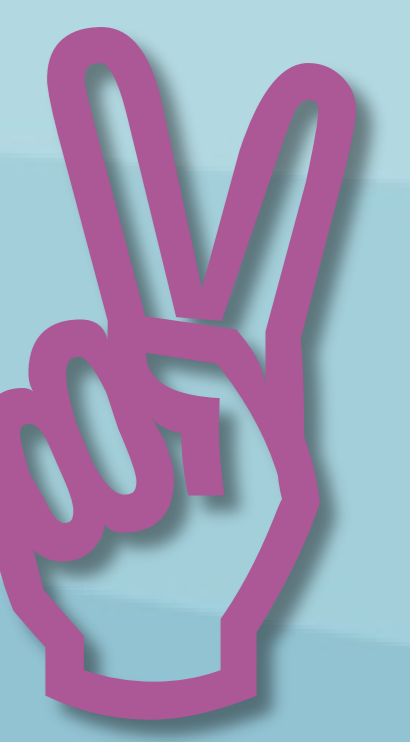


Bitter Taste of Peptides: Computational Analysis of Molecular Interactions



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Introduction:

Taste recognition is essential for survival. Mammals identify and consume nutrients and avoid toxins and indigestible substances based on gustatory cues. Bitter peptides are often generated in fermented, aged, and hydrolyzed food products and make them unfavorable for consumption. What are the details of bitter peptides taste detection and specificity towards their receptors? There are 25 subtypes of bitter taste receptors in humans, termed hTAS2Rs, with varying repertoire of ligands. These belong to the G protein coupled receptor (GPCR) family.

The present study aims to elucidate the binding mode of bitter peptide Phe-Phe-Pro-Arg with its cognate bitter taste receptors hTAS2R8 and hTAS2R39¹.

Methods and Results

1. Modeling of hTAS2R8 and hTAS2R39:

- Performing a Multiple Sequence Alignment (MSA, Figure 1).
- Manually adjusting the MSA according to the Ballesteros-Weinstein numbering (BW).
- Building the 3D homology models of taste receptors using Modeler, via Discovery Studio 3.1.
- Homology Model Verification by comparison with hTAS2R10, which was generated previously in our lab and confirmed by mutagenesis studies².

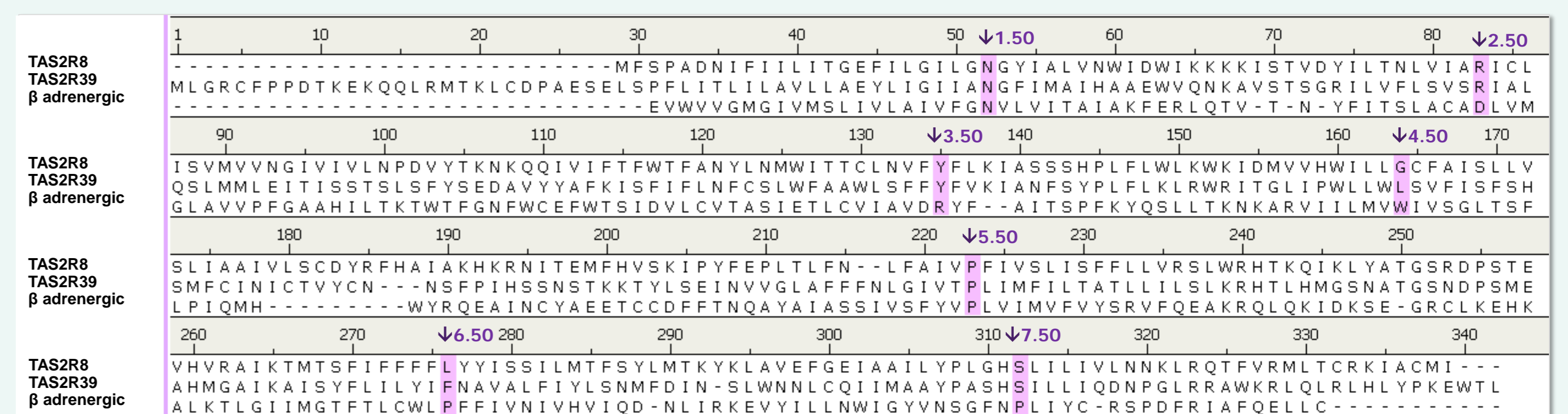
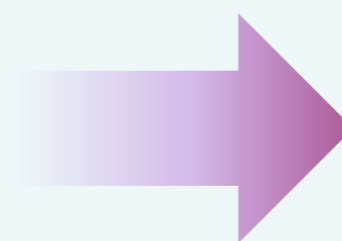


