FUNCTIONAL MECHANISM OF THE BACTERIAL EFFLUX PUMP

Allocation: GLCPC/0.43 Mnh
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EXECUTIVE SUMMARY:

Gram-negative bacteria such as *E. coli* utilize a tripartite complex system to expel toxic chemicals and antibiotics directly out of the cell. The efflux system, consisting of an inner membrane transporter, an outer membrane channel, and a fusion protein connecting the two domains, uses the proton motive force to extrude a wide variety of chemical compounds out of the cell, thus conferring resistance to a broad spectrum of antibiotics. We have built an atomic model of the tripartite complex based on the electron microscopy images of the pump and the crystal structures of its individual components, which provide a complete structure of the transport complex. Using molecular dynamics simulations, we have identified residues that may play a role in proton transport in two different pumps and determined their possible impact on the drug binding sites in the complex.

INTRODUCTION

In Gram-negative bacteria, such as *E. coli*, toxic chemicals are expelled through tripartite efflux pumps that span the inner and outer membranes [1]. The efflux system of the resistance-nodulation-division family plays a major role in the intrinsic and acquired resistance of bacteria to antibiotics. These systems provide a pathway for bacteria to export lethal concentrations of drugs and metal ions out of the cell membrane.

The complex system shown in fig. 1 is made of an energy-utilizing inner membrane transporter and an outer membrane channel that are connected by membrane fusion proteins. The inner membrane protein uses the proton motive force across the membrane to undergo conformational transitions that capture the drug from inside the cell. The substrate is then released into a tunnel that is formed by the fusion proteins and is transported all the way to the extracellular side.

The structures of individual components of the complex system have been determined by X-ray crystallography. However, it is not yet clear how the three components assemble into a tripartite system that can transport antibiotics or metal ions out of the cell. More importantly, the transport pathway of the drugs or protons through the complex and their coupling mechanism is not yet known.

METHODS & RESULTS

We used molecular dynamics simulations to construct an all-atom model of the complex system based on the crystal structures of individual protein components and a cryo-electron microscopy density map of the efflux pump from *E. Coli* [2]. The complex system involving more than a million atoms was equilibrated in explicit solvent and membrane using the program NAMD [3]. The resulting stable model provided the first atomic description of the tripartite efflux pump.

To further characterize the proton transport mechanism of the pump, we carried out extensive molecular dynamics simulations of the system for a total of several microseconds. The simulations identified possible proton transport pathways and key residues in the inner membrane protein that are involved in the process. The simulation of the complex system showed how transfer of protons through inner membrane pathways could be coupled to conformational changes of known binding sites near the fusion tunnel.

We are extending the simulations to study the transport of an antibiotic and a cancer treatment drug through the entire channel and to identify their transport pathway from the proximal binding site to the distal binding site [4] in the inner membrane protein and finally through the fusion tunnel leading to the extracellular channel. These simulations will provide insight into the underlying transport process and will determine potential barriers or binding sites along the pathway. It is imperative to include the three protein components in studying the transport process, as the presence of the fusion proteins is known to stabilize the initial drug binding sites, and in turn binding of the drug is expected to affect the opening of the extracellular channel through allosteric interactions between the three components.

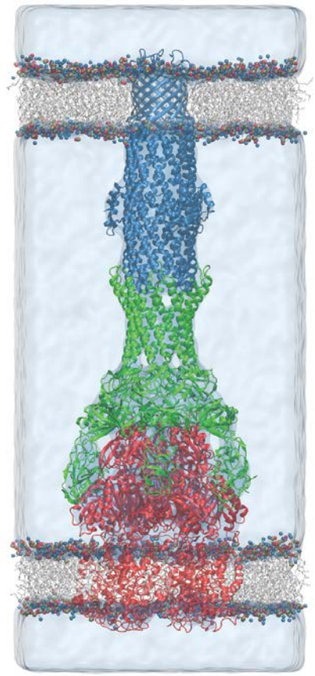

FIGURE 1: The bacterial efflux pump consisting of two lipid membranes and the tripartite assembly of AcrB/AcrA/TolC shown in red, green, and blue, respectively.

WHY BLUE WATERS?

The relatively large size of the assembled structure will make it one of the largest transport systems being studied. Obtaining meaningful statistics on the transport process are only feasible by employing the high number of cores available on Blue Waters.