# The structural basis that drives ligand efficacy at the serotonin 5-HT$_{1A}$ receptor

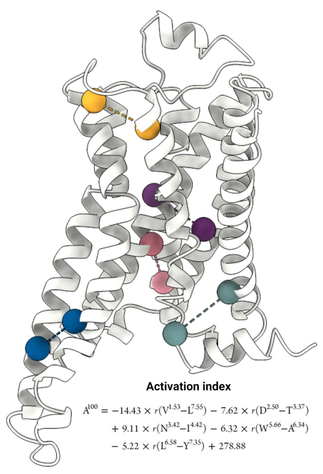
Jacqueline C. Calderón,[a] Passainte Ibrahim,[b] Dorothea Gobbo,[c] Francesco Luigi Gervasio[c] and Timothy Clark.[a]

[a] Computer-Chemistry-Center, Department of Chemistry and Pharmacy, Friedrich-Alexander-University Erlangen-Nuernberg, Naegelsbachstr. 25, 91052 Erlangen, Germany.
[b] Institute of Medical Physics and Biophysics, Faculty of Medicine, University of Leipzig, Germany.
[c] Pharmaceutical Sciences, University of Geneva, CH1206, Geneva, Switzerland.

## INTRODUCTION

G-protein coupled receptors (GPCRs) are the largest superfamily of membrane proteins in the human genome; they modulate numerous physiological responses[1]. The 5-HT$_{1A}$ receptor, a Class A GPCR, is a member of the serotonergic receptor family, which is found in the central and peripheral nervous systems and activated by the neurotransmitter serotonin (5-hydroxytryptamine, 5-HT). Although the 5-HT$_{1A}$ receptor subtype is one of the most studied, since it is an important therapeutic target for several neuropsychiatric disorders, including anxiety, depression, and schizophrenia[2], the structural basis, which involves receptor dynamics, ligand efficacy and receptor activation, is largely unknown. Here, we use a metadynamics protocol based on the general activation index[3] A$^{100}$ to study the activation of the 5-HT$_{1A}$ receptor.

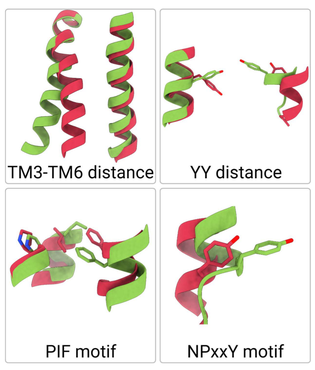
## METHODS



**Activation index**

$$A^{100} = -14.43 \times r(V^{1.53}\text{–}L^{2.55}) - 7.62 \times r(D^{2.50}\text{–}T^{3.37}) + 9.11 \times r(N^{3.42}\text{–}I^{4.42}) - 6.32 \times r(W^{5.66}\text{–}A^{6.34}) - 5.22 \times r(L^{6.58}\text{–}Y^{7.35}) + 278.88$$

**Conserved microswitches**




Active and inactive conformation is represented in green and red respectively

**Sytem preparation**
1. Structural models were based on cryo-EM structures (PDB 7E2X, 7E2Y, 7E2Z) of the 5-HT$_{1A}$ receptor bound to different ligands and the G$_i$-protein

**Molecular dynamics**
2. Systems were geometry-optimized and equilibrated for 300 ns followed by a production run of 2 µs. All simulations used the SPC/E water model and were performed using GROMACS 2019.4

**A$^{100}$ metadynamics simulation**
3. Metadynamics simulations were run in the multiple-walkers scheme using 32 walkers at 310 K. The A$^{100}$ was used as a single collective variable. Gaussian hills were deposited every 500 steps, with an initial height of 0.24 kcal mol$^{-1}$ and a bias factor of 5. The Gaussian sigma was set to 0.1 nm. Free energies were calculated using the sum hills function of the PLUMED 2.5.3 plug-in

