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Sweet and Taste Modifying Proteins – Comparative Modeling and Docking Studies of Curculin, Mabinlin, Miraculin with the T1R2–T1R3 Receptor

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Abstract

Motivation. Sweet and taste–modifying proteins and their response to the T1R2–T1R3 G–protein coupled sweet taste receptor is still a topic of discussion. In search of low caloric natural sweeteners for diabetic patients, comparative modeling of curculin, mabinlin, miraculin and T1R2–T1R3 receptor were performed as no X–ray or NMR structure was available for any one of them.

Method. Comparative modeling and docking was done using SwissModel server and GRAMM software, respectively.

Results. Docking of proteins with the sweet taste receptor was followed by analysis of protein–protein complexes with various parameters and was found nearly stable, except in the case of mabinlin.

Conclusions. Based on the results, we propose that sweet and taste modifying proteins curculin and miraculin will bind with sweet human taste receptor T1R2–T1R3 and will perform biological activity.

Keywords. Curculin; mabinlin; miraculin; T1R2–T1R3 sweet taste receptor; docking; sweet protein.

Abbreviations and notations

EXPASY, Expert Protein Analysis System.	GRAMM, Global Range Molecular Matching.
PDB, Protein Data Bank.	Proq, Protein Quality Predictor.

1 INTRODUCTION

1.1 Sweet and Taste Modifying Proteins

Diabetes mellitus is a chronic disease caused by inherited or acquired deficiency in production of insulin by the pancreas or by the ineffectiveness of insulin produced [1]. Artificial sweeteners like Saccharin, Aspartame, Cyclamate and AcesulfameK are used world–wide as low caloric sweeteners

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by patients affected by diseases linked to the consumption of sugar, *e.g*. diabetes, hyperlipemia, caries, obesity etc., but they have side effects such as psychological problems, mental disorders, bladder cancer, heart failure and brain tumors [2–6]. Sweet proteins have the potential to replace these artificial sweeteners, by acting as natural good low caloric sweeteners, as proteins do not trigger a demand for insulin in these patients whereas sucrose does.

The sweet taste in humans is mainly due to recently discovered T1R2–T1R3 receptor [7–9], the three members of the T1R class [7–9] of taste–specific receptor hypothesized to function in combination as heterodimeric sweet taste receptors. The human T1R2–T1R3 receptor recognizes natural and synthetic sweetness and T1R1–T1R3 recognizes umami taste [10–11]. So far there are seven known sweet and taste–modifying proteins, namely brazzein [12], thaumatin [13], monellin [14], curculin [15], mabinlin [16], miraculin [17] and pentadin [18]. Properties and characteristics of these proteins are illustrated in Table 1.

The key group on the protein surface responsible for biological activity has not yet been identified with certainty for any of the known sweet proteins [19]. Monellin was found to be 100000 times sweeter than sucrose on molar basis [20], followed by brazzein and thaumatin which are 500 times [12] and 3000 times sweeter then sucrose [13] respectively (both on weight basis). As no docking studies have been done so far on curculin, mabinlin and miraculin with the sweet taste receptor T1R2–T1R3. We did comparative modeling for all the three proteins and the taste receptor, as no X–ray or NMR structures were available for these proteins. Furthermore, we performed docking and brief stability check for these three docked protein–protein complexes.

Table 1. Comparison of thaumatin, monellin, mabinlin, pentadin, brazzein, curculin and miraculin

	Thaumatin	Monellin	Mabinlin	Pentadin	Brazzein	Curculin	Miraculin
Source	*Thaumatococcus danielli* Benth	*Dioscoreophyllum cumminsi* Diels	*Capparis masakai* Levl	*Pentadiplandra Brazzeana* Baillon	*Pentadiplandra Brazzeana* Baillon	*Curculingo latifolia*	*Richadella dulcifica*
Geographic distribution	West Africa	West Africa	China	West Africa	West Africa	Malaysia	West Africa
Variants	I, II, a, b, c [a]	–	I, II– a, III, IV [a]	–	–	–	–
Sweetness factor (weight basis)	3000	3000	100	500	2000	550	–
Molecular mass (active form, kDa)	22.2	10.7	12.4	12.0 [b]	6.5	24.9	98.4
Amino acids	207	45 (chain) 50 (B chain)	33 (chain) 72 (B chain)	?	54	114	191
Active form	Monomer	Dimer (A + B)	Dimer (A + B)	?	Monomer	Dimer (A + A)	Tetramer (A+A+A+A)

Source: Adapted from Kurihara [15,24,29]. [a] At least five different forms of thaumatin (Lee *et al*.) [48] and four different forms of mabinlin (Nirasawa *et al*.) have been identified [29]. [b] A chromatographic fraction containing a 12–kDa protein was sweet. This same fraction, when subjected to electrophoresis under non–reducing conditions showed bands in the region between 22 and 41 kDa, suggesting the presence of subunits

Curculin which is extracted from *Curculigo latifolia* act as good low caloric sweetener. It has taste modifying ability and its maximum sweetness is equal to 0.35 M of sucrose. The taste

modifying activity (briefed later) of protein remains unchanged when it is incubated at 50^{o} C for 1 hr between pH 3 and 11 [21]. There is no other protein available so far with both sweet taste and taste modifying abilities [22]. The molecular weight of curculin was determined by low angle laser light scattering and was found to be 27800 [22]. Its three–dimensional model has been built from the X–ray coordinates of GNA, a mannose–binding lectin from snowdrop (*Galanthus nivalis*) [23]. The three mannose–binding sites present in GNA were found in curculin but were not functional. Some well–exposed regions on the surface of the three–dimensional model of the said protein could act as epitopes responsible for the sweet–tasting properties of the protein [23]. The protein can be crystallized by the vapor diffusion method using polyethylene glycol 400 as a precipitant. The crystals belong to the orthorhombic space group P2(1)2(1)2(1) with unit cell dimensions: a = 105 Å, b = 271 Å, c = 48.7 Å. The crystals diffract X–rays to resolution of 3.0 Å and are suitable for X–ray crystallographic studies [24]. Water and sour substance elicit a sweet taste after consumption of curculin [25].

