Lipid Transport by the ABC Transporter MDR3

ABC transporters are a large family of transmembrane proteins that mediate the translocation of a wide range of substrates across the membrane bilayer. Despite recent structural advances, the exact mechanism by which ABC transporters exert their functions at the molecular level has remained elusive so far, and several studies suggest a surprising mechanistic diversity within the members of the ABC transporter family. Using molecular dynamics simulations and free energy calculations, we investigated a novel substrate translocation pathway for the highly specialized ABC transporter multidrug resistance protein 3 (MDR3).

The human ATP-binding cassette (ABC) transporter multidrug resistance protein 3 (MDR3, ABCB4) plays a vital role in bile formation. It is primarily expressed in the canalicular membrane of hepatocytes, where it translocates phosphatidylcholine across the lipid bilayer and thereby promotes phospholipid secretion into bile. Phospholipids are an essential component of biliary micelles, which represent the preferred transport vehicle for the detergent bile acids. High concentrations of free bile acids would cause a loss of the membrane integrity of biliary epithelial cells. Accordingly, mutations in the ABCB4 gene are associated with Progressive Familial Intrahepatic Cholestasis type 3 (PFIC-3), a rare hereditary disease that ultimately results in liver failure.

Despite its high sequence similarity of 86% to the well-known drug efflux pump, multidrug resistance protein 1 (MDR1, P-glycoprotein), the substrate spectrum of both transporters is fundamentally distinct: While MDR1 transports a wide range of structurally unrelated hydrophobic compounds, MDR3 is a floppase specific for lipids with a phosphatidylcholine head group [1]. Since most of the amino acids involved in substrate binding in MDR1 are identical in MDR3 [2], the lipid specificity of MDR3 must arise from a different region of the protein.

A conspicuous arrangement of non-conserved, hydrophilic amino acids in transmembrane helix 1 (TMH1) of MDR3 suggests an alternative pathway for substrate translocation that does not involve the "classical" translocation pathway via the central cavity of the transporter. Instead, we hypothesize that lipid translocation occurs along the surface of TMH1, where it is facilitated by the interaction between the phosphatidylcholine head group and the side chains of these hydrophilic residues. Transport of lipids and lipid-like molecules that partially or solely occurs along designated surface cavities has already been described for ABC transporters [3] and other lipid transporters [4].

With this project, we aim to obtain a better understanding of the molecular mechanisms by which MDR3 translocates phospholipids. Atomic-level insights into the transport process of this highly specialized ABC transporter are not yet available and could be a major step on the way towards a complete understanding of the inner workings of ABC transporters.

Methods

Molecular dynamics (MD) simulations at the classical mechanical level are at present the most appropriate way to explore the dynamics and energetics of complex biological molecules. In MD simulations, Newton's equations of motion are solved by numerical integration. They are used to estimate the equilibrium properties of biomolecular systems and to describe the quality and timescales of biomolecular processes controlled by conformational changes.

To assess whether the proposed pathway along TMH1 of MDR3 is a more efficient alternative to a spontaneous, i.e. unassisted, phospholipid flip-flop, we performed MD simulations with the Amber software suite [5]. In particular, we employed steered molecular dynamics (sMD) simulations and umbrella potential-restrained MD simulations ("umbrella sampling") to calculate if the free energy barrier of spontaneous phospholipid flip-flop decreases when moving the lipid along a pathway lined by the hydrophilic residues in TMH1 of MDR3.

Phospholipid flip-flop is accompanied by a highly unfavorable transfer of the charged lipid head group into, and then out of, the hydrophobic core of the lipid bilayer. Consequently, the free energy landscape associated with this process is steep and requires extensive and biased sampling to be accurately reproduced by MD simulations. The simulation system used to study MDR3-mediated phosphatidylcholine flip-flop (depicted in Figure 1) contains ~240,000 atoms and requires at least 2 µs of total simulation time divided into 100 umbrella windows of 20 ns length to yield converged free energy profiles. Until now, this project consumed approximately $4 \cdot 10^6$ core hours on the general-purpose cluster JURECA.

