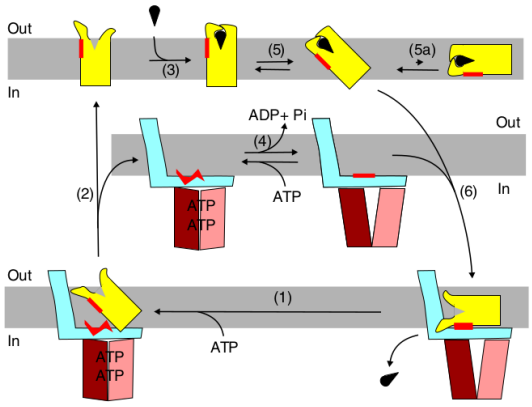
Toppling Mechanism of the S-component in Group II ECF Transporters: How Bacteria Get Their Vitamins

Benjamin R. Gilbert Literature Seminar November 24, 2020

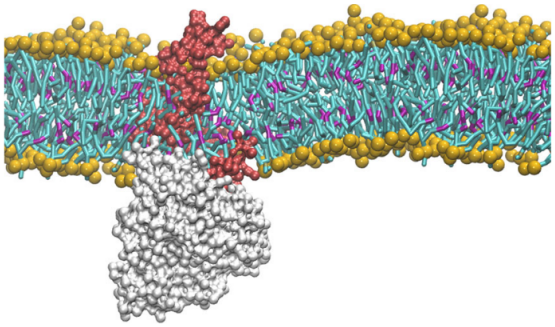
A new class of modular transporters in prokaryotes were identified in 2009 by Rodionov et al.[1] Now known as energy-coupling factor (ECF) transporters, they are of particular interest because a number of bacteria, many of them pathogenic, use ECF transporters to import essential cofactors that they are unable to directly synthesize. This paradigm is most observed in the Firmicutes phylum and may serve as a target for novel antibiotics.[1,2] ECF transporters are a type of ATP-binding cassette (ABC) transporter, but unlike the majority of ABC transporters, they use a substrate-specific small integral protein to selectively capture substrates.[1] This component of the ECF transporter is known as the S-component (EcfS). The other components are a pair of cytosolic ATPases (EcfA and EcfA') and a transmembrane component (EcfT) coupling the S-component to the EcfA-EcfA' subcomplex. The EcfA-EcfA'-EcfT complex is known as the energy-coupling module or ECF module. Computational comparative genomics were used to create functional assignments for the S-components used to transport different substrates.[1] The genomic analysis also revealed two types of ECF transporters: Group I ECF transporters with a dedicated ECF module for each substrate-specific S-component and Group II ECF transporters with a shared ECF module that can form complete ECF transporters by complexing with different substrate-specific S-components.

Some of the earliest structures for Group II ECF transporters were calculated in 2010 by Zhang et al. for the S-component for riboflavin transport, RibU, in the substrate-bound state.[3] The S-component was found to be cylindrical with the riboflavin bound within a pocket on the periplasmic face. Based on these findings, Zhang et al. hypothesized conformational changes moved the substrate down the length of the cylinder. This view was challenged when the crystal structure of a complete Group II ECF transporter from *L. brevis*, with the S-component complexed with the ECF module was calculated by Wang et al. using X-ray diffraction.[4] They found the remarkable result that unlike most other membrane proteins, the transmembrane segments of S-component lay parallel to the lipid membrane. Based on this, the ECF transporter was proposed to exist in two major conformational states, a resting state and substrate-binding state. In the resting state, the S-component is parallel to the membrane, the substrate-binding site of the S-component is directly next to the cytoplasmic side of the lipid membrane, and the ECF module is in an open conformation. ATP binding to the ECF module was proposed to close the conformation and cause a rigid-body rotation of the S-component to expose the substrate-binding site to the extracellular space. This is accomplished through the transmembrane EcfT coupling the EcfA-EcfA' subcomplex to the S-component. The S-component is then primed to receive a substrate. ATP hydrolysis in the EcfA-EcfA' subcomplex will return the ECF module to the open conformation and cause the S-component component to topple back into its resting state, before releasing the substrate into the cytoplasm.[4] In parallel to this work, Majsnerowska et al. studied the structural changes of the S-component for thiamin transport, ThiT, upon thiamin binding, using electron paramagnetic spectroscopy and molecular dynamics (MD) simulations.[5] They found two conformations, the *apo* conformation without a bound substrate, where loop L1 is extended, and the *holo* conformation, where the substrate is bound and loop L1 is folded

