Structure-function relationships of olfactory and taste receptors

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Maik Behrens[1], Loïc Briand[2], Claire A. de March[3], Hiroaki Matsunami[3], Atsuko Yamashita[4], Wolfgang Meyerhof[1] & Simone Weyand[5*]

*correspondence: sw644@cam.ac.uk

1 German Institute of Human Nutrition Potsdam-Rehbruecke, Dept. Molecular Genetics, Arthur-Scheunert-Allee 114-116, 14558 Nuthetal, Germany

2 Centre des Sciences du Goût et de l'Alimentation, AgroSup Dijon, CNRS, INRA, Univ. de Bourgogne-Franche-Comté, F- 21000 Dijon, FRANCE

3 Duke University Medical Center, Department of Molecular Genetics and Microbiology, 213 Research Dr, Durham NC, 27710, USA

4 Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, 1-1-1, Tsushima-naka, Kita-ku, Okayama 700-8530, Japan

5 University of Cambridge, Department of Biochemistry, 80 Tennis Court Road, Cambridge CB2 1GA, UK

Abstract

The field of chemical senses has made major progress in understanding the cellular mechanisms of olfaction and taste in the past two decades. However, the molecular understanding of odour and taste recognition is still lagging far behind and will require solving multiple structures of the relevant full-length receptors in complex with native ligands to achieve this goal. However, the development of multiple complimentary strategies for the structure determination of G protein-coupled receptors (GPCRs) makes this goal realistic. The common conundrum of how multi-specific receptors that recognize a large number of different ligands results in a sensory perception in the brain will only be fully understood by a combination of high-resolution receptor structures and functional studies. This review discusses the first steps on this pathway, including biochemical and physiological assays, forward genetics approaches, molecular modelling and the first steps towards the structural biology of olfactory and taste receptors.

Structural Biology, Functional Assays, Biochemistry, Taste, Olfaction, Molecular Modelling

Towards the high-resolution structures of chemosensory receptors

In recent years there has been increased success in the determination of membrane protein structures due to the development of a range of novel and improved methods. In particular, there have been a series of protein engineering breakthroughs that have facilitated the structure determination of G protein-coupled receptors (GPCRs) (Tate and Schertler 2009). This has transformed the field. The structures have been instrumental in developing a unified theory of how the conformation change in the receptor that occurs upon agonist binding results in G protein coupling on the intracellular face of the receptor (Katritch et al. 2013; Rosenbaum et al. 2009; Venkatakrishnan et al. 2016; Venkatakrishnan et al. 2013). In addition, structures of GPCRs bound to G proteins have illuminated how the subsequent signalling pathway is activated (Flock et al. 2015). High-resolution structures of specific GPCRs, such as the β_1-adrenoceptor, bound to ligands of different efficacy and potency have resulted in a detailed understanding of the relationship between the chemical structure of a ligand and how it affects the conformation of the receptor (Warne et al. 2011). It is now possible to use structure-based drug design to develop novel highly potent and subtype specific drug candidates, that will result in better drugs being available in the future for a wide range of diseases such as diabetes, neurodegeneration and cancer (Congreve et al. 2011; Mason et al. 2012). The structural biology of GPCRs is a vast field and the interested reader is guided towards the excellent reviews cited above.

Despite intense efforts in GPCR structural biology over the past decades and the crystallographic analysis of the extracellular region of a taste receptor (Nuemket et al. 2017), there is no high-resolution structure of any full-length taste receptor or olfactory receptor, which has hampered an in-depth understanding into their molecular mechanism. Problems in obtaining high-resolution structures include poor functional heterologous expression, post-translational modifications, high flexibility and low stability in detergent. The latter problem is especially important to resolve in order to allow the formation of well-diffracting crystals, which has been achieved by engineering thermostable mutants, protein fusion strategies or the formation of GPCR-G protein complexes with mini-G proteins and the addition of nanobodies (Carpenter et al. 2016; Cherezov et al. 2007; Ghosh et al. 2015; Magnani et al. 2016). Once it has been decided which strategy to use, the receptor needs to be expressed purified and crystallised, and each stage needs

