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Molecular basis of olfactory receptors: in silico modeling and in vitro validation

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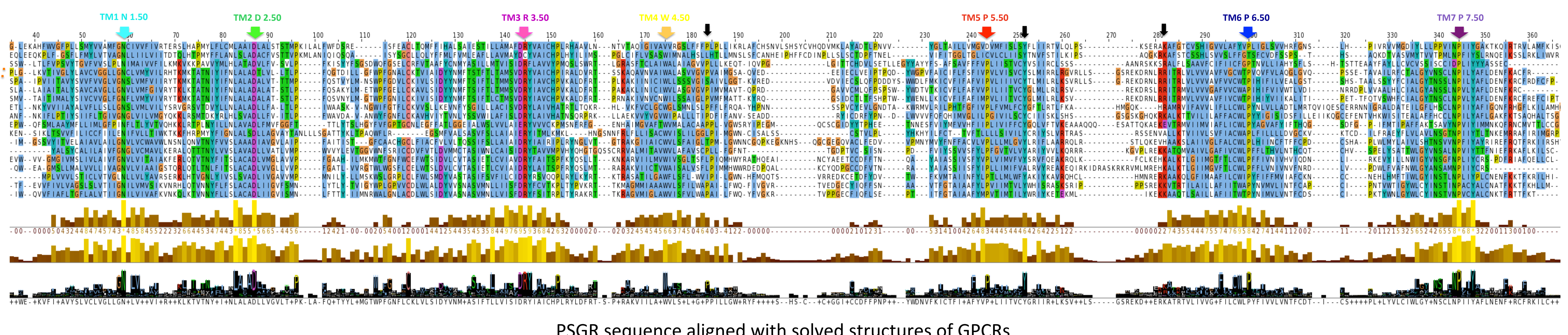
Molecular basis of Olfactory Receptors activation

In silico/in vitro studies of Prostate Specific G-coupled Receptor

Olfactory receptors (ORs) belong to the highly versatile family of G Protein-Coupled Receptor family (GPCRs)^[1-2] and account for as much as 3% of the human genome. They have been first identified in the neurons of the olfactory epithelium where they detect and signal a large panel of odorants molecules.

In addition, ORs detect steroid hormones in non-olfactory tissues^[3], regulate serotonin secretion in the gut^[4-5] or participate in cell migration & muscle adhesion^[6]. Noteworthy, some OR are overexpressed in tumors, where they promote *in vitro* cell invasiveness^[7-11]. Notably, LNCaP prostate cancer cells endogenously express an OR named PSGR, the Prostate Specific G protein-coupled Receptor^[12]. Sanz *et al* showed that stimulation of ORs expressed by LNCaP cancer cells increased the metastasis occurrence *in vivo*, in agreement with the increased cell invasiveness observed *in vitro*^[12]. All these data suggest that ORs can be stimulated by molecules in the microenvironment and could be involved in tumors. Thus, ORs could be novel therapeutic targets. As such, it is critical to identify their ligands and to unravel their specificity and activation mechanisms.

Deciphering how OR functional behaviour reconciles with structural properties is a challenge, because there is no experimental structure available for ORs and because most of them are orphans, *i.e* their physiological agonists are unknown. Despite the lack of available structures for PSGR and generally for OR, the increasing number of solved GPCR structures prompted us to investigate the molecular mechanism of PSGR activation upon agonist recognition, using molecular mechanics. We investigate *in silico* the molecular basis of folding and ligand binding in ORs. Activity tested on PSGR wild-type and mutants with α and β -ionone validated our modeling approach, including homology modeling, mutagenesis and docking.



