Protein modelling by fragmental approach: connecting global homologies with local peculiarities

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The homology modelling approaches allow to carry out structure-based studies, as ligand optimization and virtual screening, when the experimental three-dimensional structure of the biological target is unavailable. They require 3D templates can be found submitting the primary structure to search engines and performing pairwise alignments between the whole query protein and the sequences included in one or more databases of 3D structures. The sensibility of the alignment algorithms for reduced homology regions can be dramatically drawn back by sequence segments with high similarity, because the score function could overestimate the strong similarities, penalizing the alignment quality of segments with low homology. Another problem can be found submitting whole query sequences of protein families with high homology to a very limited number of 3D templates (e.g. transmembrane proteins), because the resulting models appear quite similar due to the common template and can be considered structural "clones".
In order to resolve this problem, allowing a more versatile protein modelling, we proposed a fragmental approach in which 1) the entire sequence is divided in structural/functional segments; 2) the segment folding is separately predicted using different templates favouring the local homology and increasing the alignment sensibility; 3) the global template is still used to drive the final assembly of predicted segments. This approach finds its principal application in predicting the 3D structure of transmembrane proteins due to the mentioned scarcity of resolved structures. Hence it was applied to model several structurally diverse transmembrane proteins, among which the human $\alpha4\beta2$ nicotinic receptor and human glutamate transporter EAAT1 will be discussed in this lecture.
In particular, the monomeric structures of the $\alpha4\beta2$ nicotinic receptor were derived using a fragmental approach and the heteropentameric model was assembled by protein–protein docking. The model soundness was verified docking a set of known nicotinic ligands and the results highlighted that the ligand affinity depends on key interactions involved between the ligand charged moiety and conserved apolar residues in the $\alpha4$ subunit, whereas the selectivity could be gained by the H-bond acceptor group which interacts with a less conserved and more heterogeneous residues of $\beta2$ subunit[1].
The fragmental approach allowed to generate also all segments that are assembled in the EAAT1 model, aligning them to the transporter homologue from *Pyrococcus horikoshii*. The final homotrimeric structure, obtained by protein-protein molecular docking calculations, was used to perform docking studies which allowed to discriminate among substrate inhibitors and non-substrate blockers. The docking results were further verified by generating two pharmacophore models (the former for substrates and the latter for blockers) which confirmed the features necessary for high EAAT1 activity[2].
The reported results demonstrated that the fragmental approach can be useful to explore the local properties and the agreement with the experimental data emphasize its soundness to model any kind of protein.

[1] Pedretti A., Marconi C., Bolchi C., Fumagalli L., Ferrara R., Pallavicini M., Valoti E., Vistoli G., "Modelling of full-length human $\alpha4\beta2$ nicotinic receptor by fragmental approach and analysis of its binding modes", B.B.R.C., Vol. 369(2), 648-53 (2008).
[2] Pedretti A., De Luca L., Sciarrillo C., Vistoli G., "Fragmental modeling of human glutamate transporter EAAT1 and analysis of its binding modes by docking and pharmacophore mapping", ChemMedChem, Vol. 3(1), 79-90 (2008).