Homology modeling of metabotropic glutamate receptor 2

Stefan Mordalski[(1)], Mateusz Nowak[(1)], Piotr Brański[(2)], Andrzej J. Bojarski[(1)]

(1) Polish Academy of Sciences, Institute of Pharmacology, Department of Medicinal Chemistry Krakow, Poland; stefanm@if-pan.krakow.pl

(2) Polish Academy of Sciences, Institute of Pharmacology, Department of Neurobiology, Poland

Introduction

Many studies show involvement of metabotropic glutamate receptors (mGluRs) in synaptic excitation transduction. 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.

Sequence alignment

Our approach is based on homology modeling. Since mGluRs are part of superfamily of G protein coupled receptors (GPCRs) and thus their sequence is similar to Rhodopsin, we have chosen Rhodopsin crystal structure as a template for homology modeling of mGluR2 receptor. We have prepared multiple sequence alignment using 250 sequences of Opsins and GluRs. The initial alignment has been divided into several fragments containing helices and realigned. Helical fragments were determined with PONGO server. bioinfo.pl meta server has been queried with mGluR2 sequence and mGluR alignments from literature [(3),(4)] have been acquired for comparison.

Evaluation

Since received alignments differed significantly we decided to use our version and evaluate it using mutagenesis data. Our approach is based on reproducing *in silico* Schaffhauser's [(1),(2)] site-directed mutagenesis. Several point mutations were prepared and for each a population of 100 homology models has been generated. Two ligands, LY487379 and rmlsd650, were used for flexible docking with FlexX 3.1.3. Results of docking were then scored with Cscore module of SYBYL 8.2 suite and best ligand – receptor complexes were chosen.

Mutagenesis data lead to hypothesis that three amino acids: Ser688 (4.47), Gly 689 (4.48) and Asn 735 (5.47) may participate in ligand binding. Prepared models and docking results show that only one of those amino acids (Asn 735/5.47) directly interacts with active compounds, however conformational change in the receptor caused by mutation of Ser688 (4.47), Gly 689 (4.48) disrupts the binding mode. Docking to homology models without sequence changes results with cluster of solutions deeply penetrating binding site and interacting with Asn 5.47, Phe 6.51 and Phe 6.55 whereas compounds docked to virtual mutants do not have consistent binding mode and very often results are lacking biological sense. Analysis of docking scores and total number of results returned show that models with altered sequence perform noticeably worse than non modified ones.

Conclusions

Reproduced mutagenesis positively verifies prepared sequence alignment and homology models. Docked compounds preserve orientation within binding site and key interaction with predicted (Asn 5.47) and common for GPCRs (Phe 6.51) amino acids.

Our research proves how biochemical data can be extremely helpful in developing viable virtual model.

Fig. 1 Comparison of mGluR2 and Rhodopsin sequence alignments produced in IF PAN and fetched from literature [(3)(4)] and bioinfo.pl meta server

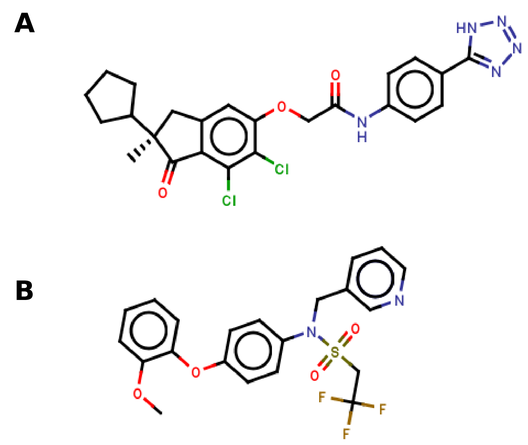

Fig. 2 Ligands used for flexible docking to mGluR2 models, rmlsd650 (A) and ly487379 (B)

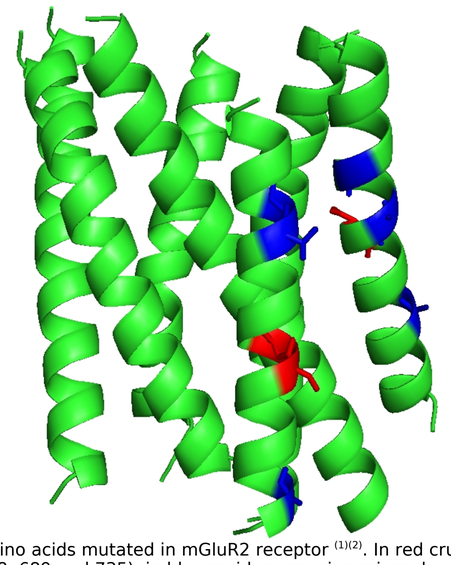

Fig. 3 Amino acids mutated in mGluR2 receptor [(1)(2)]. In red crucial for ligand binding (688, 689 and 735), in blue residues causing minor decrease in receptor response (681, 695, 696, 730, 733 and 740)

