Ultimate Molecular Theory of Bitter Taste

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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. Inspired by the sweet taste theory[1,2], this bitter taste theory conveys that bitter molecules are recognized by receptor protein helical structures. The recognition process is a dynamic action, in which the receptor protein helices have a torsion-spring-like oscillation between helical structures of 3.6 and 4 amino acids per turn. Based on the characteristics of the bitter receptor protein helix oscillation, it perfectly explains why in bitter molecules, only one unit of hydrogen donor (DH) or hydrogen acceptor (B) is enough in helping molecules to elicit bitter taste. The potential DH and B in bitter receptor are any NH or O of receptor's peptide NHs and Os, which are the ones forming intramolecular H-bonds responsible for the formation of receptor protein helical structures. Furthermore, only one unit of DH or B is allowed for structurally simple ligands. As recognition sites are only associated with a small fraction – helix structure of whole bitter receptor, multiple binding sites or multiple receptors are well expected. As the oscillation may have different extent, it translates to bitterness intensity. According to ligand-receptor binding motion, bitter receptor could be divided into two kinds of spaces, which are similar to the situation in sweet taste receptor: main and side grooves. These have been discussed in context and especially great details in paper titled deciphering aspartyl peptide sweeteners [2].

KEY WORDS: Bitter Receptor, Protein Helix, Helix Relaxing-Comeback Torsion-Spring-Like Oscillation, DH Or B, Multiple Binding Sites, Potency Versus Intensity

INTRODUCTION

Bitterness is a perception producing unpleasant taste, and one of the so-called five basic tastes (sweet, bitter, sour, salt, and umami) [3,4]. Similar to sweet taste molecules, bitter taste molecules have a large variety of different chemical structures, such as L-amino acids, peptides, amines, terpenoids, alkaloids, carbohydrates etc. When I was young, when taking bitter herbal medicine, my mother always added some sugar to the liquid to suppress the bitterness. The sweet and bitter tastes seem to be an opposite counterpart to each other psychologically and chemical structurally. In amino acids series, in general, D-amino acids are sweet and L-amino acids are bitter. It is however that the observation is not really always true. D- and L- glucose, arabinose, xylose, and others have the same sweet taste and the similar sweetness power.[5] It seems that asymmetric centers have no effect on the ability of a sugar to elicit sweet taste. Kubota and Kubo,[6] referenced to Shallenberger and Acree's AH-B theory about sweet taste, proposed that bitter tastants need intramolecular hydrogen bond after studied the bitter molecules of Isodon. Lehmunn [7] suggested the possible existence of sweet and bitter taste receptors with enantiomeric active sites. Belitz and Wieser noticed that unlike in sweet-tasting molecules, in bitter compounds, one polar group (electrophilic or nucleophilic), and a hydrophobic group[8] are required [9]. Now it is known that bitter tastants are recognized by G-protein coupled receptors T2Rs [10-12] and sweet tastants by T1R2/T1R3 [13,14-19].

In the sweet taste phenomenon, mounting evidences have pointed that sweet molecules are recognized through the sweet receptor protein helix oscillation between 3.6 and 3 amino acids per turn[1,2]. 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. Inspired by this molecular theory about sweet taste [1,2] and bitter taste structure activity relationship (SAR) in structurally various categories, this author is proposing a bitter taste molecular theory in which bitter molecules act through bitter receptor protein helix torsion-spring-like oscillation between 3.6 and 4 amino acids per turn. Current paper and the papers [1,2] dealing sweet taste theory are independent. It is however that the combined information makes both of the sweet and bitter theories' foundation stronger. Following, the bitter theory will be described in the RESULTS section, then in DISCUSSION section, bitter 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 models [20-22] 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.
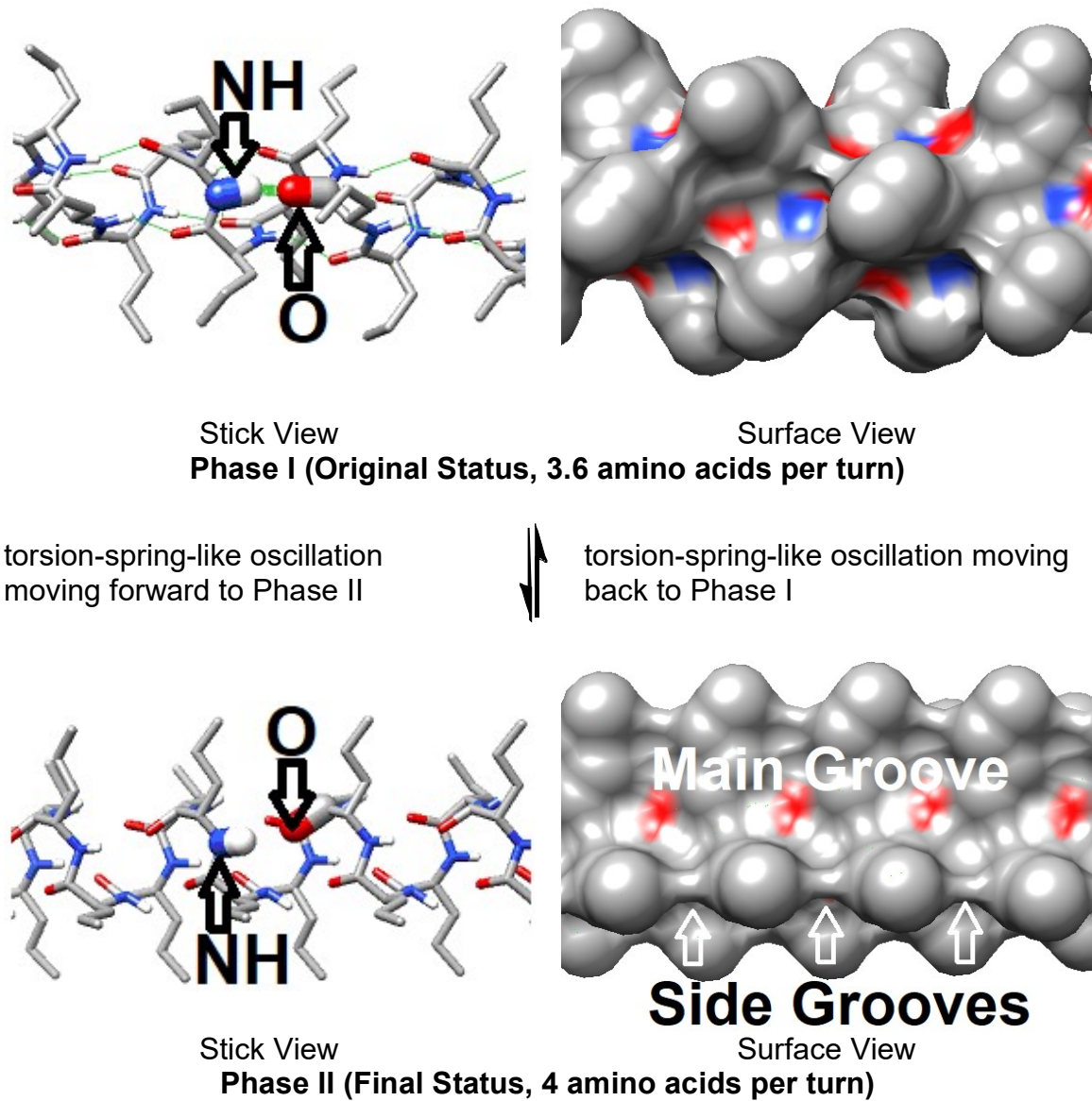
RESULTS

