AN ACTIVE CONFORMATION OF MGLU2 RECEPTOR INDUCED BY MOLECULAR DYNAMICS SIMULATION WITH C-TERMINAL G_I PEPTIDE

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- **MD simulations with Gα protein are a viable method of obtaining active conformations of G Protein-Coupled Receptors (GPCRs) [1-3]**
  - **The use of C-terminal part of Gα protein allows for simplification of the MD systems and shortening calculation time**

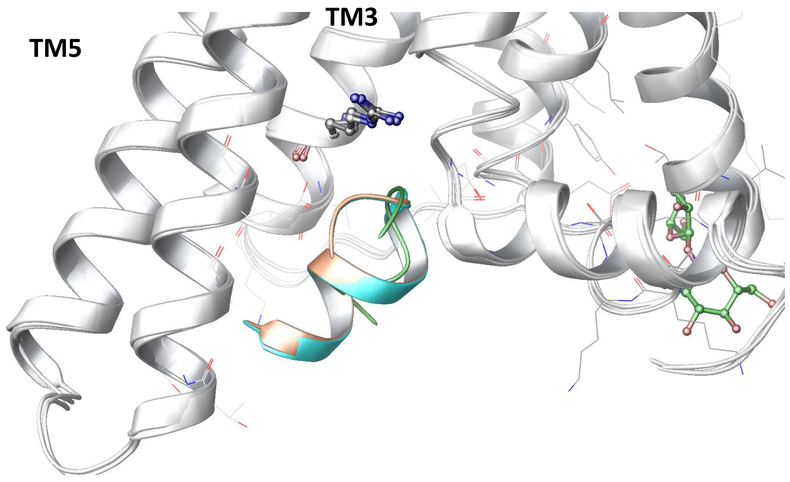

Figure 1. The superimposition of Rhodopsin structures cocrystalized with C-termianl peptides of transducin (orange, blue, PDB codes 2X72 and 4BEY, respectively) and arrestin finger loop (green, PDB 4PXF).

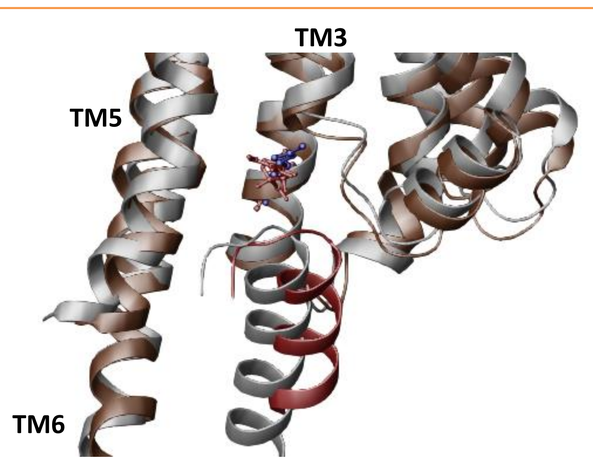

Figure 2. The MD simulation of β2AR along with C-terminal G peptide (red ribbon) compared with the respective crystal structure (white ribbon).

The crystal structures of Rhodopsin with C-terminal 10 residues long peptide of transducin (G_t) prove, that those peptides can stabilize the active conformation of the receptor. We have applied this approach to the Molecular Dynamics (MD) experiments, and verified it on the crystal structure of β2AR with G_s (PDB: 3SN6). The key interactions between G protein and Arg 3.50 and helices 5 and 6 are preserved across the simulation. RMSD of 2.91 Å is a consequence of fluctuations of the system.

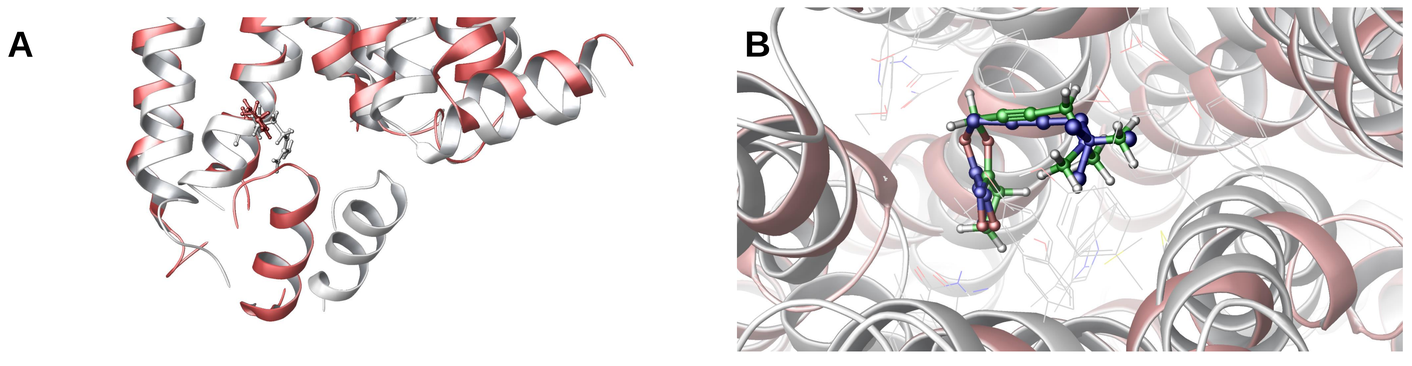

Figure 3. Results of MD simulations of inactive crystal structure of M_2R (pdb 3UON) with docked antagonist iperoxo and $G\alpha_i$ C-terminal peptide. **A:** binding of $G\alpha_i$ peptide after 179 ns of simulation (red ribbon), and interaction with key $R^{3.50}$, starting structure is represented with white ribbon. **B:** comparison between M_2R – iperoxo complex obtained from MD (red ribbons, green ligand) and crystal structure (pdb: 4MQS, white ribbon, blue ligand)

