Markov State Models to study synaptic neurotransmission at the molecular level

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Introduction

Glycine receptors (GlyRs) are pentameric ligand-gated ion channels (pLGICs) that convert a chemical signal, i.e., the binding of neurotransmitter, into an ion flux through the postsynaptic membrane [1]. They mediate inhibitory neurotransmission in the spinal cord and brainstem and have been associated with a range of neurological disorders including Hyperekplexia, temporal lobe epilepsy and chronic pain [2]. It has been shown that synaptic receptors accomplish their function by switching between (at least) three distinct conformational states: a resting state (R), where the ion pore is closed; an active state (A) stabilized by agonists, where the pore is open; and one or more desensitized states (D), where the channel shuts with the agonist still bound [3, 4]. From the structural perspective, GlyR is by far the best characterized pLGIC with more than **40 high-resolution structures** in the PDB illuminating the receptor in different conformational states and in complex with modulatory ligands

In this project, we propose to combine **All-Atom** (AA) **Molecular Dynamics** (MD) simulations with **Markov State Models** (MSM) [5] to study GlyR gating mechanism. MSMs are stochastic models that describe the temporal evolution of complex systems and they have been used to provide understanding of molecular function and regulation with atomic resolution. By capitalizing on abundant structural, functional and simulation data, we aim at illuminating the complexity underlying molecular function with atomic resolution along with a description of the energetics involved.
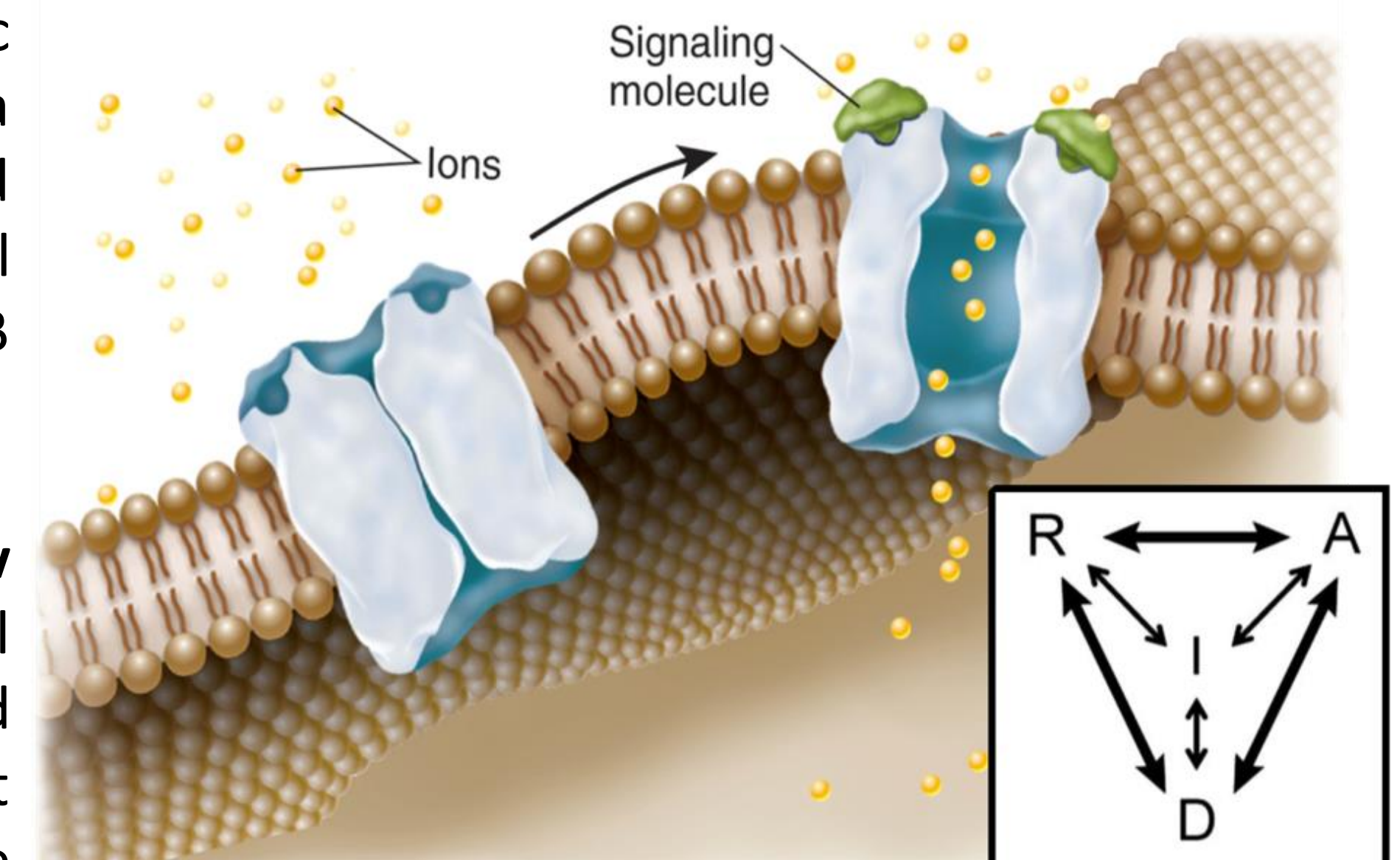
Molecular Dynamics (MD)