This theory conveys that bitter molecules are recognized by receptor protein helical structure. Recognition of bitter molecules is a dynamic process, in which the receptor protein helixes have a torsion-spring-like oscillation movement between helical structures of 3.6 and 4 amino acids per turn. In this section, receptor helices' behavior, the side chains' movement and other details will be discussed.

First thing first, there is a need to build a bitter receptor protein helix model to visualize the receptor's behavior. As the bitter taste recognition process involves a loosening helix process, the space left for bitter molecules gets squeezed and becomes narrowed. In the process of building sweet receptor model, L-leucine was used and provided a good model for the sweet taste theory's description. It is however that, as the helix with four amino acids per turn has a narrower groove than that with three amino acids per turn, if leucine is still used in building the bitter receptor model, basically the side chain of leucine is too big and it is very difficult to load bitter molecules to the bitter receptor model. The side chain size of L-α-aminobutanoic acid is in the middle of actual natural occurring amino acids; the side chain characteristics of L-α-aminobutanoic acid is relatively simple and avoids possible complication caused by selecting other much simpler or more complicated side chains for the current discussion. This model using L-α-aminobutanoic acid can satisfy basically all of the property needed to explain the recognition process for bitter molecules and will be used through out this paper.

Bitter receptor protein helices' motion in the recognition process

In Figure 1, molecular model is built [20,21,22] to demonstrate bitter 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 4 amino acids per turn. Top diagrams (Phase I) show the original status of the bitter receptor protein helix; and lower diagrams (Phase II) show the final status of the bitter receptor protein helix. Top and lower diagrams show the statuses of helices of 3.6 and 4 amino acids per turn respectively. The bitter molecule recognition process is a dynamic oscillation process between these two phases. This oscillation process initiates the bitter taste elicitation procedure.

Stick View   Surface View
Phase I (Original Status, 3.6 amino acids per turn)

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

Stick View   Surface View
Phase II (Final Status, 4 amino acids per turn)

Figure 1: Bitter receptor protein helix torsion-spring-like oscillation during the process of bitter molecules' recognition. (These bitter receptor helical models are built up using L-α-aminobutanoic acid). NH and O are highlighted and grooves are pointed out.

Bitter 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 sweet taste receptor. These two grooves have different properties in holding bitter 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 bitter-structure relationship discussion, which is very similar to the scenarios in sweet taste theory discussion. [1,2]

Bitter Receptor Hydrogen Donor And Hydrogen Acceptor DH-B

In the progress of searching for sweet taste theory, Shallenberger and Acree's AH-B theory about sweet taste was well cited. For bitter taste theory, this kind of concept was also adopted by some researchers. 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 taste theory [1,2]. In the sweet taste theory[1,2], it has been well elucidated that there are two types of hydrogen donor and hydrogen acceptor DH-B moieties for both receptor and sweet molecules, which is very different from the old understanding. For the receptor, its DH-Bs are receptor protein peptide bonds' NH-Os. . Inspired by my sweet taste theory, hydrogen donor DH and hydrogen acceptor B for bitter receptor also could be receptor peptide NH-Os. In sweet taste receptor, paired NH-O, which formed intramolecular H bond in the helix in phase I, could serve as a potential receptor NH-O. We can start from this to discuss the bitter receptor NH-Os' behavior and bitter tastants' DH-Bs implication.

In the stick view of Figure 1, the peptide bond NHs and Os can be spotted. These NHs and Os are potential receptor hydrogen donor DHs and hydrogen acceptor Bs. From now on, receptor hydrogen donor NHs and hydrogen acceptor Os will be used to refer to receptor hydrogen donor DHs and hydrogen acceptor Bs. To perturb the helix, one efficient way to do that is to act on the helix-driving force the intramolecular hydrogen-bond through complementary H-bond with ligands' DH/B. One pair of the peptide bond NH and O is highlighted, which has an intramolecular H-bond. In sweet taste receptor, it would interact with non-intramolecular ligands' DH-B. We just imagine that bitter ligand also interacts in this way tentatively. From stick views of Figure 1, when the oscillation proceeds from phase I to phase II, the paired NH and O have a zigzag-type movement. While the paired NH and O move to phase II, it can be seen that the NH and O are moved to different main grooves. When the NH and O are in different main grooves, it is unlikely for both of the bitter tastants' DH and B to continuously binding with receptor's O and NH at the same time all the way through the oscillation process. This suggests that it is unlikely that for any kind of bitter molecules' both DH and B to act on the paired intramolecular H bond NH-Os without being interrupted after the oscillation reaches phase II in general. This means that there is only one unit of DH or B is allowed for bitter tastants for simple molecules. This observation perfectly explains Belitz and Wieser [9] noticed phenomenon that only one polar group is required for molecules' bitter taste.

