1   **Understanding the molecular mechanism of umami recognition by**

2   **T1R1-T1R3 using molecular dynamics simulations**

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16   **Abstract**

17   Taste receptor T1R1-T1R3 can be activated by binding to several natural ligands, e.g., L-

18   glutamate and 5'-ribonucleotides etc., thereby stimulating the umami taste. The molecular

19   mechanism of umami recognition at an atomic level, however, remains elusive. Here, using

20   homology modeling, molecular docking and molecular dynamics (MD) simulations, we

21   investigate the effects of five natural umami ligands on the structural dynamics of T1R1-T1R3.

22   Our work identifies the key residues that are directly involved in recognizing the binding

23   ligands. In addition, two adjacent binding sites in T1R1 are determined for substrate binding,

24   and depending on the molecular size and chemical properties of the incoming ligand, one or

25   both these binding sites can be occupied. More interestingly, the binding of varied ligands can

26   lead to either closing or opening of T1R1, based on which, we further classify the five ligands

27   into two groups. This different binding effects are likely associated with the distinct umami

28   signals stimulated by various ligands. This work warrants new experimental assays to further

29   validate the theoretical model and provides guidance to design more effective umami ligands.

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31   **Author summary**

32   Umami, as the fifth basic taste, is induced by umami substances from the natural food, such

33   as L-glutamate, 5'-ribonucleotides, and peptides etc. These umami substances are widely

34   added to foods as flavor enhancers to promote food quality. However, although extensive

35   experimental and theoretical studies have been devoted to revealing the recognition

36   mechanisms of the taste receptor T1R1-T1R3 to the umami ligands, the detailed molecular

37   mechanism is still unknown, largely due to the lack of the receptor structure. Here, using a

38   new template structure different from the former theoretical studies, we constructed a more

39   accurate homology model of T1R1-T1R3. Based on this receptor model, combined with

40   molecular docking and MD simulations, we investigate how different ligands with varied

41   molecular size and chemical groups might affect the dynamics of T1R1. Our work provides the

42   structural basis for relating the dynamics of umami receptor induced by varied ligands to the

43   resulting umami signal.

44

Introduction

46 The umami, induced by binding of L-glutamic acid salt (MSG) to its taste receptor type 1 (T1R),

47 was discovered as one of the five basic tastes by Ikeda in 1908 [1-3]. T1R is comprised of three

48 members, including T1R1, T1R2, and T1R3, which can further dimerize to form either sweet

49 taste receptor (T1R2-T1R3) or umami taste receptor (T1R1-T1R3) [2, 4]. T1R belongs to the G-

50 protein-coupled receptors (GPCRs) family and is assigned to the same class as the

51 metabotropic glutamate receptor (mGluR) by previous phylogenetic tree analysis [5].

52   Human T1R1-T1R3 (hT1R1-T1R3) consists of a large extracellular N-Venus Flytrap domain

53 (VFD), and a seven-transmembrane domain (TMD), connected by a small cysteine-rich

54 domain (CRD) (Fig 1) [5]. VFD has been determined to be the ligand binding domain by former

55 site-directed mutagenesis studies using chimeric T1R constructs based on human sweet and

56 umami T1R VFD domains [3, 6], or human and rat T1R VFD domains [7-9]. Notably, apart from

57 VFD, the hT1R1 TMD is also considered as a potential substrate binding site for some umami

58 substances, such as methional [10], and S807 [6]. As a result, the T1R TMD couples with a G

59 protein inside the cell to stimulate the umami signal [11]. More interestingly, the umami can

60 be further enhanced by binding to additional 5'-ribonucleotides, such as disodium 5'-

61 guanylate (GMP) and disodium 5'-inosinate (IMP) [2, 4, 12]. This synergistic enhancement is

62 considered to be the most unique sensory feature of umami compared to other four basic

63 tastes. Notably, in addition to L-Glu, Nelson et al. and Toda et al. found that the hT1R1-T1R3

64 can also be activated by other standard L-amino acids, whereas hT1R1 are more sensitive to

65 L-Glu compared to other amino acids [2, 8]. Moreover, small peptides, especially those with

66 a molecular weight lower than 3000 Daltons, can also stimulate umami [13-15].

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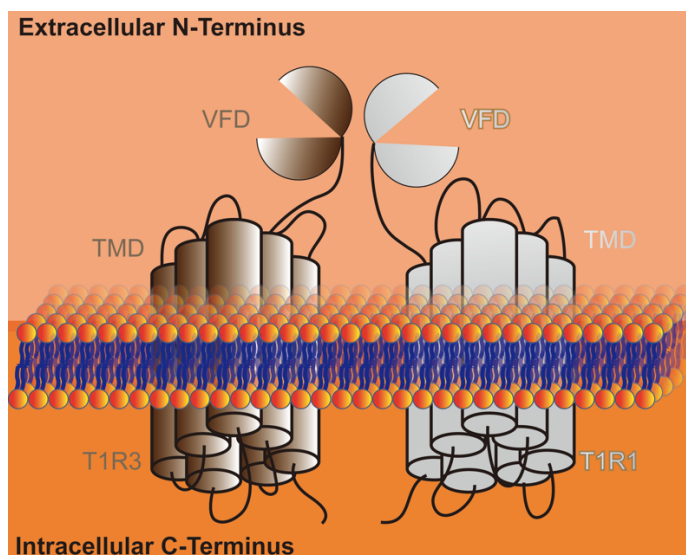
Fig 1. Schematic illustration of T1R1-T1R3 structure, which is a heterodimer with each subunit composed

of one TMD and one VFD connected by CRD.

To date, the crystal structure of hT1R1-T1R3 VFD has not been resolved. Computational

modeling has been used to construct a homology model of hT1R1-T1R3 and to understand

the molecular mechanism of umami recognition. For example, Cascales et al. [16] constructed

an hT1R1-T1R3 VFD model using the mGluR1 structure (PDB id: 1EWK) as a template. Their

MD simulations reveal that the L-Glu tends to induce the closing of T1R1 but not for T1R3.

Likewise, Dang et al. [17] also found that the T1R1 can undergo closing/opening motions in

the presence of umami peptides. In addition, Yu et al. [15] constructed a three-dimensional

quantitative structure-activity relationship model for five peptides and their umami

intensities. Moreover, Dang et al. [18] studied the molecular mechanism of the synergistic

effect between L-Glu and umami peptide using molecular docking. They revealed that after

T1R1 binds to MSG, T1R3 can adopt an open state that can recruit the peptides, therefore,

leading to the synergistic effect on promoting the umami intensity. In sharp contrast, Zhang

et al. [7] found that IMP and L-Glu can bind to hT1R1-VFD simultaneously, which provides

another explanation for the synergy. In addition, they further identified eight residues that

are critical for ligand binding using site-directed mutagenesis. Taken together, the previous

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85   theoretical studies have provided some structural basis for the ligand-bound hT1R1-T1R3

86   complexes. However, the effects of ligand binding on the dynamics of hT1R1-T1R3 are largely

87   unknow, and how structurally varied ligands might affect the dynamics of hT1R1-T1R3 in

88   different manners are also unclear. Moreover, the homology template used in former work

89   is mGluR, which has lower sequence identity (< 30 %) with hT1R1 or hT1R3 compared to the

90   recently obtained crystal structure of fish T1R2a-T1R3 [19].

91   Here, by employing homology modeling, molecular docking, and MD simulations, we

92   constructed five ligand-bound hT1R1-T1R3 VFD complexes based on the fish T1R2a-T1R3

93   structure (PDB id: 5X2M), including MSG, WSA (sodium succinate), GMP, IMP, and BMP (beefy

94   meaty peptide). We find all the ligands can reach into a cleft region of hT1R1-VFD, and more

95   interestingly, two binding sites in hT1R1-VFD are identified. Depending on the molecular size

96   and carried charges of the incoming ligands, one or both of the above two sites can be

97   occupied. Moreover, based on the effects of ligand binding on the closing and opening

98   motions of hT1R1-VFD, we further classify the ligands into two groups, which may relate to

99   their different bioactivities as umami substances. Our findings provide an atomistic-level

100   understanding of the structure-function relationship for different substrates, which further

101   guides to design more structurally diversified umami ligands.

