Modeling of a transmembrane multidrug resistance protein, P-glycoprotein

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Abstract

P-glycoprotein (P-gp) is an ATP-binding cassette (ABC) transporter, which extrudes diffusible drugs from mammalian cells using ATP hydrolysis as energy source [1, 2]. Typical examples of drug substrates include anticancer agents (to which overexpression of P-gp confers resistance) or some antibiotics (which therefore become less active against intracellular infections). Consequently there is an urgent need to understand the structure-function relationship of this protein. This knowledge would help designing inhibitors blocking drug extrusion or drugs with lower affinity for this transporter.

Experimental studies and sequence analysis proposed that P-gp consists of two homologous halves containing each six putative membrane-spanning helices and one cytoplasmic nucleotide-binding domain. Given the difficulty in crystallising membrane proteins structural information is rare. It is thus highly desirable to generate structural models. Structural and biochemical studies suggest that conformational changes occur along the catalytic cycle of P-gp. We built three-dimensional (3D) models of P-gp using an approach which combines in a rational way a series of computational methodologies. Initial models have been obtained by comparative modeling using as a template the crystallographic structure of SAV1866, a bacterial ABC transporter [3] having a topology similar to P-gp and featuring a sequence identity of about 29%. Several energy optimisations of these initial models were performed including distance-restrained potentials. These restraints were derived from data obtained from cysteine cross-linking experiments performed on P-gp in different states along its catalytic cycle [4]. The validity of the generated models is discussed in regard to the available experimental data.

Principle of comparative modeling.

Comparative modeling building consists in the extrapolation of the structure for a new target sequence from known 3D-structure of related family members (templates).
It is based on the observation that high sequence similarity is reflected by distinct structure similarity.

Alignment of sequences.

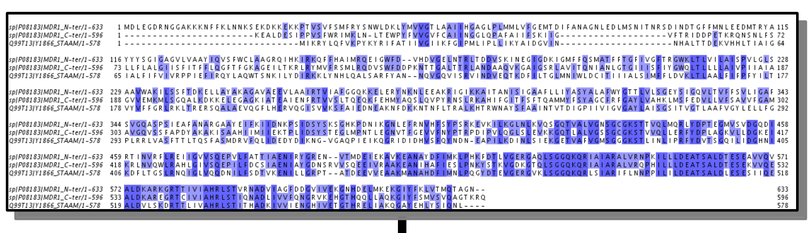


Fig. 2. Alignment of the N-ter and C-ter halves of P-gp and SAV1866 sequences using ClustalW software.

Building of the initial model.

Assessment of the initial model with biochemical cross-linking data.

Biochemical cross-linking data for P-gp with AMP-PNP.

Biochemical cross-linking data for P-gp in absence of nucleotide.

Model optimisation.

Model of nucleotide bound P-gp.

Model of nucleotide-free P-gp.

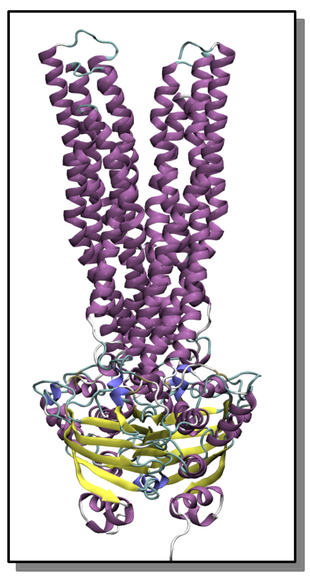


Fig. 4. P-gp model optimised with the AMP-PNP restraints.

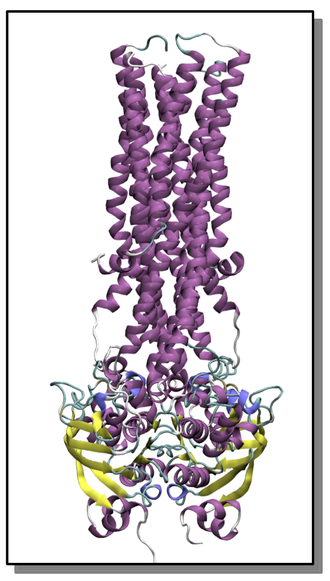


Fig. 5. P-gp model optimised with the « nucleotide-free » restraints.

Results

A. Alignment of P-gp and SAV1866 sequences.

- Accurate sequence alignment is an essential prerequisite to produce an accurate 3D model (Fig. 2).
- The sequence identity between SAV1866 and P-gp is rather low so the sequence alignment is prone to errors.
- To improve the quality of the alignment, complementary information, such as prediction of transmembrane regions, were considered.

B. Building of the initial model.

- An initial model of P-gp was built using the Modeller software.

C. Assessment of the initial model.

- Inter-residue distances measured on the model were compared with inter-residue distances derived from cross-linking experiments (Fig. 3).
- AMP-PNP is a non-hydrolysable analogue of ATP.
- 75% of the distances measured in this initial model were consistent with AMP-PNP-bound biochemical data.
- On the other hand, the initial model agreed with 80% of the abundant « nucleotide-free » cross-linking data.

D. Optimisation of the model.

- In order to achieve a better consistency with biochemical data, constraints derived from these inter-residues distances were applied through rigid-body energy minimisations and molecular dynamics simulations on our initial model.
- The model optimised with AMP-PNP restraints showed 94% of consistency with inter-residues distances derived from cross-linking data (Fig. 4).
- The optimisation applied using « nucleotide-free » restraints improved the consistency of the model with biochemical cross-linking data up to 98% (Fig. 5).

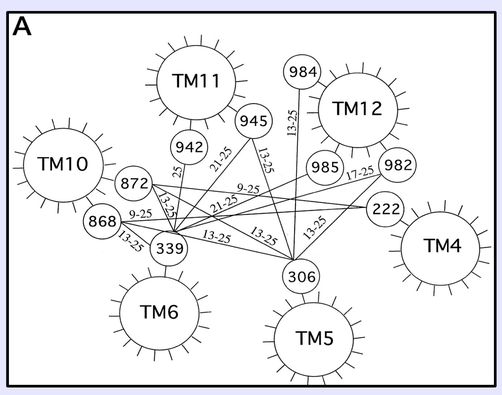
Fig. 1. Transmembrane regions of SAV1866. Extracellular helices are red, transmembrane helices are yellow and intracellular domains are blue.

The template structure : SAV1866.

The template structure shows an and corresponds to an ATP bound state. Structure was solved at a resolution of 3.0 Å. See Fig. 1 for a schematic representation.

Fig. 3. What is a cross-linking experiment ?

1. One residue is mutated into cysteine in each half-molecule of a Cys-less P-gp.
2. Those cysteines can form disulfide bonds with cross-linker reagents of known sizes.
3. Cross-linked products migrate slower than native proteins in SDS gels.



Inter-residue distances derived from cross-linking experiments. Taken from *Loo et al.*

Conclusions

- The SAV1866 structure turns out to be a good template to produce an initial model for P-gp.
- Our nucleotide-free and nucleotide-bound models fairly agree with with biochemical data.
- Residues known to be implied in the binding of drugs face the central cavity of the transmembrane region of P-gp.

References

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Perspectives

- These two models can provide insights in the power transmission from the nucleotide binding domains, the power house of P-gp, to transmembrane domains which binds and releases substrate.
- Docking of known substrates to better define a still unclear substrate binding site.