Homology modelling of Metabotropic Glutamate Receptor 2

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Introduction

Many studies show involvement of metabotropic glutamate receptors (mGluRs) in synaptic excitation transudation. The mGluR family consists of eight proteins divided into three groups corresponding to sequence similarities, pharmacology and physiological role. These groups are: I (mGluR1, -5), II (mGluR2, -3) and III (mGluR4, -6, -7, -8). Group II lies in field of our interest due to its potential as therapeutic target for stroke and pain drugs. Primary goal of this research is to create viable virtual model of transmembrane domain of mGluR2 receptor capable of binding reference ligands. This model will be used for further research.

Our approach is based on homology modeling. Rhodopsin crystal structure has been used as a template for creating mGluR2 models. Due to inconsistencies between sequence alignments found in literature our alignment has been prepared basing on experimental secondary structure prediction for mGluR1 and mGluR3 [1]. So created models were then tested against available mutational data [2,3] and by flexible docking of known active/inactive compounds.

Alignment

Most of the sequence alignments between various members of mGluR subfamily and Rhodopsin base on computational secondary structure predictions, which may be one of the reasons for such significant differences between them. We decided to rely on experimental secondary structure assignment [1]. Helices proposed were aligned to the respective ones from template pdb structure (1F88). Sequence was elongated to match length of Rhodopsin helices if needed.

Validation

A series of 100 models created with the alignment was then validated in two ways. First, the generated models were checked against the mutational data collected for mGlu receptors [3] along with mapped punctual mutations for Calcium Sensing Receptor(CaSR) [2]. Only mutations possibly affecting binding of allosteric modulators were taken into account.
Second, the docking of set of 186 ligands, taken from literature, with known but diverse affinity to mGluR2 was performed.

Results and discussion

Best model was selected on basis of number of ligands with affinity < 200 nM („actives") succesfully docked and glide docking score. Selected receptor structure docked all „actives", with 38% knowns (12 out of 32) found within first 10% of the test set. Docked poses preserve orientation within binding site and most of the mutations providing specific interactions can be found within 4 angstroms from docked ligand.

To fully benchmark the created models, there is a bigger test set needed. 186 ligands is not enough to show the real value of created models.

A

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OPSD_BOVIN   34    PWQFSMLAAYMFLLIMLGFPINFLTLYVTVQ 64
GRM2_HUMAN   561   IRWGDAWAVGPVTIACLGALATLFVLGVFVR 591

OPSD_BOVIN   71    PLNYILLNLAVADLFMVFGGFTTTLYTSLH 100
GRM2_HUMAN   597   VVKASGRELCYILLGGVFLCYCMTFIFIAK 626

OPSD_BOVIN   106   GPTGCNLEGFFATLGGEIALWSLVVLAIERYVVV 139
GRM2_HUMAN   629   TAVCTLRRLGLGTAFSVCYSALLTKTNRIARIFG 662

OPSD_BOVIN   150   ENHAIMGVAFTWVMALACAAPPLV 173
GRM2_HUMAN   677   ASQVAICLALISGQLLIVVAWLVV 700

OPSD_BOVIN   200   NESFVIYMFVVHFIIPLIVIFFCYGQ 225
GRM2_HUMAN   722   NHRDASMLGSLAYNVLLIALCTLYAF 747

OPSD_BOVIN   246   AEKEVTRMVIIMVIAFLICWLPYAGVAFYIFT 277
GRM2_HUMAN   754   ENFNEAKFIGFTMYTTCIIWLAFLPIFYVTSS 785

OPSD_BOVIN   285   PIFMTIPAFFAKTSAVYNPVIYIMMNK 311
GRM2_HUMAN   794   MCVSVSLSGSVVLGCLFAPKLHIILFQ 820
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B

