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On the human taste perception: Molecular-level understanding empowered by computational methods

Lorenzo Pallante [a], Marta Malavolta [a], Gianvito Grasso [b], Aigli Korfiati [c], Seferina Mavroudi [c], Bojan Mavkov [b], Athanasios Kalogeras [d], Christos Alexakos [d], Vanessa Martos [e], Daria Amoroso [f,g], Giacomo di Benedetto [f], Dario Piga [b], Konstantinos Theofilatos [c], Marco A. Deriu [a,*]

[a] Polito^{BIO}MedLab, Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Turin, Italy
[b] Dalle Molle Institute for Artificial Intelligence (IDSIA - USI/SUPSI), Lugano, Viganello, Switzerland
[c] InSyBio Ltd, Winchester, United Kingdom
[d] Industrial Systems Institute, Athena Research Center, Patras, Greece
[e] Department of Plant Physiology, University of Granada, Granada, Spain
[f] Enginlife Engineering Solutions, Turin, Italy
[g] 7hc srl, Rome, Italy

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ABSTRACT

Background: The perception of taste is a prime example of complex signal transduction at the subcellular level, involving an intricate network of molecular machinery, which can be investigated to great extent by the tools provided by Computational Molecular Modelling. The present review summarises the current knowledge on the molecular mechanisms at the root of taste transduction, in particular involving taste receptors, highly specialised proteins driving the activation/deactivation of specific cell signalling pathways and ultimately leading to the perception of the five principal tastes: sweet, umami, bitter, salty and sour. The former three are detected by similar G protein-coupled receptors, while the latter two are transduced by ion channels.

Scope and approach: The main objective of the present review is to provide a general overview of the molecular structures investigated to date of all taste receptors and the techniques employed for their molecular modelling. In addition, we provide an analysis of the various ligands known to date for the above-listed receptors, including how they are activated in the presence of their target molecule.

Key findings and conclusions: In the last years, numerous advances have been made in molecular research and computational investigation of ligand-receptor interaction related to taste receptors. This work aims to outline the progress in scientific knowledge about taste perception and understand the molecular mechanisms involved in the transfer of taste information.

1. Introduction

Taste is a complex phenomenon described as a gustatory sensation related to the perception of flavours, which are defined by the combination of sensations coming from the olfactory, gustatory, and trigeminal systems. Taste is one of the most critical control systems able to regulate substance intake, evaluating the healthiness and nutritional content of food and preventing the ingestion of harmful or toxic elements (Roper, 2017). The five basic commonly recognised tastes are sweet, umami, bitter, sour and salty, each associated with an essential bodily function. Sweet taste identifies the presence of sugars and carbohydrates, i.e. energetic food. Umami, described as savoury (the taste of cooked meat and broths), is linked to the food's protein content. Bitter taste is generally associated with unpleasant flavour and substances potentially dangerous to the body, such as spoiled food or poisons. However, bitter taste represents a very complex sensation, also associated with substances not harmful to the body, such as coffee, untreated olives, unsweetened cocoa, citrus peel, etc. Sour recognises acids and prevents ingestion of spoiled foods. Salty taste controls sodium and other minerals intake, which play a central role in maintaining the body

* Corresponding author.
E-mail address: marco.deriu@polito.it (M.A. Deriu).

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water balance and blood circulation.

Taste perception is an extraordinarily composite and multiscale process that involves molecular, subcellular, cellular, and tissue-level actors of the gustatory system. Taste arises from chemical substances dissolved in saliva interacting with specific proteins, i.e. taste receptors, which trigger the activation of taste receptor cells (TRCs) located on gustatory papillae, modified epithelial cells distributed throughout the oral mucosa, especially on the tongue. Specific signal transduction pathways, mediated by taste receptors, exist for each taste type: sweet, umami and bitter are determined by organic molecules, and their receptors are G protein-coupled receptors (GPCRs), while sour and salty tastes arise from the presence of ions, detected by ion channels (Munger & Zufall, 2016). The activation of the taste receptor cells triggers a specific and taste-related cascade of events reaching the nervous system and ultimately leading to taste perception. In this context, investigating how ligand-protein interactions may drive molecular events (e.g. protein conformational changes) related to activation/deactivation of taste receptors is a crucial step towards a deeper comprehension of the biological nature of taste perception and more in general human nutrition. In this context, molecular modelling, due to a detailed atomistic resolution, represents a powerful tool to shed light on the molecular mode of action of different tastants and the structure-to-function relationships driving the signal transduction at the receptor level. Molecular modelling includes several theoretical and computational methods aimed at representing or mimicking the behaviour of biomolecules, including proteins, DNA, small ligands and polymers (Barbosa, Lima, & Tavares, 2017). Molecular modelling methods are based on an atomistically-resolved description of the molecular systems, which can best be defined by direct experimental techniques. However, if the structure of interest is not already experimentally solved, it is necessary to employ some predictive method to derive a plausible molecular structure. To this end, homology modelling (HM) presents a widely-employed method to predict the 3D structures of a specific protein, called the *target,* starting from its amino acid sequence. This technique requires a solved 3D structure, the *template,* of a similar macromolecule to model the desired structure. The method accuracy depends on the sequence identity between the target sequence and the template, as well as the sequence alignment (Forrest, Tang, & Honig, 2006). Furthermore, Molecular Dynamics (MD) is a well-known *in silico* technique to investigate molecular systems' conformational dynamics. The time evolution of the system is obtained by the numerical solution of classical Newtonian dynamics, providing information on the thermodynamic and dynamic properties of the investigated system (Karplus & McCammon, 2002). Due to this atomistic resolution, MD is a crucial tool for characterising the relationship between the molecular structure of an atomistic system and its function to shed light on important molecular processes and mechanisms, including protein-ligand binding, protein folding, conformational changes driving receptor activation/inhibition, etc. (Chow, Klepeis, Rendleman, Dror, & Shaw, 2012; Dror, Dirks, Grossman, Xu, & Shaw, 2012; Hollingsworth & Dror, 2018; Karplus & McCammon, 2002; van Gunsteren et al., 2006). Along with MD simulations, several computational methods, including molecular docking, structure- or ligand-based virtual screening, virtual mutagenesis, machine learning-based methods, etc., have been developed and widely applied specifically to elucidate protein-ligand binding processes and characterise ligand properties and affinity for a specific receptor (Ben Shoshan-Galeczki & Niv, 2020; Di Pizio et al., 2017).

In the context of investigating taste receptors through molecular modelling, the first issue to be addressed is the receptors' atomistic structure definition, mainly due to the challenging nature of experimental purification of GPCRs. Indeed, only 89 out of the ~800 GPCRs in the human genome have been solved (Kooistra et al., 2020). This lack is usually compensated through HM, and good models can be obtained for template sequence identities higher than 30 % (Forrest et al., 2006). Nevertheless, literature studies highlighted that transmembrane proteins display strong conservation of structures even at low-sequence identity (below 20 %), thus suggesting that it is possible to get accurate 3D models of the TM regions by HM even in these cases (Olivella, Gonzalez, Pardo, & Deupi, 2013). In this context, several recently developed conformational and sampling prediction models have been released and customised for the GPCR structure prediction (K.-Y. M. Chen, Sun, Salvo, Baker, & Barth, 2014; Esguerra, Siretskiy, Bello, Sallander, & Gutiérrez-de-Terán, 2016).

Apart from the wider-known databases for proteins and ligands, data concerning atomistic models related to taste are collected in many dedicated databases, such as BitterDB (Dagan-Wiener et al., 2019), containing both bitter receptors and relative ligands, SuperSweet (Ahmed et al., 2011) or SweetenersDB (Chéron, Casciuc, Golebiowski, Antonczak, & Fiorucci, 2017), collecting sweet compounds.

As previously mentioned, each taste is mediated by a specific receptor, expressed on specific taste cells: sweet and umami are transduced by class-C GPCRs, bitter by class-A/class-F GPCRs, whereas sour and salty are both detected by ion channels (Töle, Behrens, & Meyerhof, 2019). Table 1 summarises the primary taste receptors involved in taste transduction and example of tastants. The table also includes information regarding available 3D structures and taste cells expressing a specific receptor. The schematic representation of the main receptor candidates for each taste is shown in Fig. 1.

Table 1
Summary of mammalian taste receptors.

	CELL TYPE	RECEPTOR(S)	AVAILABLE 3D STRUCTURES	EXAMPLES OF TASTANTS
SWEET	II	TAS1R2 + TAS1R3	No	- Natural sugars (glucose, sucrose, sucralose, maltose) - Artificial sweeteners (aspartame, neotame, monellin) - Sweet proteins (brazzein, monellin, thaumatin, curculin) - D-amino acids (D-Phenylalanine, D-alanine, D-serine)
UMAMI	II	TAS1R1 + TAS1R3, brain-mGluR1, brain-mGluR4, taste-mGluR1, taste-mGluR4, GPRC6A, CaSR and GPR92	No	- Amino acids (aspartate, L-glutamate, L-AP4, glycine, L-amino acids) - Dipeptide and tripeptide (short peptides) - Nucleotide enhancer (IMP, GMP, AMP) - organic acids (lactic, succinic, propionic acids)
BITTER	II	25 TAS2Rs	**BitterDB** (HM)	Diphenidol, Lupolon, Quinine, Benzoin, Arborescin, Noscapine, Quassin, Artemorin, Caffeine, Arglabin, Absinthin, Cucurbitacin B, Coumarin, Chlorpheniramine, Papaverine, Adlupolone and polyphenolic compounds (Vescalagin, Castalagin, protocatechuic acid).
SALTY	I*	ENaC, CALHM1/3	**RCSB** ENaC: 6WTH	Sodium chloride (NaCl), lithium chloride (LiCl)
SOUR	III	OTOP1	**RCSB** 6NF4, 6NF4, 6O84	Acids (citric acid, tartaric acid, acetic acid, hydrochloric acid)