Mabinlin, a sweet protein with the highest known thermostability [26] derived from *Capparis masaikai* is 400 times sweeter than sucrose. It consists of the A chain with 33 amino acid residues and the B chain composed of 72 residues. The B chain contains two intramolecular disulfide bonds and is connected to the A chain through two intermolecular disulfide bridges [27]. Its heat stability is due to the presence of these four disulfide bridges [28]. The sweetness of mabinlin–2 is unchanged even after 48 hour incubation at boiling point [16] and of mabinlin–3 and –4 were unchanged for 1 hr at 80^{o}C [29].

Miraculin is a taste–modifying protein and belongs to the class of sweet proteins. It is extracted from *Richadella dulcifica*, an evergreen shrub native of West Africa. The protein is a single polypeptide with 191 amino acid residues [30]. It modifies the sweet receptor in such a way that they can be stimulated by acid [31]. Miraculin has the unusual property of modifying sour taste into sweet taste [30].

Taste–modifying proteins, modifies the sweet taste receptor on binding and this behavior of the taste–modifying proteins is responsible for modification in taste of sour substance [30–31]. All acids (which are normally sour) taste sweet after consumption of these sweet–modifying proteins. The effect of these proteins is manifested for around half an hour after its consumption and intake of any sour substance will taste sweet during this period of time. The taste buds come to their normal state with time.

1.2 The Human Sweet Taste Receptor

Humans detect taste with taste receptor cells. These are clustered in taste buds. Each taste bud has a pore that opens out to the surface of the tongue enabling molecules and ions taken into the mouth to reach the receptor cells inside. There are five primary taste sensations salty, sour, sweet,

bitter and umami. Sweet and umami (the taste of monosodium glutamate) are the main attractive taste modalities in humans. T1Rs are candidate mammalian taste receptors that combine to assemble two heteromeric G–protein–coupled receptor complexes T1R1–T1R3, an umami sensor, and T1R2–T1R3, a sweet receptor [32]. Our aim is to show that it is possible to reconcile the interaction of small and macromolecular sweeteners with the same receptor, provided sweet and taste–modifying proteins interact with the T1R2–T1R3 receptor with a different mechanism with respect to small molecular weight compounds.

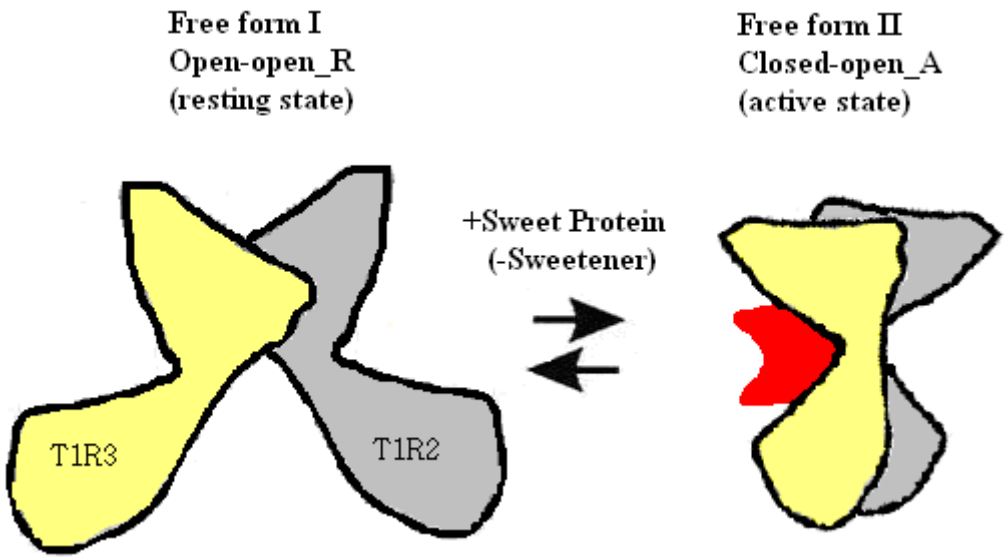

Figure 1. Diagrammatic representation of T1R2–T1R3 receptor showing possible stabilization from attachment of a sweet protein to a secondary binding site on the surface of free form II. The sweet protein is represented in red color on the left part of the free form II, preventing it from reverting to form I.

Recently, it has been shown that the T1R2–T1R3 receptor have many characteristics similar to the mGluR [33], apart from some minor differences in the active site region. The extraordinary work by Kunishima *et al*. [33] on solving the crystal structure of the N–terminal active site region of the subtype 1, in both free and complexed with glutamate in mGluR has helped a lot in understanding the mechanism of interaction between ligand and T1R2–T1R3 receptor. His structural work on mGluR and its N–terminal domain [33–34] showing considerable conformational change induced by the glutamate complexation helped him in solving the crystal structure of the N–terminal active site region. Modulation of the 'active' and 'resting' conformations of the m1–LBR, a heterodimer is modulated by dimer interface. The protomer can form 'open' or 'close' confirmations and are made up of two domains namely LB1 and LB2. The population of active conformers depends on the ligand binding, *i.e*. the so called 'closed–open_A'. The ligand–free receptor exists as two different structures, free form I (open–open_R), the 'resting' conformation with two open protomers and free form II (closed–open_A), nearly identical to the

complexed form illustrated in Figure 1.

The mechanism suggested by these structures is that the receptor is in dynamic equilibrium, and that ligand binding stabilizes the 'active' dimer. There are thus two ways, in principle, to activate the receptor: the most obvious one is to complexate form I with the proper ligand (glutamate for the mGluR, aspartame or any other small molecular weight sweetener for the T1R2–T1R3 receptor) and, secondly, by shifting the equilibrium between free form I and free form II in favor of free form II [35]. Recently it has been proposed that it is possible to reconcile the interaction of sweet proteins namely brazzein, monellin and thaumatin with the T1R2–T1R3 receptor [35]. We propose that sweet and taste–modifying proteins curculin, miraculin and mabinlin interact with the free form II of the T1R2–T1R3 receptor and stabilize it. The active site region of the sweet taste receptor, identified by us has been displayed in the Figure 5.

In order to validate this hypothesis, we have modeled the structures for three proteins (curculin, miraculin, mabinlin) and heterodimeric model of the T1R2–T1R3 receptor using 1EWT and 1ISS, in the SwissModel (oligomer mode) tool of Expasy [36–38] and docked with sweet taste receptor.

