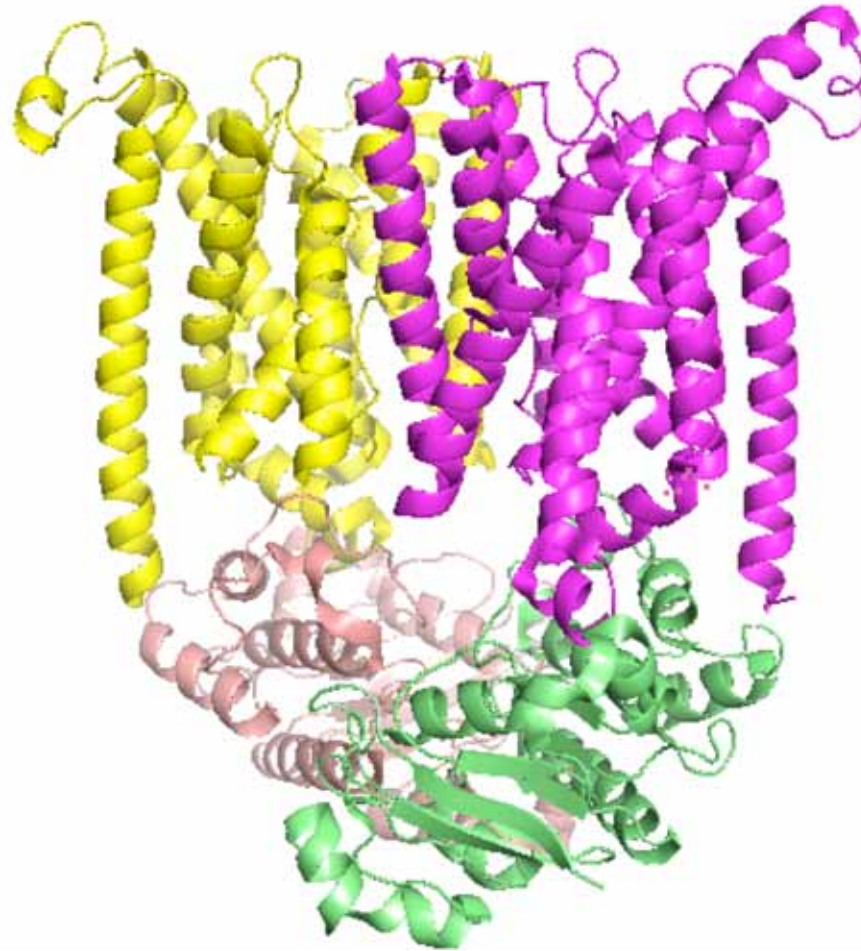


BtuCD – An ABC Transporter



Biomolecular Structures

Khadine A.Higgins

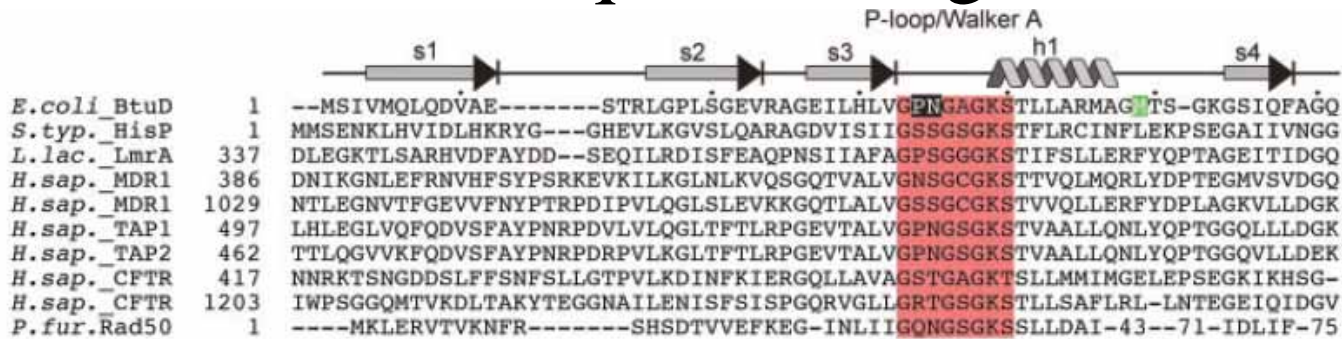
ABC Transporters

- 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 vary from a single atom to large polypeptides. The diversity of transported substrates is due to the poor sequence similarities of the membrane-spanning subunits and domains.
- In addition to transporting nutrients across the cell membrane, ABC transporters 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.
- They have been identified in organisms belonging to each of the three major domains (bacteria, archaea, and eukarya)