Figure 1 | Multiple Sequence Alignment generated by ClustalW.

The input set of query sequences: The studied receptors T8 and T39; The template crystal structure, β_2 adrenergic receptor. The BW residues X.50 are marked in each TM helix.

2. Surface mapping:

To predict GPCR hotspots that may be involved in peptide binding, we used ANCHORSmap³: an algorithm for computational mapping of amino acid side chains on protein surfaces. First we illustrated the applicability of the method using a known GPCR structure, Neurotensin receptor (NTSR1), which was solved in complex with a peptide (Figure 2).

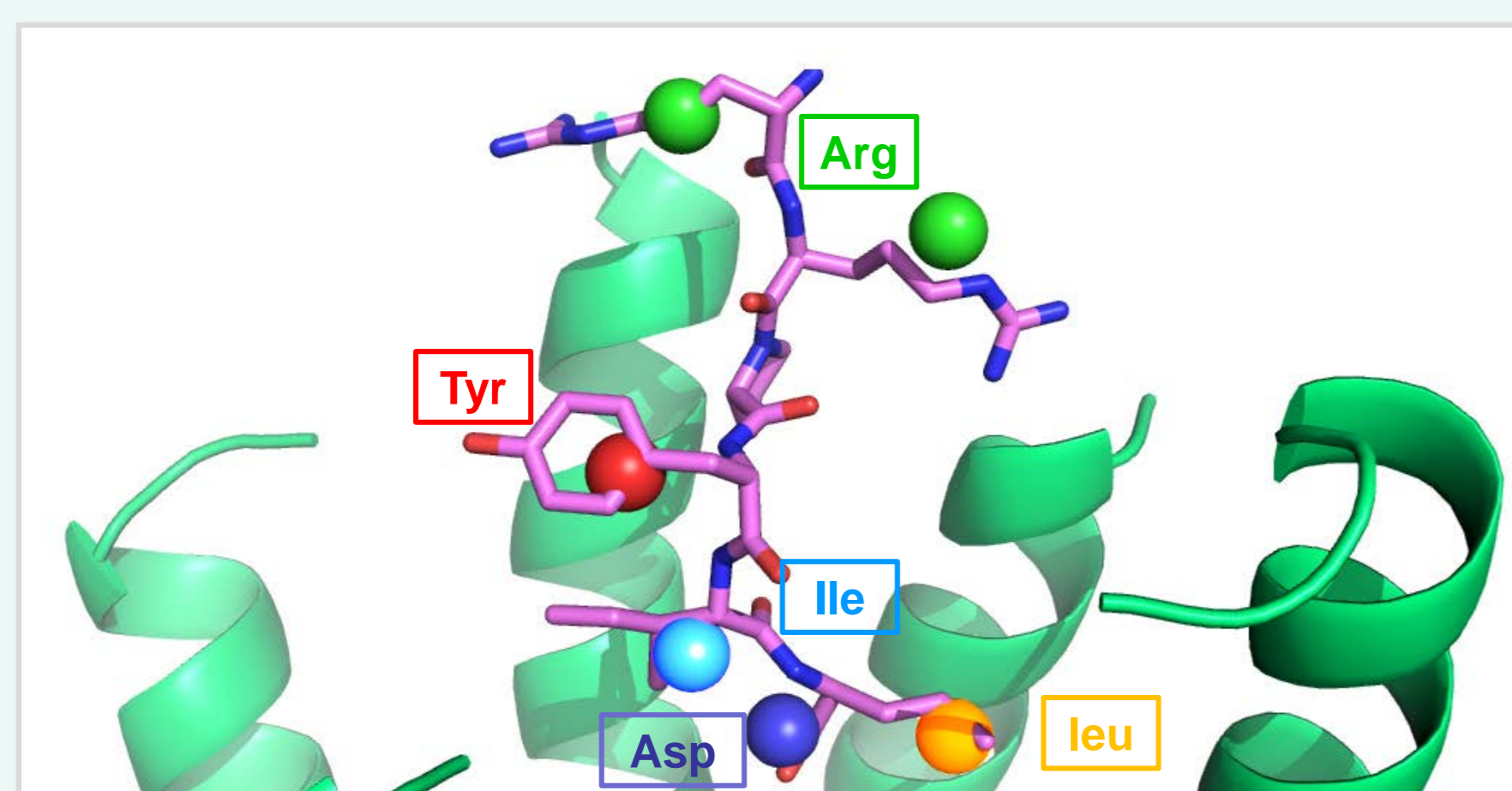


Figure 2 | Surface mapping on a solved X-ray structure of the Neurotensin receptor, NTSR1 (green ribbons), in complex with Neurotensin peptide (pink sticks) that activates. The anchoring spots maps were calculated for NTSR1 and showed that the experimental anchoring positions are accurately identified by our calculations.

