Review

Flavour and Fragrance Journal

Received: 20 September 2010; Revised: 6 January 2011; Accepted: 19 January 2011; Published online in Wiley Online Library: 18 April 2011

(wileyonlinelibrary.com) DOI 10.1002/ffj.2054

Molecular modelling and models in the study of sweet and umami taste receptors. A review.†

Gabriella Morini,[a]* Angela Bassoli[b] and Gigliola Borgonovo[b]

ABSTRACT: The mechanism of action of tastants has been under investigation for many years. For the sweet taste several models have been developed to describe the nature and topological arrangement of the glucophores of ideal sweet compounds and/or the recognition sites of the sweet taste receptor. In less than a decade our knowledge about the mechanism of sweet taste chemoreception has grown enormously, following the identification and cloning of T1Rs. The observation that receptor cells co-expressing T1R2 and T1R3 respond to a great variety of sweet compounds has been generally interpreted as an inference that there is a single heterodimeric receptor for sweet taste. The three-dimensional structure of the receptor is not known and alternative methodologies are required to model the binding of sweet compounds. Therefore homology modelling and molecular modelling become indispensable tools to suggest point mutations which further define the binding regions. Only their cooperative effect allowed researchers to determine several molecular mechanisms of the sweet taste receptors, including the modality of action of blockers and positive allosteric modulators. For umami taste, despite the general appreciation of the meaty, mouth filling and rich taste found in many foods (and the great interest of the food industry thereof), the existence of an umami receptor has been accepted only after its cloning. Probably because of this, no umami taste receptor models have been developed, while the molecular mechanism for its synergism has been unravelled before that of the sweet taste receptor. Copyright © 2011 John Wiley & Sons, Ltd.

Keywords: taste; sweet; umami; G protein-coupled receptor; molecular modelling; model

Introduction

> The human race survives only because man possesses the instinct of self-preservation and reproduction, and keenly feels the need to satisfy both. The satisfaction of a need is always accompanied by pleasure. The pleasure of self-preservation lies in the sense of taste…

This quotation from *Science in the Kitchen and the Art of Eating Well*, by Pellegrino Artusi (1899),[1] the father of modern Italian gastronomy, is just one of innumerable indications that taste has always played an important role in life. Therefore, it is easy to understand why the mechanism of action of tastants has been under investigation for many years. First of all for the relevance in the taste profile and overall hedonic outcome of foodstuffs, hence in individual food choice and, last but not least, for the impact on nutrition.[2]

Sweet and umami, together with salty, belong to the attractive taste modalities and have the function to allow mammals to identify (and then consume) nutrients.

Sweet taste appears in the list of taste modalities of Aristotle, and is one of the five *wei* of traditional Chinese medicine and one of the six *rasa* of ayurveda; nobody would present any difficult in defining 'sweet' a food that actually taste sweet. Much more recent is the official enrolment of umami as a taste: it was only in 1908 that Ikeda suggested that the sensation generated by L-monosodium glutamate (MSG), isolated from dashi (a soup made with kombu seaweeds), was different from any other of the four so-called basic tastes, and dubbed it 'umami,'[3] from the Japanese word *umai* (delicious). Since MSG alone is not pleasant, while the 'umaminess' comes out only when associated with other foods, several authors have suggested that it should be considered more a taste enhancer than a taste *per se*.

The field has achieved tremendous progress after the sequences of several mammalian genomes became publicly available, making possible the discovery and characterization of different taste receptors.[4,5]

> Although we can taste a vast array of chemical entities, it is now generally accepted that, qualitatively, they evoke few distinct taste sensations: sweet, bitter, sour, salty and savoury (or umami). This repertoire may seem modest, but it has satisfactorily accommodated the evolutionary need for an effective and reliable platform to help recognize and distinguish key dietary components.[6]

Therefore, the term 'basic taste' has been coined. But if taste serves to identify nutrients in what we eat, how come there is not a specific taste sensation for the fatty taste, if fats account for about 40% of the daily calorie intake in the West? For many years experts thought that the preference for fat was linked to

* Correspondence to: Gabriella Morini, University of Gastronomic Sciences, piazza Vittorio Emanuele, 9, I-12042 Pollenzo-Bra (CN) Italy. E-mail: g.morini@unisg.it