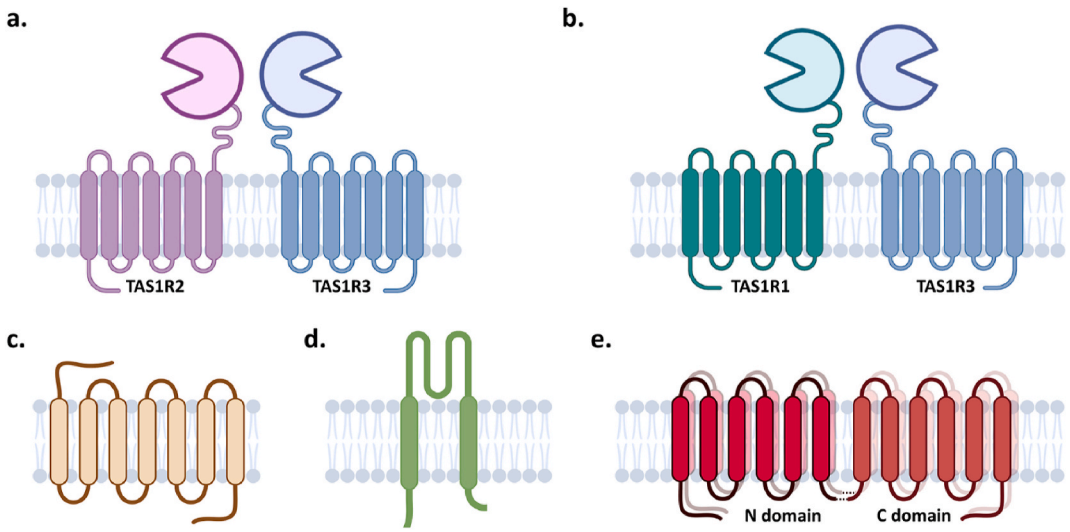

Fig. 1. Schematic representation of the main receptor candidates for each taste, (a) sweet (TAS1R2-TAS1R3, GPCR of class C), (b) umami (TAS1R1-TAS1R3, GPCR of class C), (c) bitter (TAS2Rs, GPCR of class A/class F), (d) salty (αENaC), (e) sour (OTOP1).

*Taste cells dedicated explicitly to salty taste perception are not clearly determined. In the past, several studies highlighted the absence of the ENaC expression in taste cells II and III (Chandrashekar et al., 2010), thus leading to the hypothesis that salty taste cells belong to type I (Roper & Chaudhari, 2017). However, other studies demonstrated type I cells are not-excitable and their major role is a support function (Vandenbeuch et al., 2013). Therefore, further investigations are needed to clarify the specific type of salty taste cells.

The present review aims at providing a comprehensive picture of recent molecular modelling efforts related to the main taste receptor candidates. Data regarding 3D atomic models and main findings from molecular modelling investigations will be reported and rationalised for each receptor candidate. It is worth mentioning that discussed receptors cover only a limited range of possible receptors, transducers and proteins essential to taste perception.

2. Sweet taste receptor

Sweet taste receptor is a heterodimer of TAS1R2 and TAS1R3, encoded by genes tas1r2 and tas1r3. This receptor belongs to the C family of GPCR. Its structure includes seven transmembrane helices (TMD), a large extracellular N-terminus composed of a Venus flytrap module (VFTM) and a cysteine-rich domain (CRD) connected to the transmembrane domain (Bachmanov et al., 2014; Töle et al., 2019) (Fig. 2a).

This receptor responds to many compounds, including natural sugars, such as glucose, sucrose, fructose and sugar alcohols, glycosides, e.g. stevioside and glycyrrhizin, the D-amino acids, e.g. D-tryptophan and D-phenylalanine, peptides, proteins (monellin and brazzein among the most known sweet proteins) and artificial chemical compounds, such as sucralose, aspartame, neotame, saccharin and cyclamate (Di Pizio, Ben Shoshan-Galeczki, Hayes, & Niv, 2019; K. Masuda et al., 2012). The

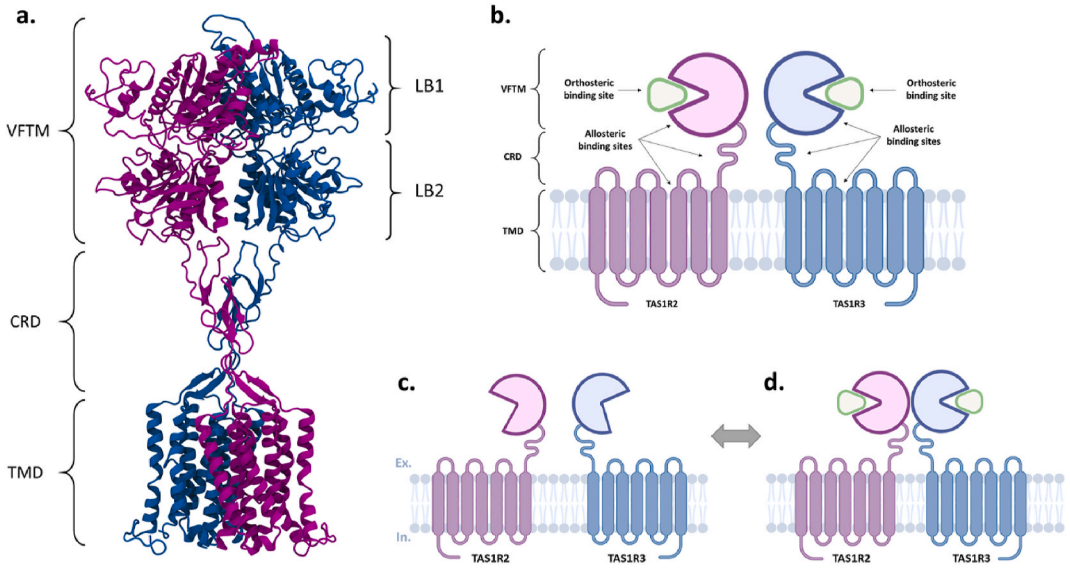

Fig. 2. (a) 3D molecular representation of the sweet receptor, in purple the TAS1R2 and blue the TAS1R3. The structure consists of the Venus flytrap module (VFTM) with the two lobes (LB1 and LB2), the cysteine-rich domain (CRD) and the transmembrane domain (TMD). (b) Representation of the main binding sites of the sweet taste receptor. The figure at the bottom right is the representation of the activation process of the sweet taste receptor. The receptor evolves from (c) the resting state (open-open conformation) to (d) the active one (close-close conformation) after the binding of the sweet tastant (green) in the VFTM binding pocket. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

sweet receptor has an active site in the VFTM, also called the *orthosteric site*, into which small sugars and different sweeteners are suggested to bind (Gravina, Yep, & Khan, 2013; K. Masuda et al., 2012). Artificial sweeteners, such as stevioside and aspartame, preferentially bind to the VFTM of the TAS1R2 subunit, whereas natural sugars, such as glucose and sucrose, bind to both VFTMs of TAS1R2 and TAS1R3 (Kim, Chen, Abrol, Goddard, & Guthrie, 2017; Nie, Vigues, Hobbs, Conn, & Munger, 2005; Töle et al., 2019). There are also allosteric binding sites within the transmembrane nucleus of the TAS1R3 subunit that can enhance sweet ligands' activity in the orthosteric site (Servant et al., 2010; F. Zhang et al., 2010). The location of the different binding sites is schematically represented in Fig. 2b. It is worth mentioning that sweet proteins, such as brazzein, monellin and thaumatin, exhibit a different mechanism of action if compared to small sweet ligands. More in detail, the CRD of TAS1R3 has a crucial role in the interaction with brazzein and thaumatin, and mutations in this region affect also receptor activity toward monellin (Assadi-Porter et al., 2010; Jiang et al., 2004; T.; Masuda et al., 2013; Ohta, Masuda, Tani, & Kitabatake, 2011).

