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Insights into the Structural Determinants of Bitter Taste Receptors from a Combined in silico and in vitro study

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Insights into the **Structural Determinants of Bitter Taste Receptors** from a Combined *In silico* and *In vitro* Study

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Why GPCR's are important?

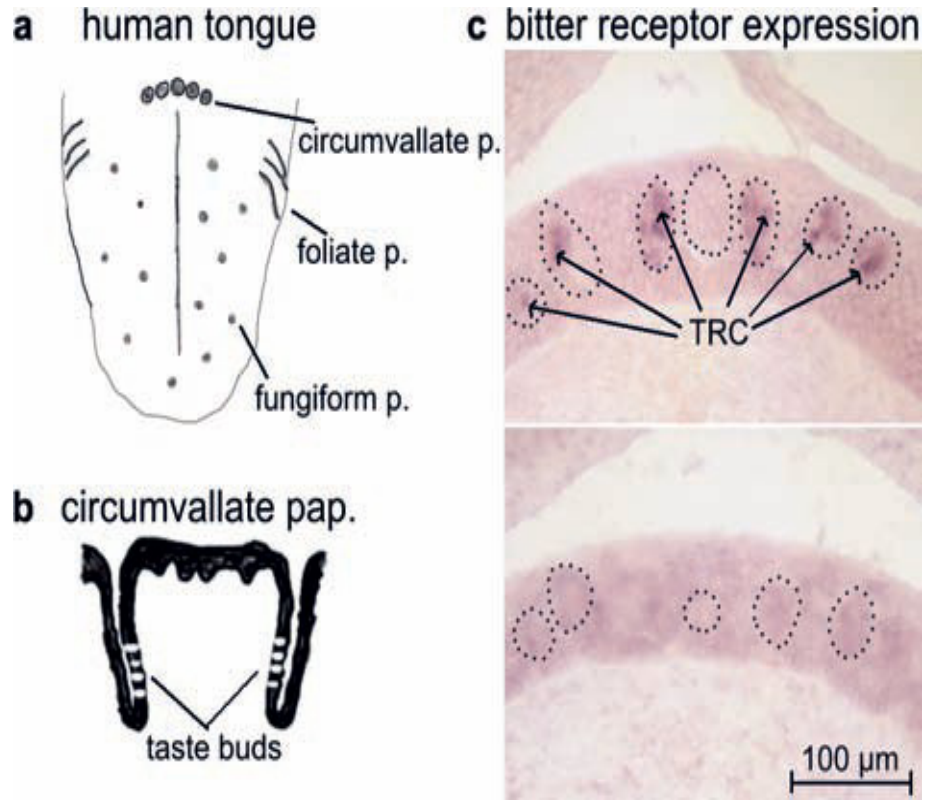
Eukaryotic 7TMR's: G-protein coupled receptors (GPCRs)

- Signaling pathways are based on the interaction with G-proteins.
- Are involved in about **~80%** of signal transduction pathways
- Constitute approximately **3-4%** of mammalian genes
- GPCRs devoted to perception (in particular bitter taste, and more, smell) are predominant (50% of all the family). In humans, more than 400 GPCRs out of 900 are odorant receptors, ~30 are bitter taste receptors , one for sweet taste (T1R2), two are umami receptors (T1R1 and T1R3) four of them (opsins) are visual receptors

The ingestion of a large variety of toxic substances has evolutionary been prevented in humans and in other mammals by an unpleasant perception for bitter tasting food

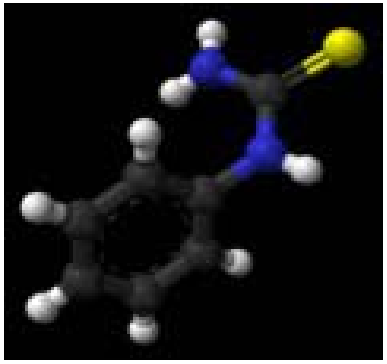
Bitter substances bind to and are discriminated by a family of roughly ~30 bitter taste receptors (TAS2Rs) expressed in taste receptor cells

Bitter compound binding to its cognate target TAS2R initiates a downstream cascade of events inside the cell typical of GPCRs signaling pathways. This cascade ultimately leads to bitter perception



M. Behrens and W. Meyerhof. Cell. Mol. Life Sci. 2006

hTAS2R38



The ability to taste phenylthiocarbamide (PTC) is a classic phenotype that has long been known to vary in human populations.

This phenotype is of genetic, epidemiologic, and evolutionary interest because the ability to taste PTC is correlated with the ability to taste other bitter substances, many of which are toxic.

Variation in PTC perception may reflect variation in dietary preferences throughout human history and could correlate with susceptibility to diet-related diseases in modern populations

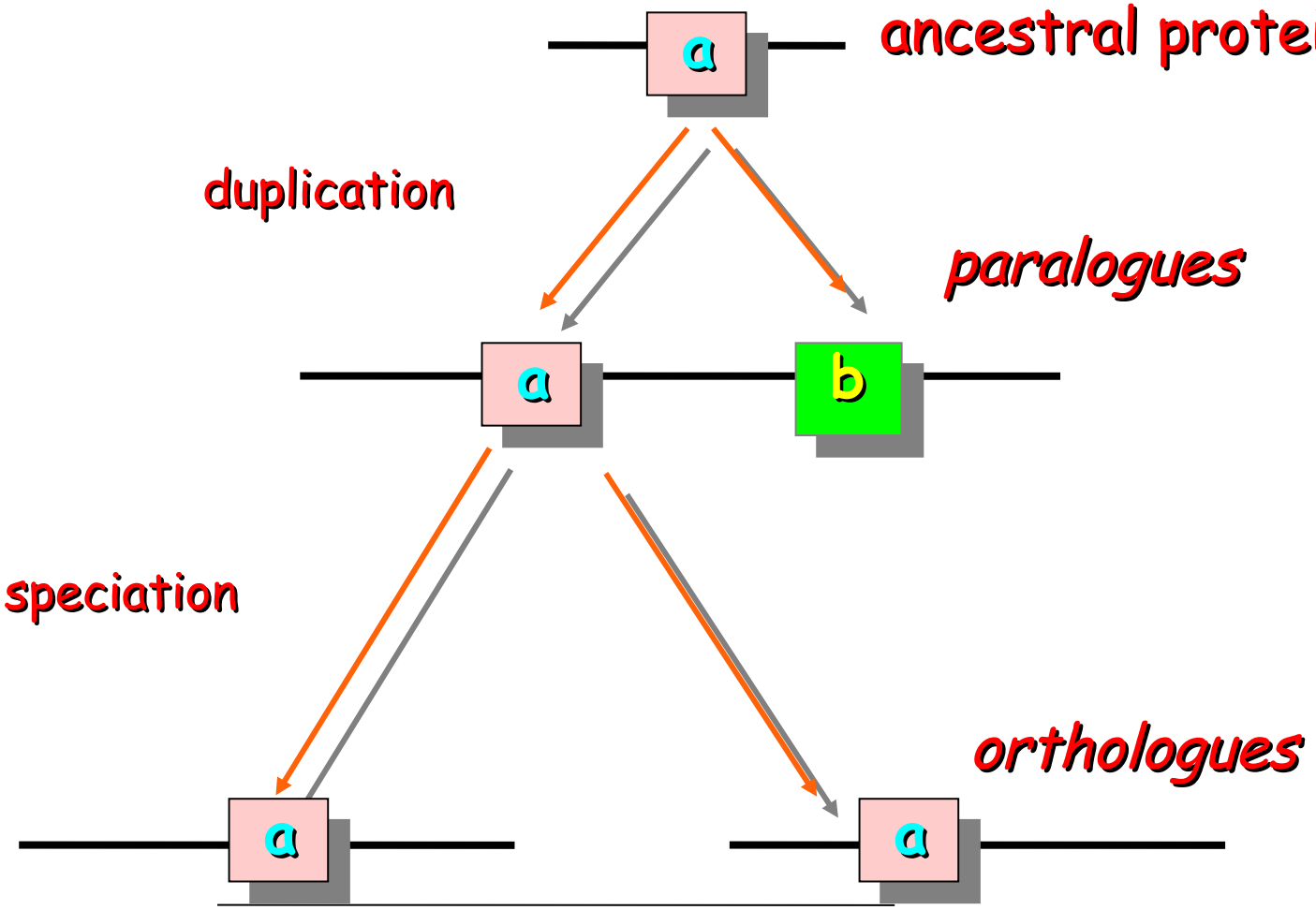
Wooding et al. Am. J. Hum. Genet. 2004

Uncovering the molecular basis of bitter perception is hence crucial for food research

Computational molecular biology methods

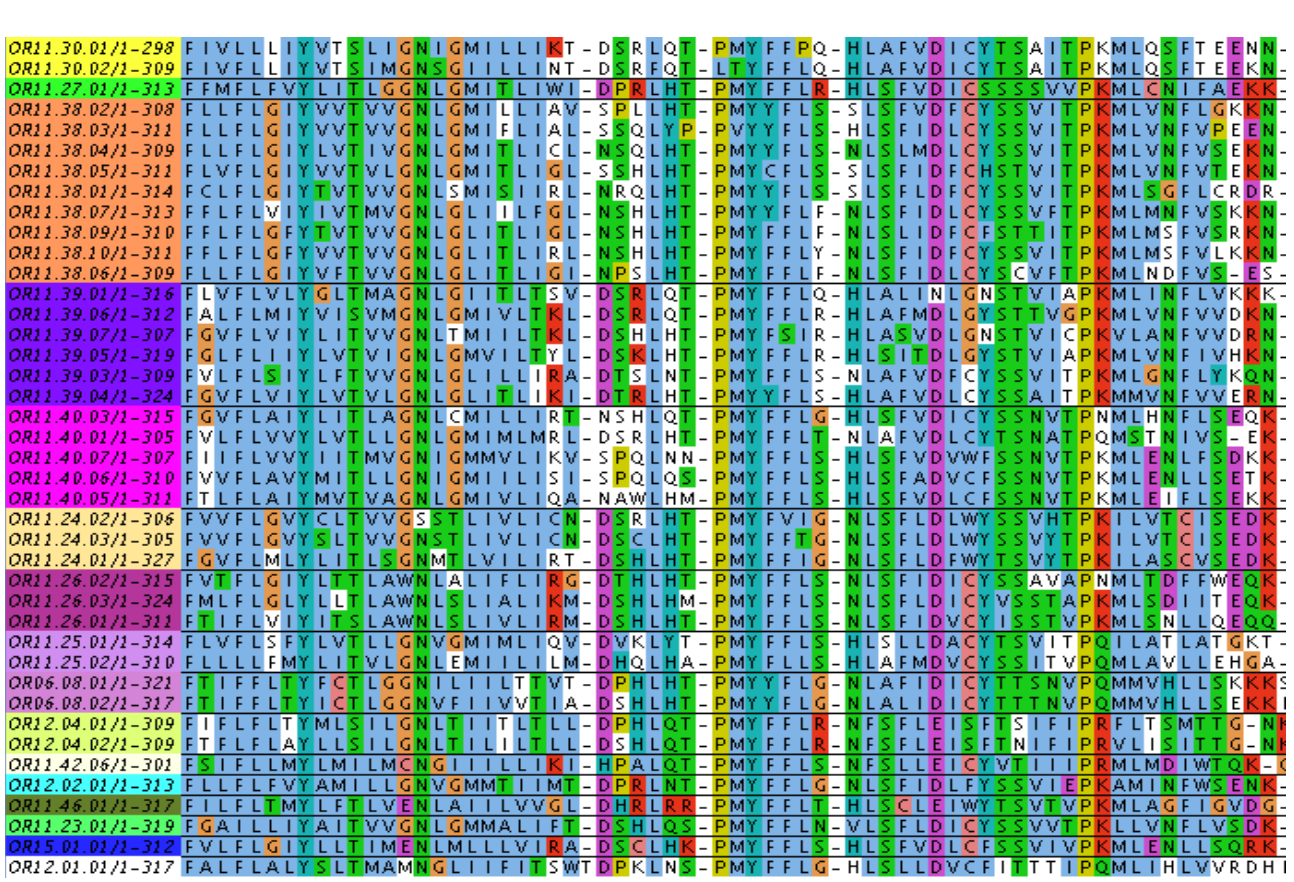
- The first strategy, the so-called ***protein bioinformatics***, is aimed at the development of computational tools that enable to decipher the information encoded in the protein sequences, thus enabling the prediction of structure and function
- The second strategy is based on the laws of physics. One of the most important methods here is ***molecular dynamics*** (MD) , which predicts structural, dynamical, and energetic (bio)molecular properties based on Newton laws of motion.