3. Ligand-Receptor interactions predicted by docking:

Molecular docking provides a commonly-used prediction of the stable complex of protein-peptide. The taste receptors and the peptide FFPR were examined using multiple approaches. Results of ClusPro2.0 docking server⁴ are shown. Some of the docking poses coincided with the ANCHORSmap-identified anchoring spots.

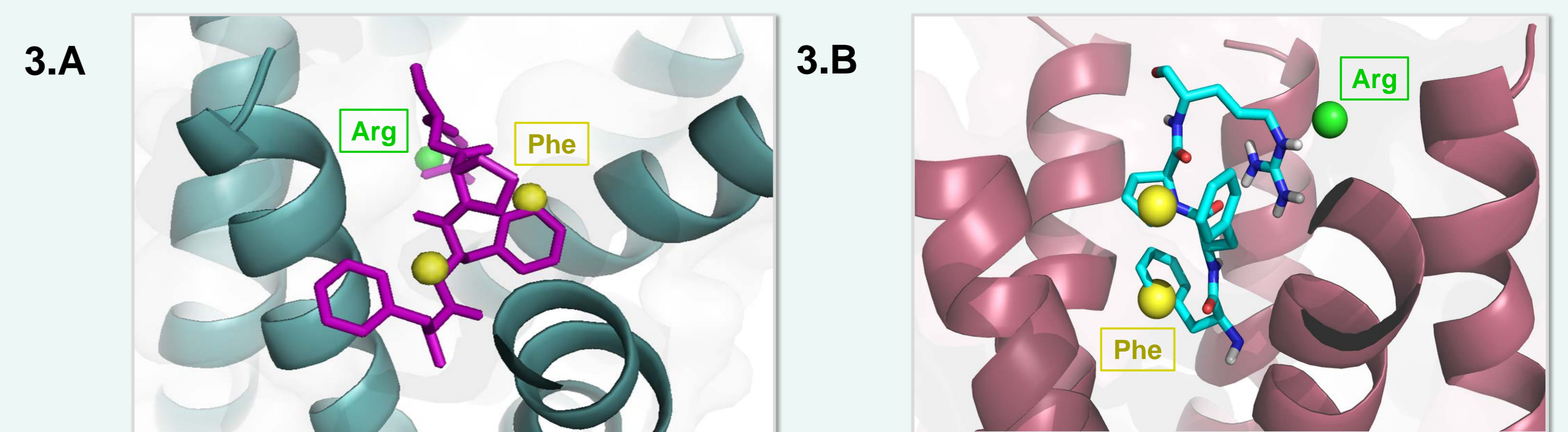


Figure 3 | Predicted anchoring spots by ANCHORSmap coincide with predicted docking positions of Arg (green) and Phe (yellow) binding positions on the protein surface of **A.** Receptor hTAS2R8 (receptor in blue ribbon, peptide in pink sticks) **B.** Receptor hTAS2R39 (receptor in pink ribbon, peptide in light-blue sticks).

