

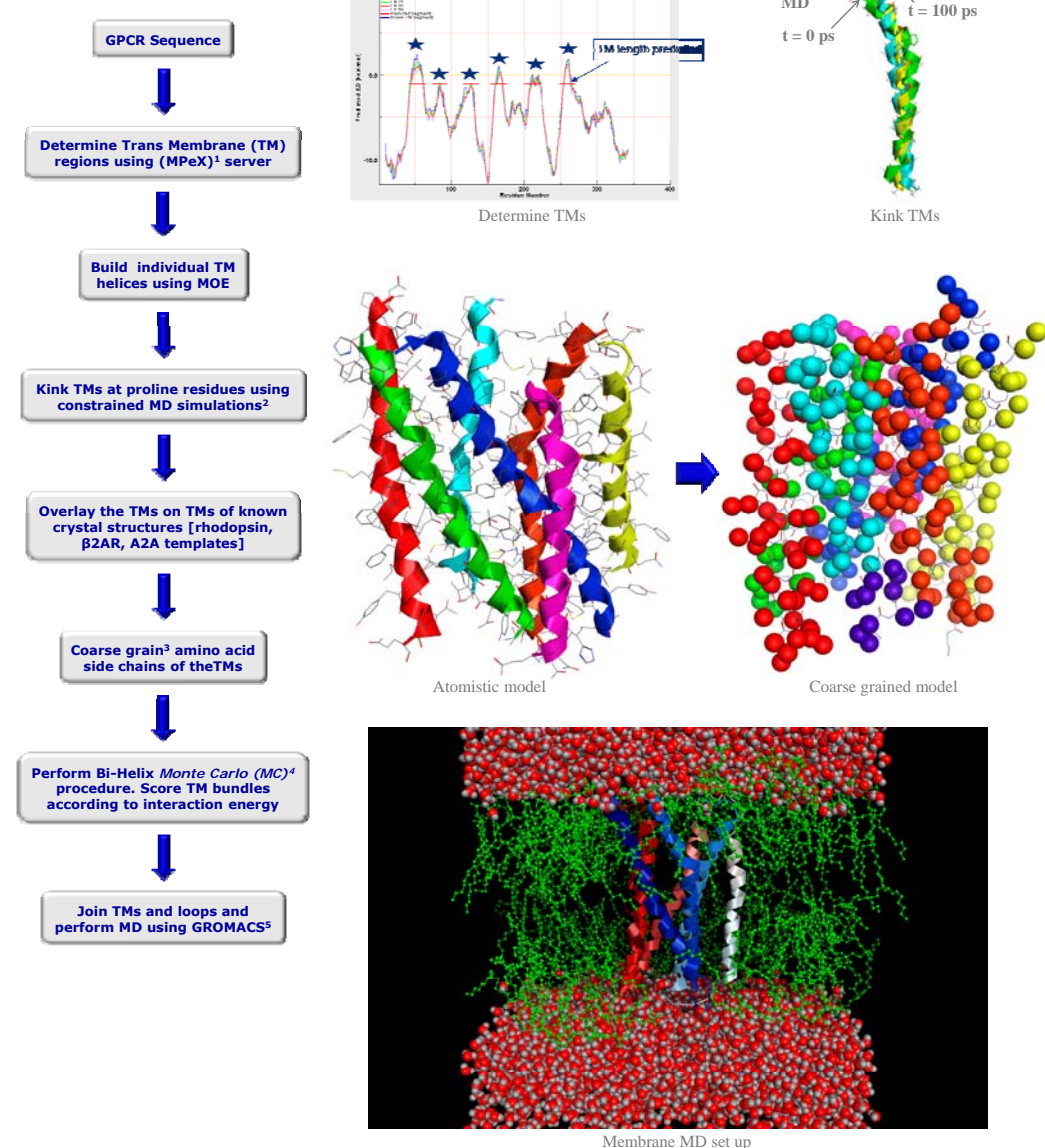
GPCR modelling from sequence to 3D structure, application on Bradykinin 1 (B1) and histamine 3 (H3) receptors

Sandeep Pal, Alexander Heifetz, Richard Law, Andreas Kahrs, Thomas Hestekamp, James Madden, Adam Davenport, Alastair Parkes, Michael Mazanetz, David Hallet and Mark Whittaker