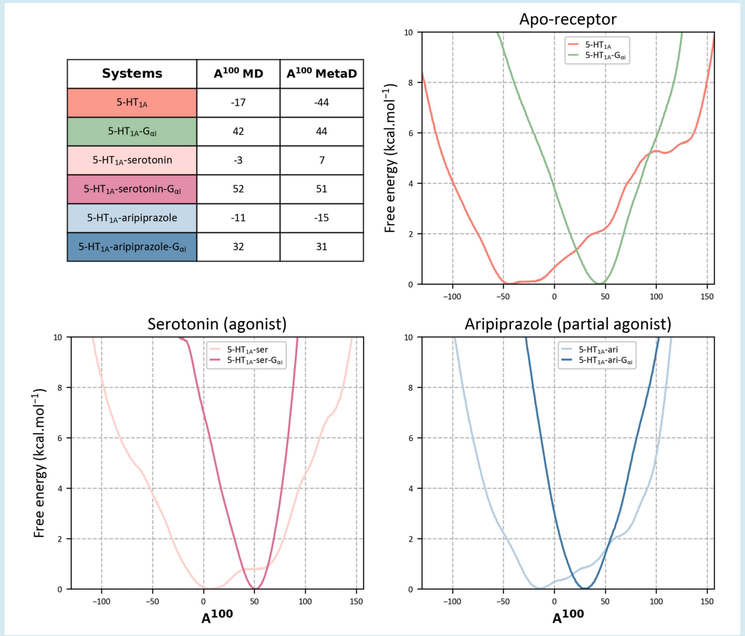
**Reweight A$^{100}$ simulation**
4. A$^{100}$ metadynamics simulations were reweighted to reconstruct free-energy landscapes with respect to the microswitches. Calculation of the reweighting factor c(t) was enabled by using the keyword CALC_RCT in Plumed

**Analysis of the trajectories**
5. Hydrogen bond interactions with a default cutoff distance and angle of 3 Å and 135°, respectively, were performed with CPPTRAJ module of AmberTools18

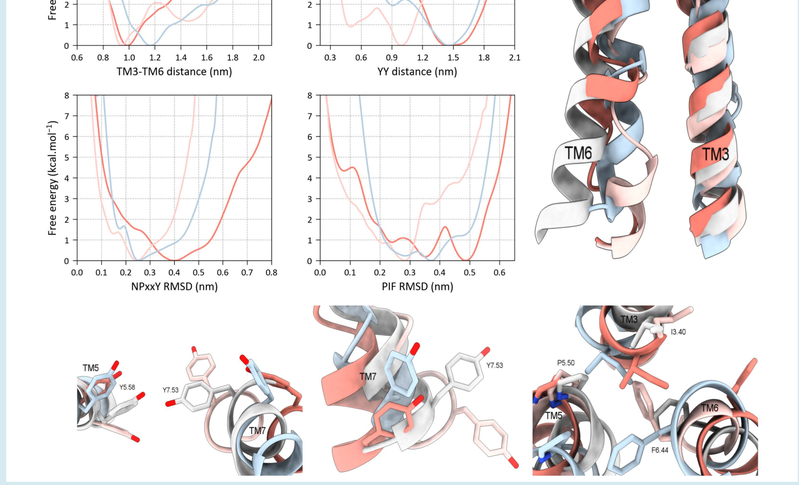
## RESULTS

The apo receptor without the G-protein shows a broad global minimum at A$^{100}$ = -43.3.
Free-energy profiles of full and partial agonist are consistent with the preference for the inactive conformation in binary complexes of receptor with agonists.
Systems with the bound G-protein show one narrow minimum at active A$^{100}$ values.

| Systems | A$^{100}$ MD | A$^{100}$ MetaD |
|---|---|---|
| 5-HT$_{1A}$ | -17 | -44 |
| 5-HT$_{1A}$-G$_{αi}$ | 42 | 44 |
| 5-HT$_{1A}$-serotonin | -3 | 7 |
| 5-HT$_{1A}$-serotonin-G$_{αi}$ | 52 | 51 |
| 5-HT$_{1A}$-aripiprazole | -11 | -15 |
| 5-HT$_{1A}$-aripiprazole-G$_{αi}$ | 32 | 31 |

