Multiscale modelling of $P2Y_{11}$, a receptor involved in heteromeric GPCR signal transduction

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Conformation-activity relationships look at the relationship between biological activities and conformational changes of biomolecules. The large number of different conformations that all classes of seven transmembrane (7TM) receptors, also known as G protein-coupled receptors (GPCRs), are able to adopt during the signaling process has made this particular family of proteins somewhat difficult to characterize using structural biology methodologies. Of the 79,851 experimentally resolved structures in the PDB database (as at 13 March 2012), fewer than 50 of these correspond to GPCR structures that include the TM domains, which contribute to the ligand-binding pocket of Class A, or rhodopsin-like, GPCRs. Six of these intact 7TM structures are of the A_{2A} adenosine receptor, a purine-activated Class A GPCR. The A_{2A} adenosine receptor structures include structures obtained in the presence of both receptor agonists and receptor antagonists, which is of use in developing and refining molecular models of receptors in both active and inactive states.

Originally believed to function as monomeric proteins, oligomerisation is a common feature amongst all classes of GPCR and GPCR protomers are able to form homomeric, heteromeric or higher order oligomeric complexes both *in vitro* and *in vivo*[1]. GPCR heteromeric complexes are of particular interest as they have unique functional properties that differ from the properties of the individual receptor subtypes. There are fewer than 10 structures in the PDB database that correspond to GPCR complexes. In all instances, these structures are of homomeric complexes; there is no structural information about GPCR heteromers available yet.

We have a long-standing interest in purinoceptor signalling with increasing emphasis on characterising receptor signalling that is a consequence of intermolecular protein-protein interactions between different purinoceptor subtypes and wished to use computer modelling based on the known A_{2A} receptor structures to identify functional determinants of heteromer purinoceptor interactions presently being characterised in the laboratory, where we are currently studying two different purinoceptor subtypes, the human A_1 adenosine receptor (hA_1) and the human and *Xenopus laevis* orthologues of the $P2Y_{11}$ receptor ($hP2Y_{11}$ and $xP2Y_{11}$, respectively). Each of these purinoceptor subtypes is capable of forming a heteromeric receptor with a common partner, the $P2Y_1$ receptor, and we have numerous species orthologues and mutants for both A_1 and $P2Y_{11}$, which provides us with extensive information about the signalling properties of each, both as monomeric receptors and when participating in the formation of a heteromeric receptor with $P2Y_1$[2-4]. These experimental resources are available to inform the modelling.

Our computational studies began with the $P2Y_{11}$ receptor. Homology modelling techniques were used to predict the structure of $P2Y_{11}$ from the limited data available for various conformational structures of the A_{2A} receptor. In the first instance, we exploited the common serpentine topology of GPCRs and the available amino acid sequences of different $P2Y_{11}$ receptor orthologues sequences to build an alignment for use as an input to homology modelling alongside the A_{2A} template structures. Ultimately, we aim to validate this with low-resolution structural data and mutation data. The homology modelling within this project was performed using MODELLER[5].

There are two discrete domains within the structure: extracellular loops and transmembrane helices. Reliable prediction of binding sites and ligand orientation within them presents a challenge. In Class A GPCRs, the

ligand-binding pocket is believed to form principally within the transmembrane domain region, although data have suggested the involvement of an extracellular loop in adenosine receptor ligand-binding[6]. In order to refine our homology models we have begun with the three extracellular loops (EL) and the seven transmembrane (TM) helices.

To investigate EL conformations, homology models of $hP2Y_{11}$ and $xP2Y_{11}$ were produced based primarily on the structure of the A_{2A} receptor (PDB structure 3EML). In areas of low homology, additional templates from a rhodopsin structure, 1F88, were included for transmembrane regions, as were templates from structures 3LCZ and 2VSA for EL2 and EL3, respectively of the $hP2Y_{11}$ model.