2.1. Receptor 3D structure and conformational dynamics

The first molecular models of sweet taste receptors came out at the beginning of the 21st century. In 2002, Temussi predicted the structure of human TAS1R2-TAS1R3 receptor starting from the free form II of a metabotropic glutamate receptor of subtype 1 (mGluR1, PDB ID: 1EWV) (Kunishima et al., 2000) and showed a stabilising effect of the active form of the receptor by docking three different sweet proteins, i.e. brazzein, monellin, thaumatin (Temussi, 2002). From these first results, several groups attempted to improve the HM process's reliability and obtain higher quality structures. In 2010, Zhang and colleagues employed the HM to predict the molecular structure of the TAS1R2-VFTM using several crystal structures of mGluR1, mGluR3, and mGluR7 (PDB IDs: 1EWK, 1EWT, 1EWV, and 3KS9 for mGluR1; 2E4U, 2E4V, 2E4W, 2E4X, and 2E4Y for mGluR3; and 2E4Z for mGluR7) (F. Zhang et al., 2010). Masuda and co-workers constructed the VFTM structures for the TAS1R2-TAS1R3 structure, both considering the active (glutamate-bound) and inactive (glutamate-unbound) forms of a mGluR1 (PDB: 1EWT and 1EWK, respectively) (K. Masuda et al., 2012). The active form of the heterodimer was constructed selecting the closed and the open forms for TAS1R2 and TAS1R3 respectively, whereas the open form of the crystal structure of mGluR1 was used to construct the inactive form of TAS1R2 and TAS1R3. Moreover, sweet small ligands were docked into the ligand-binding cleft of the TAS1R2 model in the same spot where the glutamate was in the mGluR1. Shrivastav and colleagues compared different HM and threading based methods (SWISS-MODEL, CPHmodels, Modeller, Geno3D, EsyPred 3D, HHpred, LOOPP, Phyre, I-TASSER, and Prime) (Shrivastav & Srivastava, 2013). The best tools were I-TASSER, CPH Model, SWISS-MODEL and Prime. In 2015, Maillet et al. built human TAS1R2-TAS1R3 VFTMs (open/closed and closed-open forms) by HM with MODELLER (Šali et al., 1993) starting from the mGluR1-VFTM crystal structure (PDB ID: 1EWK) (Kunishima et al., 2000) (Maillet et al., 2015). They generated missing loops with MODELLER and imposed the disulfide bonds selected from the mGluR1 structure (C67–C109, C378–C394, and C432–C439). In 2017, Kim and colleagues predicted the 3D structure of the full-length TAS1R2-TAS1R3 heterodimer, including the Venus flytrap module (VFTM) in the closed–open (co) active conformation, the cysteine-rich domains (CRDs), and the transmembrane domains (TMDs) at the TM56/TM56 interface (Kim et al., 2017). To determine the TMD structure of the sweet receptor, they predicted the ensemble of 25 stable structures for the TMD of all TAS1R1s, -2s, and -3s, and constructed the TMD heterodimer for TM45/TM45 and TM56/TM56 interfaces based on GPCR dimers from crystal structures of class A mu-opioid receptor (PDB ID: 4DKL). For the VFTM they used the structure of a mGluR1 bound to glutamate (PDB ID: 1EWK) as a template of the closed-open (co) active state and predicted the binding pose of different agonists (sucrose and stevioside). Finally, to construct the full-length heterodimer receptor, they positioned the VFDs/CRDs on top of the TMD heterodimer and coupled the bonds. In the same year, with a similar approach, Chéron et al. built a full-length sweet receptor using X-ray structures of mGluR VFD (PDB IDs: 1EWT and 1EWK), open and closed receptor states, and the mGluR1 TMD (PDB ID: 4OR2) as templates (Chéron, Golebiowski, Antonczak, & Fiorucci, 2017). In 2017, the Medaka fish TAS1R2-TAS1R3 sweet taste receptor (PDB ID: 5 × 2M) was solved by x-ray diffraction, thus providing a new, more realistic template for the VFTM (Nuemket et al., 2017). In 2019, Kashani-Amin et al. introduced a new enhanced model of the full-length sweet receptor based on the most recent templates (Kashani-Amin, Sakhteman, Larijani, & Ebrahim-Habibi, 2019). More in detail, the Medaka fish structure was chosen for the VFTM model, ensuring better models than the other tested mGluR templates, whereas PDB entry 5K5T (human calcium-sensing receptor) and 4OR2 (mGluR1) were selected for the CRD and TMD, respectively. In the same year, Perez-Aguilar and colleagues constructed and characterised a full-length structural model of the TAS1R2−TAS1R3 receptor, including both the transmembrane (TM) and extracellular (EC) domains of the heterodimer, using comparative modelling and extensive all-atom molecular dynamics simulations (Perez-Aguilar, Kang, Zhang, & Zhou, 2019). Models of the VFTM for the TAS1R2 receptor were generated by HM using the structures of the metabotropic glutamate receptors 1 (PDB ID: 1EWK) and 3 (PDB ID: 2E4U) as well as the $GABA_B1b$ and $GABA_B2$ receptors (PDB ID: 4MS4), whereas the metabotropic glutamate receptor 3 (PDB ID: 2E4U.pdb) was used for the cysteine-rich domain. Several crystallographic structures from different GPCRs were used (PDB IDs: 4GPO, 4OR2, 3ODU, 4DKL) for the transmembrane domain. It is worth mentioning that the full-length dimer structure of mGluR5 and CaSR for different activations states have recently been solved, paving the way towards more detailed and high-quality models for modelling full-length sweet taste receptors (Koehl et al., 2019; Ling et al., 2021).

The atomistic resolution of the above-mentioned molecular modelling techniques can be straightforward to shed light on the molecular mechanisms driving the activation of the taste receptors. After ligand binding into the VFTM orthosteric binding sites, the receptor undergoes a series of conformational changes evolving from an inactive/resting state to an active one. In the resting state, the VFTM domains are both in an open configuration (no ligand docked in), resulting in the so-called *open-open conformation.* On the other hand, in the active conformation, at least one sweet compound is docked into one orthosteric binding site, resulting in its closure: if both the VFTM domains are docked to the ligands (e.g. in the case of natural sugars), the active state is characterised by the *closed-closed conformation*; otherwise, if only one VFTM is docked to the sweet tastant (e.g. in the case of artificial sweeteners), the receptor structure is called *closed-open conformation* (K. Masuda et al., 2012). The transition from the resting state to the active one in the VFTM leads to the approach of the VFTMs of the two monomers, especially in the ligand-binding (LB) domain 2, and then propagates through the cysteine-rich domain to the transmembrane module. This process ultimately leads to the approaching of the TMs, which trigger the activation of the coupled G protein and the subsequent intracellular pathway (Kim et al., 2017). The activation process of the sweet taste receptor, which is fairly similar to all GPCR of class C, is schematically represented in Fig. 2c and d. Masuda and co-workers using molecular dynamics and molecular docking to characterise the modes of binding between human sweet taste receptor and low-molecular-weight sweet compounds suggesting a similar activation mechanism to that of mGluR1: the interaction at the core of lobes LB1 and LB2 appears to be essential for reception of all the sweeteners, and the interaction at the entry of LB1 and LB2 would reinforce the formation of the closed structure of the receptor for activation (K. Masuda et al., 2012). Kim and colleagues highlighted that the agonist binding into the orthosteric site of the VFTM domain of TAS1R2 leads to major conformational changes, during which the transmembrane domain (TMD) transforms from the TM56 interface to

the TM6 interface, as similarly suggested for class C mGluRs (Kim et al., 2017). After the ligand binding, the bottom part of the VFTM of the TAS1R3 is pushed toward the bottom part of the VFTM of the TAS1R2, transmitting these changes up to the TAS1R3 TMD (coupled to the G protein). Interestingly, fixing the atoms of either VFTM of TAS1R3 or CRD of TAS1R3 prevents this activation, whereas fixing CRD of TAS1R2 has no effects. Therefore, this study clarified the allosteric influence of the main structural changes of the TAS1R2 VFTM on the TAS1R3 TMD, putatively coupled to the G protein. Similarly, Perez-Aguilar and colleagues remarked that the protomers rotate respectively to each other (clockwise from the extracellular perspective), reducing the distance between the TM6 helices, especially at the extracellular helical segment (Perez-Aguilar et al., 2019). However, the authors also pointed out the importance of protein-protein contacts from each protomer's TM5 helices. Interestingly, a similar transition from the inactive state mediated by TM4 and TM5 to the TM6-driven interface in the active state was highlighted in previous literature regarding similar class C GPCRs (mGluRs) (Xue et al., 2015), and in a recently characterised mGluR5 structure (Koehl et al., 2019). It is worth mentioning that Perez-Aguilar and co-workers also suggested that, contrary to the mGluRs where full activation is proposed to be reached only when both subunits in the homodimer are bound to an agonist (Vafabakhsh, Levitz, & Isacoff, 2015), the heterodimeric receptors only require the agonist binding in one of the protomers for their full activation, according to previous literature on other class C GPCRs (Perez-Aguilar et al., 2019; Pin & Bettler, 2016).

2.2. Ligand-protein interaction investigations

The VFTM contains an orthosteric site for ligand recognition, and sweet tastant can bind both TAS1R2 and TAS1R3 with distinct affinities and structural rearrangements (Nie et al., 2005). Liu et al. identified crucial residues (S40, V66, I67, and D142 in the human model) for the species-dependent response of two artificial sweeteners, aspartame and neotame (B. Liu et al., 2011). It is worth mentioning that partially overlapping results were obtained by Zhang and co-workers, which indicated seven key residues for the sucrose and sucralose binding (S40, Y103, D142, D278, E302, P277, and R383) (F. Zhang et al., 2010). In line with these results, Masuda et al. conducted mutagenesis studies for screening the residues responsible for sweeteners recognition, highlighting 10 remarkable residues (Y103, D142, S144, S165, P277, D278, E302, D307, E382, and R383) (K. Masuda et al., 2012). The proposed model uses five acidic residues (D142, D278, E302, D307, or E382) for agonists recognition: aspartame, D-Trp, and sucralose share LB1 residues (Y103 and D142) and LB2 residues (D278, E302, and D307) for binding, but specific supplementary residues are required for ligand-specific interaction with the receptor (S144 for aspartame and P277 for sucralose). It is worth mentioning that E302 and S144 have also been previously reported as essential residues for aspartame (and neotame) recognition (Xu et al., 2004). In 2015, Maillet and co-workers ultimately identified 11 critical residues in the TAS1R2 VFTM (S40, Y103, D142, S144, S165, S168, Y215, D278, E302, D307, and R383) in and proximal to the binding pocket that is pivotal for ligand recognition and activity of aspartame (Maillet et al., 2015). More recently, Chéron et al. investigated the orthosteric and allosteric binding sites by computing the volume of TAS1R2 and TAS1R3 binding pockets and providing a list of key residues for sweeteners interactions (Chéron, Golebiowski, et al., 2017). More in detail, they remarked that the orthosteric binding pockets in the open form are big enough to allow the binding of small as well as large sweeteners and that both the TAS1R2 and TAS1R3 cavities are hydrophilic. They also identified a secondary cavity close to the main pocket, which is similar to a pocket found on mGluR4 (Acher, Selvam, Pin, Goudet, & Bertrand, 2011). On the other hand, they highlighted in the TAS1R3 TMD a principal binding pocket and a smaller one in the TAS1R2 model. This finding elucidated why the smallest ligands (e.g., lactisole and cyclamate) can fit into the TAS1R3 binding pocket but not into the TAS1R2. In the same year, Kim et al. identified the VFTM orthosteric binding sites of sucrose and stevioside, underlining strong hydrogen bonds to nearby hydrophilic residues D142 and E302, in line with the aforementioned studies. They also remarked a much stronger binding for stevioside than for sucrose, perhaps explaining why stevioside is 210–300 times sweeter than sucrose (Kim et al., 2017).