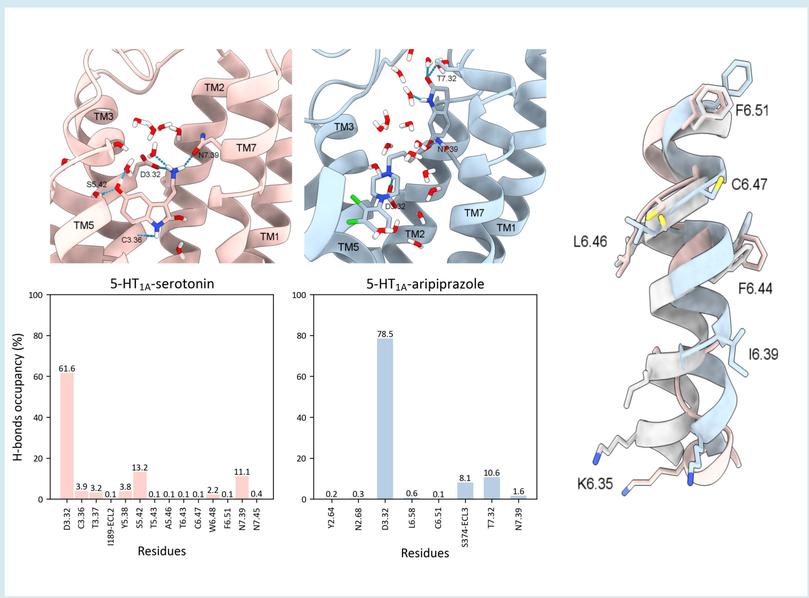



Simulation results for 5-HT$_{1A}$ systems. Calculated activation/deactivation free-energy profiles from metadynamics simulations

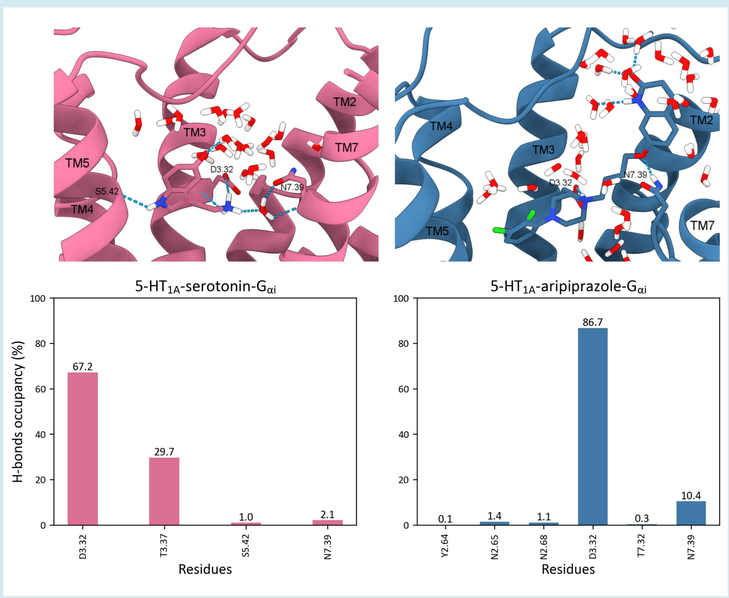



Comparison of 5-HT1A inactive systems. 1D free-energy landscapes projected as a function of the microswitches. Representation of microswitches. Cryo-EM structure 7E2Y is represented in white as reference active conformation

The apo-5-HT$_{1A}$ system shows minima compatible with inactive-like conformations relative to the active structure.
Less inactive- or even intermediate-like conformations can be adopted by 5-HT$_{1A}$-ligand systems.
Ligands can stabilize different receptor states[4].
Binding of a full agonist reduces the number of inactive-like states of the conserved microswitches in comparison to a partial agonist.




Comparison of 5HT$_{1A}$-ligand binary complexes. Representation of polar interaction network formed in the binding pocket. Hydrogen–bond occupancy. Representative view of the TM6 conformational rearrangement.

