Preferential binding odorant-olfactory receptors as a predictor of OR excitation or inhibition

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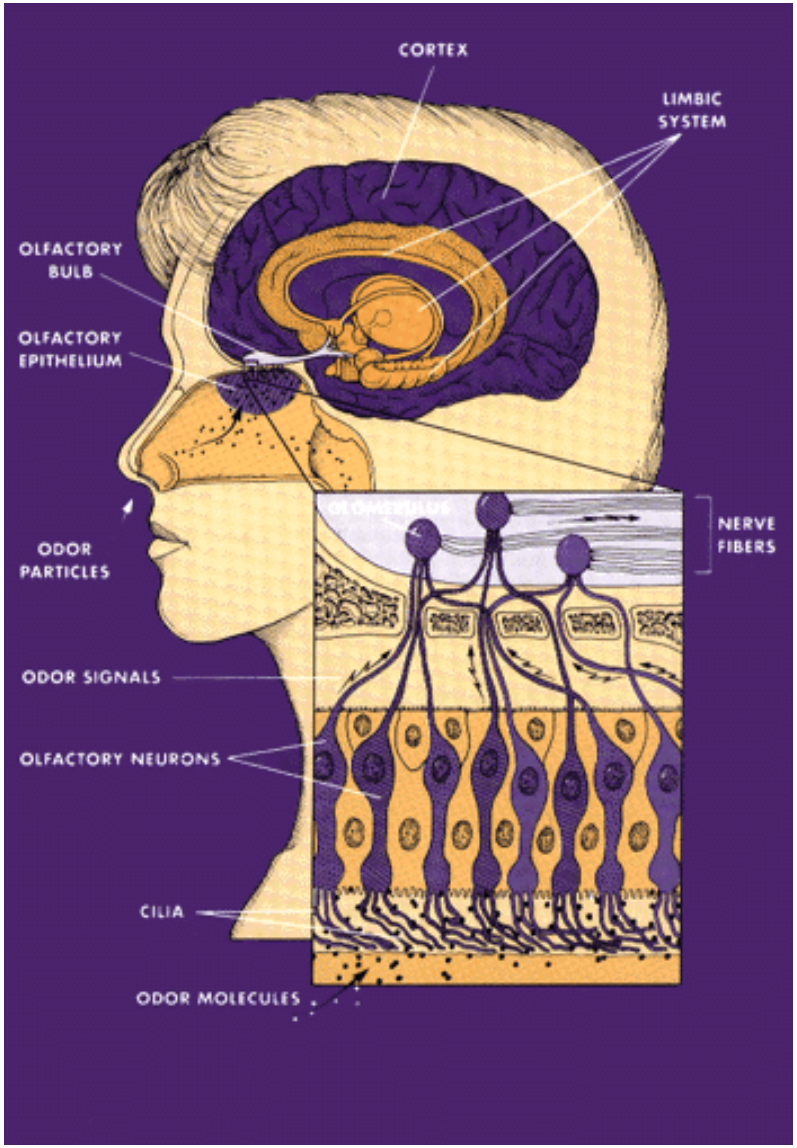
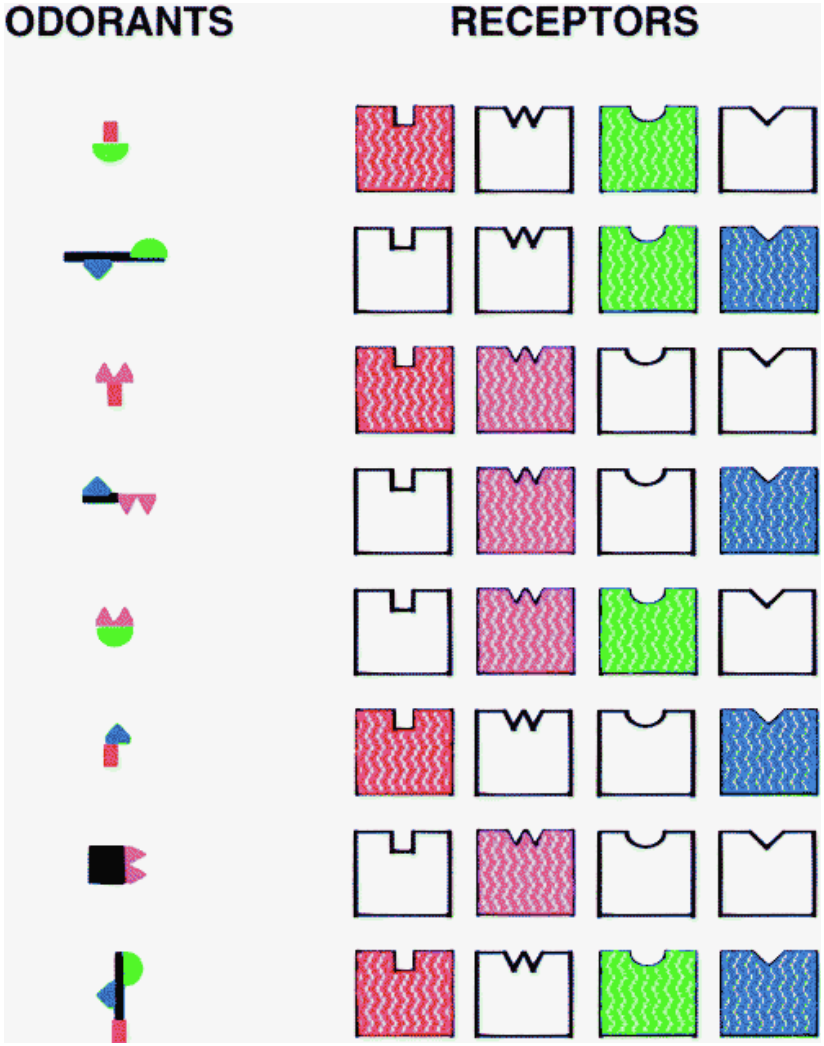
UAB Research Computing Day