Besides orthosteric ligands, positive allosteric modulators (PAMs), targeting different sites, influence taste receptors functions. These molecules are generally tasteless ligands, which bind to the periphery of the orthosteric binding sites with high selectivity, thereby changing the receptor's spatial conformation and enhancing receptor agonism by its activators. Hence, PAMs might be exploited to reduce dietary sugar intake or create high-intensity sweeteners (Servant et al., 2010; F.; Zhang et al., 2010). In this context, Yamada et al., using a massive high-throughput screening campaign boosted by molecular docking, pointed out the ability of a novel class of compounds, namely unnatural tripeptide-PAMs, to enhance the sweetness of sucrose (Yamada et al., 2019). On the other hand, several studies focused their attention on the main receptor domains specifically dedicated to the recognition of possible modulators or allosteric regulators. Particular attention has been paid to the binding sites for cyclamate and lactisole, which are sweet agonist and antagonists, respectively. Jiang et al., using both experimental and computational techniques, including chimaeras, directed mutagenesis and molecular modelling, identified key residues within the transmembrane domain of TAS1R3 that determine responsiveness to lactisole and cyclamate, interestingly finding that the two revealed binding sites are substantially overlapped (Jiang, Cui, Zhao, Liu, et al., 2005; Jiang, Cui, Zhao, Snyder, et al., 2005). Moreover, Chéron et al. characterised the structure and dynamics of the allosteric binding pocket of the TAS1R3 sweet taste receptor both in the absence and presence of cyclamate. Molecular dynamics simulations revealed significant variations in a network of conserved residues not directly implicated in the ligand-binding but unequivocally involved in the receptor function and the allosteric signalling mechanism (Chéron et al., 2019). These works suggested a critical role of the TAS1R3 transmembrane domain in receptor activation. Interestingly, Winning et al. also remarked the role of the heptahelical domain of human TAS1R3 for the activation of the sweet receptor by neohesperidin dihydrochalcone, which was shown to bind in the same binding sites as the sweetener cyclamate and the inhibitor lactisole. Residues involved in the ligand-binding are also implicated in the binding of allosteric modulators in other class C GPCRs, suggesting common architecture and function of the heptahelical domains of class C GPCRs (Winnig, Bufe, Kratochwil, Slack, & Meyerhof, 2007). Finally, Nakagita et al. characterised the molecular mechanism underlying the sweet taste inhibition of lactisole and a few of its derivatives against the TAS1R3 transmembrane domain (Nakagita et al., 2019). The higher inhibitory potency of investigated inhibitors was mainly due to stabilising interactions in the ligand pocket of the TAS1R3 transmembrane domain and increasing the hydrophobic contacts. On the other hand, Zhao et al. underlined the crucial role of the heptahelical domain of TAS1R2 in mediating the species-dependent sensitivity to sweet regulators, such as the amiloride (Zhao, Xu, Meng, & Liu, 2018). Moreover, Zhang et al. investigated the functional domains of sweet taste receptor for the interaction with enhancer molecules (F. Zhang et al., 2010). Their molecular modelling and mutagenesis studies revealed the ligand-binding pocket and the binding mode of two sweet taste enhancers, SE-2 and SE-3, into the TAS1R2 VFTM. They identified critical residues near the lips of the lobes involved in lobe-to-lobe interactions or lobe enhancer interactions and underlined a similar action mechanism to that of the umami taste enhancers. Interestingly, they remarked a cooperative binding between orthosteric and allosteric molecules: sweeteners bind near the LB1-LB2 interface, leading to an initial closure of the VFTM domain, whereas enhancer molecules bind near the opening of the pocket and further stabilise the closed conformation by strengthening

the hydrophobic interactions between the two lobes. Furthermore, Koizumi and colleagues investigated the unique behaviour of Miraculin, a homodimeric protein isolated from the red berries of *Richadella dulcifica*, which is tasteless at neutral pH but demonstrates an acid-induced sweetness: at neutral pH, Miraculin works as an antagonist, whereas the switching towards acidic pH changes the molecule into an agonist, triggering the sweet sensation (Koizumi et al., 2011). The taste-modifying activity to convert sour stimuli to sweetness was revealed by chimeric receptors and molecular modelling methods, which indicated a major role of the amino-terminal domain of the TAS1R2 for the ligand binding.

3. Umami taste receptor

The first time that the word *umami* was used was in 1908 by a Japanese chemist, DR. Kikunae Ikeda, who discovered that glutamic acid evokes a unique taste sensation. Therefore, he created the new word umami by combining two words: *umai*, delicious or savoury, and *mi*, taste (Ikeda, 2002). Only in 2002, the umami taste was recognised as the fifth basic taste.

Initially, only the class C GPCR heterodimer TAS1R1-TAS1R3 was considered as the umami taste receptor, but nowadays eight different types of receptors are accounted as umami taste receptor candidates (Jianan Zhang, Sun-Waterhouse, Su, & Zhao, 2019). Among these receptors, several class C GPCR homodimers have been proposed, such as metabotropic glutamate receptors, including *brain-mGluR1*, *brain-mGluR4*, *taste-mGluR1* and *taste-mGluR4*, the GPCR group 6 subtype A (*GPRC6A*) and the calcium-sensing receptor (*CaSR*). Finally, a non-dimeric structure, namely the GPR92, a class A GPCR, was also indicated. Since most of the above receptors belong to class C GPCRs, we decided to focus our discussion on class C GPCRs in the following.

The first molecule found to have an umami taste was monosodium glutamate (MSG); later, it was found that other amino acids such as aspartic acid and theanine also exhibit the same taste. At the end of the twentieth century, researchers observed that even small peptides could improve food taste. To date, there are 98 peptides identified as bearing umami taste, usually divided based on their number of amino acid residues (Jianan Zhang et al., 2019). A significant discovery was that nucleotides also represent significant mediators of typical umami taste, particularly inosine monophosphate (IMP) and guanosine monophosphate (GMP), which are mainly found in meat and vegetables, respectively. However, the latter two act synergistically with MSG (Spaggiari, Di Pizio, & Cozzini, 2020).

The putative binding site for these ligands is located in the extracellular part of the umami receptor. In detail, two binding sites have been distinguished: an orthosteric one, located in the TAS1R1 VFTM, and multiple allosteric binding sites that are located in the VFTM and CRD of both chains. For instance, IMP and GMP simply have the role of enhancing taste perception by creating a synergistic action with MSG.

3.1. Receptor 3D structure and conformational dynamics

Just like all receptors belonging to class-C GPCRs, they feature the same 3D architecture, comprising the VFTM, the CRD and the TMD, and also the same structure-activity relationship, switching from an active state in which the receptor is in a conformation known as '*closed-open*', to an inactive state in which the receptor is in a conformation known as '*open-open*'. The 3D molecular representation of the umami taste receptor is shown in Fig. 3.

3.1.1. TAS1R1-TAS1R3 heterodimer

Concerning the TAS1R1-TAS1R3 heterodimer, the only available structures of the human umami taste receptor stem from HM, as no crystallographic structure of this receptor exists to date. Kunishima et al. were the first to create a model of the receptor's VFTM domain from the free-form II structure of a metabotropic glutamate receptor of subtype 1

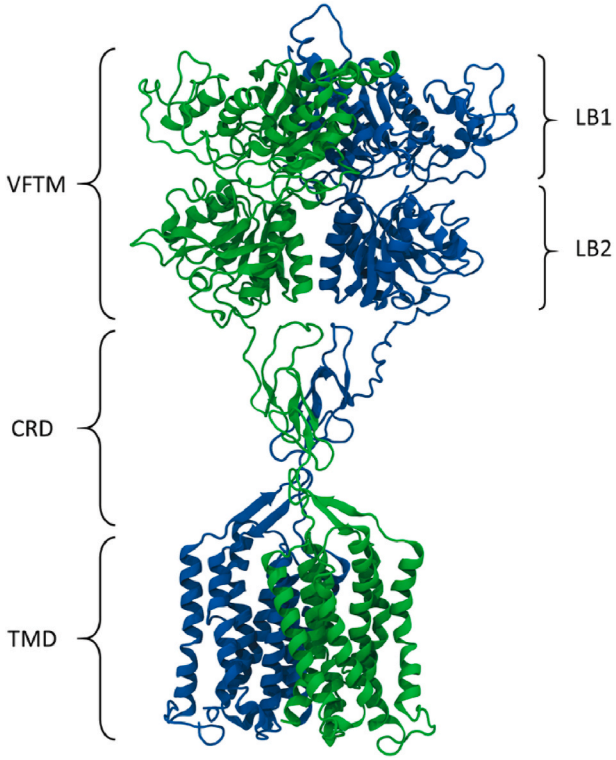

Fig. 3. 3D molecular representation of one of the main umami receptor candidates, in green the TAS1R1 and blue the TAS1R3. The structure consists of the Venus flytrap module (VFTM) with the two lobes (LB1 and LB2), the cysteine-rich domain (CRD) and the transmembrane domain (TMD). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

(mGluR1, PDB ID: 1EWK). The reference structure is not human and has an identity of around 17 % to both TAS1R1 and TAS1R3 (Kunishima et al., 2000). Many authors in the wake of these studies have continued to use mGluR1 as a reference structure; Zhang et al. have also used other metabotropic glutamate receptor subtypes such as subtype 3, mGluR3 (PDB ID: 2E4U), and subtype 7, mGluR7 (PDB ID: 2E4Z), in both open and closed forms (F. Zhang et al., 2008). The identity is 20 % and 23 % respectively. Despite low identity, the authors used the mGluR1 template since their hypothesis is based on the assumption that not only does the position of glutamate in the binding site, in the VFTM domain, between LB1 and LB2, remain the same, but also the pocket residues are conserved between the TAS1Rs and mGluRs family of proteins.

As the crystallographic structures of the extracellular part of the fish sweet receptors (PDB ID: 5X2P (Nuemket et al., 2017)) were already present, in 2019, Liu and co-workers used this template to create the umami model receptor. Unlike glutamate receptors, this template has a higher percentage of identity, around 33 % (H. Liu, Da, & Liu, 2019).

However, all the mentioned models only include homology models of the extracellular part, i.e. the VFTM. Thus, no complete model of the best-known umami receptor, the TAS1R1-TAS1R3 heterodimer, exists to this date.

3.1.2. mGluR1 (brain and taste isoform) and mGluR4 (brain and taste isoform)

These two metabotropic glutamate receptors, the mGluR1 and the mGluR4, belong to two different groups of mGluRs, based on their activity and structure: group I and group III, respectively. The two types of isoforms are a little different from each other; the taste isoform does not have the same typical opening that all other models have, indeed the VFTM part is truncated and therefore has a slightly lower affinity to L-glutamate than other receptors. This receptor's crystallographic

structures are plenty and have been used to create the TAS1R1-TAS1R3 heterodimer structure.