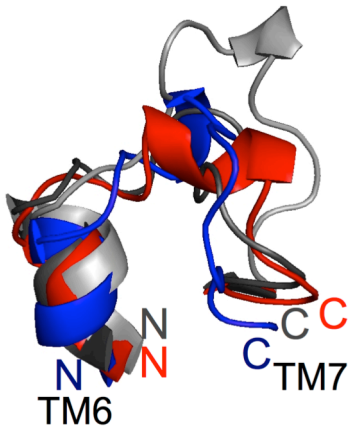

Figure 1: The model of EL3 in $xP2Y_{11}$ is believed to represent an "open configuration" of this loop. Main result (red): clustered model from 50 ns and 5 ns runs. Alternative result (grey) from a 5 ns run. The locations of TM6 and TM7 are indicated.

We developed a serial multiscale molecular dynamics approach using GROMACS[7] in order to refine the models. Loop conformation and the helices to which they are attached cannot be completely decoupled. Consequently, we ran coarse-grained simulations of the TM region in a self-assembled DPPC membrane in order to improve helix positions, tilts and hinge regions. We used the MARTINI[8] coarse-graining approach to produce 200 ns of simulation. Subsequent implicit solvent modelling (5-50 ns) with an atomistic protein model was used as a complementary method for modelling the loops of these models. The loop regions could not be modelled by homology modelling alone. EL2 and EL3 are particularly long in the human receptor, making these regions more difficult to model.

Implicit solvent simulations yielded a model of EL3 in the $xP2Y_{11}$ (Figure 1). It is partially α-helical and served as a reasonable template for EL3 in $hP2Y_{11}$. The molecular dynamics approach revealed possible differences between $hP2Y_{11}$ and $xP2Y_{11}$, particularly in the angle in the hinge region between TM7 and the intracellular C-terminal helix (Figure 2). These simulations will need to be repeated, in order to evaluate these differences. Reverse transformation from coarse-grained to atomistic models will form part of the validation process and will make final atomistic simulations of the TM regions possible.

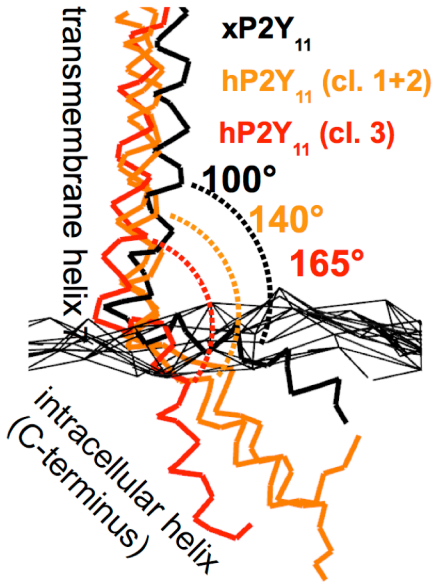

Figure 2: Comparison of the TM7 - C-terminus hinge region of hP2Y11 and its orthologue xP2Y11. Different clustered models from 200 ns coarse-grained molecular dynamics simulations are shown. The angle between TM7 and the C-terminal helix appears larger in the human receptor, potentially due to the presence of an additional proline.

Homology modelling of different species orthologues of the same receptor followed by serial molecular dynamics simulations will help to predict the structure and key dynamic features of the human $P2Y_{11}$ receptor. Critical to the building of an accurate homology model is the generation of an accurate alignment to an existing template. However, the low sequence identity between $P2Y_{11}$ and A_{2A} poses a problem. To overcome this, we attempted to subdivide the alignment into distinct helices, following the approach of Hall *et al.*[9], and

use physical properties to guide the alignment, principally by comparing the distance between central mass of the bilayer and the helix backbone with the known displacement behaviour of A_{2A}. This produced a Gaussian distribution (Figure 3), where the most closely matching distribution was shown to correspond to the most accurate alignment. By repeating this method for each TM helix it has been possible to optimise the alignment and, thus, the homology model.

