

Membrane Transport

Stephana J Cherak¹, Natalie Gugala¹ and Raymond J Turner¹

¹Department of Biological Sciences, University of Calgary, Canada

***Corresponding author:** RJ Turner, Department of Biological Sciences, University of Calgary, Alberta, Canada, Tel: 1-403-220-4308; Fax: 1-403-289-9311; Email: turnerr@ucalgary.ca

Published Date: February 10, 2016

ABSTRACT

The phospholipid bilayer is vital for maintaining cellular function and survival. The hydrophobic and hydrophilic nature of the lipid monomer confers selective permeability for many biological molecules. Transport proteins located within the phospholipid bilayer primarily act to achieve transport of solutes incapable of simple diffusion. Transport proteins are comprised of two general classes, carriers or channels, depending on their differential mechanism of action. Carrier proteins posit the capacity to cycle between conformational changes, by coupling movement of ions or molecules with or against respective concentration gradients, illustrating scenarios of either passive or active transport. There are hundreds of superfamilies of transport proteins belonging to the biological membrane, each giving rise to specific reaction equations and structural features. The largest is the major facilitator superfamily, distributed vastly among bacteria archaea and eukarya. The Major Facilitator Superfamily (**MFS**) demonstrates a generalized transmembrane helices conformation, with all members sharing the common MFS fold. Prominent conformational changes via movement in the transmembrane segments comprise the alternating access transport mechanism, essentially characterizing this superfamily as secondary transporters. The other large superfamily is the ATP-Binding Cassette (**ABC**) transporter superfamily, which utilizes chemical energy generated by ATP-hydrolysis, characteristic of primary active transporters. Transportation

of molecules by ABC members occurs in a thermodynamically unfavorable direction. Alike the MFS superfamily, ABC proteins also exhibit the alternating access model. A group of transporters of increasing medical interest is the multi-drug resistant transporters, which utilizes either the protein gradients or ATP as efficient sources of energy. In any case, membrane transport proteins exhibit a high degree of specificity for the substance transported, with the rate of transport differing considerably owing to difference in the mechanism of action.

Keywords: Carrier protein; Channel; Active transport; Facilitated diffusion; Porins; Proton/Ion gradient; Major facilitator superfamily (**MFS**); Uniport/symport/antiport; Alternating access model; ATP binding cassette (**ABC**) superfamily

MECHANISMS OF MEMBRANE TRANSPORT

Objectives

Describe the difference between a carrier protein and a channel in terms of conformational changes.

Identify the direction of solute transport for each type.

The fatty acid tails of lipid monomers comprising the hydrophobic interior of the biological membrane poses as a substantial barrier for simple diffusion. Charged, polar and bulky molecules are not able to diffuse directly from one side of the membrane to the next. In this case, membrane transport proteins specialized for such non-diffusible molecules permit transmembrane movement. Transport proteins are recognized by a slew of different names, depending somewhat arbitrarily on their mode of action: transporters, translocases, permeases, pores, channels, and pumps, just to list a few. Further, transport proteins may be classified by the substance that they transport, whether they are always open or gated (open only when stimulated), and most importantly, whether a source of free energy is required for operation.

Carriers vs. channels

Transport proteins are of two general classes: carriers or channels. A carrier protein is a fixed topology transmembrane protein, with the ability to cycle between conformational changes. In this way, a solute binding site may be accessible on either side of the membrane, depending on the current conformation of the membrane protein. An intermediate conformation may also exist, in which the bound solute is inaccessible to both, however there is never an open channel through the carrier protein all the way through the membrane. A carrier protein transports either a single or a defined number of solute molecules per conformational cycle.

Channels cycle between either a closed or an open conformation, and while open, provide a continuous pathway straight through the membrane bilayer. This allows flux of thousands of solute ions. Control of channel gating, between either the open or closed conformation, may be regulated by one of the following forms: voltage, binding of a ligand regulatory molecule, or by mechanical means such as membrane stretch (i.e. under stress) (Figure 1).

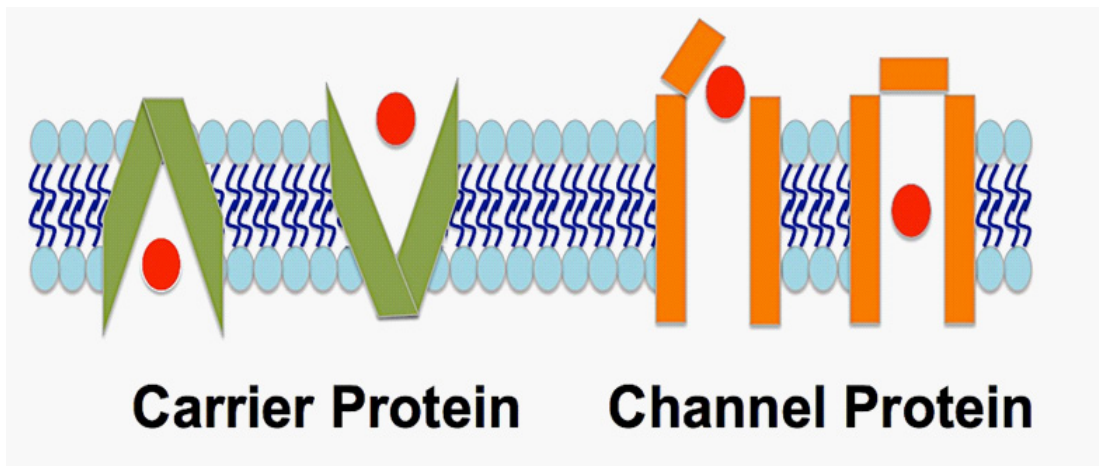


Figure 1: Classification of membrane transport proteins into either a carrier (left) or channel (right). The differential mechanisms of each are depicted, in which the carrier protein exhibits a conformational cycle while the channel alters between either open or closed.

Uniport

Carrier proteins that mediate the transport of a single solute are classified as a uniporter. These types of transport proteins facilitate the mode of diffusion by providing carrier-mediated solute transport down the concentration gradient, accelerating a reaction that is already thermodynamically favored. The uniport carrier protein allows transport of the non-diffusible solute across the membrane barrier at a rate that is much higher than passive diffusion in which the solute molecule is never in contact with the hydrophobic core of the membrane. An example is the GLUT1 glucose carrier, found within the plasma membrane of various cells. GLUT1 is a large, 12 alpha helix membrane protein permitting the facilitated diffusion of a single glucose molecule per cycle into the cytosol of the cell to be metabolized and used as energy in cellular processes (Figure 2).

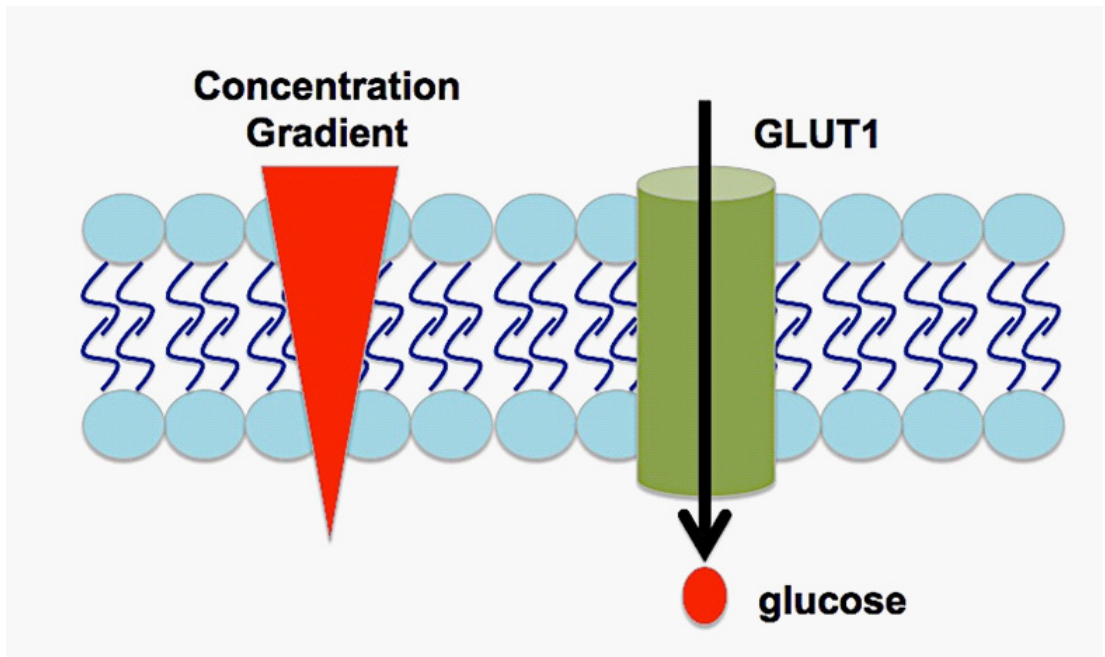


Figure 2: Mode of transport for the uniport transport protein, GLUT1. Glucose diffusion across the membrane bilayer is facilitated by the GLUT1 carrier protein.

