COMPUTATIONAL STUDIES OF ORPHAN G PROTEIN-COUPLED RECEPTORS

Thesis by

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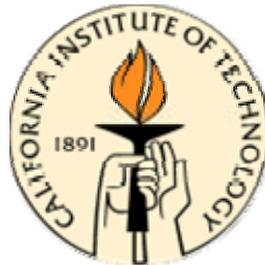
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Abstract

G protein-coupled receptors (GPCRs) play an essential role in cell communications and sensory functions. Consequently, they are involved in wide variety of diseases and are targets for many drug therapies. Particularly important is the large number of orphan GPCRs, which may play important, albeit unknown, functions in various cells. To understand their respective physiological roles, it is important to identify their endogenous ligands, and to find small molecule ligands that would serve as selective agonists or antagonists. The *mas-related gene G protein-coupled receptors* (Mrg receptors) belong to the orphan GPCR family, which is expressed in a specific subset of sensory neurons known to detect painful stimuli, suggesting that they could be involved in pain sensation or modulation.

The primary focus of this thesis is to predict the 3D structure and binding site of Mrg receptors and to identify novel ligands that would be potential agonists or antagonists. We predict the 3D structure for the mouse MrgC11 (mMrgC11) and the binding site for five chiral FMRF-NH_2 ligands. We correctly predict the relative binding observed for these five ligands. We find that Tyr110 (TM3), Asp161 (TM4), and Asp179 (TM5) are particularly important to binding the ligands. Subsequently, we carry out mutagenesis experiments followed by intracellular calcium release assays that demonstrate the dramatic decrease in activity for the Y110A, D161A, and D179A mutants predicted by our model.

The all-atom molecular dynamics simulation of the mMrgC11/F-(D)M-R-F-NH_2 complex structure in explicit water and infinite lipid membrane system shows that some conformational fluctuations are present, but no significant instability is detected, thus validating our structure prediction method.

The virtual screening with the combination of QSPR and docking methods is carried out for the predicted mMrgC11 receptor. The compounds showing the antagonistic effect are

identified by competitive functional assays. These hit compounds are certainly good staring points in designing better agonists or antagonists.

The binding site of rat MrgA receptor that shows differential binding between adenine and guanine is also predicted. The predicted binding affinity correlates with the availability of the hydrogen bonds to two Asn residues, which would be primary mutation candidates to validate the structure.

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