Metadynamics Simulations of FPR2: Using an Enhanced Sampling Method to Elucidate The Mode of Action of a Diverse Set of Ligands

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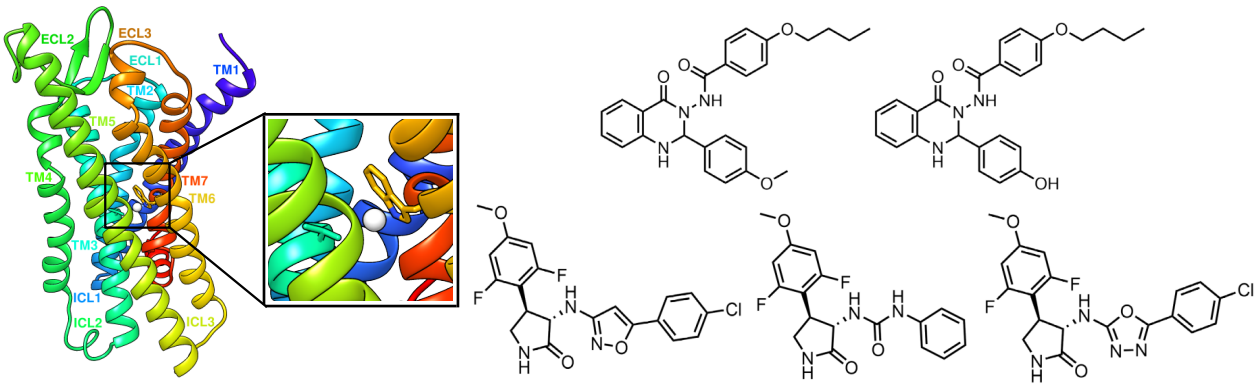

The Formyl Peptide Receptors (FPRs) belong to the class A of G-protein coupled receptors (GPCRs); the family has three members: FPR1, FPR2 and FPR3.
Seven transmembrane helices (TM) connected by three extracellular (ECL) and three intracellular loops (ICL) are characteristic for GPCRs, as illustrated in the left figure above. [1] FPRs play a key role in the host defense against microbes because they are located on immune cells like phagocytes that belong to the innate immune system. Thus, they are also involved in inflammatory diseases like Alzheimer's disease or cancer and are novel targets for the treatment of those diseases. [2] Several small molecules and peptides are known which either act as agonists, partial agonists or antagonists. [3,4] We selected some of the small molecules that represent these classes (see figure for examples) for further computational analyses including binding-mode identification, binding-pathway analysis and free energy of binding calculations using metadynamics simulations.
First, the molecules were docked into the orthosteric binding site of FPR2 of a cryo-EM structure in complex with a known peptide agonist (PDB ID 7wvw). The resulting FPR2 complex structures were used for µs-time scale metadynamics simulations conducted mainly on the Alex cluster of NHR@FAU in Erlangen using Gromacs and Plumed. The simulations were run following a protocol that was already described earlier by Saleh et al. [5]. The funnel is placed above the center between the Cα atoms of the residues Val113$^{3.40}$ and Trp254$^{6.48}$, as can be seen in the zoomed-in picture above. The residues are shown as sticks and the center is indicated by a white sphere.
With this approach, it was possible to simulate the binding/unbinding of the ligands and to determine the free energies of binding with sufficient accuracy. The global free energy minimum obtained corresponded well to the known binding poses and gave new insight into the binding mode of small-molecule ligands. This knowledge will be used to design novel ligands for FPR2. The results highlight the importance of a deep, hydrophobic pocket at the bottom of the orthosteric site and of three polar residues right above it. Furthermore, these data suggest comparable binding modes of small molecules and peptides, contrary to earlier results [6].

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