Ultimate Molecular Theory of Sweet Taste

Huazhong He, Ph. D

Molecular Recognition Organization, Elwood Rd., Delmar, NY 12054

huazhong.he@gmail.com

ABSTRACT

More than thirty years ago, I proposed a theory about sweet and bitter molecules' recognition by protein helical structures. Unfortunately the papers could not go to public platform until now. The sweet and bitter taste theory is updated and presented in separated papers^{1,2}. The sweet taste theory conveys that sweet molecules are recognized by receptor protein helical structures and the recognition process is a dynamic action, in which the sweet receptor protein helix has a torsion-spring-like oscillation between helical structures of 3.6 and 3 amino acids per turn. To help this kind of oscillation, there are two kinds of hydrogen donor and hydrogen acceptor DH-B entities for both receptor and sweet molecules: H-bond or non-H-bond. The distances between DH and B could be up to ~ 8.5 Å. The receptor H-bond type DH-B entities are the NH-O pairs forming H-bonds in protein helices; the receptor non-H-bond type DH-B entities are the ones from two pairs of NH-Os forming H-bonds which are about one turn away. To facilitate this kind of movement, the interaction of DH-Bs of a sweet molecule with those of sweet receptor, through a pair of complementary hydrogen bonds, must have hydrogen bond complementarities, which means H-bond type of ligands' DH-Bs reacts on non-H-bond type of receptor's O-NHs, and *vice versa*. As the oscillation may have different extent, it translates to sweet intensity. As recognition sites are only associated with a small fraction – helix structure of whole sweet receptor, multiple binding sites or multiple receptors are well expected.

KEY WORDS: Sweet Receptor Protein Helix, Helix Tightening-Comeback Torsion-Spring-Like Oscillation, DH-B, Hydrogen Bond, Multiple Binding Sites, Potency Versus Intensity

INTRODUCTION

A wide variety of structurally unrelated molecules provide one of the so-called five primary taste qualities (sweet, bitter, sour, salty and umami)^{3,4}: sweet taste. For example, simple inorganic ions such as beryllium salt⁵, lead salt⁶, medium size molecules as sucrose, stevioside, aspartame and up to macromolecules as monellin, thaumatin all can provide sweet tastes to human kinds. To explain this phenomenon, Cohn⁷ proposed "glucogene", like polyhydroxy, amino acids, oximes which usually in pairs, as the saporous units. Overtly-Myers⁸ elaborated the idea and proposed "glucophores" and "auxogluc" concept. Shallenberger and Acree⁹ simplified the concept and proposed a widely cited AH-B moiety, which A and B are electronegative atoms separated by a distance between 2.5 and 4 Å. Kier¹⁰ further pointed out a need for a third point. This AH-B moiety puzzled researchers by the distance requirement.^{11,12} Multiple point contacts are actually well realized.^{13,14} Now it is known that sweet tastants are recognized by G-protein coupled receptors T1R2/T1R3¹⁵⁻²¹.

Upon study of sweet taste structure activity relationship (SAR), mounting evidences have pointed that sweet molecules are recognized through the sweet receptor protein helix torsion-spring-like oscillation between 3.6 and 3 amino acids per turn. This means sweet molecules tighten up the helix a little bit and then allow the tightened helix coming back to its original status. Through this kind of dynamic process of tightening and coming back, sweet molecules initiate sweet taste elicitation procedure. Current paper and the paper titled deciphering aspartyl peptide sweeteners¹ complete the description of this sweet taste theory. And the paper about bitter taste theory² is also worthy to be referred to. These three papers^{1,2} dealing with sweet/bitter taste theory are independent with each other. It is however that the combined information makes both of the sweet and bitter theories' foundation strong and concrete. Following, the sweet taste theory will be described in the RESULTS section; then in DISCUSSION section, sweet taste-structure relationships and other aspects will be discussed in details.

METHODS

Where does the theory come from?

More than thirty years ago, upon the study of sweet/bitter-structure relationship with different structural characteristics, the theories were formed by the combined information. Through decades' efforts, the total three papers submitted at the same time basically described the reversal procedure for me to form the theory. As peers effectively blocked the publication for more than thirty years through peer-review processes, this allows the theories successfully evolved to current versions in an integrated form from the early version titled as Molecular Theory of Sweet and Bitter Tastes (unpublished).

Models used: The molecular models²²⁻²⁴ used here are to express what the theory likes to present. In a simplified way to understand the models, you can simply treat the models as hand-drawing pictures to describe the theory. As the molecular recognition process is dynamic, the models should only be considered as a moment in the dynamic process.

RESULTS

This theory conveys that sweet molecules are recognized by receptor protein helical structure. Recognition of sweet molecules is a dynamic process, in which the receptor protein helices have a torsion-spring-like oscillating movement between helical structures of 3.6 and 3 amino acids per turn. In this section, receptor helices' behavior, the side chains' movement and other details will be discussed.

First thing first, receptor protein helix model is built up using amino acid L-leucine for molecular modeling purpose. The reason to use leucine to build a helical polypeptide as a model receptor protein is that the side chain size is in the middle of actual natural occurring amino acids, the side chain is relatively simple and avoids possible complication caused by selecting other much simpler or more complicated side chains. This model can satisfy basically all of the information needed to explain the recognition process for sweet molecules. This receptor protein helix model will be used through out this paper.

Sweet receptor protein helices' motion in the recognition process

In Figure 1, molecular model is built to demonstrate sweet receptor protein helix torsion-spring-like oscillation. The left two diagrams are the molecular stick views and the right two diagrams are the molecular surface views of the helices of 3.6 and 3 amino acids per turn. Top diagrams (Phase I) show the original status of the sweet receptor protein helix; and lower diagrams (Phase II) show the final status of the sweet receptor protein helix. Top and lower diagrams show the statuses of helices of 3.6 and 3 amino acids per turn respectively. The sweet molecule recognition process is a dynamic oscillation process between these two phases. This oscillation process initiates the sweet taste elicitation procedure.

Sweet Receptor Grooves

The surface view can easily provide the grooves' views. The groove names are labeled in Phase II surface view of Figure 1. The corresponding spaces in Phase I are not easy to be pointed out directly in the diagram and can be established through the movement comparing the names labeled in Phase II. The spaces along the helix axis are called main grooves; the spaces between side chains in one turn away are named side grooves which are almost perpendicular to the main grooves, which are similar to the situation appeared in bitter taste receptor. These two grooves have different properties in holding sweet molecules due to the space size and motion during molecular recognition process. It is therefore the naming for the two grooves is also for the convenience of sweetness-structure relationship discussion.

