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Rhodopsin crystal structure: provides information on GPCR–ligand binding in general? ▼

Anyone interested in the design of potent small-molecule ligands for G protein-coupled receptors (GPCR) of therapeutic interest at some time must have been tempted to look at a computer-generated model of the structure of that receptor – at least of the helical domains.

Most models, sometimes supported by computational studies, have relied on hydropathy profiles to predict the putative transmembrane (TM) domains of the receptor. Such plots, whilst representing a 'good start', do not accurately predict such domains for the (bovine) rhodopsin protein, the details of which can now be seen from the recently published crystal structure[1] (see Box 1 for a brief overview of the findings). With the assumption that rhodopsin truly is an adequate homology model on which to base predictions for GPCRs, how does this new information impact on hypotheses of how small molecules interact with GPCRs?

A current model for Class A and B receptors is that, whilst there is considerable divergence in the way GPCR ligands might form the first interaction with the receptor (the 'collision' complex), a secondary event, with crucial involvement of residues in the TM domain, is responsible for receptor activation[2]. For simple ligands, such as the monoamines, where the homologous receptor has a short N-terminus (NT), both binding steps are thought to involve the TMs. However, for more complex ligands, such as peptides represented by corticotropin releasing factor (CRF), there is considerable evidence that the initial binding event might require predominantly the extended NT domain in consort with extracellular (EC) loops[3], while only the activation step requires the TM region. In contrast, small molecule (i.e. non-peptide) antagonists of such receptors apparently interact with the helical region. With the TM domain apparently 'protected' by the EC loops, how can this model by rationalized?

One of four interpretations come to mind. First, the crystal structure is misleading and constraints of crystal packing cause EC2 to fold deeply into the TM domain. In solution, the NT plus EC loops fold into a distinctly different structure so that access by ligands to the TM regions will be unimpeded. Alternatively, the loops might undergo a major reorganization from ligand binding to receptor activation. For example, EC2 could act as a molecular hinge capping the receptor only after ligand binding – such changes are well documented for some enzymes such as HIV protease[4].

Another possibility involves a restructuring of the pattern of disulfide bridges following ligand binding. This is particularly attractive for receptors that contain an extended NT where multiple alternatives are available (for CRF1-R, there are 8 Cys residues within the NT/EC domains). Finally, it is possible that small-molecule ligands initially dissolve into the lipid membrane that surrounds the receptor – indeed many GPCR-receptor antagonists are highly lipophilic and would favour this mechanism.

Clearly, it will be interesting to determine which of these explanations, or others not considered here, will gain experimental support. For now, I prefer to favour the second option because the precedent set by various enzymes demonstrates that large conformational changes in 'flap regions' accompanying substrate binding are to be expected.

Box 1. Crystal structure of the (bovine) rhodopsin protein

Briefly, these are the key findings of the recently published crystal structure of the (bovine) rhodopsin protein[1], which contains some surprises. Transmembrane TM1, 2, 3 and 6 are significantly longer than those predicted from hydropathy plots (30, 30, 33 and 31 compared with 25, 25, 20 and 24, respectively. For the corticotropin releasing factor receptor (CRF1-R), for example, these values are 21, 24, 24 and 20, respectively.

Another finding is the role of the ubiquitous disulfide bridge (Cys^{110}–Cys^{187} in rhodopsin), one of several 'fingerprints' within the GPCR superfamily between the second and third extracellular (EC) loops. In rhodopsin, Cys^{110} is close to the extracellular surface of TM3 and places the EC2 loop in a position that severely impedes access of ligands to the helical bundle. It is this molecular feature, in particular, that causes us to think again about the way in which small-molecule ligands approach and then interact with their receptor. Importantly, it is likely that the size and topology of the TM domains and the EC-loops will be closely conserved for the GPCR family.

Another key feature of the GPCR structure is the D(E)-R-Y(W) sequence at the intracellular boundary of TM3 of all of the subclass A receptors, which is apparently missing from class B GPCRs in general. A closer look at type B receptors reveals that a D(E)-R-X counterpart might exist if it is assumed that a glutamic acid residue towards the cytosolic boundary of TM3 (E^{209} in CRF1-R) and an arginine at the N-terminus of TM2 (R^{151} in CRF1-R) – both conserved throughout the class B sub category – are spatially positioned to fulfil this role. In the crystal structure, E^{134}-R^{135}-Y^{136} are involved in several hydrogen bonds and a salt bridge (E^{134}–R^{135}), and a reorientation of interactions in this region might be required during the transformation from the ground state of the receptor to the ligand-induced activated state.

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The optimal fragmentation principle – Reply ▲

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Response from Dale Johnson

Both Peter Bach and Ann Richard make excellent points in their replies to my letter. As Bach states, there is a need for a single platform for current predictive tools – one that enables a user to combine approaches, use different databases or add personal information. A secure web-based approach might be a reasonable solution.

Richard points out that a major limiting factor in the development of models and predictive systems is access to quality data. In many cases it is also a problem of the 'existence' of quality data. In addition to sharing proprietary information, there is also a crucial need to create new databases from mechanistic screening approaches that can be used to fill gaps in chemical space in databases or chemical libraries (especially in the drug discovery area) and to provide researchers immediate access to screens where confirmation algorithms can be applied to predictive results.

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