Symport and Antiport

Carrier proteins also hold the ability to couple the movement of one type of ion or molecule down its concentration gradient, with the movement of a second type of molecule against its concentration gradient. The ability to transport two different solutes simultaneously is termed cotransport. In this way, the transport protein mediates transport in which an energetically unfavorable reaction is coupled to an energetically favorable reaction that does not require ATP.

A carrier protein, which binds two dissimilar solute molecules to transport them across the membrane to the same side, is classified as a symport cotransport protein. Transport of the two solutes is coupled obligatorily. An example of a symport carrier protein is lactose permease, while also happen to be the first carrier protein for which an atomic resolution structure has been determined. Lactose permease catalyzes uptake of the disaccharide lactose into *E. coli* bacterial cells, along with hydrogen ions, driven by the protein electrochemical gradient.

A transport protein in which an exchange exists across the membrane, one solute for another, is classified as an antiport protein. A substrate binds and is transported across the membrane, then another substrate binds and is transported in the other direction. In this way it is an exchange, because the antiport carrier protein cannot undergo the conformational transition in the absence of bound substrate. An example of such is the adenine nucleotide translocase (also referred to as the ADP/ATP exchanger), which catalyzes a 1:1 exchange of ADP for ATP across the inner mitochondrial membrane (Figure 3).

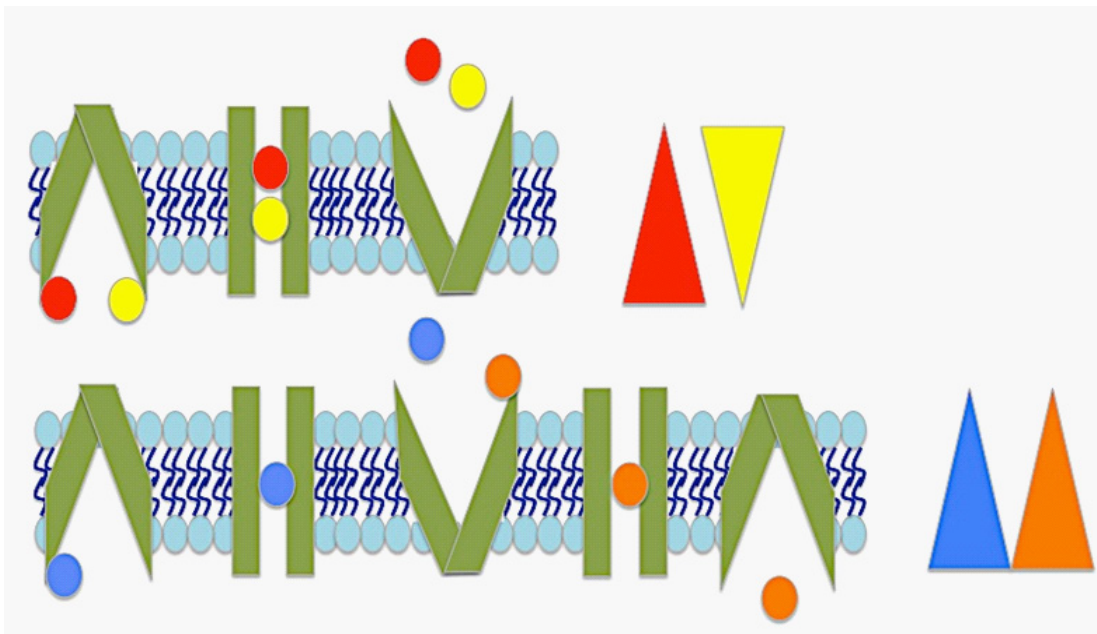


Figure 3: Mechanism of transport for symport (top) and antiport (bottom) carrier proteins. Concentration gradients for solute molecules are shown by corresponding colored triangles in which an energetically favorable reaction catalyzes that of an unfavorable reaction. Dissimilar solutes are transported in both cases, with final transport location on same side of the membrane for symport and opposite sides of the membrane for antiport. Note: the cartoon with the protein of parallel lines is a hypothetical transition state as carrier proteins are never open to both sides of the membrane at the same time.

For further reading on classification of transport proteins see [1-4]. See also the transporter classification database (<http://www.tcdb.org>).

Learning outcomes

- A carrier protein cycles through a variety of conformational states.
- Solute binding sites become alternately accessible to either side of the membrane, but a complete opening through the membrane never exists with a carrier protein.
- A channel exists between either a closed or an open conformation, providing an open channel straight through the membrane permitting flux of thousands of solute molecules.
- In uniport, the single solute transport is down the concentration gradient. In symport, dissimilar solutes are transported to the same side of the membrane, with one solute with and the other against the respective concentration gradient.
- In antiport, dissimilar solutes are transported to opposite sides of the membrane again with one solute following and the other opposing respective concentration gradients.

TYOLOGIES OF MEMBRANE TRANSPORT

Objectives

What is the main difference between passive and active transport?

Identify the environment created by a porin protein to permit facilitated diffusion.

Describe the difference in ATP utilization between primary and secondary active transport.

Name the three types of transport present within the system of glucose transport into intestinal cells.

Passive transport

When a solute moves down its respective concentration gradient, a thermodynamically favorable ($-\Delta G$) event, it is classified as passive transport. The solute is carried from an area of higher concentration to lower concentration, along the gradient, and therefore faces no resistance. Due to this lack of resistance, passive transport does not require any input of energy. Both carrier and channel proteins permit passive transport by facilitating the selective diffusion of solute molecules with their concentration gradient, termed facilitated diffusion.

Porins are beta barrel channels located in the outer membranes of bacteria, mitochondria and chloroplasts. Most currently known porins are trimers, in which each subunit is composed of 12 to 18 membrane spanning beta strands that are amphipathic. The alternating hydrophobic-hydrophilic amino acids lead to a water filled core that provides a hydrophilic passageway for the movement of ions or molecules across the membrane, without coming into contact with hydrophobic membrane. An example of a porin channel protein is *E. coli* OmpF that forms a barrel structure. In OmpF monomer is composed of 16-strands, with a single loop in each monomer folding down and into the β -barrel, preventing movement of substances greater than 600 D as well as providing a switch between open and closed conformations. When open, the OmpF protein permits passage of ions or small molecules with their concentration gradient, not requiring the usage of additional energy (Figure 4).