2 MATERIALS AND METHODS

2.1 Model Building and Energy Minimization

The theoretical models for curculin and miraculin was modeled using SwissModel server, whereas mabinlin was modeled using the oligomer mode in the SwissModel server [36–38]. Mabinlin I (SwissProt id: P80351) having A and B chain was modeled using napin Bnib (1PNB.pdb) as template which is a seed storage protein having 66% identity and 95% similarity with 1PNB:A chain and 47% identity and 66% similarity with 1PNB:B chain respectively. Curuculin (NCBI id: CAA45477) was modeled using agglutinin from daffodil (1NPL.pdb) as template having 46% identity and 57% similarity. Miraculin (NCBI id: A33872) was modeled using Amy2/Basi protein–protein complex from barley seed with D chain (1AVA.pdb, D–subunit) as template having 35% identity and 50% similarity.

The heterodimeric model of the T1R2–T1R3 receptor was built using the SwissModel tool of EXPASY in the oligomeric mode [36–38] using 1EWT.pdb and 1ISS.pdb as templates. The percentages of identical residues, between the sequence of m1–LBR of the crystal structure (1EWT.pdb and 1IIS.pdb) and the corresponding parts of T1R2 (NCBI id: AY032623_1) and T1R3 (NCBI id: AY026318_1) are 27 and 24.0 %, respectively.

The alignment between protein sequences of taste receptor T1R2, T1R3 and 1EWT.pdb; curculin and 1NPL.pdb; miraculin and 1AVA.pdb (D–subunit); mabinlin and 1PNB.pdb are illustrated

below.

Alignment between curculin and 1NPL.pdb

```
curculin          1 KFLLTILVTFAAVASLGMADNVLLSGQTLHADHSLQAGAYTLTIQNKCNL     50
                                ||:|.||:||.....|..|.|...:|..|||
1npl              1                   DNILYSGETLSPGEFLNNGRYVFIMQEDCNL     31

curculin         51 VKYQNGRQIWASNT---DRRGSGCRLTLLSDGNLVIYDHNNNDVWGSACW     97
                    |.|...:.|||:||   |||   |.|::.||||||:|...||.:|.|...
1npl             32 VLYDVDKPIWATNTGGLDRR---CHLSMQSDGNLVVYSPRNNPIWASNTG     78

curculin         98 GDNGKYALVLQKDGRFVIYGPVLWSLGPNGCRRVNGGITVAKDSTEPQHE    147
                    |:||.|..|||||...||||...|:.|.|..
1npl             79 GENGNYVCVLQKDRNVVIYGTARWATGTNIH                      109

curculin        148 DIKMVINN    155

1npl            110             109
```

Alignment between miraculin and 1AVA:d.pdb

```
miraculin         1 MKELTMLSLSFFFVSGLLAAAANPRLSAADSAPNPVLDIDGEKLRTGTNY     50
                                        ||  |.||.|.||.:||...||
1ava              1                     AD--PPPVHDTDGHELRADANY     20

miraculin        51 YIVPVLRDHGGGLTVSATTPNGTFVCPPRVVQTRKEVDHDRPLAFFPEN-     99
                    |::...|.||||||::   |.....||..|.|.........|:...|..
1ava             21 YVLSANRAHGGGLTMA---PGHGRHCPLFVSQDPNGQHDGFPVRITPYGV     67

miraculin       100 -PKEDVVRVSTDLNINFSAFMPCRWTSSTVWRLDKYDESTGQYFVTIGGV    148
                     |.:.::|:|||:.|:|.|:..|  ..||.|.:|. :.:.|:..|..|.|
1ava             68 APSDKIIRLSTDVRISFRAYTTC--LQSTEWHIDS-ELAAGRRHVITGPV    114

miraculin       149 KGNPGPETISSWFKIEEFCGSGF--YKLVFCPTVCGSCKVKCGDVGIYID    196
                    | :|.|....:.|:||::.|:..  |||:       ||...|.|:|::.|
1ava            115 K-DPSPSGRENAFRIEKYSGAEVHEYKLM-------SCGDWCQDLGVFRD    156

miraculin       197 QKGRRRLALSDKPFAFEFNKTVYF          220
                    .||.....:.:|:     ..|.|
1ava            157 LKGGAWFLGATEPY-----HVVVFKKAPPA    181
```

Alignment between mabinin and 1PNB.pdb

```
mabinlin          1 EP-LCRRQFQQHQHLRACQRYIRRRA-----QRGGLVEQRGPALR-LCCN     43
                    :| .|:|:|||.|||||||::||::.     |.|   .|:||.|| .|||
1pnb              1 QPQKCQREFQQEQHLRACQQWIRQQLAGSPFQSG---PQQGPWLREQCCN     47

mabinlin         44 QLRQVNKPCVCPVLRQAAHQQLYQGQIEGPRQVRQLFRAARNLPNICKIP     93
                    :|.|.::.||||.|:|||.....||| .||.|..::::.|:||||:|.:.
1pnb             48 ELYQEDQVCVCPTLKQAAKSVRVQGQ-HGPFQSTRIYQIAKNLPNVCNMK     96

mabinlin         94 AVGRCQFTRW    103
                    .:|.|.|...
1pnb             97 QIGTCPFIAI    106
```