[a] University of Gastronomic Sciences, Pollenzo-Bra, (CN), Italy

[b] Department of Agrifood Molecular Sciences, University of Milan, Italy

† This article is part of the Special Issue of *Flavour and Fragrance Journal* entitled "Proceedings of the Procida Workshop on Taste (April 14-17 2010)" edited by Pierandrea Temussi and Gabriella Morini.

the texture that it gives to food, so to a somatosensory sensation. The existence of specific sensors for fatty acids was demonstrated recently,[7–9] and therefore fatty taste may also be considered another basic taste.[10]

We feel therefore the need to update our vocabulary, and we propose to use the term 'receptor-mediated taste' instead of 'basic taste'. This would stress the accent on the methodological difference in studying sensations which are mediated by receptors (being them strictly tastes or chemestetic or maybe something else as astringency) and other mechanisms which are not necessarily mediate by a receptor, or for which receptors are still unknown.

Now we know that sweet and umami tastes are sensed by T1Rs, three G-protein-coupled receptors of class C, forming two heterodimeric receptor complexes: T1R2/T1R3 for the sweet taste receptor and T1R1/T1R3 for the umami taste receptor respectively. But until 2001, nothing was known about the structure of such receptors due to the failure of attempts to isolate them from human or animal tissues. There was, thus, scope for pursuing the development of models stemming from structure–taste relationship.

The present article describes the evolution of the models derived in recent years for the sweet taste receptor and gives a possible explanation for the lack of them for the umami receptor.

The Sweet-taste Receptor

One of the peculiar characteristics of the sweet-taste receptor system is its ability to recognize molecules belonging to very different classes of compounds, ranging from sugars to amino acids, peptides, proteins, and several other classes of organic compounds. Because of this, one uncertainty concerned the existence of a single receptor protein – in contrast to a multiple one – or, alternatively, a single protein with multiple binding sites.

Moreover, the behaviour of the sweet-taste receptor towards its ligands can be considered somewhat of an anomaly: it is characterized by a very low affinity towards the natural agonists, but at the same time it shows high specificity requirements. Probably the weak binding of sweet compounds in biological preparation was the cause of the failed attempts to isolate the sweet-taste receptor from human or animal tissues, the only progress toward this goal being the structural elucidation of antibodies developed against guanidinic sweeteners.[11]

If nothing is known about a receptor, the only possible information comes from the ligands that bind to it. For this reason, the derivation of models describing the nature and topological arrangement of glucophores of an ideal sweet compound and/or the recognition sites of the sweet-taste receptor has been widely used.

The first useful model was proposed in 1967 by Shallenberger and Acree.[12] They recognized the existence in almost every sweet molecule of two functional groups ('glucophores') corresponding to a hydrogen bond donor and a hydrogen bond acceptor, named AH and B, respectively. Their role in recognition involved the creation of two parallel hydrogen bonds at about 3.5 Å apart, with two complementary sites on the receptor protein (Figure 1a). Kier[13] added a third interaction site (first called X) corresponding to a hydrophobic region of the molecule (Figure 1b).

This simple model found widespread acceptance, due to its ability to explain the sweetness of many structurally different

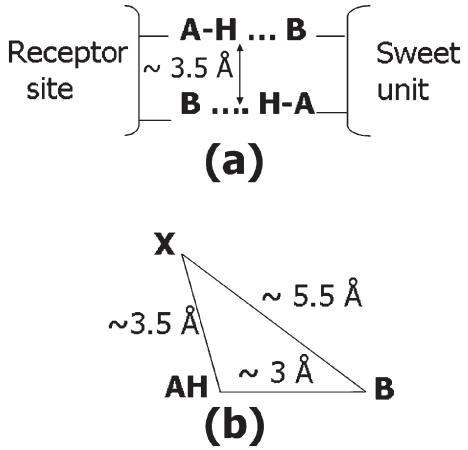

Figure 1. (a) The Shallenberger and Acree model: the AH hydrogen bond donor, and B hydrogen bond acceptor groups on the sweet compound interacting with two complementary sites on the receptor protein. (b) The Shallengerger–Acree–Kier model: a hydrophobic region of the sweet compound X (and the complementary one on the receptor) is added

compounds and also to interpret variations in sweetness due to geometrical or conformational differences, especially in sugars.

