The *E. coli* ATP binding cassette (ABC) transporter - BtuCD

Introduction

ABC transporters are ubiquitous membrane proteins that couple adenosine triphosphate (ATP) hydrolysis to the translocation of diverse substrates across the cell membrane. The size of the substrates transported varies from a single atom to large polypetides. This diversity of the transported substrates is due to the poor sequence similarities of the membrane-spanning subunits and domains.[1] In addition to transporting nutrients across the cell membrane, ABC transporters are involved in diverse processes such as signal transduction, protein secretion, drug and antibiotic resistance, antigen presentation, bacterial pathogenesis and sporulation. Some human inheritable diseases like cystic fibrosis is caused by defective ABC transport system. They have been identified in organisms belonging to each of the three major domains (bacteria, archea, and eukarya, including man)[2]

In bacteria, ABC transporters are composed of four separate protein subunits whereas in eukaryotes these four subunits are usually fused forming a single polypeptide. Bacterial ABC transporters consist of two membrane-spanning domains as well as two ATP binding cassettes (nucleotide binding domains) that are in close contact with each other. The two membrane-spanning domains have a translocation pathway for specific substrates. The ATP binding cassettes are well-conserved, water-exposed and power the transport reaction through hydrolysis of ATP.[1]

The E. coli ABC transporter-BtuCD

The *E. coli* ABC transporter, BtuCD is responsible for the uptake of vitamin B12. The two membrane-spanning subunits (BtuC) are in close contact with each other, as are the ABC subunits (BtuD). Each subunit only makes contact with its two immediate neighbors and has no interface with the diagonally positioned subunit due to the water-filled channel that in between the four subunits of the transporter.[1]

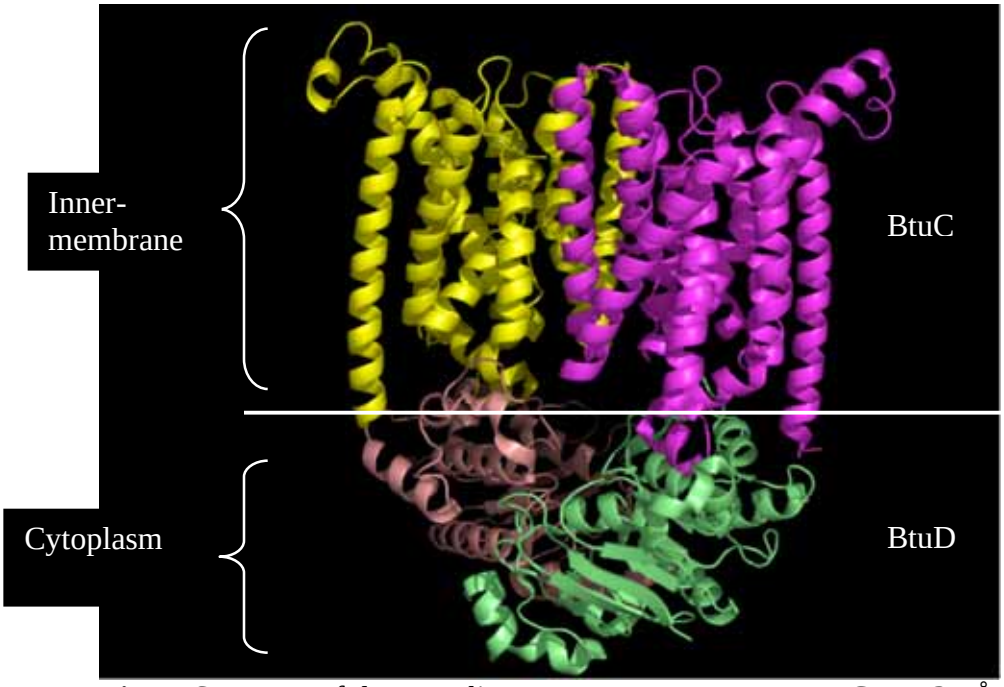

Fig. 1. Structure of the *E. coli* vitamin B_{12} transporter, BtuCD at 3.2Å resolution. The complete transporter is composed of two membrane-spanning subunits, BtuC (yellow and purple) and the two ATP binding cassettes, BtuD (pink and green).

