# Non-Class A templates in homology modeling of Metabotropic Glutamate Receptor 2

**Stefan Mordalski, Jagna Witek, Andrzej J. Bojarski**

Department of Medicinal Chemistry, Institute of Pharmacology Polish Academy of Sciences, Krakow

e-mail: stefanm@if-pan.krakow.pl

## Introduction

Homology modeling of Class C GPCRs is a challenging task. Until recently, the only available crystal templates were those of Class A receptors, rather evolutionary distant ones (with sequence identity/similarity oscillating around 19/30 percent) with both ortho- and allosteric binding sites located within heptahelical transmembrane bundle and extracellular loops. However, during the last few months three new crystals appeared, being first structures of GPCRs from other classes of the superfamily. Smoothened Receptor [Wang, 2013], Glucagon Receptor [Siu, 2013], and Corticotrophin-Releasing Factor Receptor 1 [Hollenstein, 2013] potentially open a new avenue for homology modeling studies over distant targets, including Class C GPCRs. In this research for each of those crystal templates a series of 100 homology models of mGlu2 Receptor is generated and evaluated in Virtual Screening like protocol to estimate their applicability for the screening purposes.

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.

```
CRFR1_HUMAN 115 HYHVAAIINYLGHCISLVALLVAFVLFLRAR 145
GLR_HUMAN   136 MYSSFQVMYTVGYSLSLGALLLALAILGGLS 164
OPSD_BOVIN  34  PWQFSMLAAYMFLLIMLGFPINFLTLYVTVQ 64
GRM2_HUMAN  561 IRWGDAWAVGPVTIACLGALATLFVLGVFVR 591

CRFR1_HUMAN 150 LRNIIHANLIAAFILRNATWFVVQLTMSPE 179
GLR_HUMAN   171 CTRNAIHANLFASFVLKASSVLVIDGLLRT 200
OPSD_BOVIN  71  PLNYILLNLAVADLFMVFGGFTTTLYTSLH 100
GRM2_HUMAN  597 VVKASGRELCYILLGGVFLCYCMTFIFIAK 626

CRFR1_HUMAN 184 NVGWCRLVTAAYNYFHVTNFFWMFGEGCYLHTAI 217
GLR_HUMAN   216 LSDGAVAGCRVAAVFMQYGIVANYCWLLVEGLYL 249
OPSD_BOVIN  106 GPTGCNLEGFFATLGGEIALWSLVVLAIERYVVV 139
GRM2_HUMAN  629 TAVCTLRRLGLGTAFSVCYSALLTKTNRIARIFG 662

CRFR1_HUMAN 227 RLRAWMFICIGWGVPFPIIVAWAI 248
GLR_HUMAN   263 FFSLYLGIGWGAPMLFVVPWAVVK 286
OPSD_BOVIN  150 ENHAIMGVAFTWVMALACAAPPLV 173
GRM2_HUMAN  677 ASQVAICLALISGQLLIVVAWLVV 700

CRFR1_HUMAN 266 VYTDYIYQGPMALVLLINFIFLFNIV 291
GLR_HUMAN   300 NMGFWWILRFPVFLAILINFFIFVRI 325
OPSD_BOVIN  200 NESFVIYMFVVHFIIPLIVIFFCYGQ 225
GRM2_HUMAN  722 NHRDASMLGSLAYNVLLIALCTLYAF 747

CRFR1_HUMAN 303 TSETIQARKAVKATLVLLPLLGITYMLAFVN- 333
GLR_HUMAN   342 DYKFRLAKSTLTLIPLLGVHEVVFAFVTDEHA 373
OPSD_BOVIN  246 AEKEVTRMVIIMVIAFLICWLPYAGVAFYIFT 277
GRM2_HUMAN  754 ENFNEAKFIGFTMYTTCIIWLAFLPIFYVTSS 785

CRFR1_HUMAN 338 EVSRVVFIYFNAFLESFQGFFVSVFAC 364
GLR_HUMAN   375 GTLRSAKLFFDLFLSSFQGLLVAVLYC 401
OPSD_BOVIN  285 PIFMTIPAFFAKTSAVYNPVIYIMMNK 311
GRM2_HUMAN  794 MCVSVSLSGSVVLGCLFAPKLHIILFQ 820
```

**Figure 1.** Sequence alignment between mGluR2 and Bovine Rhodopsin, Corticotrophin-Releasing Factor Receptor 1 and Glucagon Receptor with indicated point mutation for mGluR (red) and CaSR (blue). Residues in bold correspond to X.50 in Ballesteros – Weinstein notation. Helices I-IV – left panel, V-VII – right panel.