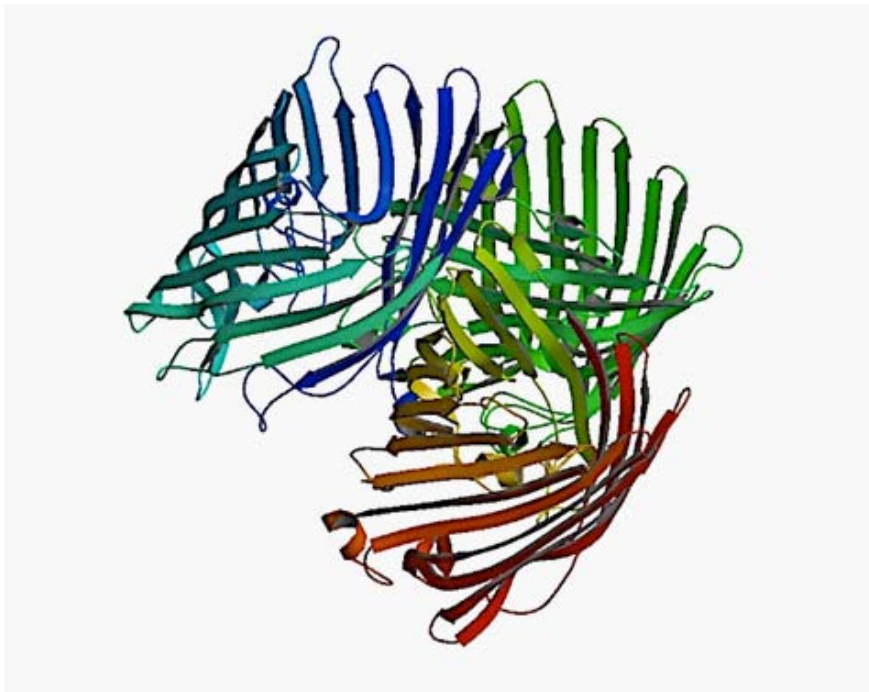


Figure 4: The *E. coli* OmpF porin. The trimeric protein is composed of 16 β strands facilitating diffusion of ions or small molecules with their concentration gradient. The porin is able to switch into an off conformation with the movement of a single loop into the hydrophilic core of each monomer (PDB 1OPF).

Active transport

During active transport, a solute is carried from low to high concentration regions, against its gradient. The solute molecule therefore faces resistance in this transport, and requires an input of energy. The conversion of energy stored in chemical bonds into energy stored in a concentration gradient defines active transport. In primary active transport, the binding and hydrolysis of ATP to $\text{ADP} + \text{P}_i$ to the membrane transport protein drives the energetically unfavorable transport reaction. Binding of ATP to the protein triggers a conformational change, with the hydrolysis and release of $\text{ADP} + \text{P}_i$ triggering another change in the conformation to generate a transport cycle, ensuring the mechanism of active transport operate only in a single direction. In secondary active transport, the uphill transmembrane movement of a substance is not directly coupled to the conversion of ATP to $\text{ADP} + \text{P}_i$. In this case, the transport protein utilizes a pre-established concentration gradient from an ATPase. The first transport protein, the ATPase, uses ATP in primary active transport to establish a concentration gradient of ions (H^+ or Na^+), with the secondary transport protein utilizing the stored energy in the established ion concentration gradient to transport solute molecules. Therefore, the secondary active transport of a solute is driven directly by the ATP-dependent formation of a gradient of a second molecule (Figure 5).

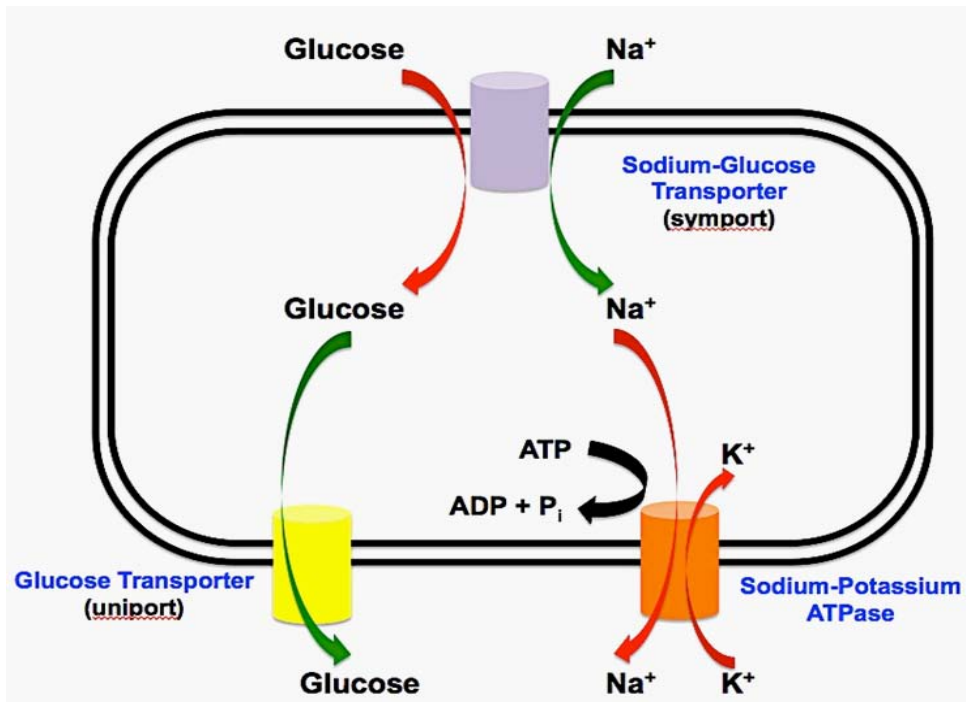


Figure 5: Glucose transport into intestinal cells. A concentration gradient of sodium ions is established via the sodium-potassium ATPase in which generates a high extracellular concentration of Na^+ . The sodium-glucose symport transport protein couples the energetically favorable movement of sodium ions into the intestinal cell, coupled with the unfavorable movement of glucose molecules also into the intestinal cell. Glucose thereby becomes concentrated inside of the cell, and exits the cell using its downhill concentration gradient via a passive glucose uniport transport protein subsequently moving glucose to the blood stream. Green arrows depict energetically favorable events with red arrows depicting energetically unfavorable events.

For further descriptions of the structures of membrane transport proteins see [5,6]. Also visit the catalogue of membrane proteins of known structures (<http://blanco.biomol.uci.edu/mpstruc/>).

Learning outcomes

- Passive transport utilized the stored energy in concentration gradient. The transport of the solute faces no resistance and thus requires no input energy.
- Active transport utilized the energy transport solutes against the resistance of their concentration gradient.
- Porins create a hydrophilic passageway through the hydrophobic membrane by their beta barrel formation.

- Primary active transport utilizes ATP by the direct binding and hydrolysis signaling the cycle through conformational changes to transport the solute molecule uphill against its concentration gradient. Movement of the solute is directly coupled to the reaction of ATP to $ADP + P_i$
- Secondary active transport is not directly coupled to ATP hydrolysis, instead the transport of a substance is driven directly by the ATP-dependent formation of a gradient of a second molecule. The transport protein utilizes the pre-established concentration gradient from an ATPase.

TRANSPORTER SUPERFAMILIES BELONGING TO THE MEMBRANE

Objectives

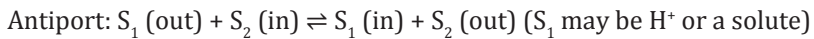
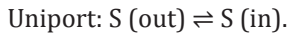
- 1) Define Major Facilitator Superfamily proteins
- 2) Provide several general structural features of MFS transporters
- 3) Describe the proposed general mechanism of transport for members of the MFS
- 4) Define the Alternative Access model
- 5) Give a general description of ABC transporters
- 6) Identify several general structural features of ABC transporters
- 7) Understand the general mechanism of ABC transport
- 8) Define the different models for ABC transporters
- 9) Give a definition of Multi-drug Resistant Proteins

Major facilitator superfamily (MFS) of transporters

In order to maintain life on earth, energy from the sun must be extracted and converted into the energy currency known as Adenosine Triphosphate (ATP). Stored solar energy in the form of chemical bonds is used to generate ATP and electron transfer. A product of the electron transport chain, or the Proton Motive Force (PMF), is the formation of proton/ion gradients across the membranes of living cells, thus driving the movement of a variety of solutes against their concentration gradients.

The mobility of solutes across the membrane of cells is vital for cell sustainability, growth and homeostasis. A limited number of molecules are capable of freely diffusing across the lipid bilayer, thus numerous solutes and substrates are transferred across the membrane via proteins generally referred to as carriers, permeases and transporters, ubiquitously found in every cell. A large number of transport proteins have evolved to mediate the exchange of ions, sugars, lipids, nucleobases, amino acids, peptides, toxic compounds and even folded proteins, in addition, these proteins may also play a role in signal transduction, energy conversion, catalysis, mechanosensing

and structural integrity. Among these, the Major Facilitator Superfamily (MFS) is evolutionarily an incredibly old and diverse set of membrane transporters that includes over 10 000 sequenced members. Presently, the MFS consists of 76 subfamilies distributed in bacteria, archaea and eukarya, yielding the largest known superfamily of secondary transporters. These membrane transporters have the capability to catalyze uniport, symport and/or antiport transport, giving rise to the generalized reactions:



Within the MFS, each family exhibits specificity for a characteristic set of related compounds that include monosaccharaides, oligosaccharides, amino acids, peptides, neurotransmitters, osmolites, vitamins, enzyme cofactors, drugs, chromophores, nucleosides, nucleotides and organic and inorganic anions and cations.