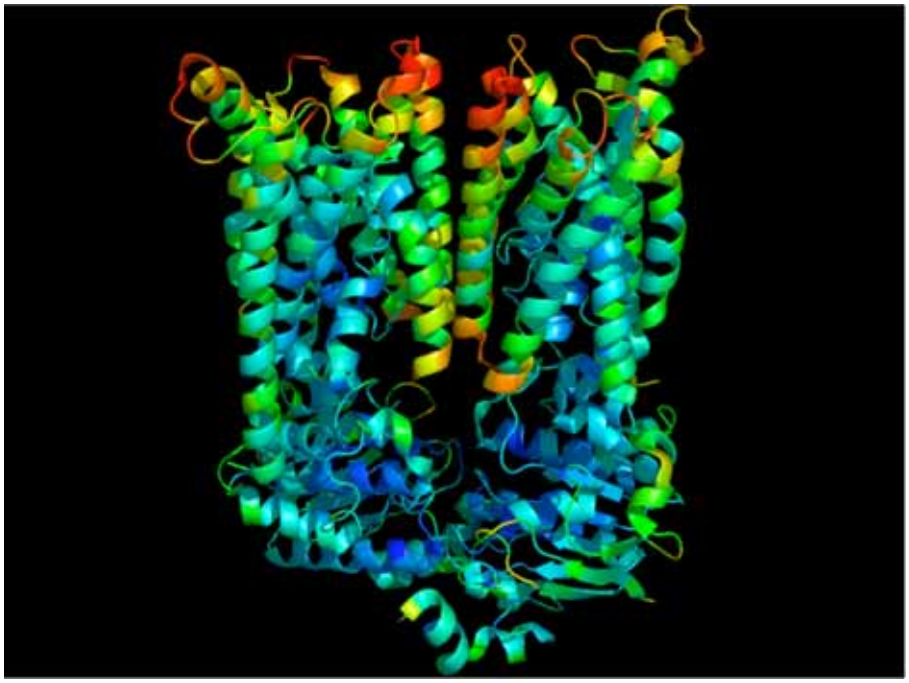

Fig. 2. Colored by b factors. The most mobile regions shown in red and yellow are at the top of the membrane-spanning domain. These residues are exposed to the periplasm. (Scene F1-nov 28part2)

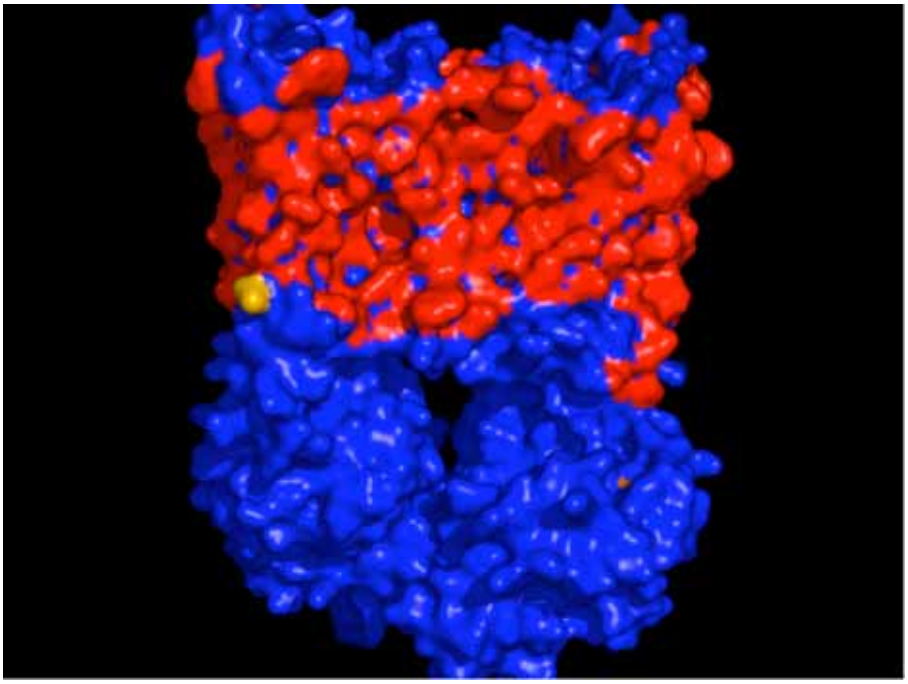

Fig 3. Hot patch image showing hydrophobic residues on the surface of the membrane-spanning domain in red. (Scene F2-nov 28part2)