Substantial improvement of techniques used to evaluate the molecular properties of organic compounds and their structure, together with the progress of molecular modelling, has provided tools from which more sophisticated models were derived. Temussi and co-workers[14–16] on the basis of extensive conformational studies of aspartame, which included NMR experiments and theoretical calculations, proposed a model for the sweet receptor. This model depicts the receptor as a hemihedral cavity with a definite shape and functionality, which includes the AH-B groups of Shallenberger and Acree. Moreover, it can explain the change from sweet to bitter taste of some enantiomeric compounds, such as some D- and L-amino acids. Modifications to this model were subsequently made by Goodman *et al.*[17] Conformational analysis and electrostatic potential calculations on some very potent sweeteners have been used by Muller *et al.*[18] to derive a three-dimensional model of the sweet taste receptor, lately used to design ultra-high potency sweeteners.[19] More recently Walters *et al.*[20] suggested a pharmacophore model derived from extensive conformational analysis of some high-potency sweeteners.

As a result of a rational approach in the design of sweet molecules Tinti and Nofre have been able to discover several series of hyperpotent sweeteners.[21] One of these series, the guanidinic sweeteners, include compounds with relative sweetness (RS) over 200 000 times that of sucrose. Comparison of the molecular properties of these extremely potent substances within qualitative structure–activity relationships of various sweeteners led to the development of an eight-site interaction model.[22,23] The multipoint attachment (MPA) model characterizes an ideal sweet compound with eight glucophores, consisting of four high-affinity sites AH, B, G (corresponding to AH, B, X of Shallenberger–Acree–Kier), and D, four secondary sites Y, E_1, E_2, (hydrogen-bond acceptor groups), and one hydrogen-bond donor group, XH (Figure 2).

The model has been successfully used to explain the sweet taste of many compounds belonging to different classes and to design compounds with high RS[24] (Figure 3).

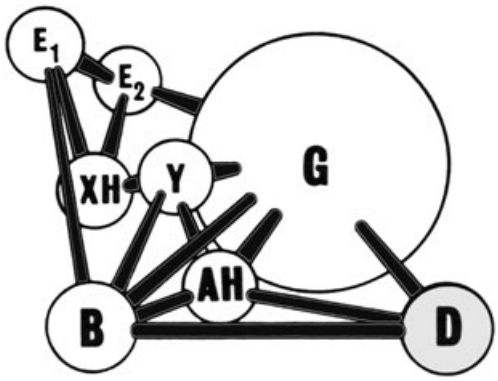

Figure 2. The multipoint attachment model (MPA) of Tinti and Nofre representing an ideal sweet compound with eight glucophores: four high-affinity sites AH, B, G (corresponding to AH, B, X of Shallenberger–Acree–Kier), and D, four secondary sites Y, E_1, E_2, (hydrogen-bond acceptor groups), and one hydrogen-bond donor group, XH

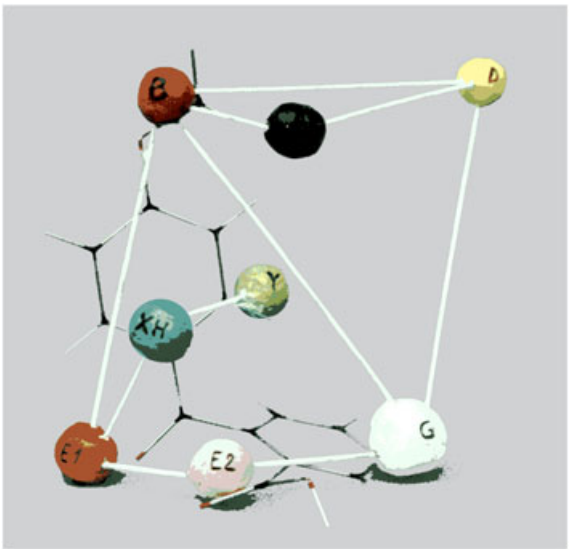

Figure 3. A multipoint attachment (MPA) model built with wooden sticks and plasticine and a sweet compound built with dreiding models: one of the systems used by our group to represent sweet taste receptor models and their interaction before comprehensive graphic molecular modelling programs were available

