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UNDERSTANDING POSITIVE ALLOSTERIC MODULATION OF THE mGLU₂ RECEPTOR BY COMBINED EXPERIMENT, LIGAND BASED DESIGN AND MD SIMULATIONS

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The metabotropic glutamate receptor 2 (mGlu₂R) belongs to class C G protein-coupled receptors (GPCRs). Activation of mGlu₂ receptors is a strategy for the treatment of psychiatric disorders. Due to the highly conserved orthosteric binding site, positive allosteric modulation (PAM) has become preferred. Here we combine experiment and modeling to understand the binding mode of mGlu₂ PAMs. We augment conventional industrial modeling approaches with molecular dynamics simulations to derive a deeper understanding of PAM-receptor interactions. Together, the experimental mutagenesis and computational simulations provide a plausible binding mode with initial indications of how the ligand allosterically activates the receptor. Altogether, this work offers new insights into the functioning of PAMs which can contribute to the design and development of new and improved drugs. Furthermore, it shows the value of incorporating MD simulations to enhance traditional ligand design protocols at GPCRs.

P08

MULTIPLE CONFORMATIONAL STATES IN RETROSPECTIVE VIRTUAL SCREENING – HOMOLOGY MODELS VS. CRYSTAL STRUCTURES. β_2 -ADRENERGIC RECEPTOR CASE STUDY

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Distinguishing active from inactive compounds is one of the crucial problems of molecular docking, especially in the context of virtual screening experiments. The randomization of poses and the natural flexibility of the protein make this active/inactive discrimination even harder. Some of the recent approaches to post-docking analysis use an ensemble of receptor models to mimic this naturally occurring conformational diversity. However, the optimal number of receptor conformations is yet to be determined. In this study, we compare the results of a retrospective screening of β_2 -adrenergic receptor ligands performed on both the ensemble of receptor conformations extracted from ten available crystal structures and an equal number of homology models. Additional analysis was performed for homology models with up to 20 receptor conformations considered.

The docking results were encoded into Structural Interaction Fingerprints [1] and were automatically analyzed by the Support Vector Machines algorithm. The use of homologs in such virtual screening

application was proved to be superior in comparison to crystal structures. Additionally, increasing the number of receptors in the profile led to enhanced effectiveness of active vs. inactive discrimination of compounds [2].

References: 1) Deng Z, Chuaqui C, Singh J, *J Med Chem.* **2004**, *47*(2), 337-44; 2) Mordalski S, Witek J, Smusz S, Rataj K, Bojarski AJ, *J Cheminf.* **2015**, *7*:13.

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CONFORMATIONAL ENSEMBLES SAMPLED BY THE CHEMOKINE RECEPTOR CXCR4 IN ACTIVE AND INACTIVE STATES

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The chemokine receptor CXCR4 is a G protein-coupled receptor (GPCR) involved in chemotaxis as well as numerous physiological processes and pathologies, such as cancer metastasis, inflammatory diseases and AIDS. The crystal structures of CXCR4 in complex with antagonists provide a static view of the inactive state. However, proteins are inherently dynamic systems and GPCRs are best described in terms of conformational ensembles [1]. The receptors sample diverse distinct conformations, each of which leading to different downstream functions and influenced by binding of different ligands. Knowledge of these conformations and of reactional paths between them is instrumental in developing drugs that exploit distinct states leading to different downstream functions. Here, we investigate CXCR4 conformational ensembles gathered from molecular dynamics (MD) simulations and the major activation/deactivation transitions typically taking place on the micro to millisecond timescale. Accelerated MD [2] is an efficient method to enhance conformational sampling and reduce the computational time necessary to observe such transitions by several orders of magnitude. Comparison of movements in active and inactive CXCR4 conformations sampled by accelerated MD provides insights into the distinctive conformational flexibility of these states. In addition, the inactivation of the active CXCR4 model highlights the time-dependent correlated conformational changes of side chains surrounding functional GPCR microswitch residues that trigger the transition from active to inactive state of CXCR4.

References: 1) Deupi and Kobilka, *Physiology*, **2010**, *25*, 293-303; 2) Markwick and McCammon, *Phys Chem Chem Phys*, **2011**, *13*, 20053-65.