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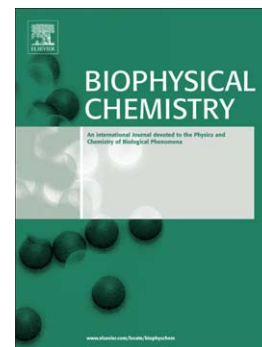
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Binding of Glutamate to the Umami Receptor

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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 in which 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.

Keywords: Glutamate, umami, taste receptor and MD simulations.

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 con-

formation. In addition, the dimer interface reorganizes in such a way that the angle between the monomers is decreased. This is illustrated schematically in Figure 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].

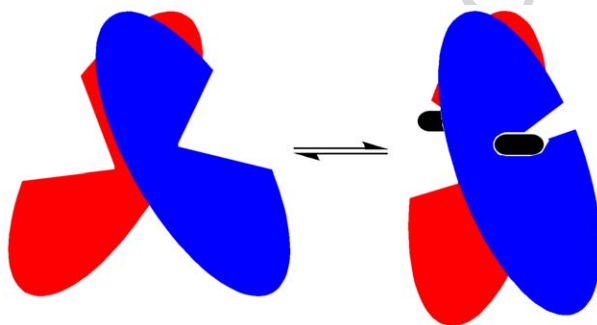


Figure 1: Schematic representation of the ligand binding domains of glutamate receptors. Left, the unliganded "open-open/resting" state, in which both monomers have open conformations. Right, the liganded "closed-open/active" state, stabilized by glutamate binding, in which one monomer has a closed conformation,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

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, 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 simula-

tions. 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 presence of glutamate at both binding sites.
2. Form 2 in presence of glutamate at both binding sites

The starting Form 1 and Form 2 conformations obtained as described above were introduced into a three dimensional periodical computational box, 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 were 95682 and 92161, 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 were 95676 and 92115, respectively. The PDB files corresponding to the two starting conformations of the two systems have been provided as Supplementary Data.

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 above, 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 298K, 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 0.8nm, 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. Figure 2 corresponding to the distance between glutamate and binding site in T1R1 (system 1) shows that 50ns of simulations are required to equilibrate the system. Similar results were seen for the other system (data not shown). Therefore, the first 50 nanoseconds of each simulated trajectory were omitted from further analysis in the present work.

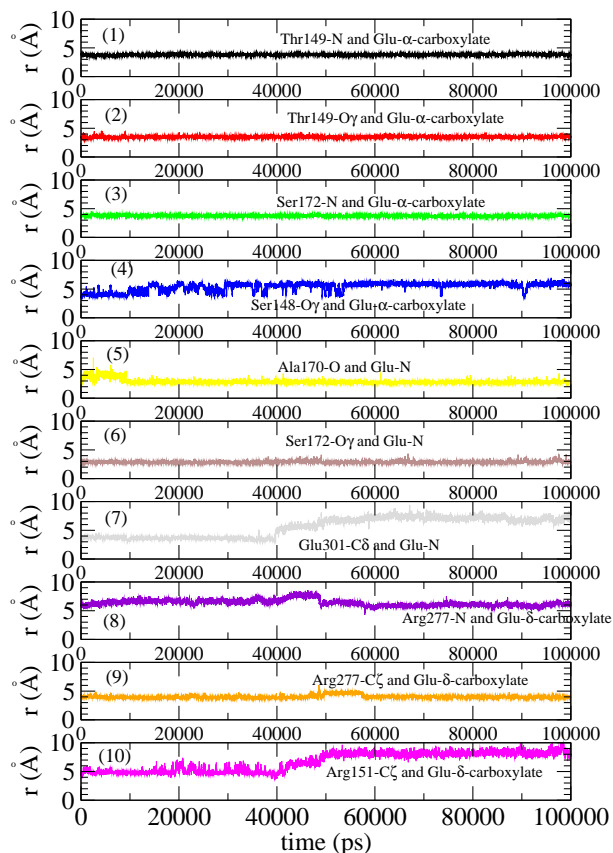


Figure 2: Distances between Glutamate and T1R1 residues in binding site 1.

