encompass *ca.* 4% of the protein-coding human genome [100] and are of paramount importance for pharmaceutical intervention, as almost 40% of currently marketed drugs target hGPCRs [3, 101]. They can be classified using two (overlapping) systems (Fig. 2A), following either classes A-F [102] or the group representatives (glutamate, rhodopsin, adhesion, frizzled and secretin, i.e., the GRAFS system) [103]. All GPCRs share a common scaffold [104] formed by seven transmembrane (TM)  $\alpha$ -helices (TM1 to TM7) connected by intracellular and extracellular loops (ICLs and ECLs, respectively) (Fig. 2B). Their tertiary structure resembles a barrel, with the seven transmembrane helices forming a cavity within the plasma membrane that serves as the ligand-binding domain, often covered by the loop ECL2 (see Fig. 2B).



**Fig. 2A** Pie diagram showing the number of members and structures of human GPCRs, grouped according to the two most commonly used classification systems: either by classes A-F [102] or by their prototypical members glutamate, rhodopsin, adhesion, frizzled and secretin (GRAFS) [103]. The taste 2 receptors have been included in class A, following phylogenetic [144] and motif conservation [145] analyses.





**Fig. 2B** Schematic representation of the basic structure of GPCRs. These membrane proteins comprise seven transmembrane helices (TM) connected by intracellular (ICL) and extracellular (ECL) loops.

Experimental structural information is available only for 46 unique receptor structures compared to the ca. 800 hGPCRs (i.e., 6%, as reported in GPCRdb [105, 106] as of Nov 15th, 2017). Most of them (39) belong to the rhodopsin (class A) family. In addition, one belonging to the frizzled (class F) family [107], four to the secretin (class B1) family [108, 109], and two to the glutamate (class C) family [110-113] have been solved. However, no structure is available for the ca. 400 olfactory receptors (ORs) and the 25 taste 2 receptors (TAS2Rs, also known as bitter taste receptors). These so-called chemosensory receptors constitute about half of the whole hGPCR superfamily and are potential novel drug targets against a myriad of extranasal and extraoral diseases [31, 114-117].

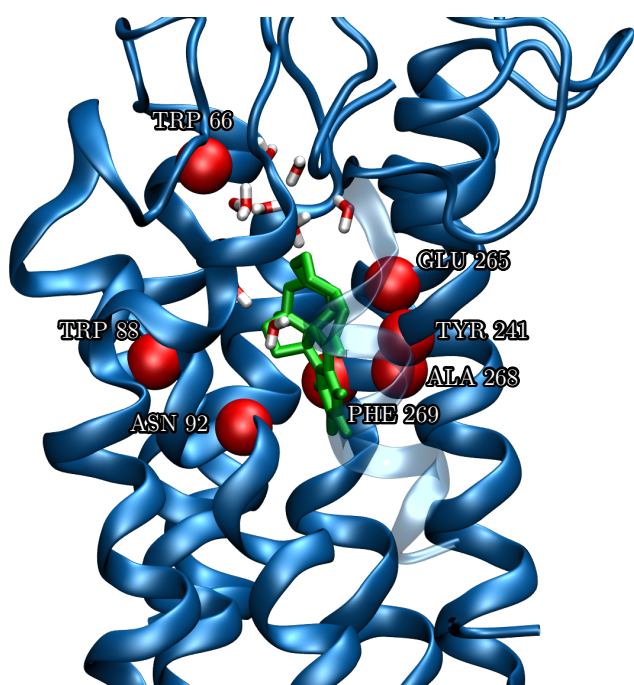
We first investigated the predictive power of MM/CG simulations on a class A hGPCR, for which both X-ray structures and extensive all-atom MD simulations were available, in order to validate our approach. Our test system was the human  $\beta$ 2-adrenergic receptor (h $\beta$ 2AR), in complex with either its inverse agonist S-Carazolol (S-Car) or its agonist R-Isoprenaline (R-Iso) [76]. We first compared the MM/CG simulations with the all-atom MD simulations performed by Vanni *et al.* [118]. Both the AA and the MM/CG simulations were carried out for 800 ns, but a 15-fold speedup was observed with the multiscale approach [118]. The MM/CG-predicted binding poses were in fair agreement with the corresponding all-atom poses in the same complex, and also reproduced the receptor/ligand interactions observed in the crystal structures used as a starting point of both types of simulations. Furthermore, we investigated the predicting power of the MM/CG method when using low-resolution homology models. To achieve this aim, a homology model of the same h $\beta$ 2AR/S-Car complex was built using MODELLER [119, 120] (using as template the crystal structure of squid rhodopsin [121], which displays a sequence identity of only 20% with the target