102

Results

Structural features of hT1R1-T1R3 VFD in complex with different umami ligands.

105   A homology model of hT1R1-T1R3 VFD was constructed based on the counterpart domain

106   from fish sweet receptor T1R2a-T1R3 (PDB id: 5X2M) complexed with one L-glutamine (L-Gln)

107   ligand. The hT1R1-VFD and hT1R3-VFD subunits share sequence identity of 33.03 % and 33.87

108   % with the corresponding subunit of fish sweet receptor, respectively, which is higher than

109   mGluR that was used in former studies [3, 8, 17]. Moreover, we constructed the phylogenetic

110   tree for T1R and mGluR based on their VFD, sourced from human, fish, mouse, and rat (S2

111   Fig). The results show that the hT1R1-T1R3 VFD has higher homology with fish T1R2a-T1R3

112   compared to mGluR1, indicating that fish T1R2a-T1R3 is a better homology template to

113   construct the hT1R1-T1R3 VFD structure. As shown in Fig 2A, hT1R1-T1R3 VFD is a

114   heterodimer, consisting of two ligand binding (LB) domains that form pseudo-symmetrical

115   lobes, and each LB domain is comprised of two parts, LB1 and LB2, resembling to the

116   structural characteristics of mGluR1 and human GABA(B) receptor [19-21].

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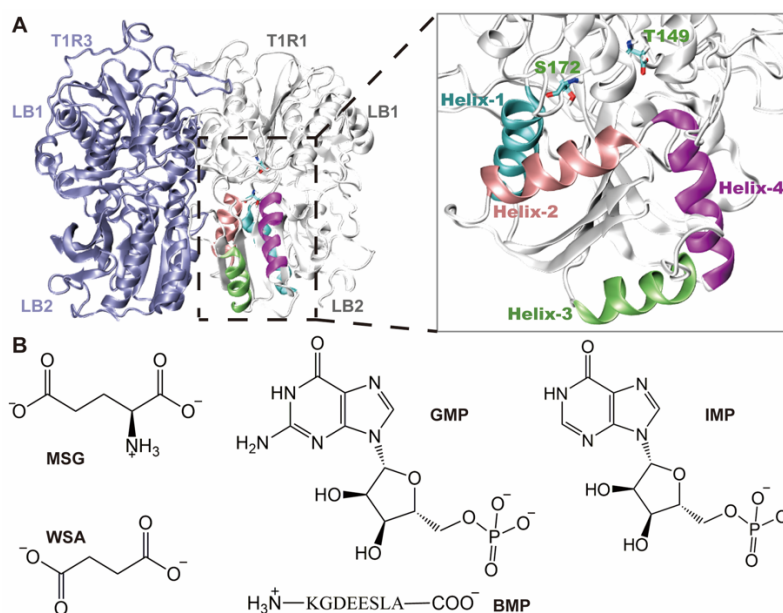
118   **Fig 2.** The constructed model of hT1R1-T1R3 VFD and chemical formulas of five umami ligands. **(A)**

119   Homology model of hT1R1-T1R3 VFD, in which hT1R1 and hT1R3 are shown in gray and blue cartoons,

120   respectively. The ligand binding site and key structural motifs of hT1R1 are highlighted in the zoom-in image,

121   in which two active site residues S172 and T149 are shown in cyan sticks, and four LB2 helices, including

122   the residues 192-205 (helix-1), 219-232 (helix-2), 256-266 (helix-3), and 277-290 (helix-4), are highlighted

123   in cyan, pink, lime, and magenta, respectively. **(B)** Chemical structures of five umami ligands studied here,

124   including MSG, WSA, GMP, IMP, and BMP.

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125   Based on the modeled structure of hT1R1-T1R3 VFD, we further constructed ligand-bound

126   hT1R1-T1R3 VFD complexes for five natural umami ligands using molecular docking strategies,

127   including MSG, WSA, GMP, IMP, and BMP (Fig 2B). Only hT1R1 was used as the docking

128   receptor since hT1R3 was found to play an auxiliary role for ligand binding [8, 22]. For each

129   ligand, the structure with the highest binding affinity was used as the final docking complex

130   for the following analysis. In the MSG-bound complex, the ligand can sit in a cleft region

131   formed by LB1 and LB2, and forms direct contacts with the residues S172, T149, A170, D147,

132   A302, R277, and Y200 (Fig 3A). In specific, MSG is sandwiched between the side-chains of

133   D147 and Y220, and forms hydrogen bonds (HBs) with S172 and T149 through its C_α-COO$^-$

134   group (Figs 2A and 3A). In addition, the C_α-NH$_3^+$ terminus of MSG can form HBs with the main

135   chain of A170, D147, and A302. The side-chain carboxylate group of MSG can establish one

136   HB with the R277 amide.

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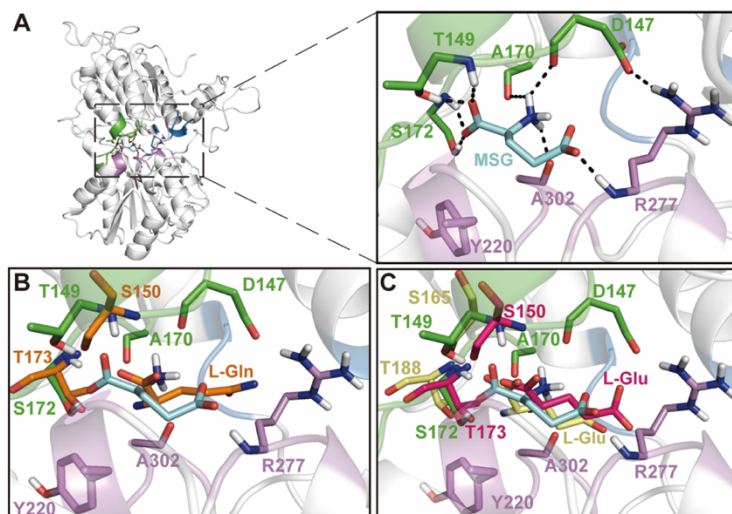
Fig 3. (A) Docking complex of MSG-bound hT1R1-VFD. The detailed interactions between MSG and nearby

residues are highlighted in the zoom-in image, MSG is shown in cyan sticks and the hT1R1-VFD residues

are shown in green/blue/violet sticks. Several key HBs are highlighted with black dashed lines. **(B)** Structural

overlay of the MSG-bound hT1R1-VFD to fish T1R2a-T1R3 (PDB id: 5X2M, ligand: L-Gln), and the ligand L-

Gln, S150, and T173 in T1R2a-T1R3 are shown in orange sticks. **(C)** Structural overlay of the MSG-bound

143   hT1R1-VFD complex to fish T1R2a-T1R3 (PDB id: 5X2P, ligand: L-Glu) and mGluR1 (PDB id: 1EWK, ligand: L-

144   Glu). L-Glu, S150, and T173 in T1R2a-T1R3 are shown in hot-pink sticks; L-Glu, S165, and T188 in mGluR1

145   are shown in pale-yellow sticks.

146   To evaluate the docking result, we compared the MSG-bound hT1R1-T1R3 VFD complex

147   with the crystal structure of fish T1R2a-T1R3 (PDB id: 5X2M, 5X2P) and mGluR1 (PDB id: 1EWK)

148   where the binding ligand is either L-Gln (from 5X2M) or L-Glu (from 5X2P, 1EWK) [19, 20], so

149   that we can compare the ligand binding modes for different complexes. We firstly

150   superimposed our modeled hT1R1-T1R3 VFD to each of the above three crystal structures by

151   their C_α atoms. Notably, the C_α-COO$^-$ group of ligands from the four complexes all reside in a

152   same pocket and lie in a similar orientation (Fig 3B and 3C). However, it is worth to note that

153   since the active site residues for different systems are not fully conserved among different

154   systems, the detailed interaction networks between the ligand and nearby residues are also

155   different (S1 Fig). For example, the residue Y220 is completely conserved in the four

156   complexes, while S172 and T149 in hT1R1-T1R3 are replaced by threonine and serine in both

157   T1R2a-T1R3 and mGluR, respectively (Fig 3B and 3C). Moreover, the residue D147 in hT1R1-

158   T1R3 is replaced by glutamine and glycine in fish T1R2a-T1R3 and mGluR, respectively (S1 Fig)

159   [23], which may lead to the fact that the taste stimuli are different for varied species. Taken

160   together, our MSG-bound hT1R1-T1R3 VFD complex provides a fairy good starting structure

161   for the following MD simulations.

