Atomistic Dynamics of Alternating Access Mechanism of an ABC Transporter

Hendrik Göddeke and Lars Schäfer

Abstract ATP-binding cassette (ABC) transporters are ATP-driven molecular machines. ATP binding and hydrolysis in the nucleotide-binding domains (NBDs) are coupled to large-scale conformational changes of the transmembrane domains (TMDs), which leads to the translocation of substrate molecules across biological membranes. The atomic details of the structural dynamics underlying the conformational transitions and the coupling of NBD and TMD motions remained largely *terra incognita*. Here, we used all-atom molecular dynamics (MD) simulations to characterize the conformational transitions underlying the working cycle of the heterodimeric ABC exporter TM287/288 from *Thermotoga maritima*. Multimicrosecond MD simulations reveal how ATP binding triggers a spontaneous conformational transition from the initial inward-facing (IF) conformation via an occluded (Occ) intermediate to an outward-facing (OF) conformation. ATP binding induces tightening of the NBD dimer, which involves closing and reorientation of the two NBD monomers. Simultaneous closure of the cytoplasmic (intracellular) TMD gate region leads to the Occ state. Subsequent wide opening of the periplasmic (extracellular) TMD gate yields the OF conformer. This distinct sequence of events imposes tight coupling of NBDs and TMDs and ensures that the cytoplasmic and periplasmic TMD gates are not open at the same time to both sides of the membrane.

1 Introduction

All-atom molecular dynamics (MD) simulations of large biomolecular systems are computationally expensive, limiting the accessible length scales and time scales to typically about 10^5 to 10^6 atoms and a few μs, respectively. Unfortunately, many biological processes occur on slower time scales and are thus inaccessible to con-

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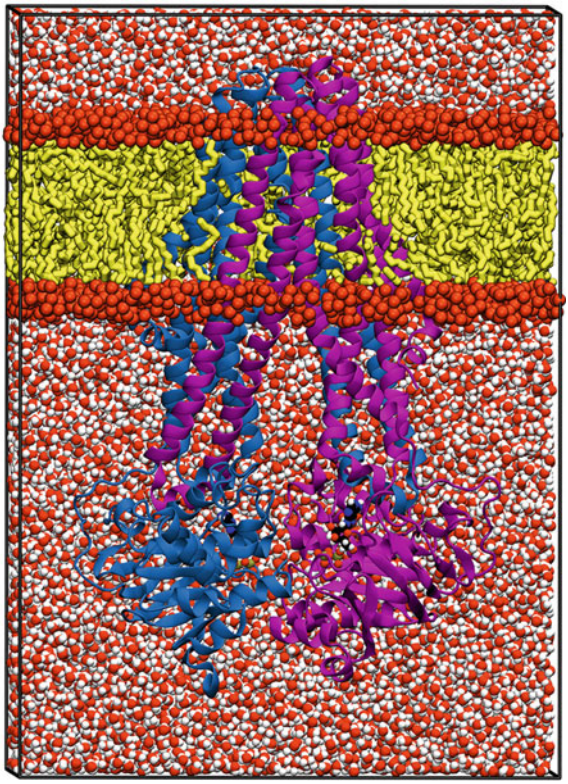

Fig. 1 MD simulation system of heterodimeric ABC transporter TM287/288 embedded in a solvated POPC lipid bilayer (ca. 132,000 atoms in total). TM287 is shown in purple, TM288 in blue. The phosphocholine headgroups of the lipids are shown as orange spheres, and the lipid hydrocarbon tails in yellow. Water oxygen and hydrogen atoms are shown in red and white, respectively

ventional MD simulations. One example for such a slow process is the large-scale conformational transition of ATP-binding cassette (ABC) transporters. ABC transporters are membrane-embedded molecular machines that use the free energy stored in ATP, the chemical energy-currency of the biological cell, to translocate a wide range of transport substrates across biological membranes [1]. They are found in all kingdoms of life and can be classified into importers, which are found exclusively in prokaryotes, and exporters, which are present in every organism. ABC exporters transport a broad range of chemically diverse molecules out of the cell, such as lipids, peptides, and drugs, also including chemotherapeutics. Hence, these proteins play a role in multidrug resistance of cancer cells and bacteria, and malfunction of ABC transporters is linked to hereditary diseases such as cystic fibrosis and neonatal diabetes [2].