In later work[25] the same authors proposed an improved model in which they included eight specific amino acids of the sweet taste receptor which were involved in 15 interactions with the glucophores. The MPA model has subsequently been re-examined and the number of interacting amino acids increased to 10.[26]

Some hyperpotent guanidinic derivatives have also been used to raise monoclonal antibodies which have been fully characterized together with some complexes between the hapten and the immunoglobulin.[27]

A peptidic receptor model consisting of an α-helix moiety has been proposed by Suami and Hough on the basis of the chirality of several sweet compounds.[28]

None of these models could give an at least semi-quantitative correlation of sweetness with structure, nor gave hints about the possible structure of the receptor. To tackle these issues, and taking advantage of the improvements in computer hardware and software, we have used a pseudo-receptor modelling approach:[29] the main features of the bioactive ligands are projected into three dimensions around their appropriately superimposed molecular framework, and the resulting map provides steric, electrostatic and lipophilic profiles which can be used to identify the type and approximate position of amino acidic residues, or their functional groups, in the true biological receptor that interacts with the ligands. In general, the sequence and arrangement of the building blocks of a pseudo-receptor (e.g. amino acids residues) and its natural counterpart will only bear little resemblance, but they should accommodate a series of ligands in a relatively similar binding pattern. The pseudo-receptor can be used for subsequent molecular modelling and allows semi-quantitative predictions of binding affinities for ligands.

This approach is generally used with very similar ligands, and indeed it has been initially applied to derive a semi-predictive model for isovanillic sweet derivatives.[29] With the prospect to derive a general pseudo-receptor model for sweet compounds (and assuming a main sweetener binding site able to host several structurally different sweet compounds) a pseudo-receptor for the guanidinic hyperpotent sweeteners has been built, and then modified using as ligands a wide group of different sweet compounds.[30] The critical point was the criterion of superimposition of different ligands, but it was overcome by taking advantage of the preceding empirical glucophoric models: the ligands were superimposed using as a template the MPA Nofre–Tinti model.

The identification and functional expression of the receptor for the sweet taste showed that the sweet taste receptor is a GPCR similar to the homodimeric mGluR1 receptor, a class C GPCRs, characterized by a large extracellular domain, the Venus fly trap domain (VFTD), containing the active site for ligands, as demonstrated by X-ray structures of mGluR1.[31] The fact that only the primary structure of the sweet receptor was (and still is) known, together with the availability of the template for the VFTD, relaunched and offered new opening in molecular modelling. Since 2001 homology models of the extracellular domain of the sweet receptor have been derived, the first being homodimers T1R3/T1R3.[32,33] After the proof that only the heterodimer T1R2/T1R3 can function as sweet receptor and that it was able to respond to all sweet taste stimuli tested, inferring that there is just one sweet taste receptor,[34] a heterodimer based on the *mouse* sequence was proposed by Temussi[35] and next used by the author to model sweet proteins–receptor interactions. The model proposed that sweet proteins, contrary to small ligands, do not bind to the 'glutamate-like' pocket but could fit a large cavity of the receptor with wedge-shaped surfaces of their structures (wedge model).

The first extensive modelling of *human* T1R2/T1R3 extracellular domain has been the one proposed by Morini *et al.*:[36] homology models for T1R2 and T1R3 on the two chains (A and B) of mGluR1 were derived and corresponding key residues of the ligand binding site in the three-dimensional structure of mGluR1 and in the T1R2 and T1R3 identified. These sites in the VFTD were not easily accessible to sweet proteins, but docking experiments *in silico* confirmed the wedge model by Temussi.[35] Moreover, the derived sites were used as an entry point for structure-based modelling by pseudo-receptor techniques, in this case used only as a tool to evaluate the reliability of the ligand binding sites identified, without essentially altering the architecture of the sites yielded by homology modelling.

Confirmation of the predictive power of the obtained models was the good prediction of the sweetness of sweet synthetic compounds which appeared in the literature after the publication of the models[37] (Figure 4).

This model provided for the first time a possible interpretation of the phenomenon of synergy (enhanced sweetness) of some sweet compounds when in combination: suggesting several possible binding sites it was proposed that to elicit sweetness is enough the binding of a sweet compound to one site, while the contemporary binding of a different sweetener to another binding site leads to enhanced sweetness.