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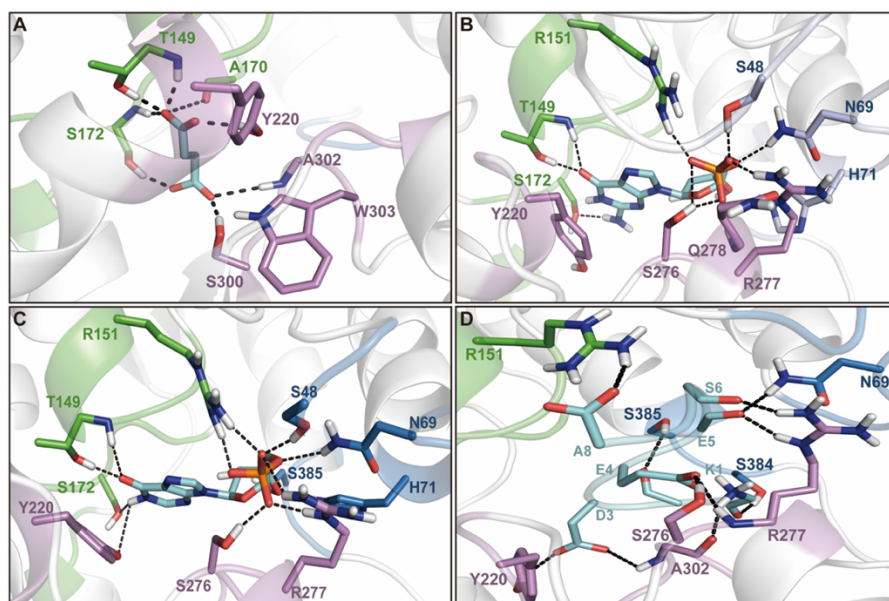
Fig 4. Interaction networks between umami ligands with hT1R1-VFD in each docking complex, including WSA **(A)**, GMP **(B)**, IMP **(C)**, and BMP **(D)**. In each complex, the ligand is shown in cyan sticks, and the key residues that form direct contacts with the ligand are shown in green/blue/violet sticks. Several key HBs are highlighted with black dashed lines.

We then docked the other four ligands (WSA, GMP, IMP, and BMP) into the same binding pocket as that for MSG. In the WSA binding complex, one of its COO⁻ group sits in the same site as the C_α-COO⁻ group of MSG, while the other COO⁻ group can form HBs with the residues A302 and S300 (Fig 4A). In addition, WSA can form hydrophobic contacts with Y220 (Fig 4A). For the GMP and IMP binding complexes, the two ligands bind to hT1R1-T1R3 VFD in a very similar mode (Fig 4B and 4C). The nucleobase of these ligands can form hydrophobic contacts with Y220 and several HBs with S172 and T149, while the phosphate group can reach into another pocket and form HBs with S276, S48, N69, and R277. Finally, in the BMP-bound complex, the peptide BMP binds in the same pocket as that for the phosphate group of GMP and IMP (Fig 4B, 4C, and 4D). In specific, the BMP can form HBs with the side-chain of R151 through its C-terminal A8. Moreover, the negatively charged residues D3, E4, E5 of BMP can also form HBs with the residues A302, Y220, S276, R277, S385, and N69 (Fig 4D).

179   Taken the above results together, two major binding sites exist in the ligand-binding pocket

180   of hT1R1-VFD, one is surrounded by the LB1 residues D147, T149, R151, A170, and S172 (Figs

181   3A and 4, shown in green sticks), termed as site A; another consists of the LB1 residues S48,

182   N69, H71, S384, and S385 (Fig 4, shown in blue sticks), termed as site B. In particular, the LB2

183   residues Y220, S276, R277, Q278, S300, A302, and W303 (Figs 3A and 4, shown in violet sticks)

184   are shared by these two binding sites.

185

186   **Effects of ligand binding on structural dynamics of hT1R1-T1R3 VFD**

187   To study the effects of the ligand binding on the structural dynamics of hT1R1-T1R3 VFD, we

188   performed MD simulations starting from the above five ligand-bound hT1R1-T1R3 VFD

189   complexes. For each system, we performed two parallel 100-ns MD simulations initiated from

190   different velocities. As a control, we performed additional 100-ns MD simulations for the apo

191   hT1R1-T1R3 VFD (without ligand). To examine whether the system reaches equilibration

192   within 100-ns MD simulation, we firstly calculated the root-mean-square deviations (RMSD)

193   along the simulation time for each system using the minimized apo hT1R1-T1R3 VFD structure

194   as the reference structure. The results show that the RMSD curves tend to level off after ~80-

195   ns for each system (S3 Fig). We thus only kept the last 20-ns simulation data of each system

196   for further analysis.

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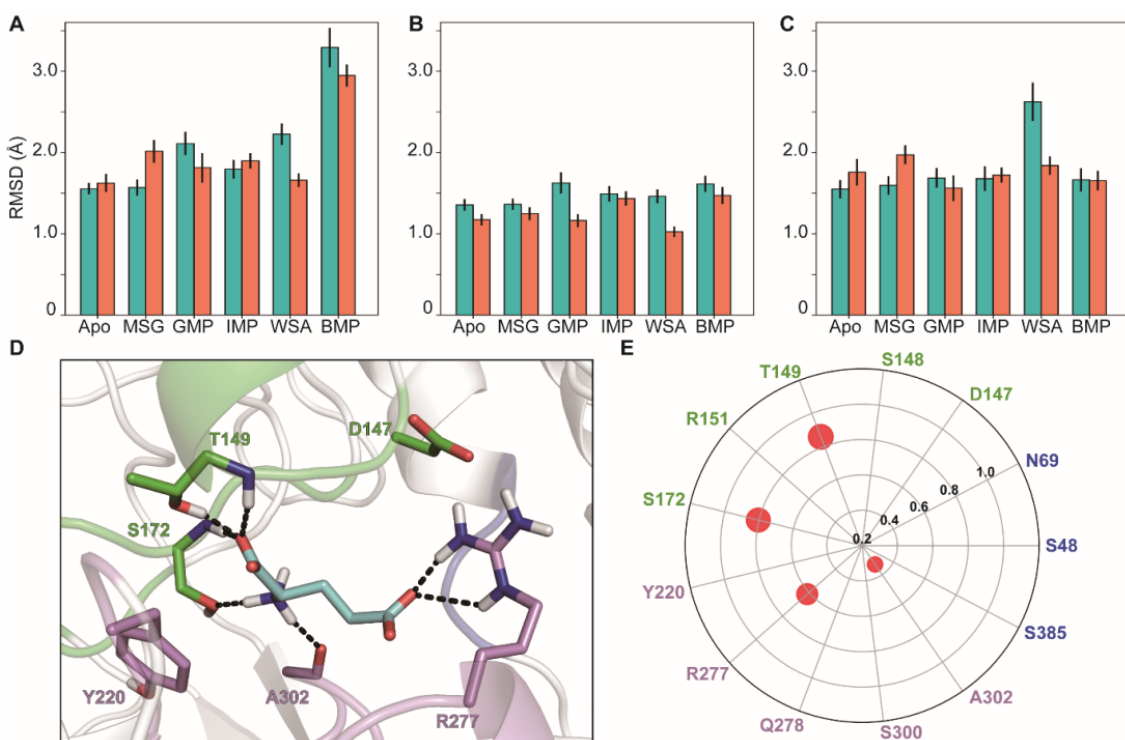
Fig 5. Effects of the ligand bindings on the receptor dynamics and HB network analysis for the MSG-bound

hT1R1-VFD complex. RMSD of LB1+LB2 **(A)**, LB1 **(B)**, and LB2 **(C)** regions calculated based on two-parallel

100-ns MD simulations (shown in cyan and coral, respectively). For each system, the mean RMSD and the

corresponding standard error were calculated for the last 20-ns simulation conformations. **(D)** The binding

mode of MSG (shown in cyan sticks) with hT1R1-VFD, the structure is derived from the last snapshot of the

100-ns MD simulation. The key residues that form direct contacts with MSG are shown in green/blue/violet

sticks. Several key HBs are highlighted with black dashed lines. **(E)** The HB network map for the MSG-bound

hT1R1-VFD complex. The residues that can form HB with MSG are highlighted with red dot, with the dot

size corresponding to the HB occupancy.