Having the bitter receptor information, bitterness potency, intensity, multiple receptor issues can be discussed.
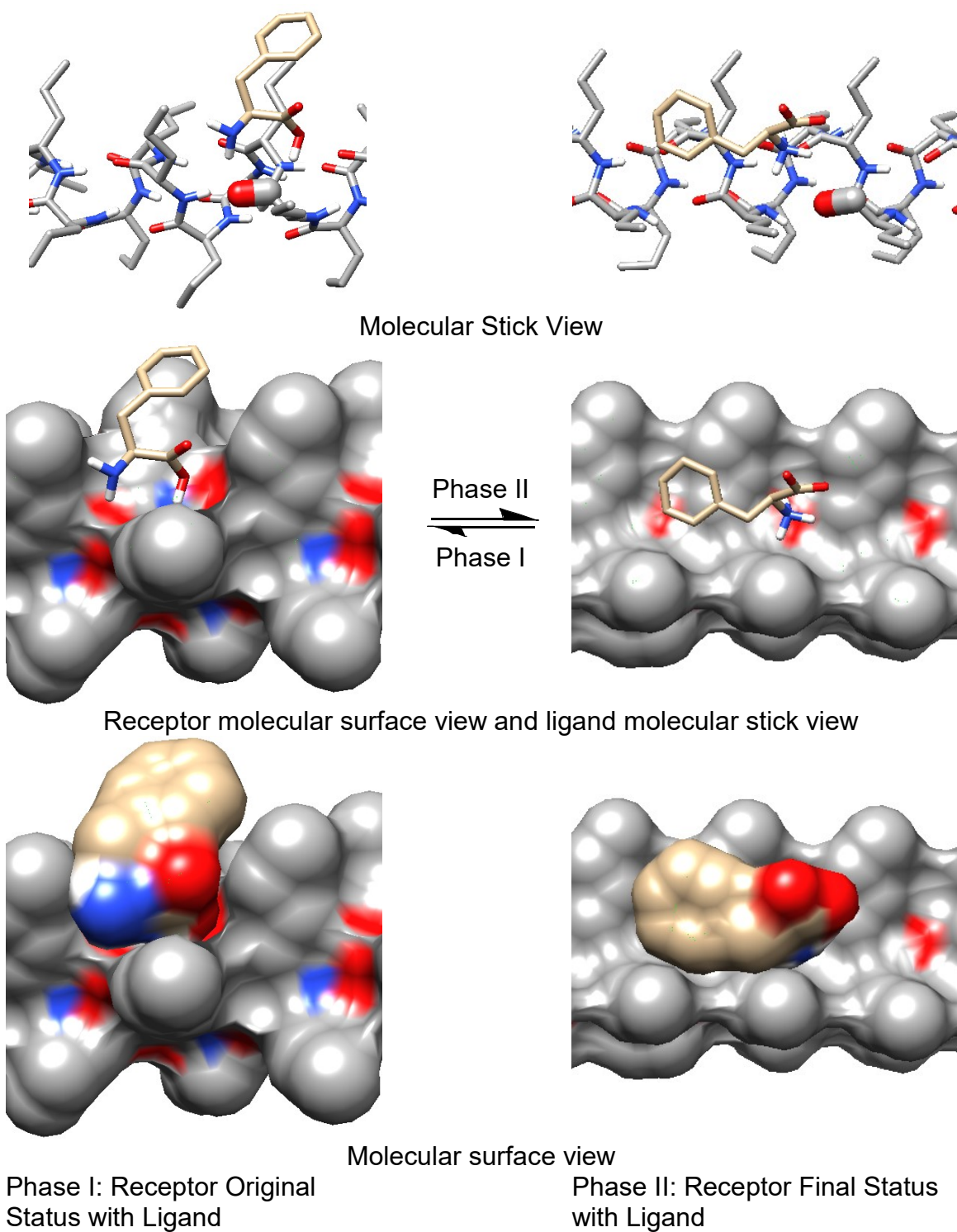
DISCUSSION

Structure-bitter taste relationships are important measurements to validate this bitter theory. let's start with amino acids, amines, strychnine and its derivatives, denatonium and its derivatives, and other series of bitter compounds.
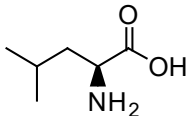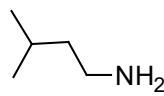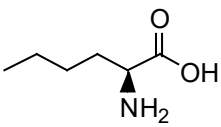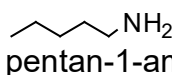
Bitter Taste of L-Amino Acids
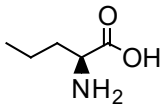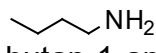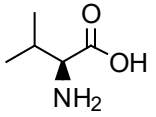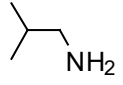
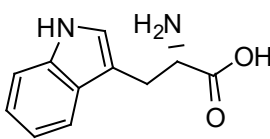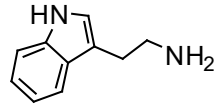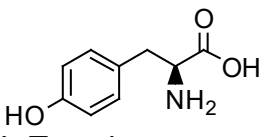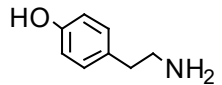In general, D-amino acids have sweet taste and L-amino acids bitter. To analyze the bitterness-structure relationship using this bitter taste theory, the oscillation process diagrams are shown in Figure 2. Here L-phenylalanine is used as an example. Upon the ligand approaching to the receptor, the ligand L-phenylalanine lets the helix moves to phase II (right) from phase I (left). The stick view, surface view and the combination are given out to show the needed details. As carboxylic and amino groups could be chosen as possible DH or B in helping the oscillation process. As discussed in the RESULTS section, there is only one unit is allowed. Which one to choose is the first issue needs to be solved.