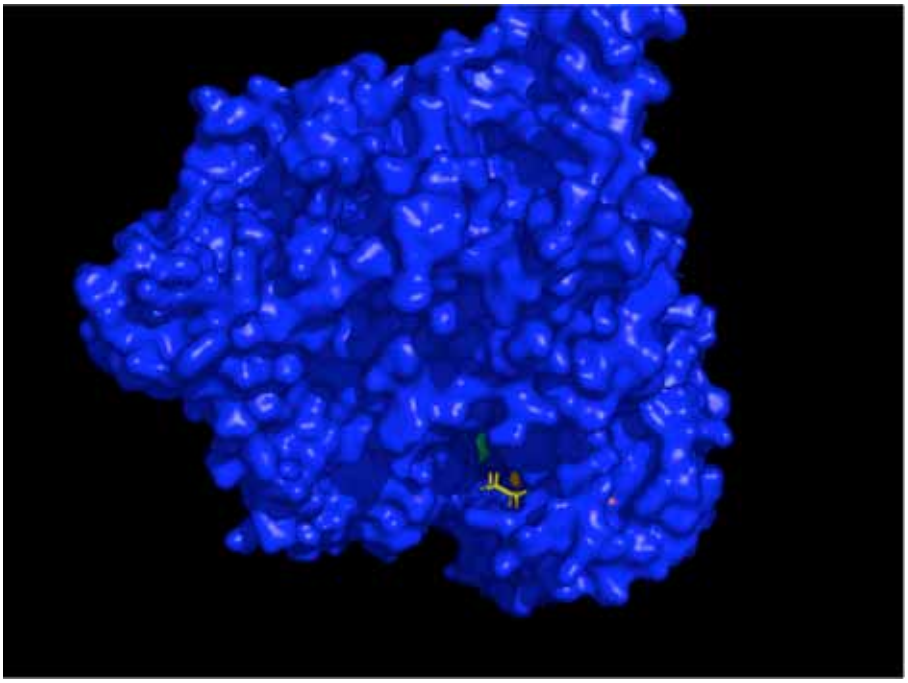

Fig. 4. Hot patch image showing the concave surfaces in the ATP binding cassette (BtuD).Concave regions located in the vicinity of the nucleotide binding site marked by cyclotetravanadate (yellow molecule). (Scene F3-nov 28part2)

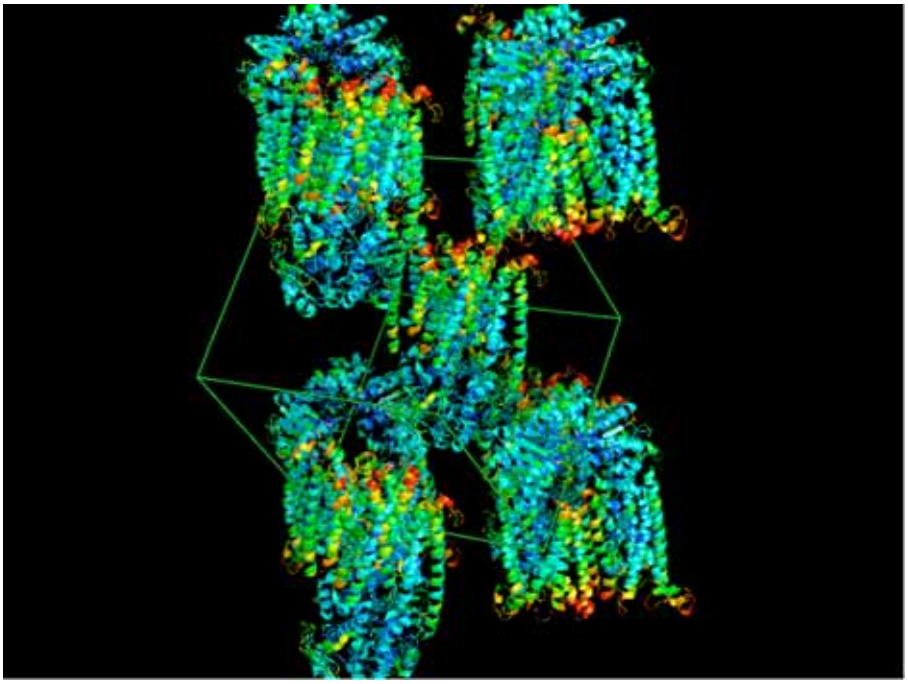

Fig 5. Structural contacts and the unit cell of the BtuCD. (Scene F4-nov 28part2)

BtuD

The two ATP-binding cassettes, BtuD is composed of a six stranded β-sheet that is surrounded by nine α-helices and a peripheral, three-stranded β-sheet. The ATP-binding cassettes have highly conserved motifs. These include the Walker A motif or P loop, the Walker B motif, a glutamine residue in the Q loop and a histidine residue in the Switch region. They also posses a D loop and a short polypeptide sequence (…LSGG…) referred to as the "ABC signature sequence."[1] Generally ABC cassettes bind and hydrolyze ATP in a similar fashion and use a common mechanism to power the substrate through the membrane-spanning domains.[1]