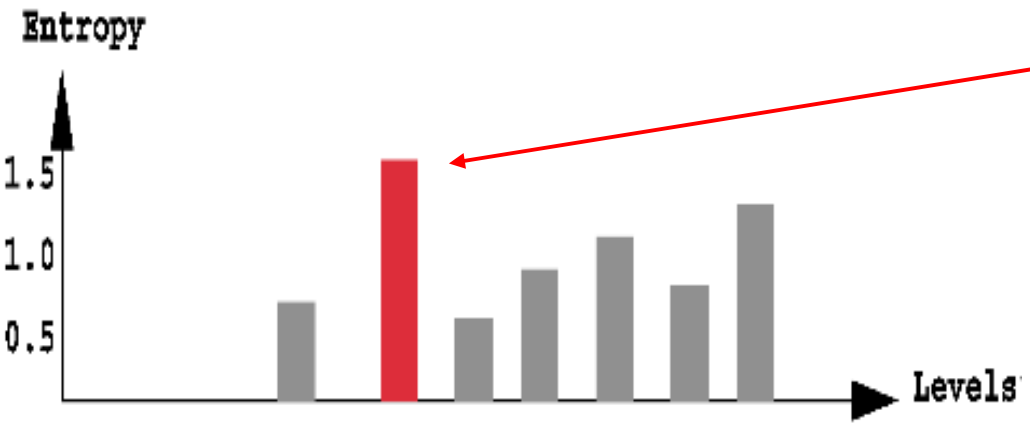
Homology based inference of protein functions

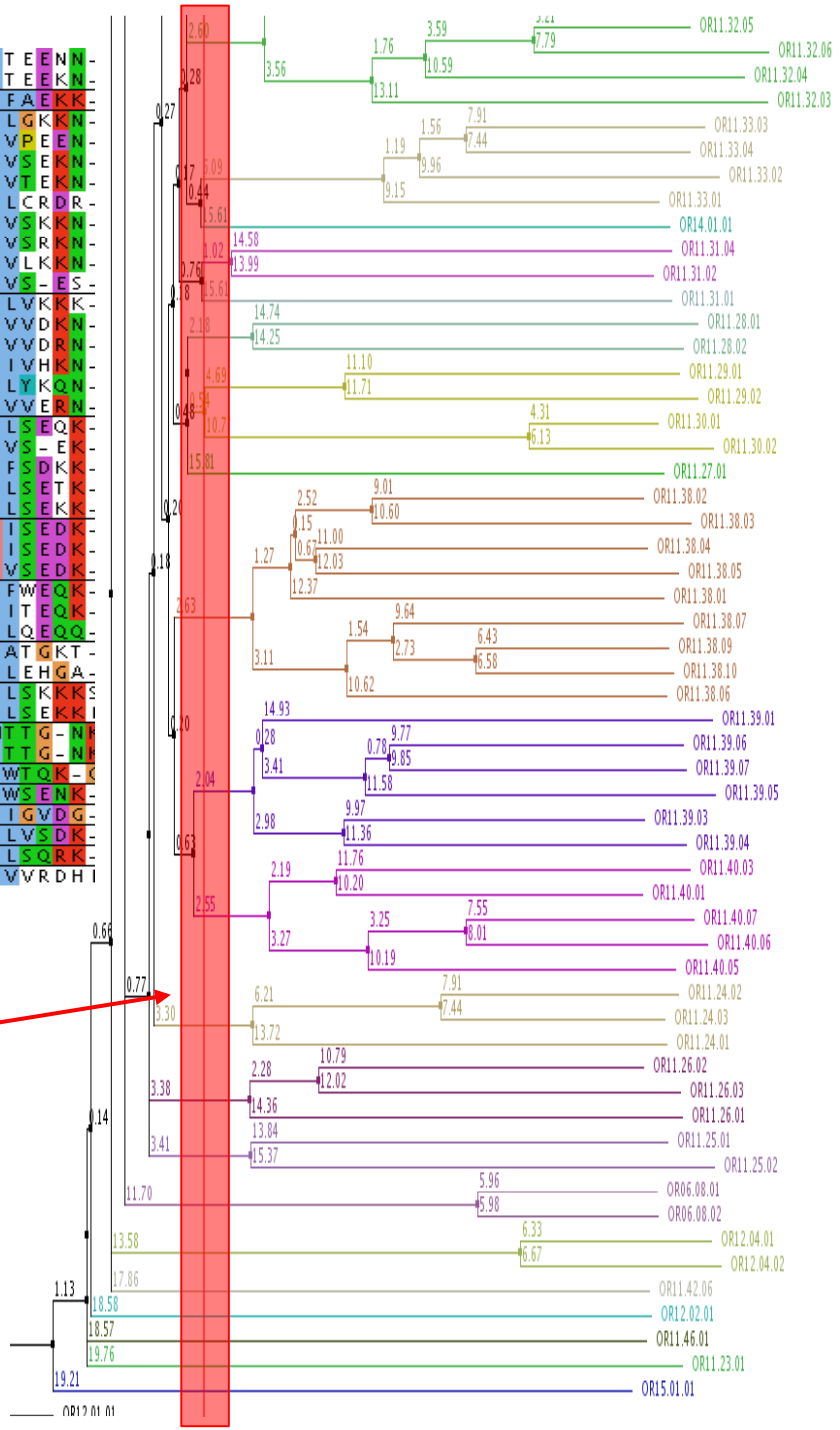


- orthologues - often have very similar functions
- paralogues - may have related functions

Identification of functional subfamilies

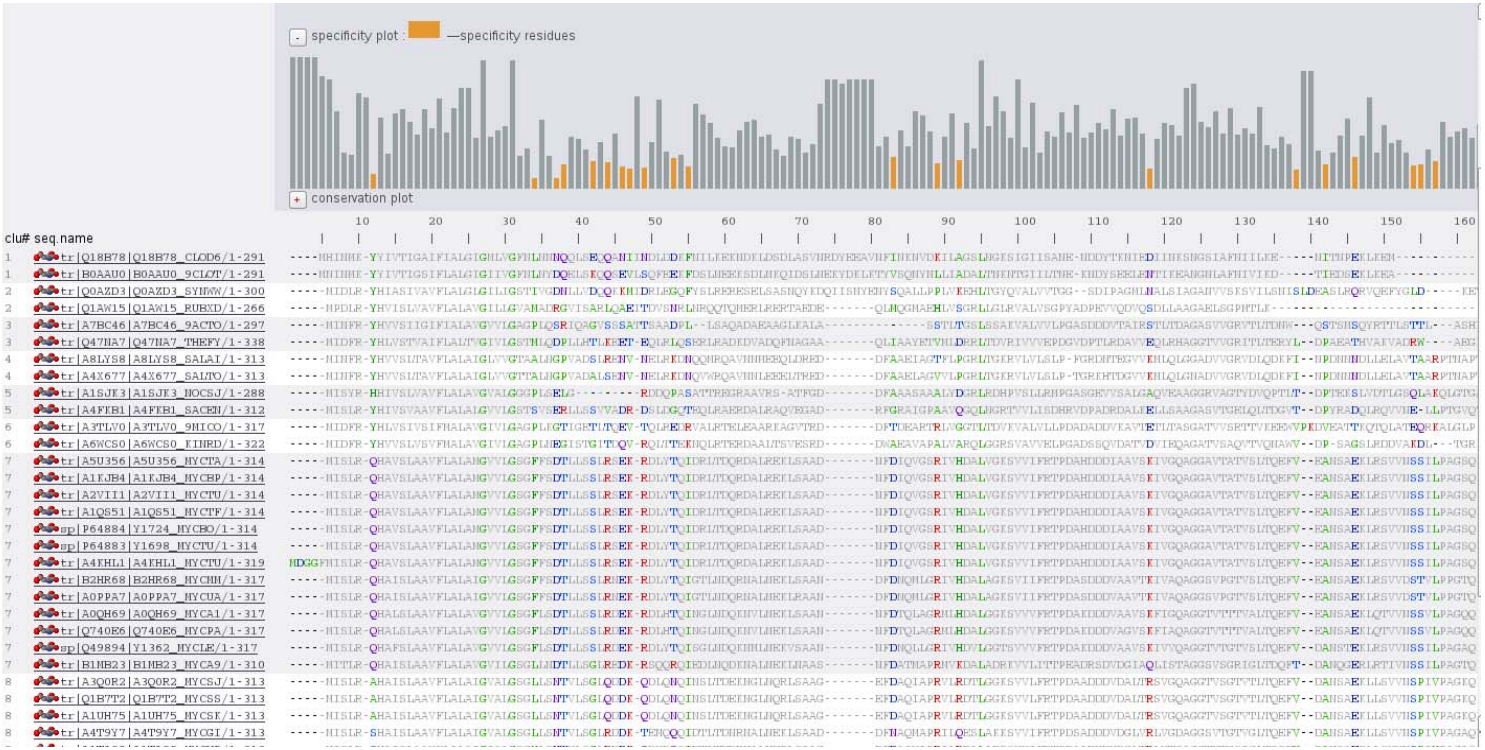




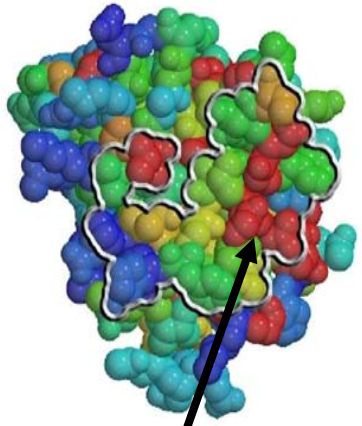


Identification of functional subfamilies

multiple sequence alignment, clustering and amino acid identification from functional subfamily