careful optimization. Overexpression systems using either insect cells or mammalian cells are the most successful, such as the baculovirus expression system (Saarenpaa et al. 2015), the use of stable inducible cell lines (Andrell et al. 2016) or the BacMam expression system (Goehring et al. 2014). Green fluorescent protein fused at the C-terminus of GPCRs is a well-established method for rapid and efficient screening of expression levels, and for distinguishing folded versus unfolded receptor by a combination of Fluorescence Size Exclusion Chromatography (FSEC), differential solubilisation and confocal microscopy (Thomas and Tate 2014). N-glycosylation may be problematic, as it may be necessary for efficient folding of the chemosensory receptors, but it interferes with the formation of well-diffracting crystals. The use of a specific mammalian cell line HEK293-GnTI$^-$, which produces truncated N-glycosylation or the treatment with the enzymes EndoH or PNGaseF can address this problem (Thomas and Tate 2014). Once large amounts of fully functional receptor have been expressed, then it needs to be purified in a functional state, and the recent development of novel mild detergents has been critical for increased successes of this step (Bae et al. 2016; Hussain et al. 2016). Crystallisation remains a real challenge, but the revival and further development of the lipidic cubic phase technique has been pivotal in the crystallisation success of GPCRs (Huang et al. 2016). Once high quality and well diffracting crystals have been obtained then the exacting procedures of crystal mounting, data collection and processing have to be performed carefully to get the maximum information from them. Usually hundreds of crystals have to be grown and screened by X-ray diffraction to determine a high-resolution structure. However, the recent rapid developments of the resolution revolution in the field of electron cryo-microscopy (cryo-EM) will undoubtedly start to have an impact on the structure determination of GPCRs, initially on the largest receptors in complex with G proteins, which will have a molecular weight of about 150-250 kDa. However, as the techniques improve, the routine structure determination of GPCRs with molecular weights less than 100 kDa will become a reality (Liang et al. 2017). The tremendous efforts accomplishing a high-resolution structure of a GPCR such as in the case of the human histamine H1 receptor in complex with the antihistaminic compound doxepin revealed the biochemical action of differences between a 1st and a 2nd generation antihistamine (Shimamura et al. 2011). A phosphate ion from the buffer used during purification, was bound in the ligand binding site together with doxepin, a 1st generation anti-histamine drug. The negatively charged phosphate ion binds exactly in the region of the binding pocket that provides receptor specificity for 2nd generation anti-histamines. Only due to the

structural insights at atomic detail it is now possible to rationally design optimized tailor made antihistaminic compounds, with maximum pharmacological potential and minimum side effects. Another example of where a GPCR structure has led to a molecular understanding of a functional phenomenon is the inactivation of the adenosine A_{2A} receptor ($A_{2A}R$) by the antibody F_{ab} fragment Fab2838. $A_{2A}R$ is a major drug target due to its role in regulating blood flow to the cardiac muscle and the regulation of glutamate and dopamine release in the brain. The high resolution structure of this receptor in complex with an allosteric antibody revealed the molecular mechanism of the underlying receptor inactivation, which is based on the prevention of agonist but not antagonist binding to the extracellular ligand-binding pocket. The structure showed that Fab2838 recognizes the intracellular surface of $A_{2A}R$ and that its complementarity-determining region, CDR-H3, penetrates into the receptor (Hino et al. 2012). $A_{2A}R$ is also a good example showing how structure-based drug design can result in the development of novel potent and specific inhibitors that have the potential for the treatment of Parkinson's disease (Congreve et al. 2012). These examples highlight the synergism of combining structural and functional studies. The initial steps for the structure determination of chemosensory receptors are still ongoing, and there is a long and rocky road to achieve the ultimate goal of determining their structures. As the 17th International Symposium on Olfaction and Taste, Yokohama, Japan, 2016 showed, there is a wide range of structure-function studies ongoing in several different laboratories around the world. The symposium "structure-function relationships of olfactory and taste receptors" brought both sides closer together and increased mutual understanding of the problems facing the field. Only a combined approach and a team effort on structural and functional studies will ultimately reveal the underlying molecular mechanisms of chemosensory receptor function.