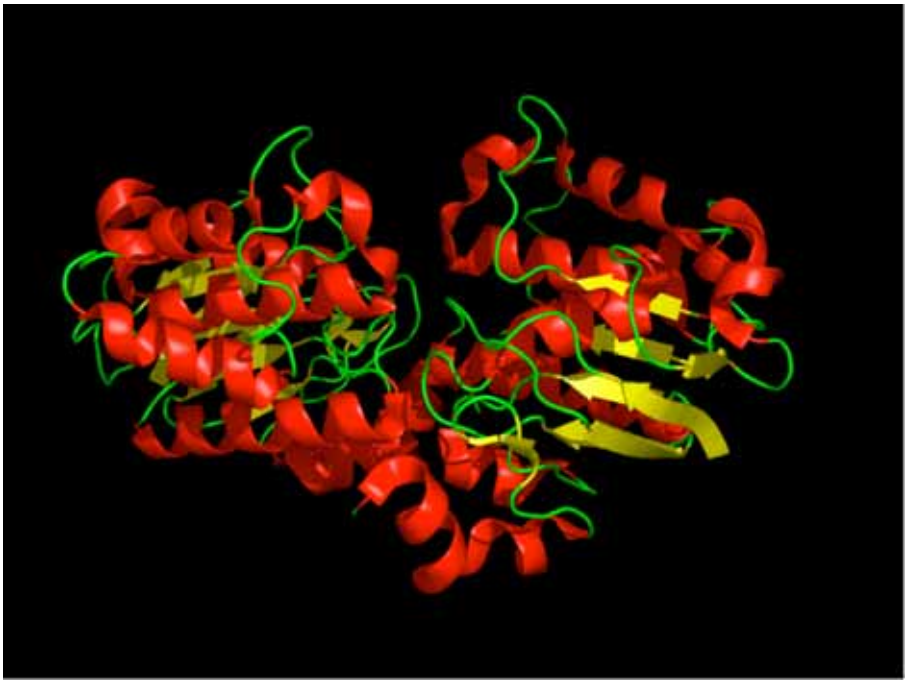

Fig. 6. The two ATP binding cassette domains, each composed of six β-sheets (yellow) surrounded by nine α-helices (red) and three β-sheets (yellow) in the periphery.

Walker A and Walker B Motifs

The Walker A motif defines the nucleotide binding site. It has been suggested to be crucial for the binding of the β and γ-phosphates of the nucleotide. The Walker A which is α-helical is linked to the Walker B which is β-strand by approximately 100 residues .[2] The walker B motif binds the attacking water molecule and Mg^{2+} ion as well as the γ phosphate.[4]

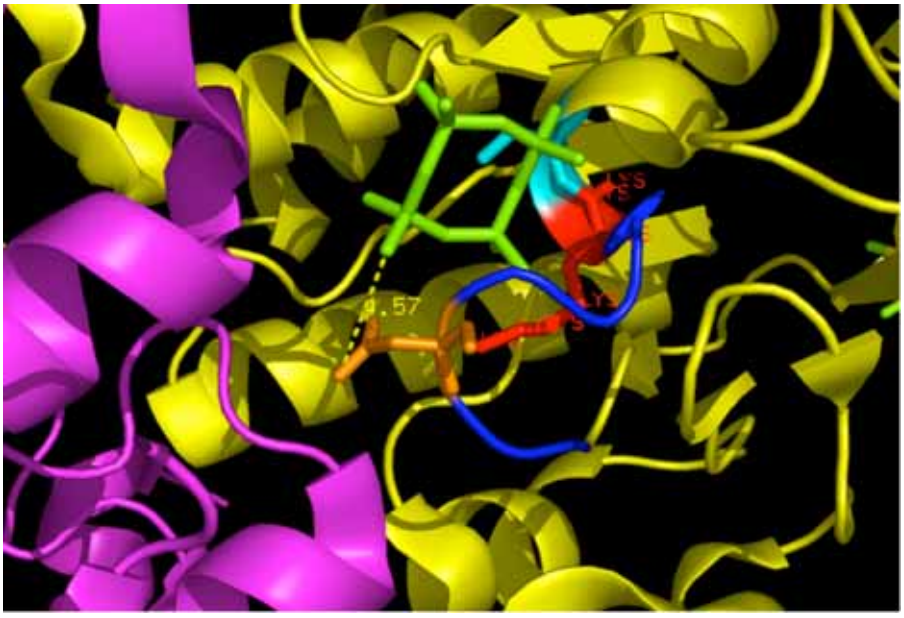

Fig. 7. The nucleotide binding site, Walker A motif that binds to the β and γ phosphates of the ATP molecule. It is a glycine rich sequence with a lysine residue at position 39 and an asparagine residue at position 39. (Scene F2-nov 28)