Phase I (Original Status) (3.6 residues of amino acids per turn)

torsion-spring-like oscillation moving forward to Phase II $\rightleftharpoons$ torsion-spring-like oscillation moving back to Phase I

Figure 1: Sweet receptor protein helix model torsion-spring-like oscillation during the process of sweet molecules' recognition. (This sweet receptor helical model is built using leucine)

Sweet Receptor Hydrogen Donor And Hydrogen Acceptor DH-B (NH-O)

In the progress of searching for sweet taste theory, Shallenberger and Acree's AH-B theory about sweet taste was well cited. In the submission process of my manuscripts in the time period of more than three decades, I also attempted to recycle this term and give it a new meaning, only adverse end consequences came out however unfortunately. As their definition for AH-B is very different from the counterpart of my sweet taste theory, hydrogen donor DH and hydrogen acceptor B (DH-B) were adopted in my new versions of sweet/bitter taste theories.^{1,2}

In the stick view of Figure 1, the peptide bond NH-Os can be spotted. These NH-Os are potential receptor hydrogen donor and hydrogen acceptor DH-Bs. From now on, receptor hydrogen donor and hydrogen acceptor NH-Os will be used to refer to receptor hydrogen donor and hydrogen acceptor DH-Bs. Figure II

illustrates sweet receptor NH-Os' motions in the process of sweet molecules' recognition. The model is demonstrated in stick views to clearly identify NH-Os and view their changes between phases. The NH-Os in interest are highlighted in bigger diameter and labeled. The green springs represent H-bonds. Left side diagram shows H-bond type of NH-O's motion; and right side diagram shows non-H-bond type of NH-O's motion of sweet receptor protein torsion-spring-like oscillation during the sweet molecule recognition process. Top two diagrams in Figure II represent the structures in phase I which are the original position, which structure is what we know from textbook about protein α helix structure. Lower two diagrams represent structure in phase II, which the helix has three amino acid residues in each turn. During the oscillation process, the NH and O have a zigzag-type movement. From left side two diagrams (diagrams A1 and B1), which has a H-bond type receptore NH-O, it can be seen that the H-bond between NH and O is getting weaker or broken while moving from phase I to phase II during the oscillation process. From right side two diagrams (diagrams A2 and B2), which has a non-H-bond type receptor NH-O, it can be seen that the two H-bonds providing a non-H-bond type NH and O are getting weaker or broken while moving from phase I (diagram A2) to phase II (diagram B2) during the oscillation process.

With the receptor NH-Os' identification, the distances between NH and O can be found which could be up to about 8.5 Å, which is the distance between NH and O of non-H-bond type receptor NH-O. As demonstrated in Figure II, there are two kinds of NH-O moieties and accordingly, there should be two kinds of sweet molecules' counterparts, and the closest ones are DH-B moieties as well. The distances between DH and B could be up to around 8.5 Å too, which are corresponding to receptor's NH-Os. As the distance between A and B of Shallenberger and Acree's AH-B is about ~2.5 – 4 Å, the DH-B in this theory is very different from the one Shallenberger and Acree proposed. The DH-Bs in this theory have two types, which does not mention in Shallenberger and Acree's AH-B theory. Another difference is that there are many possible DH-Bs in one sweet receptor, which does not imply their existence in Shallenberger and Acree's AH-B theory. Next the binding pattern of ligand DH-B with receptor NH-Os will be discussed.

Interaction pattern of sweetener DH-B with receptor NH-O moieties

In Figure III and Figure IV, the interaction manners of sweetening agent hydrogen donor - hydrogen acceptor DH-Bs and receptor NH-O moieties are described in a detailed way to reveal the actions in the course of facilitating the receptor torsion-spring-like oscillation. Figure III and Figure IV demonstrate the dynamic process of the interaction of sweet molecule non-H-bonding DH-B with receptor H-bonding NH-O and sweet molecule H-bonding DH-B with receptor non-H-bonding NH-O respectively.

Figure II: Oscillating phases of two types (intramolecular and non-intramolecular hydrogen-bond types) of receptor protein NH-Os while having torsion-spring-like oscillation. Receptor's NH-O is highlighted by showing in larger diameter bonds and labeled for clarity. Other unrelated intramolecular hydrogen bonds are omitted.

Stage I of Figure III shows the non-H-bond type of DH-B of sweeteners approaching sweet receptor H-bond type NH-O. Stage II of Figure III shows that the non-H-bond DH-B is starting interact with H-bond NH-O, and NH-O H-bond starting get weakening. When reaching to Stage III of Figure III, H-bond type of receptor NH-O is weaken or completely broken with the help of sweeteners' DH-B and the whole sweet molecules. Similar situation is true for H-bond type of sweeteners' DH-B as shown in Figure IV. There is an especial need to be mentioned about is the Stage III of Figure IV. At this stage, the sweeteners' H-Bonding could disappear. This tells an important information that as the sweeteners have a potential to have intramolecular H-bond or equivalent, the intramolecular bond will re-form and release the receptor protein's NH-O and keep the dynamic oscillating process going. Sure enough, the weakened/broken original H-bonds of receptor in both stage IIIs have a tendency to re-form and

also are driving forces for the torsion-spring-like oscillation. As demonstrated hitherto, the need of complementarities of complementary interaction of sweeteners' DH-B with receptor NH-O is an important part for facilitating receptor protein helix's torsion-spring-like oscillation. Next, structure-sweetness relationship will be discussed in detail.

DISCUSSION

A selective achiral and chiral series of compounds with H-Bond and non-H-bond type of DH-Bs will be discussed in a detailed manner. Although these molecules appear to be simple in their chemical structures, they however contain very rich information about recognition process. In this way, the receptor oscillating mode, receptor NH-O moving pattern, ligand DH-B relative position with receptor NH-O, structure-sweetness relationships and others will be discussed using above demonstrated theory. The discussion will be divided to two major groups: one is about achiral molecules; another is about chiral ones.

(I) Achiral Molecules

Representative achiral non-H-bond type DH-B compounds *meta*-nitroaniline and H-bond type DH-B compounds methyl substituted sodium

salicylates are selected to demonstrate the sweet receptor recognition process. Then the distance between DH and B is discussed using achiral sweet molecule (*E*)-3-(2,4,6-tribromo-3-carbamoylphenyl)acrylic acid.

Position isomer-sweetness relationship of achiral methyl substituted *meta*-nitroanilines (non-H-bond DH-B type sweeteners)

Achiral molecule *meta*-nitroaniline was found to be sweet by Muspratt and Hofmann in 1846.²⁵ Blanksma *et al.*²⁶⁻²⁸ about one century later published taste-structure relationships of *meta*-nitroaniline derivatives and found out that 2-substituted 5-nitroanilines are sweet and their position isomers 4-substituted 3-nitroanilines are not sweet. To decipher this series' taste-structure relationship utilizing this receptor protein helix recognition theory, the methyl substituted analogues are used (Figures V and VI). As methyl can not serve as either hydrogen provider DH or hydrogen acceptor B, there is only one possible set of DH-B moiety selection, in which NH₂ serves as hydrogen donor DH, and NO₂ as hydrogen acceptor B. This unequivocal selection is labeled and shown in Figure V. As 4-methyl-3-nitroaniline is not sweet, this molecule is not labeled with DH-B.