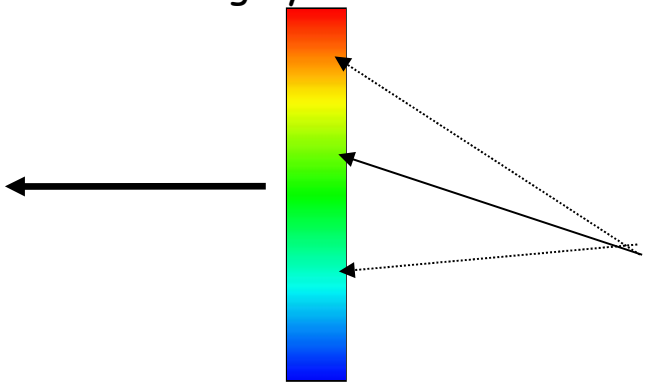


Structural model



Putative functional site

1 = highly conserved

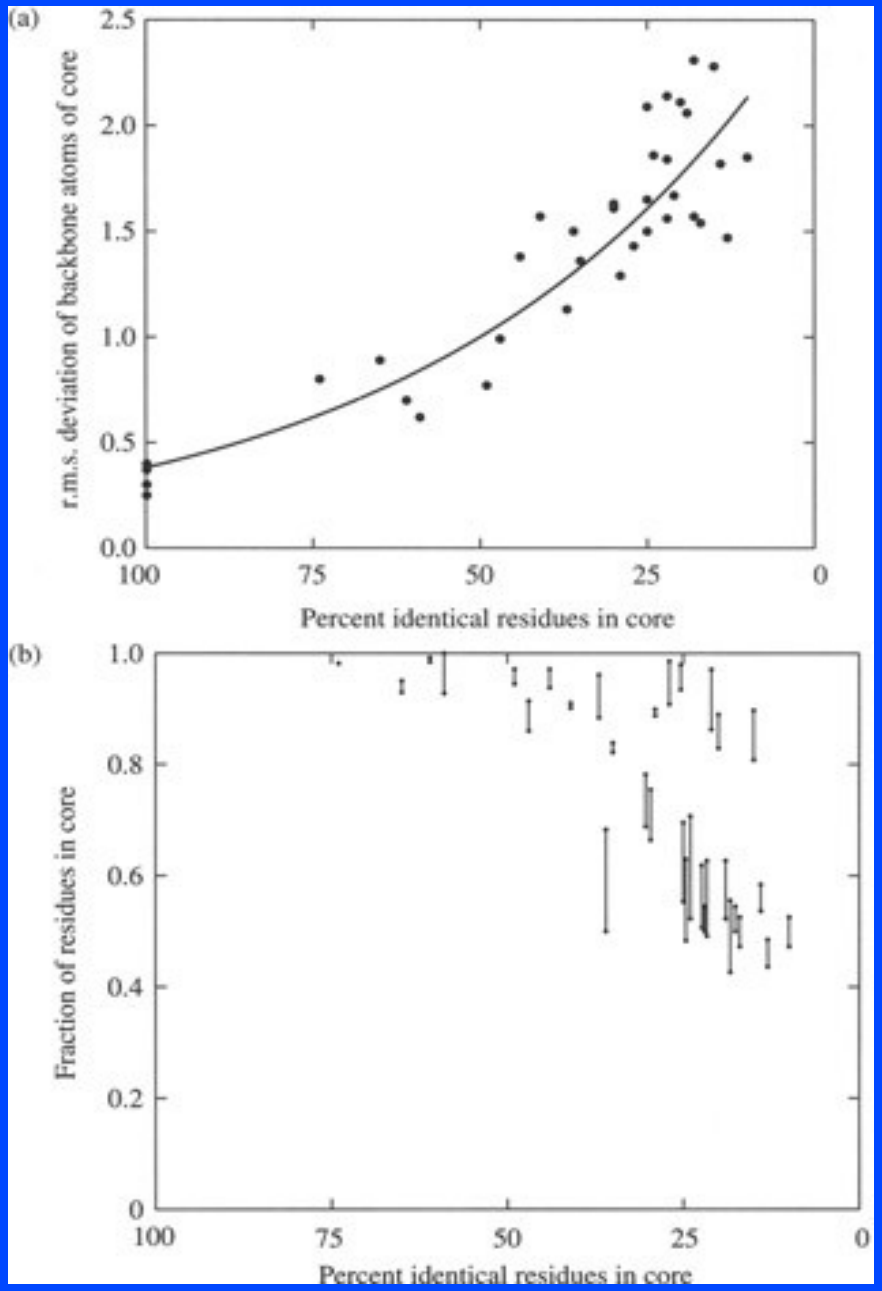


0 = unconserved

$$H = -\sum_{i=1}^{M} P_i \log_2 P_i$$

Where Pi is the fraction of residues of amino acid type i, and M is the number of amino acid types

Protein evolution



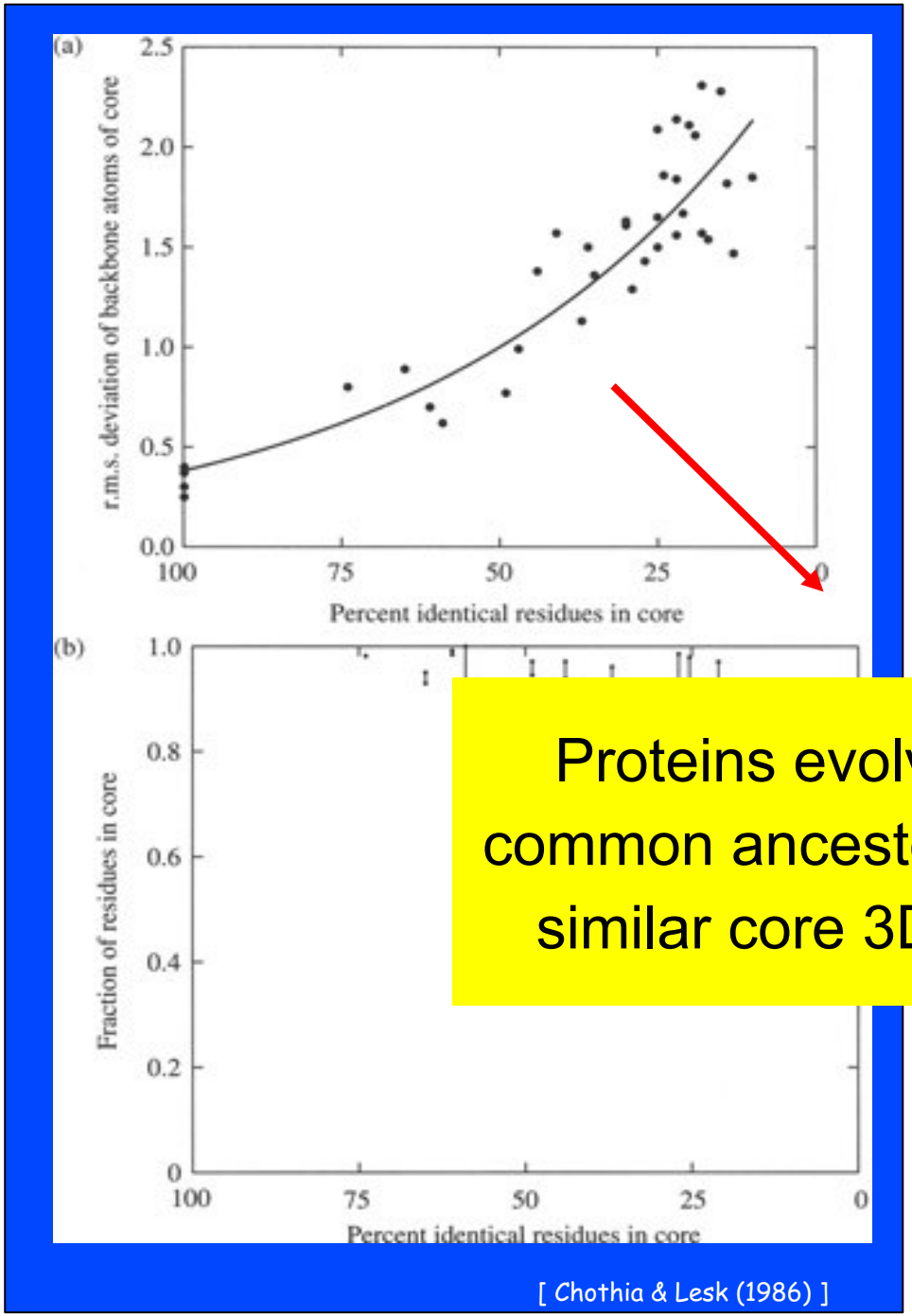
[Chothia & Lesk (1986)]

Comparative modelling

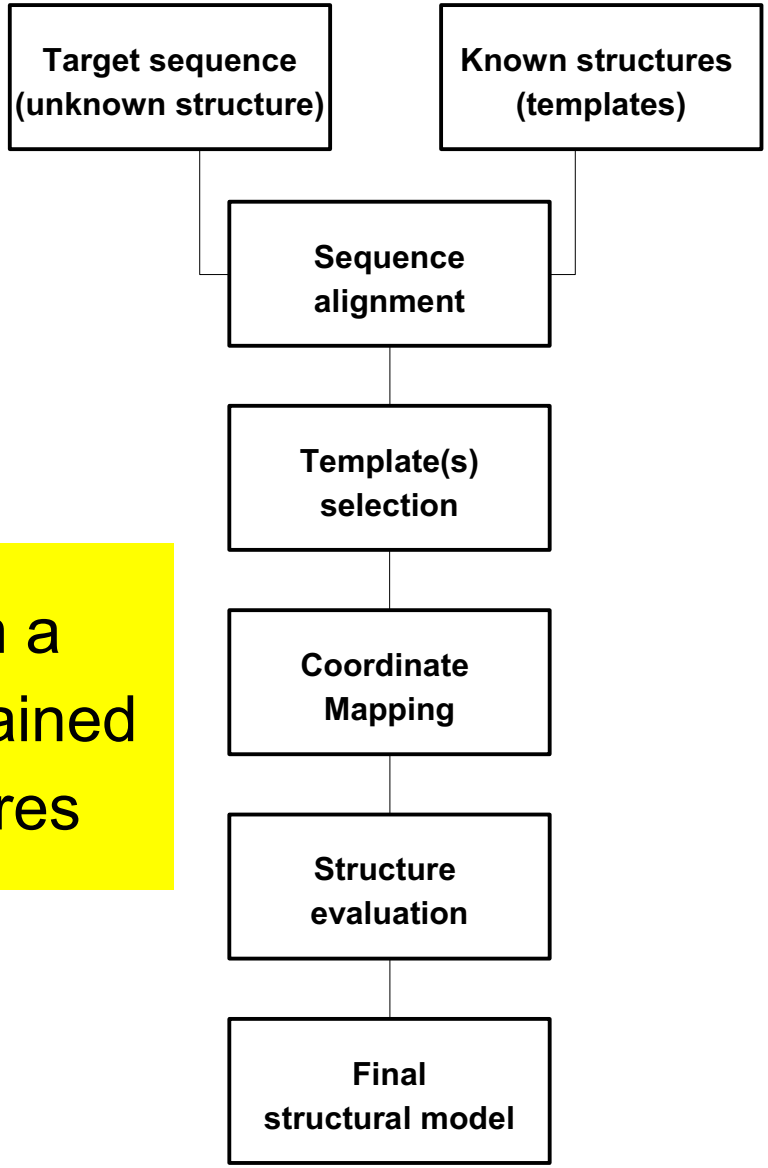
mutation of one amino acid:

- the protein does not fold any more
- the protein accommodates the replacement with minor modifications: evolutionary pressure!
- the protein folds in a completely different structure

Homology modeling



Proteins evolving from a common ancestor maintained similar core 3D structures



BITTER TASTE RECEPTORS

- Target sequence:

- Bitter taste receptor hTAS2r38

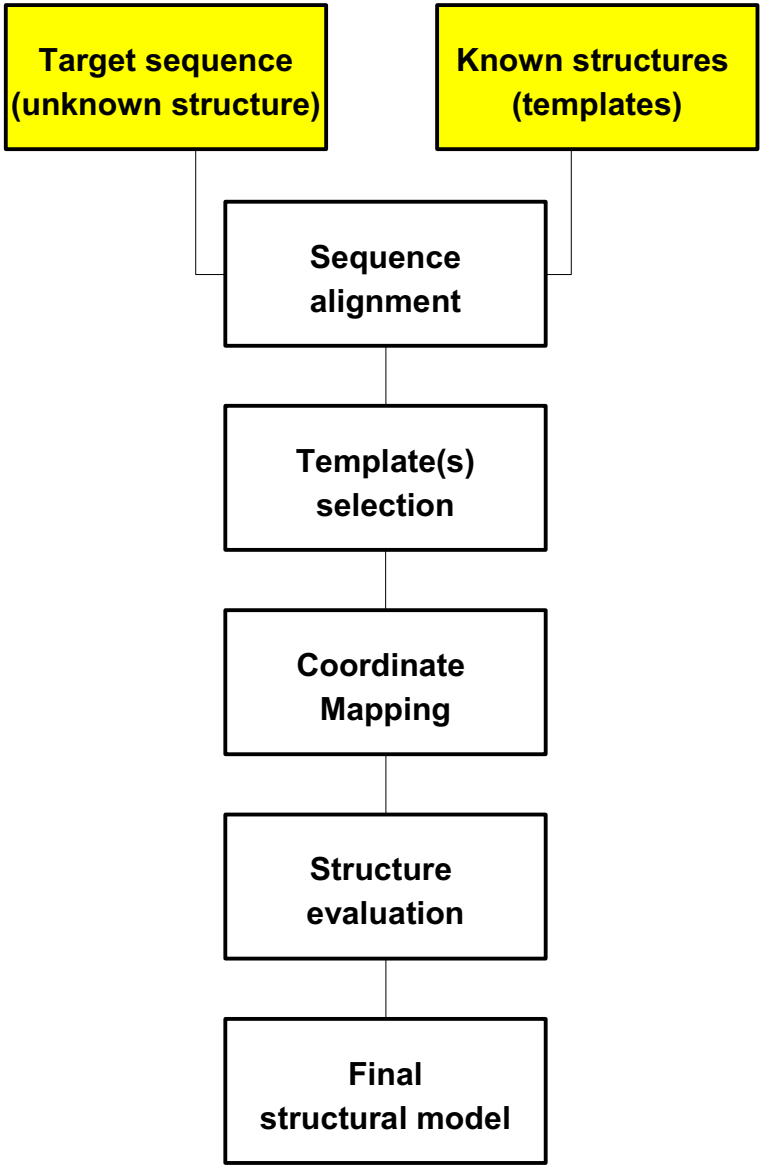
- Templates search (known structural GPCRs):

  – Bovin rhodopsin
  Palczewski et al. Science. 289,739-745. (**2000**)
  Okada et al J.Mol.Biol. 342: 571-583 (2004)

  – Human beta-2 adrenergic receptor
  PDB: 2RH1
  Cherezov et al Science 318: 1258-1265 (2007)