Introduction

GPCRs modulate the regulation of several physiological processes involved in several diseases. They represent 30-50% of the current drugs in the market. However the early stages of the drug discovery process suffer from lack of crystal structures of GPCRs. To date only three different GPCR crystal structures are solved. These crystal structures show a good 3D structural similarity. However a poor primary sequence identity between the GPCRs doesn't always result in a good homology model. We have developed an "in-house" GPCR modelling technique which is based on the minimisation of the helix bundles according to a "coarse grained Monte Carlo procedure". The method has been applied to explain the SAR of B1R antagonists and for vHTS of histamine 3 (H3) antagonists.

Methodology



Bi-Helix MC Procedure (Implemented in C++ programming language)

- Select two neighbouring helices
- Simultaneously rotate and translate them around their helical axes
- MC minimise each configuration according to Metropolis algorithm using a Boltzmann weighting criteria
- Rank MC minimised TM bundles according to the total TM bundle energy
- Residue-residue interaction energy calculated using the Miyazawa-Jernigan contact energies
- Side chain amino acid rotamer library of Richards and Ponder is used

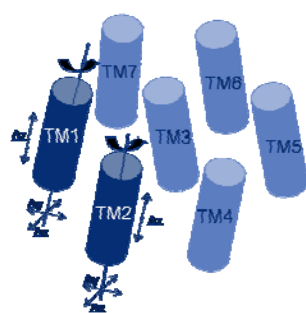


Figure 1: Bi-Helix MC procedure

Results of Bi-Helix MC procedure on known crystal structures

Energy	Helix1	Helix2	Helix3	Helix4	Helix5	Helix6	Helix7
1	-15.5855	0	0	0	0	0	0
2	-15.5252	0	0	0	-90	0	0
3	-15.4328	0	0	-30	0	0	0
4	-15.4042	0	0	-30	-90	0	0
5	-15.3933	0	0	0	90	0	0
6	-15.3754	0	0	0	-90	30	30
7	-15.3114	0	0	0	-60	0	0
8	-15.3031	0	0	0	-30	30	30
9	-15.3031	0	0	0	0	-30	30

Crystal Structure Rank 1
Bovine Rhodopsin 1U19

Energy	Helix1	Helix2	Helix3	Helix4	Helix5	Helix6	Helix7
1	-14.279	90	0	0	0	0	90
2	-14.2135	90	0	0	0	0	0
3	-14.1428	90	0	0	30	0	90
4	-14.1254	0	0	0	0	0	0
5	-14.1028	0	0	0	0	0	90
6	-14.0773	90	0	0	30	0	0
7	-13.9892	0	0	0	30	0	0
8	-13.9665	0	0	0	30	0	90
9	-13.9665	0	0	0	0	30	90

Crystal Structure Rank 2
Human beta2 Adrenergic receptor 2RH1

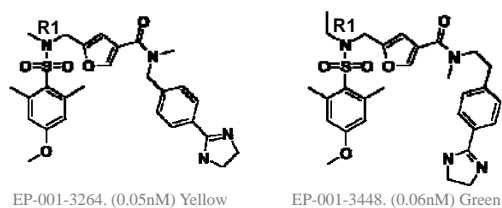
Energy	Helix1	Helix2	Helix3	Helix4	Helix5	Helix6	Helix7
1	-15.1825	0	0	0	0	0	90
2	-15.1362	0	0	0	0	-90	90
3	-15.0765	0	0	0	30	0	90
4	-15.0659	0	0	0	30	0	0
5	-15.0492	0	0	0	30	-90	90
6	-15.0366	0	0	0	0	-90	0
7	-15.0198	0	0	0	-30	0	0
8	-14.9926	0	0	0	-30	-90	0
9	-14.9926	0	0	0	-30	0	90

Crystal Structure Rank 4
Turkey beta1 Adrenergic receptor 2VT4

Modelling of human Bradykinin 1 receptor (B1R)

Figure 2 shows the sequence alignment of the B1R and beta2AR TMs. The highlighted residues in grey represent the conserved residues. The highlighted regions in red (TM1, TM2 and TM4) show that the sequence alignments near the proline residues of the two GPCRs are poor. Therefore homology modelling based on sequence alignment will result in a wrong model of B1R.

