

Modeling the Multidrug Efflux Transporter AcrB of *Escherichia coli* Using Rapid Prototyping

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Abstract

The focus of this research was to model the drug efflux protein AcrB using rapid prototyping (RP). Bacterial resistance to antibiotics can result from efflux transporters located within the cell membrane that allow bacteria to remove a variety of dissimilar antibiotics from the cell. One such transporter is the AcrA-AcrB-TolC tripartite complex found in *Escherichia coli*. The complex consists of the multidrug exporter, AcrB, outer membrane channel, TolC, and membrane fusion protein, AcrA. AcrB is the most important protein of the complex as it provides the rotating mechanism that exports the wide variety of toxic compounds. Since AcrB does not extend beyond the outer membrane, TolC serves as a channel to remove compounds from the cell. Recent experimental data suggests that AcrA plays a role in the exportation process, but its function is not completely understood. This research addressed the interactions between the three proteins while specifically focusing on the rotating mechanism of AcrB and the proton motive force that powers it. Two models of AcrB were created; the first illustrated the general structure of the protein while highlighting the interaction between three protomers, and the second focused on one protomer to further illustrate the export mechanism and proton motive force. These models can be used as teaching tools in an effort to explain the complex nature of multidrug exporters.

Keywords: drug efflux transporter, multidrug exporter, *E. coli*, AcrB, ABC complex, proton motive force, rapid prototyping

1. Introduction

Bacterial resistance to antibiotics is a growing problem in today's medical field. Antibiotic resistance is a process that has developed through the natural selection of random mutations as well as programmed evolution. Programmed evolution is similar to natural selection with the main difference being that an environmental cue has increased the number of mutations. The overuse of some drugs has increased the development of resistance. Drug efflux transporters such as the AcrA-AcrB-TolC (ABC) complex of *E. coli* are one of the few mechanisms of drug resistance. The ABC complex can export a wide variety of drugs due to its versatile binding cavity, but one of the most common is ciprofloxacin, which is a broad spectrum antibiotic marketed under the trade name Cipro® by Bayer Pharmaceuticals¹. Understanding how

drug efflux pumps operate is critical in developing more effective antibiotics. This research focused on applying rapid prototyping technology to increase this understanding.

1.1. protein structure

The structures of the proteins studied in this research were determined by various scientists and researchers using x-ray crystallography and submitted to the Protein Data Bank.

1.1.1. x-ray crystallography

X-ray crystallography is a method for determining the arrangement of atoms in crystal. The process starts with the purification of proteins. Once contaminants have been removed, the proteins can be crystallized properly, which is the most important step of the entire process. A crystal is a unique repeating structure of protein molecules that can be formed by allowing molecules to precipitate from a supersaturated solution. A crystal is necessary because one molecule alone would be too small to measure scattered x-rays. The resulting crystal is then placed under an intense beam of x-rays. The x-rays all have the same wavelength, which is about the distance between bonded atoms within the molecule. When the x-rays are passed through the crystal, they are scattered by electrons creating a distinct diffraction pattern as seen in Figure 1. The pattern is unique to the arrangement of atoms within the protein structure and is used to create an electron density map. This does not actually resolve individual atoms, so computer graphics programs are used to translate the density map into three-dimensional molecular structures. The initial models are usually less than ideal, so additional diffraction data is used to refine the structures.²

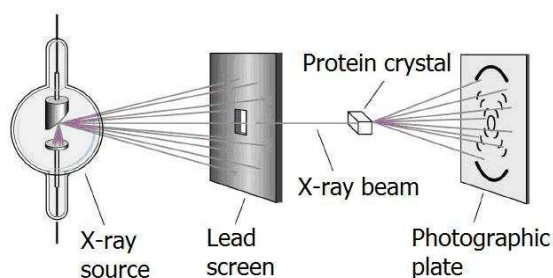


Figure 1. X-ray diffraction³

1.1.2. Protein Data Bank

The Protein Data Bank (PDB) is an online collection of three-dimensional biomolecular structures. The repository was established in 1971 by the Brookhaven National Library, and in 1998, management was transferred to Research Collaboratory for Structural Bioinformatics (RCSB). Structural data, usually obtained by x-ray crystallography, is submitted by scientists and researchers from around the world. Today, the data bank consists of over 40,000 proteins. Proteins within the PDB include information such as sequence details, atomic coordinates, crystallization conditions, and 3-D images. The PDB files can be downloaded and manipulated in a variety of computer graphics programs.⁴

1.2. rapid prototyping

Rapid prototyping (RP) is a method of creating a solid prototype from a three-dimensional computer image. The process, which is also known as solid freeform fabrication, involves using thin cross sections of a digital image to create a solid layer by layer. The thickness of the layers depends upon the detail needed for the prototype. The specific technology used for this research was Selective Laser Sintering (SLS).