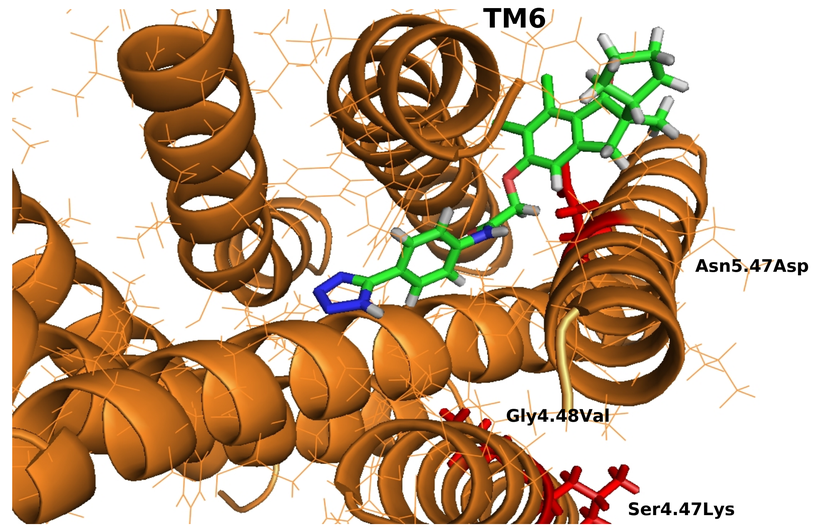

Fig. 4 Top scored result of docking rmlsd650 into mGluR2 mutant. Ligand penetrates space between helices 5 and 6. Mutated residues are in red.

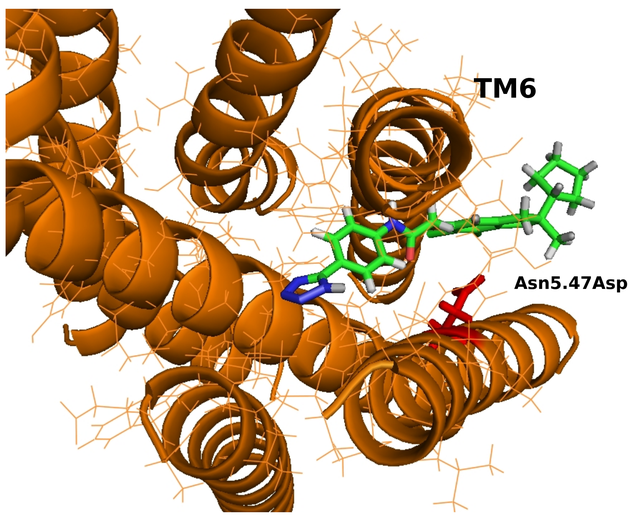

Fig. 5 Top scored result of docking rmlsd650 into mGlur2 mutant. Changing Asn735/5.47 into Asp results in lack of receptor response to this compound

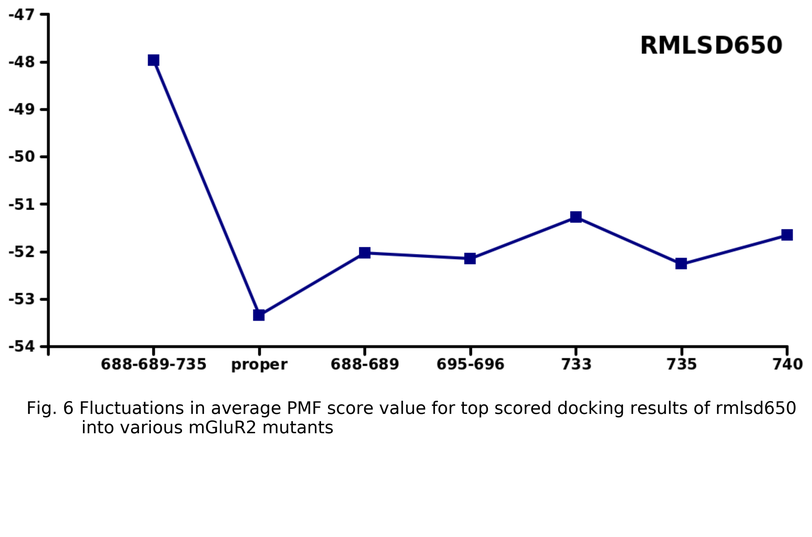

Fig. 6 Fluctuations in average PMF score value for top scored docking results of rmlsd650 into various mGluR2 mutants

Fig. 7 Cluster of five top scored results of docking ly487379 into mGluR2 model. To the right binding mode of both ly487379 and rmlsd650

Acknowledgments
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Protein homology modeling was performed with modeller 9v7, docking and scoring were executed using FlexX 3.3.1 and SYBYL 8.2 suite respectively. Visualisation were prepared with Pymol v1.1

Literature

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