General structural features

Despite the large number of sequenced members of this superfamily, only six 3D molecular structures have been reported, in chronological order of publication these are: the lactose: H^+ symporter LacY, the glycerol-3-phosphate: Pi antiporter GlpT, the multidrug: H^+ antiporter EmrD, the L-fucose: H^+ symporter FucP, the oligopeptide: H^+ symporters PepTso from *Shewanella oneidensis* and PepTst from *Streptococcus thermophilus*, and the D-xylose: H^+ symporter XylE (Figure 6).

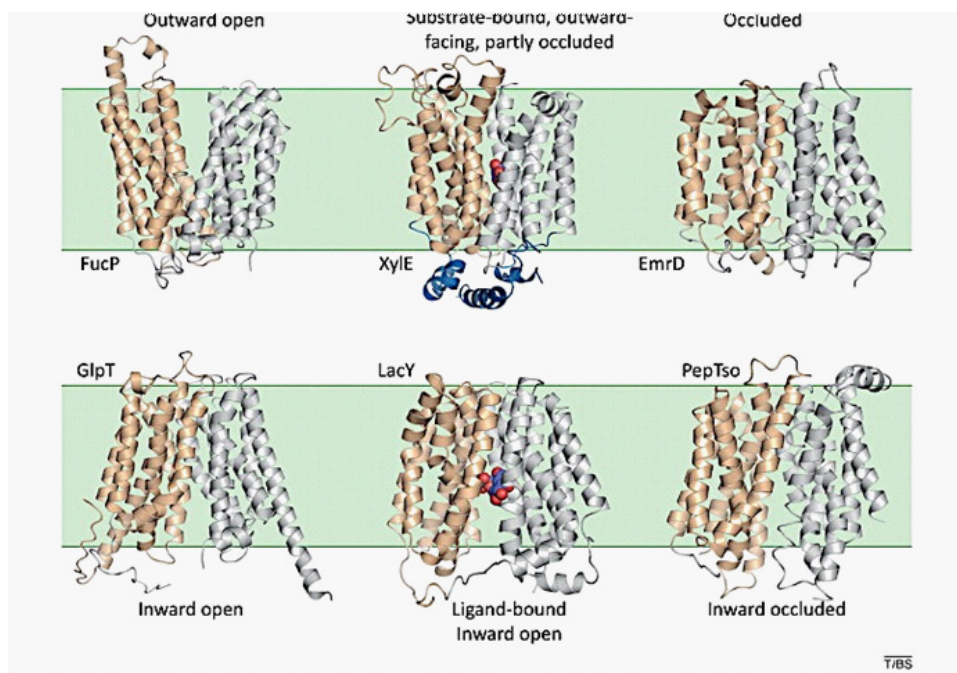


Figure 6: Conformational states of six members of the major facilitator superfamily. The N domains are coloured wheat and the C domains gray. The PDB accession codes for these structures are 3O7Q for FucP, 4GBY for XylE, 2GFP for EmrD, 2XUT for PepTso, 1PV7 for LacY and 1PW4 for GlpT. Image taken from [7].

According to bioinformatics analysis, members of this superfamily are typically comprised of 12 transmembrane (TM) helices, with some containing more. Generally, the N and C termini are located on the cytoplasmic side of the bilayer. Despite variance in amino acid sequence, substrate specificity and proton coupling mechanism, members of the MFS share a common fold, known as the MFS fold. This fold has two discrete N and C termini domains with little sequence similarity, each comprised of 6 transmembrane helices (Did you know: Experimentally you can split MFS permeases without loss of activity; check out the work done by H. R. Kaback on lactose permease [8]). Nonetheless, the two domains display two-fold pseudosymmetry with an axis perpendicular to the membrane plane. Within each domain there is an additional 3+3 inverted repeat in which the first and second 3-TM unit are correlated by a 180° axis of rotation parallel to the membrane bilayer. Therefore, an MFS fold contains four structural repeats, a repetition representing a fundamental structural unit for all members of this superfamily. It has been experimentally found that in all MFS structures transmembrane segments 1, 4, 7 and 10 are positioned in the center of the transporter and contribute the majority of residues significant for substrate transfer and proton coupling. The remaining TM segments (α -helices) loosely participate in substrate binding and proton coupling as well as structural integrity. Cytoplasmic residues generate both short and long loops dependent on the connection among the TMs, such as between neighboring TMs or

domains, respectively. Longer loops can reach up to 100 residues and exist as unstructured loops or α -helices. Despite low sequence similarity amid the loops, the conserved sequence, DRXXRR, is found at the ends of TMs 2, 3, 8 and 9 in many MFS proteins.

Mechanism of transport

To complete a round of import or export, a transporter undergoes a sequence of prominent conformational changes via movement in the TM segments and/or protein domains. In general the mechanism of all transport proteins can be simplified to an Alternating Access Model, proposed by Jardetzky more than 40 years ago [9]. In this model the substrate binding site (or sites) is exposed to sides of the membrane in an alternative fashion, entailing that the transporter has both an outward and inward conformational state for cargo upload or release, however, the substrate is never concurrently exposed to both sides of the lipid bilayer (Figure 7).

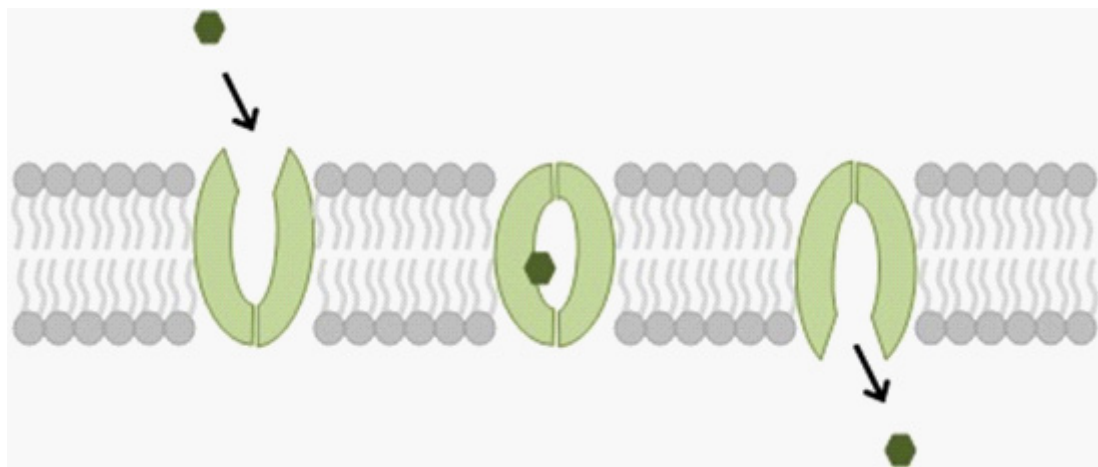


Figure 7: Schematic diagram of a MDR protein in which the substrate enters the transport protein from one end of the protein and exits from the other. Note that the substrate is never exposed to both the cytoplasm and extracellular space at one time, as described in the Alternating Access Model.

Individual conformations of certain MFS members have been determined, still, capturing multiple conformations of a single MFS protein has proven to be challenging. The use of computational, biochemical and biophysical techniques has permitted the visualization of the transport mechanism of MFS proteins.

By biochemical evidence, it has been generally accepted that MFS proteins contribute a substrate-binding cavity formed by multiple residues, located halfway through the protein and enclosed by the N and C domains. With the exception of antiporters GlpT and EmrD, all **MFS** transporters with determined structures shuttle substrates by harnessing the energy stored in the proton gradient. For symporters, the substrate and proton are co-transported in the same direction and bind to key residues in fixed stoichiometry.

