Modelling the folding of transmembrane proteins using a novel fragmental approach: the human ghrelin receptor and the glutamate transporter EAAT1

Alessandro Pedretti and Giulio Vistoli

Istituto di Chimica Farmaceutica e Tossicologica “Pietro Pratesi”, Facoltà di Farmacia, Università degli Studi di Milano, Viale Abruzzi 42, I-20131 Milano (Italy).

In these last years the number of experimentally resolved transmembrane proteins is considerably growing providing useful templates to model many other transmembrane proteins. The case of GPCR proteins can be considered paradigmatic: availability of the experimental structure of bovine rhodopsin has strongly supported GPCR modelling and the numerous models appeared in literature were successfully exploited for virtual screening and ligand optimization (1). However, the systematic use of the template (as in the case of rhodopsin), despite the clear homology among GPCRs, can lead to repetitive “clones”, which loose their structural peculiarity to forcedly comply with template structure.
In order to resolve this drawback allowing a more versatile construction of transmembrane protein models we here propose a fragmental approach in which 1) the entire sequence is divided in structural segments (namely, loops, terminal domains, transmembrane helices and so on); 2) the segment folding is separately predicted using different templates; 3) the global template (e.g., the rhodopsin for GPCRs) is still used to drive the final assembly of predicted segments. Interestingly, this fragmental approach reflects a more general trend in folding prediction which favours the local homology combining more predictive algorithms (the so-called meta-prediction).
The soundness of this approach was verified by generating the models of both human ghrelin receptor (hGHS-R1a) in its open state (2) and the human glutamate transporter EAAT1. In particular, the hGHS-R1a open state is unpredictable using the rhodopsin as unique template since the transmembrane bundle is markedly wider, whereas the fragmental approach provided a reliable open state model which was validated by docking a heterogeneous set of ligands taken from literature. The docking results are in excellent agreement with biological activities unveiling the presence of two possible binding sites with a cooperative effect. The glutamate transporter was chosen as: 1) it has a totally different topology if compared to GPCRs (the TM bundle is formed by 8 helices and two helical hairpins), 2) there is an experimental structure which can drive the final assembly ((3), the transporter homologue from *Pyrococcus horikoshii*). The fragmental approach allowed to generate a consistent EAAT1 model in its trimeric architecture and the docking results provided the pharmacophoric basis to discriminate among substrate inhibitors and non-substrate blockers.
Finally, the reported results demonstrated that the fragmental approach can be useful to explore the local properties, assuring a substantial agreement with template structure. This approach can be applied to any transmembrane protein and can be exploited in mutagenesis experiments to predict local changes in the folding of mutated proteins.

(1) Fanelli, F.; De Benedetti, P.G. Chem Rev. 2005, 105, 3297-3351
(2) Pedretti, A.; Villa, M; Pallavicini, M.; Valoti, E.; Vistoli, G. J. Med. Chem., 2006, 49, 3077-3085
(3) Yernool, D.; Boudker, O.; Jin, Y.; Gouaux, E. Nature. 2004, 431, 811-818.