1.2.1. Selective Laser Sintering

SLS uses a CO₂ laser to fuse small particles of material into a thin layer of the prototype. Once a layer is fused, the part is lowered, and a new layer of powdered material is rolled across the part and fused. The process, which can be seen in Figure 2, is repeated until the prototype is completed. A variety of powders can be used including plastics, metals, and ceramics. Specifically for this research, duraform polyamide, or nylon, was used.⁵

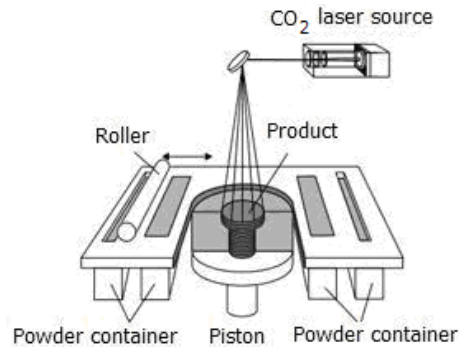


Figure 2. Diagram of SLS machine⁶

1.3. objective

The objective of this project was to research how RP could be used to promote the understanding of the mechanism of the drug efflux protein AcrB. The project aimed to create models of AcrB illustrating both the general structure of the protein and its export mechanism. A model of the protein as a whole illustrates the general structure of AcrB while showing the interaction between three individual protomers. A second model focuses on a single protomer to illustrate the mechanism of conformational changes as well as illustrate the proton motive force that provides power.

2. Mechanism

The predominant drug exporter found in *Escherichia coli* is the AcrA-AcrB-TolC tripartite complex. The complex consists of the multidrug exporter, AcrB, outer membrane channel, TolC, and membrane fusion protein, AcrA. AcrB is the most important protein of the complex as it provides the rotating mechanism that exports a wide variety of toxic compounds. Since AcrB does not extend beyond the outer membrane, TolC serves as a channel to remove compounds from the cell. Recent experimental data suggests that AcrA plays a role in the export process, but its function is not completely understood. Since the complex exports drugs against the concentration gradient, an external energy source is required. This energy is provided by a concentration gradient of protons, known as the proton motive force. An overview of the entire exporter complex can be seen relative to the inner and outer membranes of *E. coli* in Figure 3.

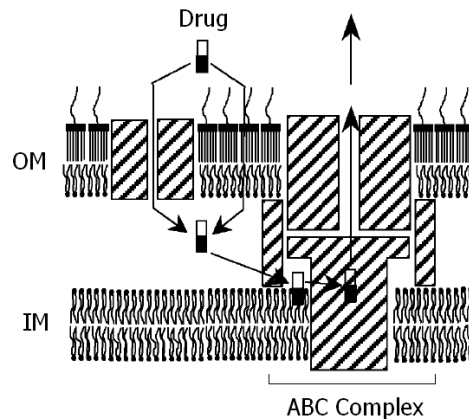


Figure 3. Overview of ABC complex⁷

The most important protein of the complex, AcrB, consists of three individual protomers, two of which are shown in Figure 4. The protomers have the same primary protein structure, but they rotate through different conformations depending on their state of the exportation process. The AcrB protein as a whole is divided into three regions or domains: the transmembrane domain, the porter domain, and TolC docking domain as seen in Figure 5⁸. The transmembrane domain spans the inner membrane of the cell and is where the proton translocation pathway of the proton motive force is located. The porter region lies between the two membranes and is where the conformational changes occur. Lastly, the TolC docking domain also lies between the two membranes and is where AcrB binds to the outer membrane channel, TolC.