3.1.3. CaSR and GPRC6A

Like TAS1R1-TAS1R3, these two receptors belong to class-C GPCRs, and are identical in structure to the umami receptor; the only difference is that they are homodimers, so the two chains are identical. Bystrova and co-workers have shown that these two receptors also respond to different ligands, including L-amino acids and peptides. Geng et al., in 2016 released the crystallography structure of human calcium receptor comprising only the extracellular part in both the active (PDB ID: 5K5S) and inactive (PDB ID: 5K5T) forms (Geng et al., 2016). More recently, the precise crystal structure of CaSR was determined for each activation states, i.e. closed-closed, open-closed, and open-open (Ling et al., 2021). CaSR was found in different tissue, including the parathyroid gland and kidney (Bystrova, Romanov, Rogachevskaja, Churbanov, & Kolesnikov, 2010).

3.2. Ligand-protein interaction investigations

Generally, in humans, the umami receptor is activated by monosodium L-glutamate (MSG). However, other amino acids can also be stimulated, such as aspartate, or by some organic acids, including lactic, succinic, and propionic acids. On the other hand, esters such as guanosine 5′-monophosphate (GMP) and inosine 5′-monophosphate (IMP) can increase the taste (Fábián, Beck, Fejérdy, Hermann, & Fábián, 2015).

As in the case of the sweet receptor, the umami receptor features an orthosteric binding site located in the VFTM of both chains, TAS1R1 and TAS1R3, as well as an allosteric binding site in the TMD and CRD, following the same scheme of the sweet receptor in Fig. 2b. When umami-enhanced peptides bind in the allosteric sites, they cause a conformational rearrangement in the receptor, which amplifies the orthosteric transduction pathway by increasing the active sites' affinity for the umami tastants. For example, Töle and colleagues reported how allosterically bound cyclamate enhances the receptor activation by L-glutamate bound in the VFTM orthosteric site (Töle et al., 2019). Also, IMP and GMP are capable of binding in the allosteric site and improving taste signal transduction by stabilising the closed conformation of TAS1R1 (Spaggiari et al., 2020). Moreover, Toda et al. showed that methional, a typical taste of cheeses, could potentially bind at two distinct sites in the transmembrane domain of TAS1R1 and served as a positive allosteric modulator (PAM) of the human umami receptor, but as a negative allosteric modulator (NAM) in mice (Toda et al., 2018).

As for the chain of conformational events beginning with ligands binding in the VFTM and ultimately leading to downstream signal transduction, different models have been proposed: Zhang et al. reported that the closure of the VFTM of TAS1R1 and TAS1R3 occurs as a two-stage process, starting with the initial positioning of glutamate in the VFTM LB1, occurring in μs timescales, followed by further positional optimisation inside the cleft, requiring ms timescales (F. Zhang et al., 2008). Cascales and his colleagues have shown with MD simulations that the closure mechanism, thus the activation of the umami receptor, is achieved by Form 1 in which the TAS1R1 chain has a closed conformation while TAS1R3 has an open conformation, as previously described. (López Cascales, Oliveira Costa, de Groot, & Walters, 2010).

4. Bitter taste receptor

Bitter taste receptors are members of another family of GPCRs called the taste 2 receptor family (*TAS2Rs*) (Chandrashekar et al., 2000). Many discussions have been carried out regarding their belonging to a specific class of GPCRs: some authors place them within the class F of GPCRs, consisting of frizzled and smoothened proteins; others place them in the broader class A of GPCRs, rhodopsin-like and, recently, the online database GPCRdb (https://gpcrdb.org/) even created a new sub-family called class T for these receptors. Due to their functional principles and the position of the binding site, they resemble those of class A GPCRs to which visual and odorant sensory receptors also belong, but this is not the case for their sequence similarity (Brockhoff, Behrens, Niv, & Meyerhof, 2010; Töle et al., 2019). Its structure includes short extracellular N-terminus and intracellular C-terminus, seven transmembrane helix (TMD) which are connected by three Extracellular Loops (ECLs) and three Intracellular Loops (ICLs) (H. Zhang et al., 2017). The most conserved component between class A GPCR and bitter receptors is the 7 TMD bundle which forms the structural core, binds ligands in the extracellular (EC) region and permit the transduction of information due to the intracellular (IC) region (Di Pizio et al., 2016). The comparison shows that important class A motifs and highly conserved disulfide bridge that facilitates GPCRs structure stabilisation are missing. On the other hand, the TAS2Rs specific conserved residue may have an essential role in stabilising the inactive conformation of bitter receptors.

The number of TAS2R genes varies largely across species (Dagan-Wiener et al., 2019; Meyerhof et al., 2009). Among the different species, not only does the number of genes coding for the bitter receptor change, but there are also differences on where the genes that encode TAS2Rs are; in humans, they are coded by chromosomes 5, 7 and 12 while in mice by 2, 6 and 15. The number of bitter compounds that humans can perceive is much larger than the number of human genes; this makes us understand that every bitter receptor responds to more than one ligand (Bachmanov et al., 2014; Meyerhof et al., 2009). TAS2Rs constitute an interesting subgroup of GPCR because they have many known agonists and few antagonists. Besides, this ligands' activity is usually in the micromolar range, higher than the typical nanomolar ranges of most GPCR ligands (Di Pizio et al., 2016).

Due to the large number of TAS2Rs, the large quantity of naturally occurring bitter-tasting substances and the presence of three *generalist* receptors - TAS2R10, TAS2R14 and TAS2R46 - recognising about one-third of all bitter compounds, heterodimerization of bitter taste receptors may not be necessary to extend their already great receptive capacity (Behrens & Meyerhof, 2018). However, *in vitro* experiments revealed that TAS2Rs bitter taste receptor form oligomers (approximately 325 homodimeric and heterodimeric receptors), but it is not yet known if TAS2Rs heteromeric receptors contribute to a broader detectable agonist spectrum (Kuhn, Bufe, Batram, & Meyerhof, 2010).

Moreover, some authors noticed that some bitter compounds could both activate the TAS2R receptor and be able to interact with the cell membrane's ion channels, so they may also function as bitter receptors (Bachmanov et al., 2014). Additionally, studies have shown that TAS2Rs are not only in the taste buds but also expressed in extra-oral tissue, including heart, skeletal and smooth muscle (Behrens & Meyerhof, 2013). The distribution of TAS2Rs is variable in different kinds of muscle cells, but TAS2R3, TAS2R4, TAS2R5, TAS2R10, TAS2R13, TAS2R19 and TAS2R50 are always present in a moderate way, while TAS2R14 is highly expressed in all the human body. Moreover, previous literature pointed out the expression of TAS2Rs on human airway smooth muscle (Deshpande et al., 2010) and smooth muscle tissue along the mouse gut and in human gastric smooth muscle cells, suggesting a possible role of TAS2Rs as targets to alter gastrointestinal motility and hence hunger sensation (Avau et al., 2015). Moreover, TAS2Rs are also related to muscles contraction or relaxation in other organs such as the bladder (Zhai et al., 2016). Bitter molecules are usually considered poisonous substances, yet there are non-toxic ones with beneficial effects on the human body. For this reason, a better understanding of the bitter taste receptor transduction may lead to the design of specific drugs with an acceptable taste and having an essential role in muscle-related diseases.

4.1. Receptor 3D structure and conformational dynamics

At present, one of the major obstacles for the molecular modelling of bitter taste receptors is the lack of experimentally solved structures representing the 25 bitter receptors. As a matter of fact, only the

molecular models by homology modelling have been developed for 23 out of 25 human bitter receptors. Those models are publicly available in the BitterDB (Dagan-Wiener et al., 2019) which also provides information concerning bitter receptors and related ligands. Only two receptors, the TAS2R45 and TAS2R19, are not included in the database. The 3D molecular representation of TAS2R3 is shown in Fig. 4a and a detailed list of all the human bitter taste receptors along with alternative names is reported in Table S1.

The 3D structure of the TAS2R14 bitter receptor stored in the BitterDB was modelled using the β2 adrenergic receptor (PDB ID: 3SN6), another class-A GPCR, as a template. This model was subsequently used as a template for the other receptors, which were built using MEDELLER (Kelm, Shi, & Deane, 2010) and then manually adjusted. Other groups followed similar homology modelling strategies using other experimental templates: Pydi et al. built the TAS2R4 receptor using Rhodopsin (PDB ID: 1U19) and opsin (PDB ID: 3DQB) as templates, in their active and inactive conformation respectively; Wang and co-workers modelled the TAS2R7 receptor using the serotonin receptor template (PDB ID: 6BQC) (Sai P. Pydi et al., 2014; Wang et al., 2019). Similarly to previous literature (Sai P. Pydi et al., 2014), bovine rhodopsin and opsin were employed as templates to model TAS2R4 and TAS2R1, whereas Squid rhodopsin (PDB ID: 2Z73) was chosen as the template for TAS2R14 (Acevedo, González-Nilo, & Agosin, 2016). Moreover, in line with the homology modelling strategy used for BitterDB, other groups used the β2 adrenergic receptor (PDB ID: 3SN6) as the template to model the TAS2R16 (Z. Chen et al., 2018) and, coherently with the GPCRdb homology modelling pipeline, TAS2R5, TAS2R7, TAS2R14, and TAS2R39 were modelled starting from the β2 adrenergic receptor (PDB ID: 3SN6), the serotonin 2B receptor (PDB ID: 5TUD), and the mu-opioid receptor (PDB ID: 5C1M) (Soares et al., 2018).

The aforementioned models were used as starting point for structure-to-function molecular studies aimed at exploring the conformational behaviour of bitter taste receptors and highlighting crucial residues/structures involved in receptor activation. For example, a *multipoint stimulation model*, similar to the one previously proposed for the sweet receptor (Mayank & Jaitak, 2015), has been suggested for the activation of bitter receptors by steviol glycosides (SG) and other water-soluble molecules: at the beginning, ligands stimulate extracellular residues and, subsequently, the allosteric modulation of the transmembrane site is triggered (Acevedo et al., 2016). Furthermore, crucial residues, i.e. H94 in helix 3 and E264 in helix 7, for the activation of the receptor driven by metallic ions, have been remarked (Wang et al., 2019).

