Supplementary Information

Inhibiting a promiscuous GPCR: iterative discovery of bitter taste receptor ligands

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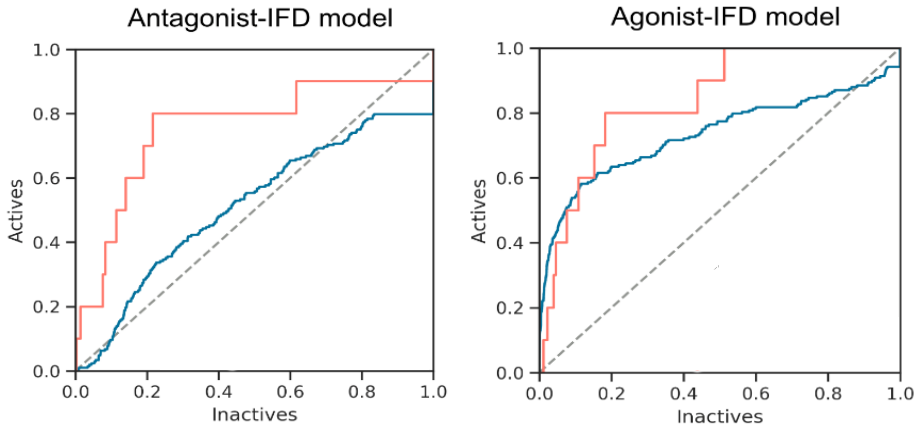
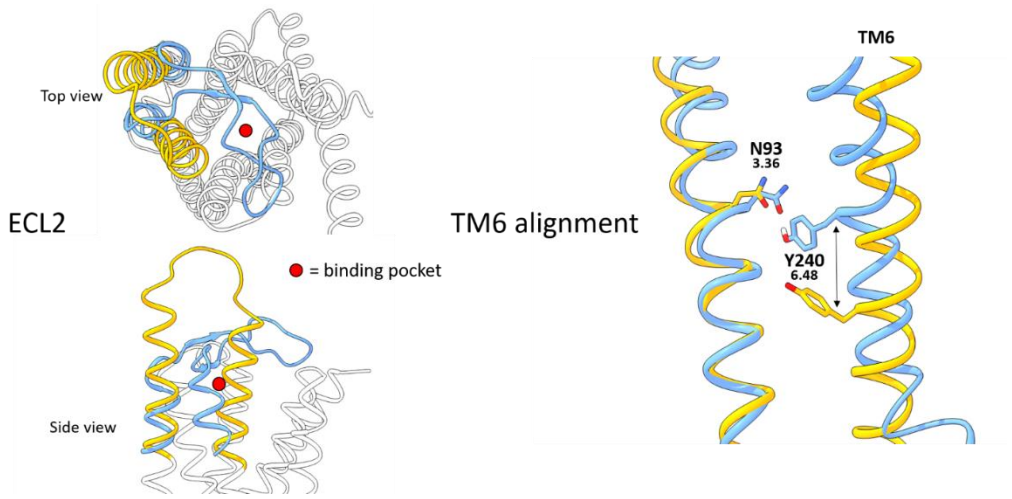


Supplementary Fig. S1 Enrichment results for iteration1. The antagonist model prioritized antagonists (orange curves) only over decoys (inactives), the agonist model prioritized both agonists (blue curves) and antagonists over decoys.

Supplementary Table S1 Functional properties of TAS2R14 activated by the agonists derived from the computational study.

	agonist effect[a]		
	EC_{50} [µM][b]	E_{max} [%][c]	N[d]
LF3	909 ± 778	77 ± 1	3
LF6	5.1 ± 2	23 ± 6	2
LF9	0.084	23.7	1
LF11	5.2±5	23 ± 20	2
LF25	16.1 ± 11	63 ± 3	3
LF26	2.8 ± 1.5	67 ± 1	3

[a]Measurement of G-protein signaling was performed applying the IP-One assay® (Cisbio) in HEK293T cells transiently co-transfected with the human TAS2R14 receptor and the hybrid G-protein G_{qi}. [b]Potency of TAS2R14 activation as mean value in µM±SEM. [c]Maximum efficacy in %±SEM relative to the full effect of flufenanmic acid. [d] Number of individual experiments all performed in triplicates.



Supplementary Fig. S2 Structural alignment between AlphaFold (orange) and IT3+ (blue) showing the differences between the two models. Within the active structure of the TAS2R46 template used to generate a homology model of TAS2R14 (not shown within this picture), the ECL2 is not solved. Therefore, our model is also missing the same loop. TM6 alignment of the homology model based on TAS2R46 overlap with the AlphaFold one.