hβ2AR) and HADDOCK (for docking S-Car) [122]. After 800 ns of MM/CG simulation time, the obtained hβ2AR protein structure was similar to the X-ray structure (2 Å RMSD of the Cα atoms), and the main protein/ligand interactions observed in the X-ray structure were reproduced as well.

These encouraging results support the notion that this MM/CG approach can be used in general for low-resolution ligand/hGPCR complexes. Hence, we applied the same procedure to chemosensory hGPCRs for which there is no experimental structural information, in particular bitter taste receptors. Unfortunately, the average sequence identity between these chemosensory hGPCR targets and the hGPCR templates with available experimental structural information is invariably 20% or lower [23, 109], making bioinformatics/docking-based structural predictions far from trivial [9, 10, 12, 13, 123-127]. Indeed, predictions of agonist binding to bitter taste and olfactory receptors using a variety of docking approaches showed that their predictive power is very limited [23], as they are not able to capture the residues shown experimentally to be important for binding. These initial binding poses can be further refined with MD simulations, in particular using our MM/CG approach, resulting in a dramatic improvement in the predictions [23, 128, 129].

We first studied the human TAS2R38 bitter taste receptor [130] in complex with its agonists phenylthiocarbamide (PTC) and propylthiouracil (PROP). The receptor structure was built by multi-template homology modeling using MODELLER [119, 120]. The sequence identity with the best templates was about 13%. Two receptor models were selected differing mainly in the highly variable extracellular loop ECL2 (Fig. 2A). To generate the initial ligand poses, the two models were funneled through an information-driven docking protocol using the HADDOCK program [122]. The resulting four docking models underwent μs-long MM/CG simulations at room temperature (300 K). These MM/CG simulations identified the protein residues involved in interactions with the ligands, in agreement with the previously reported experimental mutagenesis and functional data [130]. In particular, the interacting residues are the same as those whose mutation was found to affect the EC<sub>50</sub> values experimentally. The simulations also allowed to predict new binding site residues, and these predictions were subsequently verified by performing additional site-directed mutagenesis experiments. Altogether, the MM/CG simulations were consistent with more than 20 site-directed mutagenesis and functional calcium imaging experiments of hTAS2R38 [128]. Moreover, they pointed out key interactions between hTAS2R38 and its agonists, which were impossible to capture by using the standard bioinformatics/docking approach only [23, 128].

The MM/CG approach was subsequently used to investigate the binding structural determinants in another member of the human bitter taste receptor family, hTAS2R46, in complex with its potent agonist strychnine (Fig. 3) [129]. The initial model of the complex was built using the GOMoDo web-server [131], which combines MODELLER [119, 120] and HADDOCK [122] in a single pipeline. Also in this case, the simulation results were consistent with more than 20 site-directed mutagenesis experiments. These were either previously reported [132] or predicted on the basis of the MM/CG simulations [129]. Interestingly, the mutagenesis data could not be explained in full when considering only the protein-ligand interactions in the classical binding cavity of class A GPCRs (i.e., the so-called orthosteric site). Some of the residues experimentally verified to be important for strychnine binding were located outside this classical site, more towards the extracellular part of the receptor. This conundrum was solved with the help of the MM/CG simulations, which showed that, besides the orthosteric site, the ligand can also sample a secondary binding cavity located in the extracellular side, referred to as vestibular site [129]. This led to the conclusion that hTAS2R46 displays a two-site architecture, most likely in order to be able to discriminate the broad range of agonists recognized by this receptor. The identified vestibular binding site shares similarities with the extracellular allosteric binding site found in other hGPCRs [133-135].