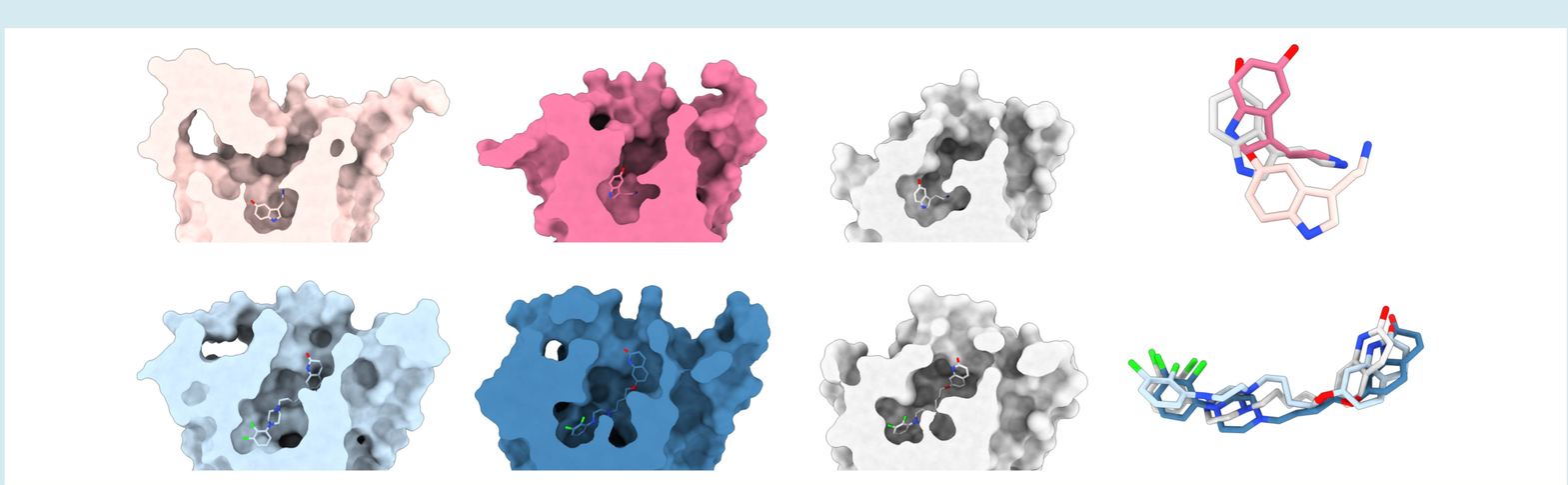



Comparison of 5HT$_{1A}$-G$_{αi}$-ligand ternary complexes. Representation of the polar interaction network formed in the binding pocket. Hydrogen–bond occupancy

Serotonin stabilizes conformational changes in TM5, TM6, and TM7 prior to G-protein coupling.
Hydrogen-bond interactions between aripiprazole and the receptor are concentrated on extracellular residues in TM7.
A TM6 counterclockwise rotation relative to the active state can be observed for 5-HT$_{1A}$-aripiprazole binary complex.
Non-optimal G-protein activation have been related to partial agonism at different sub-families of GPCRs.

## CONCLUSION

- **General metadynamics-based protocol** to simulate activation/deactivation of Class A GPCRs.
- **Computed free-energy landscapes, specific interaction analysis and structural inspection** suggest a combined mechanism that requires the action of both stabilizing intracellular and extracellular interactions in the receptor core for the full activation of the receptor.
- **Potential role of polar interaction** networks in the receptor core as a regulator in the initial stages involved in receptor activation.
- **Structural insights that rationalize the mechanism of action of ligands with different efficacy at the 5-HT$_{1A}$ receptor**.
- Findings of remarkable value that contribute to the **structure-based design** of novel ligands with desired therapeutic efficacy.



Binding sites and poses of 5-HT$_{1A}$-G$_{αi}$ ligand systems. Cryo-EM structures 7E2Y and 7E2Z are represented in white as reference active conformation

An attenuated polar interaction network in ternary complexes might be attributed to conformational changes in the orthosteric binding pocket upon G-protein binding.

1. T. K. Bjarnadóttir, et al., Comprehensive repertoire and phylogenetic analysis of the G protein-coupled receptors in human and mouse. *Genomics* **88**, 263–273 (2006).
2. N. M. Barnes, et al., International union of basic and clinical pharmacology. CX. Classification of receptors for 5-hydroxytryptamine; pharmacology and function. *Pharmacol. Rev.* **73**, 310–520 (2021).
3. P. Ibrahim, D. Wifling, T. Clark, A universal activation index for class A GPCRs. *J. Chem. Inf. Comput.* **59**, 3938-3945 (2019).
4. Bock, A.; Bermudez, M. Allosteric Coupling and Biased Agonism in G-Protein-Coupled Receptors. *FEBS* **288,** 2513–2528 (2021).