Figure V: Structure-sweetness relationship of methyl substituted-*meta*-nitroanilines. (* Sweet potency comparing to sucrose)

With this important choice, we can move on to Figure VI, which shows molecular docking models of the interactions of 2-methyl 5-nitroaniline with receptor protein helix. As the sweet molecule recognition process is a dynamic torsion-spring-like oscillation action. Presenting both original and final statuses of the recognition process is a necessary way to express the real process. Figure VI shows stick, surface and combination of both views of 2-methyl-5-nitroaniline in the process of facilitating receptor protein helices' torsion-spring-like oscillation action.

In Figure VI, in the stick view of the original status, the H-bond type of receptor NH-O in phase I is highlighted by using bigger size bond; the NH-O hydrogen-bond is highlighted by showing the bond using green spring drawing and other H-bonds are omitted. 2-Methyl-5-nitroaniline is loaded into the receptor (left diagrams). After oscillating process moving the receptor protein helix to phase II, the receptor NH-O has a zigzag type movement which can be seen from the stick view. The original H-bond gets weakening or broken. 2-Methyl-5-nitroaniline molecule sits well in phase II. Looking into the stick, and both stick & surface views of phase II, the ligand DH-B almost aligned well with the receptor

NH-O in a complementary way. It suggests that if geometry allows, NH-O/DH-B will align together to maximize the impact to the NH-O to facilitate the required helix oscillation. From surface view, it can be seen receptor's main groove can accommodate the methyl group of 2-Methyl-5-nitroaniline well. When the 2-position is substituted by propoxy group, the sweet potency can reach to 5000 time²⁷ as sweet as sucrose. As sweetener molecules land on the shallow grooves of sweet receptor, the hydrophobicity of sweetener molecule is an important trait to increase sweetness potency as a whole.

Phase I: Receptor Original Status with Ligand

Phase II: Receptor Final Status with Ligand

Figure VI: Torsion-spring-like oscillation of sweet molecule 2-methyl-5-nitroaniline (non-H-Bond Type DH-B) with sweet receptor protein helix model in stick and surface views..

Now let's take a look at the *para* position of amino group, there is not much space left on top side of the main groove, in which the receptor protein side chain needs to claim its own space there. This explains that when methyl group attached to *para*-position of NH₂ group, this position isomer turns to be non-sweet. Figure VI distinctively deciphers the structure-sweetness relationship of achiral molecules methyl substituted *meta*-nitroanilines by this helical structure recognition theory about sweet molecules.

Position isomer-sweetness relationship of achiral methyl substituted sodium salicylates (H-bond DH-B type sweeteners)

Achiral molecule sodium salicylate has a sweet taste.²⁷ Its methyl substituted derivatives^{3,29} show opposite position isomer-taste relationship as methyl substituted *meta*-nitroanilines have, which is shown in Figure VII. The tastes of methyl substituted *meta*-nitroanilines change from sweet to not sweet while methyl group moves from NH₂ (DH) to NO₂ (B) side (Figure V); the tastes of methyl substituted sodium salicylates, on the contrary, change from sweet to not sweet while methyl group moves from CO₂⁻ (B) to OH (DH) side. In Figure VII, among these compounds, methyl group on 5th and 6th positions afford sweet taste, which substituents are close to the B (COO⁻) of ligand DH-B moiety; compounds with methyl group on 3rd and 4th positions do not have sweet taste, which substituents are close to the DH (OH) of ligand DH-B moiety. The sweet ligand DH-Bs are labeled next to the structures. These pairs of DH-B belong to H-bond type. It therefore shows an opposite position-taste relationship as that of non-H-bond type compounds, such as methyl substituted *meta*-nitroanilines.

Figure VII: Position isomer-sweetness relationship of methyl substituted sodium salicylates^{3,29}.

Now let's load sweet molecule 6-Methylsalicylate into receptor model to see the details. 6-Methylsalicylate's interaction model with receptor is built in Figure VIII. Receptor non-H-bond type NH-O is highlighted using bigger diameter bond as shown in stick view; the receptor original corresponding H-bonds are highlighted using green springs. Sweetener's DH-B is only shown in Figure VII, not highlighted in the molecular model, for clarity reason. The molecule recognition process's dynamic pattern for 6-methylsalicylate is shown in Figure VIII: the left side is the original status (phase I); the right side showed the final stage of the recognition process (phase II). Upon the sweet molecule 6-methylsalicylate loading on the receptor, receptor intramolecular H-bonds are

starting weakening/breaking while the ligand is facilitating the receptor protein helix's torsion-spring-like oscillation between 3.6 to 3 amino acid residues per turn. From the phase II stick view of Figure VIII, receptor H-bonds are no more shown, which means that the original H-bonds are weaken/broken.

To accommodate the position isomer-sweetness relationship results^{3,29} of methyl substituted sodium salicylates, the phase II molecular docking model of 6-methylsalicylate on the receptor is illustrated in the right column of Figure VIII. Methyl group at 6th position has the main groove to house it. The 5th position can also allow a methyl group there and still provides a faintly sweet taste. Methyl at 3rd or 4th position however is too bulky for sweet receptor due to the two receptor side chains at lower side, which can be easily seen from surface view of phase II in Figure VIII. This model provides an explanation hereto for the position isomer-sweetness relationship.

From the phase II surface view of Figure VIII, methyl group in two positions (6th and 5th) is towards empty space of right side of main groove. The main groove has enough space to host methyl group in these two positions. It is therefore that methyl group in these two positions (6th and 5th) provide sweet molecules.

Model in Phase II of Figure VIII represents a critical intermediate moment because this model is built according to the position isomer-sweetness results^{3,29}. This moment reveals the vital behaviors of molecule's binding, H-bond breakage, re-formation of H-bond, and releasing from binding with receptor's NH-O during the process of receptor protein oscillation.