For each system, we further calculated the RMSD values for the C_α atoms of three different

parts of hT1R1-VFD, namely LB1+LB2, LB1, and LB2, by firstly fitting to the corresponding

regions to the apo hT1R1-VFD structure. The results show that comparing to LB1, the LB2 and

LB1+LB2 domains demonstrates relatively higher structural fluctuations, reflected from their

larger RMSD values, which suggests that LB1 keeps relatively rigid compared to LB2 during

the simulations (Fig 5A, 5B, and 5C). Therefore, to better characterize the recognition

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213   mechanism of these ligands, the LB1 domain of the apo hT1R1-VFD is used as a reference for

214   the further analysis. Moreover, the RMSD calculations indicate that the apo system

215   demonstrates relatively small structural fluctuations compared to the ligand-bound

216   complexes (Fig 5A, 5B, and 5C), suggesting the ligand binding can impose some structural

217   change of hT1R1-VFD. On the other hand, among the ligand-bound systems, the BMP-bound

218   complex exhibits the largest RMSD values for the LB1+LB2 regions, likely due to the relatively

219   bigger molecular size of BMP (Fig 5A). The other ligand-bound systems, however, have similar

220   structural fluctuations for the LB1+LB2 regions (Fig 5A). Notably, the paralleled MD

221   simulations for each system can reproduce the structural features of hT1R1-VFD structure

222   fairly well (Fig 5A, 5B, and 5C), we thus only used one of the two MD simulations for the

223   further analysis.

224   We next analyzed the interacting networks between each ligand and hT1R1-VFD by

225   calculating the hydrogen bond occupation (OC_{HB}). We only consider the HB that sustains

226   longer than 30 % of the simulation time for further structural analysis. In the MSG system,

227   four residues, namely T149, S172, R277, and A302, can form stable HBs with MSG, among

228   which T149 exhibits the highest OC_{HB} (85.8 %) and A302 exhibits the lowest OC_{HB} (32.9 %) (Fig

229   5E). In specific, T149 and S172 forms HBs with the C_α-COO$^-$ group of MSG in the binding site

230   A, as observed in the initial docking complex (Fig 3A). Moreover, R277 can form HB with the

231   side-chain COO$^-$ group of MSG, and notably, it can also form a salt bridge with D147, which

232   may strengthen the MSG binding (Fig 5D).
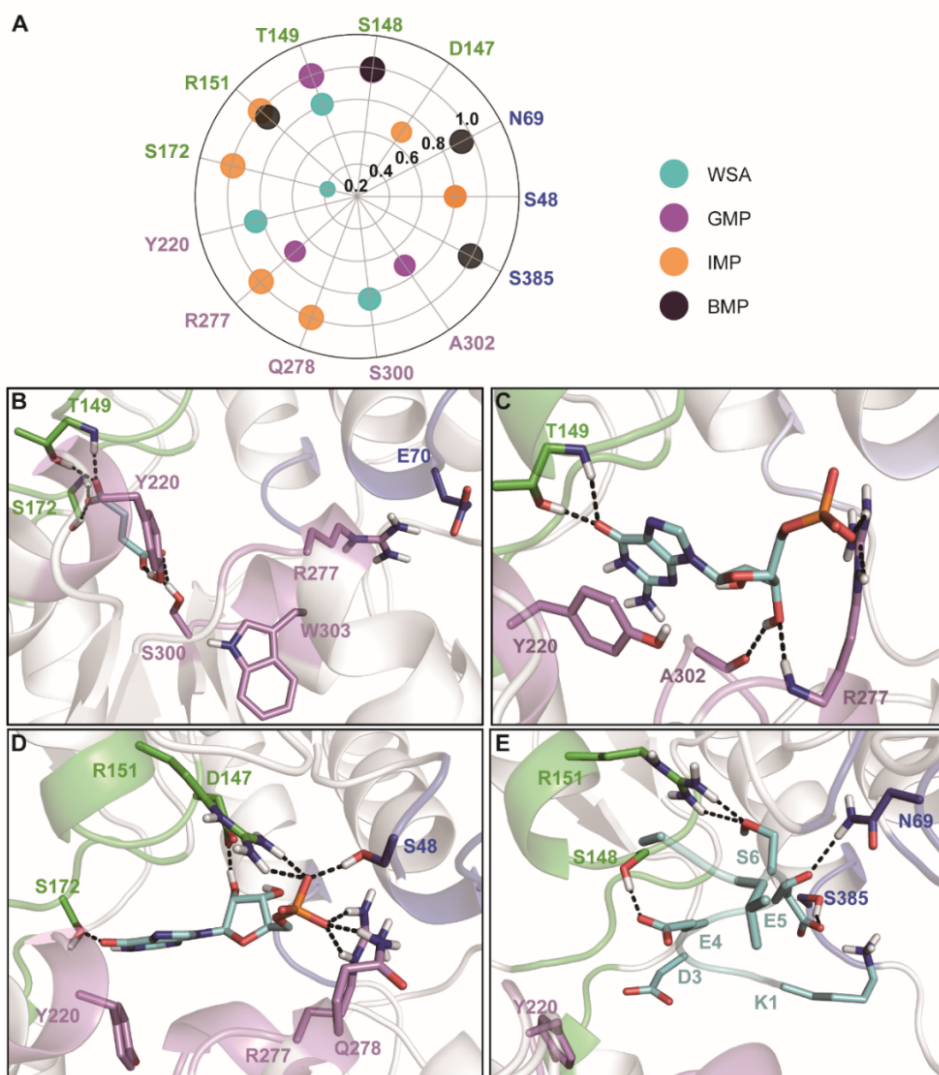
Fig 6. (A) The HB network map for four ligand-bound hT1R1-VFD complexes, including WSA, GMP, IMP, and BMP. **(B-E)** The binding mode of each ligand (shown in cyan sticks) with hT1R1-VFD, namely WSA **(B)**, GMP **(C)**, IMP **(D)**, and BMP **(E)**. The structures are derived from the last snapshot of the 100-ns MD simulation for each system. The ligand is shown in cyan sticks, and the residues that form direct contacts with the ligand are shown in green/blue/violet sticks. Several key HBs are highlighted with black dashed lines.

Similar HB analyses were performed for other ligand-bound systems. In the WSA-bound complex, four residues, Y220, S300, T149, and S172, can form stable HBs with the COO$^-$ groups of WSA, among which Y220 exhibits the highest OC$_{HB}$ (84.5 %) and S172 exhibits the lowest OC$_{HB}$ (38.8 %) (Fig 6A and 6B). In specific, T149 and S172 in the binding site A can form HBs

244   with one of the COO⁻ groups, and the other COO⁻ group can form stable HBs with Y220 and

245   S300 (Fig 6B). It is noteworthy that WSA is unable to bind to the binding site B. In the GMP-

246   bound complex, the ligand can form HBs with T149, A302, and R277, among which T149

247   exhibits the highest OC_{HB} (99.6 %) and R277 exhibits the lowest OC_{HB} (71.2 %) (Fig 6A and 6C).