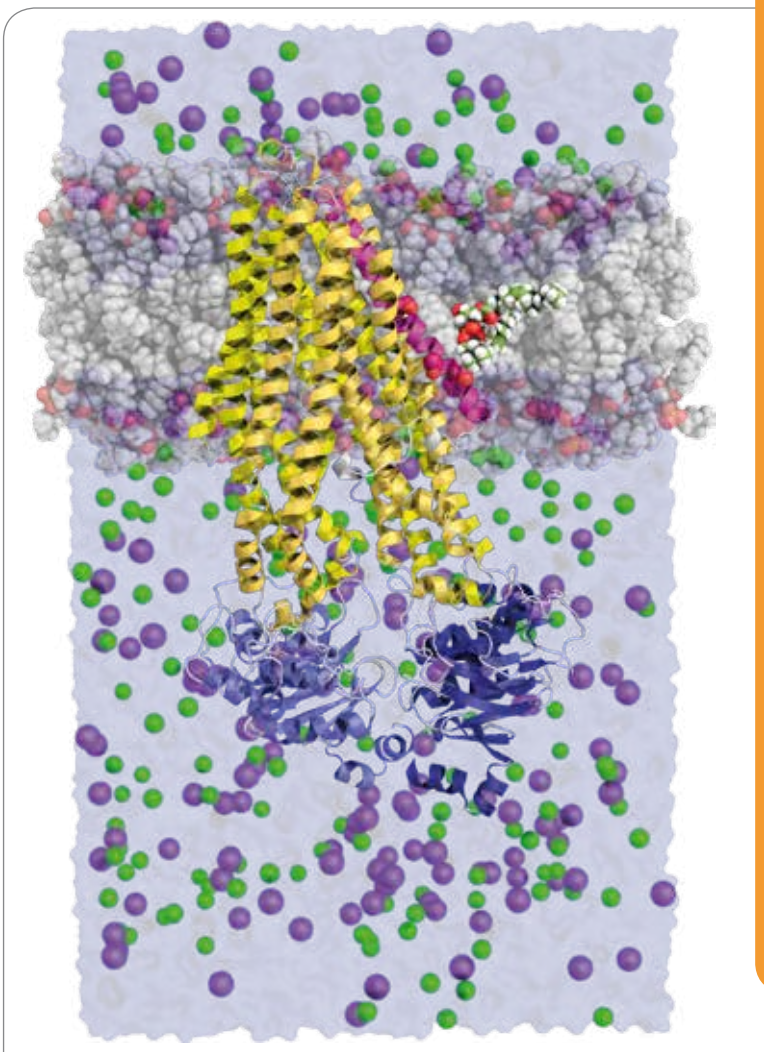

Fig. 1: Simulation system containing MDR3 (yellow and blue) embedded in a 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) bilayer (grey). NaCl (purple and green spheres) at a concentration of 154 mM mimics conditions in the intra- and extracellular space. The DOPC molecule being flipped through the bilayer is depicted in green.