System Composition (All-Atom) :

- GlyR (orange) : 1 molecule
- POPC (gray) : 308 molecules
- Waters : 41976 molecules
- NaCl (purple and green) : 150 mM
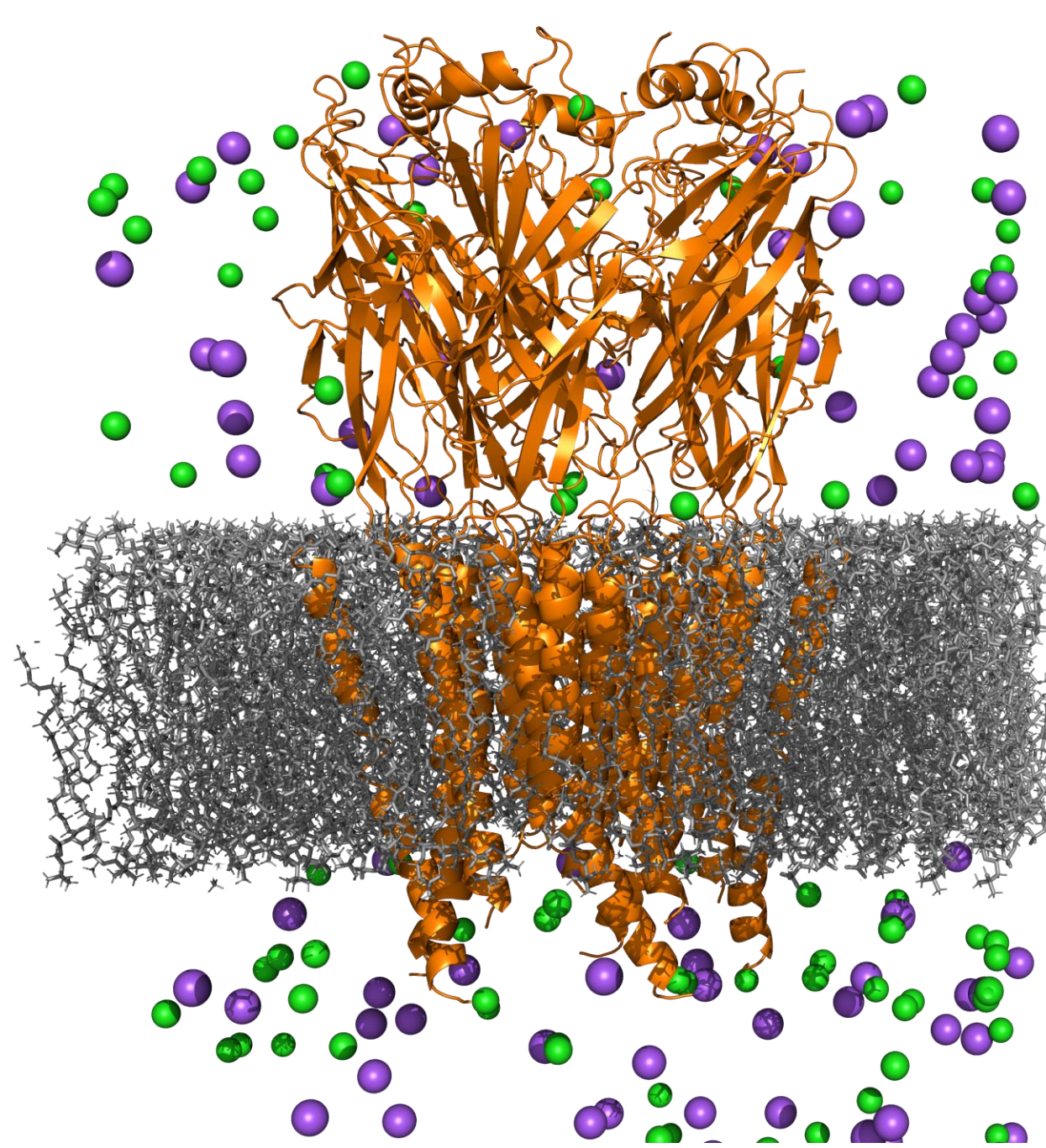
Force Field : CHARMM36m [6]