ABC transporters share a common structural architecture. As shown in Fig. 1 for the ABC exporter TM287/288 investigated here, a dimer of two nucleotide binding domains (NBDs) is connected to a dimer of two transmembrane domains (TMDs). The NBDs bind and hydrolyze ATP, while the TMDs form the substrate translocation chamber. The free energy gained from ATP binding and/or hydrolysis in the NBDs is transmitted to the TMDs, which undergo large-scale conformational changes to cycle

between an inward-facing (IF) and an outward-facing (OF) conformation, thereby providing access to both sides of the membrane in an alternating manner. This ultimately leads to substrate translocation. However, despite this common basic working principle, different ABC subfamilies can differ in terms of both structure and mechanism [3]. While bacterial ABC exporters are often homodimers, most eukaryotic exporters are heterodimers [4, 5]. Many heterodimers have one consensus and one degenerate nucleotide binding site. In the degenerate site, the catalytic residues that are essential for ATP hydrolysis [6] as well as residues in the ABC signature motif deviate from the consensus sequence, hence impairing catalytic activity.

The current mechanistic understanding of ABC transporters is largely based on high-resolution structures obtained by X-ray crystallography or cryo-electron microscopy which show static snapshots of different conformations that could play a role in the functional working cycle. However, although such structural information is of course extremely useful, it cannot provide the complete picture because the mechanism of ABC transporters is governed by dynamics. The nature of the coupled motions of the NBDs (which dimerize and partly dissociate upon nucleotide binding and unbinding, respectively) and the TMDs (which undergo large-scale conformational transitions between the IF and OF states, thereby providing access to one or the other side of the membrane) remain to be resolved.

In principle, all-atom MD simulation can provide the lacking atomic-level insights into the structural dynamics underlying the transport mechanism. However, due to the large computational effort MD simulations of complete ABC transporters in explicit membrane and water environment have been carried out only for a few systems, typically covering time scales up to a few hundred nanoseconds [7, 8]. These time scales are too short for complete conformational transitions between the IF and OF conformations to occur spontaneously. The aim of the present project was to characterize in full atomic detail the dynamics of the large-scale conformational transitions in the ABC exporter TM287/288 from the thermophilic bacterium *Thermotoga maritima*. TM287/288 is a bacterial homolog of the eukaryotic ABC exporters CFTR, which is linked to cystic fibrosis [9], and TAP, which is a key player in the immune system [10].

To overcome the above limitations in our simulations, we (i) used extensive all-atom MD sampling of more than 50 μs accumulated simulation time in total, and (ii) carried out our simulations at an elevated temperature of 375 K, which accelerates structural transitions. This was motivated by the fact that TM287/288 is from a hyperthermophile that lives under extreme conditions up to 90 °C [11], and hence the higher temperature appeared to be a natural choice.

Our MD simulations of TM287/288 are based on the X-ray structure from Hohl and coworkers [4, 5] that shows the transporter in an IF state, both for the apo protein and with the non-hydrolyzable ATP analog AMP-PNP bound to the degenerate NBD. In this work, we focus on the conformational transition, therefore we performed the MD simulations in the presence of ATP-Mg but without transport substrate. As many ABC transporters, TM287/288 shows a basal ATPase activity, i.e., the transporter cycles between different conformational states in a futile manner driven by ATP binding and hydrolysis alone [12].

