

Hierarchical GPCR modelling; application to Bradykinin 1 Receptor (B1R), Histamine 3 (H3) receptor and Melanin-Concentrating Hormone receptor 1 (MCHR1)

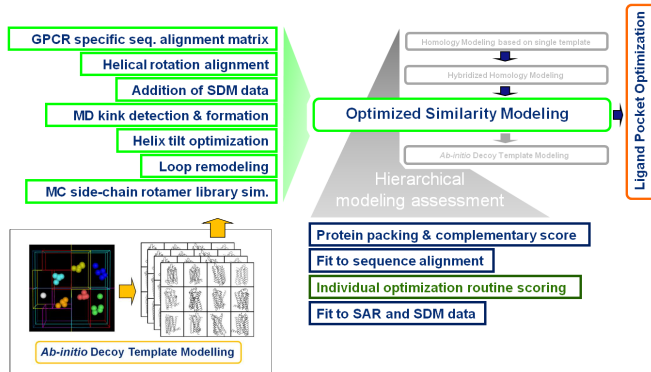
Sandeep Pal, Alexander Heifetz, Richard J. Law, Andreas Kahrs, Thomas Hesterkamp, James Madden, Adam Davenport, Alastair Parkes, Michael Mazanetz, David Hallett and Mark Whittaker

Introduction

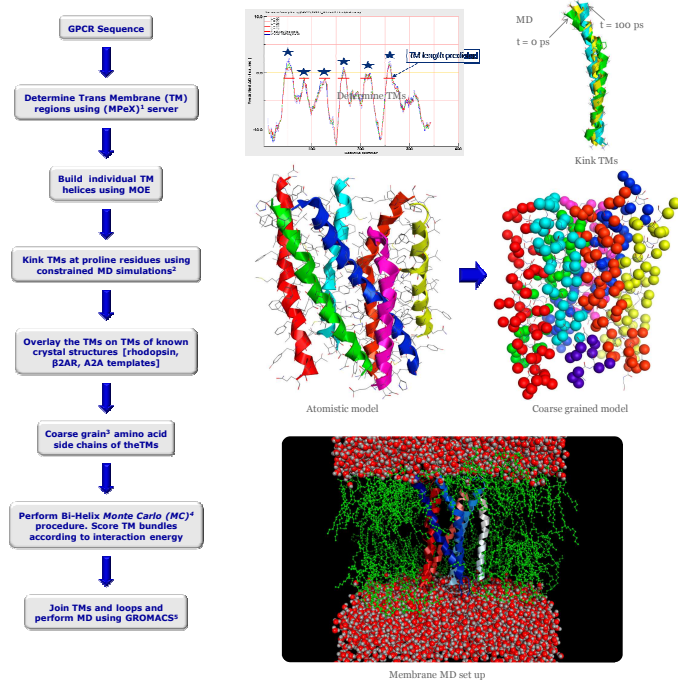
GPCRs modulate the regulation of several physiological processes involved in several diseases. They represent 30-50% of the current drugs in the market. However the early stages of the drug discovery process suffer from lack of crystal structures of GPCRs. To date only three different GPCR crystal structures 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, vHTS of histamine 3 (H3) antagonists and SAR of MCHR1 antagonists.

Overview of Hierarchical Modelling

- Model is assessed for quality at each level

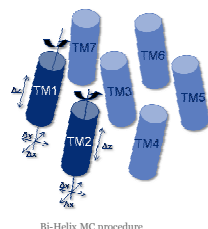


Detail of Optimized Similarity Methodology



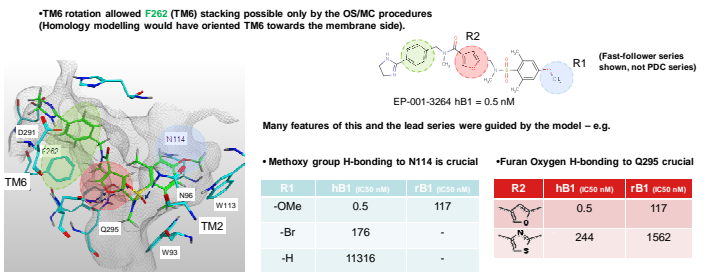
Bi-Helix MC Procedure (Implemented in C++ programming language)