Structure-function relationship of olfactory receptors

Over 40 different GPCRs have been crystallised and their structures determined in multiple different conformations by X-ray crystallography and, more recently, by electron cryo-microscopy. However, experimental structure elucidation of the largest family of GPCRs, the olfactory receptors (ORs), has remained elusive (Fig. 1) (Buck and Axel 1991; Pilpel and Lancet 1999; Probst et al. 1992).

OR sequences have characteristic motifs of conserved amino acid residues present in each of their seven transmembrane helices (TM) numbered from TM1 to TM7. Some of these motifs are also shared with non-olfactory GPCRs (indicated by * in the following listing). For each TM, the motifs are: (de March et al. 2015a)

- GN* in TM1,
- LHxPMY and LxxxD* in TM2,
- MAY, DRY* and VAICxPLxY in TM3,
- W* in TM4,
- P* and SY* in TM5,
- KAFSTCASH and FYG in TM6,
- NPxxY* in TM7.

Though hindered by the lack of experimentally verified structures, advances in computational techniques in combination with functional assessments of OR mutants have enabled investigation of ligand-receptor interactions at the atomic level. Application of these methods to ORs, guided by their similarities with non-olfactory GPCRs and experimental ligand-mediated receptor activation data, has been demonstrated to be a powerful tool for the study of their dynamics and their interactions with odorant molecules (de March and Golebiowski 2014).

Olfactory sensation begins with the detection of odour ligands by ORs. Since the human genome encodes over approximately 400 ORs, it has been thought a combinatorial code is utilized, whereby one OR can be activated by a set of odorants and one odorant can activate a combination of ORs (Malnic et al. 1999). To accommodate structurally diverse odorants, the ligand-binding cavity is supposed to be highly variable between the ORs (Fig. 1) (de March et al. 2015a; Pilpel and Lancet 1999). The chemical space associated with ORs response cover a large spectrum, leading to the denomination of narrowly and broadly tuned receptors for OR responding to a small or large number of odorants, respectively. This characteristic is influenced by the permissiveness of the OR cavity and its ability to be activated (Yu et al. 2015).

The activation mechanism of GPCRs (including ORs) is based on favouring an active state with respect to an inactive state when an agonist molecule is bound. Homology models of ORs based on conserved residues with other GPCR has successfully predicted ligand-mediated activation in cell-culture based assays, providing evidence for its accuracy in ligand binding prediction. (Floriano et al. 2000; Gelis et al. 2012; Topin et al. 2014) The calculation of affinity between an odorant molecule and the ligand-cavity of an OR is an efficient parameter for discriminating agonists and antagonists,

but high affinity is not necessarily translated into activation. Indeed, inverse agonists bind to the cavity of a receptor, but do not trigger activation (de March et al. 2015b). Once the receptor is activated, a binding site is opened in its intracellular part allowing the binding of a G protein, triggering neuronal activation (Flock et al. 2015; Nakamura et al. 2013). Assuming that all ORs share G-protein coupling as a common activation mechanism, it is reasonable to hypothesize that some residues conserved in this family control the activation (de March et al. 2015c) (Fig. 1). The structures of GPCRs are highly conserved and the mechanism of receptor activation is also highly conserved, where agonist binding causes a conformational change that results in the opening of a cleft on the intracellular surface where the G protein binds (Venkatakrishnan et al. 2013).

Despite the continued absence of an experimentally verified structure, our understanding of the structure-function relationship of ORs has dramatically increased in recent years through modelling *in silico*. However, the structure of the extracellular and intracellular loops remains unclear, because only the disulphide bridge between two Cys residues from the extracellular part of TM3 and extracellular loop 2 is conserved between ORs and non-olfactory GPCRs. The existence of a second disulphide bridge in extracellular loop 2 has also been proposed for one human OR (Cook et al. 2009).