The MD simulations reveal how TM287/288, when loaded with two ATP-Mg molecules, undergoes conformational transitions from the initial IF structure via an occluded intermediate to an OF conformation. The OF structures observed in our MD simulations were validated by EPR spectroscopy of membrane-reconstituted TM287/288. The distance distributions between spin-labeled residue pairs measured by EPR support the MD simulations and, furthermore, show that the lipid bilayer is essential for stabilizing the wide-open extracellular gate of the transporter in the OF conformation, as seen in the MD simulations. These results, which are described in Ref. [13], reveal the complete dynamic pathways of the large-scale conformational IF-to-OF transition that is underlying the working cycle of the ABC exporter. After this work was published, the group headed by Markus Seeger (Zurich University) obtained an X-ray crystal structure of the OF conformation of TM287/288 at 3.2 Å resolution (PDB 6QUZ), which very closely resembles the OF structure predicted by MD simulation [14].

2 Computational Details

2.1 Molecular Dynamics Simulations

The MD simulations were carried out with GROMACS version 5.1 [15]. The Amber99SB-ILDN protein force field [16, 17] was used together with the Berger parameters [18] for the lipids and the parameters of Meagher and coworkers [19] for ATP-Mg. The 2.6 Å resolution X-ray structure of TM287/288 in an inward-facing conformation [4, 5] with AMP-PNP bound to the degenerate site (PDB 4Q4A) was used as starting structure for the simulations, after converting AMP-PNP into ATP. A second ATP was docked into the consensus site, which was empty in the X-ray crystal structure. The resulting structure, loaded with 2 ATP-Mg molecules, was inserted into a POPC lipid bilayer [20] and solvated with water. The final simulation box contained 255 lipids, 33,370 TIP4P-2005 waters [21], and chloride ions to neutralize the periodic simulation box. The total system size was ca. 132,000 atoms. Before the MD, the system was energy-minimized with steepest descent and equilibrated for 10 ns in the NpT ensemble with harmonic position restraining potentials on all protein heavy atoms.

Short-range nonbonded interactions were treated with a buffered Verlet pair list [22] with potentials smoothly shifted to zero at a 1.0 nm cut-off. Long-range electrostatics were treated with the smooth particle mesh Ewald (PME) method [23] with a grid spacing of 0.12 nm. SETTLE [24] was used to keep the water molecules rigid, and LINCS [25] was used to constrain all other bond lengths. Virtual interaction sites were used for the hydrogen atoms [26], allowing 4 fs time steps for the integration of the equations of motion. The simulations were carried out in the NpT ensemble. Temperature was kept constant by coupling to the velocity rescaling thermostat of Bussi and coworkers [27]. For constant pressure, semi-isotropic p-coupling was applied. One hundred MD simulations, each of length 500 ns, were initiated with different random seeds for the initial velocity distribution.

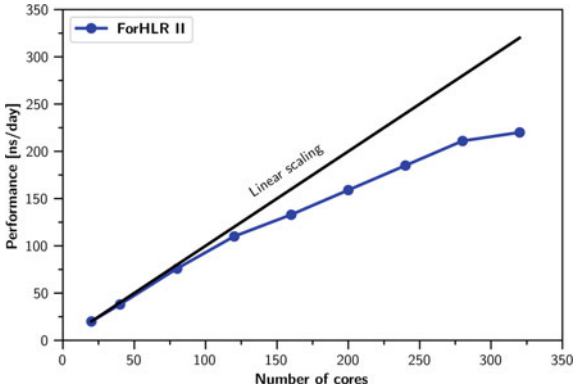

Fig. 2 Scaling of the MD simulations of the ABC transporter system shown in Fig. 1 on ForHLR II. Simulations were carried out with GROMACS version 5.1 and typically run on 200 or 280 CPU-cores (i.e., on 10 or 14 nodes) at a parallelization efficiency of 80% or 75%, respectively. The black diagonal line indicates ideal scaling

2.2 Computational Performance

The MD simulations were carried out at the Steinbuch Centre for Computing (SCC) in Karlsruhe on ForHLR II. For our simulation system comprising of ca. 132,000 atoms, a good performance was achieved on 10 nodes (200 cores) with a parallelization efficiency of 80% and an absolute performance of 159 ns/day (Fig. 2). The runtime of a typical single simulation of 500 ns was thus ca. 75 h. On 14 nodes (280 CPU-cores) the parallelization efficiency was still good (75%), and the absolute performance was 211 ns/day.