Overview of the olfactory system



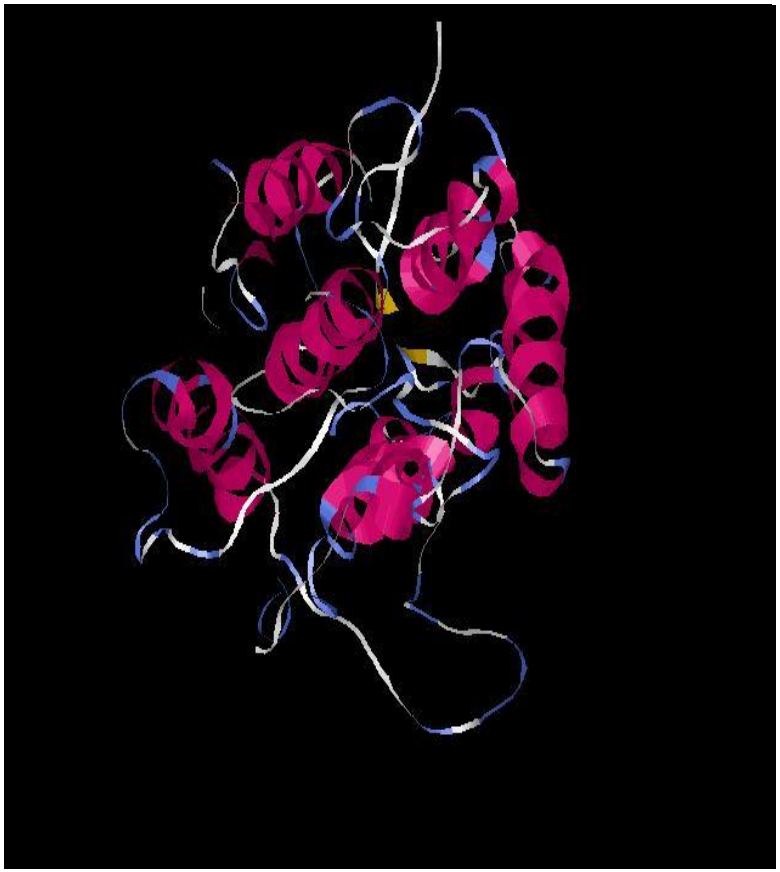
http://www.sfn.org/index.cfm?pagename=brainbriefings_smellandtheolfactorysystem

Role of Olfactory Receptors in Odor Detection



Malnic et al. (1999). Combinatorial Receptor Code for Odors. Cell, 5:713-723

Olfactory Receptors--Structurally



Top-down View

Side View

- Rhodopsin-like class A GPCRs (**G**TP-binding **P**rotein **C**oupled **R**eceptors)
- 7 transmembrane helical domains
- Extra-cellular N-terminus
- Intra-cellular C-terminus
- 6 interhelical loops
  - 3 extra-cellular
  - 3 cytoplasmic

Computational Modeling of Olfactory Receptors

- Model-Building: Secondary structure Prediction
  - Secondary structure prediction to identify transmembrane helices
  - Hidden Markov Models to identify TM helices
    - TMHMM, HMMTOP and several other programs
- Homology Modeling
  - Positioning the target protein sequence over a template and letting the structure resolve based on the template structure

Homology Modeling

- Three possible templates are currently available
  - Bovine rhodopsin (PDB ID: 1u19)
  - Beta-adrenergic receptor (PDB ID: 2r4r, 2r4s & 2rh1)
  - Adenosine A2A receptor (PDB ID: 3qak, 2ydo & 2ydv)

Issues with using Rhodopsin as a template

- The sequence identity between ORs and rhodopsin is 40% or less
- The target-template matches have to take place based on structure not sequence
- Helices in rhodopsin are longer than predicted OR helices
- Loops in rhodopsin are shorter than OR loops
- There are structure specific features for rhodopsin that need not arise in ORs

Rhodopsin (PDB Id: 1u19)

Kink present in TM 7

Making the interior of the receptor biochemically (hydrophobically) feasible

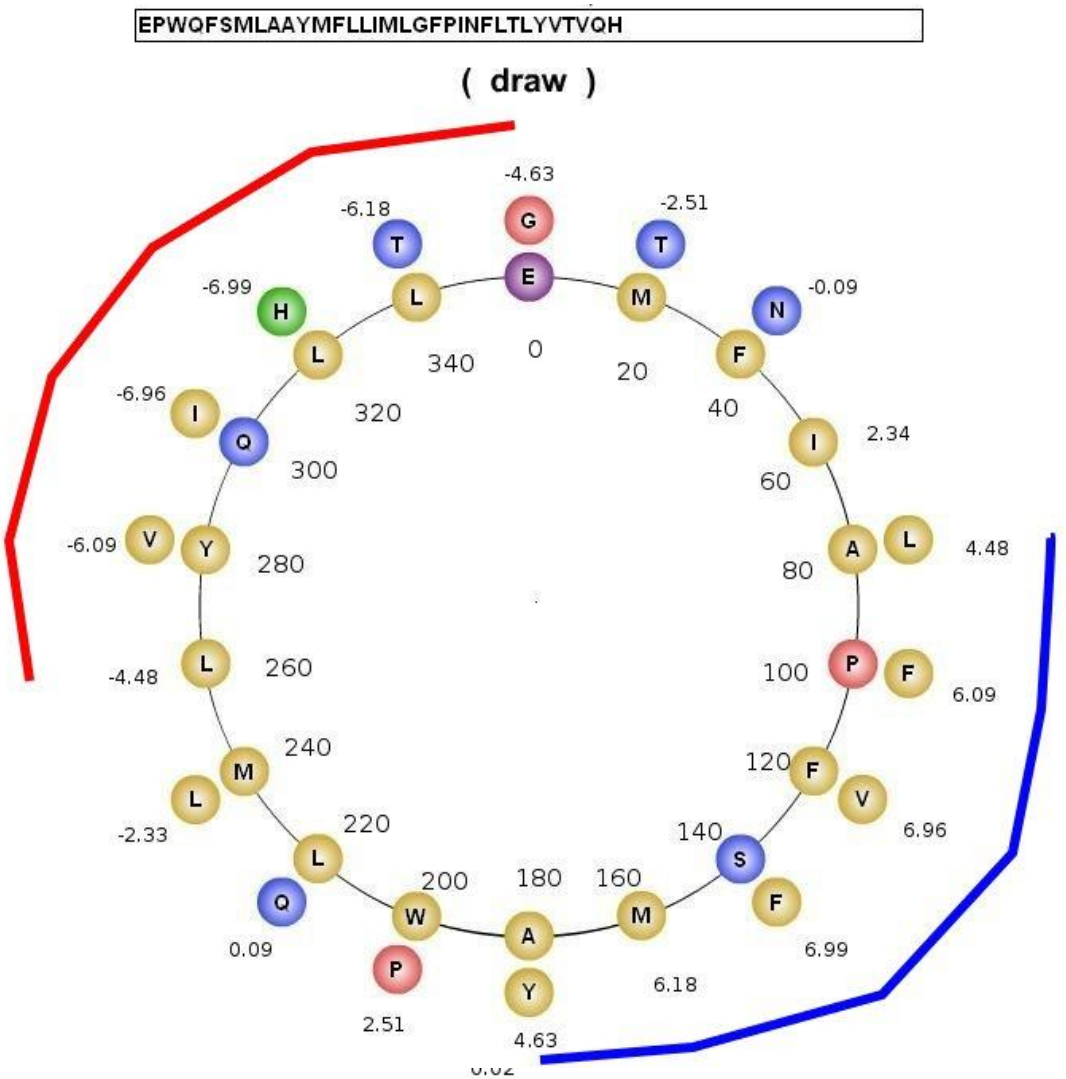
- The protein is surrounded by the lipid bi-layer of the cell membrane
- The interior of the protein tends to be hydrophilic
- Each helix then has to be repositioned such that its effective hydrophobicity is pointed in the “correct” direction—this step is post homology modeling
- The following equation is used to determine the effective hydrophobicity