Substrate binding and energy coupling represent two key, but non-distinct, aspects of MFS transport. Commonly, one or more aromatic residues are present at the substrate-binding site, adopting an open conformation in the substrate-free position. While substrate binding requires alternating exposure, proton translocation is characterized by protonation and deprotonation of residues such as Glu/Asp/His, and additionally to a lesser extent Lys/Arg/Tyr. As a result, binding of the substrate is accompanied by the presence, or relay, of H^+ ions (or Na^+), their location dependent on the type of transport, i.e. symport or antiport. Protons or sodium ions are believed to neutralize negatively charged residues located in the substrate path perhaps as a necessity to lower the energy barrier for substrate entry, facilitate domain closure or allow for proper TM conformational changes (Figure 8).

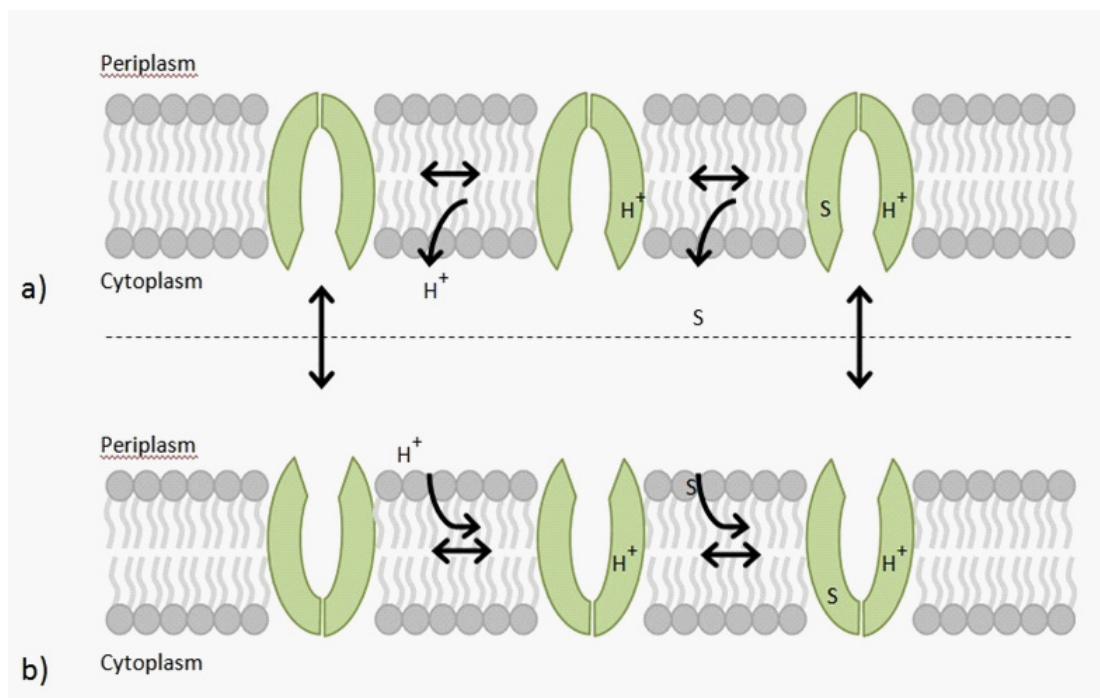


Figure 8: Schematic diagram of the proposed mechanism for MFS transporters. In the diagram the proton, denoted H^+ , and the substrate, denoted S, are released in the same direction, thus indicating the transporter is a symporter. Diagram a) represents the inward-facing conformations and diagram b) indicates the outward-facing conformation.

For a list of MFS subfamilies and peer-reviewed papers on members of this superfamily visit the site: <http://www.tcdb.org/search/result.php?tc=2.A.1>

Example - LacY

By use of biochemical and biophysical techniques, including the 3D structure solved by X-ray crystallography in 2007 [10], the general mechanism of lactose permease of *Escherichia coli* has

been determined. The transporter begins in an outward facing position, becomes protonated, recognizes and binds a lactose sugar molecule from the periplasm of the cell, this leads to changes in conformation to the inward-facing state. Next, there is release of the sugar substrate to the cytoplasm, followed by deprotonation that leads the conformation to convert back to the outward-facing state in order to begin another round (Figure 9).

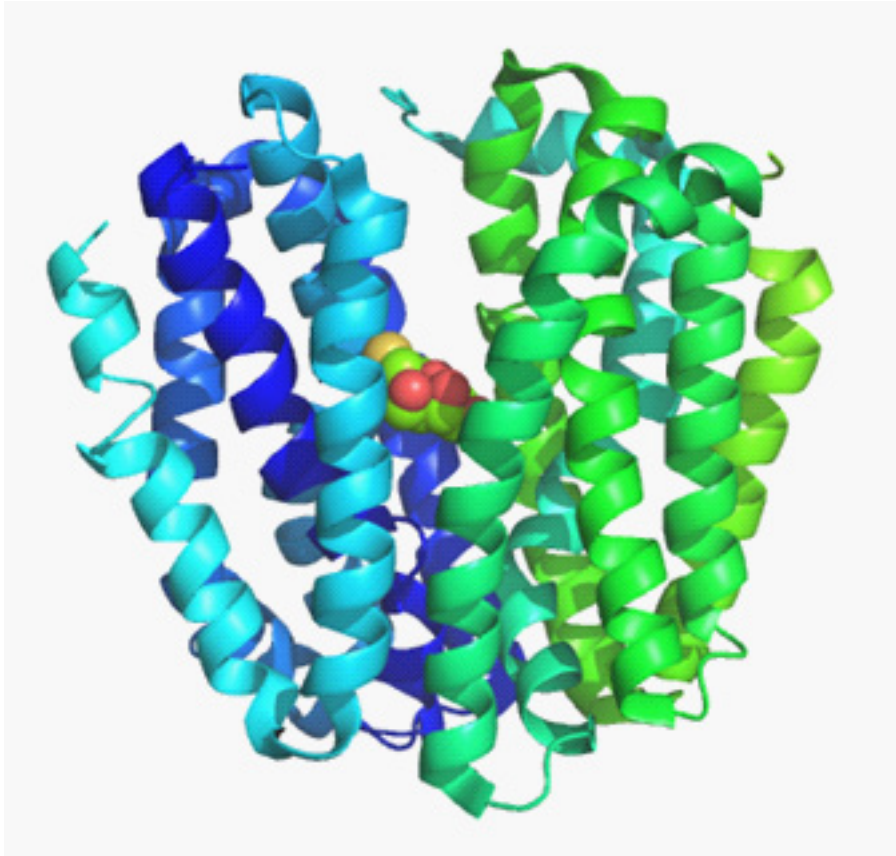


Figure 9: Reported crystal structure of lactose permease at 3.38Å, determined by Chaptal et al [11]. The structure exhibits an inward-facing conformation, covalently attached to MTS-gal. PDB 2Y5Y.

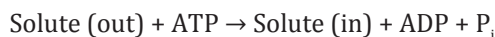
For more information on how the mechanism of this protein was determined both by use of biochemical and biophysical techniques see [12-15].

ATP Binding Cassette (ABC) superfamily of transporters

The movement of nutrients in and out of the cell requires penetration across the cellular membrane. The necessity at which this process occurs may be underestimated, for example, an actively growing *E. coli* cell must uptake ~10⁶ glucose molecules per second to support metabolic needs. To ensure transport for a diversity of molecules, many transporters are encoded in the

genomes of living organisms, like in *E. coli*, to which 10% of the genome belongs to transport proteins alone. The significance of transport across the cell bilayer cannot be undermined, but rather must be appreciated as a metabolic cost, critical for cell vitality, especially when this process is estimated to consume ~10 - 60% of the ATP requirements of bacteria and eukaryotes. Among these proteins lays one of the largest class of transporters, the ATP-binding cassette (ABC) transporter family. NOTE: that ABC is used interchangeably with nucleotide-binding domain (NBD).

