Topology-based Homology Model Construction of Metabotropic Glutamate Receptor type 4 (mGluR4), a Class C GPCR

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INTRODUCTION

Untrivial evolutionary relationships between class A and classes B & C GPCR largely impair balanced exploration of this most acknowledged pharmacological target, especially by means of rational drug design aided by computational modeling of the receptors.

Many studies show involvement of metabotropic glutamate receptors (mGluRs) in synaptic excitation transduction. Since no class B or C GPCRs are known, it becomes a necessity to employ class A receptors as templates. This is however very demanding. Shortage of experimental data, structural analyzes and ligand with known activity puts a large handicap on the alignment process.

Primary goal of this research is to create viable virtual model of transmembrane domain of mGluR4 receptor capable of binding reference ligands. This model will be used for further research.

Metabotropicvglutamate receptors

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). All mGluR receptors consist of 2 topological domains (fig. 1): an extracellular Venus Flytrap – binding glutamate or other orthosteric ligands, and heptahelical transmembrane 7TM – bearing allosteric site.
mGluR allosteric potentiators lie in field of our interest due to their potential as therapeutic target for antidepressant and anxiolytic drugs.

This research was performed on population of 100 mGluR4 models created on rhodopsin crystal structure template. Building that many virtual receptors provided us with semi-conformational search on residues assembling incriminated receptor.
The Library of 53 known allosteric modulators of group III mGluR was used for docking studies and thus forging the binding mode.

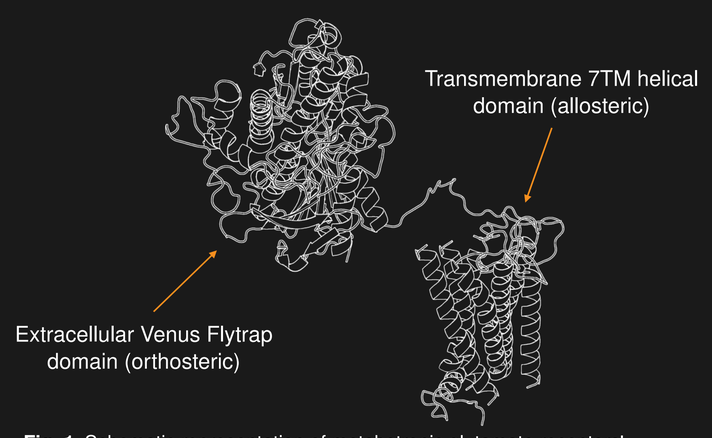

Fig. 1. Schematic representation of metabotropic glutamate receptors' domain organization.

ALIGNMENT

Buried/exposed positions map

Based on crystalographic data on GPCRs, their structural alignment and Ballesteros-Weinstein (Ballesteros, 1995) sequence description it is possible to identify positions, conserved among analyzed proteins, that remain exposed toward the binding pocket (Gloriam, 2009). This is additionally rationalized by the fact, that seven helices compose the binding site and known structural properties of a helix enable identification of relative positions within any helix where residues lay on the same side. By this approach it is possible to construct a map of buried/exposed positions in each transmembrane helix.

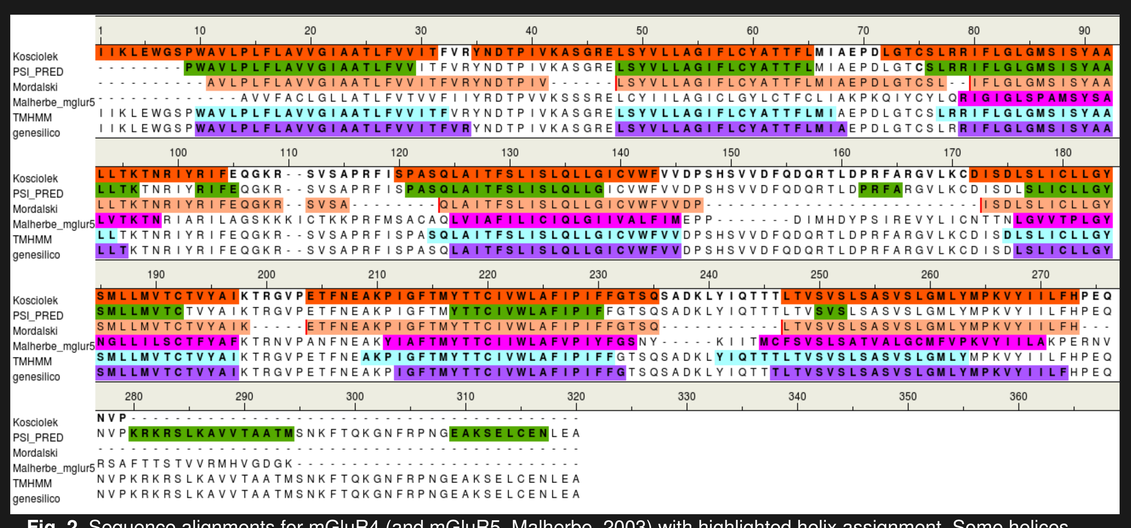

Fig. 2. Sequence alignments for mGluR4 (and mGluR5, Malherbe, 2003) with highlighted helix assignment. Some helices (i.e. TM1, TM2, TM4) have substantially different positions within the sequence. All sequences are human mGluR. Descriptions: **Kosciolek** (this work), **PSI_PRED** (PSIPRED software run with default parameters; McGuffin, 2000), **Mordalski** (Mordalski, not published results), **Malherbe_mGluR5** (Malherbe, 2003), **TMHMM** (Krogh, 2001), **genesilico** (metaserver http://www.genesilico.pl/meta2).