First let's assume that the carboxylic group serves as ligands' B. Then corresponding carboxylic acids of amino acids should be bitter. It is however that this is not the case. For example, iso-caproic acid, caproic acid, n-valeric acid, iso-valeric acid, 3-Phenylpropionic acid, 3-(4-Hydroxyphenyl)propanoic acid, which are the corresponding acids of the amino acids listed in Table 1, do not have bitter taste.[23] [24] [25] After the possibility for carboxylic group serving as ligands' B is excluded, the one left is the amino group serving as ligands' DH. It is therefore that the bitter taste of L-amino acids should be close to that of their corresponding amines, and different from that of their carboxylic acids. As receptor grooves are shallow, it is expected that hydrophobic property would increase the bitterness potency, which is similar to sweet taste situation in general. As carboxylic group is hydrophilic, it should be expected that the corresponding amines should have higher potency or lower bitterness threshold than their amino acids. Table 1 lists bitterness threshold of both L-amino acids and their corresponding amines. The left set of compounds is about L-amino acids and the right set of compounds is about their corresponding amines. Each row shows their corresponding pairs. From these data presented, the threshold value for every left entry has a higher number than the right entry. These results well match the analysis drawn above.

Molecular Stick View

Phase II

Phase I

Receptor molecular surface view and ligand molecular stick view

Molecular surface view

Phase I: Receptor Original
Status with Ligand

Phase II: Receptor Final Status
with Ligand

Figure 2: Torsion-spring-like oscillation of bitter receptor protein helixes with
bitter molecule L-Phenylalanine in stick and surface views

Table 1 Bitterness Threshold Comparison of L-amino acids and their corresponding amines [9,26]

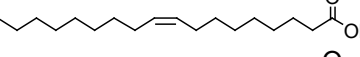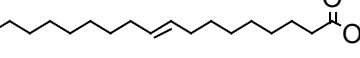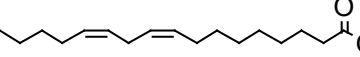| No.	Compounds	C_{tbi} (mM)	No.	Compounds	C_{tbi} (mM)
1	L-leucine	11-13	7	i-Pentylamine	3-4
2	L-norleucin	18-22	8	pentan-1-amine	1.5-2.0
3	L-norvalin	45-50	9	butan-1-amine	4-8
4	L-Valin	20-22	10	2-methylpropan-1-amine	4-5
5	L-Tryptophan	4-6	11	Tryptamine	2.0-2.5
6	L-Tyrosin	4-6	12	Tyramine	2.0-2.5
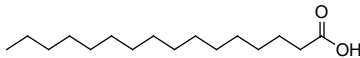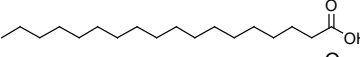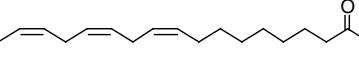
No.: Numbering of compounds; C_{tbi}: bitterness recognition threshold.

According to the bitter theory, there is only one unit of ligands' DH or B can serve as a helper for the receptor protein helix oscillation and eventually for the bitter taste for simple molecules. Even if both potential DH and B exist in one molecule, only one unit can serve as a helper and another one just serve as a regular substitute. In amino acid series, carboxylic group here happens to be an adverse hydrophilic group and provided higher bitterness threshold for amino acids comparing to their corresponding amines. This series is a perfect example about one and only one unit is allowed about DH or B according to this bitter taste theory. Carboxylic group can also serve as bitter ligands' B in helping bitter elicitation oscillation and give carboxylic acids bitter taste. It is however that the ability to elicit bitter taste seems not as powerful as their corresponding amines. Table 2 shows some bitter and non-bitter long chain aliphatic acids. So carboxylic group still have the capability to serve as ligands' B. This may be why a lot of

natural amines have very low bitterness threshold. Now let's look into one of the most bitter amine compound strychnine.
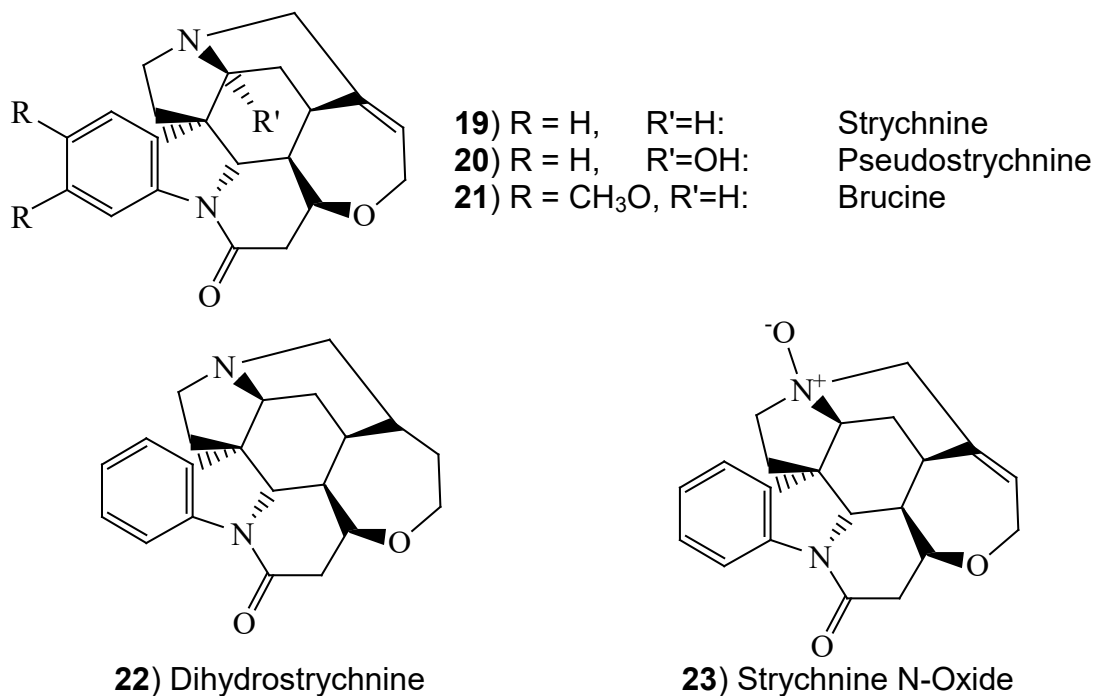
Table 2 Bitter Taste of Aliphatic acids