  – Turkey beta-1 adrenergic receptor
  PDB: 2VT4
  Warne et al Nature 454: 486 (2008)

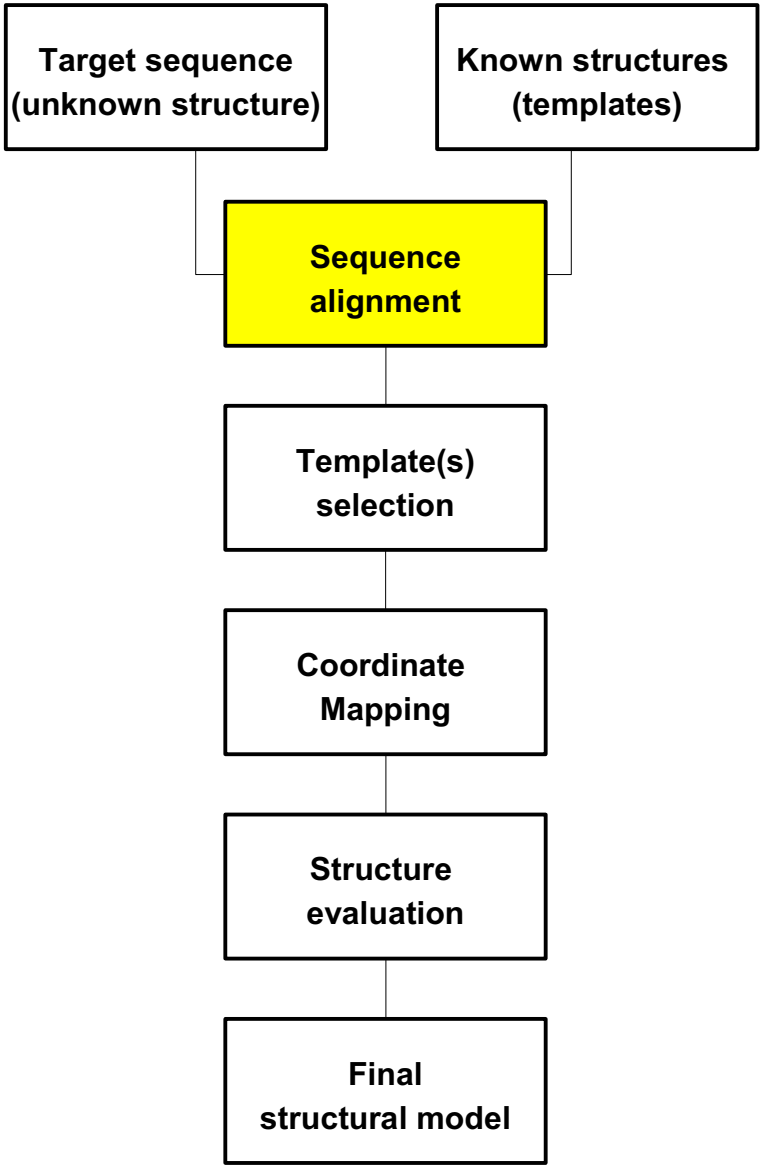
  – Human adenosine receptor A2A
  PDB: 3EML
  Jaakola *et al* Science 322: 1211-1217 (2008)



BITTER TASTE RECEPTORS

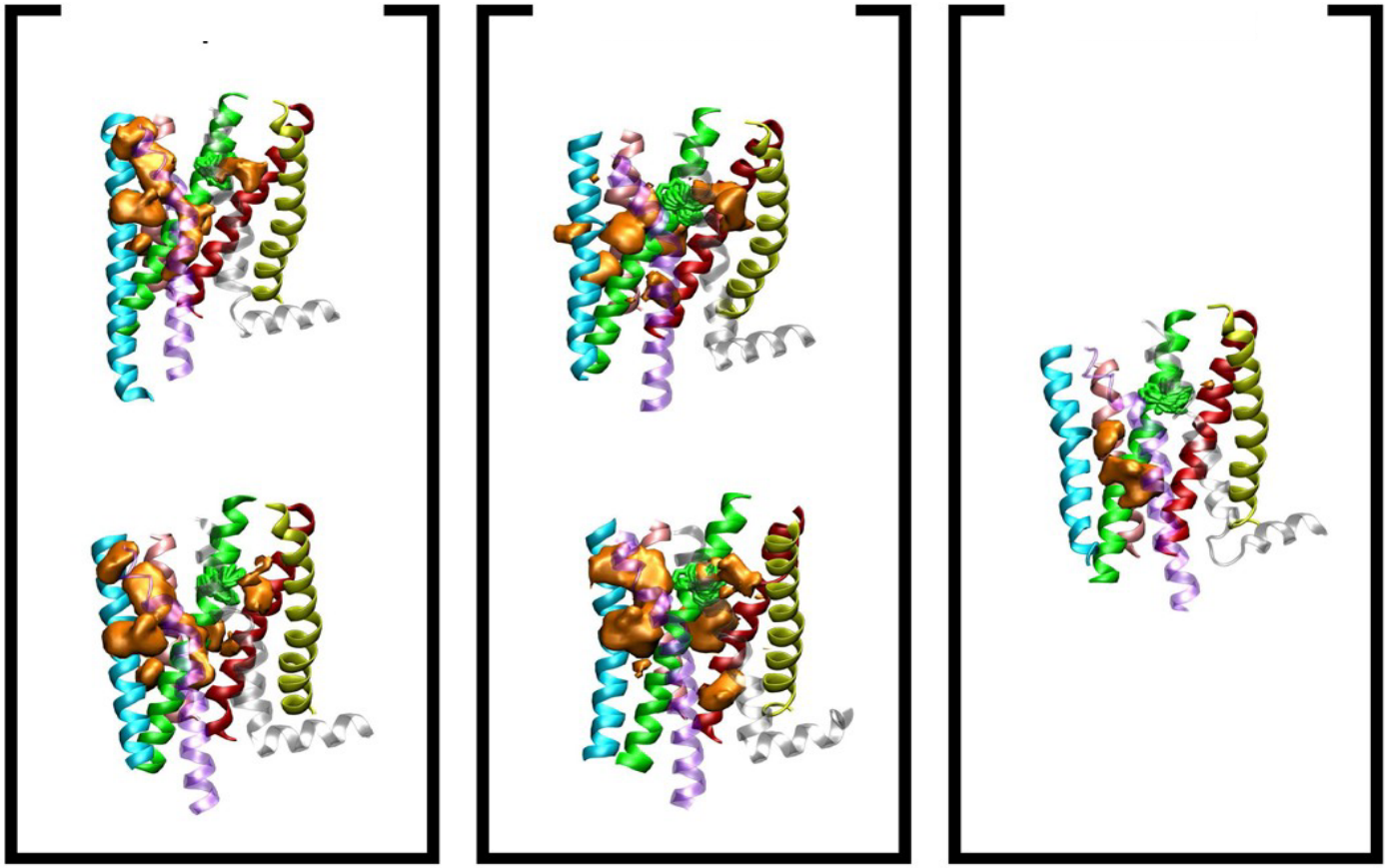
Sequence alignment:

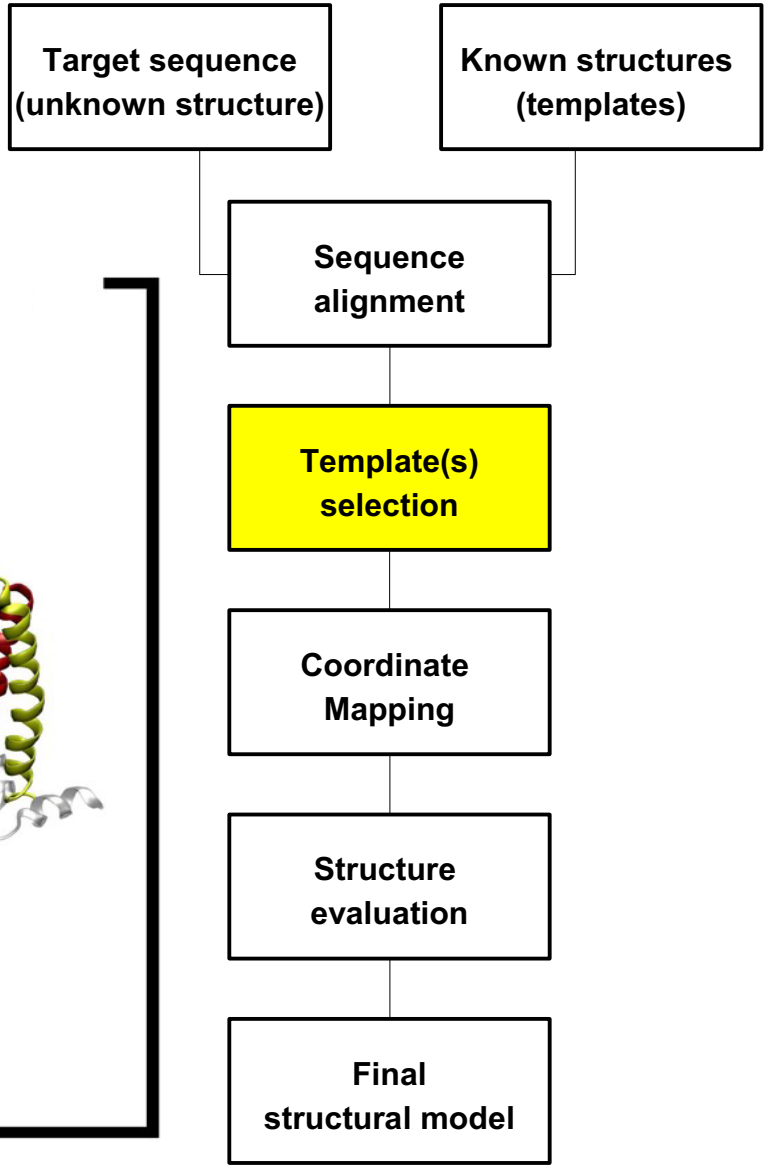
- 30 bitter taste receptors
- Alignment using PROMALS (takes into account secondary structure elements)
- Generation of the target's HMM-profile
- HMM-HMM alignments with a template database (HHsearch)
- Pairwise alignment extraction between templates and target
- Sequence identity about 13 - 16 %



BITTER TASTE RECEPTORS

Templates: different activation states





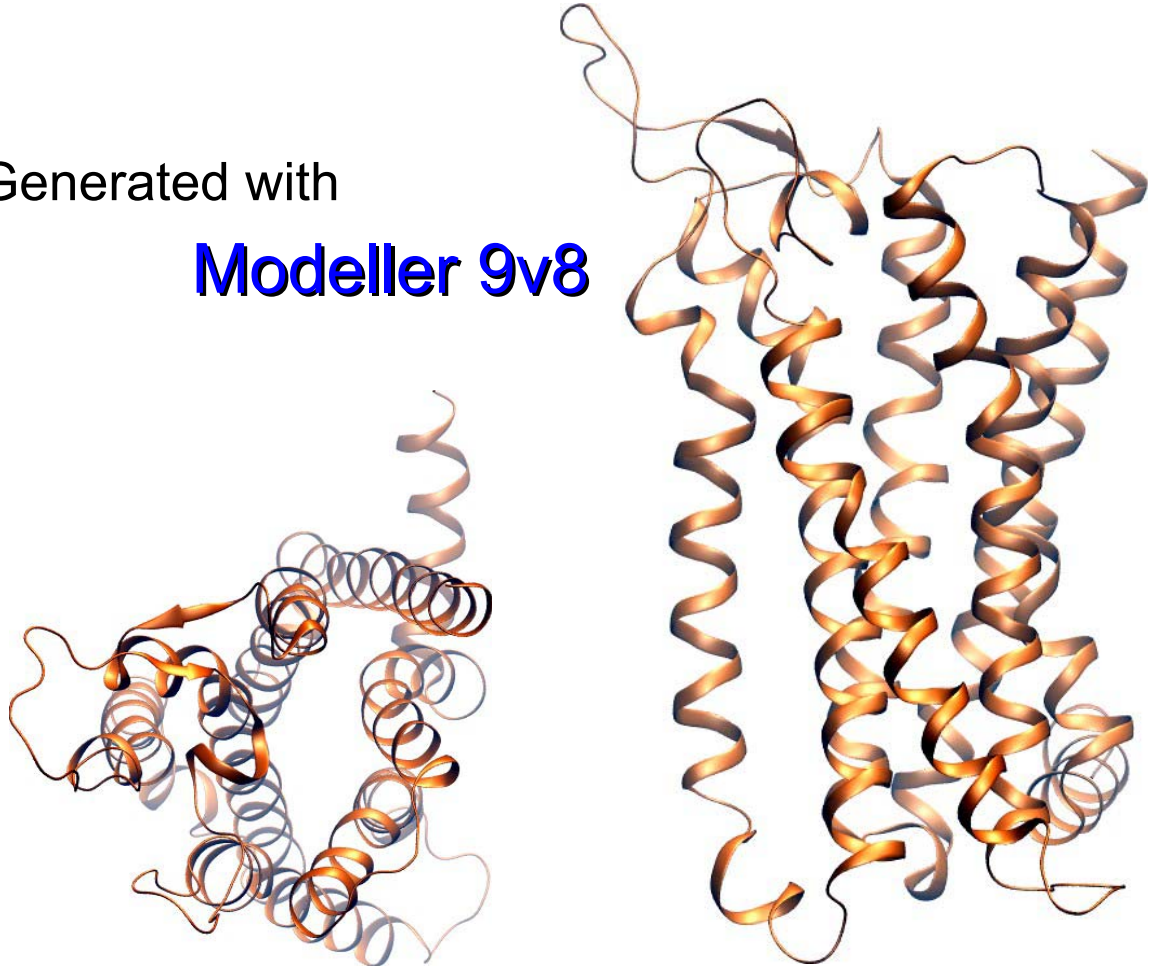
Biarnés et al.(2010). Plos One

BITTER TASTE RECEPTORS

50 structural models of hTAS2R38
based on **ALL available** templates.