3 Results and Discussion

One hundred MD simulations, each of length 500 ns, were initiated from the inward-facing (IF) crystal structure of TM287/288 with ATP-Mg bound to the degenerate NBD [4, 5]. In all simulations, the transporter was inserted into an explicit POPC lipid bilayer and a second ATP-Mg was docked into the consensus NBD that was empty in the IF X-ray crystal structure, see Sect. 2.

In 6 MD simulations, a spontaneous large-scale conformational transition from the initial IF structure to an OF conformation was observed (Fig. 3). This complex conformational transition involves formation of a tight NBD dimer, TMD closure at the cytoplasmic gate, and finally TMD opening at the periplasmic gate. The transition to OF proceeds via an occluded (Occ) intermediate, in which the NBD dimer is tightened and the cytoplasmic gate is closed, but the TMDs did not yet open at the periplasmic gate. As both TMD gates (periplasmic and cytoplasmic gate) are closed, the Occ intermediate is sealed to both sides of the membrane, ensuring that the transporter is never a fully open channel in the membrane.

In the 6 simulations in which TM287/288 completely transitioned from IF to OF, the occluded state was visited only transiently. However, in 11 other simulations,

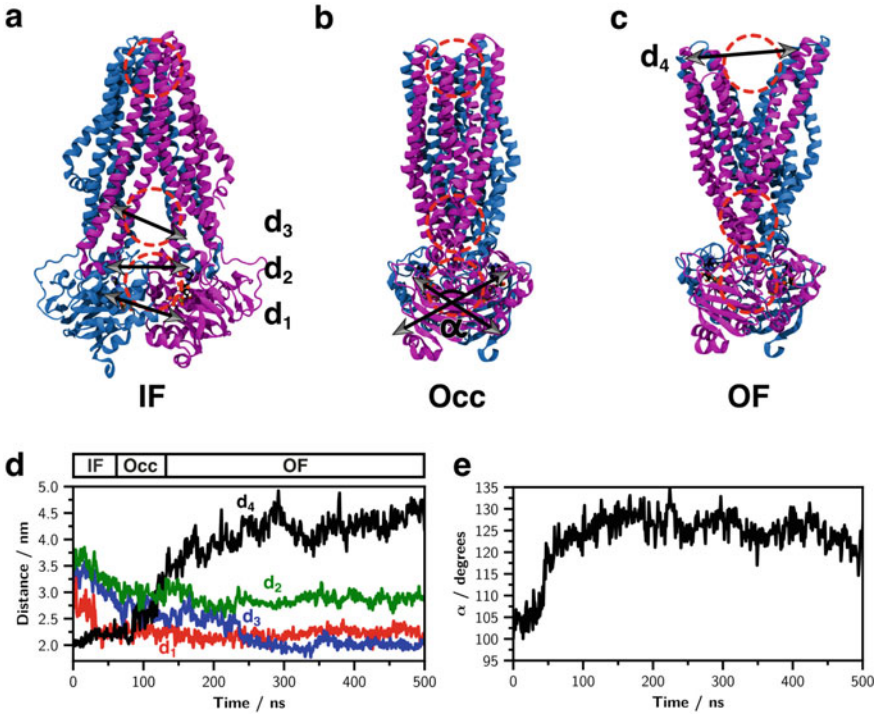

Fig. 3 MD simulation of large-scale alternating access conformational transition. (a–c) Initial IF, intermediate Occ, and final OF conformations observed during MD. The Cα–Cα distances d_1, d_2, d_3 and d_4 indicate NBD dimerization, movement of the coupling helices, closing of the cytoplasmic TMD gate, and opening of the periplasmic TMD gate, respectively. The reorientation of the two NBDs with respect to each other is described by the NBD twisting angle α. (d, e) Time traces of the four indicated distances and of the NBD twisting angle α during representative IF-to-OF transition

the transporter transitioned to Occ and got trapped in that state for the remaining simulation time, i.e., up to 500 ns. In the remaining 83 simulations, the energy barrier towards Occ was not overcome during the 500 ns simulation time, and TM287/288 remained in the initial IF conformation.