Alignment between T1R2 and 1EWT.pdb

```
t1r2         1 MGPQARTLHLLFLLLHALPKPVMLVGNSDFHLA---GDYLLGGLFTLHAN     47
                                       :|...:|   ||.::|.||::|..
1ewt         1                         SSQRSVARMDGDVIIGALFSVHHQ     24

t1r2        48 VKSVSHLSYLQVP--KCNEYNMKVLGYNLMQAMRFAVEEINNCSSLLPGV     95
               ..:.      :||  ||.|.. :..|...::||...:::||....|||.:
1ewt        25 PPAE------KVPERKCGEIR-EQYGIQRVEAMFHTLDKINADPVLLPNI     67

t1r2        96 LLGYEMVDVCYLSN-NIQPGLYFLSQIDDFLPILKDY-------------    131
               .||.|:.|.|:.|: .::..:.|   |.|.|..::|.
1ewt        68 TLGSEIRDSCWHSSVALEQSIEF---IRDSLISIRDEKDGLNRCLPDGQT    114

t1r2       132 ---SQYRPQVVAVIGPDNSESAITVSNILSYFLVPQVTYSAITDKLQDKR    178
                  .:.:..:..||||.:|..||.|.|:|..|.:||:.|||.:..|.||.
1ewt       115 LPPGRTKKPIAGVIGPGSSSVAIQVQNLLQLFDIPQIAYSATSIDLSDKT    164

t1r2       179 RFPAMLRTVPSATHHIEAMVQLMVHFQWNWIVVLVSDDDYGRENSHLLSQ    228
               .:...||.|||.|....||:.:::..:.|.::..:.::.:||........:
1ewt       165 LYKYFLRVVPSDTLQARAMLDIVKRYNWTYVSAVHTEGNYGESGMDAFKE    214

t1r2       229 RLTNTGDICIAFQEVLPVPEPNQAVRPEEQDQLDNILDKLRR--TSARVV    276
                        :|.||.|.:...::.....:...|.:|.|||.  ..||||
1ewt       215 ---------LAAQEGLCIAHSDKIYSNAGEKSFDRLLRKLRERLPKARVV    255

t1r2       277 VIFSPELSLHNFFREVLRWNFTG-FVWIASESWA-IDPVLHNLTELRHTG    324
               |.|...:::.....:.|....| |..|.|:.|| .|.|:... |:...|
1ewt       256 VCFCEGMTVRGLLSAMRRLGVVGEFSLIGSDGWADRDEVIEGY-EVEANG    304

t1r2       325 TFLGVTIQRVS----------------------IPGFSQFRVRHDKPGY    351
                  |:||:..|                     .|.|.|.|.:...||:
1ewt       305 ---GITIKLQSPEVRSFDDYFLKLRLDTNTRNPWFPEFWQHRFQCRLPGH    351

t1r2       352 RMPNETSLRTTCNQDCDACMNITESFNNVLMLSGERVVYSVYSAVYAVAH    401
               .:.|....:.     |....::.|::     :...::.: |.:|:||:||
1ewt       352 LLENPNFKKV-----CTGNESLEENY-----VQDSKMGF-VINAIYAMAH    390

t1r2       402 TLHRLLH---------CNQVRCTKQIVYPWQLLREIWHVNFT-LLGNQLF    441
               .|..:.|          |:.::    .:...:||..:...:|. :.|.:::
1ewt       391 GLQNMHHALCPGHVGLCDAMK----PIDGRKLLDFLIKSSFVGVSGEEVW    436

t1r2       442 FDEQGDMPMLLDIIQWQWGLSQNPFQSIASYSPTE-TRLTYISNVSWYTP    490
               |||:||.|...||:..|:               || .|..|:...:|:..
1ewt       437 FDEKGDAPGRYDIMNLQY---------------TEANRYDYVHVGTWHEG    471

t1r2       491 NNTV---PISMCS          500
               ...:   .|.|..
1ewt       472 VLNIDDYKIQMNKSGMVRS    490
```

Alignment between T1R3 and 1EWT.pdb

```
t1r3         1 MPALAIMGLSLAAFLELGMGASLCLSQQFKAQ--GDYILGGLF------P     42
                                .||:..|:  ||.|:|.||      |
1ewt         1                  SSQRSVARMDGDVIIGALFSVHHQPP     26

t1r3        43 LGSTEEATLNQ-RAQPNSTLCNRFSPLGLFLAMAMKMAVEEINNGSALLP     91
               .....|....: |.|           .|:....||...:::||....|||
1ewt        27 AEKVPERKCGEIREQ-----------YGIQRVEAMFHTLDKINADPVLLP     65
```

```
t1r3        92 GLRLGYDLFDTCSEPVVTMKSSLMFL---------AKVG-------SQSI    125
               .:.||.::.|:|....|.::.|:.|:          .|.|       .|::
1ewt        66 NITLGSEIRDSCWHSSVALEQSIEFIRDSLISIRDEKDGLNRCLPDGQTL    115

t1r3       126 AAYCNYTQYQPRVLAVIGPHSSELALITGKFFSFFLMPQVSYSASMDRLS    175
               ..    .:.:..:..||||.||.:|:........|.:||::|||:...||
1ewt       116 PP----GRTKKPIAGVIGPGSSSVAIQVQNLLQLFDIPQIAYSATSIDLS    161

t1r3       176 DRETFPSFFRTVPSDRVQLQAVVTLLQNFSWNWVAALGSDDDYGREGLSI    225
               |:..:..|.|.||||.:|.:|::.:::.::|.:|:|:.::.:||..|:..
1ewt       162 DKTLYKYFLRVVPSDTLQARAMLDIVKRYNWTYVSAVHTEGNYGESGMDA    211

t1r3       226 FSSLANARGICIAHEGLVPQHDTSGQQ-LGKVLDVLRQVNQSKVQVVVLF    274
               |..||...|:||||...:  :..:|:: ..::|..||: ...|.:|||.|
1ewt       212 FKELAAQEGLCIAHSDKI--YSNAGEKSFDRLLRKLRE-RLPKARVVVCF    258

t1r3       275 ASARAVYSLFSYSIHHGLSPKV-WVASESWLTSDLVMTLPNIARVGTVLG    323
               .....|..|.|.....|:.:. .:.|:.|...|.|:...:...|.:..
1ewt       259 CEGMTVRGLLSAMRRLGVVGEFSLIGSDGWADRDEVIEGYEVEANGGITI    308

t1r3       324 FLQ--------------------RGALLPEF-SHYVETHLA--LAADPAF    350
               .||                    |....||| .|..:..|.  |..:|.|
1ewt       309 KLQSPEVRSFDDYFLKLRLDTNTRNPWFPEFWQHRFQCRLPGHLLENPNF    358

t1r3       351 CASLNAELDLEEHVMGQRCPQCDDIMLQNLSSGLLQNLSAGQLHHQIFAT    400
               .........|||:             .:|:...|.:.|
1ewt       359 KKVCTGNESLEEN------------YVQDSKMGFVIN-------------    383

t1r3       401 YAAVYSVA---QALHNTLQC--NVSHCHVSEHVLPWQLLENMYNMSF-HA    444
                 |:|::|   |.:|:.| |  :|..|...:.:...:||:.:...|| ..
1ewt       384 --AIYAMAHGLQNMHHAL-CPGHVGLCDAMKPIDGRKLLDFLIKSSFVGV    430

t1r3       445 RDLTLQFDAEGNVDMEYDLK--MWVWQSPTPVLHTVGTFN-GTLQLQQS     490
               ....:.||.:|:....||:.  .:...:....:| |||:: |.|.:...
1ewt       431 SGEEVWFDEKGDAPGRYDIMNLQYTEANRYDYVH-VGTWHEGVLNIDDYK    479

t1r3       491               490

1ewt       480 IQMNKSGMVRS    490
```

Energy minimization was done by the means of program GROMOS96 [39] on the SGI O2 workstations with 500 cycles of steepest descent and 800 cycles of conjugate gradient methods for the three sweet proteins, T1R2–T1R3 receptor and the three protein–protein docked complexes respectively.