From the stick views of phase II in Figure VIII, the ligand H-bond type DH-B (OH and COO⁻) does not align up well with receptor non-H-bond type NH-O comparing it with that in the case of 2-Methyl-5-nitroaniline (Figure VI). The two molecules' volume however appears to be comparable. This suggests that the interaction process of methyl salicylate and receptor protein helix is more complicated than that in the case of 2-methyl-5-nitroaniline. During the torsion-spring-like oscillating process, H-bond type DH-B (OH and COO⁻) of sodium 6-methyl salicylate has two extra steps than that in non-H-bond type DH-B of sweeteners, like 2-methyl-5-nitroaniline: i.e. weakening/brokerage, and re-formation of DH-B (OH and COO⁻) intramolecular H-bond. Alignment of DH-B with NH-O should provide better binding. The non-alignment of the H-bond type DH-B (sodium 6-meththyl salicylate's OH and COO⁻) with receptor NH-O powerfully suggests that the importance of H-bond type DH-B is to re-form intramolecular H-bond in final stage of phase II; and this action makes DH-B losses binding with receptor NH-O; third, this kind of release makes the oscillation going back and continue to original status phase I. The model shown here is the result of re-formation of intramolecular H-bond of sodium 6-meththyl salicylate's OH & COO⁻, release of DH-B from receptor NH-O and facilitating the torsion-spring-like oscillation of receptor protein helix. This diagram gives a strong supporting picture that torsion-spring-like oscillation is the key in the elicitation of sweet taste.

Figure VIII: Torsion-spring-like oscillation of sweet molecule 6-methysalicylic Acid (H-Bond Type DH-B) with sweet receptor protein helix model.

Probing the ligands' DH-B distance from sweet molecule achiral (*E*)-3-(2,4,6-tribromo-3-carbamoylphenyl)acrylic acid

Now let's move on to address the issue about the possible distance about ligands' DH-Bs. According to this receptor protein helix recognition theory, the distance of sweeteners between DH and B could be up to around 8.5 Å in responding to the distances from the information of receptor protein helix. It

definitely can serve as supporting information for finding out some long distance's DH-B sweetener.

Figure IX: Structure of sweet molecule (*E*)-3-(2,4,6-tribromo-3-carbamoylphenyl)acrylic acid (sweet, 4000X)¹¹ (Non-H-Bond Type DH-B)

Figure X: Torsion-spring-like oscillation of sweet molecule (*E*)-3-(2,4,6-tribromo-3-carbamoylphenyl)acrylic acid (non-H-bond type DH-B) with sweet receptor protein helix model.

Gries *et. al.* in 1983 published trihalogenated benzamide sweeteners.^{11,12} The sweet taste is mild, pleasant, and more or less delayed and lingering. The greatness for our purpose to support this helical structure theory is that the distances between DH and B of this series of sweeteners. Figure IX shows the structure of one of the sweetest molecule in this series. This molecule has limited flexibility. The distance between the DH and B is around 7 Å,¹² which is far away from the distance (up to 4 Å) of A and B in the Shallenberg and Acree's theory. The sweetness potency comparing with the sucrose threshold is about 4000 times sweeter than sucrose.

To demonstrate the interaction between this trihalogenated benzamide sweetener and receptor, the binding detail is built in Figure X. The ligand's DH-B (CONH₂/CO₂H) is aligned along the main groove, and the receptor' NH-O is just well locating between the ligand's DH-B. The whole molecule also fits well in the receptor main groove. Now let's move to chiral molecules to discuss these characteristics.

(II) Chiral Molecules

In general, D-amino acids are sweet and L-amino acids are not sweet.³⁰ It is however that this is not the whole story about the configuration-sweetness relationship of amino acids. Some small side chain amino acids have comparable sweetness potency regardless of its configuration as shown in Table I. And L-proline is sweet and D-proline is not sweet.³⁰ The capability to explain this subtle phenomenon is a reality check for the theory's mightiness. This phenomenon will be explained using this theory in the following sections.

Table I. Sweetness Threshold of D- and L- amino acids³¹

Compounds	T*	Compounds	T*
D-Alanine	14.7	D- α -Amino butyric acid	13.3
L-Alanine	13.8	L- α -Amino butyric acid	14.5

T*: Threshold [mmole/L]

Explanation for D-amino acids sweetness (non-H-bond type DH-B sweeteners)

As sweet taste for D-amino acids is the major feature about their sweet taste phenomenon, D- phenylalanine is used to represent D-amino acids for the docking. Figure XI shows the docking result. From the molecular stick view of phase II, it can be seen that the receptor's H-bond type NH-O is aligned well with the D- phenylalanine's DH-B, as it does in the 2-methyl 5-nitroaniline case. The phenyl ring extends towards the main groove and there is a plenty of spaces for bigger groups. This explained why D-amino acids are sweet.

Why do some small side chain L-amino acids (Table I) have also sweet taste then? Let move to Figure XII, which uses L-alanine as ligand, to see what happens when small side chain L-amino acids is docked into receptor helix.

Figure XI: Torsion-spring-like oscillation of sweet molecule D-phenylalanine (non-H-Bond Type DH-B) with sweet receptor protein helix model.

Explanation for L-alanine sweetness (non-H-bond type DH-B sweetener)

Figure XII: Torsion-spring-like oscillation of sweet molecule L-alanine (non-H-Bond Type DH-B) with sweet receptor protein helix model.

The interaction modeling of L-alanine with receptor helix is built in Figure XII. From the stick view and surface view in the phase II, it can be seen that the methyl group is well tolerated. As L-alanine is small comparing to the groove width, it allows certain size's group in the methyl position of L-alanine. The "unexpected" sweet L-amino acids are due to the space available for the side chain of amino acids. The side chains of L-amino acids only have the space in the partial groove's width size to manipulate to still have sweet taste. The D-amino acids, on the contrary, have a space along the main groove, which has much larger space to hold the side chains of D-amino acids. Next another "unexpected" case proline issue will be explained in the following.

Explanation for L- proline sweetness (H-bond type DH-B sweetener)

For proline case, L-proline is sweet and D-proline is not sweet, which is different from other amino acids' configuration-sweet taste relationship. From the theory proposed by this author, L-proline belongs to H-bond type DH-B sweetener. With the guidance of this theory, this phenomenon prompts this author to locate H-bond data about amino acids. Sure enough, Jeffrey and Maluszynska³² surveyed amino acids' hydrogen bonds and found out that proline's amino and carboxylic groups have the shortest N-H...O=C hydrogen bond. Bingo! It is therefore proline belongs to H-bond type DH-B and L-proline is sweet and D-proline is not sweet, which is different from other amino acids' configuration-sweet taste relationship.

So far, through achiral and chiral sweet molecules model building, DH-B distance, two types of DH-B moieties and their steric fitting into the receptor have been thoroughly discussed. These discussions are related to sweetness potency. Now let's move to other two aspects of sweet taste: sweetness intensity and sweet receptor.

Sweetness Intensity

In RESULTS section, the sweet theory has stated that the recognition process is an oscillation action of the sweet receptor protein helix. The oscillation process happens between the helices of 3.6 and 3 amino acids per turn. This oscillation is the key for the sweetness elicitation. A question could be raised out of this statement: does the interaction of sweet molecule with sweet receptor protein helix have the perfect oscillation as shown in Figure 1 in all the occasion? The answer, of cause, is negative. This would immediately pop up another question: what does that mean then? This introduces a term called intensity. Different extent of the sweet receptor protein helix oscillation results in different sweetness intensity.