Software : GROMACS 2021 [7]

Temperature / Pressure : 300 K / 1 bar

Nb of trajectories : 61

Nb of starting structures : 24

Simulation time / trajectory: 500 ns

Markov State Models (MSM)

- **Stochastic model** to describe a sequence of events where the probability of each event only depends on the previous state
- In MD, an MSM describes the different **structural states** of the system and the **transition mechanisms**
- The molecular system needs to be **markovian** (memoryless) and the dynamics **reversible**
- The aim is to decipher the **structural**, **kinetic** and **thermodynamic** properties of the system


Markov state model
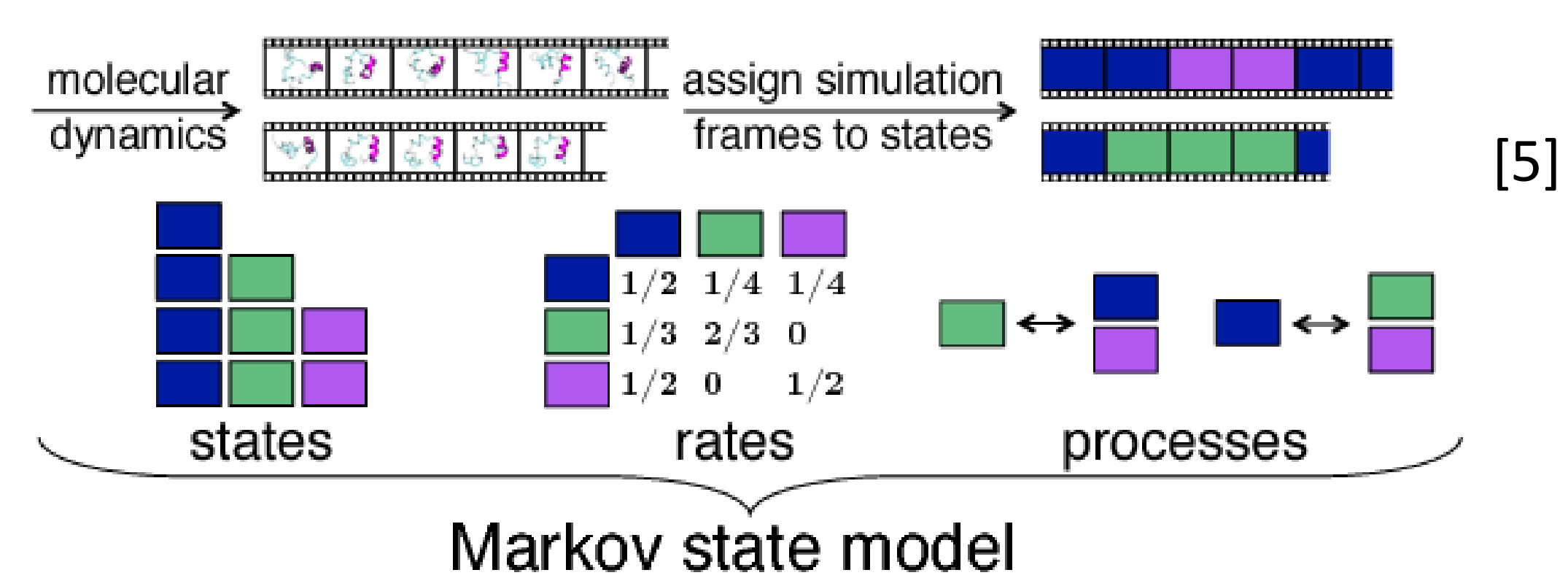
MSM were constructed using PYEMMA [8] and DeepTime [9] softwares

Preliminary results

Goal : construct the first complete model of ion gating in a human synaptic receptor

- We have produced **30,5 μs** of MD simulations of the GlyR in all known conformational states

- We have determined an **ensemble of features** capable of clustering the structures in biologically relevant state

- We have constructed a **preliminary model** that distinguishes four relevant states: resting, pre-active, active and desensitized