2.2 Docking with the T1R2–T1R3 receptor

Our next step was the process of docking. Docking calculations between sweet proteins and the heterodimeric model of the T1R2–T1R3 receptor were performed by means of the program GRAMM in the low–resolution mode [40–41] with a grid step of 6.8 Å, a repulsion parameter (ro used in GRAMM for low–resolution docking) of 6.5 and an angle of rotation of 20°. Protein–protein interactions play a major role in protein functions. The detailed studies of these interactions are important for better understanding of protein function and for the subsequent applications of this

knowledge to protein engineering and drug design [42]. Progress in understanding the principles of protein recognition leads to computation methods for protein docking. The principle draw back of existing docking technologies is sensitivity to structural inaccuracies and one such example for inaccuracies is conformational changes upon the formation of complexes. Among the two program GRAMM and HEX [43–44] used for docking, the program GRAMM was found to overcome many of above problems in low resolution (as the three sweet proteins and the taste receptor taken by us are theoretical models). It allows docking at variable resolution depending on the accuracy of the structural component to be docked. The low resolution docking is very fast and may tolerate structural inaccuracies on the order of 7 Å which is a precision characteristic of many protein models [42]. The parameters for low resolution docking were fixed before docking. The docking was performed with the modeled structures of the three proteins with the T1R2–T1R3 receptor in low resolution mode.

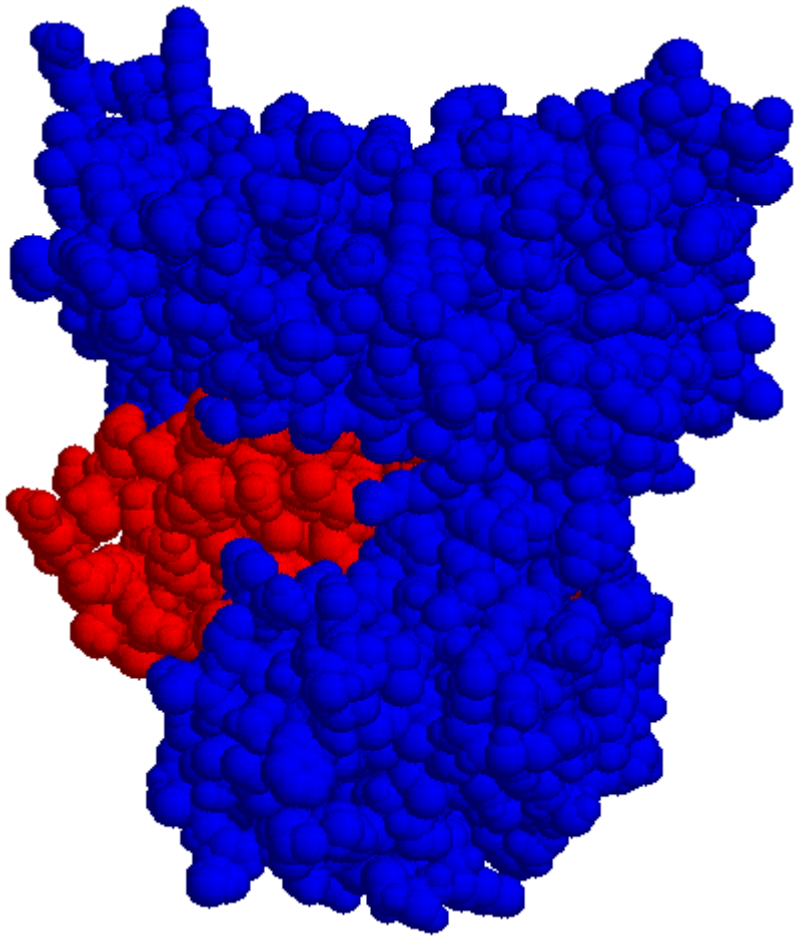

Figure 2. Representation of the docked model of T1R2–T1R3 receptor with curculin shown in the space filling model. The curculin is represented in red color.

NACCESS [45] was used for finding the change in the surface accessibilities for protein before and after docking conditions. NACCESS is a stand alone program that calculates the accessible area of a molecule from a PDB format file. It can calculate the atomic and residue accessibilities for both

proteins and nucleic acids. The hydrogen bonds between sweet proteins and taste receptor were calculated using HBPLUS [46].

3 RESULTS AND DISCUSSION

The free energy of binding values for the docked complexes was obtained from the GRAMM software. The energy value for curculin–receptor, miraculin–receptor and for mabinlin–receptor was –919, –915, –706 kJ respectively.

After docking, we analyzed the stability of the three protein–protein complexes (Figures 2, 3, 4) using various structure validation parameters. It was found that curculin and miraculin showed good results for all structure validation checks while mabinlin was not showing much stability on various parameters.