From time to time, it could be heard why sweeteners do not appear to be as sweet as the number tells? This is also a question before this author entered into this research area. When I was very young, there had enthusiastic chats among our kids about what does a high potency sweetener mean. The point of the chats were initiated by the fancy Chinese name "Tang-Jing" which means extreme/essence/spirit of sugar for then available high potency sweetener and

“Tang-Jing” does not appear to be that sweet comparing to sugar as we expected. Anything becoming “Jing”, which contains some deified meaning, always could be raised as a topic in interest among our kids. Subsequently I went to my parents looking for a more trustworthy answer. My mother could not give any suggestion. My father however told me that “Tang-Jing” is so sweet that it becomes bitter, as he enjoyed pretty much about then available sweetener(s) saccharin and/or cyclamate, and the taste experience of that (those) was what the conclusion was based on I think. This answer was not too far away from our kids’ speculation. I carried this explanation for a long time until Professor Zeng told me that the bitterness of “Tang-Jing” is called aftertaste. What a piece of mind-blowing information. This kind of question still can be heard from time to time in my daily life. To the best of my knowledge, curiously enough, it seems not many serious professional papers confront this issue or probably this should not be a question for most of people in this research area. Anyway I strongly however believe that this kind of question has been haunting in a lot of regular people’s mind. Do we have simple words to give to those confused regular people?

On the way to report sweetener information, sweetness^{33,34}, potency^{35,36}, intensity³⁷⁻³⁹ have been used to describe the relative sweetness potency comparing to reference sweetener such as sucrose, at certain concentrations such as threshold¹¹, 2%³⁶, 5%^{34,35} etc. Relative sweetness potency of a sucrononic acid has reached to 200,000 times as sweet as sucrose.⁴⁰

Sweeteners have another property, which is paid less attention to.⁴¹⁻⁴⁶ Especially noteworthy work in this aspect was performed by DuBoise *et. al.*⁴⁶ They trained panelists to give sweetness intensity ratings on sweeteners such as sugars (sucrose, glucose, fructose, fructo-oligosaccharide sweetener), sugar alcohols (maltitol, lactitol, isomalt), terpenoid glycosides (monoammonium glycyrrhizinate, stevioside, rebaudioside-A), dipeptide derivatives (aspartame, alitame), a sulfamate (sodiumcyclamate), a protein (thaumatin), a chlorodeoxysugar (sucralose), two N-sulfonylamides (sodium saccharin, acesulfame-K), a dihydrochalcone (neohesperidin dihydrochalcone), and an amino acid (glycine). They found out that sucrose and other natural carbohydrate sweeteners have higher maximum sweetness intensities than high potency sweeteners. In other words, if we attempt to obtain sweetness potency comparing to a sucrose solution with maximum sweetness intensity, the answer would be smaller than one for basically all of the high potency sweeteners. Luckily human beings generally do not enjoy/consume sucrose at their maximum intensity, their sweetness potencies are reported comparing to threshold or reasonably consumable concentration. In this way, sweetness potencies at certain times as sweet as sucrose are widely obtained and reported.

Now the critical terminologies of sweetness potency and sweetness intensity have to be clarified and emphasized. For the above-mentioned threshold, 2% and 5% sucrose concentrations, the differences among them are those in sweetness intensities. The relative sweetness results comparing to certain concentration through dilution are sweetness potencies. It is therefore that sweetness intensity should refer to the strength, force or power of sweetness

of a sweetener at certain concentration; and sweetness potency should refer to that determined by concentration ratio comparing to certain sweetener (such as sucrose) at certain concentration (such as threshold, 2%, 5% etc.).

With the understanding and discussion of potency and intensity concept, the hidden meaning of the question in the beginning of this section is that the high potency sweetener's intensity at its maximum intensity does not appear to be that many fold as sweet as sucrose as reported. The answer is that this is a question about sweet potency comparing to sweetness intensity, which is therefore a scientifically invalid, but easily confusing question for regular people. For the psychology of regular people, when talking about sweetness, one would think about sweetness intensity instead of sweetness potency. Normally reported data to public is about sweetness potency. Sweetness intensity data is not easy and basically never reported to regular people. This is really still confusing researchers in this area. Fortunately enough, a paper written by DuBoise⁴⁷, who clearly pinpointed this issue about intensity versus potency, was published in 2016. In lieu of the difference between potency versus intensity, current so-called high intensity sweeteners should be called high potency sweeteners.⁵¹ As have discussed before, intensity is related to concentration. When talking about sweeteners' intensity in general comparison, it should refer to maximal intensity. With this understanding, to explicitly express the meaning, sucrose could be called high intensity sweetener; and saccharin, aspartame, neotame and other sweeteners should be called high potency sweeteners.

Different concentrations of a sweet compound acting on a receptor or different sweet compounds could make the oscillation to a different extent, which translates to different sweetness intensity. Sweetness potency and intensity are two characteristics for sweet compounds. Now let's deal with sweet receptors.

Sweet Receptor

This whole theory presentation is about sweet molecules recognized by helical structures of sweet receptors. As a helix is only a small part of a receptor complete structure, multiple binding sites and/or multiple receptors are well expected from the beginning of the theory. Thirty years ago, peer challenged the existence of helical structure. At that time, there was no information about receptors. I do not think people in this area would still respect the challenge today. Sweet receptor is now believed to be a heterodimer of taste receptors type 1 subtypes 2 and 3 (T1R2 and T1R3).¹⁵ Almost at the same time, the sweet receptor has also been identified in other laboratories.¹⁶⁻²¹ T1R2 and T1R3 are Class C G-coupled protein receptors (GPCRs). In generally, GPCRs have an intracellular carboxylic-terminal domain, seven trans-membrane domain (TMD) and extracellular amino-terminal domain. In the description of sweet receptor, the general name of extracellular amino-end domain can be divided into N-terminal domain (NTM), and cysteine-rich domain (CRD) which links TMD and NTM. So far there are five locations identified on the heterodimer of taste receptors T1R2 and T1R3 serve as binding sites: sucrose, glucose, sucralose bind in the NTM of both T1R2 and T1R3;⁴⁸ cyclamate^{49,50} and neohesperidin dihydrochalcone⁵¹ in the TMD of T1R3; aspartame^{52,53}, neotame⁵², saccharin, acesulfame-K, D-

tryptophan in the NTM of T1R2;⁴⁹ neoculin in the NTM of T1R3;⁵⁴ brazzein and thaumatin in the CRD of T1R3;^{55,56} monellin at both the NTM of T1R2 and the CRD of T1R3;⁵⁵ S819, an alkoxyaryl thiourea sweetener in the TMD of T1R2;⁵⁷ perillartine in the T1R2.⁵⁸ Perillartine and P-4000 only need T1R2 for *in vitro* screen sweet response.^{59,60} The difference between T1R2 alone versus heterodimer of taste receptors T1R2 and T1R3 is that the later screen⁶⁰ is more consistent with real taste experience.