$$\Theta_\theta = \sum_{i=\theta}^{360-\theta} \mu_\theta . \cos i$$

Hydrophobicity profiles in G protein-coupled receptor transmembrane. helical domains. Crasto C. Journal of Receptor, Ligand and Channel Research, 2010. 3:123-133.

Effective hydrophobicity

$$\Theta_\theta = \sum_{i=0}^{360-\theta} \mu_\theta . \cos i$$



http://www.site.uottawa.ca/~turcotte/resources/HelixWheel/

Completing the OR model and introduction to docking

- Loops are then added back to join the helices
- The energy of the entire structure is minimized
- Ligands that are known to activate a receptor (for ORs, these would be odorant molecules)—are then docked into the binding region of the receptor. This is static docking

Simulation Studies of Interactions between Olfactory Receptors and Odorant Molecules—tracing the path of an odor in a receptor

There are disadvantages to static docking

- Static docking provides a single snapshot of what occurs within a protein's binding region
- Protein-ligand interactions, and any other processes that follow from it, are always dynamic
- These Interactions are also not restricted to one amino acid residue in the protein and the ligand
- Different interactions might occur at different times during a ligand's tenure in the proteins binding pocket, following docking

Chem. Senses 30: 781–792, 2005
doi:10.1093/chemse/bji070
Advance Access publication October 21, 2005

Structural Activation Pathways from Dynamic Olfactory Receptor–Odorant Interactions

Peter C. Lai[1], Michael S. Singer[2] and Chiquito J. Crasto[3]

Cell, Vol. 96, 713–723, March 5, 1999, Copyright ©1999 by Cell Press

Combinatorial Receptor Codes for Odors

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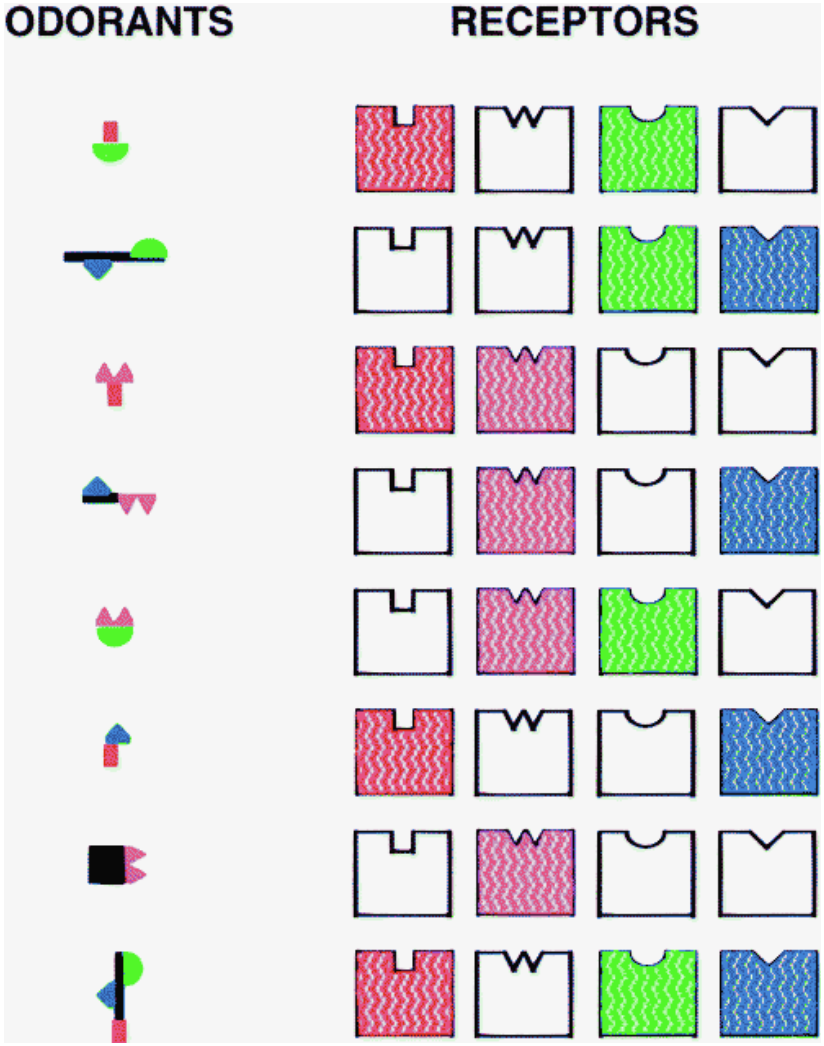
- One of the first papers on functional analysis of olfactory receptors
- 14 mouse olfactory receptors were analyzed to test responses to 22 odorants (organic compounds) with different lengths and functional groups and for different concentrations