- 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

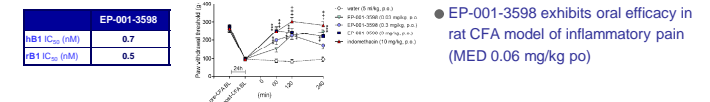


Modelling of human Bradykinin 1 receptor (B1R)

- Modeling of B1 - supported H2L and LO
- Simple homology modeling was unable to produce a model that could explain our SAR (even though it did explain some published B1 SAR)
- Due to unavoidable error in structure – i.e. incorrect proline driven kink in TM2
- Optimized similarity modeling corrected the TM2 error, also TM6 rotation different to homology model



- Helped to drive LO of potent B1 antagonists
- Process repeated for rat B1 and was able to drive the production of compounds equipotent in rat & human – a very important milestone in the project



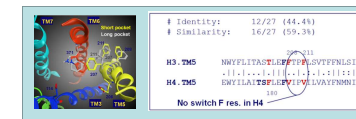
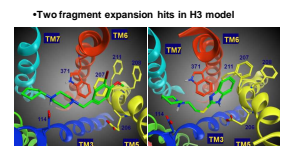
H3/H4 receptor Modelling and Fragment Screen

- A random set of 1,700 fragments (out of 20,000) was tested in quadruplicates
- At 2µM and 20µM in functional Ca²⁺ flux assays (then IC₅₀s for actives)
- On cell lines expressing either the histamine receptors H1, H3, or H4 to identify sub-type specific antagonists
- Hit expansion VS performed using H3/H4 GPCR models

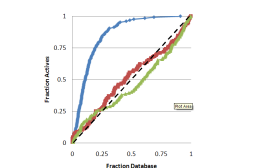
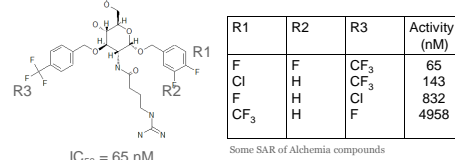


	Fragments	VS total
# Compounds screened	1,700	172
Confirmed hits	106	123
Hit rate	6%	72%
H3 selective hits	64	20
H4 selective hits	21	5

- VS produced very good enrichment
- Successful selection of H3 selective compounds
- Much less good selection of H4 selective compounds



Modelling of MCHR1 receptor (collaboration with Alchemia)



- ROCS search with min. energy Alchemia active
- ROCS search with docked Alchemia active
- Docking VS with MCHR1 model

References

- [1] Jayaraman, S., Hindson, K., White, S. H. Energetics, stability, and prediction of transmembrane helices. *J. Mol. Biol.* 2001, 312, 927-934.
- [2] Frenkel, D., Smit, B. Understanding Molecular Simulations from algorithms to applications. 2002. Academic Press. Frenkel, D., Klein, M., Parrinello, M., and Smit, B.
- [3] Heryk, P.; Hubbard, R. E. A reduced representation of proteins for use in restraint satisfaction calculations. *Proteins* 1993, 17, 310-324.
- [4] Goddard, W. A., III; Kim, S. K.; Li, Y.; Trzaskowski, B.; Griffith, A. R.; Alrod, R. Predicted 3D structures for adenosine receptors bound to ligands: comparison to the crystal structure. *J. Struct. Biol.* 2010, 170, 10-20.
- [5] www.gromacs.org
- [6] Ha, S. N.; Hey, P. J.; Ranson, R. W.; Harrell, C. M., Jr.; Murphy, K. L.; Chang, R.; Chen, T. B.; Su, D. S.; Markowitz, M. K.; Bock, M. G.; Freidinger, R. M.; Hess, F. J. Binding modes of dihydroquinolines in a homology model of bradykinin receptor 1. *Biochem. Biophys. Res. Commun.* 2005, 331, 159-166.