One of the reasons why OR structures have not been determined is that heterologous expression of ORs for large-scale production is notoriously difficult. ORs are not efficiently transported to the cell surface when expressed in cells other than olfactory sensory neurons (Gimelbrant et al. 1999; Lu et al. 2004; Sharma et al. 2017; Wu et al. 2012). The features controlling this surface expression are thought to be dictated by certain amino acids within the ORs sequences. It could rely as well as on the interaction of the OR with the RTP1S (facilitator of OR trafficking) (Wu et al. 2012) or with the membrane composition. Understanding the mechanisms underlying the surface expression of ORs could facilitate the development of new ways to produce large quantities of functional ORs for structural studies.

Structure-function relationship of sweet and umami taste receptors

The vertebrate gustatory system is an indispensable tool for the selection of edible food items. Five taste qualities (sweet, sour, salty, umami (typically elicited by the L-amino acid, glutamate), and bitter) are generally accepted as the basic tastes. Each taste has a specific role to evaluate the caloric content, the electrolyte concentration and the presence of carbohydrate- and protein-rich foods or

potentially harmful substances. Detection of the corresponding food components is mediated by taste receptors expressed in sensory cells residing in the oral cavity (Behrens and Meyerhof 2015). Sweet tasting compounds are perceived through the activation of a heterodimeric GPCR composed of, TAS1R2 (taste receptor type 1, member 2) and TAS1R3 (taste receptor type 1, member 3) (Fig. 2). Umami tasting compounds are perceived through a GPCR heterodimer composed of TAS1R1 (taste receptor type 1, member 1) and TAS1R3 (taste receptor type 1, member 3). The sweet and umami taste receptors (TAS1Rs) are members of the small family of class C GPCRs that includes the metabotropic glutamate receptors (mGluRs), the γ-aminobutyric acid receptor B ($GABA_BR$) and the calcium-sensing receptor (CaSR). In addition to the characteristic heptahelical transmembrane domain (TMD), TAS1Rs possess a large extra-cellular domain, composed of the Venus Flytrap (VFT) domain followed by a short cysteine-rich region. Cellular assays, molecular docking and site-directed mutagenesis studies have revealed that the VFT domain of TAS1R2 (TAS1R2-VFT) or TAS1R1 (TAS1R-VFT) contains the primary binding site for most of the sweet ligands including natural sugars artificial sweeteners and natural sweeteners or umami ligands including amino acids and purine 5' nucleotides (Li et al. 2002; Nelson et al. 2002; Nelson et al. 2001; Xu et al. 2004) . However, some studies have revealed that the VFT, the cysteine-rich region and the TMD of T1R3 interact with some sweeteners including sweet-tasting proteins (Jiang et al. 2005a; Jiang et al. 2005b; Jiang et al. 2004; Maitrepierre et al. 2012; Winnig et al. 2007). To elucidate the contribution of TAS1R2-VFT to sweet tastant binding, Briand and co-workers heterologously expressed human TAS1R2-VFT in *Escherichia coli* (paper in preparation). In agreement with previously published data (Nie et al. 2005), ligand binding studies using intrinsic tryptophan fluorescence revealed micromolar and millimolar range affinities of TAS1R2-VFT with natural sugars and various sweeteners, confirming the functional state of the recombinant protein. Site-directed mutagenesis combined with fluorescent binding assays demonstrated that TAS1R2-VFT binding is specific. The interaction of TAS1R2-VFT with the two recombinant sweet-tasting proteins, brazzein and monellin, was measured using Bio-Layer Interferometry (BLI). This optical technique analyses the signal variations in the interference pattern generated from visible light reflected from an optical layer and a biolayer containing the immobilized protein of interest. BLI experiments demonstrated that TAS1R2-VFT binds these two sweet-tasting proteins with affinities in agreement with the physiological range. The strategy for the large-scale

production of functional VFT domains of TAS1Rs will be used in future work to investigate the structure-function relationships of these taste receptors using NMR or crystallographic approaches.