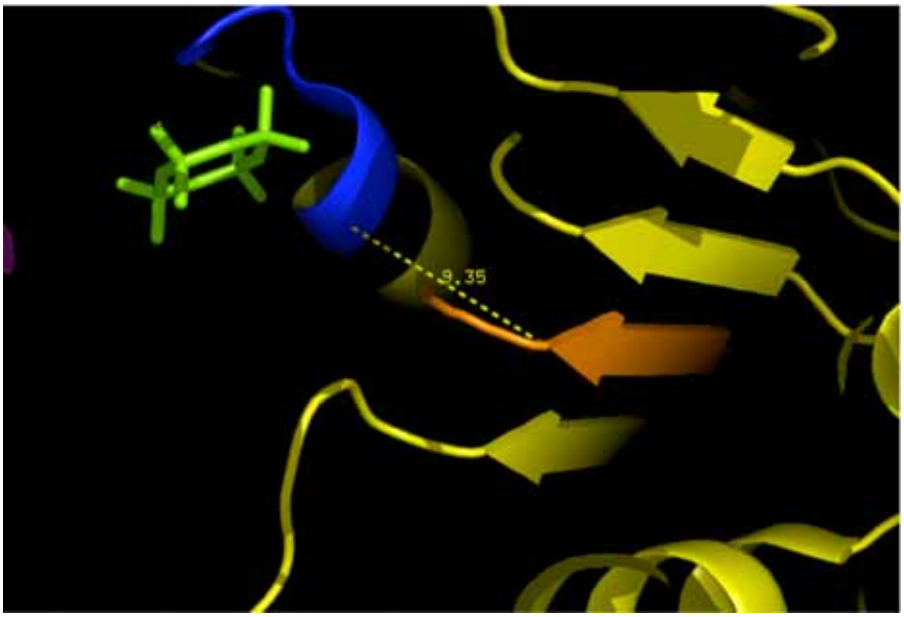

Fig. 8. Showing the location of the Walker B (orange) with respect to the Walker A. (Scene F4-nov 28)

The D-loop

The D loop is located five residues away from the Walker B. It is thought that the D loop is responsible for linking ATP hydrolysis in one dimer to ATP hydrolysis in the other dimer.[6]

The ABC family signature motif

The ABC family signature motif (LSGGE) of each cassette completes the ATP-binding site of the opposing cassette such that the two ATP molecules are buried in the dimer interface. It is believed that the movement of the signature motif is responsible for ATP hydrolysis.[3] The signature is located in between the Walker A and B motifs. The ABC family signature motif is responsible for linking ATP hydrolysis to functionally critical conformational changes in the ATP binding cassette.[6] The activation of ATP hydrolysis may be associated with the movement of the signature motif into contact with the phosphates of ATP.[7] Likewise the release of ADP and P_i following hydrolysis may require the withdrawal of the signature motif.[8] The ATP molecules are buried in the dimmer interface sandwiched between the P loop of one BtuD subunit and the conserved signature motif from the opposing subunit. The signature motif binds to an ATP γ-phosphate O via the Ser-109 sidechain OH and the Gly-111 main chain N.[6]