In addition to its use *in silico* studies *per se*, molecular modelling has become a very useful complementary tool to *in vitro* tests and therefore for molecular biology and mutagenesis studies. Owing to the difficulty of direct structural studies, modelling of sweeteners and sweet taste modulators in complex with their receptor active sites is a perfect way to suggest further mutations and therefore has contributed to the identification of the different ligand binding sites of the sweet taste receptor.[38–43]

The discovery of positive allosteric modulators[44] and the identification of the molecular mechanism of the sweet taste enhancers[45] (Figure 5) can be classified among the most recent and relevant results of the interplay of modelling and wet laboratory studies.

The Umami-taste receptor(s)

As already mentioned in the introduction, the umami taste has had a much more troubled history, and only after the

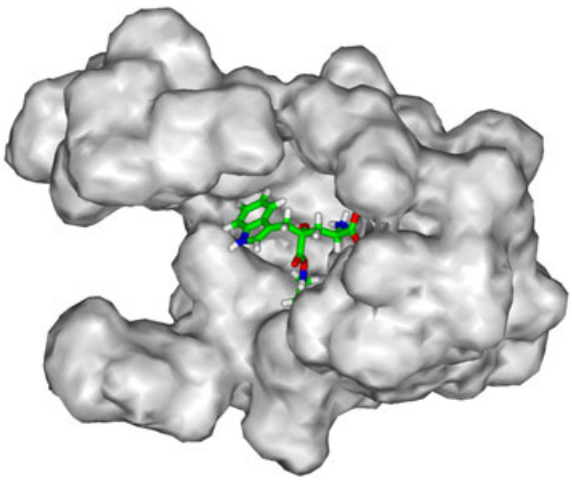

Figure 4. The ligand binding site in the VFTD of T1R2 proposed by Morini *et al.*[36] with a sweet carboxamide derivative of monatin whose sweetness was well predicted and fitted into it

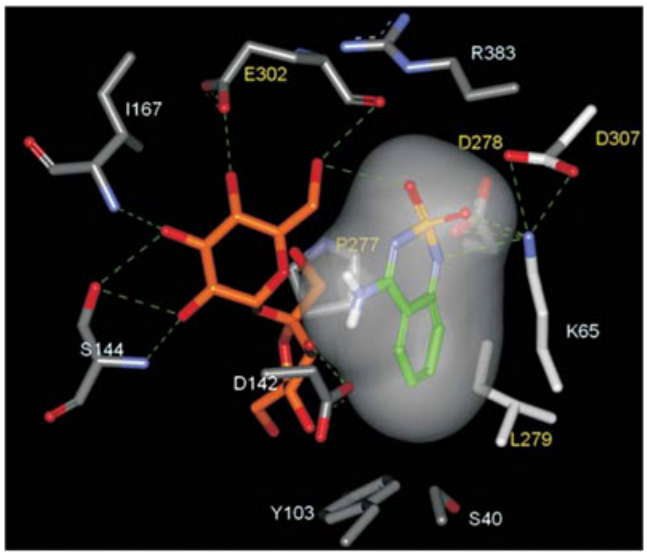

Figure 5. A molecular model of the T1R2 VFT domain containing sucrose (in gold), from Servant *et al.*[44] Lower lobe residues are labelled in yellow letters and upper lobe residues in grey letters