Another important factor regarding the structure-function relationship of TAS1Rs is dimerization. Class C GPCRs constitutively exist as homo- or heterodimers, and heterodimerization of TAS1Rs are indeed essential for receptor functions for umami and sweet taste perception (Li et al. 2002; Nelson et al. 2002; Nelson et al. 2001; Zhao et al. 2003). The previous crystallographic structure analyses of mGluR1 VFT domains revealed dimer structures with two different arrangements. One is a compact arrangement like an inverted "U"-shape with interprotomer interactions at each VFT region. The other arrangement is a more open inverted "V"-shape resulting from a scissoring rearrangement of each protomer (Kunishima et al. 2000). The 'U' and 'V' conformations were considered to be an active state and a resting state, respectively, and the observed dimer rearrangement has been presumed to underlie the receptor activation. In theory, these conformation changes could induce rearrangements of the transmembrane regions, thus resulting in receptor activation. It should be noted, however, that the crystal structures of the other class C GPCR VFTs exhibited various types of conformational changes (Geng et al. 2013; Geng et al. 2016; Muto et al. 2007).

So far, purification of a full length TAS1R heterodimer has been unsuccessful. However, Nango and co-workers succeeded in purifying the recombinant heterodimeric TAS1R VFT domains, composed of TAS1R2 and TAS1R3 from the medaka fish (*Oryzias latipes*), and revealed the manner of heterodimerization and conformational change by integrating several biophysical analyses such as electron microscopy, Förster resonance energy transfer, and small-angle X-ray scattering (Nango et al. 2016). They found that TAS1R2/3-VFT heterodimer has a compact structure in the presence of compounds that induce TAS1R2/3 receptor responses in fish e.g. amino acids. In contrast, the dimer becomes more open in the absence of amino acids, presumably through a rearrangement of the dimer interface (Fig. 2B). They also confirmed that the conformational change was accompanied by binding of amino acids to the protein. The results suggested that the mechanism of taste signal transduction by TAS1R taste receptor is similar to that proposed for the other class C GPCRs. Recently, the same group succeeded in determining the crystal structures of the fish TAS1R2/3-VFT in the presence of amino acids, and provided the structural basis for taste substance recognition (Nuemket et al. 2017). The TAS1R2/3-VFT was found to share a similar overall architecture, including the primary ligand-binding pocket, with those of VFTs in other class C GPCR members such as mGluR (Fig. 2B). The arrangement of the dimer with any amino acid bound was always compact and very

similar to the conformation observed in the active state of mGluR. This reflects both the conserved mechanism of receptor activation in Class C GPCRs and also the broad ligand specificity of fish TAS1R2/3. Further structural studies are required to elucidate detailed molecular mechanisms of TAS1Rs, such as receptor activation induced by the binding of taste substances.

Structure-function relationship of bitter taste receptors

The taste receptor 2 (TAS2R) family are GPCRs and facilitate the detection of countless structurally diverse bitter substances. The human TAS2R gene family encompasses ~25 members which, like odorant receptors, possess different ligand profiles. Whereas the majority of the TAS2Rs responds to only a moderate number of agonists, three receptors (TAS2R10, TAS2R14, and TAS2R46) are exceptional because they are sensitive to an extraordinarily large array of bitter substances (Behrens and Meyerhof 2015). Somewhat surprisingly, structure-function analyses of TAS2R46 revealed the existence of a single orthosteric ligand binding pocket that accommodates all the different agonists (Brockhoff et al. 2010). Recently, an additional vestibular binding site in TAS2R46 was proposed (Sandal et al. 2015). Here, ligands bind only transiently *en route* to the orthosteric site, which may help to pre-filter ligands from the bulk of irrelevant compounds present in complex food matrices. Focusing on the TAS2R10, Born and colleagues concluded that this receptor evolved to interact with many agonists weakly at the expense of binding only a few compounds with high affinity (Born et al. 2013). Amino acid residues in at least three positions within the binding pocket exert ambivalent contributions to the receptor's activation by different agonists. Whereas these residues support binding of some compounds, they decrease the affinity of binding of other agonists. Experimental studies have involved generating inter-receptor chimeras and using functional assays to identify regions within TAS2Rs that are involved in agonist interactions (Brockhoff et al. 2010). In efforts to expand on these initial data, more recent studies *in silico* have relied on molecular modelling to suggest amino acid residues located in the receptor binding pocket (e.g. (Biarnes et al. 2010; Born et al. 2013; Marchiori et al. 2013; Sandal et al. 2015)). However, since TAS2Rs are only remotely related to the other GPCR-families (Fredriksson et al. 2003), the current homology models are all based on crystal structures of distantly related receptors. Hence, TAS2R structure-function research would benefit more than any other chemoreceptor-family from the successful crystallization of at least one member of this highly interesting group of receptors. Initially efforts to characterise TAS2Rs were focussed on the human receptors (Meyerhof et al. 2010), but in recent years numerous receptors from other species have been characterised and their ligands identified (Behrens and Meyerhof 2016). Most recently, Lossow and co-workers added the functional characterization of the majority