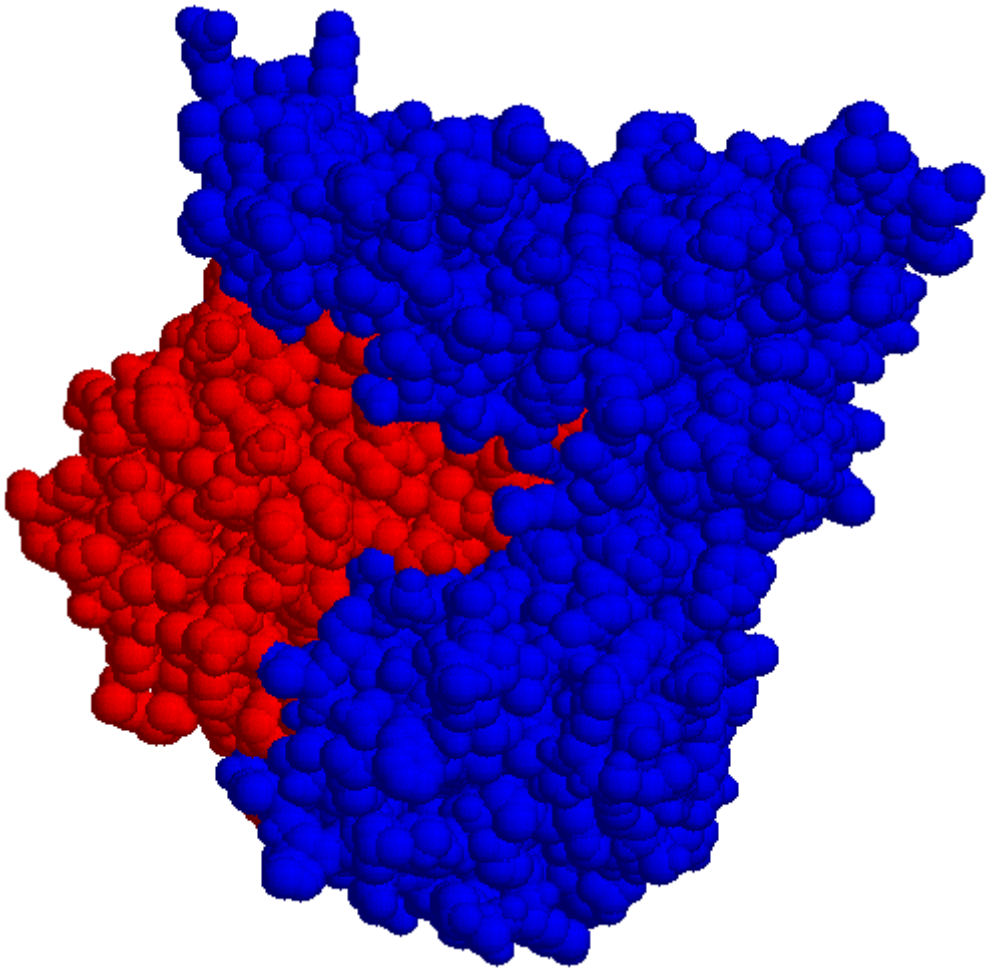

Figure 3. Representation of the docked model of T1R2–T1R3 receptor with miraculin shown in the space filling model. The miraculin is represented in red color.

RAMPAGE server [47] was used for calculating the Ramachandran plot values for structures of curculin, mabinlin, miraculin, T1R2–T1R3 receptor and the three docked complexes. The plot

values for all the proteins and docked structures were in the allowed regions except in the case of mabinlin and mabinlin–taste receptor complex (Table 2). Values for the three protein–protein complexes were slightly lower than the expected 90%, as both the protein and the receptor were theoretical models. Apart from this, the structures of three sweet proteins and taste receptor were validated by the SAVS server in which all the validation parameters were in the expected range.

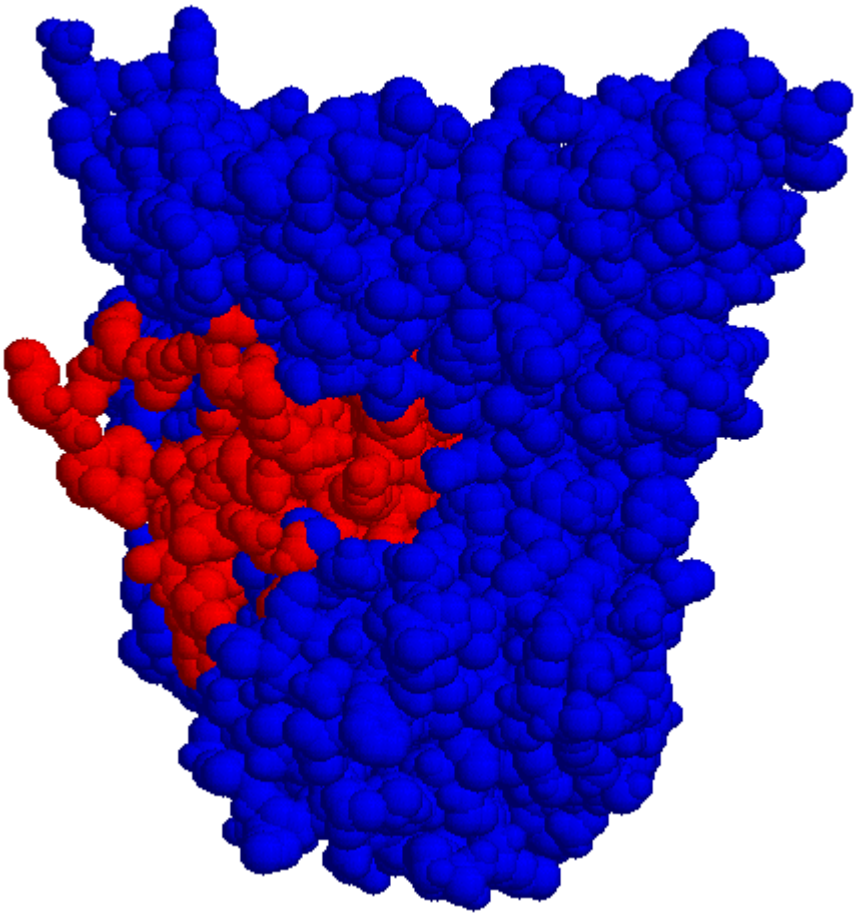

Figure 4. Representation of the docked model of T1R2–T1R3 receptor with mabinlin shown in the space filling model. The mabinlin is represented in red color.

Table 2. Ramachandran plot values for sweet proteins, taste receptor and three docked complexes

Proteins	No of residues in allowed region (%)
Curculin	99.0
Miraculin	90.4
Mabinlin	78.0
Taste receptor	97.1
Curculin–Taste receptor complex	88.2
Miraculin–Taste receptor complex	85.6
Mabinlin–Taste receptor complex	83.9

The change in the accessibility of the residues in the taste receptor protein before and after

binding with the sweet proteins were calculated using NACCESS [45]. When the sweet protein binds with taste receptor, there is a marked decrease in the accessibility of the residues which are in contact with the sweet proteins. The accessibility was calculated for all atoms, total size, main chain, non–polar and all polar. Table 3, shows the decrease in the accessibility of the protein in the bound state than unbound state which indicates that sweet proteins have strongly bound with the taste receptor.