As only a short helix fragment of receptor is responsible for sweet molecule recognition, then multiple location binding (on protein helices) undoubtedly is possible and definitely serves as a powerful indirect support for the receptor protein helix recognition theory. This kind of helix tightening-comeback torsion-spring-like oscillation movement is like the pronunciation of word "sweet". No matter where you say it, as long as you can let the down stream hear it, it will be understood that it means "sweet". For further studies on binding sites of all kinds of sweeteners, it is very likely to discover that sweeteners bind on one or more than one domains; one domain could have a couple of sub-domains for one sweetener or different sweeteners, which would appear to be "pretty messy", but will reveals the true story about helix recognition.

CONCLUSION

More than thirty years ago, I proposed a theory about sweet and bitter molecules' recognition by protein helical structures. Unfortunately the papers could not go to public platform until now. Both of the theories are updated. Total three papers are presented.^{1,2} The processes for the theories' formation are basically the reversal procedures for the information presented in these papers. The total three papers are independent and as a whole make the foundation for the theories very strong.

This paper started with introducing sweet receptor protein helix recognition theory. The theory reveals that sweet molecules' recognition process is a dynamic action, in which the receptor protein helix has a torsion-spring-like oscillating movement between helical structures of 3.6 and 3 amino acids per turn. To help this kind of oscillation, receptor protein helix driving force H-bonds' NH-Os are important points to be perturbed, which are called receptor's hydrogen donor and hydrogen acceptor NH-Os. The corresponding counterparts for sweet molecules are named as DH-Bs. There are two kinds of hydrogen donor - hydrogen acceptor DH-B entities for both sweet molecules and sweet receptor (NH-O is used for receptor's DH-B): hydrogen-bond and non-hydrogen-bond types, which means one kind of DH-B is that DH forms intramolecular hydrogen bond with B or equivalent and another is that DH does not form intramolecular hydrogen bond with B respectively. The distances between DH and B could be up to ~ 8.5 Å, which are in corresponding to receptor NH-Os' distances. Receptor's NHs and Os are the ones forming intramolecular H-bonds, which are the driving force responsible for the formation of receptor protein helical structures. The receptor H-bond type NH-O entities are the NH-O pairs forming H-bonds in protein helices; the receptor non-H-bond type NH-O entities

are the ones from two pairs of NH-Os forming H-bonds which are about one turn away.

The interaction of DH-B of a sweet molecule with NH-O of sweet receptor, through a pair of complementary hydrogen bonds, must have hydrogen bond complementarities. This means that an DH-B entity of a sweet molecule, which forms intramolecular hydrogen bond, interacts with NH-O of sweet receptor protein helix, which does not form intramolecular hydrogen bond, and *vice versa*. Comparing with Shallenberger and Acree's AH-B moiety, which distance is about between 2.5 and 4 Å, it is necessary to point out that this theory's DH-Bs are very different from this well-cited one in three aspects: types they could have, distance between D and B, availability number implied in a receptor. In corresponding to ligand-receptor binding manner and motion, beyond receptor's NH-Os, sweet receptor could be roughly divided into two kinds of spaces to accommodate sweet molecules. One is named as main grooves, which is the grooves following receptor protein carbon to nitrogen end helix axis (refer to helix with 3 amino acids per turn and its corresponding space in helix with 3.6 amino acids per turn); another is named as side grooves, which are the grooves between side chains and almost perpendicular to the main grooves.

To elucidate this theory with real examples, achiral and chiral molecules are utilized. Non-H-bond type DH-B achiral molecules meta-nitroanilines and H-bond type DH-B achiral molecules substituted salicylates show the opposite position-sweet taste relationships. It is well explained using this theory. Sweet molecule (E)-3-(2,4,6-tribromo-3-carbamoylphenyl)acrylic acid only has one DH-B choice separated by ~7 Å. The distance of the DH-B is well within this theory's distance of DH-B, which allows up to around 8.5 Å. This distance is far away from the limit (up to 4 Å) of A and B in the Shallenberger and Acree's theory.

Chiral sweet molecules D- and L-amino acids, are then dealt with. First, D-amino acids' sweet taste is explained. It is then explained that some small side chain D and L-amino acids have comparable sweet taste potencies, although D-form sweet, L- amino acids are not sweet in general. This capability to explain such a subtle exception, undoubtedly serves as a strong support for the theory. For another perplexed two enantiomers D- and L-proline, which L- form is sweet and D- form is not sweet, the reveal of H-bond type DH-B of proline and non-H-bond type DH-B of other amino acids undoubtedly strongly supported the existence of two kinds of DH-B moieties and eventually the authenticity of this theory.

The issue about potency versus intensity is a question raised spontaneously from the torsion-spring-like oscillation movement. From the clarification of this issue, it has been point out that there is a need to distinguish between sweetness potency versus intensity. From the sweetness discussion, it is very likely that natural sweet receptors' helix side chains and lengths are optimized during evolution and adapted to sucrose and other carbohydrates, which are the nutrients needed by living beings. In this way, sugar can have higher maximum sweetness intensity than other sweeteners, although other sweeteners can activate sweet receptor and could have high sweetness potencies. As sweetener molecules land on the shallow grooves of sweet

receptor, the hydrophobicity of sweetener molecule is an important trait to increase sweetness potency as a whole. The identification of multiple locations about sweeteners binding undoubtedly is a strong indirect support about helices' recognition about sweeteners.

With the guidance of this theory, it is unprecedented to be able to explain sweetness-structure relationships of achiral & chiral molecules, two types of DH-Bs/NH-Os & their distance information, potency vs intensity, multiple binding sites etc.

ACKNOWLEDGEMENT

I am grateful to Dr. Fengxia Deng for her decades-long encouragement and support for completing this research. I would like to thank Mr. Charlie He for his help in organizing data. I would also like to thank the late Professor Guangzhi Zeng (Kuangchi Tseng) for leading me into this fascinating area.

CONFLICT OF INTEREST

The author declares no conflicts of interest with the contents of this article.

FUNDING

There is no funding for this research.