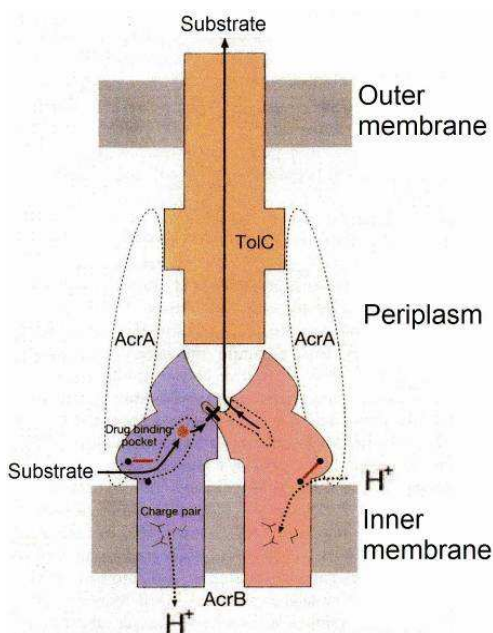


Figure 4. AcrA-AcrB-TolC complex⁹

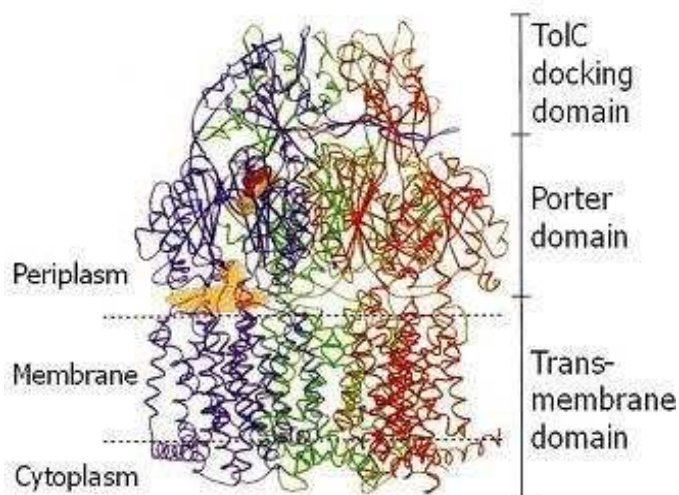


Figure 5. AcrB domains¹⁰

2.1. conformational changes

The basis for the drug exportation mechanism within AcrB is a series of conformational changes within the porter domain. Each protomer is in one of three different conformations: access, binding, or extrusion¹¹. A substrate binding pocket exists within each conformation, but its binding affinity varies among the three. In the access conformation, there is a large laterally accessible pathway to the binding pocket. The conformation itself seems to be an intermediate between the neighboring binding and extrusion states but does not interact with the two. In the binding conformation, the laterally accessible pathway remains, and the binding pocket is optimally configured for hydrophobic interaction with a substrate. There is also an interaction between the binding and extrusion states as an inclined alpha helix of the extrusion conformation blocks the exit of the binding conformation. In the extrusion conformation, the entrance to the binding pocket is blocked, but the inclined alpha helix opens an exit toward TolC, the outer membrane channel. The binding pocket is compressed, thus forcing the substrate into the channel.

The conformational changes within AcrB are similar to the mechanism of peristaltic pump, as the protein moves a drug forcibly through a channel in a manner similar to how a peristaltic pump moves a fluid through a tube. Specifically, AcrB compares to a rotary peristaltic pump in which a rotor spins to compress a flexible tube and thus move a fluid. The protein AcrB works in a similar manner in that the conformational changes within each protomer compress a binding pocket to force a drug through; however, the individual protomers do not revolve. The three different conformations revolve between the three protomers, but the actual protomers do not rotate. They simply bend to change conformations. A schematic of the rotating conformations can be seen in Figure 6.