4.2. Ligand-protein interaction investigations

Although all bitter taste receptors are characterized by one single binding site for bitter ligands, the high number of TAS2R receptors allow for the recognition of a huge number of bitter compounds (Meyerhof et al., 2009). More in detail, bitter taste receptors are activated by a wide variety of chemically different agonists. This affinity toward a huge range of chemical structures may be achieved with various interaction types between different ligands in the binding pocket (Brockhoff et al., 2010). Bitter taste receptors can be divided into *promiscuous*, activated by a multitude of chemically different compounds, and *selective*, activated by few chemicals (Meyerhof et al., 2009). Examples of promiscuous receptors are the TAS2R10, TAS2R14 and TAS2R46. Each TAS2R receptor has specific patterns for the recognition of related bitter substances, but numerous compounds can activate several TAS2Rs (Meyerhof et al., 2009). The selectivity and promiscuity profile of bitter taste receptors and their ligands has been recently explored by chemoinformatics approaches (Di Pizio & Niv, 2015). More in detail, results highlighted that almost all selective bitter receptors are activated only by promiscuous compounds, i.e., those ligands targeting more than one TAS2R. Instead, promiscuous receptors are activated by both promiscuous and selective binders (Di Pizio & Niv, 2015). The relevance of the ligand promiscuity investigation lies primarily in the possibility of a rational ligand design specifically aimed at modifying their chemical structure according to specific needs. On the other hand, characterisation of the molecular features defining receptor promiscuity may be pivotal for understanding the ability of bitter receptors to identify the huge variety of bitter tastants. The receptor promiscuity can be accessed with the so-called promiscuity index (PI), i.e. the number of bitter compounds that activate the receptor divided by the total number of molecules considered. On the other hand, the diversity of the ligand set can be measured with another promiscuity index, namely the PI_{NUS}, calculated as the number of unique scaffolds (NUS) for each receptor divided by the total number of NUS (Di Pizio & Niv, 2015; Levit, Beuming, Krilov, Sherman, & Niv, 2014). Previous literature on class A GPCR identified a correlation between the binding site characteristics and the variety of antagonists. In particular, the number of unique scaffolds, that measures the number and variability of antagonists, was demonstrated to be correlated to the exposure and hydrophobicity of the binding site and opposed to the number of hydrogen bond donors (Levit et al., 2014). Interestingly, despite the lack of structural data that limits a full investigation of TAS2Rs, Di Pizio et al. suggested that the

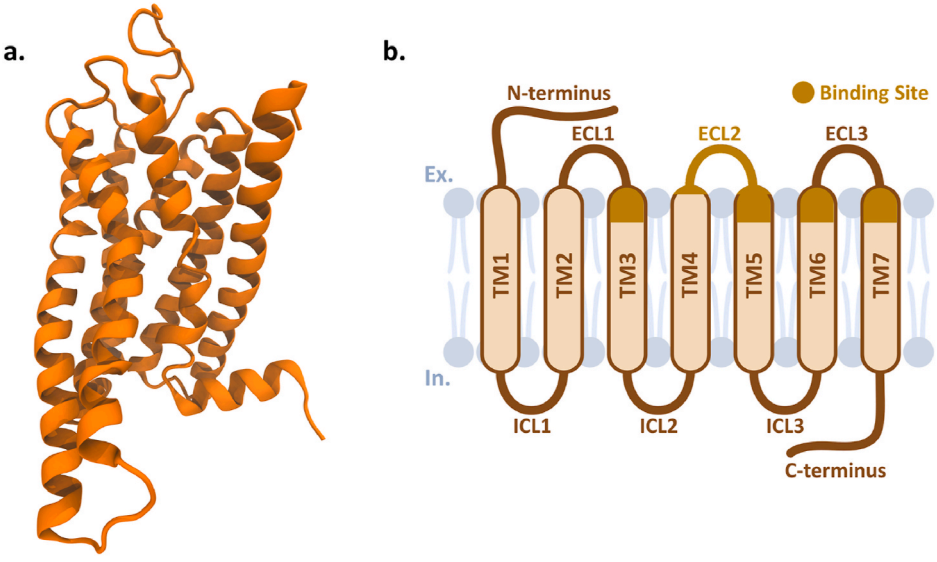

Fig. 4. *(a) 3D homology model of the TAS2R3 bitter receptor (PDB from BitterDB (Dagan-Wiener et al., 2019). (b) Schematic representation of the bitter taste receptor, including the extra- and intra-cellular loops (ECLs and ICLs), the transmembrane (TM) helices, and the main structures involved in the ligand binding.*

aforementioned properties of the binding site correlate also with the TAS2R-promiscuity (Di Pizio & Niv, 2015).

The ability of the bitter receptors to detect a huge variety of ligands is made possible by single point mutations in the binding pocket that can improve or reduce affinity towards a specific ligand (Born, Levit, Niv, Meyerhof, & Behrens, 2013). Despite the raised hypothesis that bitter receptors could have more than one binding site to accept the huge variety of bitter agonists, Slack and colleagues demonstrated the existence of a unique binding pocket (Slack et al., 2010). Several studies were also performed to identify the binding pocket of bitter receptors through the use of point mutations on TAS2R16. These studies highlighted the binding site involves seven residues belonging to TM III, V and VI and in particular at least three of them interact directly with salicin (Sakurai et al., 2010). This prediction was also confirmed by experimental studies and functional analyses on mutant receptors that led to the identification of residues responsible for the agonist selectivity and activation of TAS2R46, TAS2R43, and TAS2R31 (Brockhoff et al., 2010). Most structure-function studies involving bitter taste receptors have confirmed the binding pocket of TAS2Rs is located in the extracellular side of the TM bundle, between TMs III, V, VI and VII (as shown in Fig. 4b), which is the canonical site of class A GPCRs. Indeed, several investigations on TAS2R14, TAS2R10 and TAS2R46, the most examined receptors, experimentally confirmed the involvement of residues present in the above mentioned TMs (Born et al., 2013; Brockhoff et al., 2010; Nowak et al., 2018), but also suggested an involvement of TM II for TAS2R14 and TAS2R46 receptors, which might be explained by the more spacious pocket shape, as already reported for TAS2R14 (Karaman et al., 2016). It is worth mentioning that the residue composition of the above-mentioned binding site is highly different in every TAS2Rs, suggesting the possibility of the detection of different ligands with a variety of agonist-specific interactions patterns (Born et al., 2013). Several investigations highlighted that residues belonging to the ECL2, the longest loop in the extracellular side of the receptor, significantly contribute to ligand binding and activation of TAS2Rs: Liu and colleagues demonstrated that residues N167, T169 and W170 could influence ligand binding in TAS2R7 (K. Liu, Jaggupilli, Premnath, & Chelikani, 2018), and previously Karaman et al. showed that residues N163 and N172, located in ECL2, present the same function in TA2R14 (Karaman et al., 2016). Moreover, computational studies highlighted the type of interactions between the receptors and some ligands and major conformational changes related to ligand-driven activation. For example, Chen and colleagues investigated the possible activation mechanism of TAS2R16 in the presence of its agonist and antagonist, i.e. salicin and probenecid respectively, docked into its active pocket (Z. Chen et al., 2018). Acevedo and co-workers investigated steviol glycosides (SG), non-caloric sweeteners derived from plants, which demonstrated in *in vitro* studies a specific affinity towards TAS2R4 and TAS2R14. This ability makes these compounds able to generate, in addition to their sweetening effect, also an unpleasant bitter taste (Acevedo et al., 2016). They showed that SGs have only one site for orthosteric binding and SGs only bind to TAS2R4 and TAS2R14 and not to TAS2R1. Moreover, they remarked a negative correlation between protein-ligands binding energies and bitterness intensity, but again not for TAS2R1. Therefore, this research pointed out that the binding site of TAS2R1, mainly inserted in the transmembrane region, is not tailored for this type of sweeteners and other water-soluble molecules, e.g. caffeine or quinine. They also observed a crucial role of the ligand size compared to the dimension of the binding site, underlining that SGs with more sugars have less affinity for bitter taste receptors. Moreover, steered molecular dynamics simulations highlighted a major difference in affinity between stevioside and rebaudioside A: the former is characterised by stronger interaction with the receptor if compared to the latter due to the formation of more hydrogen bonds at the binding site of both receptors (Acevedo et al., 2016). Other bitter ligands particularly important for their nutritional properties are polyphenols, which are present for example in coffee, wine, or red fruits. Soares and his colleagues investigated the bitterness of different classes of 16 polyphenolic compounds through the activation of TAS2Rs and pointed out their stimulation on bitter taste receptors. They also noticed that the condensed tannins, a subclass of the flavonoids/flavanols, specifically activates the TAS2R5, whereas the hydrolyzable tannins, in particular the ellagitannins, triggers the TAS2R7 (Soares et al., 2018).

In literature, it is reported that bitter receptors may have only one binding site for agonists and antagonists, due to the type of interactions with a selected residue depending on the ligand nature (K. Liu et al., 2018). However, some studies suggest that there may be an additional *vestibular binding site* located in the extracellular part of the receptor. Sandal and co-authors proposed that agonists can transiently occupy this site and be prefiltered before the introduction into the canonical binding site and that these two sites may have a role in discrimination of different agonists of TAS2R46 (Sandal et al., 2015).

The interaction between TAS2Rs and bitter tastants also depends on several factors, e.g. type of ligands, membrane lipids and movements of TMs and ECLs. Indeed, Pydi et al. suggested cholesterol sensitivity of T2Rs and remarked a crucial role of cholesterol in the cell membrane for the interaction between amino acid (Sai Prasad Pydi et al., 2016).