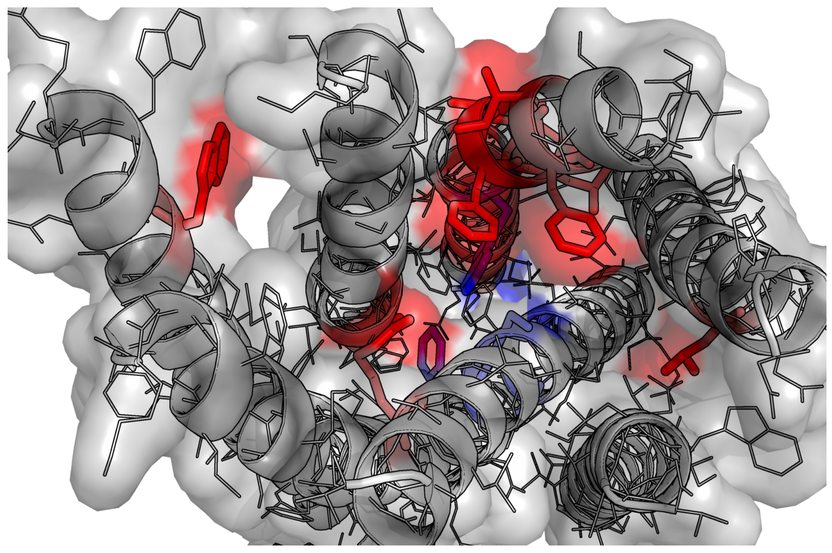

Fig. 1
(A): Sequence alignment of helical regions between Rhodopsin and mGluR2. Amino acids in bold face are residues .50 in Ballesteros-Weinstein notation. Residues in blue are punctual mutations of mGluR receptors [3], in red are mapped mutations from CaSR receptor [2]. **(B)**: Mutations from (A) visualised on mGluR2 model, colours corespond to sequence alignment scheme.

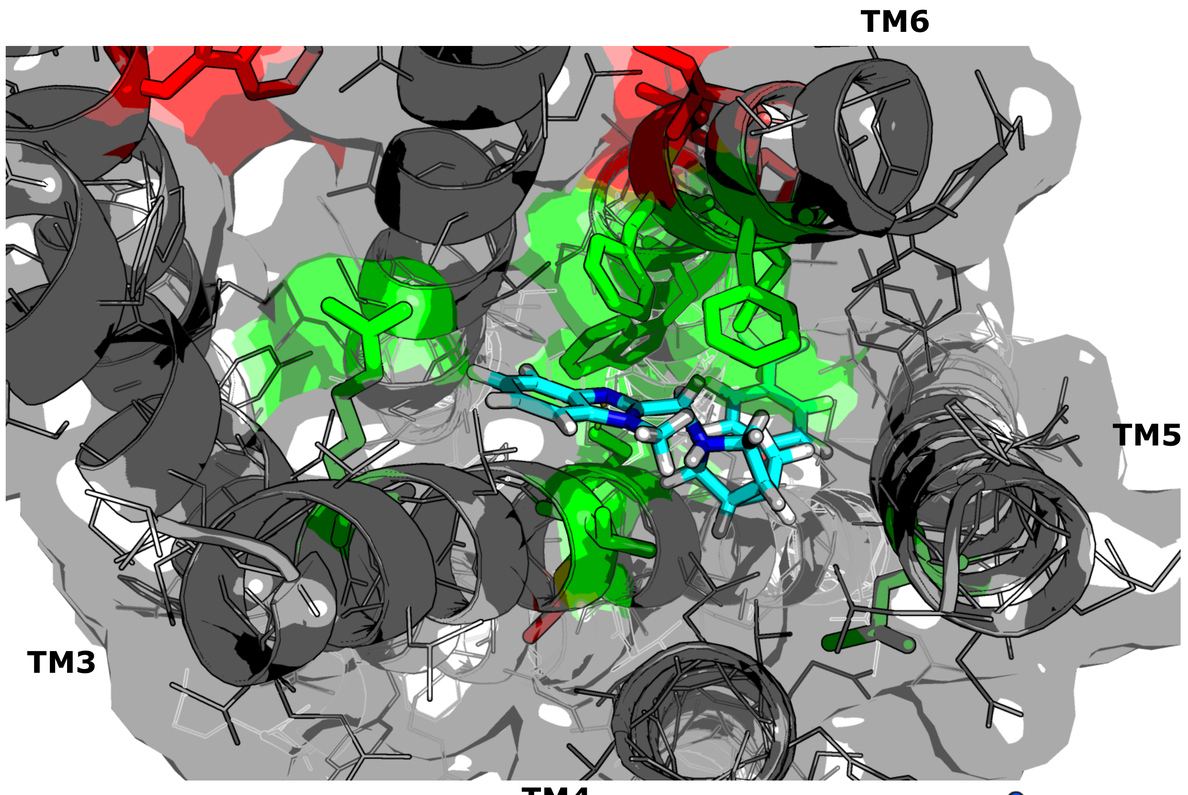

Fig. 2
(A): Top ranked model with ligand of highest score (which appeared to be active compound with EC50 of 85 nM. Amino acids in green are mutants taking part in forming a binding site. Residues in red are mutations not participating in binding site formation either by being too far from ligand or by not facing it. (B): Residues forming binding site (within 4A radius from ligand). In blue amino acids pointed by mutagenesis of mGluRs and CaSR.

Protein homology modeling was performed with modeller 9v8, docking and scoring were executed using Glide from Schrodinger Suite 2010. Visualisation were prepared with Pymol v1.2 and self scripts.

Acknowledgments

The study was partly supported by a grant PNRF-103-AI-1/07 from Norway through the Norwegian Financial Mechanism

Literature

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