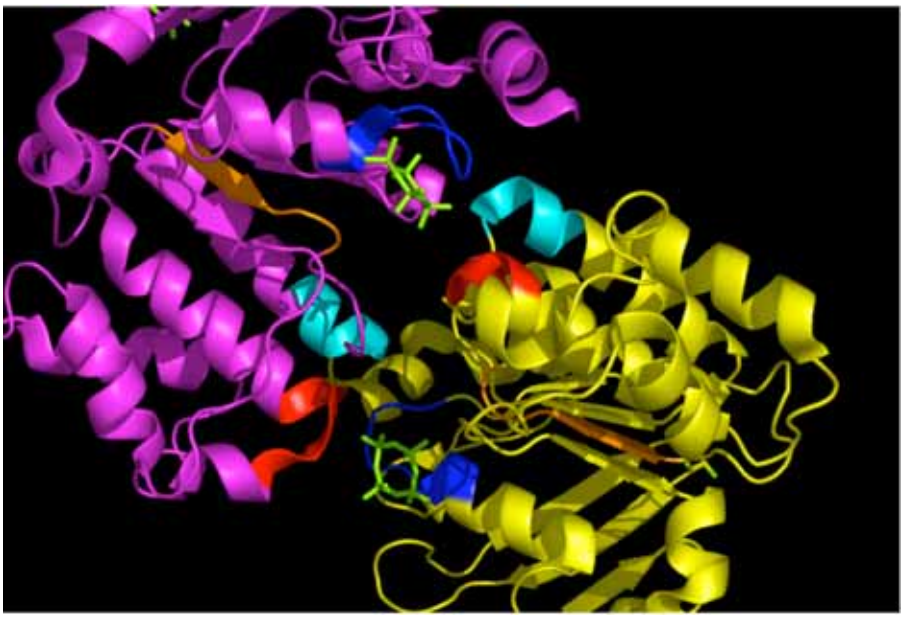

Fig 9. Showing the Walker A motif (blue), Walker B (orange), signature motif (red), D loop (cyan). Cyclotetravanadate (green) is bound in the nucleotide binding region. (Scene F5-nov 28)

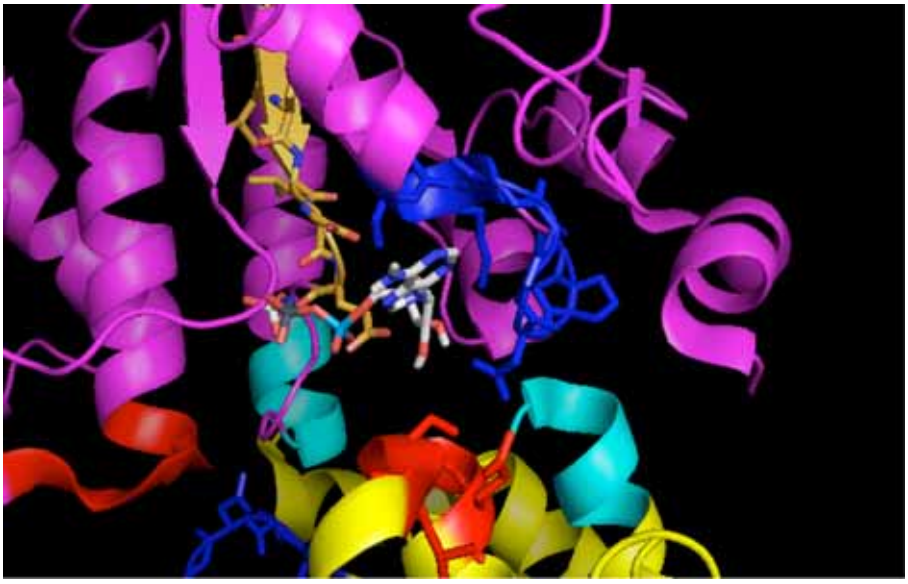

Fig. 10. Showing the nucleotide binding region with AMPNP.
(Scene F6-nov 28)

The switch region

The switch region is located approximately 25 residues down stream of the Walker B motif. It contains a histidine residue that is very well conserved in both E. coli and in yeast ABC-ATPases. Mutations of this histidine residue in some prokaryotic ABC-ATPases abolished transport function but not necessarily ATPase activity, suggesting a role for this region in coupling ATP hydrolysis to transport.[5]

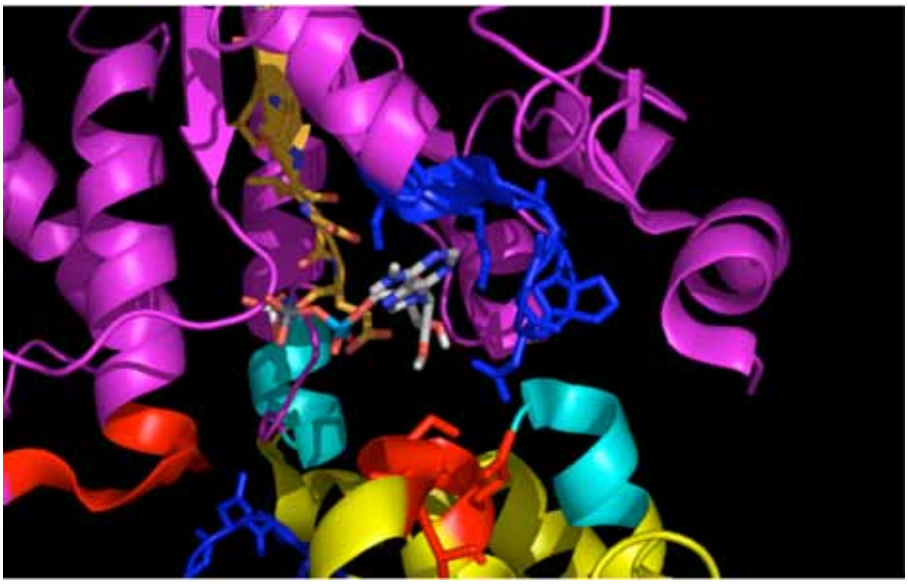

Fig 11. The conserved histidine residue in the switch region is shown in green.
(Scene F7-nov 28)