of mouse TAS2Rs (Lossow et al. 2016). Intriguingly, it was found that the TAS2R repertoire of mice contains fewer generalist receptors and more narrowly tuned receptors. Despite general assumptions anticipating functional conservation among human and mouse orthologues, Lossow et al. demonstrated that such orthologues rarely share common ligands. The combined data from functional experiments performed with TAS2R repertoires of different species and careful structure-function analyses enables insights into the evolutionary history and the selective forces acting on TAS2R gene repertoires of human and mouse. It is believed that species-specific expansion of TAS2R genes serves an important role in the adaptation of species to the presence of specific bitter substances encountered by each species in their environment (Shi et al. 2003; Di Pizio and Niv 2015). One of these gene expansions occurred after the separation of the rodent and primate lineages, and led to muroid cluster I; this contains five homologues in mice and only one (TAS2R10) in humans. The amino acid residues identified previously as crucial for TAS2R10 agonist interactions (Born et al. 2013) were different in all the mouse muroid cluster I receptors, suggesting that the mouse receptors had different selectivity and bound different agonists. Hence, permutation of these positions along with gene expansion appears to be an effective evolutionary strategy to rapidly diversify pharmacological properties. In contrast, the human TAS2R38 receptor that binds phenylthiocarbamide (PTC) (Biarnes et al. 2010; Marchiori et al. 2013) has not been duplicated during mammalian evolution. It was hypothesized that the exquisite PTC-sensitivity of primate TAS2R38 developed after separation of rodent and primate lineages, whereas the orthologous mouse TAS2R138 lost PTC-responsiveness and developed other binding specificities (Lossow et al. 2016).

Remarkably, despite pronounced amino acid sequence divergence, striking functional similarities between bitter taste receptors and other class A GPCRs exist (Di Pizio et al. 2016) suggesting that they activate G proteins in a similar manner.

Future perspectives

The ISOT symposium with the title “Structure-function relationships of olfactory and taste receptors”, which took place on the 07.06.2016 in Yokohama, Japan, demonstrated the importance and necessity of high resolution structures of chemosensory receptors. Moreover, it clearly showed that the joint effort of structural and functional studies is essential and necessary to achieve this goal. Given the enormous efforts in all aspects of pharmacological, cell and molecular biological, neurobiological and modelling studies, the structural studies can now be performed in a more

rational way by extracting and combining the crucial information of each of the fields presented and letting the structural work being guided by this prior knowledge. Eventually any chemosensory high-resolution structure will shed light on the molecular mechanism of any receptor within this class of proteins.