Figure 3 shows the residues around the B1R active site W98, which is considered to be important for binding antagonists, faces TM1 and not towards binding site. B1R model published by Ha *et al.*⁶ has an unphysical proline kink in TM2 which results in W98 pointing towards ligand binding site.



TM	B1R	beta2AR
TM1	24	24
TM2	70	70
TM3	108	108
TM4	147	147
TM5	188	188
TM6	231	231
TM7	287	287
TM8	346	346

Figure 2: Bi-Helix MC procedure

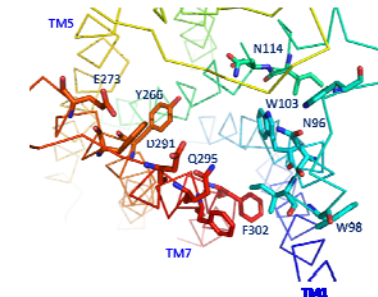


Figure 3: Residues around the B1R binding site

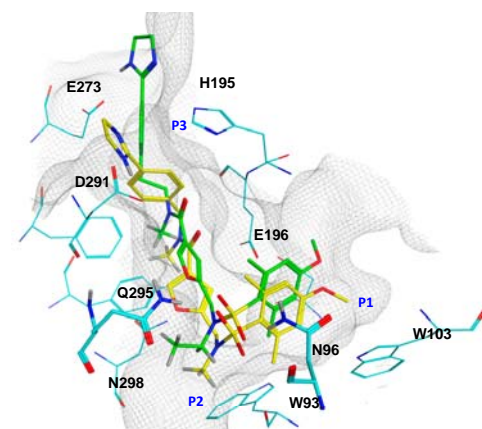


Figure 4: Docking poses of Evotec's furan series in the modelled B1R

Our B1R modelling elucidates the anomalous SAR between the two Evotec Sub-nanomolar active compounds (Figure 4) belonging to the furan series. Some important questions are:

- Why changing an ethyl group to methyl group at position -R1, compound 3448 losses activity by approximately 17 times?
- Why presence of a bulkier group (e.g. cyclopropyl group) at position -R1 for the compounds is not tolerated?
- What about different substitutions around the furan ring?

Modelling of H3 receptor

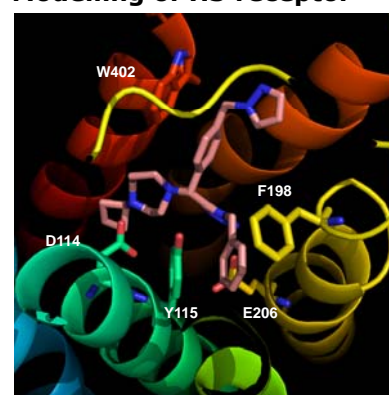
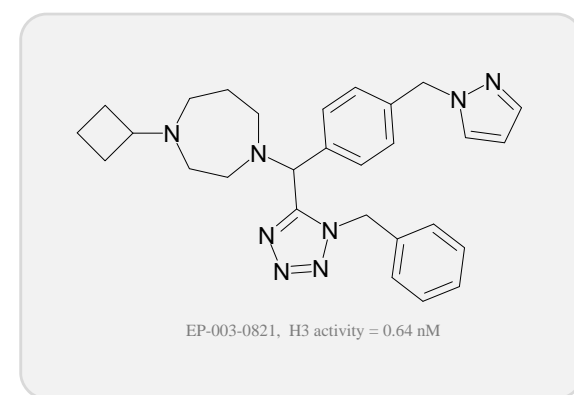


Figure 5: Compound EP-003-0821 docked in the modelled H3 receptor



	Fragment screening	Shape and pharmacophore similarity (Types Topomer)	GPCR Modelling
# Compounds screened	1,702	145	172
H3 Confirmed hits	84	63	79
H3 Rate of confirmed hits	2.7%	43.4%	45.9%
H4 Confirmed hits	21	28	68
H4 Rate of confirmed hits	1.2%	19.3%	40.1%

Figure 6: Comparison of hit rates from various screening methods

Summary

- The Bi-Helix MC procedure has been successfully applied on human B1R and H3 receptors
- Further work is in progress to understand the selectivity between human and rat B1R antagonists
- GPCR modelling is currently being used in several other projects

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