Role of Olfactory Receptors in Odor Detection



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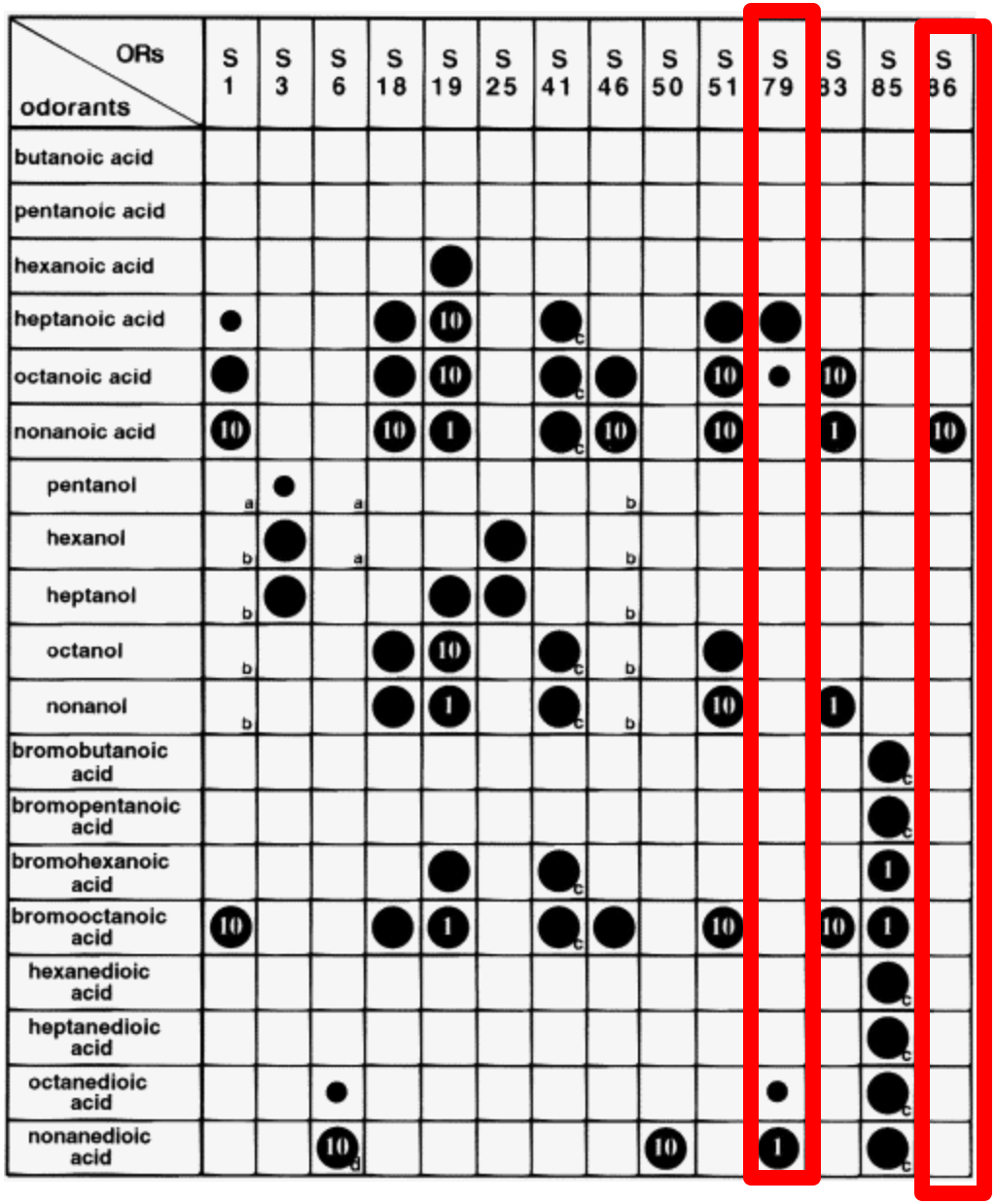


ORs / odorants	S 1	S 3	S 6	S 18	S 19	S 25	S 41	S 46	S 50	S 51	S 79	S 83	S 85	S 86
butanoic acid														
pentanoic acid														
hexanoic acid					●									
heptanoic acid	●			●	10		●c			●	●			
octanoic acid	●			●	10		●c	●		10	●	10		
nonanoic acid	10			10	1		●c	10		10		1		10
pentanol	a	●	a					b						
hexanol	b	●	a			●		b						
heptanol	b	●			●	●		b						
octanol	b			●	10		●c	b		●				
nonanol	b			●	1		●c	b		10		1		
bromobutanoic acid													●c	
bromopentanoic acid													●c	
bromohexanoic acid					●		●c						1	
bromooctanoic acid	10			●	1		●c	●		10		10	1	
hexanedioic acid													●c	
heptanedioic acid													●c	
octanedioic acid			●								●		●c	
nonanedioic acid			10d						10		1		●c	

Figure 6. The Recognition Profiles of 14 Olfactory Neurons and the ORs They Expressed