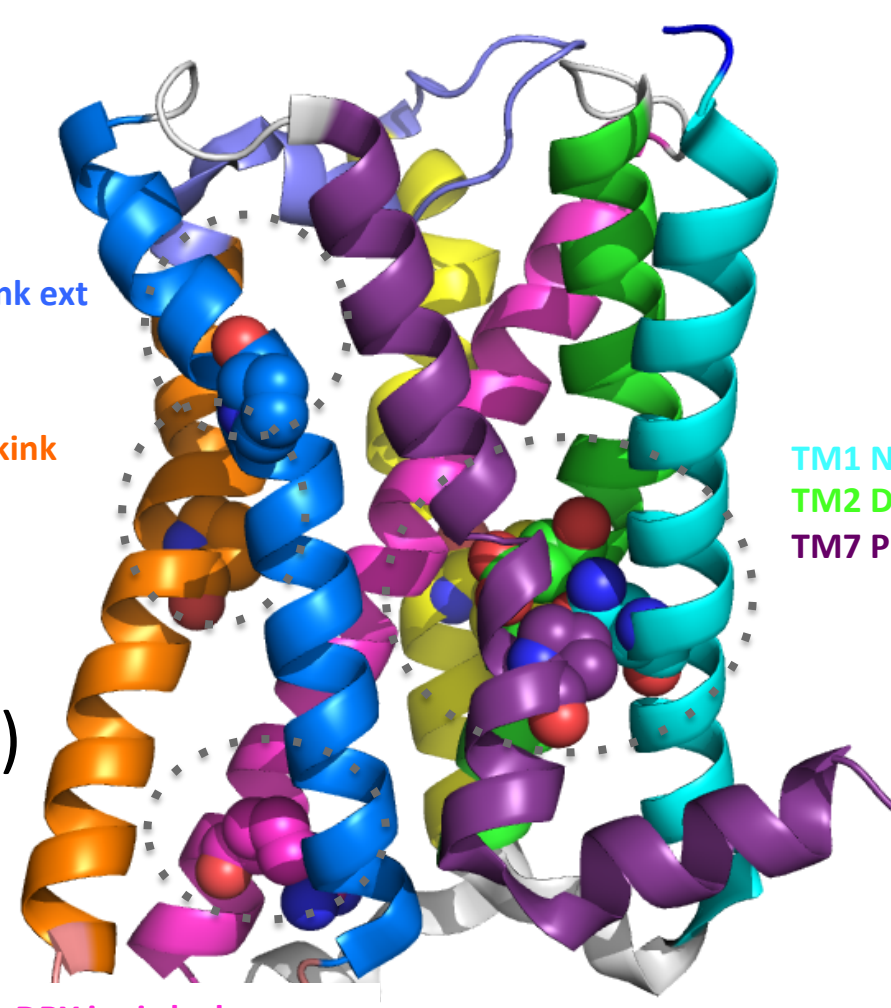
PSGR sequence aligned with solved structures of GPCRs

Sequence analysis of ORs

- ⊛ Olfactory proteome non redundant & checked => 437 ORs.
- ⊛ Clustering with nrdb 90 (alignment at 90% d'id min) => 377 ORs.
- ⊛ Alignment with Clustalw and analysis using HMM profiling of TM segments

Conservation of Ballesteros-Weinstein^[14]:

TM	GN	DRY ₅ P	GN
TM1 1.50	N de GN	R de DRY ₅ P	N de « GN » (-1/377)
TM2 2.50	D	R de DRY ₅ P	D/E (-8/377)
TM3 3.50	R de DRY ₅ P	R de DRY ₅ P	R de DRY ₅ P (-46/377)
TM4 4.50	W	W/Y/hydrophobe	
TM5 5.50	PX ₇	P/D/C/S/TX ₆ SY	
TM6 6.50	P de K/RX ₁₇ P	P/S/T de KX ₁₇ P (X ₁₇ conserved)	
TM7 7.50	P de NP	P de NP	



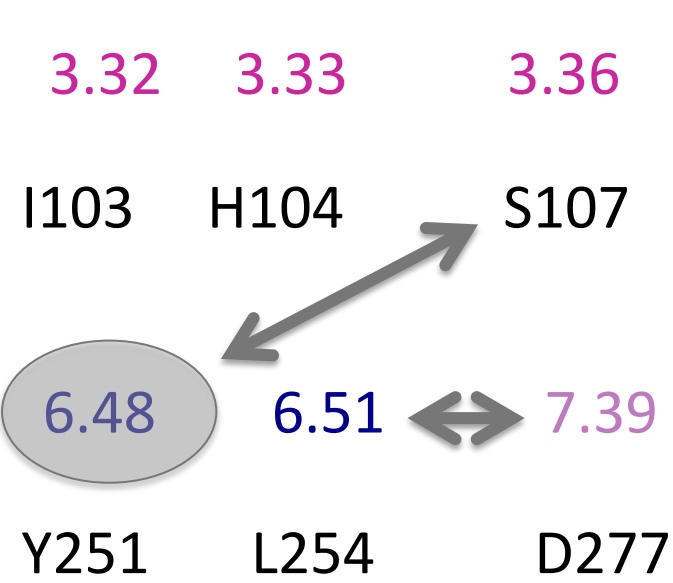
TM4-TM6 less conserved in GPCR but highly conserved among ORs Specificity of ORs.

Homology modeling

- ⊛ For each solved structure of GPCR that defines a template, 100 homology models of PSGR were computed using Modeller^[14], in respect with the multiple alignment, described above.
- ⊛ Active and inactive conformations were modelled.
- ⊛ For each series of PSGR structure, 5 models were selected, on the GABE and DOPE scores, and analyzed with Molprobit. They were then minimized with Charmm forcefield^[15] 5,000 it, 0,01 RMS and Smart Algorithm from DiscoveryStudio©.