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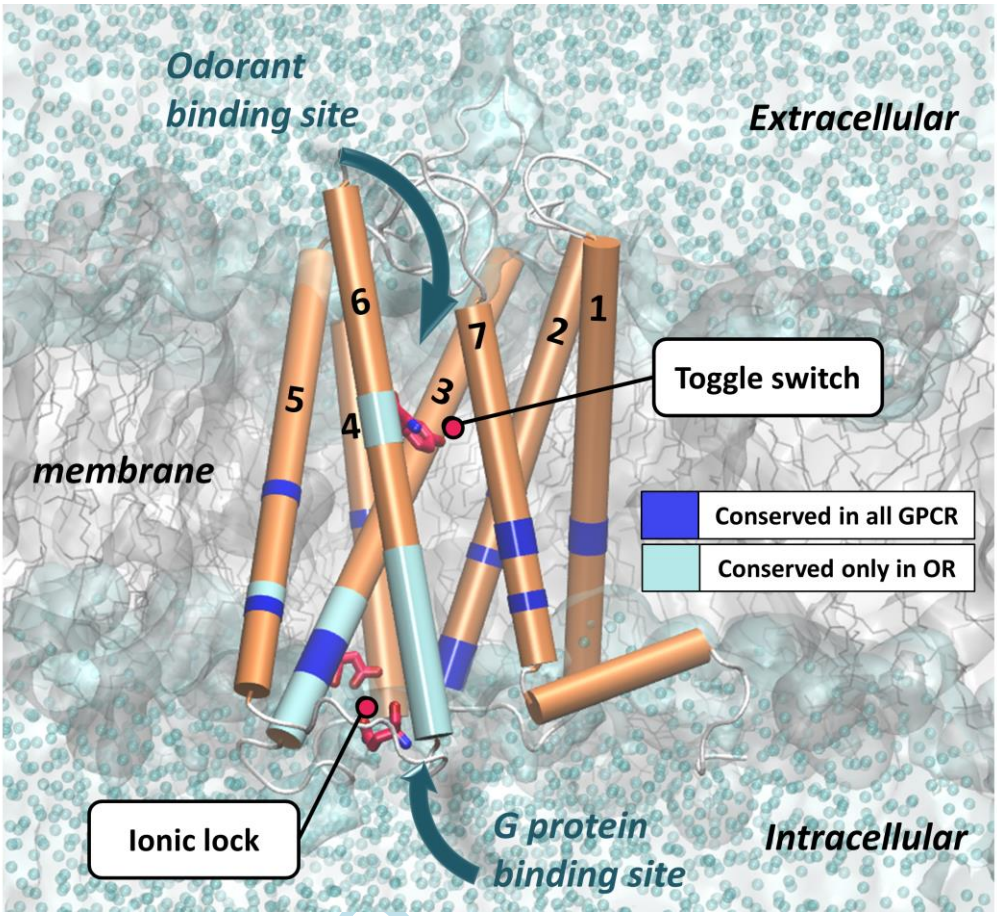

Figure 1: Homology model of MOR256-3 embedded in a membrane and solvated in water. The backbone of the receptor is represented in orange and the toggle switch and the ionic lock are detailed in pink. The conserved residues locations are highlighted in dark blue for those common to all the class A GPCR and in light blue for those specific to the OR family. The predicted odorant binding site is located at the extracellular part of the receptor close to the toggle switch. The binding site of the G protein is at the opposite side of the bundle, in its intracellular part.