- We are currently working on sampling the transition between the open and desensitized state to construct a **partial MSM** describing the **desensitization mechanism** along with the energetics involved
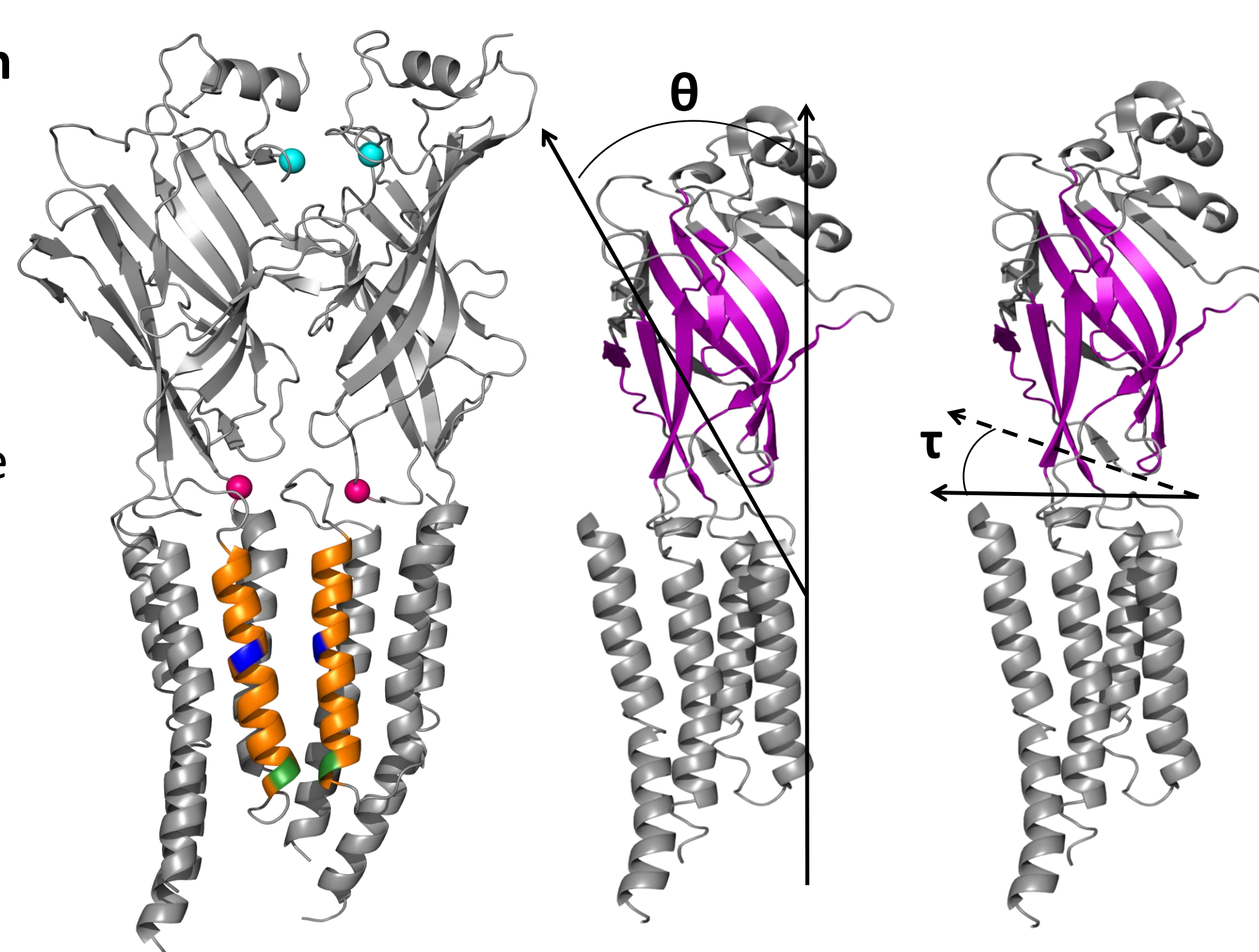
On the left, ensemble of structural features selected for MSM construction: W110-D102 distance (cyan), W159-Q235 distance (magenta), M2 helix tilt (orange), pore size at L9' (blue), pore size at P-2' (green), blooming angle (θ, purple) and twisting angle (τ, purple). On the right, preliminary energy landscape for the WT GlyR in APO state. The trajectories are projected into IC1 and IC2, two reaction coordinates obtained with TICA, a dimensionality-reduction algorithm commonly used in MSM construction [10]. The colour is related to the free energy value. The points are the projection of the experimental structures used as starting structures for the MD simulations: resting (red, 6PXD), pre-active (green, 6PM3), active (cyan, 6PM2) and desensitized (orange, 6PM1).