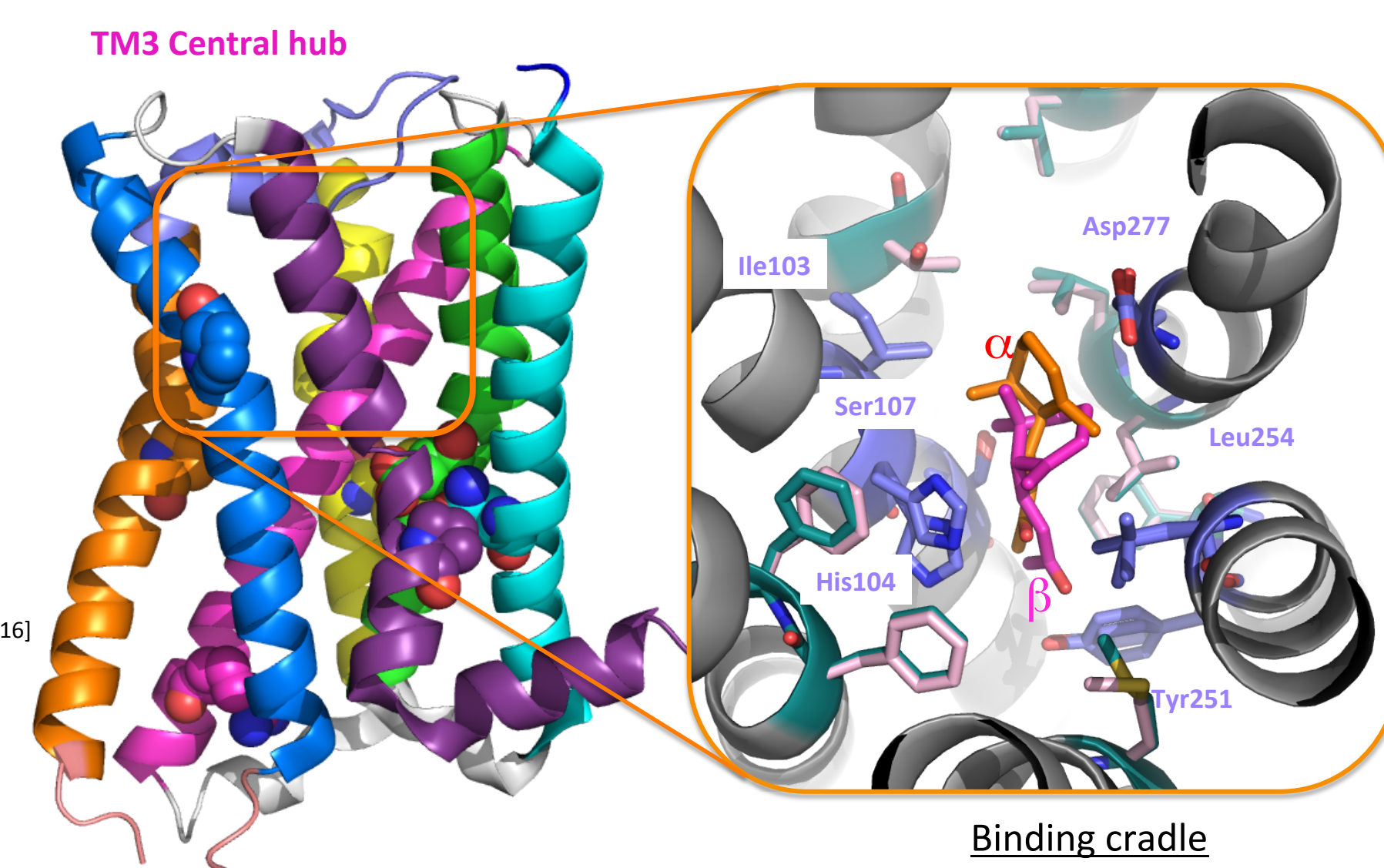
By analogy with the solved GPCR^[16], the binding cradle was proposed to be composed of I103, H104 & S107 of TM3, Y251 & L254 of TM6 and D277 of TM7 and subjected to alanine site-directed mutagenesis.

Docking of ligands



Position 6.48 is part of the transmission switch^[16]

« Upon agonist binding, a rearrangement of a cluster of conserved hydrophobic and aromatic residues occurs deeper in the receptor core. »