Table 3. Comparison of changes in surface accessibility of protein in bound and unbound state

Complex name	All atoms	Total size	Main chain	Non polar	All polar
Taste receptor	20836.0	18207.1	2628.9	13774.4	7061.6
Taste–curculin	18324.6	15973.6	2351.1	11866.0	6458.7
Taste–miraculin	18314.4	15955.7	2358.7	11822.7	6491.6
Taste–mabinlin	19190.2	16704.4	2485.8	12535.2	6655.0

The CASTp server was used for finding all possible pockets in the taste receptor. The active site pocket was found to be overlapping in the A and B chains of the taste receptor. The functionally relevant residues in the pocket were 112 Ser, 127 Lys, 130 Asp, 132 Arg, 134 Phe, 165 Val, 170 Asp, 171 Asp, 174 Arg, 178 His, 201 Glu, 212 Lys, 389 Gln, 449 Thr, 450 Thr, 451 Glu, 452 Glu, 505 Glu, 510 Met, 529 Ser, 530 Ser, 538 Lys, 551 Ser, 562 Thr, 563 Phe, 599 Asp, 600 Asp, 603 Arg, 607 Ser, 628 Gln, 658 Ala, 659 Arg, 682 Glu.

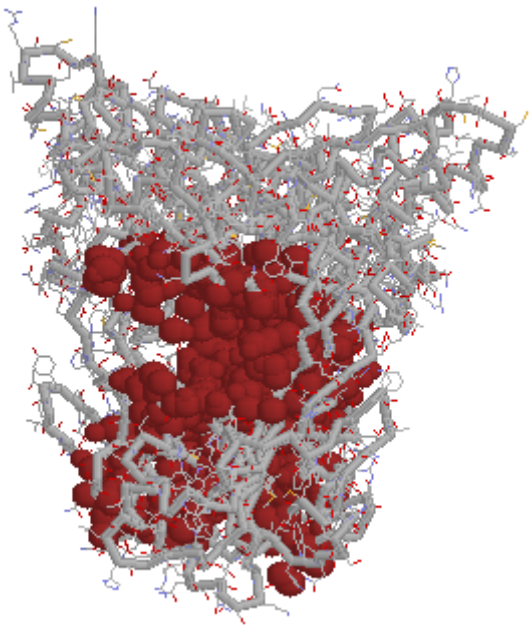

Figure 5. The active site region of the taste receptor protein.

The center of gravity for taste receptor was 11.777 Å (X coordinate of the center of gravity), 30.717 Å (Y coordinate of the center of gravity), 46.468 Å (Z coordinate of the center of gravity) where as the center of gravity (X, Y and Z coordinates) for the taste receptor and curculin complex

were 14.411 Å, 31.829 Å, 46.336 Å and for the taste receptor and miraculin complex were 20.144 Å, 30.35 Å, 48.377 Å respectively. There was less shift in the center of the gravity of the T1R2–T1R3 receptor after complexation, which indicates that curculin and miraculin did not affect the stability of the T1R2–T1R3 receptor after docking. The omega angle for the curculin and miraculin were found to be 4.9 and 5.0 respectively. The omega angle for docked structures of miraculin and curculin have standard deviations of omega values agreeing with the expected values. The stability of the docked complex was also analyzed using ProQ server and it was found that curculin and miraculin lies in the range of values satisfying good models.

Hydrogen bonds play an important role in predicting the stability of protein–protein complexes. The important residues in the taste receptor which are involved in hydrogen bonding with the sweet proteins were calculated using HBPLUS [46]. The key residues are listed in Tables 4, 5, and 6.

Table 4. List of potential hydrogen bonds between taste receptor protein and curculin

Taste receptor protein			Curculin		
Num	Res	Atom	Num	Res	Atom
A127	LYS	N Z	B61	LEU	O
A127	LYS	N Z	B84	ASP	OD1
B81	CYS	N	A130	ASP	O
B81	CYS	SG	A130	ASP	O
A132	ARG	NH2	B76	VAL	O
A133	ARG	NH2	B76	VAL	O
A133	ARG	NE	B65	ASN	OD1
B62	SER	OG	A171	ASP	O
A174	ARG	NH1	B60	LEU	O
A174	ARG	NH2	B60	LEU	O
A174	ARG	NH2	B84	ASP	OD1
A212	LYS	NZ	B110	PRO	OXT
A389	GLN	NE2	B79	SER	OG
A449	THR	OG2	B44	ILE	O
A450	THR	N	B95	ASP	OD1
A450	THR	OG1	B95	ASP	O
B94	LYS	NZ	A505	GLU	O
B94	LYS	NZ	A509	THR	OG1
A529	SER	OG	B96	GLY	O
A531	GLU	N	B86	GLY	O
A538	LYS	NZ	B100	ILE	O
B91	VAL	N	A551	SER	OG
B12	GLN	NE2	A598	SER	O
A607	SER	OG	B109	GLY	O
A628	GLN	N	B13	THR	O
B18	HIS	N	A628	GLN	O
A658	ALA	N	B21	GLN	OE1
A659	ARG	N	B19	SER	OG1
A659	ARG	NE	B28	THR	OG1
A659	ARG	NH1	B15	HIS	O
A663	SER	OG	B18	HIS	NE2
B21	GLN	NE2	A682	GLU	O

Table 5. List of potential hydrogen bonds between taste receptor protein and miraculin

Taste receptor protein			Miraculin		
Num	Res	Atom	Num	Res	Atom
B59	ARG	NH2	A112	SER	O
B190	ASN	ND2	A127	LYS	O
A129	ARG	NH2	B13	ASP	OD2
B32	ARG	NH2	A131	LYS	O
A133	ARG	NH2	B191	LYS	O
B20	ARG	NH2	A179	LEU	O
A183	ARG	NH2	B17	GLU	OE2
B20	ARG	NH2	A185	THR	OG1
A185	THR	OG1	B78	ASP	OD2
A186	ASN	ND2	B77	GLU	O
B76	LYS	NZ	A188	ASP	OD2
A450	THR	N	B40	VAL	O
A450	THR	OG1	B63	ASP	OD2
B39	THR	N	A452	GLU	OE1
A454	THR	OG1	B34	HIS	O
A454	THR	OG1	B36	GLY	O
A456	ASN	N	B60	LYS	O
B58	THR	OG1	A457	GLN	OE1
B57	GLN	NE2	A459	THR	OG1
B56	VAL	N	A460	GLN	O
A463	GLY	N	B41	SER	OG
A466	SER	OG	B46	ASN	OD1
B96	CYS	SG	A468	LEU	O
B97	ARG	NH2	A471	PHE	O
A500	TYR	OH	B96	CYS	O
A551	SER	OG	B95	PRO	O
B91	SER	OG	A599	ASP	OD1
A600	ASP	N	B195	PHE	O
A628	GLN	NE2	B89	ASN	OD1
B21	THR	OG1	A630	ASP	OD2
A632	SER	OG	B70	PHE	O
A633	GLN	NE2	B73	GLU	OE1
B46	ASN	N	A738	GLU	OE2
A752	CYS	SG	B169	TRY	O
B114	GLY	N	A754	TYR	OH