**Fig. 3.** Close up of the orthosteric ligand binding site of the hTAS2R46 receptor in complex with its agonist strychnine [129]. The atomistic part of the protein is displayed as blue ribbons and the ligand in green sticks; only the water molecules closest to the ligand are shown for the sake of clarity. The red spheres represent the C $\alpha$  atoms of the residues interacting with the ligand in the MM/CG simulations [23], which coincide with the residues experimentally determined to be involved in ligand binding by mutagenesis and functional data [132].

## 4. Perspectives

Calculations are now underway for all chemosensory hGPCRs for which functional, site-directed mutagenesis and ligand SAR experiments are available [23]. In addition, several new features are being implemented in the MM/CG code in order to increase the accuracy of the ligand binding pose prediction.

In particular, the currently implemented united atom force fields (i.e., GROMOS43a1 [91] for the protein and PRODRG [136], optionally with RESP charges [137] for the ligand) are being complemented by the all-atom AMBER14SB force field [138, 139] for the protein and the GAFF force field [140] for the ligand. The electric field of the protein frame, not present in the CG Gō potential, is being included by using the multipolar reconstruction approach reported in Ref. [141]. This may refine the binding pose for ligands with net charge or dipole moment, as well as improve the orientation of the water network inside the receptor.

Furthermore, one may want to remove possible artifact on protein dynamics imposed by the upper hemisphere wall (see Fig. 1), in order to enable rigorous calculations of binding affinities. We are addressing this issue by introducing a coarse-grained water reservoir around the system that exchanges water molecules with the atomistic region [142], using the so-called Hamiltonian Adaptive Resolution Scheme (H-AdResS) [143]. In its original implementation, the H-AdResS method was used only for liquid water [143], but recently we investigated its reliability in the presence of proteins [142]. Fully atomistic cytoplasmatic proteins and their surrounding water droplets were coupled to the CG water reservoir, so that the MM water molecules diffuse to and from CG waters by changing their resolution on-the-fly. Notably, the structure and dynamical properties of both protein and water were well reproduced [142]. Within our current efforts to improve the MM/CG code, we are now combining the MM/CG scheme with H-AdResS by replacing the upper hemisphere capping the water droplet (Fig. 1) with the dual-resolution solvent. This will result in a grand-canonical ensemble for the MM/CG system, and thus in a correct statistical ensemble for calculating ligand binding free energies.

As long-term future extensions of the code, we are considering the application of our MM/CG approach to other non-GPCR membrane proteins. This will require the implementation of further modifications in the MM/CG code in order to account for the different number and size of the MM and CG regions. Specifically, in ion channels the process of interest may be ion binding and conduction, and thus the region of interest consists of the residues lining the channel pore. As a

result, a larger number of atoms needs to be treated atomistically, which may pose a challenge to the current parallelization of the code. In addition, some ion channels are composed of several subunits, but the current version of our coarse-graining script (used to set up the MM/CG system) does not allow a straightforward treatment of such oligomeric systems yet. Finally, the lack of electrostatics of the Gō-like model might affect the accuracy of the ion channel simulations more than for GPCRs. Given the structural diversity of non-GPCR membrane proteins, the list of possible improvements needed in our MM/CG code is not exhaustive and will have to be re-assessed specifically for each type of system.

In conclusion, we anticipate that the MM/CG code may become a useful and rather general tool to predict both the pose and the affinity of ligands binding to any membrane protein whose structural determinants are not experimentally known. So far, our MM/CG approach has been tested extensively only for hGPCRs, since the current implementation of the MM/CG code has been tailored to study ligand binding to GPCRs. As of today, as many as 94% of the total number of receptors of this biologically and pharmacologically fundamental membrane protein class do not have an experimental structure available. Therefore, we expect that further applications of the MM/CG code will be useful to the hGPCR community and that, in the future, our MM/CG approach could be extended to other non-GPCR membrane proteins.

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