

# Sweet Protein Crystallography in Post-AlphaFold Era

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Sweet proteins have been studied as safe replacements for artificial low-calorie small-molecule sweeteners which have harmful side effects [1]. So far, three intensely sweet proteins (22kDa thaumatin, 11kDa monellin & 6.4kDa brazzein with thousand times sweet potency compared to sucrose) and four taste-modifying sweet proteins (12kDa curculin, 14.5kDa egg white lysozyme, 12kDa manbinlin & 12kDa pentadin) were discovered from nature [2]. Especially, crystal structures of thaumatin [3], monellin [3], brazzein [4], curculin [5] and manbinlin [6] have been studied to reveal the mystery of 'sweetness' of sweet proteins. Since the discovery of thaumatin and monellin, sweet proteins have shown neither sequence homology nor structural similarity between them while they share very specificity for the sweet taste receptor, T1R2-T1R3 heterodimer, both of which are G-protein coupled receptors (GPCRs). Further difficulty may arise if we consider how both small molecules such as dipeptides (0.3-0.5kDa) and large sweet proteins (6-22kDa) can activate the same sweet taste receptor. Unlike other tastes (saltiness, sourness, bitterness & umami which were known to be elicited by salt, pH & small molecules respectively), sweetness has been known to come from the intakes of diversely sized molecules which target the same sweet taste receptor [1]. To explain such a huge size-diversity in the sweetener-receptor complex, the lack of structural knowledge of human sweet taste receptor and its complex structures with known sweeteners has been a problem. Based on both the free and the complex structures of metabotropic glutamate receptors (mGluR) which is another GPCR family protein and shares many characteristics to taste receptors, a multi-binding site model (wedge model) has been proposed [1,2].

Recently, we took the advantage of 'timely' release of human T1R2 and T1R3 structures from AlphaFold database together with our own crystal structures of brazzein mutant D29K (sweetness-enhanced) and R43A (sweetness-diminished), reporting that they might bind to different sites of sweet taste receptor [7]. Because we failed to have any meaningful outcome in docking simulations before AlphaFold, we think that AlphaFold database definitely provided higher-quality structure of sweet taste receptor than homology-based sweet taste receptor structures as previously reported. Further we believe that this heralds an era of 'unreasonable effectiveness' [8] of AlphaFold and such computing efforts for protein design. We would rather invent 'artificial' sweet proteins than 'synthetic' sweet peptides in the post-Alpha fold era. Furthermore, computational screening of protein-protein interaction between 'invented' sweet protein and sweet taste receptor can be an interesting and profitable research. Recognizing that computational predictions and experimental validations have different roles at different stages [8], we hope that protein crystallography will work as Quod Erat Demonstrandum (QED) for protein design in the manner of mathematical proofs where QED has been used as a 'proud' closing symbol for such intellectual efforts.

## Acknowledgements

This work was supported by the Korea Research Institute of Bioscience and Biotechnology (KRIBB) Research Initiative Program and National Research Council of Science and Technology (Grant No. NTC0232211).

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