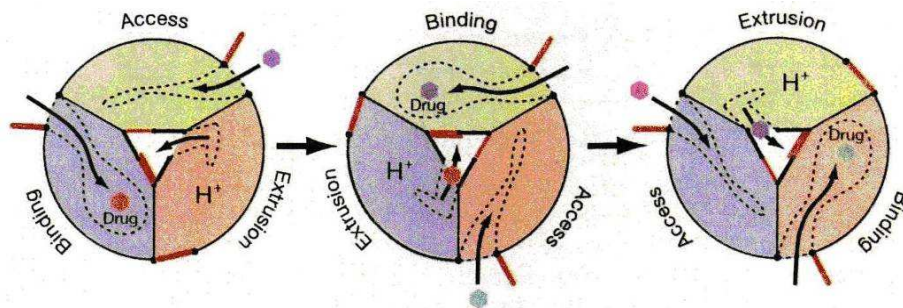


Figure 6. Rotating conformations¹²

The conformational changes within AcrB result from the movement of subdomains with the porter region. Each protomer contains four subdomains: PN1, PN2, PC1, and PC2, each structurally consisting of two β - α - β sandwiches. In the binding conformation, PN2 and PC1 move away from PN1 and PC2 as seen in Figure 7. This expands the binding pocket and arranges the aromatic rings of phenylalanine residues in a configuration optimal for hydrophobic interaction with a drug substrate. The movement of PN2 and PC1 away from PN1 and PC2 also opens an exit toward TolC, but it is blocked by the inclined alpha helix of the neighboring extrusion conformation. In the extrusion conformation, PN2 and PC1 move toward PN1 and PC2 as seen in Figure 7 as well. This compresses the binding pocket and moves phenylalanine residues within range to interact with each other rather than the substrate. As a result, the substrate is forced out through an exit opened by the conformation's inclined alpha helix.¹³

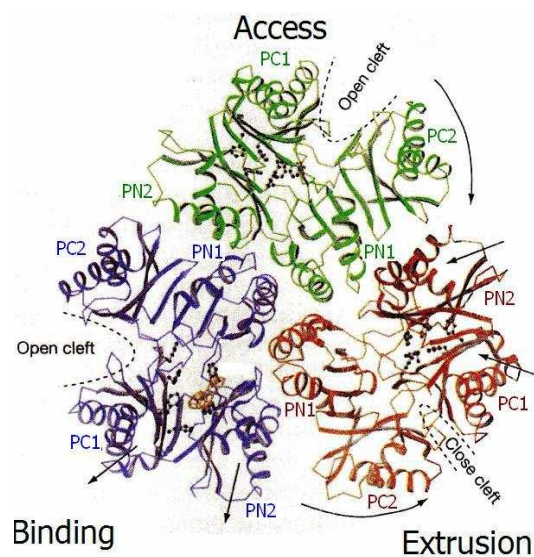


Figure 7. Movement of subdomains with porter domain of AcrB¹⁴

2.2. proton motive force

Since AcrB exports drugs from a lower concentration to a higher concentration, an external energy source is needed. This energy is provided by the proton motive force, which is energy released by allowing charged hydrogen atoms, or protons, to pass through the inner membrane from high concentration within the periplasm to low concentration within the cytoplasm.¹⁵ This source of energy is similar to hydroelectric power, which results from the potential energy of dammed water. The cell membrane is similar to the dam itself as it serves as a barrier between two different concentrations of protons. The release of protons then releases energy similarly to how released water turns a turbine to create electricity. The nature of this

mechanism would destroy the concentration gradient of protons if left unchecked, so adenosine triphosphate (ATP) is consumed to move excess protons from the cytoplasm back into the periplasm.

Because the proton motive force requires a membrane, the process occurs in the transmembrane domain of AcrB. The diffusing protons follow a translocation pathway of three negatively charged amino acids: aspartic acid 407, aspartic acid 408, and lysine 940.¹⁶ The negatively charged amino acid side chains attract the positively charged hydrogen ions as they pass through the membrane. How the energy released within the transmembrane domain is translated to conformational changes within the porter domain is not entirely understood, but the proposed mechanism involves the twisting of transmembrane alpha helices.¹⁷ This twisting within the transmembrane domain results in the movement of the aforementioned subdomains within the porter region.

3. Model Construction

Two RP models of AcrB were created as part of this research: one of the three interlocking protomers and the other of a single protomer highlighting the porter domain's subunits and the proton translocation pathway. For both models, PDB files of the AcrB protein were downloaded from the Protein Data Bank. These files were opened in a molecular graphics program called RasMolTM and manipulated to illustrate various components of the protein. The manipulated files were then converted to STL file formats so that the 3D models could be produced by the rapid prototyping machines. The final models were approximately 10⁷ times larger than the actual proteins.

