Structure, Dynamics, and Energetics of ATP Hydrolysis by ABC Transporters

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Molecular dynamics simulations provide atomic-level details of the structure, dynamics, and energetics underlying the ATP hydrolysis reaction by ABC transporters.

Adenosine triphosphate (ATP)-binding cassette transporters (ABC transporters) are transmembrane proteins present in cells of every living organism. These proteins pump specific substrate molecules across cell membranes, transferring the substrates against the concentration gradient. The necessary free energy to drive this thermodynamically unfavorable process comes from the ATP hydrolysis that takes place at the ABC transporters active sites.

Both ABC importers (which pump molecules into the cells) and ABC exporters (which pump molecules out of the cells) can be found in nature. Prokaryotes generally have both, and eukaryotes have almost exclusively exporters. ABC transporters are the largest family of proteins in certain organisms, such as Escherichia coli, representing up to 5% of their whole genome.

ABC transporters contain at least two nucleotide binding domains, where ATP is hydrolyzed, and two very variable transmembrane domains (TMDs) that form the dynamic channel through which the substrates enter or leave the cell (Figure 1).

In an oversimplified description of this process, the TMDs open one channel end for a substrate loading, change their conformation to move the substrate through the channel, and change their conformation again to open the opposite end and allow the substrate to exit.

Despite the progress in this field, several stages of the transmembrane transport still need a full and clear atomic-level description: (i) specific conformational changes in TMD that induce the unidirectional substrate transport against the concentration gradient; (ii) molecular events (substrate and ATP binding, ATP hydrolysis, product release) that provide the “power stroke” and trigger each of these conformational changes in TMD; (iii) the way these conformational changes couple with ATP binding and hydrolysis.

In addition to the fundamental biochemical interest in understanding such extraordinary molecular-level mechanical pumps, their role in the emerging multidrug resistance to chemotherapy (by pumping drugs out of cancer cells) grants them a prominent place in biochemical research.

In this issue of ACS Central Science, Schäfer and co-workers present an elegant work devoted to one of the most studied...
bacterial ABC importers, BtuCD, whose role is to pump vitamin B$_{12}$ across the inner cell membrane of bacteria.$^3$ The studied system contained a BtuCD−“binding protein” complex (Btu-F), where the protein specifically binds vitamin B$_{12}$ and BtuCD, delivering vitamin B$_{12}$ to the ATP importer. The work focuses on the mechanism of ATP hydrolysis in BtuCD and how this mechanism couples with the conformational changes of its transmembrane domains. The authors have computationally modeled a system consisting of the complete BtuCD−BtuF complex (using known X-ray structure of the complex$^4$) inserted into a solvated lipid bilayer (Figure 1).

In general, the mechanism of ATP hydrolysis should involve the following steps: the deprotonation of a lytic water molecule by a base; the attack of the lytic water molecule on the ATP γ-phosphate; and the elimination of the phosphate. However, at the atomic-level description, many questions remain open: (i) does the reaction follow an associative, dissociative, or concerted mechanism? (ii) is there a specific basic group (among several basic groups in the protein and ATP phosphate) that deprotonates the lytic water molecule? (iii) at which stage of the reaction cycle does the water deprotonation take place? (iv) is the hydrolysis reaction inside the protein exergonic? The reaction is exergonic in aqueous solution under physiological conditions, but inside the protein, free energy of the reaction might be quite different. This last question is very important to understand the contribution of the ATP hydrolysis reaction to the overall thermodynamically unfavorable process of vitamin B$_{12}$ uptake.

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Schäfer and co-workers offer possible answers to these questions. Given the strict conservation of the nucleotide binding domains of the ABC transporter proteins, it is expected that most of the answers can be extrapolated to other members of the ABC family of proteins.

In a classical molecular dynamics simulation used in this work, a water molecule penetrated into the active site of the protein and became ideally placed to carry out the hydrolysis. The probability of water molecules adopting such hydrolysis-competent configuration was ~15%. Resorting to computational molecular dynamics at the quantum mechanical/classical mechanics level, a set of possible reaction coordinates were explored using the umbrella sampling method. The quantum mechanical part consisted of 49 active site atoms described using the “MPW1B95-D3/6-31+G(d,p)” density functional/basis set, which was shown in the past to be very precise for phosphate hydrolysis.$^6$ The coupling of a high-level hybrid-meta density functional with the sampling of the conformational space provides robustness to these simulations that require enormous computing efforts. Free energy profiles for several possible reaction steps were obtained through the above-mentioned simulations, and the dominant profiles were chosen based on their more favorable kinetics (lower free energy barriers).

The results have shown that the attack of the water molecule on the AT γ-phosphate is concerted, but asynchronous, with the water deprotonation. The base that deprotonates the lytic water has been identified as the highly conserved E159 residue that, despite being Mg$^{2+}$-coordinated, is very well positioned to facilitate such process. In a second step, E159 restores its initial state by transferring the proton to HPO$_4^{2−}$, yielding H$_2$PO$_4^{−}$. Finally, H$_2$PO$_4^{−}$ rearranges its coordination to the active site Mg$^{2+}$, replacing a coordinated bond of Mg$^{2+}$ with a protonated oxygen atom by a coordinated bond of Mg$^{2+}$ with a negative oxygen atom. Figure 2 summarizes this reaction mechanism.

![Figure 2. Proposed mechanism of ATP hydrolysis by ABC transporters. Reprinted with permission from ref 3. Copyright 2018 American Chemical Society.](https://doi.org/10.1021/acscentsci.8b00631)

The overall rate constant calculated from the free energy profile matches the experimental value very well. More importantly, the free energy of the reaction inside the protein is close to zero (± 1.8 kcal/mol), which indicates that the ATP hydrolysis itself does not confer the “power stroke” to induce conformational changes in the TMD. In this sense, it is proposed that ATP binding induces dimerization of the nucleotide binding domains and provokes the transition of the TMD from the inward conformation (open to the bacterium cytoplasm) to the outward conformation (open to the periplasm and ready to be loaded with vitamin B$_{12}$ by Btu-F). ATP hydrolysis is proposed to induce the return of the TMD conformation to the inward conformation, allowing for the uptake of vitamin B$_{12}$ and resetting the conformational cycle.
This working mechanism contrasts with some aspects of earlier proposals, which state that BtuCD−Btu-F is already open outward before ATP binding and hydrolysis, and changes to the inward opening after ATP binding and hydrolysis. Given the enormous molecular complexity of the phenomenon, it is natural that several interpretations still coexist, even though our understanding of the phenomenon is progressing.

The work of Schäfer et al. opens many research avenues. From a computational point of view, one of the most evident future studies would be the direct simulation of the coupling between ATP binding and the transition from the inward to the outward conformation, and the coupling between ATP hydrolysis/unbinding and the reverse TMD conformational transition. Despite the large time scales in which these phenomena take place, such simulations would represent the ultimate goal of “seeing” this amazing molecular transporter at work.

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Notes
The authors declare no competing financial interest.

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