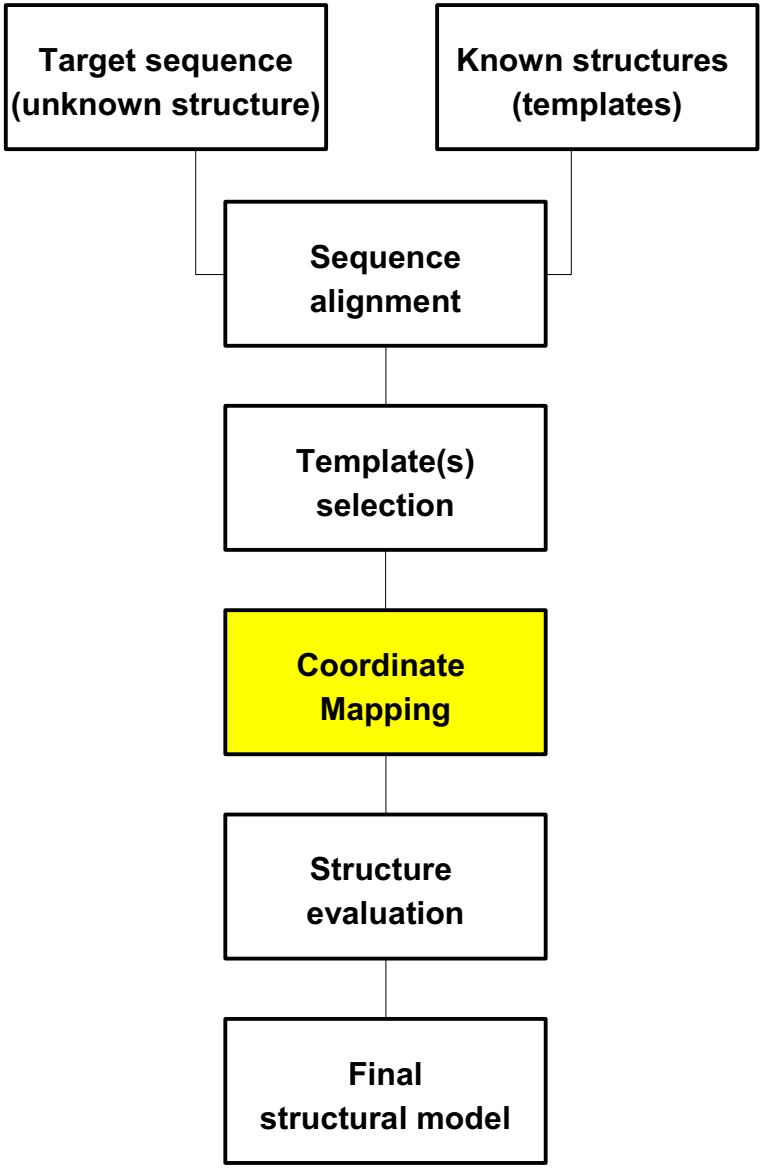
Generated with

Modeller 9v8



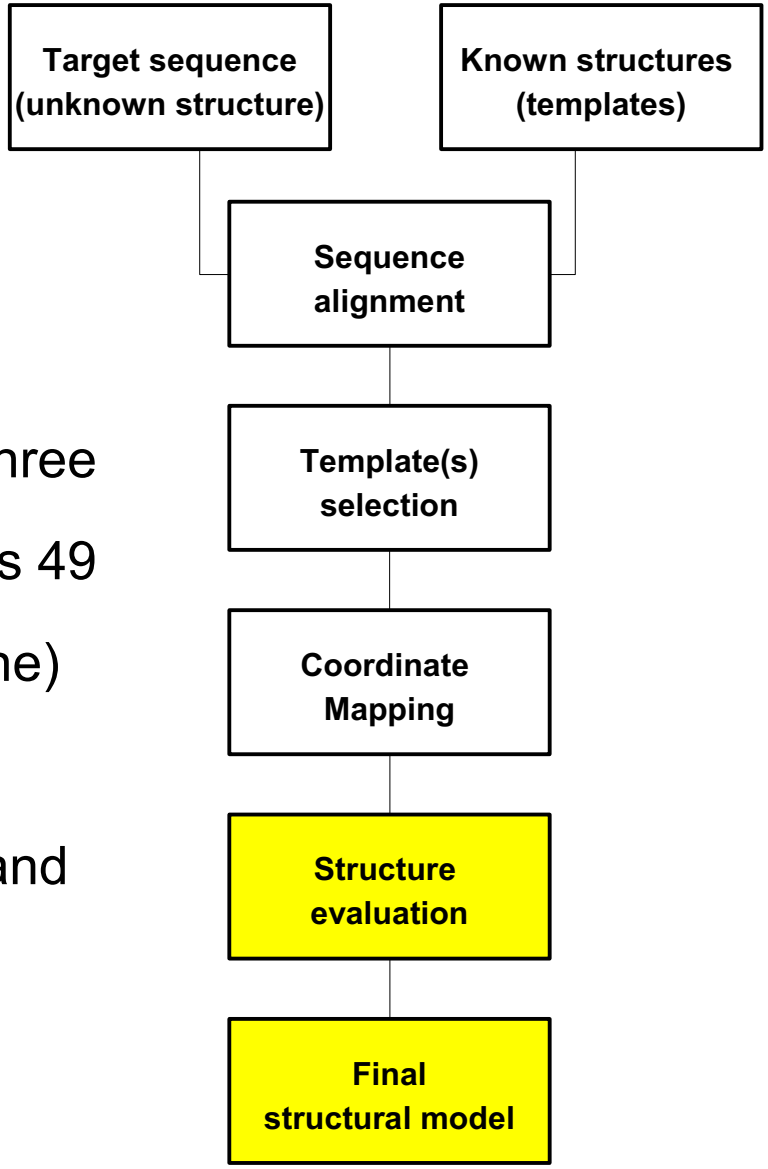
top view

side view



BITTER TASTE RECEPTORS

Experimental validation



Most of the variation in this gene is explained by three common **amino-acid polymorphisms** at positions 49 (encoding proline or alanine), 262 (alanine or valine) and 296 (valine or isoleucine) that determine two common isoforms: proline–alanine–valine (PAV) and alanine–valine–isoleucine (AVI)

Site directed mutagenesis is needed.