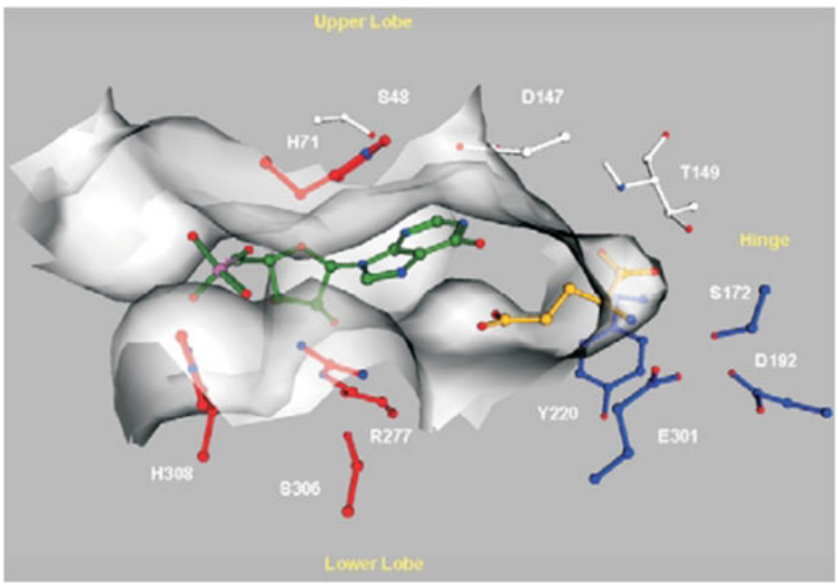

Figure 6. A molecular model of T1R1 VFT domain containing MSG and IMP. Key residues for MSG (blue) and for IMP (red) binding are indicated, from Zhang *et al.*[52]

identification of specific GPCRs that respond to MSG and exhibited nucleotide potentiation, it has been accepted as a taste. Notwithstanding, some authors still favour the idea that umami should be considered more a taste enhancer than a taste *per se*, and that the sensation it provokes is mainly due to taste and olfactory pathways converging in the brain.[46] For sure, umami is a much more complex taste, since MSG alone is not pleasant (and, in any case, very few people have tasted it) while it is certainly able to improve the overall taste of food[47] and also to strongly interact with other taste modalities, salty and sweetness in particular. One motive of the complexity of umami taste may lie in the fact that for its detection several receptors have been proposed,[48] while is still unclear how their activation is translated into a neural code.[4] It is probably because of all these reasons that, in the past, no models have been proposed for the umami taste. Moreover, there are a few small compounds able to elicit the umami taste, while it is known that many oligopeptides are umami:[49] it would be very difficult to derive ligand-based information through molecular modelling because it would be necessary to solve the complicated task of finding (elusive) peptide conformations before to proceed to any further analysis.

There are few structure activity relationship studies about 5′-nucleotides, the more recent one being that of Manitto *et al.*[50] After 2001 no homology models of the heterodimer T1R1/T1R3 have been published, although many have probably been calculated (i.e. our unpublished work), until the very recent one by Walters *et al.*[51]

For sure, the work to unravel the molecular mechanism of the umami taste has been very active, as judged from the great number of patents, due to the relevance of umami compounds in the overall taste of foods and in the possibility to drastically reduce the NaCl content of food, while still having a good appreciation of it, when MSG or other umami tasting compounds are added. The breakthrough came in 2008 when Li *et al.* published the paper 'Molecular mechanism for the umami taste synergism'[52] in which a cooperative ligand binding model involving the VFTD of T1R1 (the subunit present only in the umami receptor) was proven using *in vitro* tests with chimeric receptors, together with molecular modelling. Homology modelling and preliminary docking studies allowed the identification of residues likely to be involved in the binding site of MSG and IMP. Mutation of these residues, followed by more molecular modelling, made it possible to propose a mechanism for a positive allosteric modulator (Figure 6) never reported before for GPCRs, lately confirmed for the sweet taste receptor.[45]

Summary and Outlook

Understanding of the molecular mechanism at the basis of sweet and umami taste perception has seen extraordinary developments in the last decade, thanks to the synergistic action of molecular biology and molecular modelling.

These collective efforts will further improve in the near future leading to more refined definition of ligand binding sites. The information derived and their use will provide specific indications for the design of tastants, taste modifiers able to modulate, turn on or off, or tune sweet and umami taste sensations. We are aware that models are simplified representations of complex systems, and as such present advantages but, regardless of their degree of sophistication, also intrinsic limitations. Notwithstanding, they will be used over and over again and will provide important hints until the whole picture of taste perception will be unravelled.

> Life has two principal functions: nourishment and the propagation of the species. Those who turn their minds to these two needs of existence, who study them and suggest practices whereby they might best be satisfied, make life less gloomy and benefit humanity. They may therefore be allowed to hope that, while humanity may not appreciate their efforts, it will at least show them generous and benevolent indulgence.[1]

Acknowledgement

The authors thank the Ministry of Foreign Affairs of Italy (MAE), Direzione Generale per la Promozione e la Cooperazione Culturale, the University of Milano (FIRST 2007 and 2008, PUR 2009) and the University of Gastronomic Sciences for support of their researches quoted in this article.