3 Results and Discussion

3.1 Receptor conformation.

The mGluR1 template structure is a homodimer, while the umami receptor is a heterodimer. Since the active form is asymmetric, we first considered the two possible models: Form 1, in which T1R1 is in the closed conformation and T1R3 is in the open conformation, and Form 2, in which T1R1 is in the open conformation and T1R3 is in the closed conformation. Our molecular dynamics simulations provide good evidence that Form 1 is the correct choice.

Over the course of the 100 ns simulation of Form 1, both glutamate molecules remain in their binding sites. In contrast, during the Form 2 simulation, the glutamate in the open (T1R1) subunit consistently diffused away from its binding site, as is illustrated in Figure 3. To validate this conclusion, this simulation was repeated three times starting with different velocity profiles of the atoms at the beginning of the simulation, and analogous results were attained: glutamate migrated from the open binding site in Form 2, but not in Form 1. Thus, based on these simulation results, we propose that the active conformation of the umami receptor has the T1R1 ligand binding component in a closed conformation, and the T1R3 ligand binding domain in an open conformation. We note that Zhang et al. recently modeled glutamate binding to T1R1 in the closed conformation [14]; however, they did not consider glutamate binding to T1R3. Our results are consistent with binding of glutamate to both T1R1 and T1R3 in the "closed-open/active" state, with T1R1 closed and T1R3 open (which we have termed Form 1), so our subsequent investigation centers on this Form 1 of the umami receptor.

3.2 Binding sites

Our molecular dynamics simulation of two glutamates bound to Form 1 shows a number of interactions to be important in the glutamate binding sites. These are shown graphically in Figure 4. The interactions were analyzed quantitatively by monitoring distances between glutamate and receptor

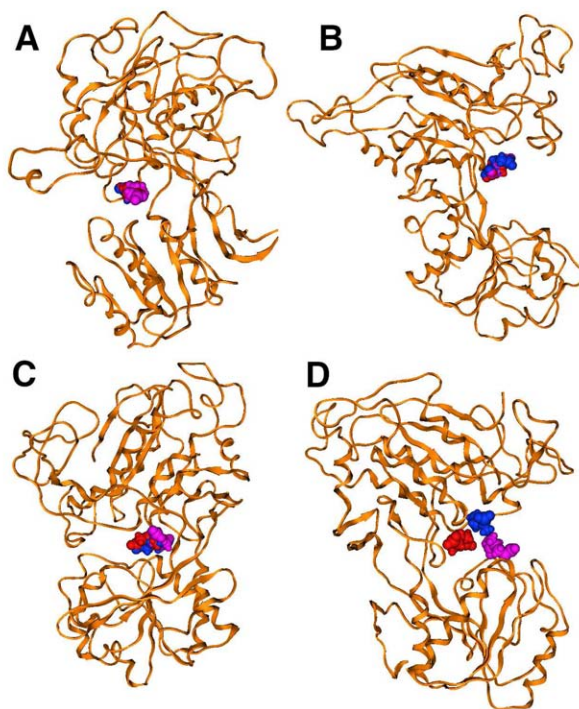


Figure 3: Glutamate diffuses away from the open site of Form 2, but not from other sites. Protein backbone is shown as an orange ribbon. Glutamate position at early, middle, and late time points in the simulation are shown as red, blue, and magenta spacefilling structures, respectively. A, Closed subunit of Form 1. B, Open subunit of form 1. C, Closed subunit of Form 2. D, Open subunit of Form 2.

atoms over successive 1 nanosecond periods during the simulation. Figure 5 shows the normalized distribution function for a number of important distances in the T1R1 site during the course of the simulation, and Figure 6 shows the normalized distribution function for distances in the T1R3 site.

In the T1R1 binding site, the glutamate α -carboxylate group maintains a number of favorable interactions. There is consistent ionic hydrogen bond interaction with both the backbone -NH and the sidechain -OH of Thr149.