Although the MD simulations did not draw on any structural information about a desired target structure, the final OF conformation obtained after IF-to-OF transition closely resembles the OF X-ray structure published later [14]. The low Cα-RMSD of ca. 0.3 nm for the entire transporter reflects thermal fluctuations around an average structure that is close to the X-ray crystal structure (Fig. 4).

The above mechanism is in line with the ATP switch model [28] in that ATP binding, and not hydrolysis, suffices to trigger the IF-to-OF conformational transition. After release of the transport substrate, ATP hydrolysis and unbinding of the reaction products ADP + P_i would thus merely be required to reset the conformational cycle back to the IF state that is again receptive for binding of transport substrate in the

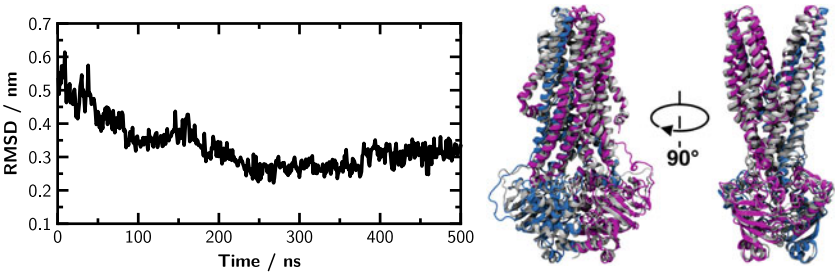

Fig. 4 The OF conformer predicted from MD simulations after IF-to-OF transition is compared to the X-ray structure of the OF conformer [14]. (Left panel) Time trace of Cα-RMSD of entire TM287/288 transporter with respect to the OF X-ray structure during representative IF-to-OF transition. The similarity of the structures is visualized by a superposition of the final snapshot after 500 ns of MD simulation (shown in color) with the X-ray structure (shown in grey)

TMDs and of ATP-Mg in the consensus NBD [6]. In a ABC heterodimer such as TM287/288, catalytic activity occurs predominantly in the consensus site. Thus, one ATP molecule would always remain bound to the degenerate site and mediate interactions between the two NBD monomers, precluding them from fully dissociating during the cycle.

4 Conclusions and Outlook

The conformational transitions between IF and OF states of the heterodimeric ABC exporter TM287/288 were studied by all-atom MD simulations in explicit membrane and water environment. The MD simulations reveal the conformational changes underlying the working cycle of the ABC transporter and provide an atomistic picture of the alternating access mechanism. First, ATP binding to both nucleotide binding domains triggers tightening of the NBD dimer by a concerted closing and twisting motion, that is, upon dimerization the two NBD monomers reorient to form a fully closed dimer. At the same time, the cytoplasmic TMD gate closes, which leads to an occluded intermediate. Finally, the periplasmic TMD gate opens to yield the OF conformation. The MD simulation results are supported by X-ray crystallography and EPR spectroscopy [13, 14].

To fully characterize the functional working cycle, substrate molecules should be explicitly considered. Due to the inherently dynamic nature of the process, mapping out the pathways along which substrates are shuttled through the ABC exporter is a formidable challenge that has not been achieved at atomic resolution up to now. The present results are a key step in this direction. As the conformational transitions occur spontaneously in our simulations, it might be possible to extract a small set of suitable collective variables for free energy simulations of the IF/OF transition in the presence of transport substrate molecules.

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