No.	Compound Name	Compound Structure	C_{tbi} (mM)	
			Wieser et al[27]	Lainer et al[28]
13	Palmitic acid		NA	0.81
14	Stearic acid		n. bi. (22)	0.81
15	Oleic acid		9-12	0.98
16	9t-Octadecenoic acid		n. bi. (22)	NA
17	Linoleic acid		4-6	0.93
18	α-Linolenic acid		0.6-1.2	0.28

NA: not available; n. bi.: not bitter

**Bitter Taste of Strychnine and Its Derivatives**

Strychnine (**19**) is a very toxic compound. [29] Its oral LD_{50} in dogs, cattle, horses, and pigs is 0.5 - 1 mg/kg, and in cats is 2 mg/kg. Strychnine (**19**) is a very bitter compound (bitterness threshold: 1:130,000 (Merck Index, 1989)). Its dihydro compound (**22**) has a bitter taste similar to that of strychnine (**19**). [30] Its dimethoxy compound brucine (**21**) has a lower bitterness threshold: 1:220,000 (Merck Index, 1989). A very interesting compound pseudostrychnine (**20**), which has a hydroxyl group at the α-carbon of the free amine's nitrogen, has no bitter taste. [31] The pKa values of pseudostrychnine (**20**) and strychnine (**19**) are 5.50 and 7.37 respectively. [32],[33] This suggests that at physiological pH, the tertiary amine nitrogen of pseudostrychnine (**20**) can not be protonated and no NH^+ is formed. If the OH group does not interfere the capability for possible NH^+ to serve as DH, pseudostrychnine (**20**) should have bitter taste at around pH 5.5 or lower. If pseudostrychnine (**20**) would be still not bitter at pH 5.5 or lower, that still would be an interesting piece of information, because it could mean that the hydroxyl group is interfering with the binding/oscillation process. In Human Bitter Taste Receptor hTAS2R46 assay, strychnine (**19**) and strychnine-N-oxide (**23**) show thresholds at 0.1 and 30 μM respectively. The difference is 300 times. This also tells that one atom change makes a big impact. Only reliable explanation for this is that the capability to form ligand DH (protonated tertiary amine NH^+) in strychnine (**19**) completely disappeared in strychnine-N-oxide (**23**); the binding mode of strychnine-N-oxide (**23**) is different from that of strychnine (**19**) and makes it have a very different bitterness potency. This is another series of example about ligand's DH. Now let's move on to grooves for their impacts on bitterness potency.
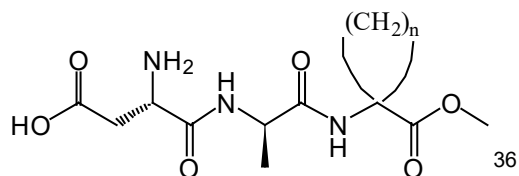
19) R = H,   R'=H: Strychnine
20) R = H,   R'=OH: Pseudostrychnine
21) R = CH$_3$O, R'=H: Brucine

22) Dihydrostrychnine   **23**) Strychnine N-Oxide

Figure 3 Structures of Strychnine and its derivatives

Bitterness - Ring Size Relationship

In the papers about sweet taste, [1,2] there are many examples that could be used to address the groove issues. About bitter receptor protein helix grooves, there are less examples which could be used to deal with them systematically. In the limited data, Table 3 lists two sets of aspartyl peptide compounds. When the C-end ring size get larger, the taste of the peptide changes to bitter gradually, then becomes tasteless, although left side set of peptide is dipeptides and right side set of peptide is tripeptide. For sweeteners, the binding pattern is clear, the rings in different sets bind at relative different sites. With this helix recognition theory, it is very easy to understand the trend, because they act on main groove. Only difference is that they act on different relative spots. From compound **27** to **28**, the taste from bitter to very bitter is due to the increase in hydrophobicity. Compound **29** turns to tasteless when one more –CH$_2$- group is added due to ring's contribution towards' binding/oscillation. It is worthy to remind that the groove for ligands to tackle receptor is very shallow. When a larger part of a molecule is not contributing to the binding/oscillation, the molecule may loss the ability to do that at all. This kind of trend will be able to see in cycloalkanones and 1-amino-cycloalkane-1-carboxylic acid series.

Table 3 Ring size-sweetness relationship of L-aspartyl peptides

L- aspartyl-α-aminocycloalkane-carboxylic acid methyl esters
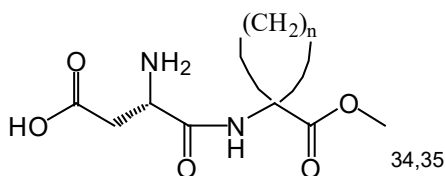
L-Asp-D-Ala-AA-OCH$_3$

No.	n	Taste	No.	n	Taste
24	3	Sweet	30	3	Sweet
25	4	Sweet	31	4	Sweet
26	5	Sweet	32	5	Sweet
27	6	Bitter	33	6	Sweet
28	7	Very Bitter	34	7	Bitter
29	8	TL	35	8	Bitter

TL: Tasteless.