Unlike members of the Major Facilitator Superfamily, which are characterized as secondary transporters, ABC transporters utilize the favorable source of chemical energy generated directly by ATP-hydrolysis, identifying these proteins as primary active transporters. Translocation of molecules by ABC members, both in and out of the cell, occurs in a thermodynamically unfavorable direction, as anticipated due to the use of chemical energy. The generalized reactions of ABC transporters can be described by the equation:



ABC proteins are found in all kingdoms. Importers are exclusive to prokaryotes, enabling the uptake of amino acids, sugars, ions, peptides and even essential metals. Approximately 50 exporters are found in humans, imperative in the movement of lipids, fatty acids and cholesterol {P-glycoprotein (PGP) is an ABC transporter that maintains the cholesterol distribution across the leaflets of plasma membranes, however PGP also removes other lipophilic compounds like chemotherapeutic agents, resulting in tumor cell multi-drug resistance}. Other members of ABC transporters participate in antigen presentation, multidrug and antibiotic resistance and mitochondrial iron regulation. Cystic fibrosis, hypercholesterolaemia, macular degeneration, Tangier disease, diabetes and others have been associated with mutations in proteins belonging to this superfamily.

General structural features

All ABC proteins comprise of a core ABC transporter imbedded in the membrane consisting of two types of units, which include a pair of TM segments or the ABC domain enclosing a defined pathway, and a pair of peripheral components or nucleotide-binding domains (NBD), that bind and hydrolyze ATP. In prokaryotes (importers) these domains are usually encoded separately or as variants that are fused. For ABC proteins that export compounds (both eukaryotes and prokaryotes), the core is typically encoded by a single gene.

Each TM domain is usually composed of 6 α -helices that span the membrane, resulting in a total of 12 for exporters. Importers, which are larger, have additional domains hence are found to have between 10 and 20 helices. The TM segments between members of this family are not well conserved in sequence, length and number. However, a central pathway is observed in many

crystal structures, but as seen with MFS proteins, substrate access is only available from one side. There are three sets of folds currently recognized: type I, II and exporter folds.

The nucleotide-binding domain, also called the ABC domain, contains several conserved sequence motifs, contrary to the TM segments, and are not embedded in the membrane contrary to the TM domains. Some of these motifs include the phosphate binding region called the P-loop/Walker-A motif, the LSGGQ motif that contacts the nucleotide in the ATP-bound state, the hydrophobic stretch of amino acids called the Walker-B motif, which provides a highly conserved glutamate residue resulting in nucleophilic attack on ATP by water, the D-loop associated in NBD contact and many more. In addition to conservation of residues, the arrangement of the NBDs is also conserved. In the ATP-bound state, two ATP molecules are found at the interface of the NBD-NBD site, giving the domains a head to tail orientation. In this way, the ATP bridges between the two NBD domains, bringing them in close contact.

Mechanism of transport

Although there are many proposed mechanisms of transport (Figure 10), a common mechanism characteristic of conformational changes result in translocation of the molecule either in or out of the cell. As seen in MFS proteins, the general mechanism can be described as an Alternating Access Model, in which the protein shifts between an outward/inward-facing conformation, ensuring that there is never an open channel.

For ABC proteins it is a requirement to prevent the uncoupling process from occurring in the favorable direction, which would occur by leakage of the accumulated product causing a futile cycle. In general, ATP is bound in the outward conformation of the transporter, followed by hydrolysis that drives the conversion from the outward to the inward; ADP is now found in the inward conformation. The fundamental part of ABC transport is to keep the rate of ATP hydrolysis minimal until the proper substrate is bound, at which point ATP hydrolysis will drive the transport via proper protein conformational changes.

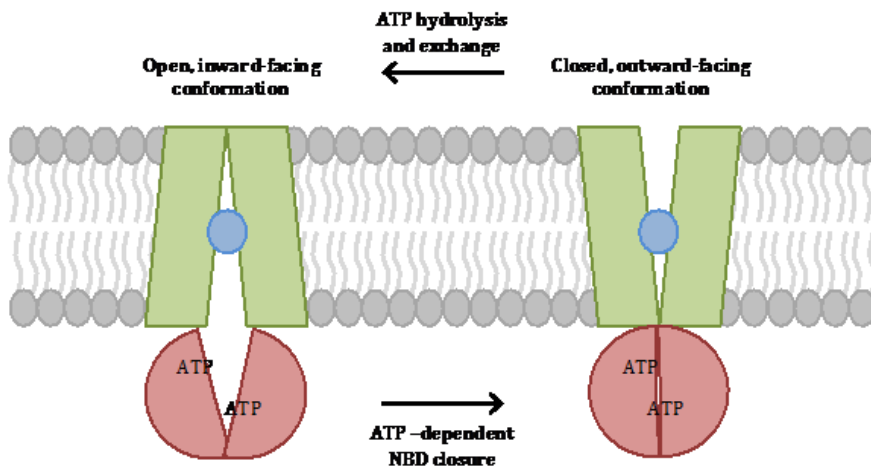


Figure 10: Schematic diagram of the characteristic mechanism of ABC transporters. The closure/dimerization of the NBDs (in red), located in the cytoplasm, delivers the energy needed for the power-stroke that pulls the TMDs (in green) from the inward to outward conformations. Hydrolysis of the ATP returns the conformation back to the inward orientation.

The relevant ratio of ATP molecules per substrate is most likely 2, although this ratio has only been observed *in vitro*. The hydrolysis of ATP involves the company of two properly positioned groups, one for phosphate binding and the other for the activation of water and attack on the phosphate of ATP. The Walker A and Walker B motifs are involved in nucleotide phosphate binding and Mg^{+} coordination, respectively. In addition two residues are also positioned in the active site, functioning as catalysts for the rate of ATP hydrolysis and turnover. One ATP is bound to the Walker A loop of one NBD and a second motif, with the signature sequence LSGGQ, on the second NBD, and vice versa, thus bridging the two NBD domains close together.

In general the mechanism follows the elementary steps: binding of substrate and Mg^{+}/ATP , dimerization of the NBD followed by conformational changes characterized by ABC domain switching between outward/inward conformations (depending on direction of transport), ATP hydrolysis, substrate, phosphate and ADP release and regeneration of the protein to the ground state in preparation for the next cycle. It is important to remember that the order at which these events occur may vary from protein to protein, regardless, experimental and physical evidence has shown that these steps take place at some point.

Twister model: the ABC exporter Sav1866

The structure of this bacterial ABC exporter (Did you know: this protein is a bacterial homolog of the human ABC transporter MDR 1, which is known to cause multidrug resistance in cancer cells) from *Staphylococcus aureus* was determined in 2006 by Dawson and Locher. A large cavity

was found to be present at the interface of the two TMDs and the presence of several hydrophilic, polar and charged residues were representative of a translocation pathway. The 3D structure revealed a tight interaction upon dimerization of NBDs when ATP was bound, consequential in coupling to the outward-facing conformation. This would then result to release of the product to the cell exterior via a conformational change akin to a twisting action. Upon hydrolysis the protein is expected to return to the inward-facing ground state. Conformational changes in this model include the domain swapping and subunit twisting presented in Figure 11.

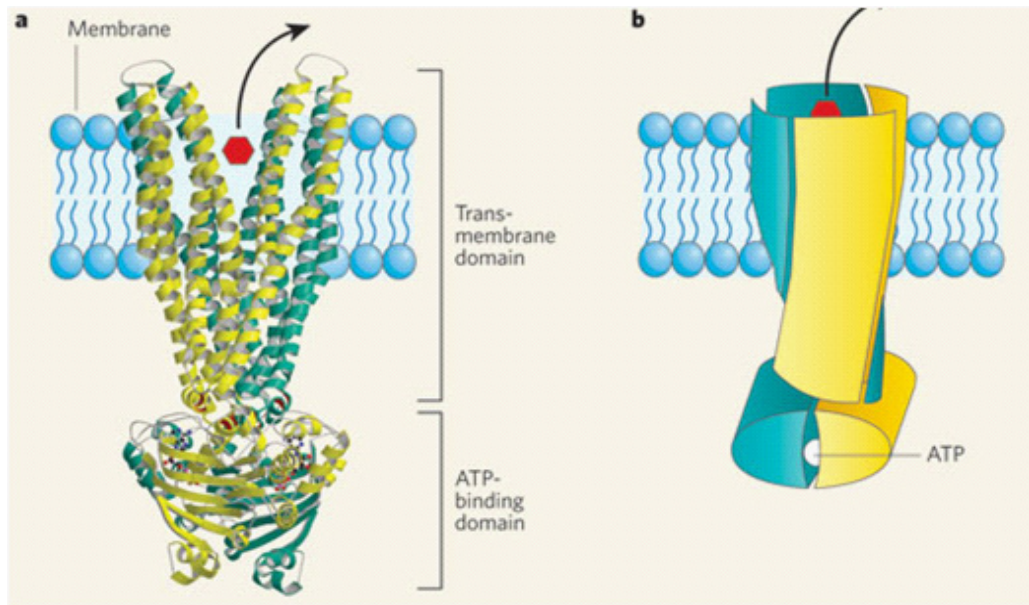


Figure 11: a) Reported structure of Sav1866 in ribbon structure transporting a drug. The two domains are coloured in turquoise and yellow. b) The interaction of the 4 domains shown as a schematic diagram. Image from [16]. For more information on the 3D structure and proposed mechanism of transport of Sav1866 see [17].

