G-protein coupled receptors (GPCRs) are in the limelight of both academia and pharmaceutical industry since they mediate plurality of biological processes from signaling to transport. Computational structure prediction of “free” receptors and their complexes with ligands is a pivotal stage in design of new drugs that target these proteins. GPCR B family comprises receptors of secretin, glp1r, gastrin, vasoactive intestinal peptide (VIP) and other neuropeptides. VIP, which is synthesized in central and peripheral nervous system, is a potent immunomodulator and inhibitor of inflammation, and thus is a perspective agent for treatment of autoimmune diseases (such as rheumatoid arthritis and Crohn’s disease) and local inflammatory conditions that are associated with, e.g., transplantation.

Molecular modeling of complex structure of VIP receptor with the peptide (VPAC1–VIP) is an essential step towards design of more effective and selective VIP analogs for novel therapies.

In this study, six structures of six GPCRs from family A (rhodopsin family) have been elucidated; although no members of B family (family of secretin receptor) have known complete 3D structure. For six of GPCR-B receptors structure of extracellular (EC) domains has been determined, but neither structure of transmembrane (TM) domain nor its mutual orientation with EC-domain, nor mechanism of peptide receptor “capturing” and receptor activation are known (fig. 1).

Here, we describe a homology model of TM-domain of VIP receptor (VPAC1). The main complication was to construct unambiguous amino acid alignment between model and the template given that very low homology level (~10%) hampers use of common alignment algorithms (such as CLUSTAL). To overcome this obstacle, we employed an iterative procedure of alignment “selection” guided by assessment of packing “quality” of corresponding 3D-models. This was done by means of the “membrane score” approach (S3) that has proved itself capable to identify close-native structures of membrane proteins (MPs) and also to detect alignment errors (see “box”, fig. 1). This is especially important in drug design-related tasks [2].

Model building & refinement
Rhodopsin crystal structure was chosen as a template for VPAC1 TM-domain modeling. Sequence homology level of alignment pool was extended from literature or built with the “membrane score” approach. Calculated alignment is “selected” from pool of combined alignments derived from several distinct initial variants, collected from literature or built using common tools. Then, “alignment pool” was extended by independent shifts of each TM-heks of the model by ±1 or 0 residues (for seven independent regions this yields 33=2187 variants). Each alignment variant was scored by assessment of protein packing “quality” in the resulting models. The “quality” (reasonableness) of computer-generated models of TM-domain was checked employing the “membrane score” approach (S3) (see “box”, [1]).

Refinement of modelled transmembrane proteins structure
An example of VIP receptor (GPCR B)
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The “Membrane score” approach

Application of packing “quality” and conformation property analysis for optimization of models of TM-domain structure. (template-model) is equally low (1-2%) for rhodopsin and any other available template. The “optimal” alignment was iteratively “selected” from a pool of combined alignments derived from several distinct initial variants, collected from literature or built using common tools. Then, “alignment pool” was extended by independent shifts of each TM-heks of the model by ±1 or 0 residues (for seven independent regions this yields 33=2187 variants). Each alignment variant was scored by assessment of protein packing “quality” in the resulting models. The “quality” (reasonableness) of computer-generated models of TM-domain was checked employing the “membrane score” approach (S3) (see “box”, [1]). The top-ranking alignment was an input for repeated “selection” procedure with sequence shifts, and the final alignment (fig. 2) was established after shift correction optimization by variant population analysis. Conformational sampling with molecular dynamics confirmed that the “final” model has statistically better packing quality than several intermediate structures.

Analysis of VPAC1 model
In the model several residues that are suggested from mutagenesis studies [2] to be important for VIP binding and/or receptor activation, line up (fig. 3): R188(TM2)–Q380(TM7)–N229(TM3), and this proximity is further confirmed by cooperative and anti-cooperative activation effects that appear in double mutants built from reciprocal residue exchanges. This allows to develop a hypothesis for activation of VPAC1 and, presumably, other receptors of this family.

VPAC1 model permits rationalization of available experimental data (point mutagenesis and location of possible dimerization site in family B receptors), and this marks the “membrane score” approach as useful during building and refinement of computer models of membrane proteins, which find their application in drug design-related tasks [2].


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