Multiple Sequence Alignment

All metabotropic glutamate receptors are closely related to each other. They share large sequence similarity. Therefore, it is plausible, they should share common requirements for ligand binding. Basing on this rationale, a Multiple Sequence Alignment (MSA) may be constructed (fig. 3). This MSA may then aid choosing corresponding residues (i.e. between mGluR5 and mGluR4).

In principle, helix ranges derived from aligning mGluR5 onto rhodopsin sequence were then transferred to mGluR4 basing on the MSA constructed (fig. 2).

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Fig. 3. Multiple Sequence Alignment (MSA) of all metabotropic glutamate receptors transmembrane regions. Highlighted in **orange** are parts of sequence identified as TM helices.

Mutagenetic data

There are no mutagenetic studies concerning directly allosteric site of mGluR4. It is therefore necessary to rely on data emerging from other, closely related receptors. These receptors belong to group I mGluR (Malherbe, 2003, 2006). All unique mutations, with their corresponding Ballesteros-Weinstein ids are gathered in the table below.

receptor	mutation	B-W
mGluR5 (rat)	P654S	**3.36**
	Y658V	**3.40**
	W784A	**6.48**
	F787A	**6.51**
	A809V	**7.47**
mGluR1 (rat)	F801A	6.51
	Y805A	**6.55**
	T815M	**7.39**

MODELS

Loops

Since proven evidence exists (Jamroz, 2010; Fiser, 2000), that modeling loops of 8+ amino-acids imposes serious bias on the structure and significantly low target-template homology occurs in coil regions, only TM helices are considered while the model is built. This is in order to avoid presence of ill-predicted loops blocking the active-site, as well as, far-fetched assumptions towards existence of disulfide bonds (i.e. between TM3 and ec2) for which there is no clear evidence.

Generating models

Separate helices alone were modeled using Modeller 9v4 with bovine rhodopsin (pdb id: 1U19) as a template. A population of 100 models was generated in order to thoroughly explore side-chain conformational space. This enables searching for optimal interactions while performing docking of reference compounds.

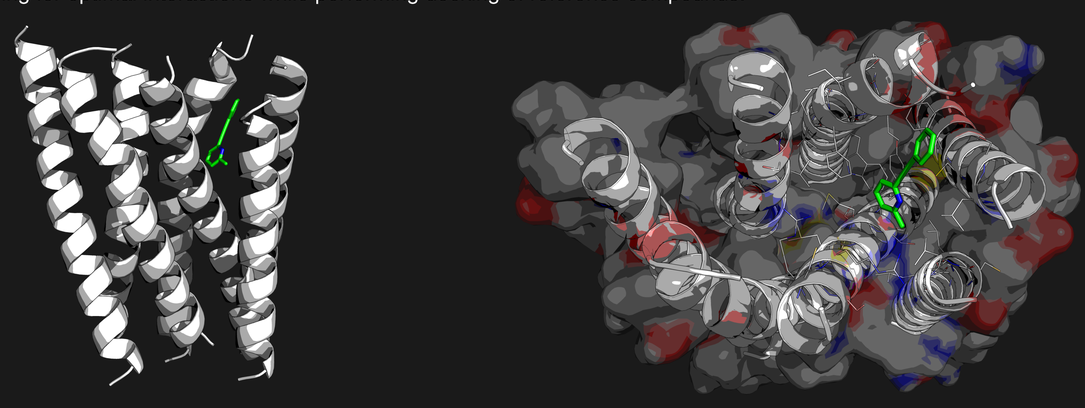

Fig. 4. A representative ligand-receptor complex with MPEP docked. (left) depth of binding pocket. (right) binding site with a surface and interacting amino acids. (TM1 - TM7 from left counterclockwise.)

Binding Site composition

Generated population of protein-ligand complexes (fig. 4) was then systematically analyzed by means of binding mode consistency, correct activity vs. score correlation and agreement with experimentally determined crucial interactions. Binding site amino acids were identified utilizing structural interaction fingerprints (SIFt).

Structural interaction fingerprints aid identification of critical residues involved in ligand binding, especially in presence of large amount of data. Due to scarce experimental data utilizing SIFt also allows focusing model analysis on highly conserved interactions. This data can be easily compared with experimentally available information on the receptors.

CONCLUSIONS

Obtained models present very good agreement with available data. Inspection of docked ligand poses allow to identify differences in their affinity and rationalize observed SAR (i.e. methyl in MPEP's *meta* and *para* positions). Picture of residues engaged in ligand binding obtained by SIFt analysis, stays in good agreement with experimental data (only residue 7.47 is not interacting with ligand. This might be due to its flexibility and peripheral position within a helix).
It is however still a matter of future to improve obtained models on the basis of extensive docking with a set of decoys. Creating such database is difficult due to shortage of compounds' activity data, especially concerning inactive ligands.

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