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Review Article

Multi-scale simulations of membrane proteins: The case of bitter taste receptors

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ABSTRACT

Human bitter taste receptors (hTAS2Rs) are the second largest group of chemosensory G-protein coupled receptors (25 members). hTAS2Rs are expressed in many tissues (e.g. tongue, gastrointestinal tract, respiratory system, brain, etc.), performing a variety of functions, from bitter taste perception to hormone secretion and bronchodilation. Due to the lack of experimental structural information, computations are currently the methods of choice to get insights into ligand–receptor interactions. Here we review our efforts at predicting the binding pose of agonists to hTAS2Rs, using state-of-the-art bioinformatics approaches followed by hybrid Molecular Mechanics/Coarse-Grained (MM/CG) simulations. The latter method, developed by us, describes atomistically only the agonist binding region, including hydration, and it may be particularly suited to be used when bioinformatics predictions generate very low-resolution models, such as the case of hTAS2Rs. Our structural predictions of the hTAS2R38 and hTAS2R46 receptors in complex with their agonists turn out to be fully consistent with experimental mutagenesis data. In addition, they suggest a two-binding site architecture in hTAS2R46, consisting of the usual orthosteric site together with a "vestibular" site toward the extracellular space, as observed in other GPCRs. The presence of the vestibular site may help to discriminate among the wide spectrum of bitter ligands.

1. Introduction

The 25 human bitter taste receptors (hTAS2Rs) [1,2] constitute the second largest group of chemosensory G-protein coupled receptors (GPCRs), in turn the largest membrane protein superfamily, with about 850 members in humans. hTAS2Rs are found in many different tissues of the human body [3–5]. These include the plasma membrane of the type II taste receptor cells (from which their name, TAS2Rs, comes from), located in the taste buds of the tongue [1,6–8], the respiratory system [9–11], the gastrointestinal tract [12,13] the endocrine system [13] and the brain [14]. Hence, hTAS2Rs play different roles, ranging from perception of bitter taste, to detection of toxins [15], to bronchodilation [16], and to hormone secretion [17]. hTAS2Rs can recognize hundreds of structurally diverse agonists using a combinatorial coding scheme [18,19]. One hTAS2R is able to recognize more than one agonist [20,21], and one agonist can be recognized by more than one hTAS2R [20]. Understanding the details of hTAS2Rs–agonists interactions may provide important hints on the effect of genetic variability on bitter taste perception, and new opportunities for

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designing more subtype-specific ligands [22,23] and novel therapies against diseases related to hTAS2Rs' dysfunction, e.g. asthma or chronic rhinosinusitis [4,5].

hTAS2Rs, as all GPCRs, are made up of seven transmembrane helices arranged in a helix-bundle shape and connected by three extracellular loops (ECLs) and three intracellular loops (ICLs) [24]. Agonist binding to the receptor's binding site (called orthosteric site) facilitates conformational changes towards an "active state". The latter allows the activation of downstream effectors [7,25]. The location of the orthosteric site in hTAS2Rs is similar to other receptors of the largest GPCR class, class A [26–31]. However, because of the low sequence similarity between hTAS2Rs and the other GPCRs, it has not been clearly established yet if hTAS2Rs belong to class A [32–34] or class F [27,35,36] GPCRs. These proteins could even constitute a different family [27].