Sewing Machine model: The DNA repair enzyme, Rad50.

In 2000, Hopfner and colleagues characterized the structure of Rad50 from *Pyrococcus furiosus*. This enzyme has ATP-dependent binding and partial DNA unwinding activities and its fold closely resembles that of ABC transporters. In the absence of a nucleotide, Rad50 is a monomer however, and once ATP is bound this protein becomes stable. The dimer clamps the triphosphate of ATP between the Walker A and Walker B motifs of one domain and the LSGGQ sequence found on the second domain. This formation is in agreement with ABC transporters that demonstrate stabilization between the TM domains and the NBD, in addition to ABC activity, a type of power-stroke that drives solute transport. In this model, the transmembrane movement of the substrate is supplemented by variation in the depth of the α -helical TM segments within the membrane upon the binding of two ATP molecules. It is predicted that the hydrophobicity of the

transmembrane segments would be required to change by rotation of the helices and exposure of the appropriate regions at a given moment in order to allow the segments to push the ligand through the membrane, bobbing up and down like the needle of a sewing machine, reset by ATP hydrolysis and ADP release. For more information on this type of ABC transport see [18].

Clothes-pin model: the type II transporter, BtuCD

The structure of BtuCD was determined in 2002 using X-ray crystallography by researchers Locher et al. [19]. This protein belongs to *Escherichia coli* and mediates the uptake of vitamin B12 past the membrane and into the cell. In the case of ABC importers, the substrate-binding site and ATP-binding site are positioned on opposite sides of the membrane unlike what is found in ABC export proteins, in which both are located on the cytoplasmic side. This denotes that the signal from the periplasmic binding site must be communicated through the TM segments to the NBD in the cytoplasm. ATP hydrolysis is then coupled with substrate passage. The long-range conformational changes, powered by binding and hydrolysis of ATP, in the NBD region control the gating region of the spanning TMs. The large body movements that mimic a clothespin-type spring illustrate the movement of the substrate. The spring symbolizes the interface between the TM domains and the pressure produced by the bilayer. Separation in the domains would then be expected to close the periplasmic entrance and the ligand would be released through the BtuCD interior across the membrane and past the gate. Passage through the tight core of the protein might occur via diffusion where the entrance is blocked by α -helix belonging to one of the TMDs. Through the release of ADP, the protein is able to return to its ground state (Figure 12).

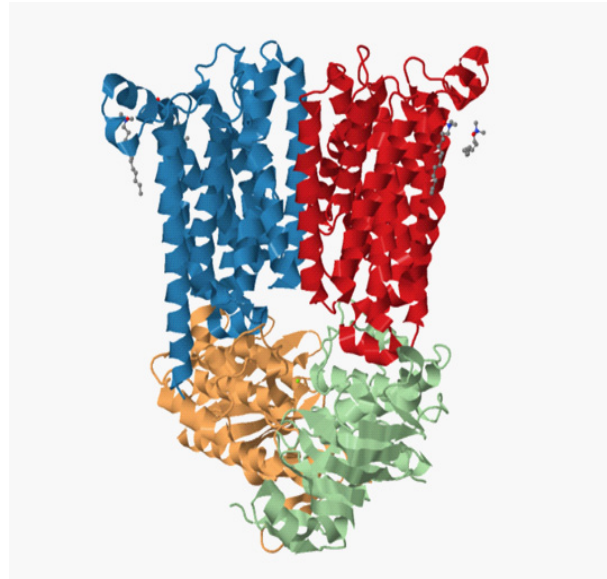


Figure 12: Ribbon diagram of BtuCD. The colouring of the diagram represents the distinctive domains. Domains coloured in red and blue signify the TMDs imbedded in the membrane, while the domains coloured beige and green (NBDs) would be located in periplasm. The PDB accession number is 4R9U.

For more information on the structure and mechanism of this importer see [19,20].

Multi-Drug Resistant (MDR) proteins

This family of transporters was coined in 1992 by researchers Cole and Deeley upon successful cloning of the multidrug resistance-associated protein gene known as MRP 1. Multi-drug resistant (MDR) transporters can be divided into two classes based on the source of energy utilized to extrude compounds: Secondary transporters, which use protein gradients and ATP-dependent primary active transporters. Secondary transporters are sub-divided into four families that include the Resistance/nodulation/division family, the Multidrug/oligosaccharidyl-lipid/polysaccharide Flippase family, the Drug/metabolite Transporter Superfamily and the Major Facilitator Superfamily (See 3.1). Presently there are three major groups of primary active ABC transporters involved in multidrug resistance: the P-glycoprotein MDR1, the multidrug resistance associated family and ABC half-transporters such as ABCG2.

In tumor cells, multidrug resistance is repeatedly correlated with an ATP-dependent decrease in cellular drug accretion resulting in the over-expression of particular ABC transporters. Although multi-drug resistant (MDR) proteins are commonly involved in drug resistance, as their name implies, there is also evidence that these proteins have importance in tissue defense and maintenance of barrier function. An element of such transporters that distinguishes them from other transport proteins is their ability to accept many different molecules as substrates belonging to a similar structural class. MDR proteins are able to transport lipophilic cationic, anionic and neutrally charged drugs, carcinogens, pesticides, metals, metalloids and lipid peroxidation products.

Example: Multidrug efflux pump: AcrAB

This MDR transporter from *E. coli* functions alongside TolC in order to extrude antimicrobial agents, antibiotics, detergents, dyes and organic solvents from the cell into the external environment. AcrB, a member of the Resistance/nodulation/division family, is found in the bacterial inner membrane, recognizes and captures its substrates and transports the compounds directly to the external media using the outer membrane channel TolC, an interaction believed to be mediated by AcrA. TolC, ArcA, and AcrB are both found as homotrimers within the periplasmic space.

Reconstitution indicates that AcrB is a protein-substrate antiporter composed of an assembly of monomers, with 12 TM segments each, governed by a rotating three-step process. The general mechanism of AcrB is access to the binding site by the substrate, followed by substrate binding and then extrusion.

Based on several crystal structures of AcrB a precise mechanism of transport for this protein has been postulated. The binding cavity begins as a partially open pocket, slightly shrunk in size. The binding pocket of AcrB then expands to accommodate the substrate followed by passage through the channel and binding to different locations, which are saturated with various aromatic

residues. At this point the exit site is blocked by a helix found in the extrusion protomer of AcrB. Once the binding pocket closes the helix is pushed away, allowing for exit of the drug into the extracellular space. These changes are assumed to be coupled with protonation and deprotonation events of groups within the transmembrane domains (Figure 13).