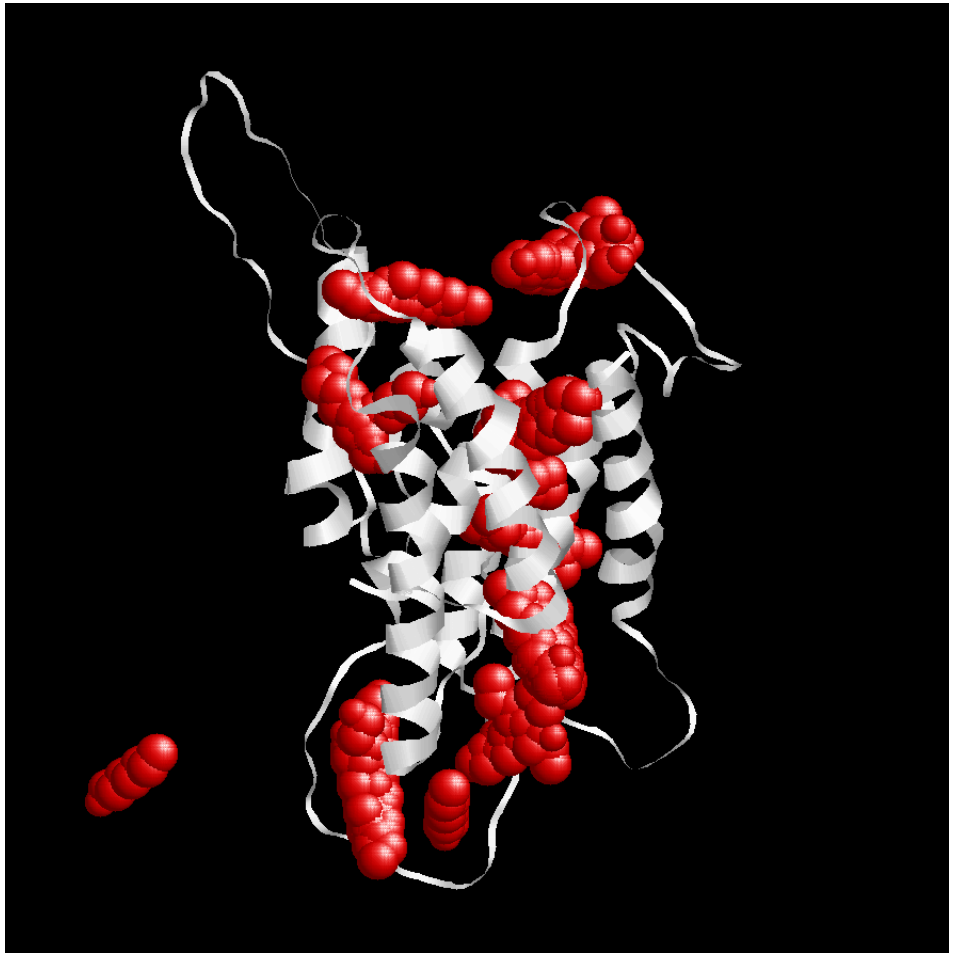
Test odorants are shown on the left, and the ORs identified in the responsive neurons are shown on top. Filled circles indicate responses to 100 μM odorants, with smaller circles indicating a relatively weak response (less than half the change in fluorescence intensity elicited by KCl). Responses that were also obtained at 1 or 10 μM odorant are indicated by a 1 or 10 inside the filled circle. a, not tested; b, tested at 10 μM, but not 100 μM; c, not tested at 10 μM or 1 μM; d, not tested at 1 μM.

S79 (octanoic acid, heptanoic acid, nonanedioic acid, heptanol)

S86 (nonanoic acid, heptanoic acid)

