Binding pose prediction using Free Energy Perturbations

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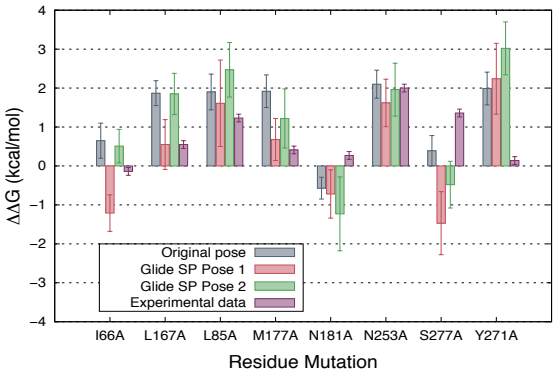
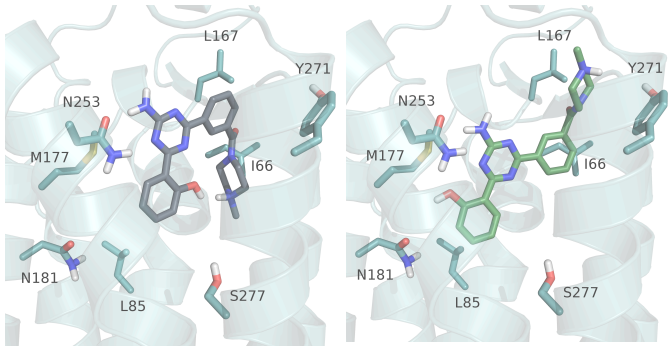
Background: Our group has recently published a protocol for the computation of the effect of site directed mutagenesis (SDM) on ligand-binding affinities, based on the free energy perturbation (FEP) methodology. [1–3] The protocol was thoroughly applied to characterize both agonist (NECA) and antagonist (ZM241385) binding to the Adenosine A_{2A} Receptor, with excellent results.

Objective: To characterize the binding mode and SAR of novel A_{2A} antagonist scaffolds recently published. [4-5]

Methodology: We use our FEP protocol in combination with exhaustive docking. The *in silico* exploration is integrated with all available experimental data publicly available for the compound series reported by Heptares. This includes crystal structures, pharmacological data/SAR and biophysical mapping (BPM) data on three ligand series for 8 alanine mutations.





Results: We initially characterized the effect of the 8 binding-site mutations on the binding affinity of two 1,2,4-triazines, starting from the corresponding crystal structures (PDB codes 3UZA/3UZC). [4] The calculated values are in good qualitative correlation with experimental data (not shown). We thereafter applied this protocol to predict the binding poses of compounds where no crystal structure is available.

The second scaffold explored was compound 15 from the preceding 1,3,5-triazines hit series, a highly potent and moderately selective antagonist for the A_{2A}-receptor for which BPM data is available. [5] We considered different binding modes, including one proposed in the original publication and two additional docking poses obtained with GLIDE-SP. [6] For all binding modes, we calculated the effect on ligand affinity for each of the 8 mutations. The binding mode proposed in the original publication [5] was revealed as the most promising. Unfavourable interactions with Asn253$^{6.55}$ suggested a rotation of the phenol group, in a conformation stabilized by an internal hydrogen bond. As illustrated in the figure (grey), this binding mode showed the best correlation with available experimental data. However, the effect on the N181A$^{5.42}$ mutation was still incorrectly predicted, similar to co-crystallized 1,2,4-triazines. This is most probably due to the indirect effect of this mutation, involved in inter-helical contacts between TM5 and TM6 bridged through a water molecule.

Conclusions: A binding mode for the 1,3,5-triazine series was successfully proposed based on the best explanation of the BPM data with our combined docking/FEP protocol.

Future perspectives: We are currently generating a semi-automated workflow to characterize the effect of point mutations on class A GPCRs to characterize binding modes of additional compounds.

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