4. Identifying important residues for the ligand binding:

To unravel the molecular basis of tastant recognition by bitter taste receptors, docking of FFPR peptide to each of the receptors was carried out and important residues in the putative binding site are shown. From previous works^{5,6} several positions are conserved and appear to contact diverse ligands in nearly all class A GPCRs. The consensus contacts that are important for bitter taste receptors were checked in these docking results.

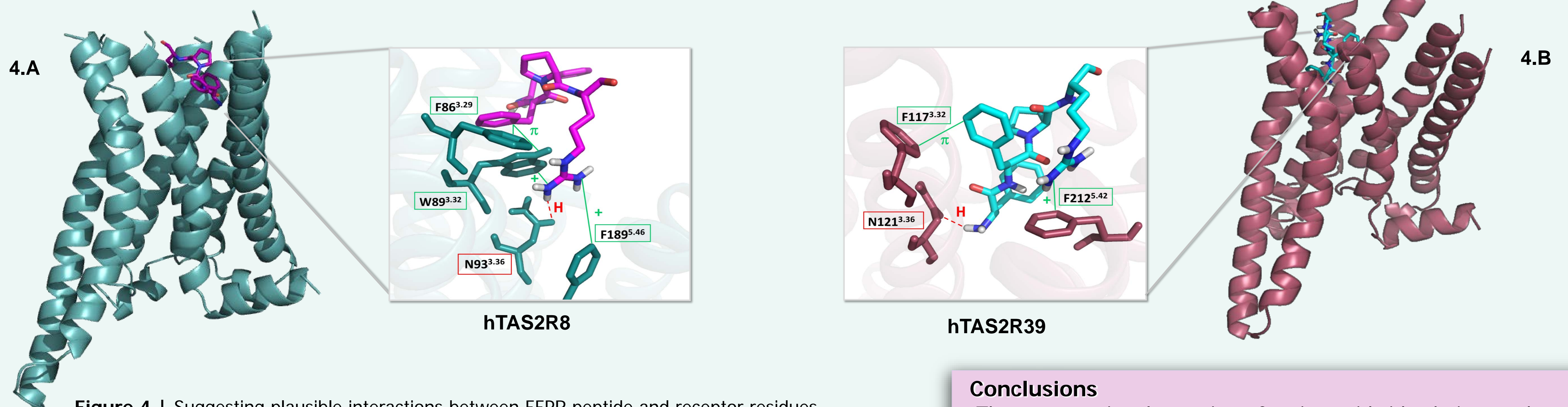


Figure 4 | Suggesting plausible interactions between FFPR peptide and receptor residues **A.** F86^{3.29}, W89^{3.32}, N93^{3.36} and F189^{5.46} of hTAS2R8. **B.** F117^{3.32}, N121^{3.36} and F212^{5.42} of hTAS2R39. Hydrogen bonds are shown as dashed red lines, Pi-Pi interactions are shown in light green lines and interaction type (cation-pi as '+' or pi-pi as 'π') is indicated.

Conclusions

- The computational mapping of amino acid side chains on the protein surfaces using ANCHORSmap coincided with computational poses docking.
- hTAS2R8 and hTAS2R39 have different binding sub-sites and binding poses of the FFPR peptide, but some of the interaction types are similar.
- Same ligand – different binding pose strategy has been already observed in other bitter taste receptors² and may represent a common trait.

References:

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