The Q loop

The Q loop also known as the γ-phosphate switch contains a glutamine that binds Mg^{2+} ion and the attacking water. It also involved in the structural contacts with the membrane spanning domains. The presence or absence of ATP in the nucleotide binding site influences the confirmation of the Q loop as well as the interactions it makes with the membrane spanning domain thereby providing a pathway for the coupling of ATP binding and hydrolysis with the rearrangements with the transmembrane regions.[4]

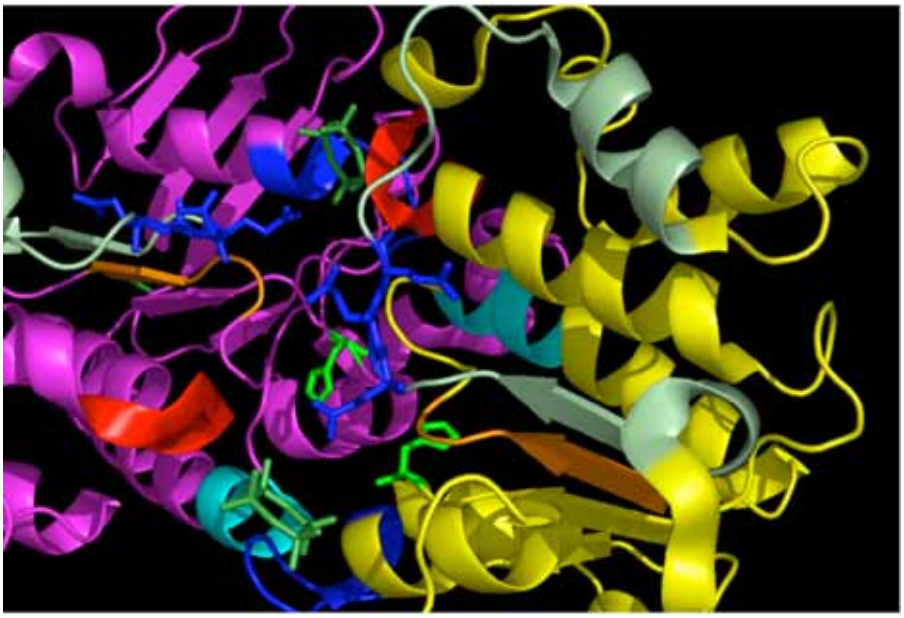

Fig. 12. The Q loop (light cyan) has three glutamine residues (blue), one of which binds Mg^{2+} ion and the attacking water. The Q loop forms structural contacts with the L loop of the membrane-spanning domain BtuC. (Scene F8-nov 28)

BtuC

Each BtuC subunit contains ten transmembrane helices that are packed intricately, crossing at different angles and sometimes breaking formation before reaching the opposite side of the membrane.[3]