References

1. P. Artusi. *Science in the Kitchen and the Art of Eating Well*. University of Toronto Press: Toronto, **2003**.
2. B. Lindemann. *Physiol. Rev.* **1996**, *76*, 718.
3. K. Ikeda. *Inventor and Assignee*. Japanese Patent **1908**, 14805.
4. N. Chaudari, S. D. Roper. *J. Cell Biol.* **2010**, *190*, 285.
5. D. A. Yarmolinsky, C. S. Zuker, N. J. P. Ryba. *Cell* **2009**, *139*, 234.
6. J. Chandrashekar, M. A. Hoon, N. J. P. Ryba, C. S. Zuker. *Nature* **2006**, *444*, 288.
7. F. Laugerette, P. Passilly-Degrace, B. Patris, I. Niot, M. Febbraio, J. P. Montmayeur, P. Besnard. *J. Clin. Invest.* **2005**, *115*, 3177.
8. A. Sclafani, K. Ackroff, N. A. Abumrad. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2007**, *293*, R1823.
9. P. Wellendorph, L. D. Johansen, H. Bräuner-Osborne. *Mol. Pharmacol.* **2009**, *76*, 453.
10. R. D. Mattes. *Annu. Rev. Nutr.* **2009**, *29*, 305.
11. L. W. Guttad, L. Shan, C. Broomell, P. A. Ramsland, Z. Fan, J. M. Anchin, D. S. Linthicum, A. B. Edmuson. *J. Mol. Biol.* **2000**, *302*, 853.
12. R. S. Shallenberger, T.E. Acree. *Nature* **1967**, *216*, 480.
13. L. B. Kier. *J. Pharm. Sci.* **1972**, *61*, 1394.
14. P. A. Temussi, F. Lelj, T. Tancredi. *J. Med. Chem.* **1978**, *21*, 1154.
15. M. A. Castiglione Morelli, F. Lelj, F. Naider, M. Tallon, T. Tancredi, P. A. Temussi. *J. Med. Chem.* **1990**, *33*, 514.
16. J. Kamphuis, F. Lelj, T. Tancredi, C. Toniolo, P. A. Temussi. *Quant. Struc. -Act. Relat.* **1992**, *11*, 486.
17. T. Yamazaki, E. Benedetti, D. Kent, M. Goodman. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1437.
18. G. W. Muller, D. L. Madigan, J. C. Culberson, D. E. Walters, J. S. Carter, C. A. Klade, G. E. DuBois, M. S. Kellogg. In *Sweeteners Discovery, Molecular Design and Chemoreception*, D. E. Walters, F. T. Orthoefer, G. E. DuBois (eds.). American Chemical Society: Washington DC, **1991**, 113.
19. G. E. DuBois, D. E. Walters, M. S. Kellogg. In *Sweet Taste Chemoreception*, M. Mathlouthi, J. A. Kanters, G. G. Birch (eds.). Elsevier: London, **1993**, 237.
20. D. E. Walters, I. Prakash, N. Desai. *J. Med. Chem.* **2000**, *43*, 1242.
21. J. M. Tinti, C. Nofre. In *Sweeteners Discovery, Molecular Design and Chemoreception*, D. E. Walters, F. T. Orthoefer, G. E. DuBois (eds.). American Chemical Society: Washington DC, **1991**, 88.
22. J. M. Tinti, C. Nofre. In *Sweeteners Discovery, Molecular DesignaAnd Chemoreception*, D. E. Walters, F. T. Orthoefer, G. E. DuBois (eds.). American Chemical Society: Washington DC, **1991**, 206.
23. C. Nofre, J. M. Tinti. In *Sweet Taste Chemoreception*, M. Mathlouthi, J. A. Kanters, G. G. Birch (eds.) Elsevier: London, **1993**, 205.
24. C. Nofre, J. M. Tinti. *Food Chem.* **2000**, *69*, 245.
25. C. Nofre, J. M. Tinti, D. Glaser. *Chem. Senses* **1996**, *21*, 747.
26. D. Glaser, M. Wanner, J. M. Tinti, C. Nofre. *Food Chem.* **2000**, *68*, 375.
27. L. W. Guttad, L. Shan, J. M. Anchin, D. S. Linthicum, A. B. Edmuson. *J. Mol. Biol.* **1994**, *236*, 247.
28. T. Suami, L. Hough. *J. Carbohydr. Chem.* **1994**, *13*, 1079.

