

Automatic modeling of mammalian olfactory receptors and docking of odorants

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ABSTRACT

We present a procedure that (i) automates the homology modeling of mammalian olfactory receptors (ORs) based on the first six three-dimensional (3D) structures of G protein-coupled receptors (GPCRs) available and (ii) performs the docking of odorants on these models, using the concept of colony energy to score the complexes.

ORs exhibit low-sequence similarities with other GPCRs and current alignment methods often fail to provide a reliable alignment. Here, we use a fold recognition technique to obtain a robust initial alignment. The procedure is then applied to a human OR we had previously functionally characterized.

The results, supported by receptor mutagenesis and functional assays, suggest that antagonists dock in the upper part of the binding pocket whereas agonists dock in the narrow lower part. We propose that the potency of agonists in activating receptors depends on their ability to establish tight interactions with the floor of the binding pocket.

We developed a web site that allows the user to upload a GPCR sequence, choose a ligand in a library and obtain the 3D structure of the free receptor and ligand-receptor complex (<http://genome.jouy.inra.fr/GPCRautomodel>).

INTRODUCTION

Olfactory receptors (ORs) belong to the GPCR class R ('rhodopsin-like') family, the only one for which structural information is currently available. 6 GPCR structures (14 by now) have been solved by X-ray crystallography, and these high-resolution structures make it possible to combine traditional homology modeling techniques with ligand-based pharmacophore modeling.

METHOD

We use a fully automated method to perform homology modeling of GPCR structures. Homology modeling techniques are critically dependent on the initial sequence alignment between the query sequence and the sequence of a receptor whose 3D structure is known (the template). Receptors of the class R family exhibit relatively low-sequence identity, even within the rhodopsin subfamily of GPCRs.

- The alignment step was improved from our previous threading method FROST (Marin et al. 2002), intended to detect and align remote homologs with low-sequence identities, using fold recognition.
- 3D structure was built with MODELER (Sali & Blundel, 1993).
- Selected ligands were docked on the modeled receptor using VINA (Trot & Olson, 2010), and the conformations of the resulting complexes were scored using a new procedure.

These three steps are included in a pipeline available on the Web (GPCRautomodel).

RESULTS

Evaluation of the homology procedure

Queries (sequences)	Targets (3D templates)				
	2rh1A	Iu19A	2v4A	3em1A	3oduA
2rh1A	2.17–2.47	0.80–0.91	2.14–2.37	2.17–2.42	1.51–1.82
Iu19A	2.04–2.32	2.11–2.36	2.50–2.84	2.14–2.50	1.75–2.19
2v4A	1.17–1.28	2.26–2.45	1.96–2.18	2.44–2.67	1.68–1.96
3em1A	2.08–2.27	2.36–2.76	1.95–2.24	2.68–3.07	2.25–2.64
3oduA	2.42–2.72	2.16–2.35	2.29–2.53	2.60–3.10	1.94–2.48
3pb1A	1.38–1.54	1.68–1.96	1.56–1.80	2.22–2.70	1.82–2.18

Modeled and experimental receptor structures are compared using Cα rmsd. Positions taken into account for this calculation are identical to those in Table II.

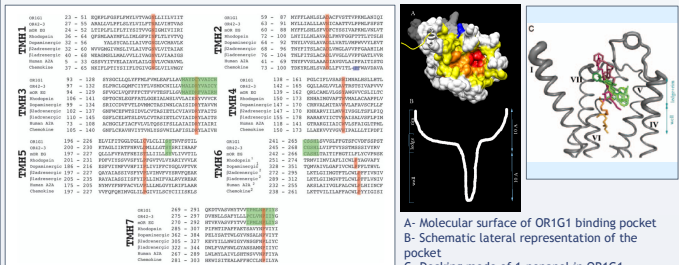
Evaluation of the ligand docking procedure using known complexes

PDB code	Complex*	Ligand type	Predicted binding mode		No. of contacts with ECL2
			<i>I_{bind}</i>	<i>M_{bind}</i>	
2rh1	b2AR/carazolol	Inverse agonist	1.20	0.84	4
2v04	b1AR/sabutamol	Partial agonist	1.66	0.99	0
2v01	b1AR/dobutamine	Partial agonist	2.89	1.84	0
2v03	b1AR/sopentolol	Agonist	3.20	1.88	0
2v02	b1AR/carnoterol	Agonist	4.95	3.78	4
3pb1	D3DR/eticlopride	Antagonist	3.59	1.44	4
2ydv	A2AR/NECA	Agonist	6.52	3.66	14
3em1	A2AR/ZM241385	Antagonist	9.95	5.71	13
3odu	CXCR1/11	Antagonist	8.81	3.35	16

The predicted docking mode is the one with the smallest colony energy (columns 4–5). The last column indicates the number of atomic contacts between the ligand and the second extra-cellular loop. A contact corresponds to a distance <3.6 Å between an atom of the ligand and an atom of ECL2.

Application to olfactory receptors

OR1G1 was modeled based on six templates. Alignment of OR1G1 sequence with the sequence of the structural template is presented below (left). Several odorants: 1-nonanol, octanal and tridecanal were docked in these models.



A- Molecular surface of OR1G1 binding pocket
B- Schematic lateral representation of the pocket
C- Docking mode of 1-nonanol in OR1G1

The functional response of OR1G1 to these odorants was characterized.

Ligand	Formula	Function	Response	Odor
1-nonanol	CH ₃ (CH ₂) ₈ OH	alcohol	good agonist	citrus
octanal	CH ₃ (CH ₂) ₇ CHO	aldehyde	weak agonist	fruit-like
tridecanal	CH ₃ (CH ₂) ₁₂ CHO	aldehyde	good agonist	citrus

Functional response of OR1G1 to the odorants.

A) Docking mode of 1-nonanol in OR1G1 (green helices). 1-nonanol is involved in a network of hydrogen bonds, notably Ser255 (broken line)
B) Docking mode of octanal in OR1G1 (magenta helices). Lys 80 interacting with the aldehyde function is shown on top of the figure (its nitrogen is shown in blue)

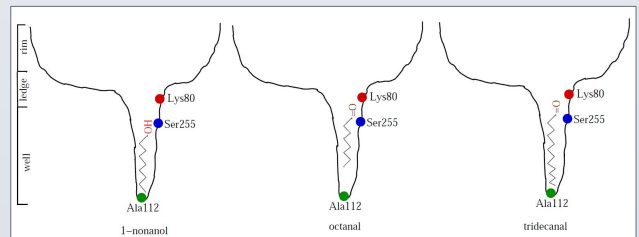
Some site-directed mutations, on Ala112 at the bottom of the well of OR1G1, were made to test the models of the OR-odorant complexes:

- mutation A112S decreases the response of receptor with 1-nonanol, tridecanal and nonanonal
- mutation A112V slightly enhances the response of the receptor to octanal (raises the well floor)

Surprisingly, octanal has a better docking energy than 1-nonanol

Two features must be taken into account to explain OR activation:

- The binding free energy (but antagonists can also bind tightly to the receptor)
- The position in the binding pocket (cf. below)



We propose that the potency of agonists in activating OR1G1 depends on their ability to establish tight contacts with the floor of the binding pocket.

CONCLUSION

In the absence of experimental structures, molecular modeling and computer simulations can suggest a number of hypotheses to be validated experimentally, for instance by mutagenesis, heterologous expression of receptors and functional assays. This interplay between simulations and experimental validations is crucial to progress in our understanding of the signal transduction by ORs or, more generally, GPCRs. The method developed in this work, GPCRautomodel, should constitute a useful starting point for this endeavor.

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