5. Sour taste receptor

Sour sensing is particularly important in the taste system for monitoring the functional state of body fluids. Even if a lot of progress has been made in the studying and discovery of the molecular mechanisms behind sweet, bitter and umami tastes, sour taste is still poorly understood (Ishimaru et al., 2006). Sour taste is detected by type III cells and it is essential in regulating the intake of H^+ ions (Roper, 2017).

During the past decades, several membrane ion channels have been proposed as sour taste transducer, including epithelial sodium channel (ENaC), Acid-Sensing Ion Channel (ASIC), two-pore domain potassium (K2P) channels, H^+ gated calcium channels. In the recent past, the polycystic kidney disease 2-like1 ion channel (PKD2L1) was identified as a putative sour taste receptor (Huang et al., 2006; Ishimaru et al., 2006; LopezJimenez et al., 2006). However, a direct role for PKD2L1 or its partner, the PKD1L3, in sour transduction was not supported by subsequent studies on knocked out mice (Horio et al., 2011). Nevertheless, PKD2L1 is still considered a useful marker for sour taste cells (type III cells) (Ye et al., 2016).

More recently, a tremendous breakthrough was achieved from Tu and co-workers, who have discovered that transduction of sour taste in mice involves permeation of H^+ through a proton selective ion channel, a protein named Otopetrin1 (OTOP1) (Tu et al., 2018). OTOP1 is specifically expressed in type III taste cells, it generates a proton current across the membrane in response to extracellular acidification, and it is sensitive to Zn^{2+}, which is a crucial factor for the proton current related to sour perception (Tu et al., 2018). Using PKD2L1 as a molecular identifier for sour-responsive taste cells, different research groups (Teng et al., 2019; Zhang et al., 2019) confirmed OTOP1 as the necessary transduction channel underlying sour taste. OTOP1 belongs to the Otopetrins family, which also comprises two other ortholog proteins, i.e. OTOP2 and OTOP3 (Tu et al., 2018). Human OTOP1 (hOTOP1) forms a channel with similar properties to murine OTOP1, and murine OTOP2 and OTOP3 share 30–34 % amino-acid identity with murine OTOP1(Tu et al., 2018).

5.1. Receptor 3D structure and conformational dynamics

Only a few 3D atomistic structures are currently available in the RCSB database for the Otopetrin family. As far as we know, the main structures are the zebrafish OTOP1 and the chicken OTOP3 (PDB entries: 6NF4 and 6NF6) (Saotome et al., 2019), and the Xenopus Tropicalis OTOP3 (PDB entry: 6O84) (Q. Chen, Zeng, She, Bai, & Jiang, 2019).

Recently, Chen et al. characterised the first molecular structure of the

OTOP family due to the cryo-EM experimental determination of the *Xenopus Tropicalis* OTOP3 (XtOTOP3) (PDB entry: 6O84) (Q. Chen et al., 2019). They highlighted that XtOTOP3 adopts a unique two-pore architecture forming a homodimer: each subunit is composed of 12 transmembrane helices divided into two structurally homologues halves representing the C and the N domains, which surround a highly hydrophobic tunnel filled with lipids. It is worth mentioning that, from Wheatley, half of the plasma membrane, both subunits contain solvent-accessible cavities that are enclosed by TMs 2–6 (N-pore) and TMs 8–12 (C-pore), respectively.

Subsequent studies analysed and compared zebrafish OTOP1 (zfOTOP1) and chicken OTOP3 (chOTOP1), which are 30 % identical to each other by sequence and share 44 % and 59 % identity with human OTOP1 and OTOP3, respectively (Saotome et al., 2019). Their results were achieved due to the direct analysis on the full-length OTOP1 (PDB entry: 6NF4), represented in Fig. 5a and b, and OTOP3 (PDB entry: 6NF6) (Saotome et al., 2019). Observed structures are very close to the ones highlighted by Chen et al., thus suggesting a common topological organisation to all Otopetrin family members.

The receptor function allowing the proton transfer across the membrane is still under debate. It is supposed that protons can flow through a 'hopping' mechanism along a hydrogen-bonded network made by water molecules and/or amino acid side-chain moieties. Two structurally analogous vestibule-shaped openings in each OTOP1 and OTOP3 subunits could represent loci for proton permeation, one housed by the N domain and the other by the C domain (Saotome et al., 2019). Interestingly, the same pattern is shared by XtOTOP3 (Q. Chen et al., 2019). Both domains contain numerous polar and charged residues: the region of hydrophobic residues could potentially be a hydrophobic plug that regulates water/ions accessibility. Another feature of the putative permeation pathways within the N and C domains is the *constriction triads* composed of glutamine-asparagine-tyrosine, which we abbreviate as the QNY triad. Respectively for the N- and the C-pore, they are formed by residues:

- Q174/N204/Y268 and Q433/N528/Y571 in zfOTOP1
- Q175/N205/Y266 and Q429/N503/Y546 in chOTOP3
- Q232/D262/Y322 and Q558/N623/Y666 in XtOTOP3

Their side chains are sufficiently close to interact directly or through intervening waters. The function of these triads is uncertain, but a role in proton transfer seems possible considering it is conserved in both N and C domains for both zfOTOP1 and chOTOP3 (Saotome et al., 2019). In XtOTOP3, instead, even if both pores could potentially function as proton permeation pathways, the C-pore constriction triad could probably be more crucial in determining channel activity and proton permeation in XtOTOP3 (Q. Chen et al., 2019). Further investigations are needed to clarify the specific role of each triad in both pores and to possibly depict a general working mechanism for all the otopetrin family members.

Another aspect to be considered deals with water permeation from the extracellular *milieu* through the N and C domain vestibules. During MD simulations, the presence of water is continuously observed at the intrasubunit interface of the N and C domains in zfOTOP1 and chOTOP3, but not at the intersubunit interface, where water permeation through the central tunnel was completely blocked by the cholesterol molecules (Saotome et al., 2019). Similar behaviour was also reported for the XtOTOP3 (Q. Chen et al., 2019). The stochastic formation of a water wire during molecular dynamics simulation suggests also that proton conduction could occur through a water-hopping mechanism (Saotome et al., 2019).

In conclusion, molecular dynamics simulations shed light on the molecular mechanisms for proton conduction, pointing to three main possible mechanisms: aqueous vestibules in the N and C domains, and the intra-subunit interface (Saotome et al., 2019). However, it is still unclear which of these three pathways or their combination allow the flux of proton currents.

6. Salty taste receptor

Salty taste controls sodium and other mineral intakes, which play a central role in maintaining the body water balance and blood circulation. In this context, the sodium ion (Na^+) is an essential mineral regulating the osmolality of the extracellular fluid and takes part in many physiological processes. Since Na^+ is constantly excreted from the body, it is paramount to properly integrate the ion's loss to effectively maintain the bodily balance through the diet. Na^+ specifically elicits the salty taste sensation, which guides the intake of this important mineral

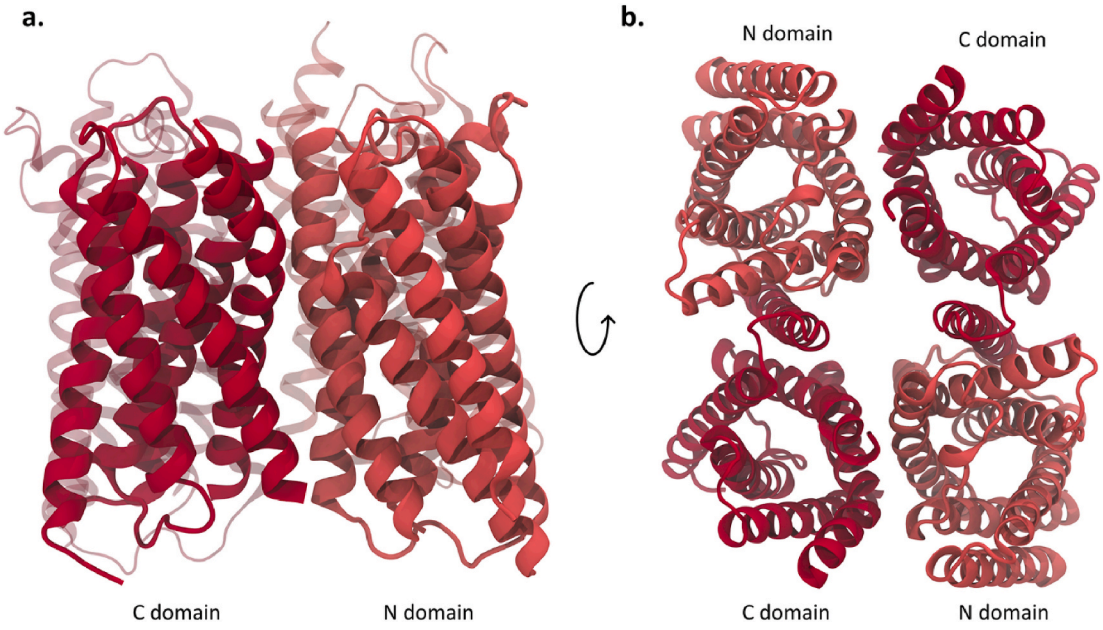

Fig. 5. Frontal (a) and top (b) views of the 3D molecular structure of OTOP1 (PDB entry: 6NF4). Each subunit is formed by two structurally homologous domains, i.e. the N domain (light shade) and C domain (dark shade). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

(Bigiani, 2020). Salty perception may trigger both attraction and repulsion towards the source. At high concentrations, saltiness usually results in a negative reaction, whereas at low to moderate concentrations, saltiness is attractive (Lindemann, 2001). Chemically, the salt that is usually regarded as the main trigger of a salty perception is sodium chloride (NaCl) and other salts also feature more compound gustative footprints, for example by triggering also bitter or sour sensations.