The approach was tested on a set of crystal structures of M_2 receptors (pdb 3UON – inactive, 4MQS – agonist bound stabilized by a nanobody). Crystalized agonist (iperoxo) was docked into the inactive conformation, and the 500ns MD simulation was performed with $G\alpha_i$ peptide. Results show the binding of the peptide into intracellular pocket (Fig. 3A), and conformation of the ligand similar to one from crystal structure (Fig. 3B). The RMSD between modelled complex and 4MQS was 3.3 Å overall, while for the ligand structure it was 1.15 Å.

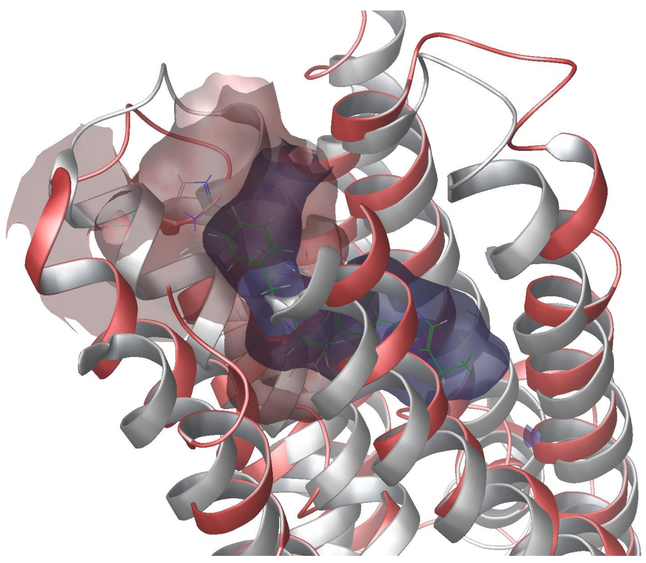

Figure 4. The superimposition of active (red) and inactive (white ribbons, blue surface) conformations of mGluR2 receptor with docked positive allosteric modulator.

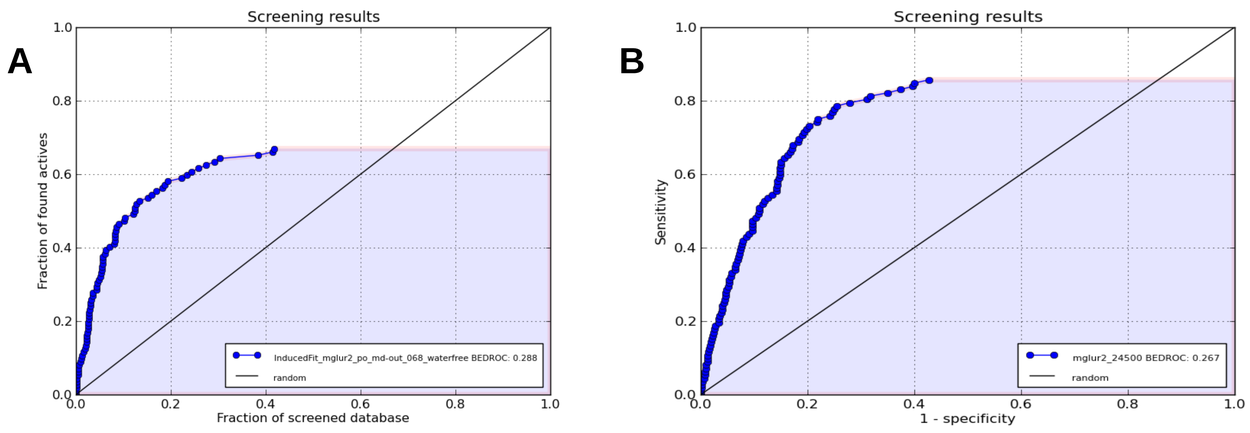

Figure 5. The results of screening 112 positive allosteric modulators (PAMs) of mGluR2 against 10978 DUD-like decoys ; **A**: for an inactive conformation after Induced Fit Docking. **B**: for a conformation activated with $G\alpha_i$ peptide.

The application of methodology of simulating GPCR along with Gα C-terminal peptide allowed us to receive the active conformation of the mGlu2 receptor from inactive homology model based on mGluR1 crystal (PDB: 4OR2).

References

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Acknowledgements

The study was partially supported by the Polish-Norwegian Research Programme operated by the National Centre for Research and Development under the Norwegian Financial Mechanism 2009-2014 in the frame of the Project PLATFORMex (Pol-Nor/198887/73/2013). www.platformex.eu