Malnic et al. (1999). Combinatorial Receptor Code for Odors. Cell, 5:713-723

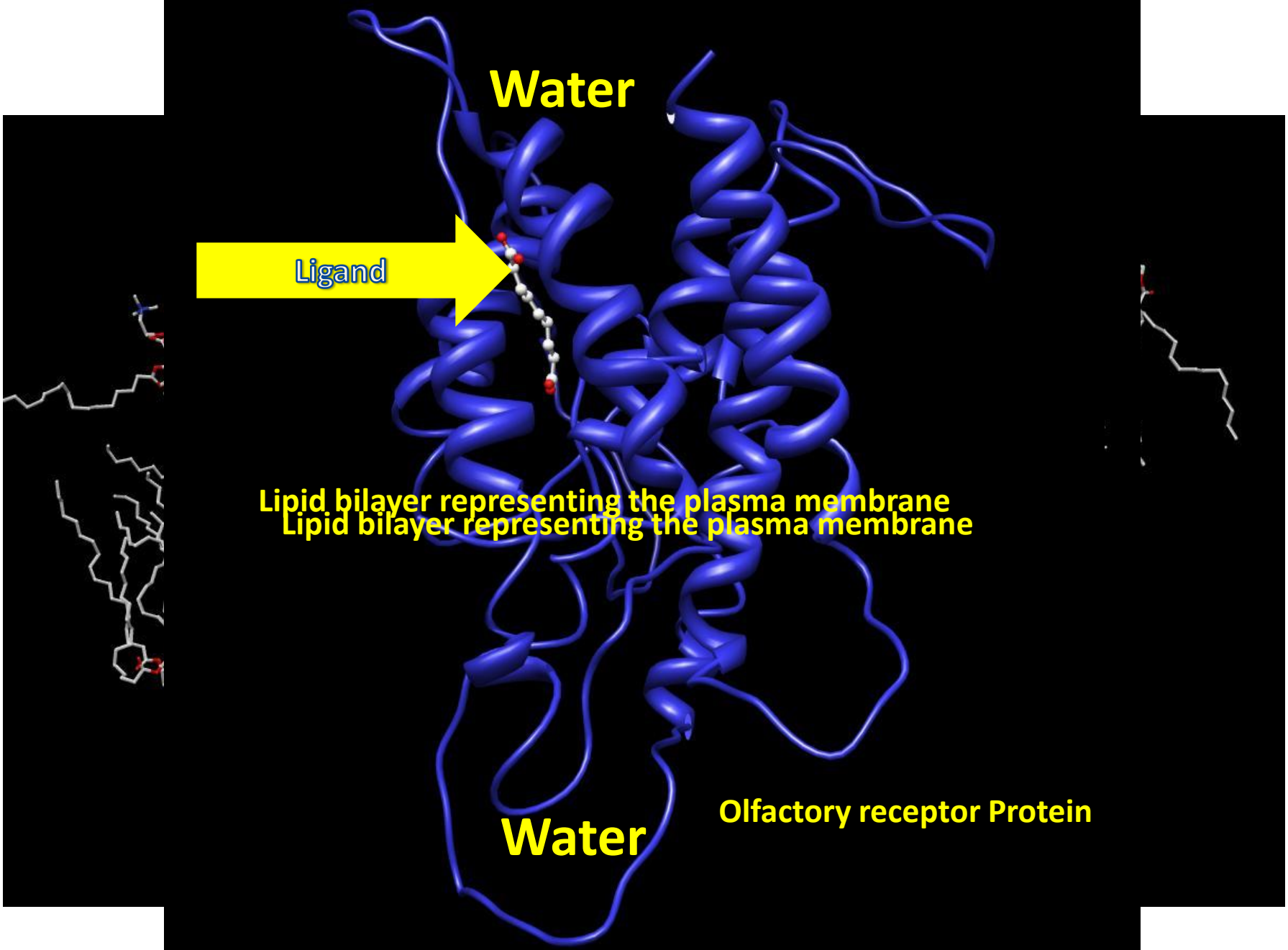
Raw file, post-docking



GRAMM was used to dock the odorant ligands. (http://vakser.bioinformatics.ku.edu/main/resources_gramm.php)
(Center for Bioinformatics, University of Kansas)

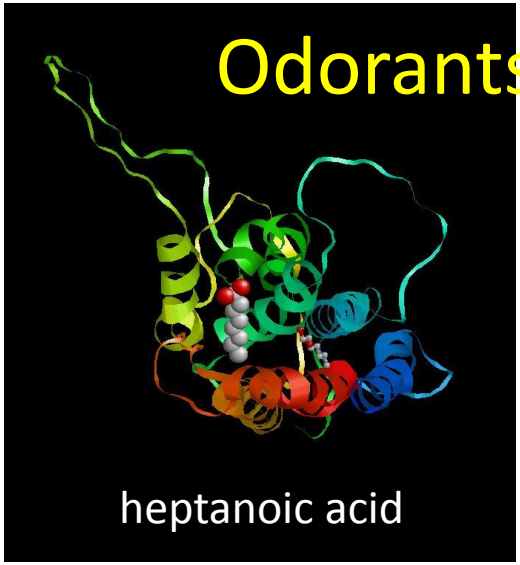
High Performance Computing Critical to Creating a properly simulated biological system

- Olfactory receptor protein bound to a ligand
- The 7 transmembrane domains of the receptor can be seen embedded into a lipid bilayer consisting of 230 palmitoyloleoylphosphatidylcholine (POPC) molecules and close to 22,000 explicit 3-site water molecules.
- There are a more than 100,000 atoms being simulated in this system.

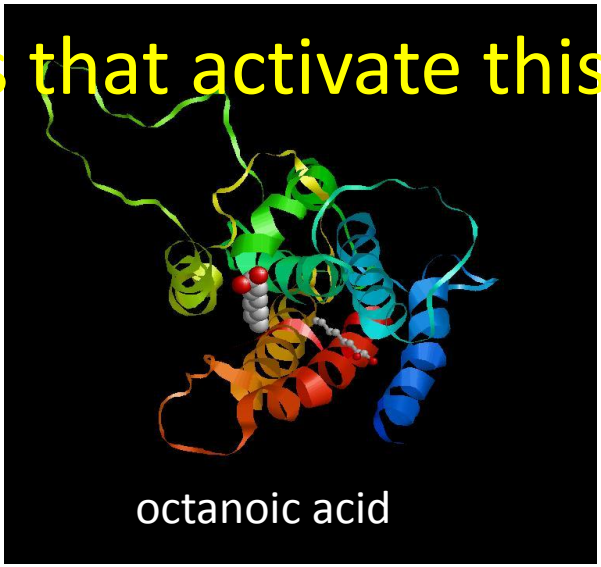

Water
Ligand
Lipid bilayer representing the plasma membrane
Lipid bilayer representing the plasma membrane
Water
Olfactory receptor Protein