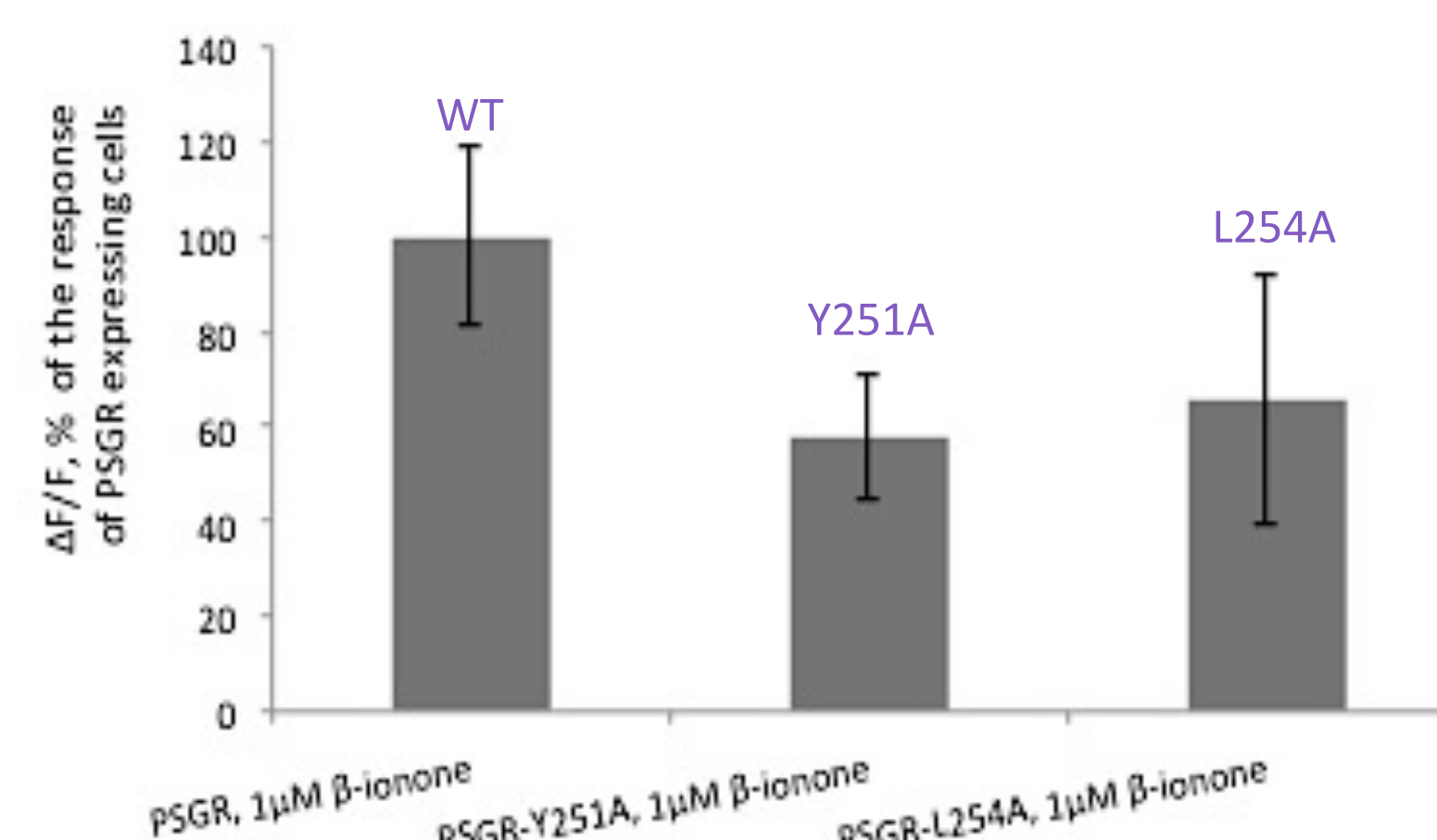


- ⊛ α -ionone, β -ionone, testosterone and other ligands were alternatively docked inside the PSGR binding cradle that defines the receptor site. Multiple positions were tested and ranked with Charmm FF, during when the PSGR was kept rigid. The complexes, ranked as best, were minimized and their total potential energy plus interaction energy were computed. Data with α & β ionones are below.

Ligand	Energy (kcal/mol)	Interaction Energy (kcal/mol)
α -ionone in 3PQR	-24776	-86.1
β -ionone in 3PQR	-24763	-98.6

Experimental validation of TM6 mutants

Activity test



- ⊛ HEK 293 cells expressing PSGR wild type or mutants were seeded onto a black 96-well plate. 24 hours later, cells were loaded with 2.5 μ M of fluo-4 acetoxymethyl ester and stimulated with 1 μ M β -ionone or buffer with 1 μ M of DMSO (control).
- ⊛ Calcium responses are expressed as the mean fluorescence variation $\Delta F/F$ relative to that of control.

We designed homology models of active & inactive conformations of PSGR, in respect with the available template structures of GPCR.

Six residues that putatively define the agonist binding site were mutated into alanine residues. Notably, four of them result in PSGR functional impairment, thus validating our computational analysis, including sequences alignment, homology modeling and docking. We now step further to identify the molecular determinants that discriminate agonist from antagonist binding in the PSGR. Ligand specificity in ORs will then be more largely addressed. Eventually, this work is dedicated to develop a more accurate version of our GPCR automodel webservice^[17].

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