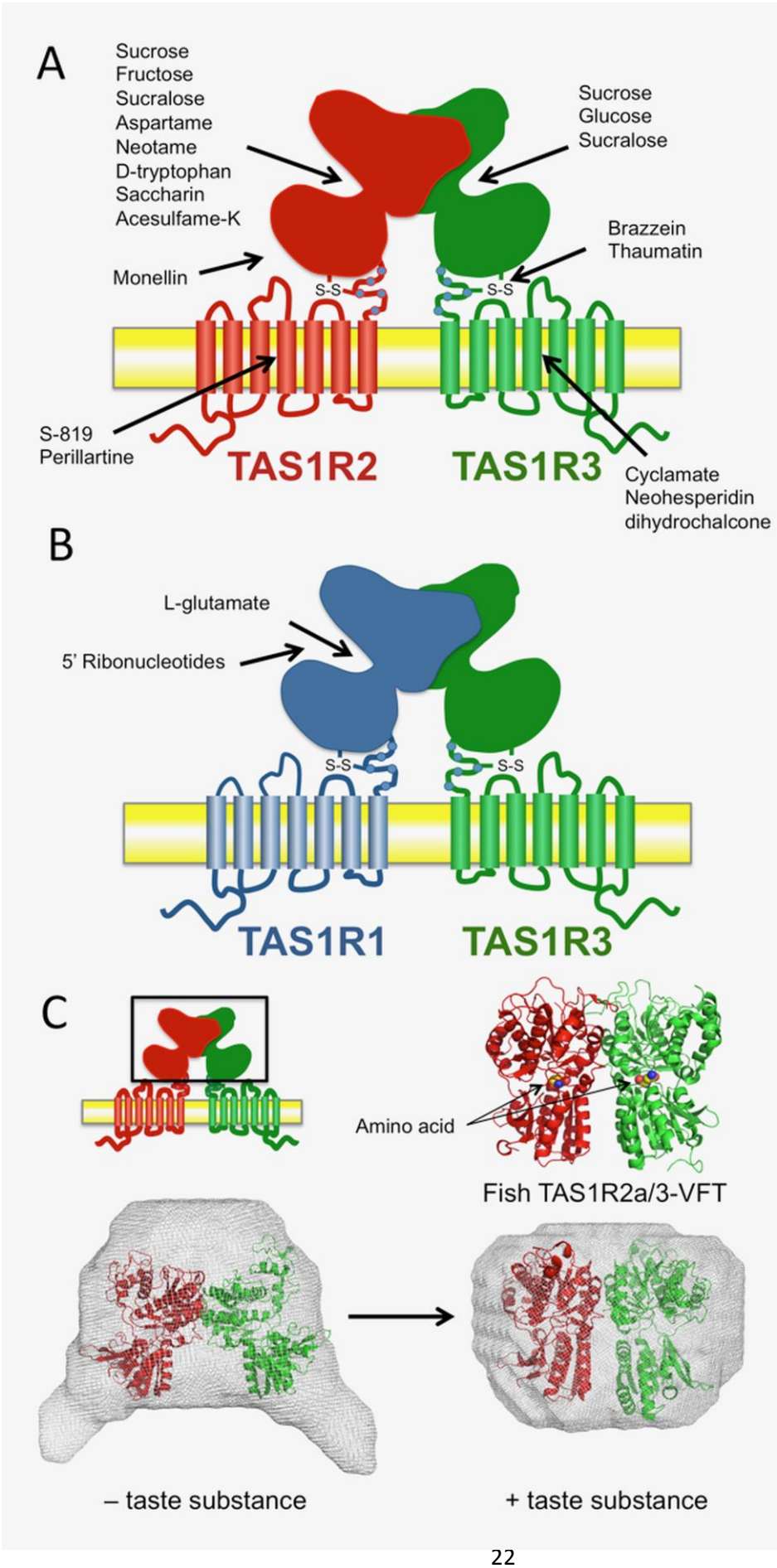

Figure 2: Sweet and umami taste receptors (TAS1Rs) structure. (A) The sweet taste receptor possesses multiples binding sites. The sweet taste receptor is composed of two subunits named TAS1R2 and TAS1R3. The VFT domain of TAS1R2 contains the primary binding site for most of the sweet ligands including natural sugars and, artificial and natural sweeteners, including the sweet tasting protein monellin. Four additional binding loci are indicated. (B) The umami taste receptor is composed of two subunits named TAS1R1 and TAS1R3. The VFT domain of TAS1R1 contains the primary binding site for umami ligands including L-glutamate and 5'-ribonucleotide. (C) Conformational change of the VFT domains upon taste substance binding. Molecular envelopes displayed representative low-resolution models reconstructed from the SAXS data of fish TAS1R2a/TAS1R3-VFT heterodimer. The crystal structure of mGluR1-VFT dimer in a resting (PDB: 1EWT) and an active (PDB: 1EWK) state was superimposed on the restored model in the absence (left) and presence (right) of a taste substance, respectively (Adapted from Nango et al. 2016).