248   In the IMP-bound complex, however, six residues, Q278, R151, R277, S172, S48, and D147,

249   form stable HBs with the substrate, and D147 exhibits relatively low OC_{HB} (68.3 %) compared

250   to other five residues (all > 80 %) (Fig 6A and 6D). Notably, GMP and IMP both sit in the same

251   pocket, with their phosphate group forming HB with R277 in the binding site B. Whereas

252   several HBs can be formed between IMP and R151, which is absent in GMP complex (Fig 6C

253   and 6D). Finally, in the BMP-bound complex, BMP largely sits in the binding site B, and forms

254   HBs with S148, S385, R151, and N69, with the OC_{HB} value all higher than 80 % (Fig 6E). The

255   above HBs are formed through the BMP residues E4, E5, and S6. In specific, the side-chain

256   COO⁻ of E4 forms HB with the side-chain of S148, and BMP E5 forms HBs with N69 and S385

257   (Fig 6E). In addition, BMP S6 can form HB with the side-chain of R151, and an intermolecular

258   HB can also be formed in BMP between K1 and E5.

259   Our HB analysis is consistent with our previous conclusion that, in general, the ligands

260   largely interact with two binding sites in hT1R1-VFD, and in each site, several polar residues

261   within the binding pocket that can potentially form HBs with the incoming ligand, as shown

262   in Figs 5E and 6A. Based on varied molecular size and chemical groups of the incoming ligand,

263   one or both of the above two sites can be occupied, i.e., MSG and WSA largely sit in the site

264   A, while BMP binds to the site B; GMP and IMP, on the other hand, can occupy both binding

265   sites. Therefore, we can expect that varied ligands may impose structural changes of hT1R1-

266   VFD in different manners, which in turn can induce distinct umami intensities.

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268 **Effects of ligand bindings on the opening/closing motions of hT1R1-VFD**

269 Previous studies have suggested that the opening/closing motions of VFD are critical for

270 ligand binding [20]. To measure the effects of the ligands on the opening/closing motions of

271 hT1R1-VFD, we calculated the radius of gyration (Rg) values for the two identified ligand

272 binding sites (A and B) in hT1R1-VFD, which can roughly measure the extents of the hT1R1-

273 VFD opening/closing. Both sites contain the LB2 residues 219-221, 276-278, 300-303, and in

274 site A, we also include the LB1 residues 146-151, 170-173 (shown in green and violet in Fig

275 7A), and in binding site B, the LB1 residues 47-49, 68-71, 384-386 are included (shown in blue

276 and violet in Fig 7A). For each binding site, we then projected the MD conformations onto

277 two reaction coordinates: Rg and RMSD, calculated for the binding site residues (Fig 7B and

278 7C).
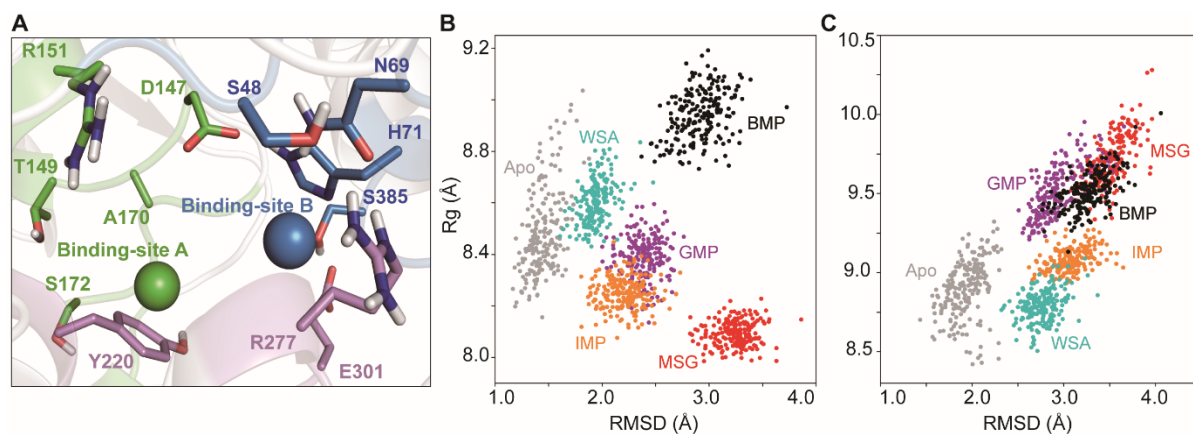
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280 **Fig 7.** The opening/closing motions of hT1R1-VFD induced by ligand binding. **(A)** To investigate the closing

281 and opening motions of hT1R1-VFD, two binding sites (A and B) are defined and represented with green

282 and blue sphere, respectively. Each sphere represents the centroid of C_α atoms of residues within each

283 binding site. In specific, both binding sites include the LB2 residues 219-221, 276-278, 300-303 (shown in

284 violet). In addition, the site A also contains the LB1 residues 146-151, 170-173 (shown in green), and the

285 site B contains the LB1 residues 47-49, 68-71, 384-386 (shown in blue). For the binding site A **(B)** and B **(C)**,

286 we mapped the MD conformations onto two reaction coordinates: one is the Rg of the residues belonging

287 to each site, another one is the corresponding RMSD.

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288   For binding site A, by treating the apo system as a ground state, we can group the five

289   ligands into two classes (Fig 7B). One group includes WSA and BMP that prefer to increase

290   the Rg value of the binding site A, suggesting that they can potentially promote the opening

291   of hT1R1-VFD. In contrast, the other group, consisting of MSG, GMP, and IMP, can tighten the

292   site A, reflected from the reduced Rg values comparing to the apo system. However, it is

293   worth to note that within each group, the ligands also act differently on the protein dynamics,

294   reflected from both the Rg and RMSD calculations. In specific, BMP exerts much higher

295   structural disturbances on hT1R1-VFD than WSA, with an increased Rg and RMSD values, and

296   has stronger effects on promoting the opening of the binding site A compared to WSA. For

297   the second group, MSG tightens the binding site A the most compared to GMP and IMP,

298   reflected from its lowest Rg values (Fig 7B). GMP and IMP, on the other hand, can affect the

299   protein structure in a similar manner.

300   On the other hand, for binding site B, only WSA can slightly tighten the binding site B,

301   reflected from the reduced Rg value comparing to the apo system (Fig 7C), which is likely due

302   to the fact that WSA is unable to reach the binding site B. The other ligands, however, can all

303   promote the opening of binding site B, reflected from the increased Rg values comparing to

304   the apo system (Fig 7C). In specific, MSG, BMP, and GMP exert relatively larger effects on

305   promoting the hT1R1-VFD opening compared to IMP. It is noteworthy that although MSG

306   largely binds to site A, it tightens binding site A, whereas opens up binding site B (Fig 7B and

307   7C), we speculate that this is because MSG attracts more interactions from the LB2 residues,

308   e.g., Y220, R277, and A302, which induces the movements of LB2 towards site A.

309   In conclusion, depending on the various incoming ligands, the hT1R1-VFD can undergo

310   either opening or closing motions. In particular, MSG, GMP, and IMP tend to promote the

311   closing of binding site A, while opening of binding site B. BMP, however, can promote the

312   opening of both two binding sites. Notably, WSA can also promote the opening of binding site

313   A, whereas slightly induce the closing of site B.

314

315   **Effects of ligand bindings on hT1R1 LB2 dynamics**

316   The hT1R1-VFD opening/closing induced by ligand binding may result in a structural

317   rearrangement of either LB1 or LB2 domain. Our previous analyses indicate that LB1 keeps

318   relatively rigid compared to LB2, therefore, we further examine the effects of ligand binding

319   on the dynamics of LB2. We thus pinpointed two helices (termed as 1 and 2) near to the

320   binding site A, and two other helices (termed as 3 and 4) near to the binding site B for the

321   following dynamic cross-correlation map (DCCM) analysis (Fig 2A). DCCM describes the

322   correlated motion for a residue pair, with a positive value representing positive correlation

323   (moving towards the same direction), while a negative value indicating the residue pair