over and occludes the substrate-binding pocket. The most comprehensive structural study of Group II ECF transporters was performed by Swier et al. in 2016 for the folate-specific ECF transporter from *L. delbruecki*.[6] Based on their findings, they developed a transport model in which the S-component could associate and dissociate from the energy-coupling module during the transport cycle (Figure 1a). As recently as 2017, there was still uncertainty about the role ATP hydrolysis plays in the toppling[7] and whether solitary S-components can topple without an associated ECF module.[8]



(a) Proposed transport model.[6]



(b) Snapshot of CG MD simulation of ECF module in the lipid membrane.[9]

Figure 1: Proposed transport model of Swier et al. and snapshot of a CG MD simulation of the ECF module embedded in the membrane by Faustino et al.

Seeking to answer some of the questions about the unique mechanism of Group II ECF transporters, Faustino et al. used MD to investigate the mechanism.[9] Specifically, they analyzed the toppling mechanism of the S-component and the association and dissocation of the S-component with ECF module for the folate-specific ECF transporter.[6] MD simulations were performed for a variety of membrane compositions using a coarse-grained representation (CG) based on the Martini force field.[10] Simulations of solitary S-components in the membrane showed that the toppled orientation was more stable in the substrate-bound *holo* conformation versus the *apo* conformation. Additional CG MD simulations were performed on S-components for other substrates and the same orientational stability for toppled states in different membrane compositions was observed. Next, CG MD simulations of the ECF module in a membrane were performed and it was found that the ECF module imparted a local negative curvature on the side of the membrane where the S-component can associate (Figure 1b). This was further confirmed by backmapping the CG representation to an all-atom representation and performing an atomistic simulation of the system. To investigate the influence of the induced curvature on transport, CG MD simulations of the *apo* S-component complexed with the ECF module were performed. The complex remained stable for 32 μs and because unbinding of the S-component is likely an ATP-activated process, umbrella sampling was used to trigger the dissociation and estimate the free energy profile of the process (Figure 2).

Their most interesting finding was that an additional binding free energy of 55 kJ mol^{-1} can be attributed to the membrane curvature induced by the ECF module and they concluded that the toppling mechanism is membrane-assisted.[9] They then proposed two pathways for transport: 1) spontaneous self-toppling of the S-component and subsequent association of the toppled S-component and ECF module

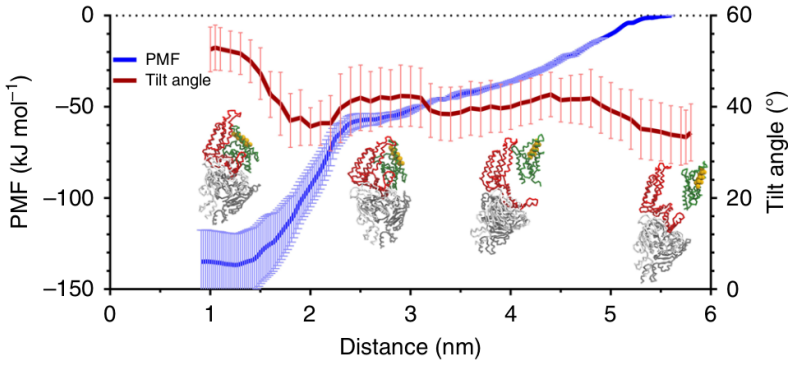


Figure 2: Results from Faustino et al. showing dissociation of the S-component from the ECF module[9] - Potential of mean force of ECF module and S-component (blue). Average tilt angle between helix 5 and positive z-direction along the dissociation pathway (red). Toppled state is on the left and canonical state is on the right.

or 2) membrane-assisted toppling of the S-component during the association process. Their results suggest that the function of Group II ECF transporters are strongly dependent on the membrane composition, which has implications for the kinetic modeling of cells in systems biology. Additionally, the functional dependence on a shared ECF module in Group II ECF transporters remains a possible target for the development of novel antibiotics, and further mechanistic insight aids this endeavor.

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