REFERENCES

- 1 He, H. Deciphering Aspartyl Peptide Sweeteners Using the Ultimate Molecular Theory of Sweet Taste. To Be Submitted.
- 2 He, H. Ultimate Molecular Theory of Bitter Taste. To Be Submitted.
- 3 Zeng, G. & Wei, S. *Molecular recognition of Gustation* (Science Press (China) 1984).
- 4 Moncrieff, R. W. *The Chemical Senses*. (CRC Press, 1967).
- 5 Vauquelin, L.-N. "De l'Aiguemarine, ou Béril; et découverte d'une terre nouvelle dans cette pierre" [Aquamarine or beryl; and discovery of a new earth in this stone]. *Annales de Chimie* **26**, 169 (1798).
- 6 Hernberg, S. Lead Poisoning in a Historical Perspective. *American Journal of Industrial Medicine* **38**, 244-254 (2000).
- 7 Cohn, G. *Die Organischen Geschmacksstoffe*. (Fritz Siemenroth: Berlin, 1914).
- 8 Oertly, E. & Myers, R. G. A New Theory Relating Constitution to Taste. [Preliminary Paper.] Simple Relations Between The Constitution of Aliphatic Compounds and Their Sweet Taste. *Journal of the American Chemical Society* **41**, 855-867, doi:10.1021/ja02227a001 (1919).
- 9 Shallenberger, R. S. & Acree, T. E. Molecular Theory of Sweet Taste. *Nature* **216**, 480-482 (1967).
- 10 Kier, L. B. A molecular theory of sweet taste. *J Pharm Sci* **61**, 1394-1397 (1972).

- 11 Gries, H. *et al.* Trihalogenated benzamides — a new class of artificial sweeteners. *Zeitschrift für Lebensmittel-Untersuchung und Forschung* **176**, 376-378, doi:10.1007/bf01057731 (1983).
- 12 Belitz, H.-D., Rohse, H., Stempfl, W. & Gries, H. Trihalogenated benzamides: structure taste relationships. *Zeitschrift für Lebensmittel-Untersuchung und Forschung* **190**, 319-324, doi:10.1007/bf01184500 (1990).
- 13 Zeng, G. Natural Carbohydrate Sweeteners and Synthetic Analogues. *Huaxue Tongbao* **11**, 18-23 (1989).
- 14 Nofre, C. & Tinti, J.-M. Sweetness reception in man: the multipoint attachment theory. *Food chemistry* **56**, 263-274, doi:http://dx.doi.org/10.1016/0308-8146(96)00023-4 (1996).
- 15 Nelson, G. *et al.* Mammalian Sweet Taste Receptors. *Cell* **106**, 381-390, doi:10.1016/S0092-8674(01)00451-2 (2001).
- 16 Bachmanov, A. A. *et al.* Positional cloning of the mouse saccharin preference (Sac) locus. *Chem Senses* **26**, 925-933 (2001).
- 17 Kitagawa, M., Kusakabe, Y., Miura, H., Ninomiya, Y. & Hino, A. Molecular genetic identification of a candidate receptor gene for sweet taste. *Biochem Biophys Res Commun* **283**, 236-242, doi:10.1006/bbrc.2001.4760 (2001).
- 18 Li, X., Inoue, M., Reed, D. R., Huque, T., Puchalski, R. B., Tordoff, M. G., Ninomiya, Y., Beauchamp, G. K., Bachmanov, A. A. High-resolution genetic mapping of the saccharin preference locus (Sac) and the putative sweet taste receptor (T1R1) gene (Gpr70) to mouse distal Chromosome 4. *Mamm Genome* **12**, 13-16 (2001).
- 19 Max, M. *et al.* Tas1r3, encoding a new candidate taste receptor, is allelic to the sweet responsiveness locus Sac. *Nat Genet* **28**, 58-63, doi:10.1038/88270 (2001).
- 20 Montmayeur, J. P., Liberles, S. D., Matsunami, H. & Buck, L. B. A candidate taste receptor gene near a sweet taste locus. *Nat Neurosci* **4**, 492-498, doi:10.1038/87440 (2001).
- 21 Sainz, E., Korley, J. N., Battey, J. F. & Sullivan, S. L. Identification of a novel member of the T1R family of putative taste receptors. *J Neurochem* **77**, 896-903 (2001).
- 22 Pettersen, E. F., Goddard, Thomas D., Huang, Conrad C., Couch, Gregory S., Greenblatt, Daniel M., Meng, Elaine C., Ferrin, Thomas E. UCSF Chimera—A visualization system for exploratory research and analysis. *Journal of Computational Chemistry* **25**, 1605-1612, doi:10.1002/jcc.20084 (2004).
- 23 Trott, O. & Olson, A. J. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry* **31**, 455-461, doi:10.1002/jcc.21334 (2010).
- 24 Sanner, M. F. Python: a programming language for software integration and development. *J Mol Graph Model* **17**, 57-61 (1999).

- 25 Muspratt, J. S. & Hofmann, A. W. Ueber das Nitranilin, ein neues Zersetzungsproduct des Dinitrobenzols. *Justus Liebigs Annalen der Chemie* **57**, 201-224, doi:10.1002/jlac.18460570208 (1846).
- 26 Blanksma, J. J. & van der Weyden, P. W. M. Relationship between taste and structure in some derivatives of meta-nitraniline. *Recueil des Travaux Chimiques des Pays-Bas* **59**, 629-632, doi:10.1002/recl.19400590705 (1940).
- 27 Blanksma, J. J. & Hoegen, D. The sweet taste of 4-nitro-2-aminotoluene, 4-nitro-2-aminobenzoic acid and 2-nitro-4-aminobenzoic acid. *Recueil des Travaux Chimiques des Pays-Bas* **65**, 333-337, doi:10.1002/recl.19460650504 (1946).
- 28 Blanksma, J. J., van den Broek, W. J. & Hoegen, D. On the sweet taste of the 1-halogeno-2-amino-4-nitrobenzenes. *Recueil des Travaux Chimiques des Pays-Bas* **65**, 329-332, doi:10.1002/recl.19460650503 (1946).
- 29 Zeng, Guangzhi (Tseng, Kuangchih) Structural chemistry of sweet molecules and synthetic sweeteners. *Chinese Journal of Applied Chemistry* **7**, 11-26 (1990).
- 30 Kawai, M., Sekine-Hayakawa, Y., Okiyama, A. & Ninomiya, Y. Gustatory sensation of (L)- and (D)-amino acids in humans. *Amino Acids* **43**, 2349-2358, doi:10.1007/s00726-012-1315-x (2012).
- 31 Nofre, C., Sabadie, J. & Bal, D. Sweetening properties of L-alanine tert-butyl ester. *Naturwissenschaften* **61**, 404-405 (1974).
- 32 Jeffrey, G. A. & Maluszynska, H. A survey of hydrogen bond geometries in the crystal structures of amino acids. *International journal of biological macromolecules* **4**, 173-185, doi:http://dx.doi.org/10.1016/0141-8130(82)90048-4 (1982).
- 33 Birch, G. G. Sweetness and sweeteners. *Endeavour* **11**, 21-24, doi:http://dx.doi.org/10.1016/0160-9327(87)90165-7 (1987).
- 34 Ariyoshi, Y., Yasuda, N. & Yamatani, T. The structure-taste relationships of the dipeptide esters composed of L-aspartic acid and β -hydroxy amino acids. *Bulletin of the Chemical Society of Japan* **47**, 326-330 (1974).
- 35 Janusz, J. M. *et al.* High potency dipeptide sweeteners. 1. L-aspartyl-D-phenylglycine esters. *J Med Chem* **33**, 1052-1061 (1990).
- 36 Muller, G. W., Walters, D. E. & DuBois, G. E. N,N'-disubstituted guanidine high-potency sweeteners. *J Med Chem* **35**, 740-743 (1992).
- 37 Kawai, M., Chorev, M., Marin-Rose, J. & Goodman, M. Peptide sweeteners. 4. Hydroxy and methoxy substitution of the aromatic ring in L-aspartyl-L-phenylalanine methyl ester. Structure-taste relationships. *J Med Chem* **23**, 420-424 (1980).
- 38 Acton, E. M., Leafer, M. A., Oliver, S. M. & Stone, H. Structure-taste relations in oximes related to perillartine. *Journal of agricultural and food chemistry* **18**, 1061-1068 (1970).
- 39 Kinghorn, A. D. & Soejarto, D. D. Intensely sweet compounds of natural origin. *Med Res Rev* **9**, 91-115 (1989).
- 40 Nofre, C., Tinti, J. M. & Chatzopoulos-Ouar, F. Agents édulcorants dérivés des acides n-phénylguanidinoacétique et n-phényléthanamidinoacétique