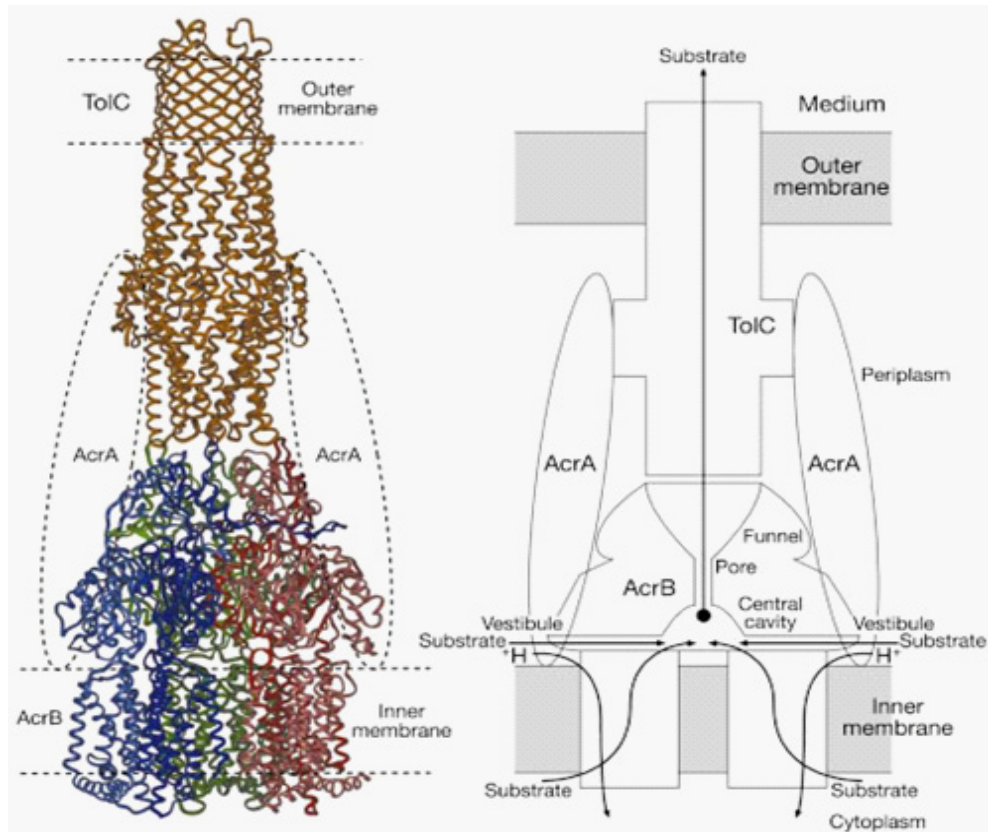


Figure 13: Ribbon (left) and schematic (right) diagram of the AcrAB and TolC complex. The diagram on the right represents a proposed mechanism of transport by use of a protein gradient across the inner membrane. Photo taken from [21].

Learning Outcomes

1. Define Major Facilitator Superfamily proteins

- Secondary transporters that are distributed throughout all phyla that are capable of symport, antiport and uniport transport mechanisms

2. Provide several general structural features of MFS transporters

- Generally comprised of 12 TM helices that generate an MFS fold, in which there are two domains, the N and C domains, that have pseudosymmetry about an axis that is perpendicular to the membrane plane

- Within the N and C domains are 3 + 3 repeats and conserved for all MFS protein is the role of particular TM segments, such as substrate transfer and proton coupling vs. structural integrity
3. Define the Alternative Access model
- This model is based on the idea that a substrate binding site(s) can only be exposed to one side of the membrane at a given moment
4. Describe the proposed general mechanism of transport for members of the MFS
- This mechanism is based on a charge relay system in which the charge of precise residues change
5. Give a general description of ABC transporters
- These are membrane transport proteins that utilize the favourable source of energy generated by ATP hydrolysis directly i.e. primary active
6. Identify several general structural features of ABC transporters
- These importers are generally encoded by a several genes as separate entities, exporters however are usually encoded by a single gene
 - Each TMD is typically composed of 6 α -helices and are not well conserved in sequence and in length
 - The NBDs, which are not embedded in the bilayer, contain numerous stretches of amino acids that generate well conserved motifs
7. Understand the general mechanism of ABC transport
- The elementary steps are: binding of substrate and Mg^{+}/ATP , dimerization of the NBD followed by conformational changes characterized by ABC domain switching between outward/inward conformations (depending on protein nature), ATP hydrolysis, substrate, phosphate and ADP release and regeneration of the protein to the ground state in preparation for the next cycle (Note: the order of these steps may vary, however the general mechanism for all ABC transporters characterized includes each step)
8. Define the different models for ABC transporters
- Twisting model: Upon ATP binding there is close NBD contact in the out-ward facing conformation, via a twisting motion the substrate is released in the in-ward facing position, followed by ATP hydrolysis in order to reset the system
 - Sewing machine model: Upon ATP binding, domain stabilization occurs between the TMDs and NBDs, a power-stroke action drives the substrate through the transporter as the variation in the depth of the TMS within the bilayer changes like the bobbing of sewing machine needle. This system reaches the ground state through ATP hydrolysis followed by ADP dissociation

- Clothes-pin model: The transporter forms a clothes-pin type spring in which long-range conformational changes, powered by ATP binding and hydrolysis, control the gating. Separation of the domains releases the product and dissociation of ADP resets the system

9. Give a definition of Multi-drug Resistant Proteins

- Ability to transport multiple substrates rather than a unique single substrate.
- Energetics may be PMF or ATP.

References

1. Thatcher J D. Transport Proteins. *Science signaling*. 2013; 6: tr3.
2. Goswitz VC, Brooker R.J. Structural Features of the Uniporter/symporter/antiporter Superfamily. *Protein science*. 1995; 4: 534-537.
3. Griffith JK, Baker ME, Rouch DA, Page MG, Skurray RA, Paulsen IT, et al. Membrane Transport Proteins: Implications of Sequence Comparisons. *Current Opinion in Cell Biology*. 1992; 4: 684-695.
4. Ter Horst R, Lolkema JS. Rapid Screening of Membrane Topology of Secondary Transport Proteins. *Biochimica biophysica acta*. 2010; 1798: 672-680.
5. Mishra NK, Chang J, Zhao PX. Prediction of Membrane Transport Proteins and their substrate specificities using Primary Sequence Information. *PloS one*. 2014; 9: e100278.
6. Vinothkumar KR, Henderson R. Structures of Membrane Proteins. *Quarterly reviews of biophysics*. 2010; 43: 65-158.
7. Yan N. Structural advances for the major facilitator superfamily (MFS) transporters. *Trends in Biochemical Science*. 2013; 38: 151-159.
8. Guan L, Kaback HR. Lessons from Lactose Permease. *Annu Rev Biophys Biomol Struct*. 2006; 35: 67-91.
9. Jardetzky O. Simple allosteric model for membrane pumps. *Nature*. 1966; 211: 969-970.
10. Guan L, Mirza O, Verner G, Iwata S, Kaback HR. Structural determination of wild-type lactose permease. *Proc Natl Acad Sci*. 2007; 104: 15294-15298.
11. Chaptal V, Kwon S, Sawaya MR, Guan L, Kaback HR, Abramson J. Crystal structure of lactose permease in complex with an affinity inactivator yields unique insight into sugar recognition. *Proc Natl Sci*. 2011; 108: 9361-9366.
12. Kaback HR, Sahin-Tóth M, Weinglass AB. The kamikaze approach to membrane transport. *Nat Rev Mol Cell Biol*. 2001; 2: 610-620.
13. Guan L, Kaback HR. Lessons from lactose permease. *Annu Rev Biophys Biomol Struct*. 2006; 35: 67-91.
14. Kaback HR1, Smirnova I, Kasho V, Nie Y, Zhou Y. The alternating access transport mechanism in LacY. *J. Membrane Biol*. 2011; 239: 85-93.
15. Abramson J, Smirnova I, Kasho V, Verner G, Kaback HR, Iwata S. Structure and mechanism of the lactose permease of *Escherichia coli*. *Science*. 2003; 301: 610-615.
16. Schuldiner S. Structural Biology: the ins and outs of drug transport. *Nature*. 2006; 443: 156-157.
17. Dawson RJ, Locher KP. Structure of a bacterial multidrug ABC transporter. *Nature*. 2006; 443: 180-185.
18. Thomas PJ, Hunt JF. A snapshot of Nature's favourite pump. *Nature Struc Biol*. 2001; 8: 920-923.
19. Locher KP, Lee AT, Rees DC. The *E. coli* BtuCD structure: A framework for ABC transporter architecture and mechanism. *Science*. 2002; 296: 1091-1098.
20. Korkhov VM, Mireku SA, Veprintsev DB, Locher KP. Structure of MAP-PNP-bound BtuCD and mechanism of ATP-powered vitamin B12 transport by BtuCD-F. *Nat Struct Mol Biol*. 2014; 21: 1097-1099.
21. Murakami S, Nakashima R, Yamashita E, Yamaguchi A. Crystal structure of bacterial multidrug efflux transporter AcrB. *Nature*. 2002; 419: 587-593.