324   moving towards the opposite direction (Fig 8).
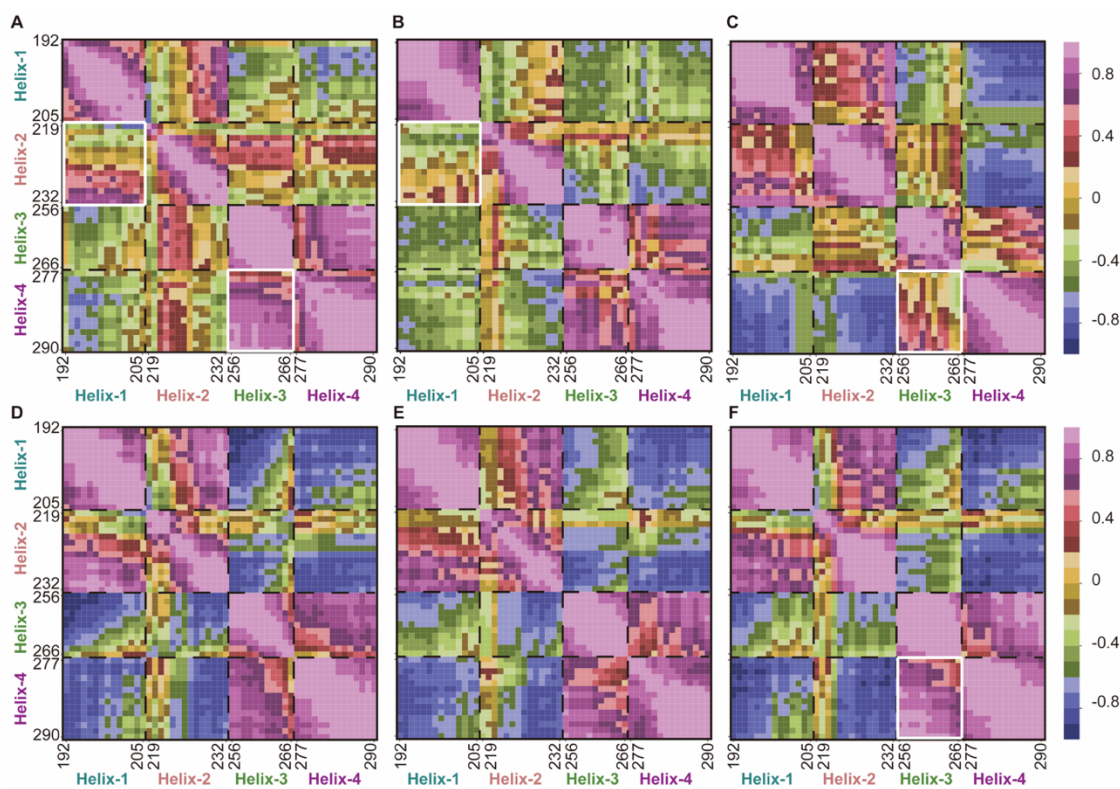
325

326   **Fig 8.** Effects of ligand bindings on hT1R1-VFD dynamics. Cross-correlation matrices of four LB2 helixes for

327   apo hT1R1 **(A)**, and five ligand-bound hT1R1-VFD complexes, namely MSG **(B)**, WSA **(C)**, GMP **(D)**, IMP **(E)**,

328   and BMP **(F)**. The correlation values were calculation based on the last 20-ns MD simulation dataset for

329   each system, with the value equal to 1 representing a positive correlation between two motions, and the

330   value equal to -1 representing a negative correlation between two motions.

331   The DCCM shows that the five ligands indeed exert different effects on the dynamics of the

332   LB2 domain. For the apo system, helix-1 and 2 have a weak correlation, and helix-3 and 4 have

333   a strong positive correlation (Fig 8A). In comparison, the MSG binding can further weaken the

334   correlation between helix-1 and 2, while has no obvious effect on the helix 3 and 4 (Fig 8B),

335   suggesting that MSG can interfere the interacting networks between helix 1 and 2. In addition,

336   GMP and IMP demonstrate very similar correlation values, suggesting that these two ligands

337   impose similar stresses on the binding sites in hT1R1-VFD, which is consistent with previous

338   results (Fig 8D and 8E). For WSA and BMP, their bindings give rise to a stronger positive

339   correlation between helix 1 and 2 compared to the apo system (Fig 8C and 8F), consistent

340   with the above analysis that they can both open up binding site A (Fig 7B). Moreover, WSA

341   tents to decorrelate helix 3 and 4 compared to all other systems, suggesting its potential role

342   in interrupting the interaction network between helix 3 and 4.

343   Taken the above results together, the ligand binding within the interface between LB1 and

344   LB2 can indeed affect the structure dynamics of LB2, likely through either tightening or

345   opening up the binding sites. In particular, the closing of binding site tends to weaken the

346   couplings between the adjacent helices, such as the effects of MSG on helix 1 & 2 and WSA

347   on helix 3 & 4.

Discussion

349 One of our major findings is to identify the key residues that directly recognize the five umami

350 ligands, which warrants further experimental studies to validate our theoretical models.

351 Former modeling and mutagenesis work has already identified several critical hT1R1-VFD

352 residues for MSG, such as S172, D192, Y220, E301, A170, and A302; moreover, H71, R277,

353 S306, and H308 were identified to be critical for GMP and IMP binding [3, 8]. This result is

354 well consistent with our theoretical models in which S172, A302 can form direct contacts with

355 MSG, and R277 is essential roles in recognizing GMP and IMP. In addition, Y220 can form

356 hydrophobic contacts with all the above three ligands (Figs 5D, 6C, and 6D). Therefore, the

357 constructed model of hT1R1-T1R3 VFD using the fish T1R2a-T1R3 as a template is reliable to

358 derive the binding complexes for umami ligands. Moreover, we can predict more mutational

359 effects on the substrate binding, such as WSA can form important contacts with S172, T149,

360 Y220, and S300 (Fig 6B); the residues T149 and A302 are found to be critical for GMP binding

361 (Fig 6C); five residues R151, S148, N69, Y220, and S385 can directly interact with BMP (Fig 6E).

362 Notably, we can see that the afore-mentioned residues are not highly conserved among

363 different species, such as, S48, N69, D147, R151, A170, Q278, and S385 in hT1R1-VFD are

364 completely non-conservative in fish, rat, and mouse (S1 Fig). Interestingly, former

365 luminescence intensities assay have shown that L-Glu can induce higher umami intensities to

366 hT1R1-T1R3 than mT1R1-T1R3, indicating the sequence differences in the two receptors

367 indeed influences the umami responses [8]. Therefore, our model suggests that these non-

368 conservative residues may contribute to the distinct umami intensities for different species.

369 More importantly, we find that hT1R1-VFD contains two major binding sites for the umami

370 ligands, and the molecular size and carried charges of the incoming ligand determine its

371 favored binding sites. In specific, MSG and WSA can largely sit in the binding site A, BMP binds

372   to the binding site B, while GMP and IMP can occupy both sites (Figs 5 and 6). From a

373   structural perspective, the site B has a larger binding pocket than site A, which allows the

374   binding of relatively bigger chemical groups (Figs 3A and 7A). On the other hand, to further

375   understand the charge preference for different binding sites, we calculated the electrostatics

376   potentials for hT1R1-VFD using Adaptive Poisson-Boltzmann Solver (APBS). The results

377   indicate that the binding site B is more positively charged compared to binding site A (S4 Fig).

378   In summary, we thus speculate that ligand with large molecular size and negatively charges

379   tends to bind to binding site B, for example, E4 and E5 in BMP, and the phosphate group of

380   GMP and IMP (Fig 6C, 6D, and 6E).

381   Former experimental studies have shown that the umami threshold value of BMP (1.41

382   mM) is lower than MSG (1.56 mM), indicating that the taste receptor is more sensitive to BMP

383   than MSG [24]. Moreover, Zhang et al., using the fluorescence imaging plate reader (FLIPR),

384   studied the molecular mechanism of umami synergism for GMP and IMP [3]. They found that

385   both ligands can enhance the umami stimuli of L-Glu. These studies indicate that different

386   umami substances might impose different effects on the protein dynamics. As shown above,

387   different umami ligands can occupy one or both of the two binding sites, leading to distinct

388   closing/opening motions of hT1R1-VFD. For example, BMP promotes the binding site A

389   opening, while MSG tends to close the binding site A. Considering these two ligands have

390   different umami threshold values, we thus speculate the umami properties of the two ligands

391   may relate to their different effects exerted on the protein dynamics. On the other hand, GMP

392   and IMP can impose similar structural influences on the receptor, i.e., they can both induce

393   the closing of binding site A, which lead to a similar dynamic properties of the hT1R1 LB2

394   region (Fig 8D and 8E). This finding may explain the experimental observation that GMP and

395   IMP have similar umami intensities. Taken together, our work provides a structural basis for

396   relating the effects of ligand binding to the resulting umami signals.