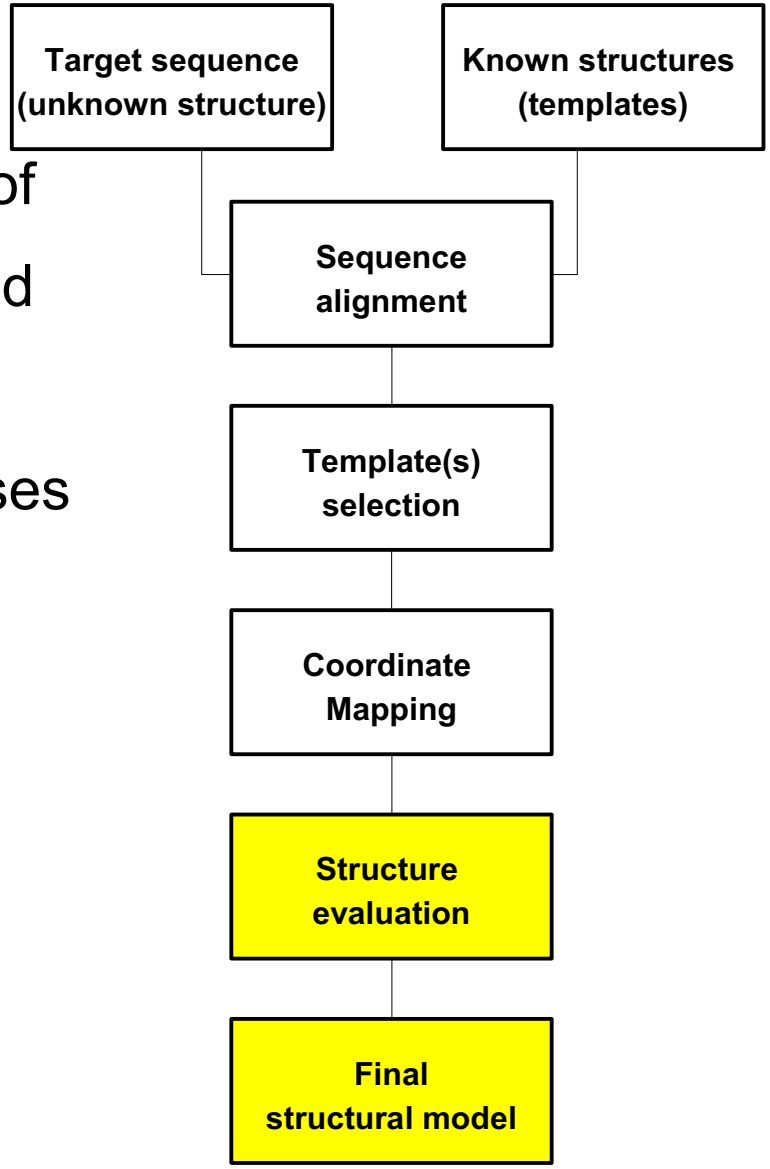
BITTER TASTE RECEPTORS

Functional assay: Calcium imaging

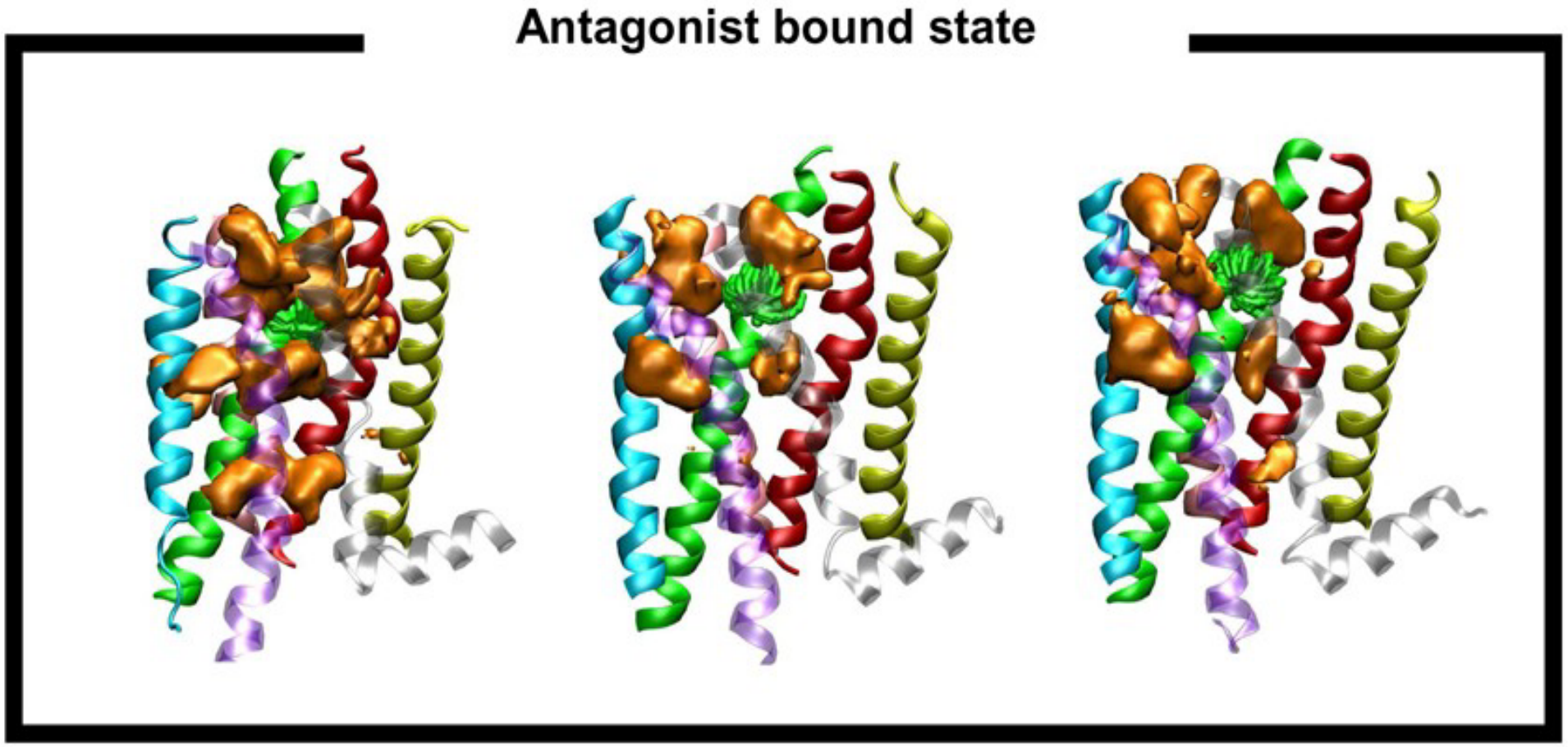
*Ba*sed on the fluorescence emission increase of intracellular probes, Fluo4-AM dye, when bound to Ca^{2+}.

- Cytoplasmatic Ca^{2+} concentration increases upon GPCRs activation, the increase of fluorescence of the probes inside cells is associated with activation by agonist

- Positive (PAV variant) and negative controls have been performed.



BITTER TASTE RECEPTORS



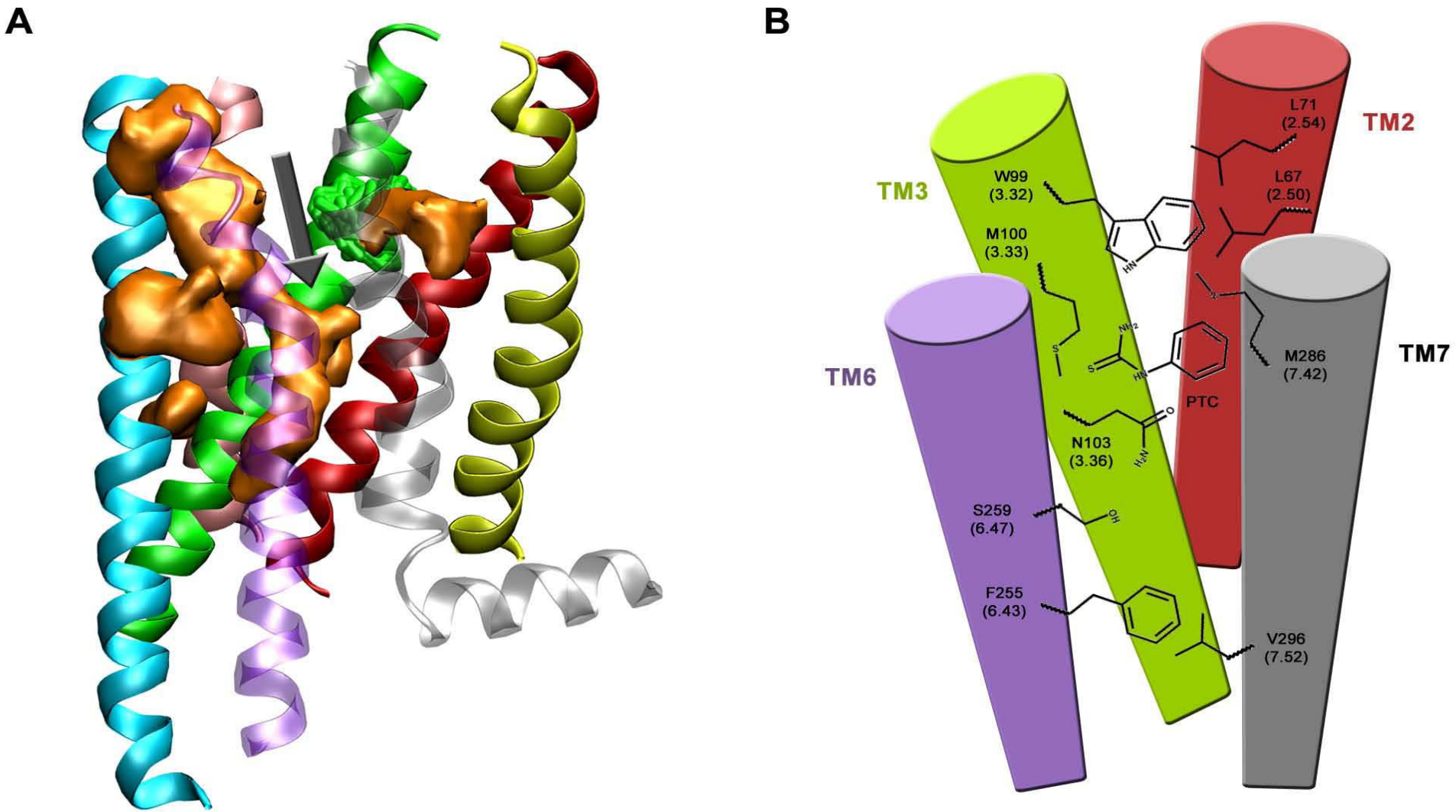
hTAS2R38/PTC using Autodock.

Lamarckian Genetic Algorithm for configurational exploration

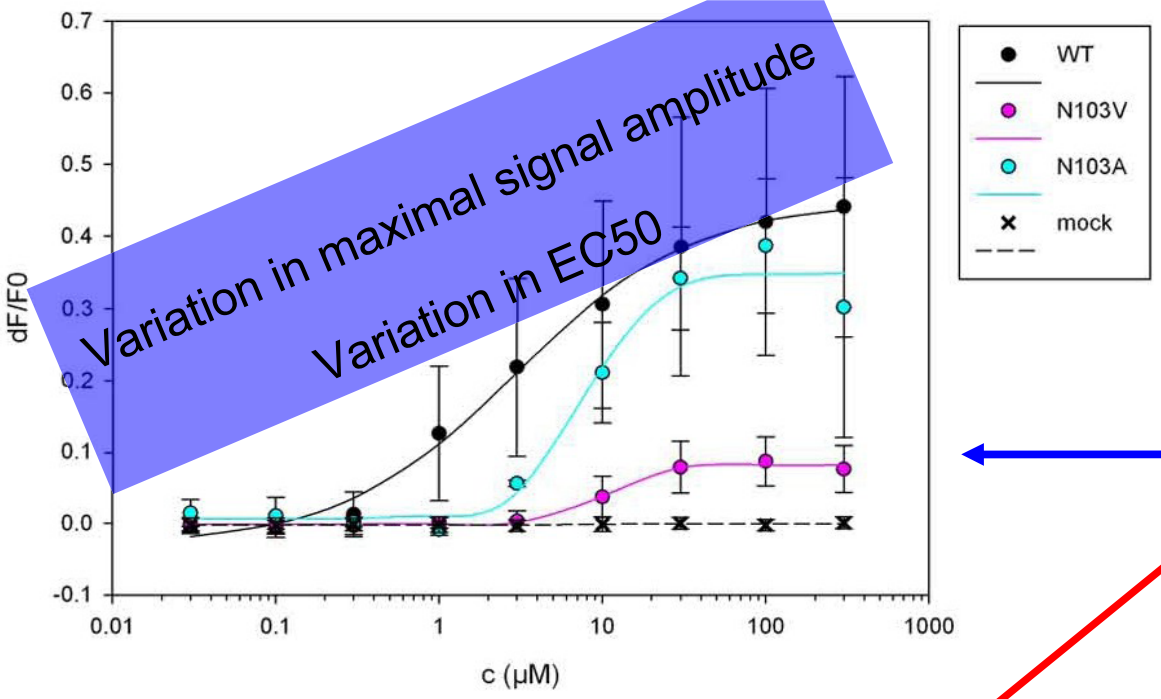
100 decoys of PTC compound binding to 50 hTAS2R38 conformations.

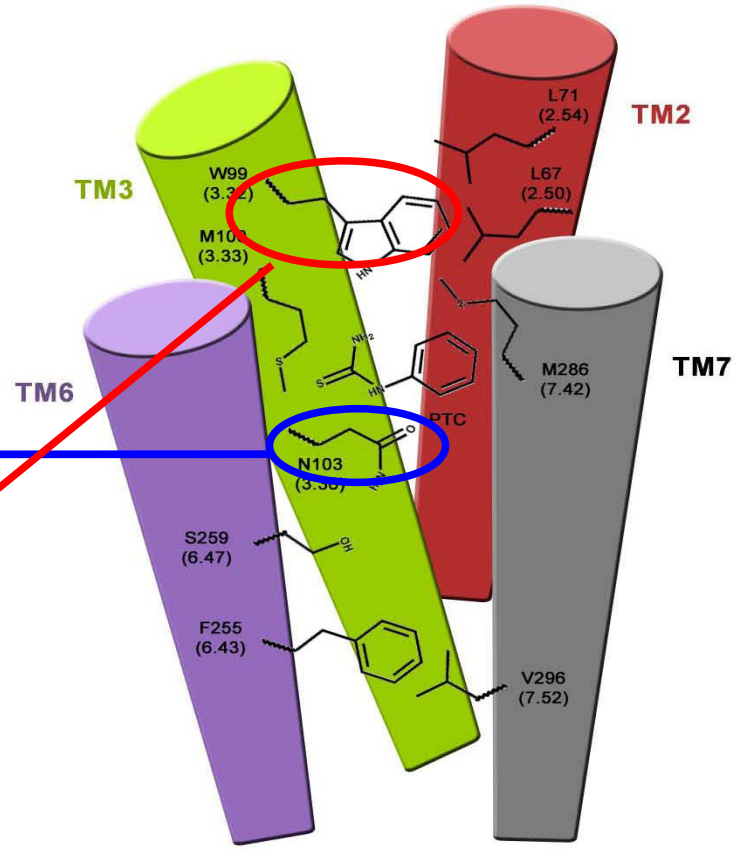
Clustering according to the three dimensional localization of the ligand.

