Simulating beyond FRET experiments

The C-terminus in GPCRs: from FRET to ensembles

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In Short

- Structure-function relationship of the β2-Adrenergic receptor's C-terminus
- Highly parallel MD simulations
- Exploit FRET-data to bias the MD-exploration via adaptive sampling
- Markov State Modeling 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. Kobilka, currently BIH-Einstein visiting fellow at Charité - Universitätsmedizin Berlin[2].

Recently, single-molecule, Försters-Resonance-Energy-Transfer (FRET) experiments by the Kobilka lab[5] 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[3] 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[4]) to interpret and express the obtained structural and kinetic data of the β2AR-CT interplay, which is otherwise scarcely characterized.

The first round of adaptive simulations produced an aggregated 10 μs of MD-simulation data and showed remarkable conformational flexibility of the

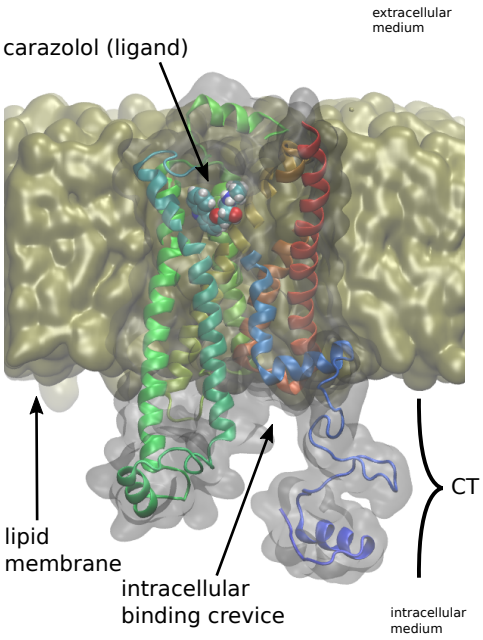

Figure 1: *Rendering of the β2-Adrenergic receptor (B2AR) embedded in a lipid bilayer. The ligand carazolol is depicted in the binding pocket near the extracellular medium, which is on upper side of the membrane. On the lower side, we see the intracellular segments leading to the G-protein binding crevice as well the C-terminus (CT). The goal of this project is to elucidate the conformational ensembles accessible to the usually unstructured CT.*

CT even within the short trajectories. The starting bias towards a known distance was quickly *forgotten* during each individual simulation and different conformations were sampled by the CT.

While this initial dataset was distributed across 50 different, short, trajectories and was off-equilibrium, it richness was enough to detect and later experimentally confirm key-residues in the CT-β2AR interaction[6].

However, the extraction of a meaningful kinetic model of the CT-β2AR system, from which metastable states can be extracted, remained challenging: the data was split into four *basins* with no exchange of population between them.

To solve this problem, and based off the observed flexibility of the CT, in the second funding period we carried out three long, unbiased simulations to check whether transitions between basins would occur spontaneously after attainable simulation lengths, given that each individual adaptive-sampling trajectory had been only 200ns long. Unfortunately, these simulations have not connected the basins, as is shown in Fig. 2. There, we show the new data together with the old data, projected onto Time-

Independent-Component (TIC) space.

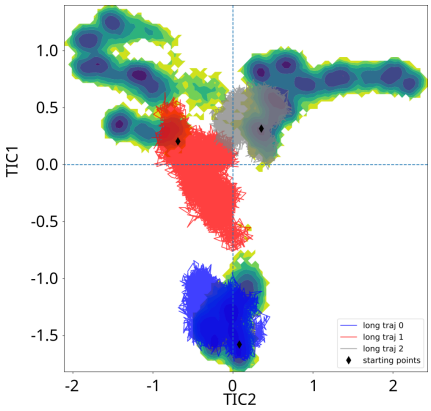

Figure 2: *Time-Independent-Component (TIC) plot of the simulated MD-data. As contour-plot we see the adaptive-sampling data of the first round of simulations. Superimposed are the time-traces of three new long trajectories (blue/red/gray), each started from a different basin (black diamonds)*

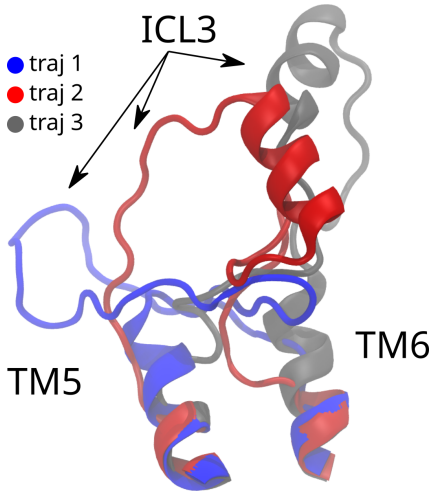

Figure 3: *Representative frames of the TM5-ICL3-TM6 region of the TM-bundle of the three new trajectories. The differences in helicity can be clearly appreciated and are representative of the whole trajectory*

Upon further inspection, we could clearly attribute the disconnectivity to the intracellular side of the TM-bundle, in the TM5-ICL3-TM6 region. Particularly in TM6, the α-helix secondary-structure is either elongated or shortened by a few turns. We have selected representative snapshots where these differences are very visible in Fig. 3.

This (un)folding is comparatively slow with respect to other processes and might need some bias to re-equilibrate. On the other hand, the new trajectories only ran fur just 1, and simply prolonging them might spontaneously lead to transitions between these states. That is what we intend to find out in the next phase of our project. 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.

Finally, we used analysis software developed initially for this project elsewhere and acknowledged HLRN in the publication (7, Hildebrand, Sauer and Langenhan are all corresponding authors).

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https://biophysik.medizin.uni-leipzig.de/forschungresearch/prof-dr-peter-hildebrand/

More Information

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Project Partners

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