S79

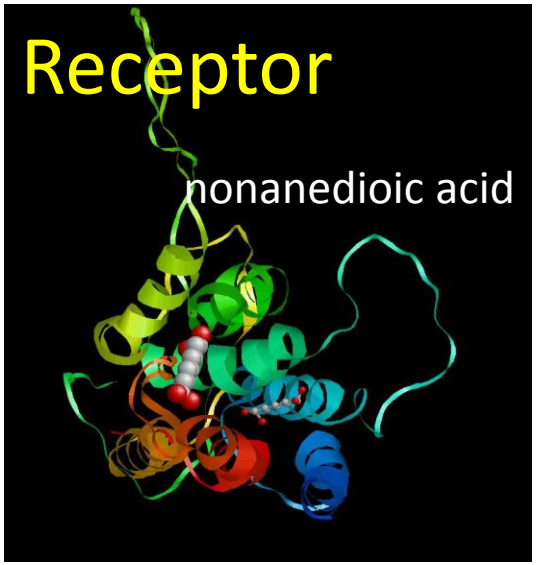
Odorants that activate this Receptor



heptanoic acid

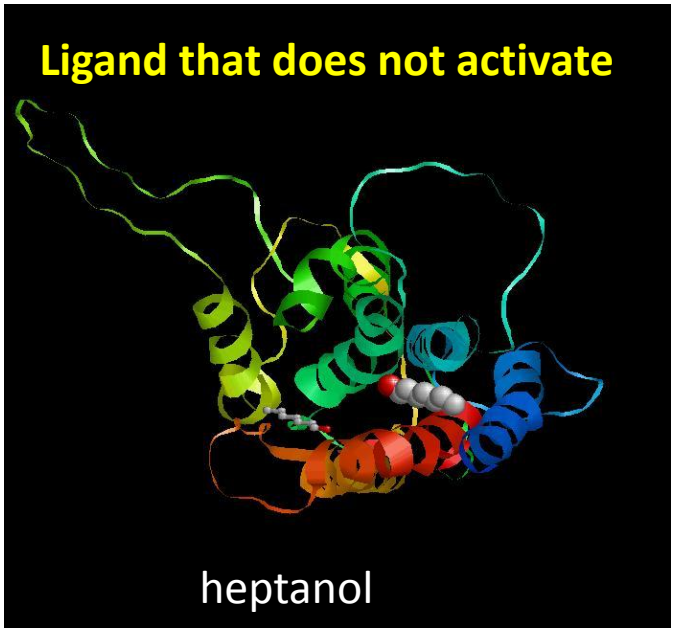


octanoic acid



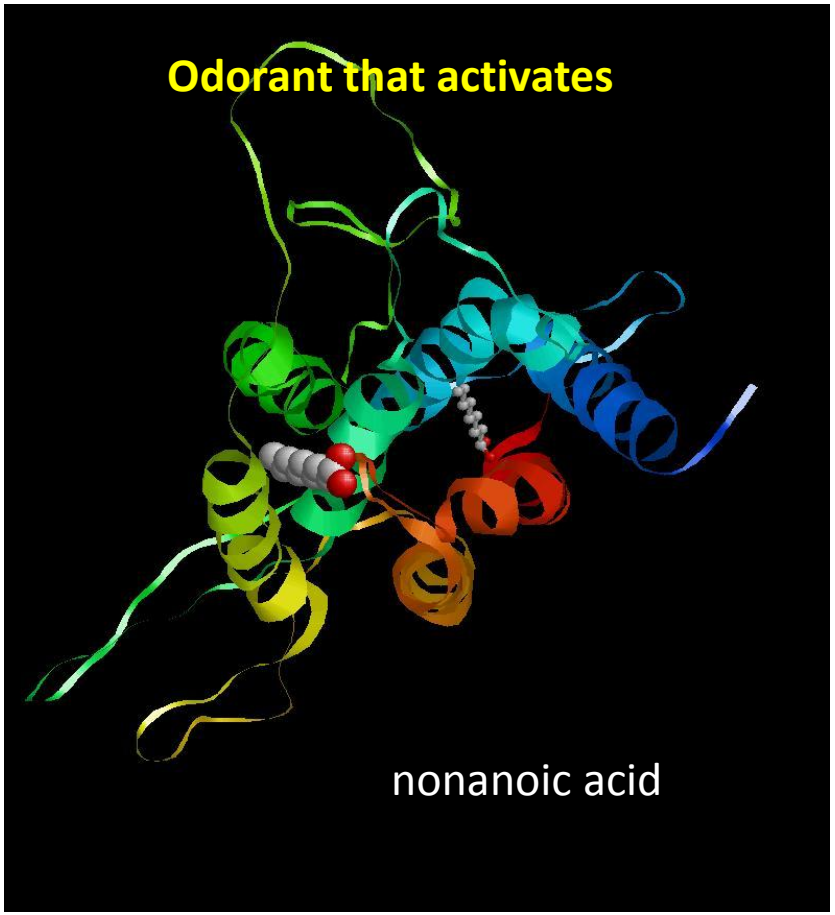
nonanedioic acid

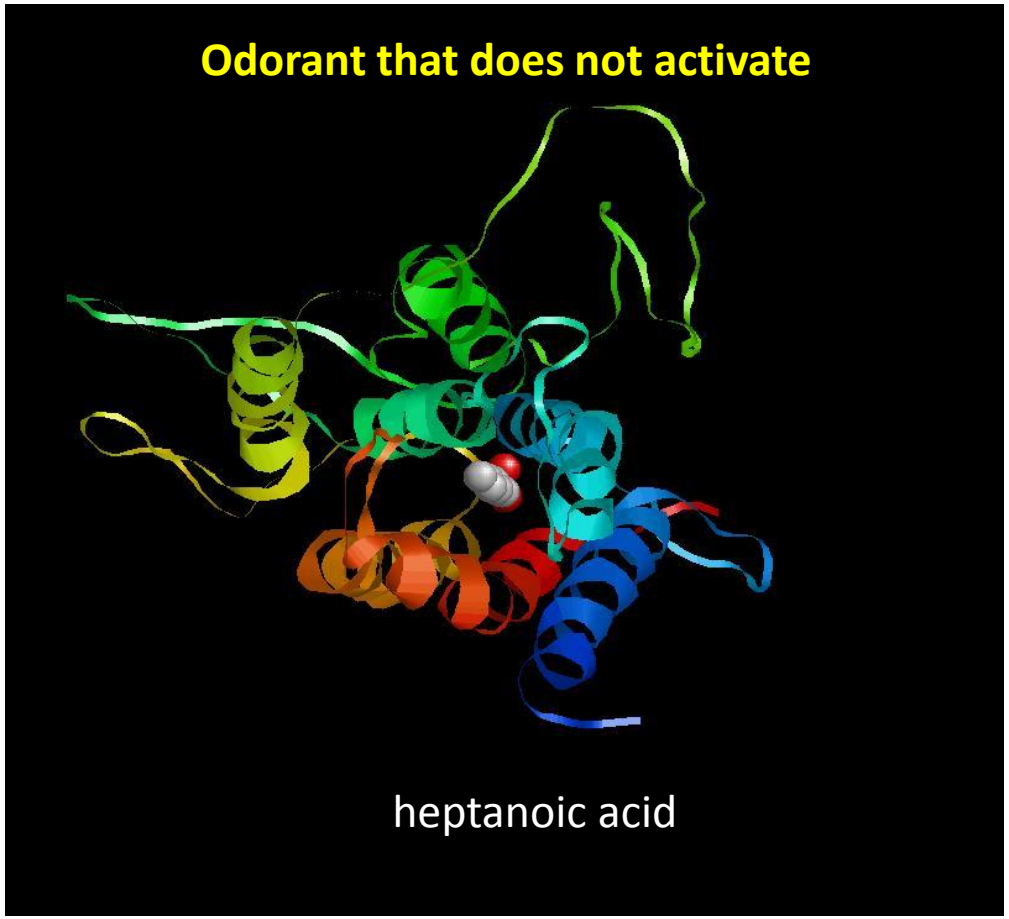
Ligand that does not activate



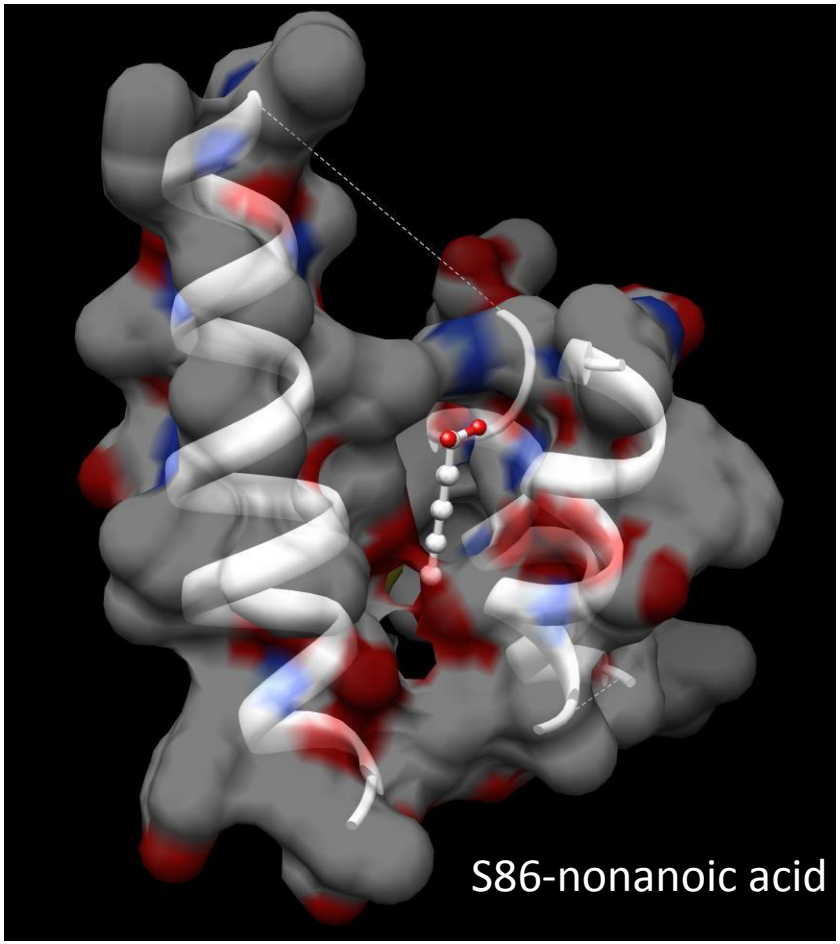
heptanol

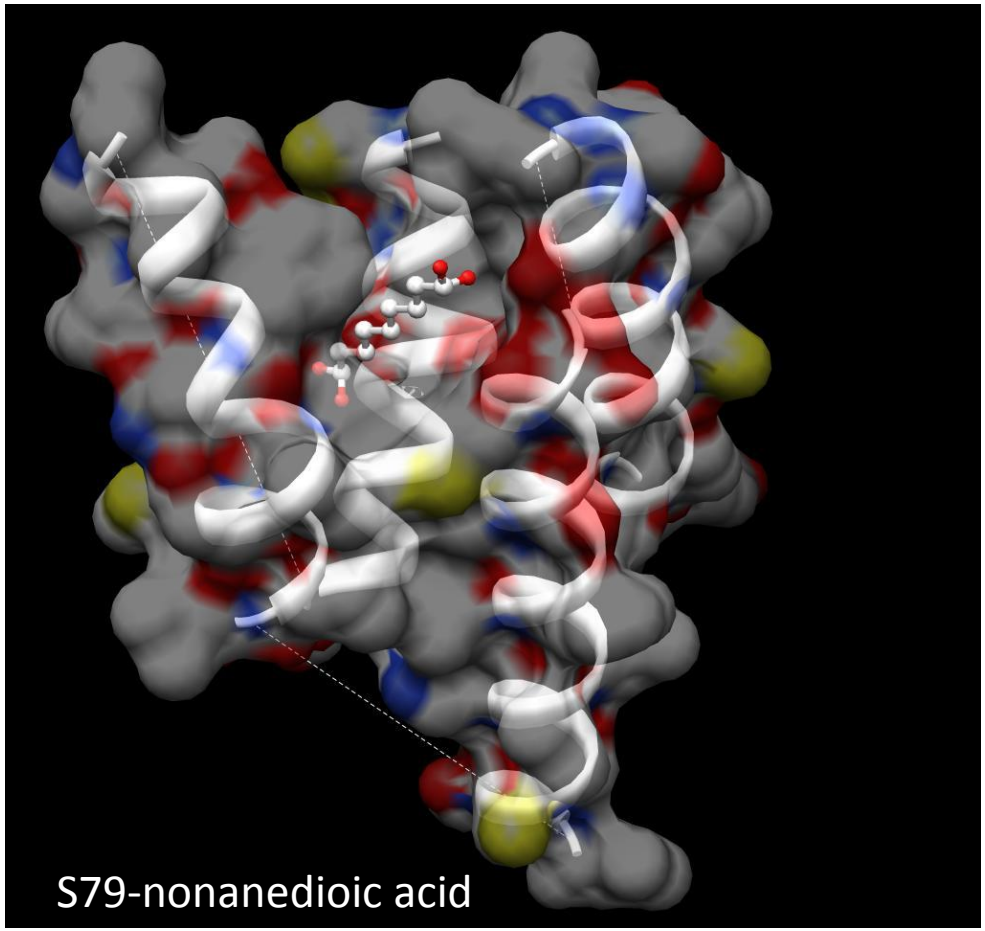
S86


Odorant that activates
nonanoic acid


Odorant that does not activate
heptanoic acid

Study of the Interior of the binding region





We can simulate an odor ligand “visiting” different binding sites.

- This clip demonstrates an all-atom molecular dynamics simulation of a previously modeled human olfactory receptor, hOR17-209 docked with its activating ligand, iso-amyl acetate.
- This clip shows the first 5 ns of simulation time in order to highlight ligand binding pocket sampling, the entire simulation lasted 10 ns.
- The simulation was carried out on the UAB HPC cluster using Gromacs 4.5.4 and CHARMM (Chemistry at HARvard Molecular Mechanics) all-atom force field
- Using 232 of the "gen3" compute nodes, the entire single-precision simulation took 8 and half hours to complete, having used 471 GFlops of computing power.

Movie of simulation

- http://www.youtube.com/watch?v=z8UPl_wP8K8

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Thank you