In mice, the attractiveness of salty sensation is selectively triggered by sodium and inhibited by amiloride. Since amiloride is a potent inhibitor of the ENaC, it has been proposed as a crucial component of the salty receptor machinery (Lindemann, 2001; Yoshida et al., 2009). The expression of ENaCs in humans is mostly on the apical surface of epithelial tissues throughout the body. ENaC belongs to the ENaC/Degenerin (DEG) family, which include also well-known ASIC. These receptors are characterised by subunits that consist of short intracellular N- and C-termini, two membrane-spanning helices, and a large cysteine-rich extracellular domain (ECD) that can form homo- or heterotrimeric ion channels (Jasti, Furukawa, Gonzales, & Gouaux, 2007; Noreng, Bharadwaj, Posert, Yoshioka, & Baconguis, 2018). The ENaC receptor has three homologous subunits α, β and γ or δ (Hanukoglu & Hanukoglu, 2016). This ion channel allows the passage of Na ions, maintaining the right concentration of salt and water in the body.

In mice, the salty attraction is mediated by the α subunit of the epithelial sodium channel (α-ENaC) (Chandrashekar et al., 2010) and exhibiting sensitivity to amiloride (Halpern, 1998). Therefore, in rodents, attraction to low sodium is blocked by amiloride, and knockout mice lose this attraction (Chandrashekar et al., 2010). On the other hand, appetitive salty taste is not sensitive to amiloride in humans (Halpern, 1998), and an additional ENaC gene, the δ gene, is found in their genomes, leading to the expression of both the amiloride-sensitive α- and the less sensitive δ-ENaC subunits in human taste cells (Stähler et al., 2008). Moreover, in rodents' model, ENaC should be found at the apical membrane of taste cells (Witt, 2019), whereas some pieces of evidence suggest that only the δ-subunit localises to the taste pore region in human taste buds and other ENaC subunits seem to be segregated in the basolateral compartment, thus suggesting the δ-subunit as a possible salty taste receptor. In light of these considerations, it is still under debate if all the subunits are required to form a functional sodium receptor (Lossow, Hermans-Borgmeyer, Meyerhof, & Behrens, 2020). In conclusion, the ENaC is probably involved in human sodium detection, but no certain evidence has defined in which stage of the perception process. The lack of the amiloride effect (Halpern, 1998) and the presence of α-, β-, and γ-subunit only in the basolateral portion of taste buds (Stähler et al., 2008) seem to favour a role for ENaC downstream of the initial receptive events (Bigiani, 2020).

Very recently, Nomura et al. showed that sodium taste signalling in mice is independent of Ca^{2+} concentration (in contrast to the taste perception mediated by type II and type III cells) and only voltage-dependent (Nomura, Nakanishi, Ishidate, Iwata, & Taruno, 2020). This study demonstrates that the Na^{+} entry through ENaC leads to depolarization, driving the subsequent generation of the action potential by voltage-gated ion channels. Interestingly, the authors showed that the co-expression of the voltage-gated neurotransmitter-release channel (CALHM1/3) and ENaC, both required for amiloride-sensitive salty taste transduction, is essential to identify salty taste cells. These findings represent a big step forward in the salty taste pathway perception, notwithstanding ENaC has still to be proven as the principal sensor for salty taste in humans (Halpern, 1998; Schiffman, Lockhead, & Maes, 1983; Stähler et al., 2008) and the apparent insensitivity to amiloride of salty taste in humans has not been explained yet (Liman, 2020).

6.1. Receptor 3D structure and conformational dynamics

The first crystal structure of ENaC was solved by cryo-electron microscopy (cryo-EM) at a nominal resolution of 3.9 Å (PDB entry: 6BQN). The ion channel is composed of a large extracellular domain and a narrow transmembrane domain, characterised by a 1:1:1 stoichiometry of α:β:γ subunits arranged in a counter-clockwise manner (Fig. 6) (Noreng et al., 2018).

The same group recently solved the molecular structure of ENaC by cryo-EM at 3 Å (PDB entry: 6WTH), showing that the α subunit has a primary functional module consisting of the finger and the Gating Release of Inhibition by Proteolysis (GRIP) domains, which strongly separate the behaviour of this receptor from close relative ASICs. The module is bifurcated by the α2 helix dividing two distinct regulatory sites: Na^{+} and the inhibitory peptide. Removal of the inhibitory peptide perturbs the Na^{+} site via the α2 helix highlighting the critical role of the α2 helix in regulating ENaC function (Noreng, Posert, Bharadwaj, Houser, & Baconguis, 2020). However, the experimental resolution of the transmembrane domain (TMD) and the cytosolic domain (CD) is still missing. Future improvements on the above-mentioned structures might pave the way towards the full-length channel and gain fruitful insight to understand the mechanistic link between the removal of inhibitory peptides in the cysteine-rich extracellular domain (ECD) and channel gating.

7. Conclusions

In this work, we provided a comprehensive review of the main findings in the molecular modelling of taste receptors. The work is focused on the main candidates commonly discussed in the literature, i.e. GPCRs for sweet (TAS1R2-TAS1R3), umami (TAS1R1-TAS1R3) and bitter (TAS2Rs), OTOP1 for sour and ENaC for salty. It is worth mentioning that discussed receptors cover only a limited range of possible receptors, transducers and proteins essential to the taste perception process. Just to name a few, sugars are also transduced by sugar transporters (Yee et al., 2011), the salty taste has amiloride-sensitive and amiloride-insensitive components (Nomura et al., 2020), and sour taste most certainly involves mechanisms other than OTOP1, such as intracellular acidification and blockage of KIR2.1 (Ye et al., 2016). The presence of other key players, as well as the identification of other possible basic tastes, makes the understanding of the taste perception still incomplete and lacking, and a lot of work is still needed to get to a more granular and comprehensive knowledge. Interestingly, the existence of a sixth taste quality linked to fat perception has been recently highlighted (Besnard, Passilly-Degrace, & Khan, 2016; Khan, Keast, & Khan, 2020; D.; Liu, Archer, Duesing, Hannan, & Keast, 2016). In addition, some studies have remarked that the ability to detect fatty acids is reduced in response to a high-fat diet (Newman, Bolhuis, Torres, & Keast, 2016). In this context, the fat taste seems

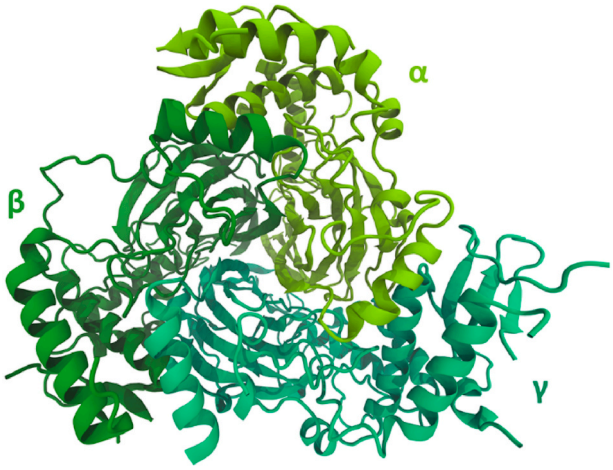

Fig. 6. Representation of the 3D molecular structure of the trimeric ENaC (PDB entry: 6WTH), comprising the α:β:γ subunits arranged in a counter-clockwise manner.

pivotal for the connection between fat intake and health status, specifically linked to overweight or obesity. Therefore, further studies related to fat taste may provide new bases for controlling the development of obesity, one of the main causes of global disease burden, including cardiovascular diseases, cancer and diabetes (Ng et al., 2014).

At present, the main findings on the receptor function come from computational and/or combined computational/experimental studies focusing on the structure-to-function relationships and ligand-protein binding investigations. We deeply discussed the need for developing high-quality molecular structures as a crucial step in molecular modelling and described the most recent experimentally-solved and *in silico*-derived structures for each taste receptor candidate. Out of the mentioned players of taste transduction, the only available experimental structure is the VFTM domain of the sweet receptor of the medaka fish, which represents a fundamental starting point for most computational investigations of sweet taste transduction mechanisms (Nuemket et al., 2017). Conversely, umami and bitter receptors have been studied through HM relying on experimental templates sharing some degree of sequence similarity. Homology models for bitter receptors are publicly available from BitterDB. Of note, the comparably low reported sequence identities for these models to their respective templates, compared to other HM applications, are not detrimental to the quality of the reported studies, due to the nature of the involved receptors. Lastly, some experimentally obtained molecular structures of both human and non-human salty and sour receptors are currently available in the RCSB, and pioneered many computational studies investigating their molecular mechanisms.

A better comprehension of taste receptor molecular behaviour and ligand-driven activity modulation is a crucial scientific challenge in the wider research concerning the complex mechanisms that drive toward the cascade of supramolecular, cellular, and tissue-level events emerging as an elaborated taste sensation. The molecular-scale investigation is a first, irreplaceable step and computational molecular modelling, due to its atomistic resolution, represents a powerful tool to explore receptor structure-to-function relationships and to elucidate ligand roles in driving taste receptor activity. This type of investigation allows to quantitatively characterise the ligand-binding process, thermodynamics and kinetics of the binding mechanism, binding modes, and ligand-target interaction properties, along with quantitative measures of receptor activation/inhibition, local and global protein rearrangements, correlations between receptor domains, transition pathways between active-resting conformations, etc. Ligand-receptor binding investigations allow the evaluation of food molecular constituents in terms of specificity, selectivity and multi-target features and shed light on the natural role of taste receptors in preserving life by discriminating between healthy and dangerous foods. Despite the enormous progress made in recent years, especially in molecular research and in the computational investigation of ligand-receptor interaction related to taste receptors, the scientific knowledge remained rather granular and unable to explain the latter in a holistic fashion. Thus, it remains of crucial interest to correctly frame the mechanisms involved in the transfer of taste information from the chemistry level, where food molecular constituents bind taste receptors, to molecular-, supramolecular- and cellular-level events, which ultimately manifest as a composite perception strongly linked to the food organoleptic profile. This vision is embraced by EU-supported research actions, such as the VIRTUOUS project (https://www.virtuoush2020.com), which aims at creating an intelligent computational platform by integrating molecular modelling methods, drug discovery techniques, machine learning classifiers, algorithms for big data, cloud computing, and experimental data to predict the organoleptic profile of selected types of food based on their chemical composition. In the VIRTUOUS project, molecular-level information and modelling outcomes represent a key data source feeding higher scale level modelling such as protein-protein cascade models, cell activation models, and ML-driven predictors.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tifs.2021.07.013.

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