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# Binding of glutamate to the umami receptor

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ABSTRACT

The umami taste receptor is a heterodimer composed of two members of the T1R taste receptor family: T1R1 and T1R3. It detects glutamate in humans, and is a more general amino acid detector in other species. We have constructed homology models of the ligand binding domains of the human umami receptor (based on crystallographic structures of the metabotropic glutamate receptor of the central nervous system). We have carried out molecular dynamics simulations of the ligand binding domains, and we find that the likely conformation is that T1R1 receptor protein exists in the closed conformation, and T1R3 receptor in the open conformation in the heterodimer. Further, we have identified the important binding interactions and have made an estimate of the relative free energies associated with the two glutamate binding sites.



## 1. Introduction

The umami taste receptor is a Class C G protein coupled receptor (GPCR [1]). In humans, it responds to L-glutamate and, to some extent, L-aspartate; in other species, it serves as a more general detector of L-amino acids in the diet [2]. The receptor is a heterodimer composed of the T1R1 and T1R3 members of the T1R family.

The T1R taste receptors are related to the metabotropic glutamate receptors (mGluR) of the central nervous system as well as to other Class C GPCR receptors. The Class C GPCR family has seven transmembrane helical segments, like all GPCRs. In addition, these receptors have a large N-terminal ligand binding region (>500 amino acids) with a "clamshell" or "venus flytrap" fold, having two ligand binding domains that can open or close. This N-terminal region is linked to the transmembrane segment by a smaller (~70 amino acids) cysteine-rich domain [3]. Class C GPCRs may function as homodimers or heterodimers.

X-Ray crystallographic studies of the ligand binding region of some mGluRs with and without bound glutamate show that these domains can exist in both "open" and "closed" conformations [4–6]. In the unliganded form, both of these domains are in an open conformation; ligand binding stabilizes a "closed-open/active" state [4]. In this state, one protomer binds glutamate in the closed conformation, and the other binds glutamate in the open conformation. In addition, the dimer interface reorganizes in such a way that the angle between the monomers is decreased. This is illustrated schematically in Fig. 1. Homology modeling of the closely related sweet taste receptors (T1R2 + T1R3) has facilitated an understanding of the interactions of sweeteners with their receptor [7–12].

During the last two decades, the technique of molecular dynamics simulation (MD) has emerged as a valuable tool to provide information related to the dynamic and static properties of biophysical systems with atomic detail. Two factors have contributed to this: improvements in simulation algorithms, and significant improvement in computing power, permitting simulation of larger systems for longer trajectory times [13].

Here we report homology modeling of the ligand binding domain of the umami receptor, and molecular dynamics based evaluation of the binding of glutamate to the two likely binding sites. Recently, Zhang et al. [14] have modeled the binding of glutamate to T1R1. However, it is known that metabotropic glutamate receptors bind two molecules of glutamate, one in each protomer, and that these exhibit negative cooperativity [15]. Cooperativity has been postulated for the T1R family taste receptors as well [16]. Therefore we have modeled the ligand binding domains of the umami receptor with a glutamate molecule bound in each monomer. This approach should provide useful information about a possible second binding site, although it is unlikely that simulations can be run long enough to provide details about cooperativity.

## 2. Methods

### 2.1. Setting up the molecular structures

All molecular modeling was carried out using Molecular Operating Environment (MOE, version 2007.09, Chemical Computing Group,

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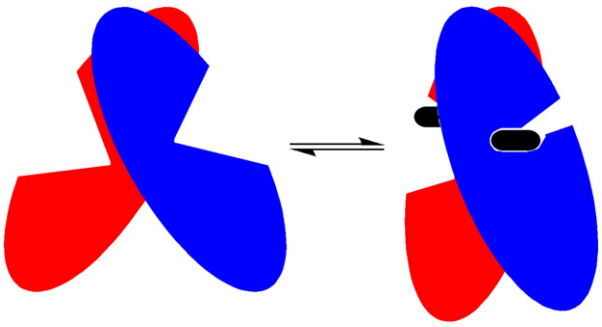

**Fig. 1.** Schematic representation of the ligand binding domains of glutamate receptors. Left, the unliganded "open-open/resting" state, in which both monomers have open conformations. Right, the liganded "closed-open/active" state, stabilized by glutamate binding, in which one monomer has a closed conformation, and the interface between monomers has rearranged so as to decrease the angle between the monomers.

Montreal). Homology modeling of the ligand binding region of the umami receptor was carried out as described previously for the sweet taste receptor [12], using the "closed-open/active" state of mGluR1 as the template (PDB code 1EWK [4]). The ligand binding domain of the mGluR1 has 26.8% sequence identity with human T1R1 and 24.1% identity with human T1R3. The sequence alignments used are included as Supplementary data. Homology modeling was carried out with all histidines in the protonated state.