From compound **36** to **40**, cycloalkanones increase their –CH$_2$-, increase their hydrophobicity and eventually increase the bitterness potency. When reach to **43**, as mentioned before, the ring size becomes too big to contribute to the binding/oscillation. It is therefore that **43** is neutral. From compound **44** to **51**, the sweet taste potency becomes higher (lower threshold) (**44** to **46**), then lower (**47** to **48**), then no more sweet taste (**49** to **51**); the bitter taste potency becomes higher (lower threshold) (**45** to **48**), then lower (**49**), then no more bitter taste (**50** to **51**); For the bitter taste, as cycloalkanones can only provide ligands' B and amino-cycloalkane-1-carboxylic acids should only use their ligands' DH as discussed in amino acid and amine bitter taste series, their binding manners are different, but they show similar trend. This is because they act on receptor protein helix main grooves no matter dealing with sweeteners or bitter tastants.

Table 4 Taste-Ring Size Relationships of Cycloalkanones [9] and 1-amino-cycloalkane-1-carboxylic acids [37,38]

$$(CH_2)_{n-1} \quad C=O \quad _9 \qquad (CH_2)_{n-1} \quad \overset{NH_3^+}{\underset{COO^-}{C}} \, _{37}$$
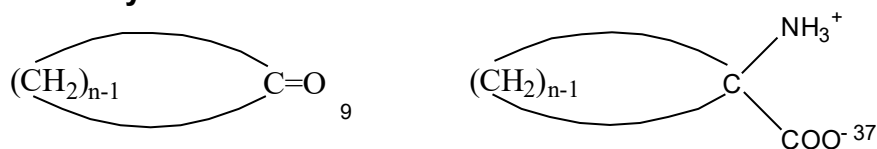
n	No.	C_{tbi} (mM)	No.	C_{tsw} (mM)	C_{tbi} (mM)
4	**36**	neutral	**44**	20-30	NA
5	**37**	10-15	**45**	3-6	95-100
6	**38**	6-10	**46**	1-3	45-50
7	**39**	5-6	**47**	2-4	13-15
8	**40**	2-4	**48**	2-4	2-5
9	**41**	NA	**49**	N.S.(50)*	20-50
11	**42**	NA	**50**	N.S.(20)*	N.B. (20)
12	**43**	neutral	**51**	N.S.(20)*	N.B. (20)

C_{tsw}: sweetness recognition threshold; NA: not available; N.S.: not sweet; N.B.: not bitter, in parenthesis maximum concentration tested.

It is the helix recognition theory makes the comparison possible for the trend of sweet versus bitter compounds; and compounds with hydrogen donor DH type versus hydrogen acceptor B type. Next let's see more examples about main groove, and also side groove in denatonium series compounds.

Bitter Taste of Denatonium and Its Derivatives

Now many examples have been provided to deal with main grooves. What is the side groove's contribution in bitterness elicitation? In the aspartyl peptide sweetener series,[2] there are many examples discussed. As there is not many bitter compounds can deal with the information, we are going to only briefly discuss about side grooves. For more in-depth details, intermolecular weak interactions and side groove sections in aspartyl sweeteners paper [2] should be read. Luckily enough, Saroli's four papers [39,40,41,42] about denatonium derivatives have showed some information about this issue.
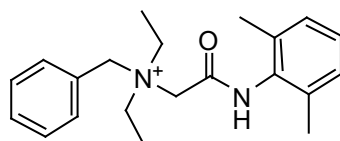
Denatonium

Denatonium salts have been used as aversive agent, which are not known to pose any long-term health risks; its LD_{50}s for various species range from 500 to 1400 mg/kg.[43] Denatonium salts are one of the most bitter compounds. Saroli synthesized and found out the bitterness thresholds about dozens of compounds which structures are related or close to denatonium salts (some just dipeptides or amino acid amide). The contents are briefly summarized as: the bitterness for the

compounds basically are guaranteed, which means there is no critical atom or group identified for the bitter taste: the length for the compounds basically have some flexibility; having or not having the amide part is not important; halide or benzoate basically having the same threshold; hydrophobility provided higher activity (lower threshold); both ends with a benzene ring would provide higher activity.

For the sweetness-structure relationship of Aspatyl peptide stweeteners, the aspartyl part is basically an essential structure and retained through most of sweetener exploration, although other parts could have very flexible variation. The reason has been discussed in the paper titled deciphering aspartyl peptide sweeteners [2] that in aspartyl structure there is critical hidden information H-bonding-like DH-B moiety. From Saroli's research on bitter-structure relationship of denatonium analogues, it was found that, the bitterness almost exist in all the cases no matter how fundamental changes on both sides, chain length, etc. In bitter molecules, there is no such crucial factor as subtle DH-B-like moiety in helping bitter receptor protein helix oscillation.

Table 5 Sweetness-Structure Relationship of N-Substitute Aspartame and Bitterness-Structure Relationship of Denatonium Analogues				
R	No.	Sweetness * [44]	No.	C_{tbi} (M) [39]
C_5H_{11}	NA	NA	55	$2-4 \times 10^{-5}$
C_6H_{13}	NA	NA	56	$4-8 \times 10^{-5}$
C_8H_{17}	NA	NA	57	$4-8 \times 10^{-5}$
$C_{10}H_{21}$	NA	NA	58	$4-8 \times 10^{-5}$
$C_6H_5CH_2$	NA	NA	59	$1-2 \times 10^{-7}$
$C_6H_5CH_2CH_2$	52	Faintly SWEET	60	$2-4 \times 10^{-5}$
$C_6H_{11}CH_2CH_2CH_2$	53	0	NA	NA
$C_6H_5CH_2CH_2CH_2$	54	1000	61	$2-4 \times 10^{-5}$
NA: not available; * Sucrose = 1.				