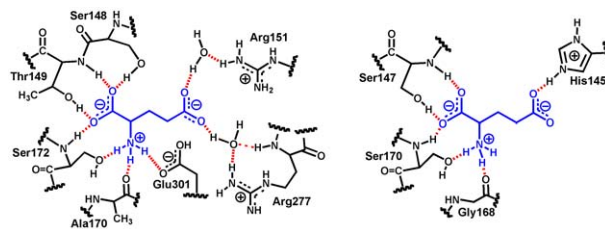


Figure 4: Important binding interactions that are maintained over the course of the simulation. The left side shows binding of glutamate in the closed T1R1 site. The right side shows binding of glutamate in the open T1R3 site. The bound glutamate is shown in blue.

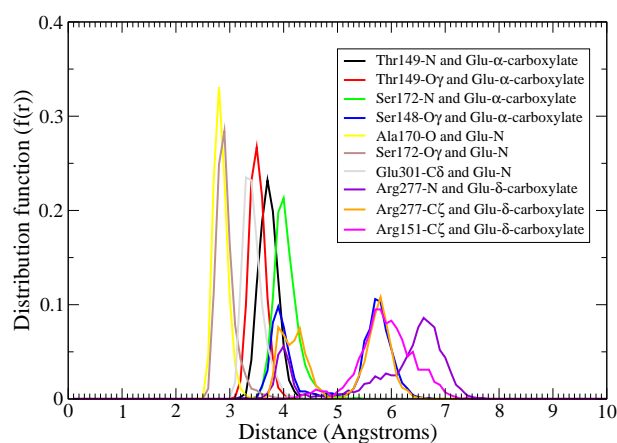


Figure 5: Distance distribution functions for selected pairs of atoms between the closed T1R1 site and the bound glutamate.

The distance from Thr149-N to the carboxylate carbon is centered at 3.69 Å and the distance from Thr149-sidechain O to the carboxylate carbon is centered at 3.49 Å. The α -carboxylate also binds to the backbone -NH of Ser172, with the distance from Ser172-N to carboxylate carbon centered at 3.98 Å. In addition, there is interaction with the sidechain -OH of Ser148 about 50% of the time; the distance distribution function shows peaks centered at 3.91 and 5.73 Å.

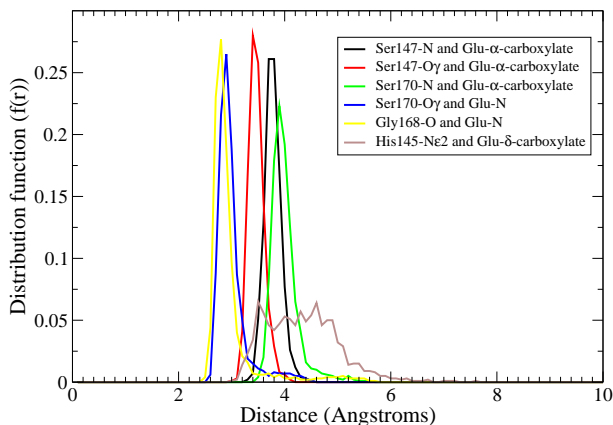


Figure 6: Distance distribution functions for selected pairs of atoms between the open T1R3 site and the bound glutamate.

The glutamate amine nitrogen also maintains three favorable interactions, with the backbone-O of Ala170 (O - N distance centered at 2.81 Å), the sidechain-O of Ser172 (O - N distance centered at 2.91 Å), and the sidechain carboxylate of Glu301 (C - N distance centered at 3.34 Å). In contrast to the mGluR1 crystal structure, the amino group of the glutamate does not interact significantly with the aryl ring of Tyr220 (corresponding to Tyr235 in mGluR1) [4].

The γ -carboxylate of the glutamate is less firmly bound to this site. At different time points during the simulation, we can observe this carboxylate interacting either directly or through a bridging water molecule with the backbone -NH of Arg277, the sidechain guanidinium group of Arg277, and the sidechain guanidinium group of Arg151. These two arginines are sufficiently far apart that the carboxylate cannot interact with both simultaneously; it appears to alternately bind one, then the other. This is reflected in the

distance distribution functions for these interactions. The distance between Arg277 backbone N and carboxylate carbon has peaks centered at 4.00 Å and 6.60 Å; the distance between Arg277-C ζ and carboxylate carbon has peaks centered at 3.87 Å, 4.29 Å, and 5.79 Å; the distance between Arg 151-C ζ and carboxylate carbon has a small peak centered at 4.62 Å and a broad peak centered at 5.77 Å.