Table 6. List of potential hydrogen bonds between taste receptor protein and mabinlin

Taste receptor protein			Mabinlin		
Num	Res	Atom	Num	Res	Atom
B15	ARG	NH2	A450	THR	O
B15	ARG	NH1	A452	GLU	OE1
B15	ARG	NH2	A452	GLU	OE1
A504	SER	OG	B8	ARG	O
A509	THR	OG1	B13	GLU	OE1
A530	SER	N	B10	CYS	SG
A562	THR	N	B32	GLN	OE1
A599	ASP	N	B49	PHE	O
A601	TYR	N	B52	ALA	O

The residues in the interface for each complex were calculated using STING server. The list of all residues participating in the interaction between sweet proteins and the sweet taste receptor (as determined by docking) are presented in Tables 7, 8 and 9, respectively.

Table 7. List of residues in the interface of taste receptor and curculin

Taste receptor	Curculin
Asn100, Ser103, Ser108, Leu115, Met137, Lys131, Tyr172, Glu195, Lys212, Trp366, Glu452, Pro506, Glu531, Thr536, Arg556, Leu557, Ser558, Asp559, Arg560, Glu561, Arg603, Leu625, Gln634, Asp630, Ala656, Gly704, Arg708, Tyr765, Gln772, Ala779, Asn819	Asn6, Val7, Gly11, Gln12, Thr13, Leu14, His15, Leu20, Ala24, Tyr25, Leu27, Ile 29, Gln30, Asn31, Lys32, Cys33, Asn34, Leu35, Val36, Lys37, Tyr38, Gln43, Ile44, Trp45, Asn48, Leu60, Gly64, Ser79, Ala80, Trp82, Asn85, Ala89, Gln93, Phe96, Gly102, Pro103, Leu108, Pro110

Table 8. List of residues in the interface of taste receptor and miraculin

Taste receptor	Miraculin
Arg129, Asp130, Phe134, Pro135, Ala136, Pro142, Ser143, Ala144, Ile148, Glu149, Asp170, Gly173, Leu184, Thr185, Asn186, Thr187, Asp188, Pro365, Trp366, Glu388, Gln389, Leu445, Ala473, Cys749, Ser750, Ile755, Asn767, Leu768, Lys538, Phe539, Arg556, Asp559, Arg574, Tyr667, Ser668, Ile669, Leu740, Glu741, Glu742, Cys749, Ser750, Asn767, Leu768, Ser769.	Leu12, Asp13, Ile14, Asp15, Gly16, Glu17, Lys18, Arg20, Thr21, Gly22, Asn24, Tyr25, Tyr26, Ile27, Val28, Pro29, Val30, Val40, Ser41, Ala42, Thr43, Thr44, Pro45, Gly47, Phe49, Val50, Cys51, Pro53, Arg54, Val55, Val56, Gln57, Thr58, Lys60, Asp65, Leu68, Ala69, Phe70, Phe71, Pro75, Asp85, Ile88, Phe90, Ser91, Phe93, Met94, Pro95, Cys96, Arg97, Trp98, Thr99, Ser101, Val103, Trp104, Arg105, Leu106, Asp107

Table 9. List of residues in the interface of taste receptor and mabinlin

Taste receptor	Mabinlin
Tyr113, Ile124, Leu128, Pro135, Arg174, Ser181, Glu195, Pro226, Gly443, Leu444, Phe445, Thr502, Cys503, Ser504, Ser553, Phe540, His622, Glu623, Ser663, Phe655, Trp684, Leu689, Met691, Leu740, Gln751, Phe778, Leu814	Glu1, Pro2, Leu3, Arg6, Gln9, Gln10, His11, Gln12, Leu14, Arg15, Cys17, Arg19, Tyr20, Ile21, Arg22, Arg23, Arg24, Arg27, Gly29, Leu30, Arg35, Ala38, Leu39, Arg40, Leu41, Cys42, cys43, Asn44, Gln45, Leu46, Asn50, Lys51, Pro52, Cys53, Val54, Cys55, Pro56, Val57, Leu58, Arg59, Gln60, His63, Leu66, Gln68, Gln70, Pro74, Arg75, Gln76, Arg78, Gln79, Leu80, Ala84, Arg85, Asn86, Leu87, Asn89, Ile90, Lys92, Ile93, Gly97, Cys99, Gln100, Phe101, Thr102, Arg103

4 CONCLUSIONS

Even though our work involved theoretical models for sweet and taste–modifying proteins with the taste receptor protein, we got good, reliable results through our computer aided design and stability checks. The validity of the theoretical models was explored by studying the interaction of taste receptor and sweet proteins. The docking results indicate that sweet proteins bound nicely with the ligand binding region of the taste receptor. Based on our work and results obtained, we are proposing that curculin and miraculin obeying all parameters for docking and stability checks to be one of the best low caloric sweeteners.

But at the same time, the relationship between sweet taste and binding can be analyzed only through experimental techniques (wet lab) and it can validate our hypothesis that, curculin and miraculin are the best sweeteners among the three. We have plans to work further and validate our results (hypothesis).

Scope of Further Work

Sweet and taste modifying proteins can be used as natural low caloric sweeteners by people suffering with diseases linked to consumption of sugar *e.g*. obesity, diabetes, hyperlipemia. The interaction of sweet protein with the human sweet taste receptor T1R2–T1R3 is a positive sign. As it has been found that sweet proteins are thousands of times sweeter than sugars and are of low calorie value. The work can be further continued by solving the structures for the proteins and taste receptor by X–ray diffraction or NMR with a view to increasing the efficiency of these low calorie sweeteners. Proteins can be checked for biological activity with the human taste receptor. Also certain kinds of mutations can be induced in these sweet proteins to analyze the changes in physical, chemical and biological properties of the sweet proteins.

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