For sweet taste of N-R-Aspartame (Table 5), structurally close counterparts of **54** (R=$C_6H_5CH_2CH_2CH_2$) have much less sweetness potency: **53** (R=$C_6H_{11}CH_2CH_2CH_2$, 0), **52** (R=$C_6H_5CH_2CH_2$, faintly sweet). Compound **53** does not have flat ring, the cyclohexyl group can not insert into the side groove of sweet receptor protein; and **52**, the bridge (CH_2CH_2) is too short, the phenyl ring can not or can not fully reach the side groove. Due to the difference in binding pattern or inability in binding, **54** has a much better sweetness potency than **52** and **53**. The importance for the phenyl ring here is that the side chains of side groove have a movement like sliding on the phenyl ring. Non-plane structure of cyclohexyl group can not serve the purpose.

For bitter taste in denatonium analogue series (Table 5), Compound **59** (R=$C_6H_5CH_2$) has a much higher potency (>100 times) than its close

counterparts (**55 – 58**, **60**, **61**). For compounds **55 – 58** (R = C_5H_{11}, C_6H_{13}, C_8H_{17}, $C_{10}H_{21}$), they do not have a flat phenyl ring. Their R groups can not insert into the side groove of bitter receptor protein. For compounds **60**, **61**, the bridges (CH_2CH_2, $CH_2CH_2CH_2$ respectively) are too long. Their phenyl ring can not optimally reach the side groove. This explains the difference about main grooves and side grooves in structural requirement and the enhancement effects for bitterness potency. Only with this helix recognition theory, it is possible to discuss sweet taste of aspartyl peptide and bitter taste of denatonium analogues side by side.

With Saroli's work, some SAR information about main grooves can also be discussed and compared with L-aspartyl peptide sweet SAR. In L-Asp-D-Ala-NHR sweetener series, the R groups act on receptor main groove. When R=$C_6H_3(2,6-(CH_3)_2)$ (**66**), the sweetness activity is better than its corresponding phenyl ring (**65**) or open chain **63** (R=$CH_2CH_2CH_3$) compounds. In denatonium derivatives series, they also have similar structure-bitterness relationship. When R=$C_6H_3(2,6-(CH_3)_2)$ (**71**), the bitterness activity is better (lower threshold) than its corresponding phenyl ring (**70**) or open chain **67** (R= CH_3), **69** (R= $CH_2CH(CH_3)_2$) compounds. In these cases, phenyl rings or open chains do not provide drastic change in sweetness/bitterness. Again, the sweet and bitter theories allow the structure - sweet/bitter taste relationship discussion side by side.

Table 6 Some SAR Similarity Between Sweet L-Asp-D-Ala Amide And Bitter Denatonium amide side.

		L-Asp peptides	**Denatonium Derivatives**	
		L-Asp-D-Ala-NHR	Cl^- ⟨phenyl⟩$-C(H_2)-N^+(CH_3)(CH_3)-CH_2CONH-R$	
No.	R	Sweetness* [45]	No.	C_{tbi} (M)** [41]
62	CH_3	NA	67	$2\text{-}4 \times 10^{-5}$
63	$CH_2CH_2CH_3$	72	68	NA
64	$CH_2CH(CH_3)_2$	NA	69	$1\text{-}2 \times 10^{-6}$
65	C_6H_5	38	70	$4\text{-}8 \times 10^{-6}$
66	$C_6H_3(2,6-(CH_3)_2)$	500	71	$1\text{-}2 \times 10^{-7}$
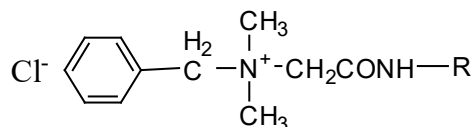
Bitterness Intensity

In RESULTS section, the bitter theory has stated that the recognition process is an oscillation action of the bitter receptor protein helix. The oscillation process happens between the helices of 3.6 and 4 amino acids per turn. This oscillation is the key for the bitterness elicitation. A question could be raised out of this statement: does the interaction of bitter molecule with bitter receptor protein helix have the perfect oscillation as shown in Figure 1 in all the occasion? The answer, of cause, is negative. This would pop up another question: what does that mean then? This introduces a term called intensity. Different extent of

the bitter receptor protein helix oscillation results in different bitterness intensity. Most reports deal with threshold or the like. Those are about the potency.

There are plenty of research dealt with bitterness intensity issues: for example, researches on iso-α-acids' bitter intensity in different concentration [46], tasters & non-tasters' bitter intensity of caffeine and quinine at four concentration levels [47], super-tasters, medium-tasters, non-tasters' bitter intensity of 6-n-propylthiouracil (PROP) in different concentration [48], phenylthiocarbamide (PTC) bitterness intensity to predicts TAS2R38 genotype [49], and others. Different concentrations of a bitter compound acting on a receptor or different composing amino acids in receptors responding to a bitter compound will make the oscillation to a different extent, which translates to different bitterness intensity. Bitterness potency and intensity are two characteristics for bitter compounds. Now let's deal with bitter receptors.

