Structural and Functional Analysis of Human Bitter Taste Receptors

STRUCTURAL AND FUNCTIONAL ANALYSIS OF HUMAN BITTER TASTE RECEPTORS

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Introduction

Taste perception is a prime example of complex signal transduction at the subcellular level. The investigation thereof at the nanoscale is empowered by Computational Molecular Modelling, herein employed to investigate bitter human taste receptors, which protect against the ingestion of toxins. Bitter perception involves TAS2Rs receptors, A-family GPCRs composed of an extracellular N-terminus, an intracellular C-terminus, 7 transmembrane (TM) α-helices, 3 Extracellular (ECLs) and 3 Intracellular Loops (ICLs). TMs and ECLs form the binding site. This work investigates the 25 human bitter taste receptors by using computational methods to underline structural and mechanical differences, as well as functional motions distinguishing *promiscuous* and *selective* receptors.

Methods

The 3D structures of the 25 human bitter receptors were downloaded in PDB format from the BitterDB [1]. Protein-membrane complexes were built with CHARMM-GUI. GROMACS was used to run Molecular Dynamics (MD) simulations. Principal Component Analysis (PCA) was carried out on the MD trajectories to analyze simulation data and distinguish the receptors' main motions. To underline collective atomic motions related to a specific function, Functional Mode Analysis (FMA) was used. The binding pocket volume was analyzed to evaluate promiscuous and selective receptors' opening, with the fpocket tool.

Results

To distinguish the 25 receptors as *promiscuous (PRO)* vs. *selective (SEL)*, different analyses were performed, choosing only 6 of them (TAS2R14, TAS2R10 and TAS2R46 for the promiscuous receptors and TAS2R3, TAS2R5 and TAS2R9 for the selective ones). The analyses were focused on the secondary structure, the identity between receptors, and the residue types: none of those underlined a substantial difference between the two groups. We found the binding pocket volume to be bigger on average for *PRO* receptors, as shown in Figure 1. FMA analysis highlighted a relationship between the ICL3 - C-terminus distance and receptor selectivity: this is in line with previous work highlighting the role of ICL3 in TAS2Rs activation [2]. Ensemble-weighted Maximally Correlated Motions (ewMCMs) were analyzed in every receptor, and the *SEL* receptors were found to have a different behaviour in the ECL2, in the form of greater ewMCM RMSF values with respect to *PRO* ones. The ECL2, located in the extracellular site and near the binding pocket, has a crucial role in the ligand-binding in GPCRs due to its position [3]. RMSF values computed from classical MD simulations confirmed these results.

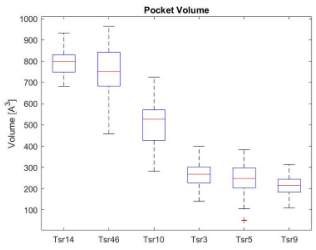

Figure 1: Box-plot of binding pocket volumes of the six receptors. The first three on the left side are the volumes of promiscuous receptors while the others are the volumes of the selective ones.

Discussion

The analyses of the *PRO* vs. *SEL* bitter taste receptors were aimed at functionally linking the intracellular domain, where the G-protein binds to the receptor, to the extracellular domain, where ligand-binding occurs. Binding pocket volume was found to be a differentiating factor between *PRO* and *SEL* receptors. PCA showed the importance of ICL3 and C-terminus fluctuations, the former having previously been found to be fundamental in the activation of the TAS2R receptors [2]. Hence, ICL3 – C-terminus distance was chosen as the function for FMA: ewMCMs were analyzed to investigate the fluctuation related to this function. The main difference between *PRO* and *SEL* receptors appears in the residue fluctuation in ECL2, higher in the selective ones. Such higher fluctuations in ECL2 in SEL receptors may partially explain the receptor selectivity. Also, the analysis of the loop's RMSF confirms this hypothesis. Such structure-function relationship findings represent an important starting point for future analyses, focused on the receptors' capacity to bind ligands in a promiscuous vs. selective fashion.

References

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