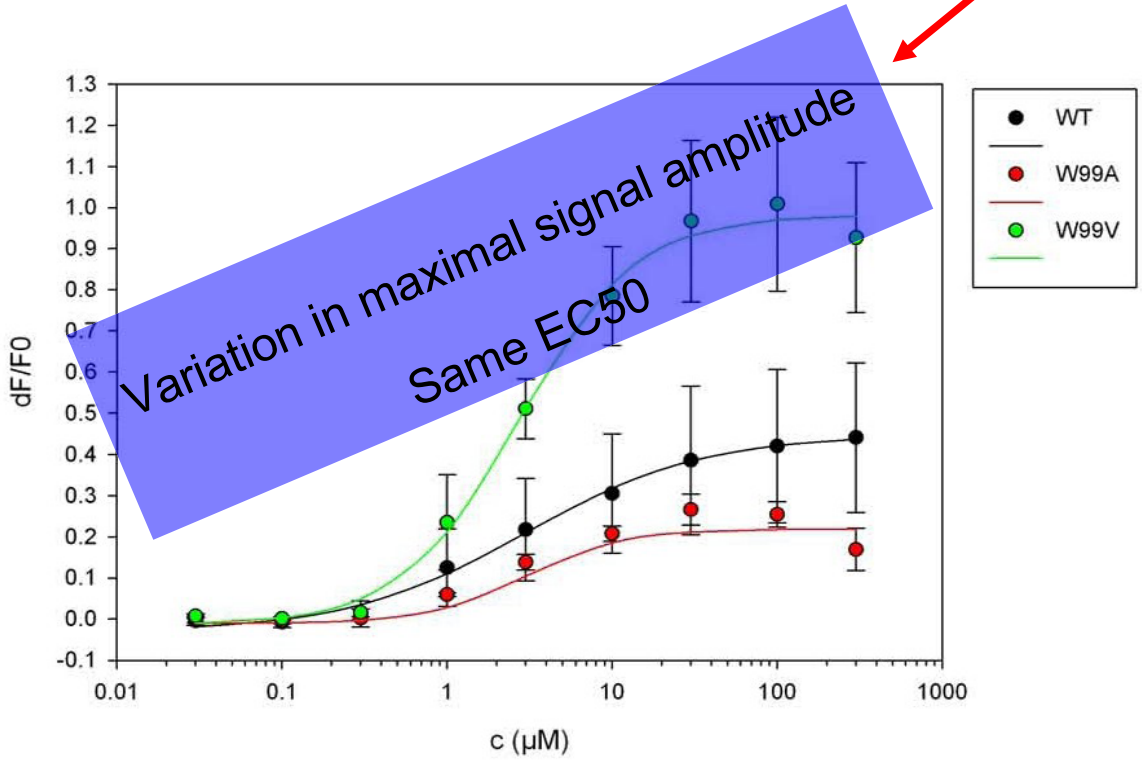
BITTER TASTE RECEPTORS



Biarnés et al.(2010). Plos One

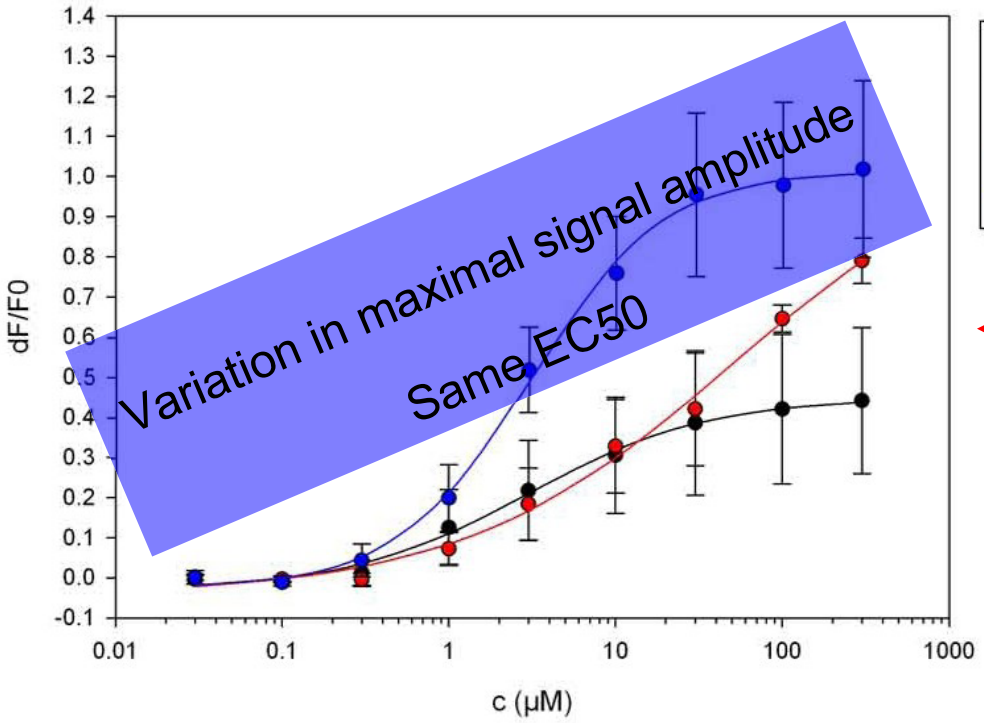


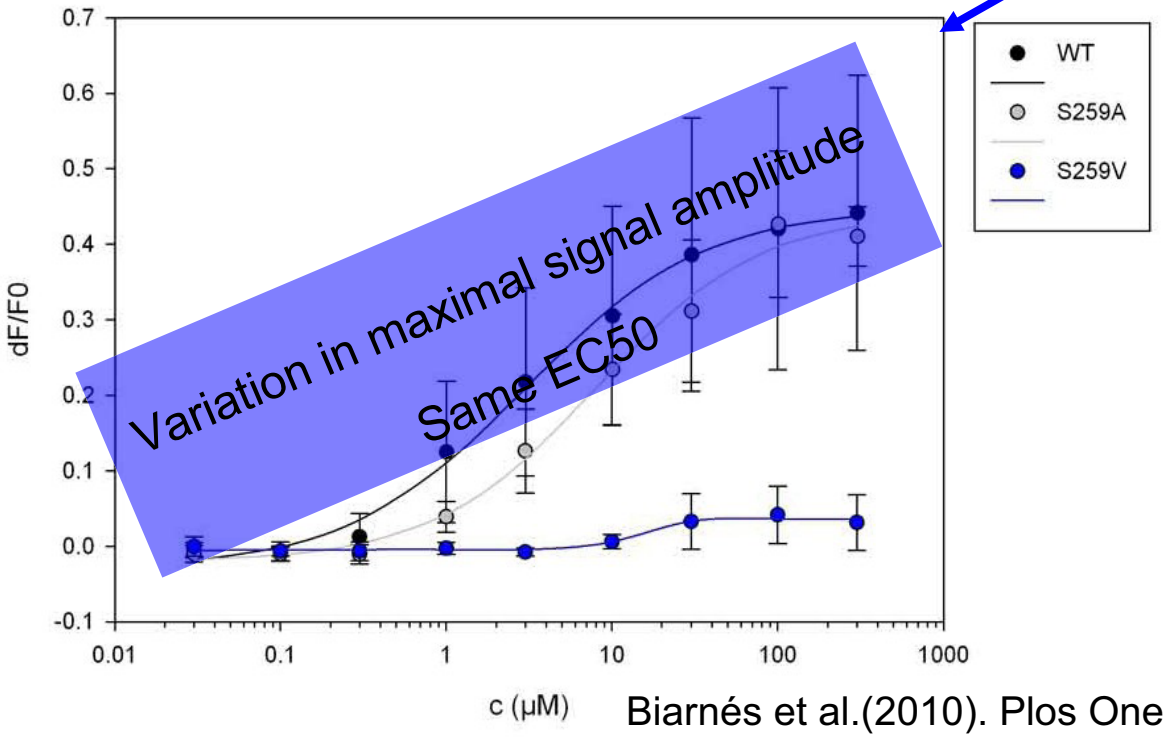


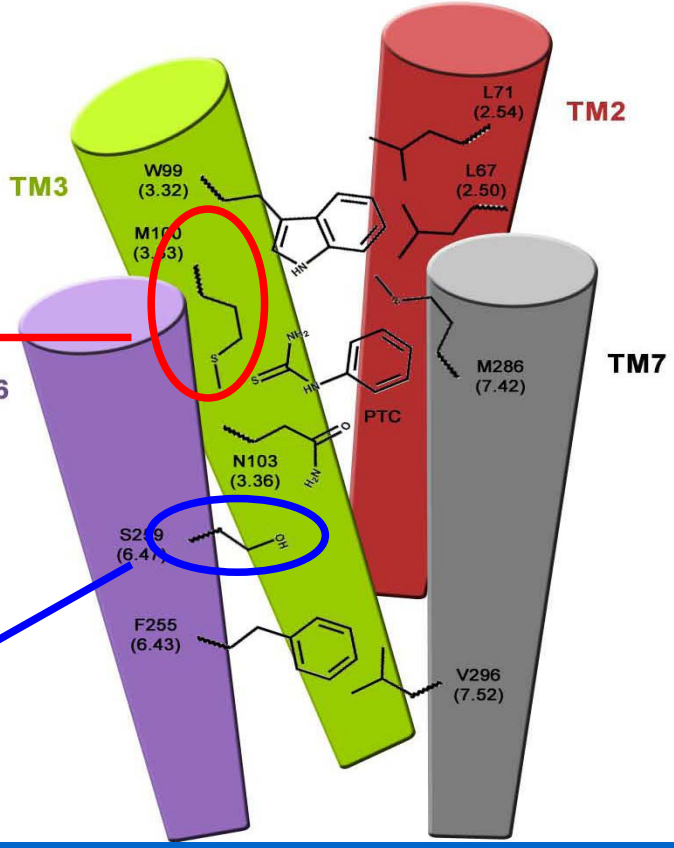


Variant	Helix	EC_{50} (μM)	Max. Activity (dF/F0)
PAV		3	0.47
W99A	TM3	4.25	0.25*
W99V	TM3	2.7	1.12*
N103V	TM3	15*	0.09*
N103A	TM3	8*	0.38

Biarnés et al.(2010). Plos One

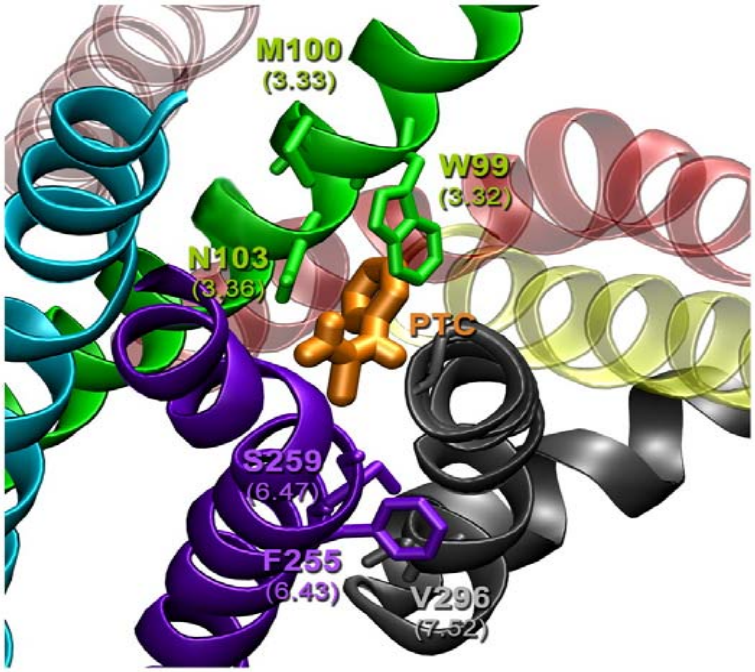


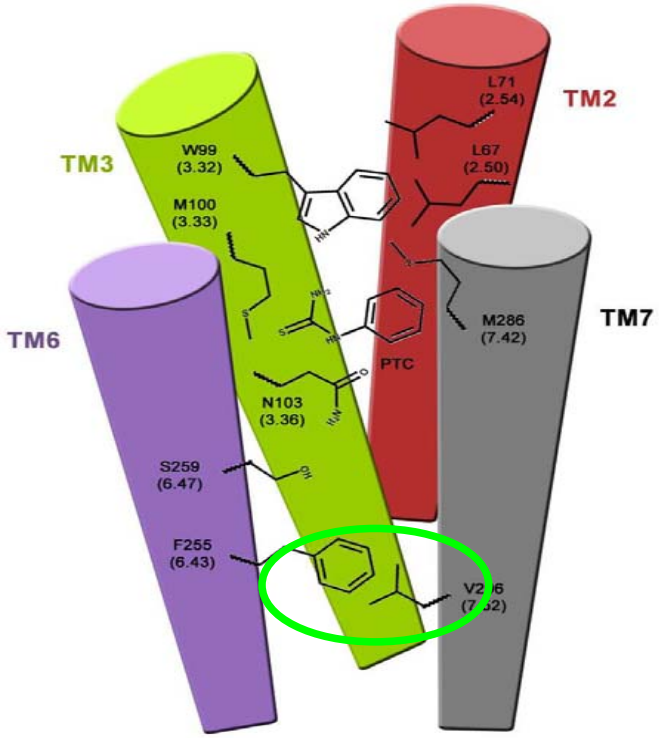




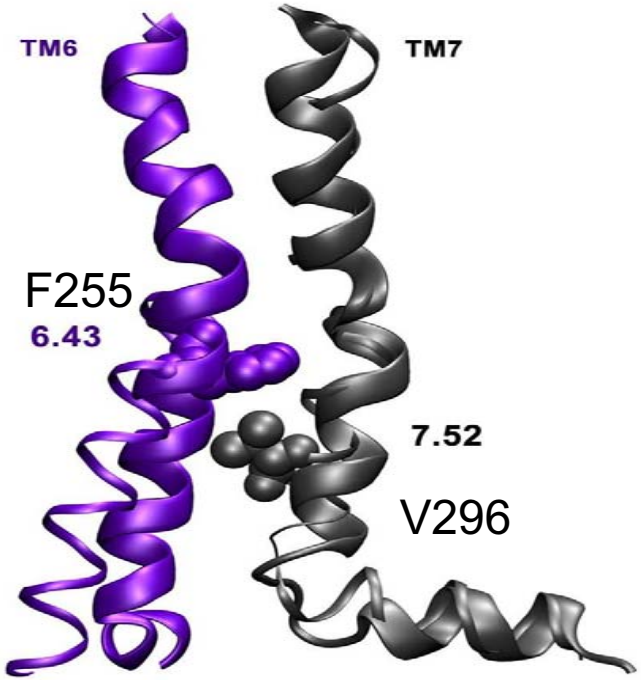
Variant	helix	EC_{50} (μM)	Max. Activity (dF/F0)
PAV		3	0.47
M100V	TM3	10 §	0.79
M100A	TM3	3	1.01*
S259A	TM6	5.4*	0.42
S259V	TM6	27*	0.04*

Biarnés et al.(2010). Plos One





F255 and V296 interaction may be critical for receptor activation. This result is consistent with the observation that the PVI variant (holding the V296I mutant) is less sensitive to PTC than the PAV variant



Biarnés et al.(2010). Plos One

Conclusions:

Model of activation?