## Methods

The sequence alignment of the templates and mGluR2 were developed using structural alignment between each crystal and the structure of bovine rhodopsin (PDB: 1u19), and existing alignment of mGluR2 and the latter. Manual adjustments were applied to ensure proper orientation of the amino acids involved in ligand binding [Gregory, 2010].

Homology models were generated with Modeller 9.12 [Sali, 1993] with restrains for the secondary structure of the helices (excluding bending regions).

Docking experiments were conducted with Glide 5.7 (SP mode) using a set of 112 known allosteric modulators of mGluR2 acquired from ChEMBL database and a set of 1000 drug-like decoys from Schrödinger. The analysis of the docking results was performed with home made scripts and Schrödinger Suite 2013.

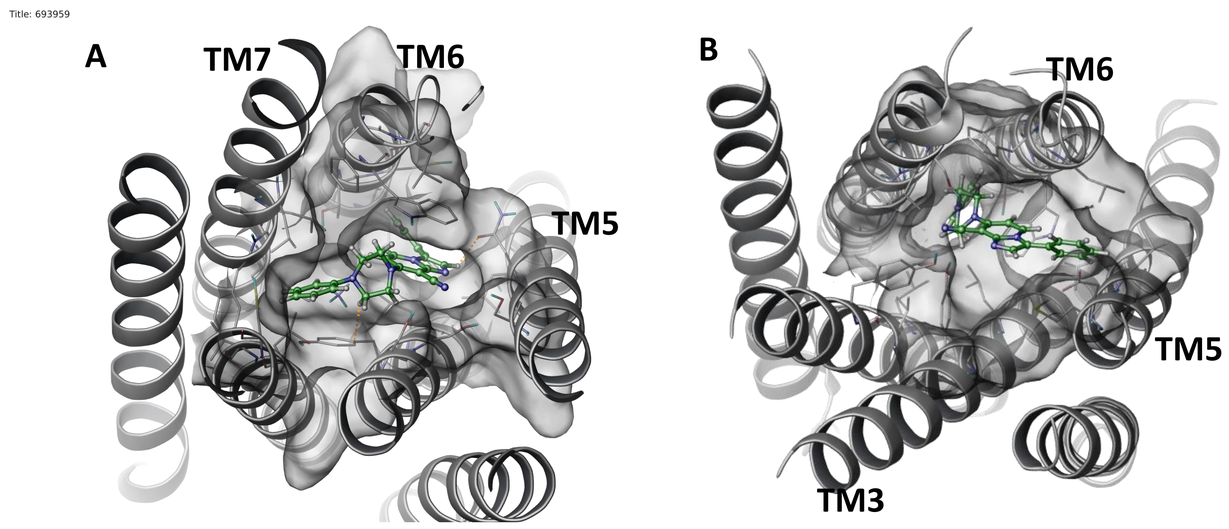


**Figure 2.** Binding sites of models obtained fr different templates: CRFR1 (**A**) and GLR (**B**).

## Results

For all of the investigated templates, a sequence alignments with mGluR2 were created (Fig. 1). Unfortunately for the Smoothened Receptor, the resulting models were not in accordance with the mutational data for mGluR2 and so those models were rejected from further studies.

Docking experiments performed led to up to 110 out of 112 active compounds docked for the Glucagon Receptor template and 100 for CRF Receptor 1 mold. Shapes of received binding sites are presented on Figure 2, and enrichment curves obtained for docking active compounds along with decoys are on Figure 3.