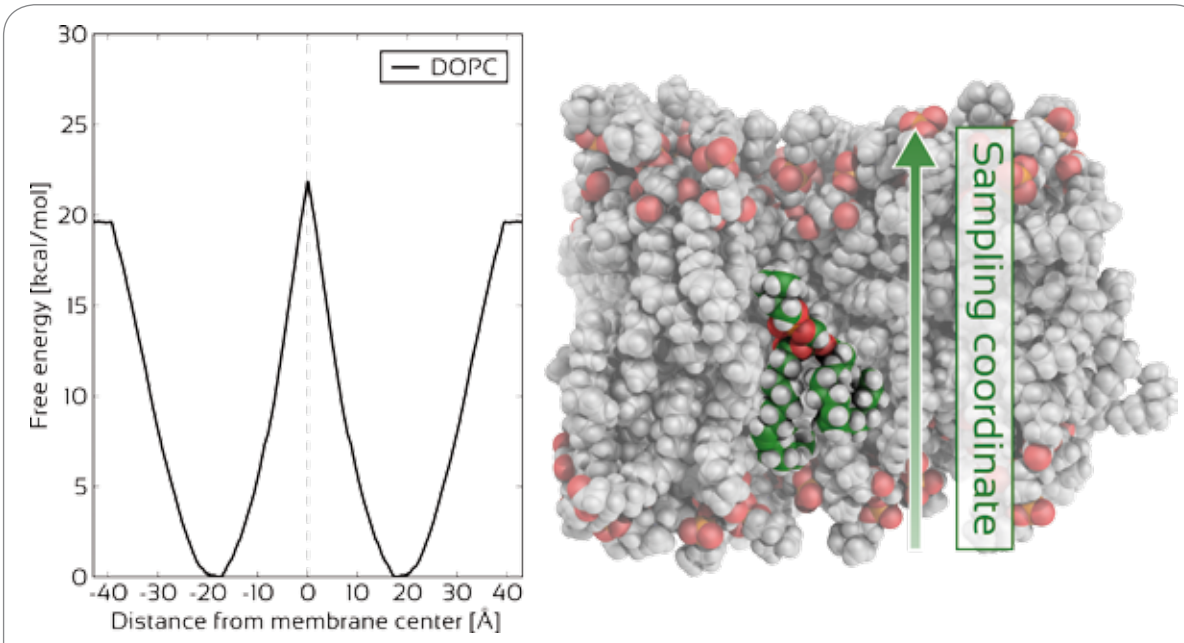

Fig. 2: Free energy profile of spontaneous DOPC flip-flop in a homogeneous DOPC bilayer. The simulation system is depicted as space-filling model. The flipped lipid is highlighted in green.

Results

Free energy profile of spontaneous flip-flop. As a reference for spontaneous phospholipid flip-flop, we calculated the free energy profile for the passage of a single 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) through a homogeneous DOPC bilayer. Figure 2 depicts the obtained profile, which displays an energy barrier of 20.8 kcal mol^{-1} and agrees both qualitatively and quantitatively with calculated profiles for related lipid species [6].

Free energy profile of MDR3-assisted flip-flop. The free energy profile obtained for DOPC flip-flop along TMH1 of MDR3 is remarkably different from the reference profile. As shown in Figure 3, the barrier height is decreased by 7.0 kcal mol^{-1}, rendering MDR3-mediated flip-flop approximately five orders of magnitude faster than spontaneous flip-flop. According to our hypothesis, this effect should primarily be attributable to salt bridge and hydrogen bond interactions between the DOPC head group and the side chains of the hydrophilic amino acids in TMH1. Indeed, the free energy profile of MDR3-mediated flip-flop starts to diverge from the reference profile once the phosphatidylcholine head group reaches a "buffer zone" in which interactions with the hydrophilic serine and threonine residues are possible. A similar profile calculated along TMH7 of MDR3, which is the structural equivalent of TMH1 in the second pseudohalf of the transporter but does not show hydrophilic amino acids exposed to the