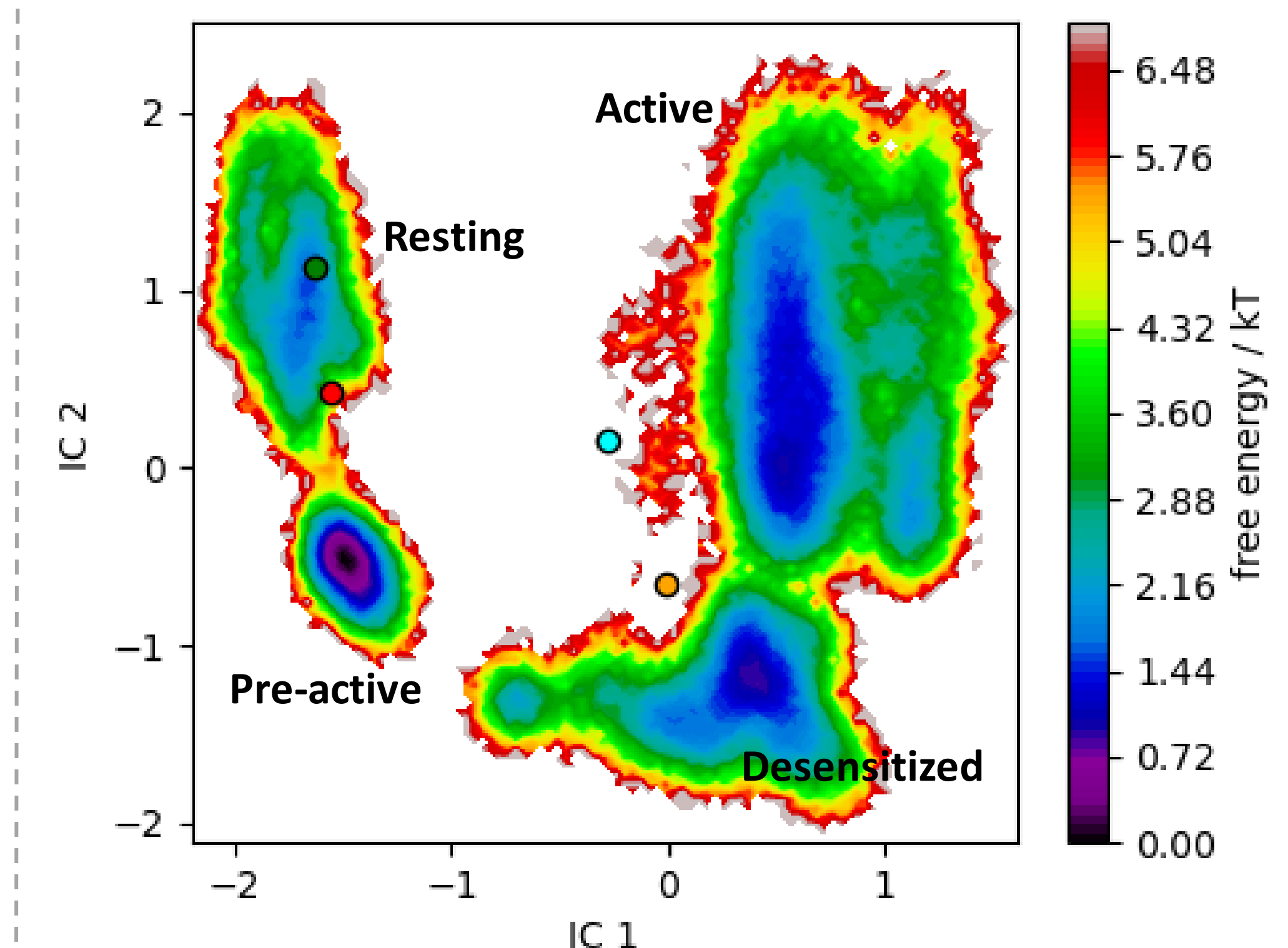
Future perspectives

1. Provide a description of the **conformational transition pathways** underlying synaptic receptor activation and desensitization with atomic resolution

2. Reveal the existence of **structural intermediates** which are not accessible experimentally

3. Quantify the **free energy changes** and **barriers** between the physiological states of the receptor.

4. Construct a **second model** in presence of **agonist** (taurine)

5. Provide quantitative understanding to the **allosteric modulation of gating** via the changes in free energy changes involved upon ligand binding.

6. Establish a **general framework** to rationalize the allosteric modulation of synaptic receptor by binding events and/or pathogenic mutations that could open to the development of **drug therapies** against neurological disorders and cancer.

Acknowledgments

References

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