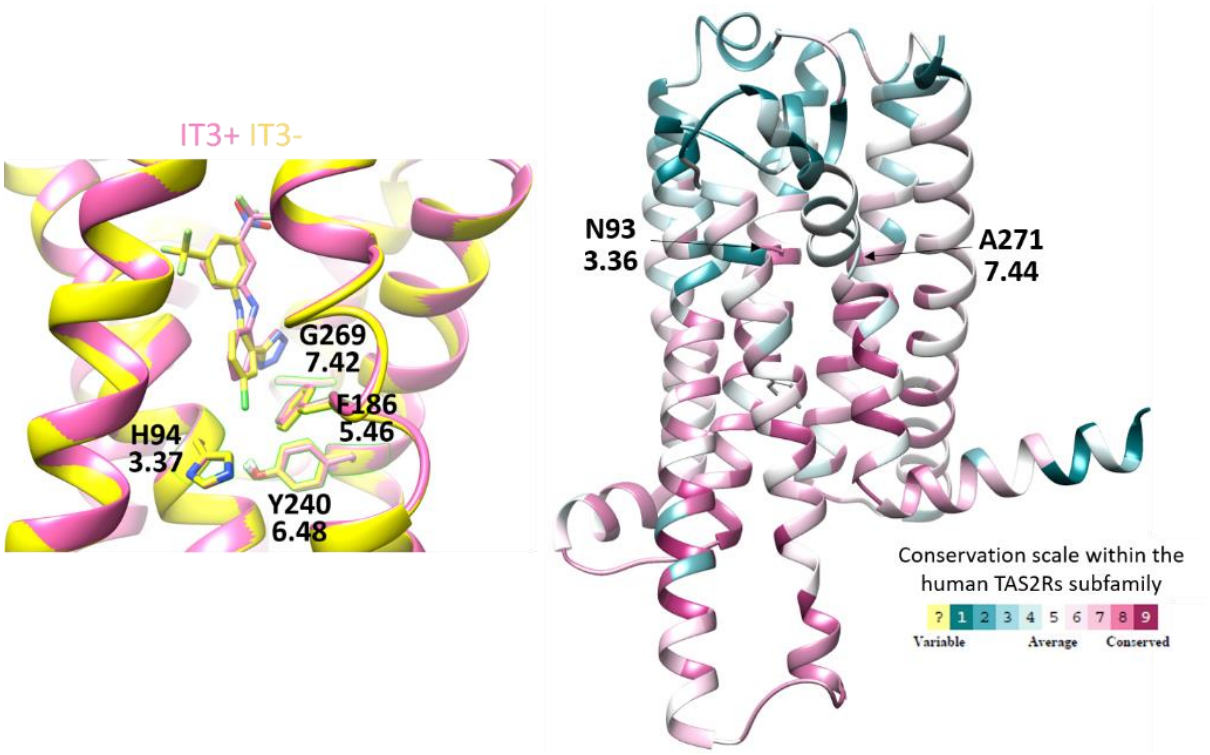
Supplementary Text S1: Additional residues potentially involved in agonist vs antagonist discrimination according to different analysis.

Mutagenesis data: vast mutagenesis data for TAS2R14 is available from [1] showing that the same mutation may affect the receptor in a ligand-dependent manner. Since many of the new agonists and antagonists are derivatives of the FFA, it is reasonable to focus on the mutations that lead to total lack of activation by FFA (non-detectable EC_{50} values). These are H94E$^{3.37}$, F186L$^{5.46}$, Y240A$^{6.48}$, and G269I$^{7.42}$. The potential role of Y240$^{6.48}$ in agonist/antagonist discrimination is discussed in the "Agonist/antagonist model comparison", and "Receptor activation" paragraphs of the main paper. As shown in Supplementary Fig. S2 (left panel), the other critical residues (according to the EC_{50} values of their mutations) are located within the same region.

The orientation of H94$^{3.37}$, F186$^{5.46}$, and G269$^{7.42}$ is identical between IT3+ and IT3-. This could be due to the shortcomings of our models, or suggest that these residues are not directly involved in binding or activation mechanisms.

Conservation analysis: TAS2Rs have a low sequence identity within the subfamily, e.g. TAS2R14 sequence identity with other human TAS2Rs is between 21% and 48%. 5 additional TAS2Rs (TAS2R1, 13, 39, 40, 41) simultaneously conserve N3.36 and Y6.48 - the two residues we suggest as involved in agonist/antagonist discrimination. Only TAS2R13 conserves also positions H94$^{3.37}$, F186$^{5.46}$ and G269$^{7.42}$. Therefore, the details of activation mechanism may be similar between TAS2R14 and TAS2R13, but this hypothesis requires further study, and detailed understanding of activation mechanisms probably must await future advances in experimental determination of TAS2R structures. The region expected to discriminate between agonists and antagonists, located in the deeper region of the binding site, shows only a few conserved positions including N93$^{3.36}$ and A271$^{7.44}$ (right panel of Supplementary Fig. S2).

Knowledge derived from class A GPCRs: positions 3.36 and 6.48 are well known in many GPCRs to be involved in transferring the information carried by the ligand (agonist/antagonist) into receptor activation/inactivation. Other positions involved in the same mechanism, such as 6.52 and 5.47 in S1PR [2] do not change their orientation within our models.



Supplementary Fig. S3: Left panel shows residues whose mutation brings to receptor's inactivation according to [1]. All of these residues are topologically close, confined within the same area. No major differences in side chain orientations were observed between IT3+ (pink) and IT3- (yellow) for those residues. Right figure: residues conservation across human TAS2Rs. As shown within the figure legend, most conserved positions are in dark magenta, less conserved in green (prepared using Consurf [3]). N93 and A271 are the most conserved positions next to the ligand region and may be involved for relaying agonist-induced activation pathway.

References

1. Nowak S, Di Pizio A, Levit A, Niv MY, et al (2018) Reengineering the ligand sensitivity of the broadly tuned human bitter taste receptor TAS2R14. Biochimica et Biophysica Acta (BBA)-General Subjects 1862(10): p. 2162-2173.
2. Maeda S, Shiimura Y, Asada H, Hirata K, et al (2021) Endogenous agonist–bound S1PR3 structure reveals determinants of G protein–subtype bias. Science Advances 7(24): p. eabf5325.
3. Ashkenazy H, Abadi S, Martz E, Chay O, et al (2016) ConSurf 2016: an improved methodology to estimate and visualize evolutionary conservation in macromolecules. Nucleic Acids Research 44(W1): p. W344-W350.