Bitter Receptors

This whole theory is about bitter molecules recognized by helical structures of bitter receptors. This kind of helix relaxing-comeback torsion-spring-like oscillation movement is like the pronunciation of word "bitter". No matter where you say it, as long as you can let the down stream hear it, it will be understood as "bitter". 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. Bitter receptors are G-protein coupled receptors, which are seven trans-membrane helical structure proteins. Two research groups discovered bitter taste receptors in human and mouse in 2000. [10-12] Bitter taste in humans is believed to be mediated by 25 receptors (hT2Rs, or TAS2Rs). [50] Each receptor responds to certain bitter compounds: for example, hT2R38 for phenylthiocarbamide (PTC) and 6-n-propylthiouracil (PROP), [51,52] hT2R61 for 6-nitrosaccharin, hT2R44 for denatonium and 6-nitrosaccharin, [53] hT2R47 for denatonium in micromolar concentration, hT2R7 for strychnine, quinacrine, chloroquine and papaverine [54]. In the bitter receptor oscillation process, the main groove is getting narrower. This may make the receptor more sensitive to bitter molecules' size, shape, and other properties, and need different receptors to sense different bitter molecules. The "bitter blindness" phenomenon, such as Phenylthiocarbamide (PTC)'s taster and non-taster issue,[55] may be also due to this same reason. In sweet molecules' recognition process, it has been known that different molecules could bind to different spots of sweet receptor (T1R2/T1R3). Could one receptor bind with bitter molecules at different spots? Very likely, if not absolutely, the answer is positive.

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. Inspired by the sweet taste theory[1,2], the bitter taste theory is also updated. The ideas for the formation of the sweet and bitter taste theories are basically came from the study of sweetness-structure relationships, although the bitterness-structure relationship played some roles too in the process. The reason is that the systematic researches on sweeteners are much more extended than those on bitter compounds. The processes for the theories' formation are basically reversal procedures for the information presented here and in other papers [1,2]. The total three papers are independent and as a whole make the foundation for the theories very strong.

In the very beginning of this paper, the bitter theory has described as that the bitter molecule recognition process is a torsion-spring-like oscillation action of the bitter receptor protein helix. The oscillation process happens between the helices of 3.6 and 4 amino acids per turn (helix relaxing-comeback torsion-spring-like oscillation). This oscillation is the key for the bitterness elucidation. 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 bitter molecules are named as DH-Bs.

In sweet taste theory, it was found out that there are two types of DH-B/NH-O entities for sweet compounds and receptor respectively, which play important role in sweetness elicitation. To look into the similar counterparts in bitter molecules and receptor, the receptor protein helix oscillation is carefully examined. From the observation of the helices' oscillation pattern, the intramolecular H bond paired hydrogen donor NH and hydrogen acceptor O in helix of 3.6 amino acids per turn locate at different main grooves after oscillation process is moved to helix of 4 amino acids per turn. This unprecedented finding suggests that only one unit of receptor NH or O is involved for the bitter molecules' action on the bitter receptor; and only one unit of DH or B is allowed for structurally simple molecules. In this way, based on the characteristics of the bitter receptor protein helix oscillation, it perfectly explains why in bitter molecules, only one unit of hydrogen donor (DH) or hydrogen acceptor (B) is enough in helping molecules to elicit bitter taste. The potential DH and B in bitter receptor are any NH and O of receptor's peptide NHs and Os, which are the ones forming intramolecular H-bonds responsible for the formation of receptor protein helical structures. In helping bitter taste elicitation, the hydrogen bonding interaction between one unit of NH or O of receptor and one unit of B or DH of bitter taste ligands is sufficient, which is very different from the sweet receptor/ligand NH-Os/DH-Bs' interactions which are more complicated. Experts in this area should treat this alone as an important contribution to bitter taste research area.

In the process of building a bitter receptor helix model, the size of amino acid side chain becomes an issue. As the helix of 4 amino acids per turn has a

narrower width of main groove than that of helix of 3 amino acids per turn, L-leucine used in building sweet receptor model is too big for bitter receptor model building, which means that it is not easy to put bitter molecules into the grooves. It is therefore that the bitter receptor model is built using L-aminobutanoic acids.

According to ligand-receptor binding manner and motion, bitter receptor could be roughly divided into two kinds of spaces to accommodate bitter molecules: one is named here as main grooves which is the grooves following receptor protein carbon to nitrogen end helix axis (referring to the helix with 4 amino acids per turn in which it is easier to be identified); another is named as side grooves which are the grooves between side chains and almost perpendicular to the main grooves, which is similar to the situation appeared in sweet taste receptor. The contents discussed above basically lead to the explanation of bitterness potency.

From the discussion of the bitterness of L-amino acids, amines, strychnine derivatives, it demonstrated the importance of bitter molecules DH. From bitter tastes of carboxylic acids and cycolketones, it shows the function of bitter ligands' B. By comparing sweet/bitter taste trends of cyclo-compounds, it probed the main grooves, which not just the bitter receptor's main groove, but also sweet receptor' main grooves width. The groove impacts on sweet and bitter taste are similar, but not the same. Only through these theories about sweet and bitter taste, it is possible to have this kind of comparison side by side. Again, only with these theories about sweet and bitter taste, it becomes possible for the comparison about Denatonium derivatives' SAR similarities with L-aspartyl sweeteners. Bitter receptor's side groove issue is briefly gone through using denatonium derivatives' SAR information. As bitter compounds' SAR information is not as rich as that in sweeteners. For more in-depth discussion, one should refer to intermolecular weak interactions and side groove sections in aspartyl sweeteners paper [2]. Another characteristics for bitter taste is bitterness intensity, which is due to the extent of helix oscillation.

Similar to sweet taste receptor, multiple bitter receptors or multiple binding sites on a single receptor are well expected. It is because the recognition process only involves a very small helix piece, which is a small fragment of a whole receptor. It has been known that different molecules could bind to different spots of sweet receptor (T1R2/T1R3), although it seems not clearly stated in bitter receptors. In bitter receptor case, there are 25 bitter receptors. Multiple binding sites in one receptor are very likely. The finding of multiple bitter receptors will be another supporting evidence for this theory.

As the bitter receptor's torsion-spring-like oscillation makes the grooves get narrower. It makes the receptor more sensitive to bitter molecules' size, shape, and other properties, and may need different receptors to sense different bitter molecules.

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