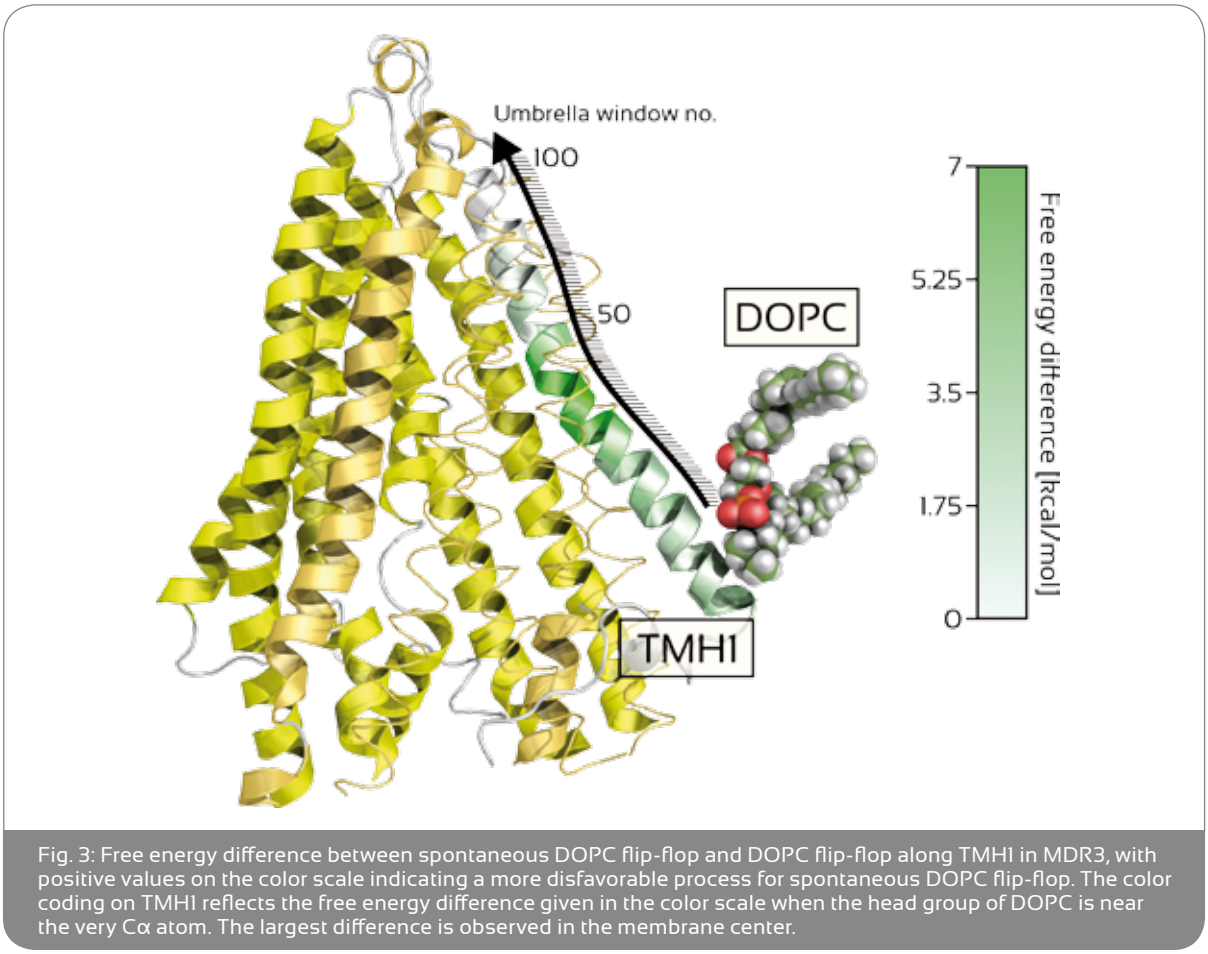

Fig. 3: Free energy difference between spontaneous DOPC flip-flop and DOPC flip-flop along TMH1 in MDR3, with positive values on the color scale indicating a more disfavorable process for spontaneous DOPC flip-flop. The color coding on TMH1 reflects the free energy difference given in the color scale when the head group of DOPC is near the very Cα atom. The largest difference is observed in the membrane center.

membrane, does not display any reduction in barrier height. Thus, the obtained profile along TMH1 strongly supports our hypothesis of an alternative substrate translocation pathway for the lipid floppase MDR3.

Comparison to experimental data. While experimentally determined rates of spontaneous phosphatidylcholine flip-flop amount to 0.04 h^{-1} [7], the ATPase activity of MDR3 has been determined as 828 h^{-1} [8]. Assuming that one molecule of DOPC is translocated during each ATPase cycle, MDR3-mediated DOPC transport is thus sped up by a factor of ~2 * 10^4, relating to a lowering of the energy barrier by about 6 kcal mol^{-1} at 300 K. This value is in excellent agreement with our computed barrier height decrease of 7.0 kcal mol^{-1}.

Ongoing research / outlook. In order to rule out the possibility of a "classical", cavity-mediated phospholipid translocation in MDR3, additional 3 µs of unbiased MD simulations on different MDR3 systems were performed. Using

configurations from these MD simulations as templates, we aim to construct free energy profiles for cavity-mediated DOPC transport in a similar way as presented for TMH1-mediated transport. The results obtained from our simulations will furthermore serve to guide mutation experiments for biochemical validation of our predictions.

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