In the T1R3 binding site, the glutamate α -carboxylate maintains favorable interactions with the backbone -NH groups of Ser147 and Ser170. The distance from Ser147-N to the carboxylate carbon is centered at 3.75 Å, and the distance from Ser170-N to the carboxylate carbon is centered at 3.90 Å. The sidechain -OH of Ser 147 also interacts, with the distance from sidechain O to the carboxylate carbon centered at 3.41 Å.

The glutamate amine nitrogen maintains favorable interactions with the backbone carbonyl oxygen of Gly168 and the sidechain -OH of Ser170, with O-N distances centered at 2.80 Å and 2.90 Å, respectively. The glutamate gamma-carboxylate group maintains only a limited interaction with the His145 sidechain. The distance from His145-N ϵ 2 to the carboxylate carbon ranges from 3 to 5 Å.

Comparison to the mGluR1 structure [4] shows that binding of the α -carboxylate and the α -amino group are quite similar in the umami receptor, with serine and threonine backbone and sidechain groups playing important roles. This is consistent with the function of the umami receptor as a

sensor for α -amino acids. In contrast, the γ -carboxylate binding is substantially different between mGluR1 and the umami receptor. In mGluR1, the γ -carboxylate interacts with Lys409, Arg78, and (in the closed protomer) Arg323. In the closed T1R1 site, the γ -carboxylate interacts mainly with Arg277 through a water molecule bridge, and in the open T1R3 site, the γ -carboxylate interacts with His145. Since, in most species, the umami receptor is a fairly non-specific detector of α -amino acids, it is not surprising that the ligand's side chain has fewer and less specific interactions than do the α -carboxylate and the α -amino groups.

4 Binding free energy

To understand the glutamate-receptor binding process, the relative free energies were estimated for the two glutamate binding sites of Form 1.

For the studied association reaction $L + P \rightleftharpoons LP$ between protein P and ligand L the potential of mean force as assessed by the umbrella sampling simulations [25] reflect the ratio of bound and unbound states as:

$$\Delta G_{bind} = -k_B T \ln \frac{[LP]}{[P]} \quad (1)$$

where k_B is the Boltzmann constant and T the temperature. Thus, from calculating the potential mean force (PMF) of the binding process, an estimation of the binding free energy i.e. of the affinity of glutamate for the binding sites of the T1R1 and T1R3 is obtained. We focused our efforts on evaluating

the PMF for the two binding sites described above. We used the umbrella sampling method [25] to compute the potential of mean force (PMF), where an artificial biasing potential is added to glutamate to force it to sample the regions of interest, from the binding site in the receptor to bulk solution. For convenience, the origin of the coordinate system was placed at the position of the glutamate after 100 ns of simulation, with the z-axis along the path away from the binding site. Negative values of z represent movement deeper into the binding site, and positive values represent movement away from the receptor, into bulk solvent. For each binding site, 32 independent simulations of 30 ns each were performed. For each successive simulation, the glutamate molecule was shifted along the z-axis by 0.1 nm, and the center of mass of the glutamate was restrained to that position by an harmonic potential on the z-axis, leaving it to move it freely in the xy plane. When the 32 simulations were completed, the weighted histogram analysis method (WHAM) [26] was used to obtain the potential of mean force (PMF). The zero of free energy was placed in the bulk solution.

To confirm that the entropic contribution converged during our simulation times, a study of ΔG was performed for different simulation lengths. For simulations of 10ns, the free energy converged to the same values as for simulations of up to 30ns length.