- et compositions contenant de tels agents édulcorants. EP 0241395A2 (1987).
- 41 Stone, H. & Oliver, S. M. Measurement of the Relative Sweetness of Selected Sweeteners and Sweetener Mixtures. *Journal of food science* **34**, 215-222, doi:10.1111/j.1365-2621.1969.tb00922.x (1969).
 - 42 Moskowitz, H. R. Sweetness and intensity of artificial sweeteners. *Perception & psychophysics* **8**, 40-42, doi:10.3758/bf03208930 (1970).
 - 43 Fry, J. C., Yurttas, N., Biermann, K. L., Lindley, M. G. & Goulson, M. J. The sweetness concentration-response of r,r-monatin, a naturally occurring high-potency sweetener. *J Food Sci* **77**, S362-364, doi:10.1111/j.1750-3841.2012.02885.x (2012).
 - 44 Hoppe, K. & Gaßmann, B. Bestimmung der Mißgeschmacksschwellen von Saccharin, Cyclamat, Acesulfam und Aspartam. *Food / Nahrung* **29**, 417-420, doi:10.1002/food.19850290431 (1985).
 - 45 Schiffman, S. S., Lindley, M. G., Clark, T. B. & Makino, C. Molecular mechanism of sweet taste: relationship of hydrogen bonding to taste sensitivity for both young and elderly. *Neurobiol Aging* **2**, 173-185 (1981).
 - 46 DuBois, G. E. *et al.* in *Sweeteners* Vol. 450 *ACS Symposium Series* Ch. 20, 261-276 (American Chemical Society, 1991).
 - 47 DuBois, G. E. Molecular mechanism of sweetness sensation. *Physiol Behav* **164**, 453-463, doi:10.1016/j.physbeh.2016.03.015 (2016).
 - 48 Nie, Y., Vignes, S., Hobbs, J. R., Conn, G. L. & Munger, S. D. Distinct contributions of T1R2 and T1R3 taste receptor subunits to the detection of sweet stimuli. *Curr Biol* **15**, 1948-1952, doi:10.1016/j.cub.2005.09.037 (2005).
 - 49 Xu, H., Staszewski, L., Tang, H., Adler, E., Zoller, M., Li, X. Different functional roles of T1R subunits in the heteromeric taste receptors. *Proc Natl Acad Sci U S A* **101**, 14258-14263, doi:10.1073/pnas.0404384101 (2004).
 - 50 Jiang, P. *et al.* Identification of the cyclamate interaction site within the transmembrane domain of the human sweet taste receptor subunit T1R3. *J Biol Chem* **280**, 34296-34305, doi:10.1074/jbc.M505255200 (2005).
 - 51 Winnig, M., Bufe, B., Kratochwil, N. A., Slack, J. P. & Meyerhof, W. The binding site for neohesperidin dihydrochalcone at the human sweet taste receptor. *BMC Struct Biol* **7**, 66, doi:10.1186/1472-6807-7-66 (2007).
 - 52 Masuda, K. *et al.* Characterization of the modes of binding between human sweet taste receptor and low-molecular-weight sweet compounds. *PLoS One* **7**, e35380, doi:10.1371/journal.pone.0035380 (2012).
 - 53 Maillet, E. L. *et al.* Characterization of the Binding Site of Aspartame in the Human Sweet Taste Receptor. *Chem Senses* **40**, 577-586, doi:10.1093/chemse/bjv045 (2015).
 - 54 Koizumi, A. *et al.* Taste-modifying sweet protein, neoculin, is received at human T1R3 amino terminal domain. *Biochem Biophys Res Commun* **358**, 585-589, doi:10.1016/j.bbrc.2007.04.171 (2007).

- 55 Jiang, P. *et al.* The cysteine-rich region of T1R3 determines responses to intensely sweet proteins. *J Biol Chem* **279**, 45068-45075, doi:10.1074/jbc.M406779200 (2004).
- 56 Masuda, T. *et al.* Five amino acid residues in cysteine-rich domain of human T1R3 were involved in the response for sweet-tasting protein, thaumatin. *Biochimie* **95**, 1502-1505, doi:10.1016/j.biochi.2013.01.010 (2013).
- 57 Zhang, F. *et al.* Molecular mechanism for the umami taste synergism. *Proceedings of the National Academy of Sciences* **105**, 20930-20934, doi:10.1073/pnas.0810174106 (2008).
- 58 Cai, C. *et al.* Characterization of the Sweet Taste Receptor Tas1r2 from an Old World Monkey Species Rhesus Monkey and Species-Dependent Activation of the Monomeric Receptor by an Intense Sweetener Perillartine. *PLoS One* **11**, e0160079, doi:10.1371/journal.pone.0160079 (2016).
- 59 Slack, J. P. Use of a T1R2 nucleic acid sequence to identify tastants. US8124360 (2012).
- 60 Servant, G. & Servant, N. Screening assays to identify compounds which modulate T1R associated taste modalities which eliminate false positives. US9804157 (2017).