29. A. Bassoli, L. Merlini, G. Morini, A. Vedani. *J. Chem. Soc. Perkin Trans. 2* **1998**, 1449.
30. A. Bassoli, M. G. B. Drew, L. Merlini, G. Morini. *J. Med. Chem.* **2002**, *45*, 4402.
31. N. Kunishima, Y. Shimada, Y. Tsuji, T. Sato, M. Yamamoto, T. Kumasaka, S. Nakanishi, H. Jingami, K. Morikawa. *Nature* **2000**, *407*, 971.
32. M. Max, Y. G. Shanker, L. Huang, M. Rong, Z. Liu, F. Campagne, H. Weinstein, S. Damak, R. F. Margolskee. *Nat. Genet.* **2001**, *28*, 58.
33. D. E. Walters. *Pure Appl. Chem.* **2002**, *74*, 1117.
34. X. Li, L. Staszewski, H. Xu, K. Durick, M. Zoller, E. Adler. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 46924.
35. P. A. Temussi. *FEBS Lett.* **2002**, *526*, 1.
36. G. Morini, A. Bassoli, P. A. Temussi. *J. Med. Chem.* **2005**, *48*, 5520.
37. A. De Capua, M. Goodman, Y. Amino, M. Saviano, E. Benedetti. *ChemBioChem* **2006**, *7*, 377.
38. H. Xu, L. Staszewski, H. Tang, E. Adler, M. Zoller, X. Li. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 14258.
39. P. Jiang, M. Cui, B. Zhao, L. A. Snyder, L. M. Benard, R. Osman, M. Max, R. F. Margolskee. *J. Biol. Chem.* **2005**, *280*, 15238.
40. M. Winning, B. Bufe, W. Meyerhof. *BMC Neurosci.* **2005**, *6*, 22.
41. P. Jiang, M. Cui, B. Zhao, L. A. Snyder, L. M. Benard, R. Osman, M. Max, R. F. Margolskee. *J. Biol. Chem.* **2005**, *280*, 34296.
42. M. Winning, B. Bufe, N. A. Kratochwil, J. P. Slack, W. Meyerhof. *BMC Struct. Biol.* **2007**, *7*, 66.
43. F. M. Assadi Porter, E. L. Maillet, J. T. Radeck, J. Quijada, J. L. Markley, M. Max. *J. Mol. Biol.* **2010**, *398*, 584.
44. G. Servant, C. Tachdjian, X. Tang, S. Werner, F. Zhang, X. Li, P. Kamdar, G. Petrovic, T. Ditschun, A. Java, N. Brune, G. E. DuBois, M. Zoller. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 4746.
45. F. Zhang, B. Klebansky, R. M. Fine, H. Liu, H. Xu, G. Servant, M. Zoller, C. Tachdjian, X. Li. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 4752.
46. C. McCabe, E. T. Rolls. *Eur. J. Neurosci.* **2007**, *25*, 1855.
47. G. K. Beauchamp. *Am. J. Clin. Nutr.* **2009**, *90*, 723S.
48. N. Chaudhari, E. Pereira, S. D. Roper. *Am. J. Clin. Nutr.* **2009**, *90*, 738S.
49. G. E. DuBois, J. De Simone, V. Lyall. In *The Senses: A Comprehensive Reference*, Volume *IV*. *Olfaction and Taste*, D. Smith, S. Firestein (eds.). Elsevier: London, **2008**, 27.
50. P. Cairoli, S. Pieraccini, M. Sironi, C. F. Morelli, G. Speranza, P. Manitto. *J. Agric. Food Chem.* **2008**, *56*, 1043.
51. J. J. López Cascales, S. D. Oliveira Costa, B. L. de Groot, D. E. Walters. *Biophys. Chem.* **2010**, *152*, 139.
52. F. Zhang, B. Klebansky, R. M. Fine, H. A. Pronin, H. Liu, C. Tachdjian, X. Li. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 20930.