The free energy profile associated with the glutamate binding process was calculated, and Figure 7 depicts the free energy profile associated with

each binding site. The error bars were calculated after splitting the 30ns of simulated trajectory

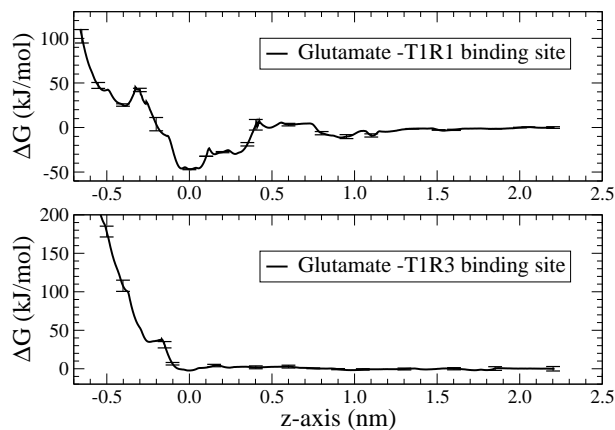


Figure 7: Free Energy profile (ΔG) of binding glutamate to the T1R1 and T1R3 binding sites respectively. Error bars were calculated from 3 subtrajectories of 10ns of simulation.

From Figure 7 was derived a minimum of ΔG of -47.3 kJ/mol associated with binding site of T1R1, compared with the value of -2.66 kJ/mol obtained for binding site of T1R3. From these values of ΔG , we conclude that the binding of glutamate to T1R1 is highly favorable whereas binding to T1R3 is expected to occur only transiently. We can find no experimental data for free energy of glutamate binding to taste receptors, to validate our simulations results. However, Morini et al [9] report free energies for various sweeteners binding to the closely related T1R2/T1R3 sweet receptor, with values ranging from -25 to -57 kJ/mol, that are in line with our value calculated for T1R1.

5 Conclusion

Homology modeling and molecular dynamics simulation provide a model for the ways in which glutamate may interact with the umami taste receptor. We have identified probable binding sites and, at the atomic level, specific interactions likely to be involved in glutamate binding.

From thermodynamic calculations, the binding free energy of glutamate for the two binding sites was estimated. Binding to T1R1 was found to be highly favourable, whereas only transient binding to T1R3 would be expected based on our calculations.

6 Acknowledgment

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Supplementary Data for Lopez Cascales et al., Binding of Glutamate to the Umami Receptor

Figure 1. Sequence alignment for ligand binding domains of mGluR1 (.pdb file 1EWK), human taste receptor T1R1, and human taste receptor T1R3.

CLUSTAL

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1EWK          -----QRS-VARMDGDVIIIGALFSV-HHQPPAEKVP---
sp|Q7RTX1|TS1R1 MLLCTARLVGLQLLI SCCWAFACHSTESSPDFTLPGDYLLAGLFPL-HSGCLQVRHRP--
sp|Q7RTX0|TS1R3 MLGPAVLGLSLWALLHP--GTGAPLCLSQ-QLRMKGDYVLGGLFPLGEAEEAGLRSTRP

1EWK          -ERKCGE--IREQYGIQRVEAMFHTLTKINADPVLLPNITLGSEIRDSCWHSSVALEQSI
sp|Q7RTX1|TS1R1 EVTLCDRSCSFNEHGYHLFQAMRLGVVEEINNSTALLPNITLG YQLYDVCSDSA-NVYATL
sp|Q7RTX0|TS1R3 SSPVCTR---FSSNGLLWALAMKMAVEEINNKSDDLPLGLRLGYDLFDTCSEPVVAMKPSL

1EWK          EFIR---KP-----IAGVIGPGSSSVAIQVQNLQLFDIPQIAYSATSIDL
sp|Q7RTX1|TS1R1 RVLSLPGQHHIELQGDLLHYSPTVLAVIGPDSTNRAATTAALLSPFLVPMISYAASSETL
sp|Q7RTX0|TS1R3 MFLAKAGSRDIAAYCNYTQYQPRVLAVIGPHSSELAMVTGKFFSFFLMPQVSYGASMELL

1EWK          SDKTLYKYFLRVVPSDTLQARAMLDIVKRYNWTYVSAVHTEGNYGESGMDAFKELAAQEG
sp|Q7RTX1|TS1R1 SVKRQYPSFLRTIPNDKYQVETMVLVLLQKFGWTWISLVGSSDDYGQLGVQALENQATGQG
sp|Q7RTX0|TS1R3 SARETFPSFFRTVPSTRVQLTAAAEELLQEFGNWVAALGSDDEYGRQGLSIFALAAARG