The template structure is a homodimer, with the two chains adopting two different conformations, so we generated two models for the umami receptor: Form 1 has T1R1 in the closed conformation and T1R3 in the open conformation, and Form 2 has T1R1 in the open conformation and T1R3 in the closed conformation. The template structure has a disordered segment, residues 125-153, located near the top of the upper "clamshell" [4]. This segment contains a cysteine residue (Cys140 in mGluR1) that is known to form a disulfide linkage between the two subunits. In the T1R family, this region contains a cysteine (Cys129) in T1R3, but T1R1 and T1R2 do not have a cysteine in this region, so it was not necessary to model such a disulfide. Since the disordered regions are at least 30 Å from the binding sites, we consider that they are unlikely to have a significant impact on calculated binding interactions.

The template structure includes a glutamate bound to each subunit, so we initially considered those two glutamate orientations. We also wished to account for the possibility of glutamate binding conformations different from those of the mGluR crystal structure, so alternate glutamate binding orientations were generated using the Dock module of MOE. First, a stochastic search method was used to generate a database of 220 L-glutamate conformations. Then the alpha-triangle method implemented in MOE was used to produce a series of docked poses in each binding site. Finally, minimization was carried out to produce starting points for molecular dynamics simulations. Ultimately, the starting points with the greatest number of favorable interactions with the binding sites corresponded to those of the template crystal structure.

### 2.2. Setting up the MD simulations

Two different systems were simulated in this study:

1. Form 1 in the presence of glutamate at both binding sites.
2. Form 2 in the presence of glutamate at both binding sites.

The starting Form 1 and Form 2 conformations obtained as described earlier were introduced into a three dimensional periodical computational box, and hydrated with a layer of water 1 nm thick, using a box containing 216 equilibrated SPC water molecules [17]. Thus, after solvating the proteins, the total number of atoms of the systems 1 and 2 was 95,682 and 92,161, respectively. To balance the positive charge of the systems, 2 chloride ions were introduced into the system by substitution of 2 water molecules in both cases. No other salt ions were included in the simulations. Thus, the final number of atoms of the systems was 95,676 and 92,115, respectively. The PDB files corresponding to the two starting conformations of the two systems are available from the corresponding author.

GROMACS 3.3.3 was the engine for all of the MD simulations [18,19] and the OPLS force field [20] implemented in GROMACS was the force field used in all simulations. Once the starting configurations were generated as described earlier, the systems were subjected to a steepest descent minimization process to remove any existing strain or overlap between neighboring atoms. A time step of 2 fs was used in all of the simulations. Due to the fact that all the simulations were performed under the NPT thermodynamic conditions, the systems were coupled to an isotropic external pressure and temperature bath of 1 atm and 298 K, using Berendsen's algorithm [21]. The temperature and pressure constants used in our simulations were 0.1 and 0.5 ps respectively. All the bond lengths in the system were constrained using LINCS [22]. Steric interactions were modeled with the Lennard–Jones potential, with a cut-off of 0.8 nm, and electrostatic interactions were modeled with the Ewald algorithm [23,24].

Once the systems were set up, MD simulations of 100 ns each were carried out. To determine when the system had achieved an equilibrated state, the pair distances between glutamate and receptor were followed as a function of time. Fig. 2 corresponding to the distance between glutamate and binding site in T1R1 (system 1) shows that 50 ns of simulations are required to equilibrate the system. Similar results were seen for the other system (data not shown).

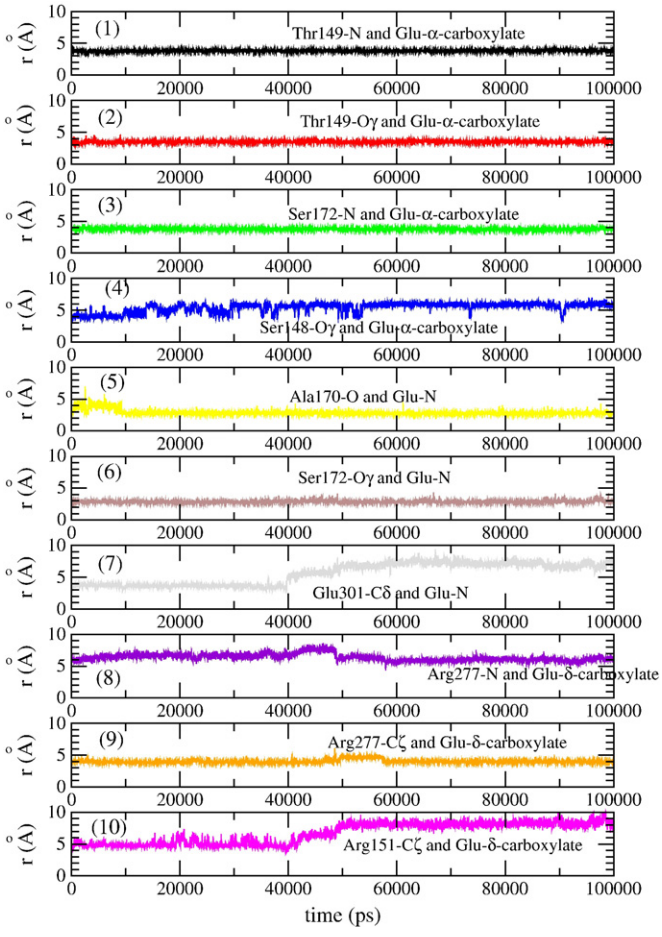


**Fig. 2.** Distances between Glutamate and T1R1 residues in binding site 1.