3.1. model 1: AcrB backbone showing all three protomers

The first model created was of the AcrB backbone using Selective Laser Sintering. The backbone refers to the central chain of carbon atoms. All of the amino acid side chains were omitted in order to clearly see the structural features of the three protomers, most notably the many alpha helices. RasMolTM was used to remove unwanted atoms and insert supports that would allow the model to maintain its shape. Each protomer was created separately, but a program called Magics was used to manipulate the individual STL files so that the three resulting models would fit together. The model, which is shown in Figure 8, can be used as an educational tool to explain the general structure of AcrB. The most noticeable difference between the different conformations is the position of the PC1 and PC2 subdomains. In the extrusion conformation, they are significantly closer to each other than in the access and binding conformations.

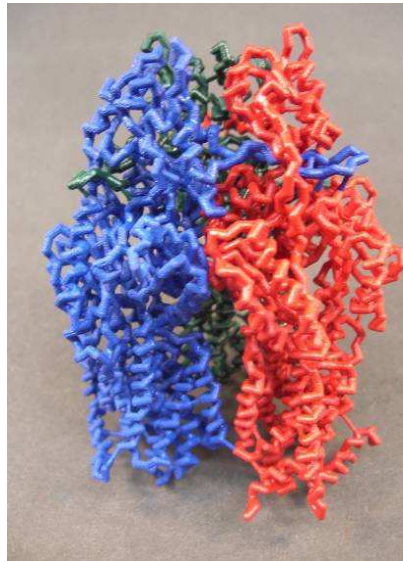


Figure 8. Model 1

3.2. model 2: single protomer of AcrB backbone

For the second model, which can be seen in Figure 9, SLS was used to create a model of a single protomer's backbone structure. Magnets were inserted so that the model could be separated into the TolC docking, porter, and transmembrane domains. These domains can be seen in Figure 10. Within the porter domain, each of the four subunits (PN1, PN2, PC1, and PC2) was colored a different color to illustrate the basis for the conformational changes. Within the transmembrane domain, wireframe structures of the amino acids aspartic acid 407, aspartic acid 408, lysine 940, and threonine 978 were included to illustrate the proton translocation pathway. The first three amino acids are proton acceptors whereas the last amino acid plays a role in the twisting of transmembrane helices. The three most important helices with regard to transferring motion to the porter domain are colored separately as well.

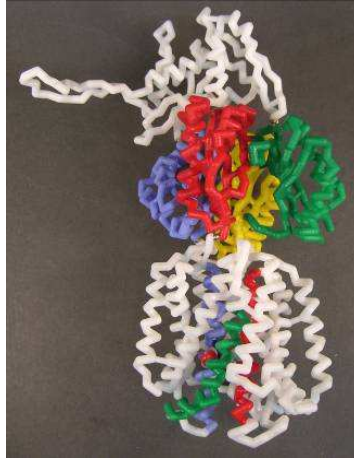
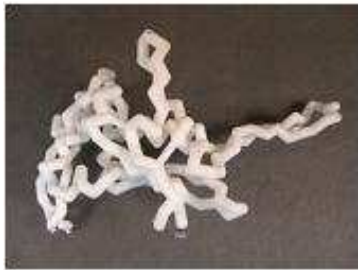


Figure 9. Model 2



TolC Docking Domain



Porter Domain



Transmembrane Domain

Figure 10. Model 2 domains

4. Conclusion

This research will contribute to understanding the mechanism of the drug efflux protein AcrB. The models created can be used to help understand not only the complex mechanism of the AcrA-AcrB-TolC complex, but also many similar drug exporters found in various bacteria. Such exporters include the MtrCDE complex of *N. gonorrhoeae*, the MexXY-OprM complex of *P. aeruginosa*, and the BpeAB-OprB complex of *B. pseudomallei*.¹⁸ All of these exporters consist of tripartite protein complexes that operate with similar mechanisms to the AcrA-AcrB-TolC complex of *E. coli*. Furthermore, this research has helped extend the

limits of rapid prototyping technology. RP is an efficient way to create detailed biomolecular models that can be very helpful in understanding complex proteins. Fully understanding proteins such as the various multidrug exporters is a step toward developing more effective antibiotics.

5. Disclaimer

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7. References

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