397

Conclusions

399   In this work, by employing homology modeling, molecular docking, and MD simulations, we

400   constructed the ligand-bound hT1R1-T1R3 VFD complexes for five natural umami ligands and

401   revealed how the ligand bindings affect the dynamics of hT1R1-VFD. We identified the key

402   residues that play essential roles in recognizing the ligands, some of the results are supported

403   by former site-directed mutagenesis studies [3, 8]. Further interacting network analysis

404   indicates that two major adjacent binding sites exist in the cleft region of hT1R1-VFD. More

405   interestingly, we find that depending on the molecular size and chemical properties, the

406   incoming ligand can sit in one or both of these two binding sites, which in turn can regulate

407   the closing and opening motions of hT1R1-VFD. In conclusion, our work reveal, at an

408   atomistic-level, the structural basis for the functional roles of different umami ligands in

409   regulating the dynamics of hT1R1-T1R3, and provides further guidance for designing more

410   effective umami ligands.

411

Methods

413   **Constructing the hT1R1-T1R3 VFD structure using homology modeling**

414   By employing the structure of fish taste receptor T1R2a-T1R3 (PDB id: 5X2M) as a homology

415   template, we constructed the model of hT1R1-T1R3 VFD using SWISS-MODEL software [25].

416   The target amino acid sequences of hT1R1-T1R3 VFD was acquired from the universal protein

417   resource knowledgebase (UniProKB) (HT1R1, Q7RTX1; HT1R3, Q7RTX0). The fish T1R2a-T1R3

418   was used for the homology modeling because it exhibits the highest sequence identity with

419   hT1R1-T1R3 VFD compared to other homologues (S1 Fig). Moreover, a phylogenetic tree was

420   constructed to further prove that the fish T1R2a-T1R3 is the best template so far for the

421   model construction. In specific, the T1R and mGluR were selected to construct the

422   phylogenetic tree based on their VFD, sourced from human T1R1 (h-T1R1, UniProtKB:

423   Q7RTX1), human T1R2 (h-T1R2, UniProtKB: Q8TE23), human T1R3 (h-T1R3, UniProtKB:

424   Q7RTX0), mouse T1R1 (m-T1R1, UniProtKB: Q99PG6), mouse T1R2 (m-T1R2, UniProtKB:

425   Q925I4), mouse T1R3 (m-T1R3, UniProtKB: Q925D8), rat T1R1 (r-T1R1, UniProtKB: Q9Z0R8),

426   rat T1R2 (r-T1R2, UniProtKB: Q9Z0R7), rat T1R3 (r-T1R3, UniProtKB: Q923K1), fish T1R2a (f-

427   T1R2a, PDBID: 5X2M), fish T1R3 (f-T1R3, PDBID: 5X2M), human mGluR1 (h-mGluR1,

428   UniProtKB: Q13255), human mGluR4 (h-mGluR4, UniProtKB: Q14833), mouse mGluR1 (m-

429   mGluR1, UniProtKB: P97772), mouse mGluR4 (m-GluR4, UniProtKB: Q68EF4), rat mGluR1 (r-

430   mGluR1, UniProtKB: P23385), and rat mGluR4 (r-mGluR4, UniProtKB: P31423).

431

432   **Obtaining initial ligand-bound hT1R1-T1R3 VFD complex using molecular docking**

433   By using Gauss View 5 and AMBER 14 software [26, 27], the structure of five umami ligands

434   were constructed, including MSG, WSA, GMP, IMP, and BMP. Then, we performed molecular

435   docking for the above five ligands to hT1R1-VFD using the AutoDock vina-1.1.2 software

436   package with the default scoring function [28]. The docking cube grid box size is set to 30 Å,

437   and the center of grid box is set as the center of mass of L-Gln in the homology template

438   structure. The PDBQT of receptor and ligands was calculated by AutoDock Tools-1.5.6

439   package [29, 30].

440

441   **Setup of MD simulations**

442   Each ligand-bound hT1R1-T1R3 VFD complex was centered in a cubic box with the size of 10

443   Å, and filled with TIP3P water. A appropriate number of Na^+ and Cl^- were added in the water

444   box by randomly replacing the solvent waters to neutralize the system and ensure an ionic

445   concentration of 0.15 M. The cutoff distances for Van der Waals and short-range electrostatic

446   interactions were set to 12 Å, and the long-range electrostatic interactions were treated using

447   the Particle-Mesh Ewald (PME) [31]. The SHAKE algorithm was used to constrain all the

448   chemical bonds [32]. The AMBER14SB fore field was employed to describe the hT1R1-T1R3

449   VFD structure, and the AMBER force fields of ligands were generated using the antechamber

450   module implemented in the Amber-Tools package, and the RESP charges were calculated

451   using the Hartree-Fork methods under the basis set 6-31+g (d) [15, 33, 34].

452   Energy minimization was firstly performed for each complex by combing the steepest

453   descent and conjugate gradient methods, consisting of the following three steps: (1) with all

454   the heavy atoms of the system constrained with a force constant of 10 kcal/mol/$Å^{-2}$; (2) With

455   all the C_α atoms of the system constrained with a force constant of 10 kcal/mol/ $Å^{-2}$; (3) All

456   the atoms are fully relaxed. After minimization, the system temperature was gradually

457   increased from 0 k to 300 k within 100 ps. Then, we performed two 100-ps constrained MD

458   simulations for each complex, with all the heavy atoms constrained with 10 kcal/mol/ $Å^{-2}$ and

459   5 kcal/mol/ $Å^{-2}$, respectively. Finally, two parallel non-constrained 100-ns NPT MD simulations

460   were conducted under NVT ensemble, each initiated with different velocities. The

461   temperature was kept at 300K using the Langevin Thermostat [35].

462

463   **HB and DCCM analysis**

464   One HB was defined as that the distance between that acceptor heavy atom A and a donor

465   heavy atom D is less than 3.5 Å, and the angle formed by atom A, donor H atom and atom D

466   is greater than 135°. The OC_{HB} were determined by the following equation:

467    $$OC_{HB} = \frac{n}{N} \times 100\%$$

468   Where n indicates a number of frames that formed HB during MD simulation, N indicates the

469   total number of frames. The PYMOL 2.0 (The PyMOL Molecular Graphics System, Version 2.0

470   Schrödinger, LLC.) and Visual Molecular Dynamics (VMD 1.9.4a12) [36] were used to analyze

471   and visualize the ligand-bound complex.

472   DCCM was performed to investigate the correlated motions between four helices from

473   hT1R1 LB2 region. Before constructing the covariance matrix (C_{ij}), all the MD configurations

474   were fitted to the reference structure, then C_{ij} is determined by the following equation:

475    $$C_{ij} = \frac{< \Delta r_i \cdot \Delta r_j >}{\sqrt{< \Delta r_i \cdot \Delta r_i >< \Delta r_j \cdot \Delta r_j >}}$$

476   Where the angle bracket represents the average of coordinate over the MD simulation

477   trajectory, and Δr_i indicates the deviation of the C_α atom of the ith residue from its mean

478   position [37, 38]. The value of C_{ij} fluctuates from −1 to 1. When the value of C_{ij} is positive, it

479   represents a positive correlated motion between the ith residue and the jth residue,

480   otherwise, it is negatively correlated motion.

