Hunting for Structures in an Unstructured Region

Structural and Kinetic Investigation of the C-Terminus in GPCRs: from FRET to Ensembles

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In Short

• Unknown structure-function relationship of the β2-Adrenergic receptor's C-terminus
• Exploit FRET-data to bias the MD-exploration via adaptive sampling
• Highly parallel MD simulations
• Markov State Modelling to interpret and express structural and kinetic information

G-protein coupled receptors, GPCRs, are a large class of proteins that act like antennae that help cells sense their environment. These receptors can detect and transmit information about a wide range of stimuli, and comprise about 50% of FDA approved pharmaceutical drug targets [1].

Across varying families and sub-families of receptors, the same scaffolding structure is repeated: a bundle of seven alpha-helices spanning the cellular membrane, with a binding pocket near the extracellular side and a binding crevice near the intracellular side. The elucidation of this particular structure-function relationship, for stimuli as different as hormones and light, was awarded with the Nobel Prize in chemistry in 2012 to Robert J. Lefkowitz and Brian K. Koblika, currently BfH-Einstein visiting fellow at Charité - Universitätsmedizin Berlin [2].

Recently, single-molecule, Försters-Resonance-Energy-Transfer (FRET) experiments by the Koblika lab [3] have shed light on specific structural features of the otherwise poorly characterized structure of the C-terminus (CT) of the β2-Adrenergic receptor (β2AR, Fig. 1).

In this project, we intend to exploit this information by using adaptive sampling methods [4] to bias classical molecular dynamics (MD) simulations of the β2AR. We will combine this enhanced sampling method with the use of Markov-State-models (MSMs [5]) to interpret and express the obtained structural and kinetic data of the β2AR-CT interplay, which is otherwise scarcely characterized.

The adaptive-sampling paradigm is particularly well-suited for massively parallel computing architectures like the HLNR, because the computational effort is not concentrated in one single, long, MD-trajectory, but rather distributed across a high number of shorter trajectories. These trajectories are i) independent of each other and ii) launched together in so-called epochs, which parallelize perfectly across an arbitrary number of nodes. The adaptive part comes from the epochs being launched one after the other, with new starting frames for the subsequent epoch chosen via a user-defined fitness-criterion. Encoded in that criterion will be the structural information obtained from FRET-experiments. By repeating this selection process many times, the ensemble of trajectories is biased towards a given distribution, without having manipulated any particular degree of freedom during simulation time.

Furthermore, and precisely because of the high number of independent trajectories, and their off-equilibrium nature, MSMs offer the perfect framework to analyze and express our data, given that i) MSMs are based on conditional transition probabilities (which need not come from the same trajectory) and ii) MSM-estimation exploits the constraint of physical reversibility to weight the off-equilibrium ensemble back to an equilibrated (i.e.
Boltzmann-distributed) ensemble. In other words, the re-weighting, post-processing step needed by most enhanced sampling methods is carried out automatically by the MSM estimation.

Using a smaller test-system of the membrane embedded β2AR, we have carried out our intended simulations at a smaller scale in equivalent HPC architectures. By exploiting the FRET data mentioned above in an adaptive sampling setup, we have been able to arrive at a preliminary, yet interpretable MSM of the the CT’s dynamics near the receptor’s binding crevice, which we partially show in Fig. 2.

We hope that with access to HLRN, we can scale up the system-size and generate ten-times more simulation data. The MSMs will be much closer to the physical reality of the CT’s dynamics and be statistically more accurate. Given the shared nature of most of the structural scaffolding in the GPCR family, it is likely that many insights achieved by this project be exportable to other GPCRs beyond the β2AR.

WWW
https://biophysik.charite.de/forschung/ag_modellierung/

More Information


[3] B. Kobilka, internal communication


Project Partners
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