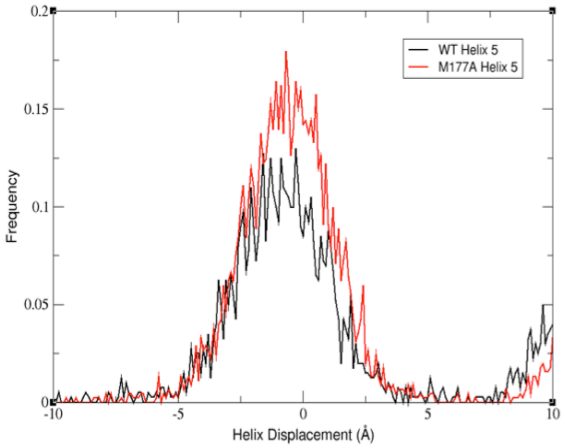

Figure 3: Helix displacement distributions for TM5 of the wild type and Met177Ala mutant A_{2A} receptor. The displacement was measured from central mass of helix backbone to the central mass of the bilayer and normalized to the frequency. No significant displacement difference is seen.

Our initial results have used species orthologues and mutational data to construct an alignment and an initial homology model of the human $P2Y_{11}$ receptor. Our results suggest that the regions of the receptor involved in oligomerisation may differ from those identified for the A_{2A} receptor.

In addition, we also looked at A_{2A} mutation data to evaluate the effect of specific residues upon helix location within the membrane. These motions and changes can be interpreted alongside evidence for conformational change to allow us to understand the role and mechanisms of experimentally-important amino acid residues. TM5 is of particular interest in the A_{2A} receptor which undergoes homodimerization via the PxxxM motif (Figure 4)[10]. However $P2Y_{11}$ lacks this motif, having instead the more widely recognized binding motif GxxxG, (Figure 4). Studies of these motifs, including assessment of helix dimerising simulations, are currently underway to compare the ability of A_{2A} and $P2Y_{11}$ to form TM5 homodimers.

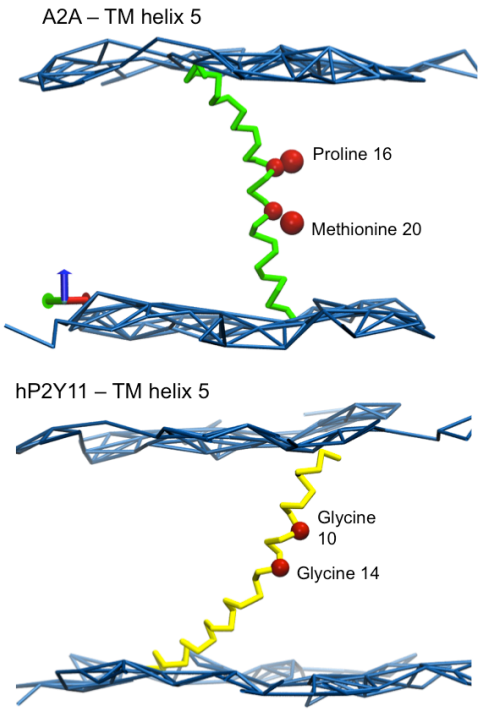

Figure 4: (top) The putative PxxxM dimerization motif of TM5 (A_{2A}). (bottom) Alignment of TM5 in $hP2Y_{11}$, showing the GxxxG motif.

References

[1]Selent, J. and Kaczor, A.A. (2011) Current Med. Chem. 18:4588-4605, [2]Yoshioka, K. *et al*. (2001) Proc. Natl. Acad. Sci. 98:7617-7622, [3]Ecke, D. *et al*. (2008) Biochem J. 409:107-116, [4]Cai, Q. and Townsend-Nicholson, A. (2008) Purinerg. Signal. 4:S77-78, [5]Eswar, N. *et al*. (2006) Current Protocols in Bioinformatics Supp 15:5.6.1-5.6.30, [6]Olah, M.E. *et al*., (1994) J. Biol. Chem. 269: 24692-24698, [7]Hess, B. *et al*. (2008) J. Chem. Theory and Comput. 4:435-447, [8]Monticelli, L. *et al.* (2008) J. Chem. Theory and Comput. 4:819-834, [9]Hall, B.A. *et al*., (2011) PLoS Comput. Biol. 7:e1002204, [10]Thevenin, D. *et al*. (2005) Protein Sci. 14:2177-2186