481

482   **Supporting information captions**

483   **S1 Fig. Sequence alignment of the N-Venus Flytrap domain (VFD) in T1R and mGluR1.** The

484   amino acid sequences of VFD from human T1R1 (HT1R1, UniProtKB: Q7RTX1), human T1R3

485   (HT1R3, UniProtKB: Q7RTX0), rat T1R1 (RT1R1, UniProtKB: Q9Z0R8), rat T1R3 (RT1R3,

486   UniProtKB: Q923K1), fish T1R2a-T1R3 (FT1R2a-T1R3, PDBID: 5X2M), and mGluR1 (PDBID:

487   1EWK) were aligned by blast module of NCBI, and adjusted by ESPript 3.0. The residues 219-

488   221, 276-278, 300-303 are highlighted in violet border; the residues 146-151, 170-173 are

489   highlighted in green border, and the residues 47-49, 68-71, 384-386 are highlighted in blue

490   border. Moreover, the residues that form direct contacts with the five ligand are highlighted

491   with red star. In addition, for FT1R3, the residues that are critical for L-glutamine's binding

492   are highlighted with coral rectangle. In mGluR1, the residues that play essential roles in

493   recognizing the L-glutamate are highlighted with blue ellipse.

494   **S2 Fig. Phylogenetic tree for T1R and mGluR from different species.** The phylogenetic tree

495   was constructed using the amino acid sequences of T1R and mGluR VFD, sourced from human

496   T1R1 (h-T1R1, UniProtKB: Q7RTX1), human T1R2 (h-T1R2, UniProtKB: Q8TE23), human T1R3

497   (h-T1R3, UniProtKB: Q7RTX0), mouse T1R1 (m-T1R1, UniProtKB: Q99PG6), mouse T1R2 (m-

498   T1R2, UniProtKB: Q925I4), mouse T1R3 (m-T1R3, UniProtKB: Q925D8), rat T1R1 (r-T1R1,

499   UniProtKB: Q9Z0R8), rat T1R2 (r-T1R2, UniProtKB: Q9Z0R7), rat T1R3 (r-T1R3, UniProtKB:

500   Q923K1), fish T1R2a (f-T1R2a, PDBID: 5X2M), fish T1R3 (f-T1R3, PDBID: 5X2M), human

501   mGluR1 (h-mGluR1, UniProtKB: Q13255), human mGluR4 (h-mGluR4, UniProtKB: Q14833),

502   mouse mGluR1 (m-mGluR1, UniProtKB: P97772), mouse mGluR4 (m-GluR4, UniProtKB:

503   Q68EF4), rat mGluR1 (r-mGluR1, UniProtKB: P23385), and rat mGluR4 (r-mGluR4, UniProtKB:

504   P31423).

505   **S3 Fig. Effects of the ligand bindings on the receptor dynamics.** Root mean square deviations

506   (RMSD) of LB1+LB2, LB1, and LB2 regions calculated based on two-parallel 100-ns MD

507   simulations (named run-1 and run-2, respectively). For each system, the RMSD along the

508   simulation time was calculated for the 100-ns simulation dataset.

509   **S4 Fig. The electrostatics potential of hT1R1-VFD.** Positive and negative potential is shown in

510   blue and red, respectively.

511

Author Contributions

513   **Conceptualization:** Hai Liu, Lin-Tai Da, Yuan Liu.

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516   **Funding acquisition:** Yuan Liu.

517   **Methodology:** Hai Liu, Lin-Tai Da, Yuan Liu.

518   **Project administration:** Yuan Liu.

519   **Supervision:** Yuan Liu, Lin-Tai Da.

520   **Writing - original draft:** Hai Liu.

521   **Writing – review & editing:** Hai Liu, Lin-Tai Da, Yuan Liu.

522

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642

Extracellular N-Terminus

VFD

VFD

TMD

TMD

T1R3

T1R1
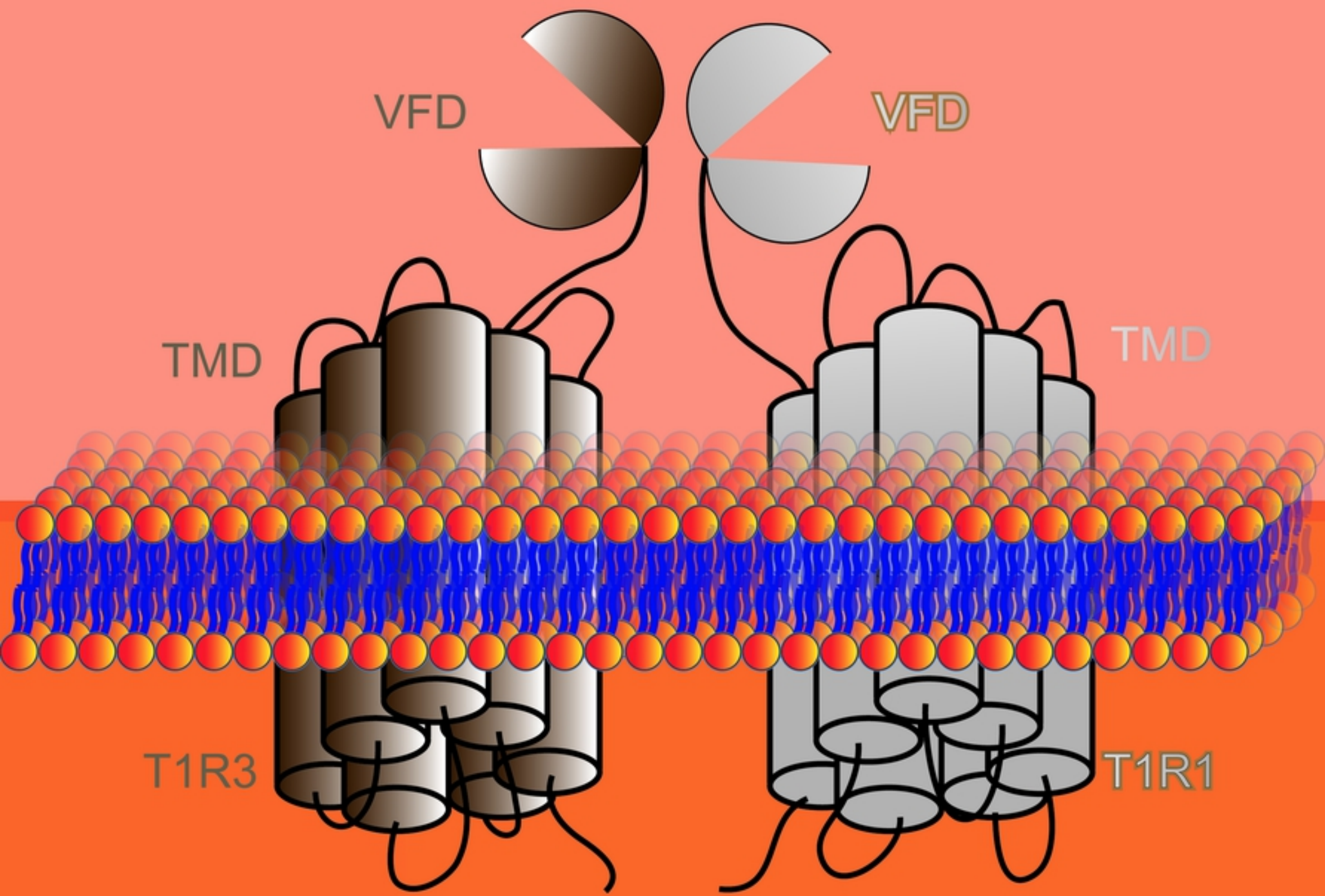
Intracellular C-Terminus

Fig1

A

T1R3   T1R1

LB1   LB1

LB2   LB2

S172   T149

Helix-1   Helix-4

Helix-2

Helix-3

B

MSG

WSA

GMP

IMP

H₃N⁺—KGDEESLA—COO⁻   BMP

Fig2

Fig3

Fig4

Fig5

Fig6

Fig7

Fig8

Sequence alignment (labels): HT1R1, HT1R3, RT1R1, RT1R3, FT1R2a, FT1R3, 1EWK_A, 1EWK_B

Secondary structure annotations: β1, β2, β3, β4, α1, β5, α2, β6, α3, η1, β7, η2, β8, α4 (Helix-1), β9, α5 (Helix-2), β10, α6 (Helix-3), β11, (Helix-4) α7, β12, η3, η4, β13, α8, β14, β15, α9, α10, β16, β17, β18, β19, η5

S1

T1R

mGluR

S2

S3

S4