At present no experimental structural information is available for hTAS2Rs. Therefore, any attempt at understanding the hTAS2R-agonist complexes has to rely on computational approaches. Bioinformatics techniques, such as homology modeling [37], along with molecular docking [38–40], could in principle provide insights into agonist/antagonist binding. Unfortunately, however, the sequence identity between bitter taste receptors and the possible templates is extremely low (~10–17% with any of the 42 unique X-ray structures as of February 2017 (http://blanco.biomol.uci.edu/mpstruc/)). As a consequence, the construction of reliable alignments between the target sequence and the available structural templates is challenging [41–43]. Moreover, even with a good sequence alignment, the orientation of the side chains in the orthosteric binding site, which is key for protein–ligand interactions, is not accurately modeled [44,45]. This hinders the correct prediction of docking poses. In addition, current bioinformatics and docking algorithms face at times limitations (such as the lack of protein flexibility and hydration [46,47]), which may further limit the power of the predictions, especially in light of the fact that factors such as conformational dynamics [48] and water molecules [49,50] play a crucial role for ligand binding and receptor activation. A way to overcome these difficulties is to combine these static computational approaches with molecular simulation techniques, such as molecular dynamics (MD) and enhanced sampling [51–53]. These methods may explore efficiently the conformational space, including hydration and ligand–protein interactions. All-atom MD has been successfully used in high quality homology models (i.e. based on a template with sequence identity above 60%) [48,54,55]; however, it may provide far less satisfying results when the protein structure is a homology model based on a low sequence identity template (as it is the case for hTAS2Rs). Here, the side chains' rotamers are poorly predicted and often their relaxation requires longer time scales that cannot be reached with atomistic MD. Coarse-grained (CG)-based MD can be used to sample longer timescales [56–59], yet it cannot describe in detail the molecular recognition events between protein and ligand. A way to overcome these limitations is represented by the combination of the two aforementioned techniques [60–67]. In this context, our group has developed a hybrid "Molecular Mechanics/Coarse-Grained" (MM/CG) method for refinement of GPCRs homology models [63,68,69]. Here, the system is modeled at two different resolutions. While ligand, binding site residues and surrounding water molecules are treated using an atomistic force field, the rest of the protein is described at a CG level. A coupling scheme is then used to connect the two regions at the boundary. This MM/CG method maintains the atomistic resolution needed to describe correctly the protein–ligand interactions at the binding site, while allowing a larger conformational sampling and a reduced computational cost compared to an all-atom simulation. The presence of the membrane is mimicked by introducing five repulsive walls. Two planar walls coincide with the height of the head groups of the membrane lipids, two hemispheric walls set a limit on the extracellular and intracellular ends of the protein and the last wall follows the initial shape of the interface between protein and membrane [70–72].

The accuracy of the MM/CG method in reproducing binding poses and protein fluctuations was established in our early work [68]. Here, we will present more recent predictions for widely studied hTAS2Rs, which were successfully validated against extensive mutagenesis data [73–75]. Specifically, we investigate hTAS2R46 [76], a promiscuous bitter taste receptor [20,73,77] that can detect bitter molecules belonging to several different chemical classes, and hTAS2R38 [74,75], a receptor that recognizes agonists containing an isothiocyanate or thiourea group [20,77,78]. Given their different receptive range, these two receptors constitute excellent contrasting test cases to assess the applicability of the MM/CG methodology to study ligand binding in human bitter taste receptors.

2. Materials and methods

Our web-server GOMoDO [79] performs automatically both the homology modeling and molecular docking steps, by combining state-of-the-art bioinformatics tools for GPCRs. In particular, GOMoDO uses the profile–profile HMM algorithm (for database search and target-template alignment) and the MODELLER program [80] (for protein homology model construction), followed by information-driven flexible docking of ligands through the HADDOCK program [81]. This protocol was used to produce the initial model of hTAS2R46 in complex with one of its agonists, strychnine, as well as the models of hTAS2R38 in complex with its two agonists, namely propylthiouracil (PROP) and phenylthiocarbamide (PTC). Specifically, the MODELLER algorithm [80] was used to generate 200 models of hTAS2R46 and hTAS2R38, applying a single-template or multiple-template approach, respectively [74–76]. Then, a clustering analysis was performed to identify "representative" receptor models, using as criteria both the MODELLER quality scores and available experimental site-directed mutagenesis data. In the case of hTAS2R46, one single model was taken as representative, whereas for hTAS2R38 two models were selected, which mainly differ in the conformation of the ECL2. The agonists, strychnine for hTAS2R46 and PROP and PTC for hTAS2R38, were docked into the modeled receptor structures using HADDOCK [81]. Information about the putative binding residues was used to drive the docking. For hTAS2R46 the putative binding residues were predicted using FPOCKET [82], whereas for hTAS2R38, they were selected based on previous bioinformatics and site-directed mutagenesis studies [74]. In the docking protocol, first 1000 structures were generated in the rigid body step and, then, the top scoring 200 complexes were further optimized using a flexible simulated annealing step, followed by a final refinement step in explicit water. Next, a clustering analysis was performed to identify the best initial model, that is, the structure of the most populated cluster with the lowest binding energy. The best docking models then underwent MM/CG simulations [63,68,69]. In these multiscale approach, ligand, binding site residues and surrounding water molecules were treated using the GROMOS96 atomistic force field [83], whereas the rest of the protein was described at a CG level, including only the Cα atoms of the amino acids and using a Go-like model [84]. For hTAS2R46, the model of the receptor–strychnine complex was used to set up three replicas, with different initial velocities; a 1 μs-long MM/CG simulation was run for each [76]. For hTAS2R38, the models for each receptor-agonist complex (PROP and PTC) were submitted to MM/CG simulations; for each complex,