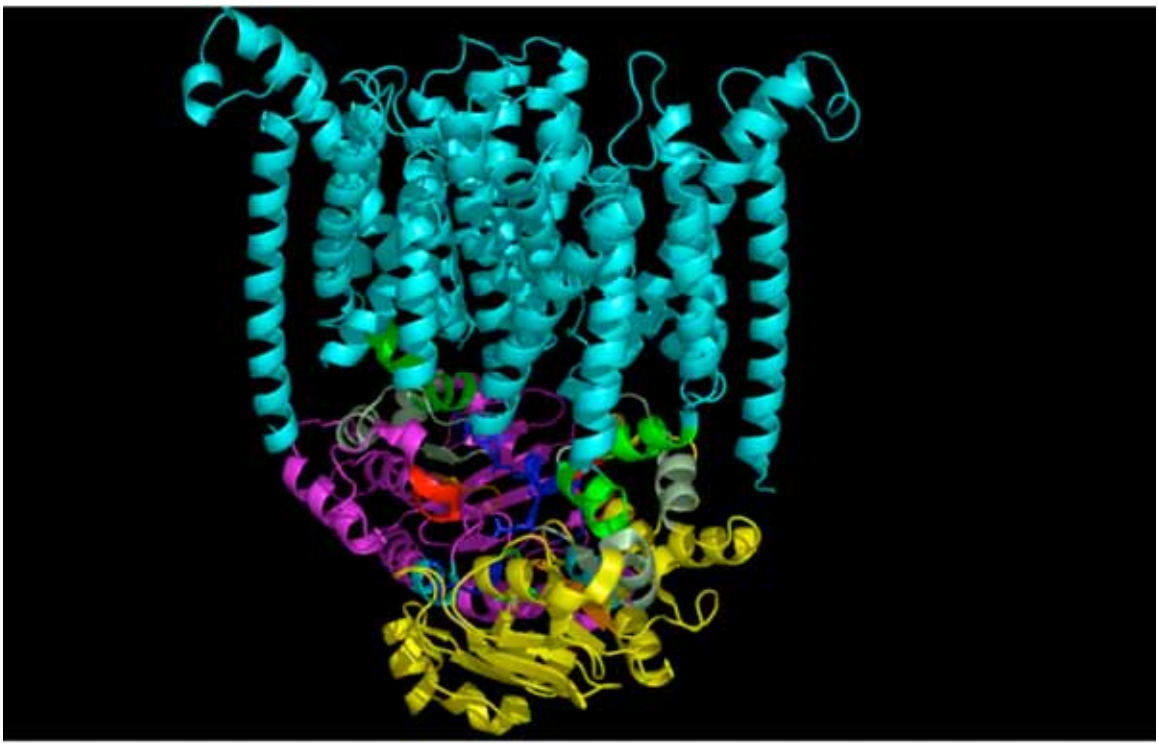

Fig. 13. The membrane-spanning subunits, BtuC (cyan). The L loop of BtuC (green) makes structural contacts with the ATP binding cassette (BtuD) via the Q loop (light cyan). (Scene F11-nov 28)

Model for the action of ABC-transporters

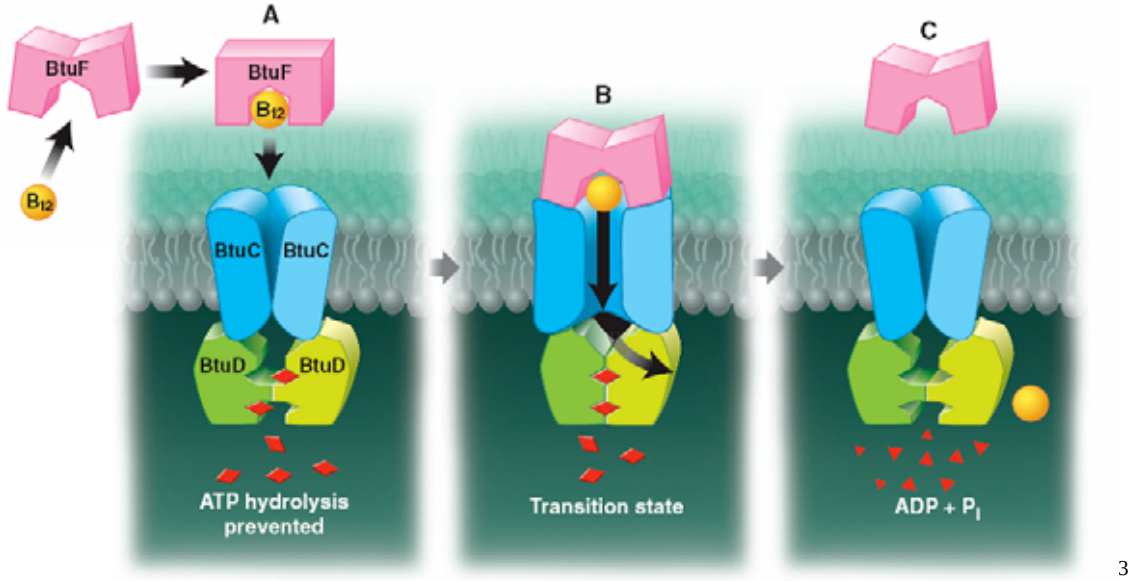

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Fig. 14. Model showing how ATP hydrolysis in the ATP cassette causes conformational changes in the membrane-spanning domains thus allowing the transport of vitamin B12 into the cytoplasm.

- ATP hydrolysis in the ATP binding cassette is coupled to conformational changes in the membrane-spanning domains.
- BtuF delivers vitamin B12 to the mouth of the transporter and binds to BtuC stabilizing the transporter in the transition state.
- BtuD motif (ABC signature) has moved together to complete the ATP-binding sites. That is the signature motif moves towards the Walker A burying the ATP molecule in the dimer interface. Movement of the ATP binding cassette is communicated to the membrane spanning domain through the contacts that the Q loop makes with the L loop.
- BtuF releases its grip on vitamin B12 and rearrangements in the transmembrane helix open a translocation pathway from the binding protein to the cytoplasm through a vestibule located in between the 4 subunits.
- After ATP hydrolysis and vitamin B12 release the transporter returns to the original state and BtuF is released.

References

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