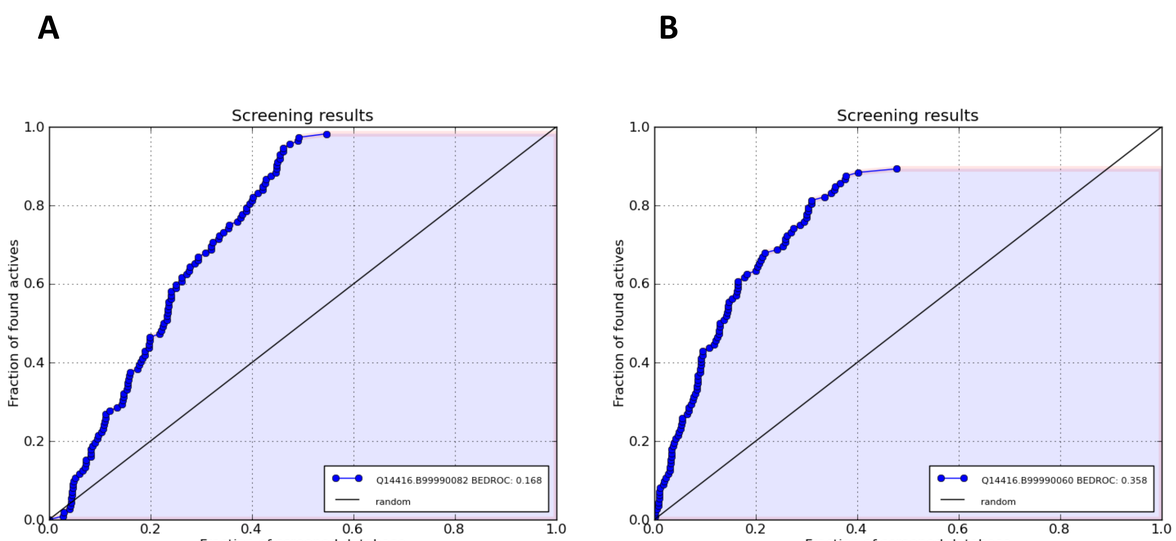


**Figure 3.** The results of screening like experiments for mGluR2 models built with different templates: CRFR 1 (**A**) and Glucagon Receptor (**B**).

## Conclusions

The results of VS like experiments show the potential of models on non Class A templates for further research. Satisfactory compatibility with mutational data, along with high number of known ligands recognized prove, that new Class B GPCR crystal templates are indeed of better use for distant targets such as mGluR2.

## References

- Gregory KJ, Dong EN, Meiler J, Conn PJ. **Allosteric modulation of metabotropic glutamate receptors: structural insights and therapeutic potential.** *Neuropharmacology*. 2011;60(1):66-81.
- Hollenstein K, Kean J, Bortolato A, Cheng RK, Doré AS, Jazayeri A, Cooke RM, Weir M, Marshall FH, **Structure of class B GPCR corticotropin-releasing factor receptor 1.** *Nature*. 2013 Jul 25;499(7459):438-43.
- Sali A, Blundell TL. **Comparative protein modelling by satisfaction of spatial restraints.** *J Mol Biol*. 1993;234(3):779-815.
- Siu FY, He M, de Graaf C, Han GW, Yang D, Zhang Z, Zhou C, Xu Q, Wacker D, Joseph JS, Liu W, Lau J, Cherezov V, Katritch V, Wang MW, Stevens RC, **Structure of the human glucagon class B G-protein-coupled receptor.** *Nature*. 2013 Jul 25;499(7459):444-9.
- Wang C, Wu H, Katritch V, Han GW, Huang XP, Liu W, Siu FY, Roth BL, Cherezov V, Stevens RC, **Structure of the human smoothened receptor bound to an antitumour agent.** *Nature*. 2013 May 16;497(7449):338-43.

## Acknowledgements

This study was supported by project UDA-POIG.01.03.01-12-063/09-00 co-financed by European Union from the European Fund of Regional Development (EFRD); http://www.prokog.pl

INNOVATIVE ECONOMY
NATIONAL COHESION STRATEGY

EUROPEAN UNION
EUROPEAN REGIONAL DEVELOPMENT FUND