1EWK          LCIAHSDKI--YSNAGEKSF--RLLRKLRLRRLPKARVVVCFCEGMTVRGLLSAMRRLGV
sp|Q7RTX1|TS1R1 ICIAFKDIMPFSAQVGDERMQ--CLMRHLAQA--GATVVVVVFSRQLARVFFESVLTNL
sp|Q7RTX0|TS1R3 ICIAHEGLVPLPRADDSRLGKVQDVLHQVNQS--SVQVLLFASVHAAHALFNYSISSRL

1EWK          VGEFSLIGSDGWADRDEVIIEGYEVEANGGIT- IKLQSPVRSFDDYFLKLRDLTNTNRPW
sp|Q7RTX1|TS1R1 TGK-VWVASEAWALSRHITGVPGIQRIGMVLGVAIQKRAVPGLKA-FEEAYARADKKAPR
sp|Q7RTX0|TS1R3 SPK-VWVASEAWLTSDLVMGLPGMAQMGTVLGFLQGAQLHEFPQ-YVKTHLALATDPAF

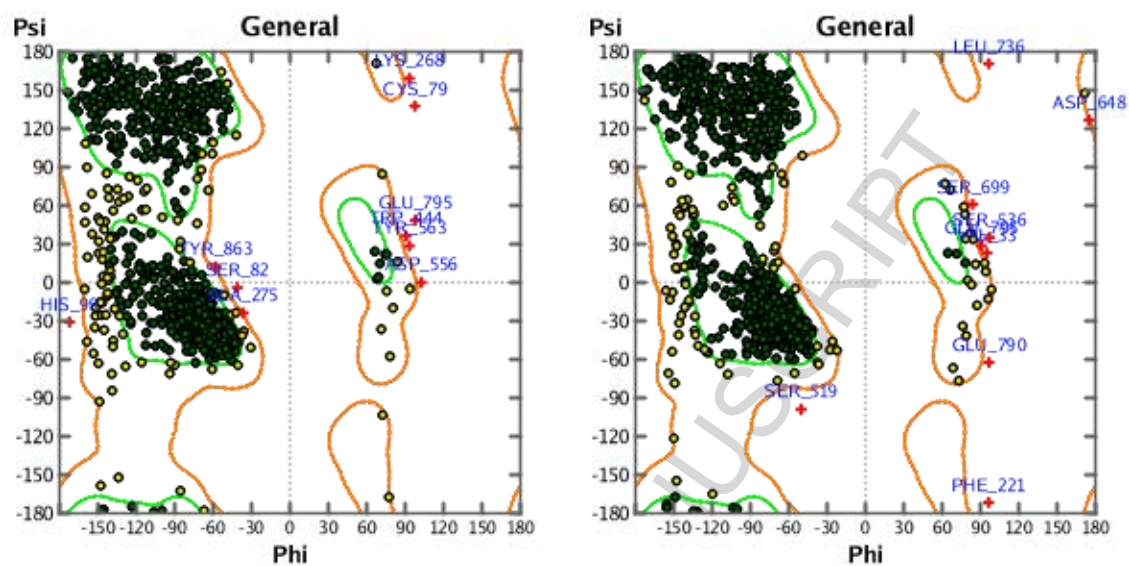
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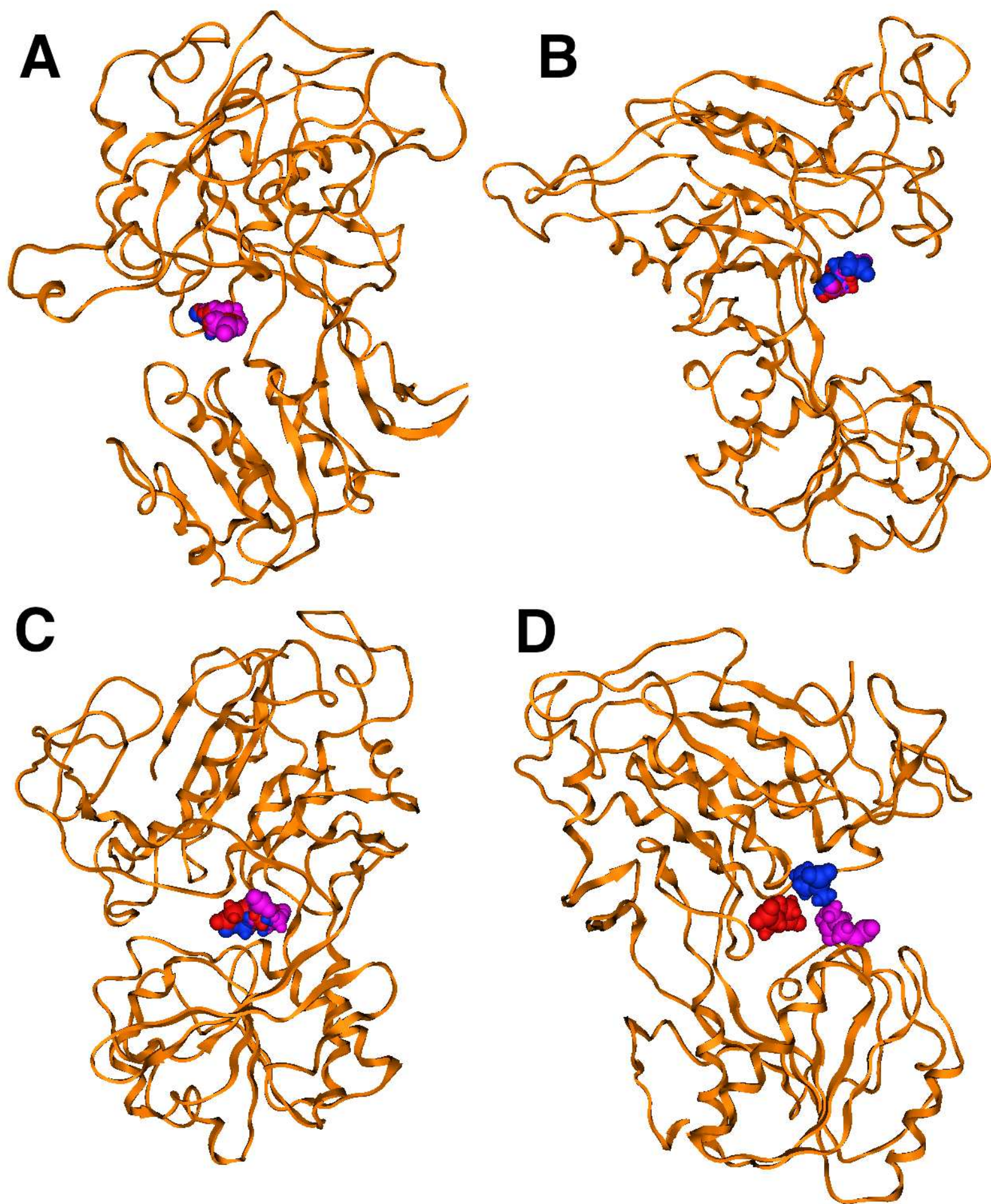
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sp|Q7RTX0|TS1R3 --HNTLQCNASGCPAQDPVKPWQLLENMYNLTFFH-VGGLPLRFDSSGNVDMEYDLKLWVW

1EWK          TEANRYDYVHVGT--WHEGVLNIDDYKI
sp|Q7RTX1|TS1R1 NGPK-WTFTVLGSSTWSPVQLNINETKI
sp|Q7RTX0|TS1R3 QGSV-PRLHDVGR--FNGS-LRTERLKI

```

Figure 2. Ramachandran plots of T1R1+T1R3 models. Left, Form 1. Right, Form 2.





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Binding of Glutamate to the Umami Receptor

Research Highlights

- A new model of the ligand binding domain of the umami taste receptor (T1R1 + T1R3) has been prepared
- Molecular dynamics simulations have provided detailed descriptions of the two binding sites for glutamate
- The free energy of binding has been estimated for glutamate in each of the two sites