What we know

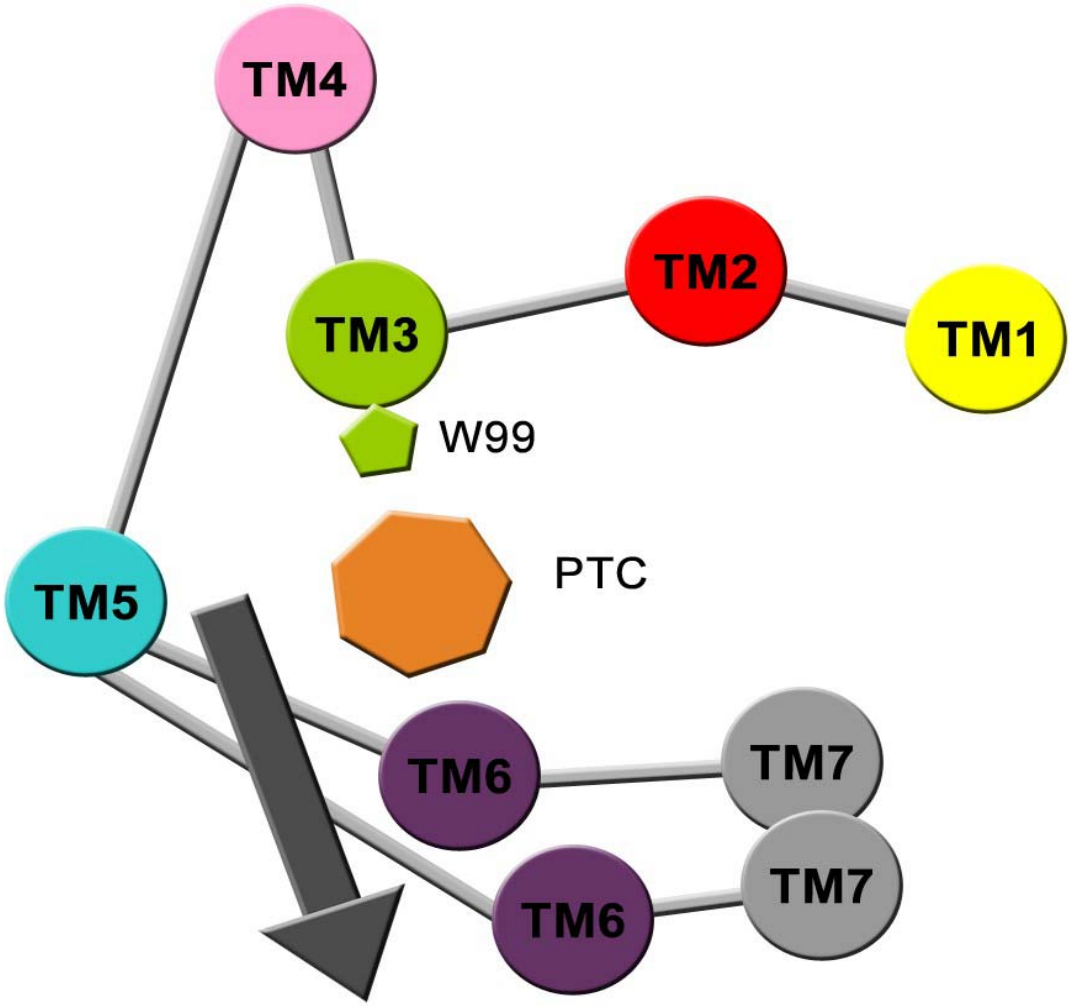
N103 implied in binding

W99 shaping the cavity and interacting with residues located in other helices.

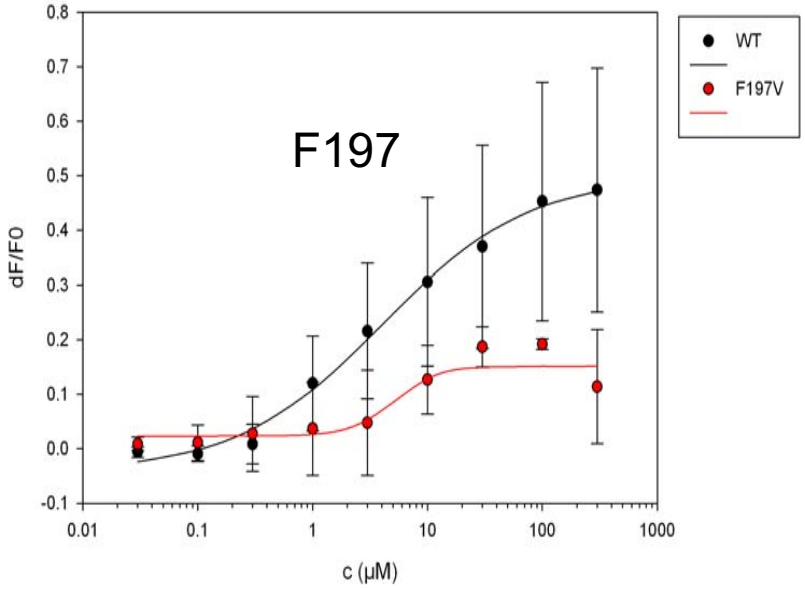
M100 and S259: close to the binding cavity, perhaps shaping it

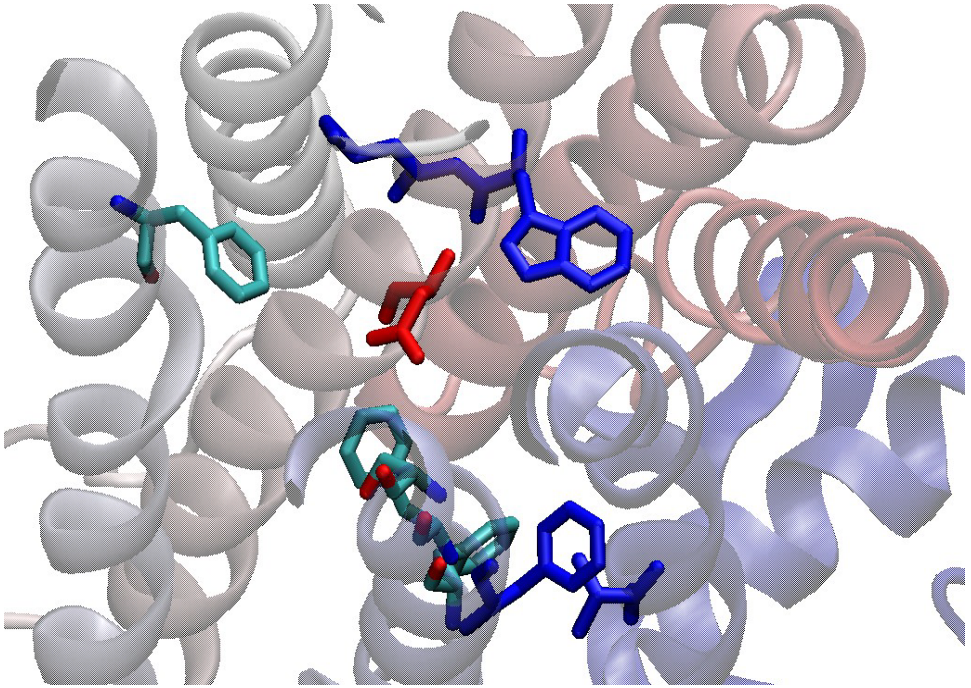
F255 interacts with V296 and actively participates in activation.

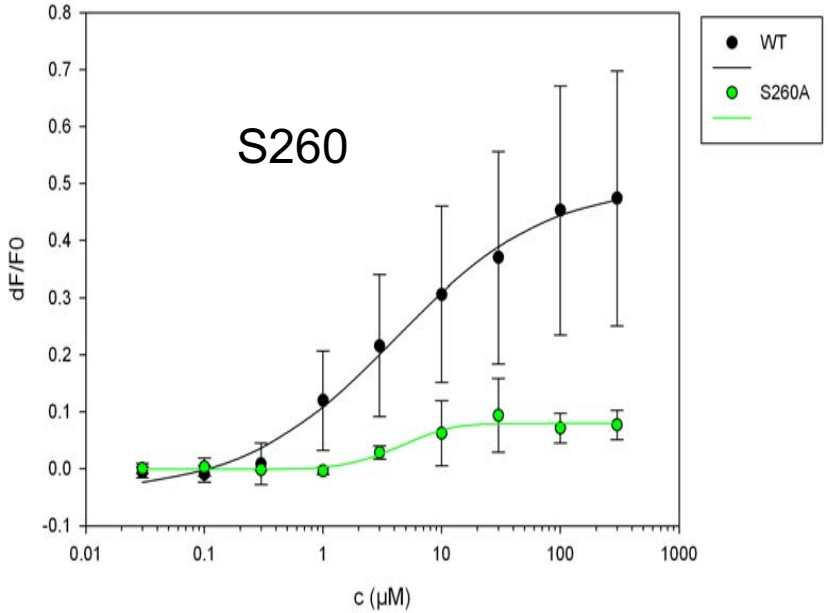
What we WOULD like to know

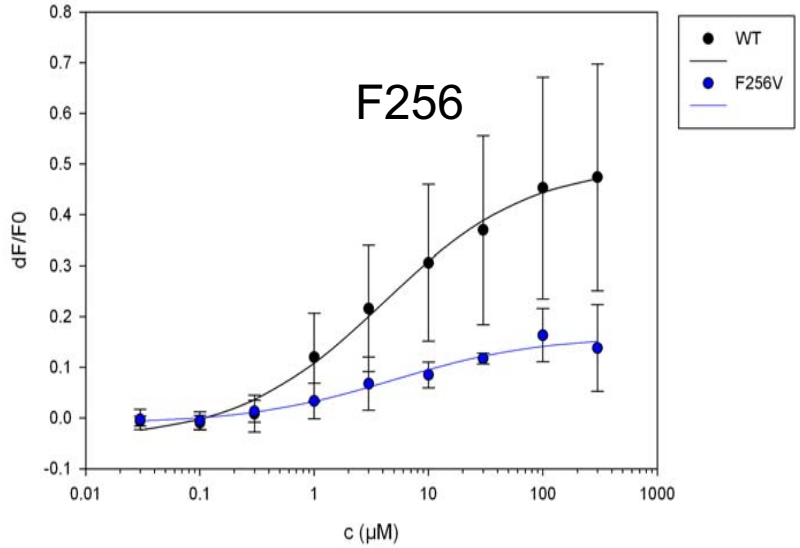


2nd Iteration: New mutants









And now??

Perspectives and future work.

Our predictions were fully validated by experimental data. This makes us confident that by an extensive use of our protocol (as proposed here) we will able to provide relevant insights on the structural determinants of hTAS2R38.

The future work will be divided in two principal phases, each of them including rounds of modelling, docking and experimental validation (rejection)

A last word: predictive or not predictive?

- Although low resolution models are at the limit of validity of Homology modelling techniques, state-of-art bioinformatic tools allow the possibility of building and analysing thousands of models, thus allowing the identification of residues critical for the functioning of the receptor.

- The combined computational/experimental approaches permitted the design of a few experiments aimed at a clear characterization of the residues involved, not only in ligand binding, but also in receptor activation

Acknowledgements

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