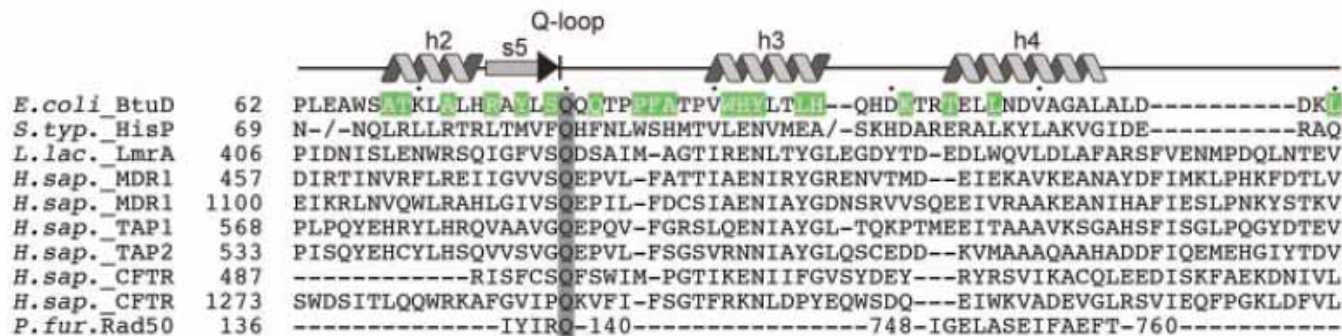
ABC Transporters

- In bacteria ABC transporters are composed of four separate protein subunits whereas in eukaryotes they 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.
- ABC cassette has many highly conserved motifs, some of which are involved in the binding and hydrolysis of ATP, such as the P loop or Walker-A motif, the Walker-B motif, a glutamine residue in the Q loop, a histidine residue in the Switch region, and a D loop. The ABC signature sequence, a short polypeptide stretch, (LSGGE).

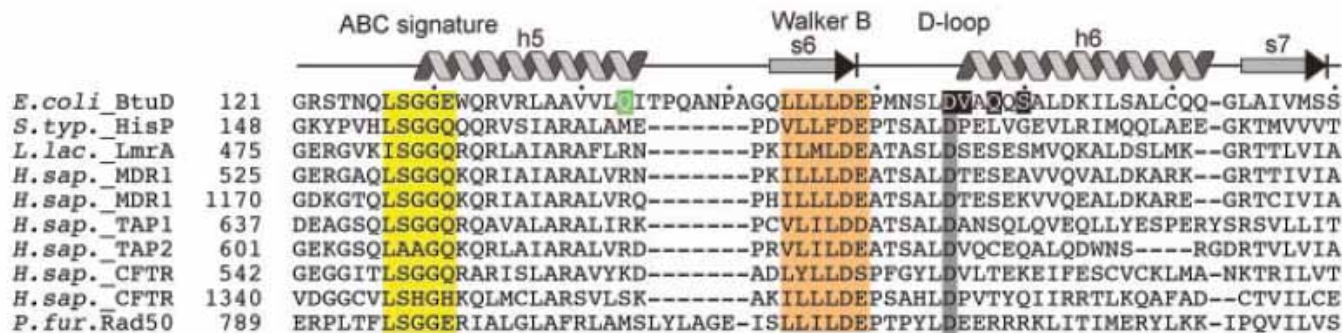
Sequence Alignment



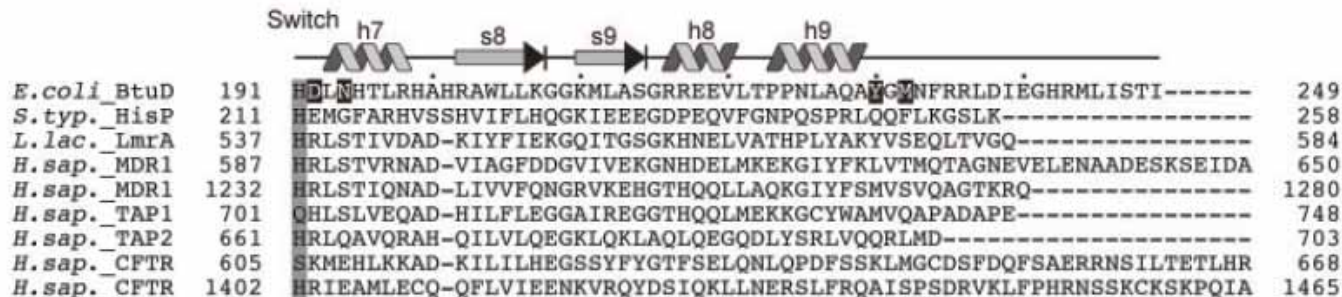
- 30% or more amino acid identity between ABC transporters



- Different folds



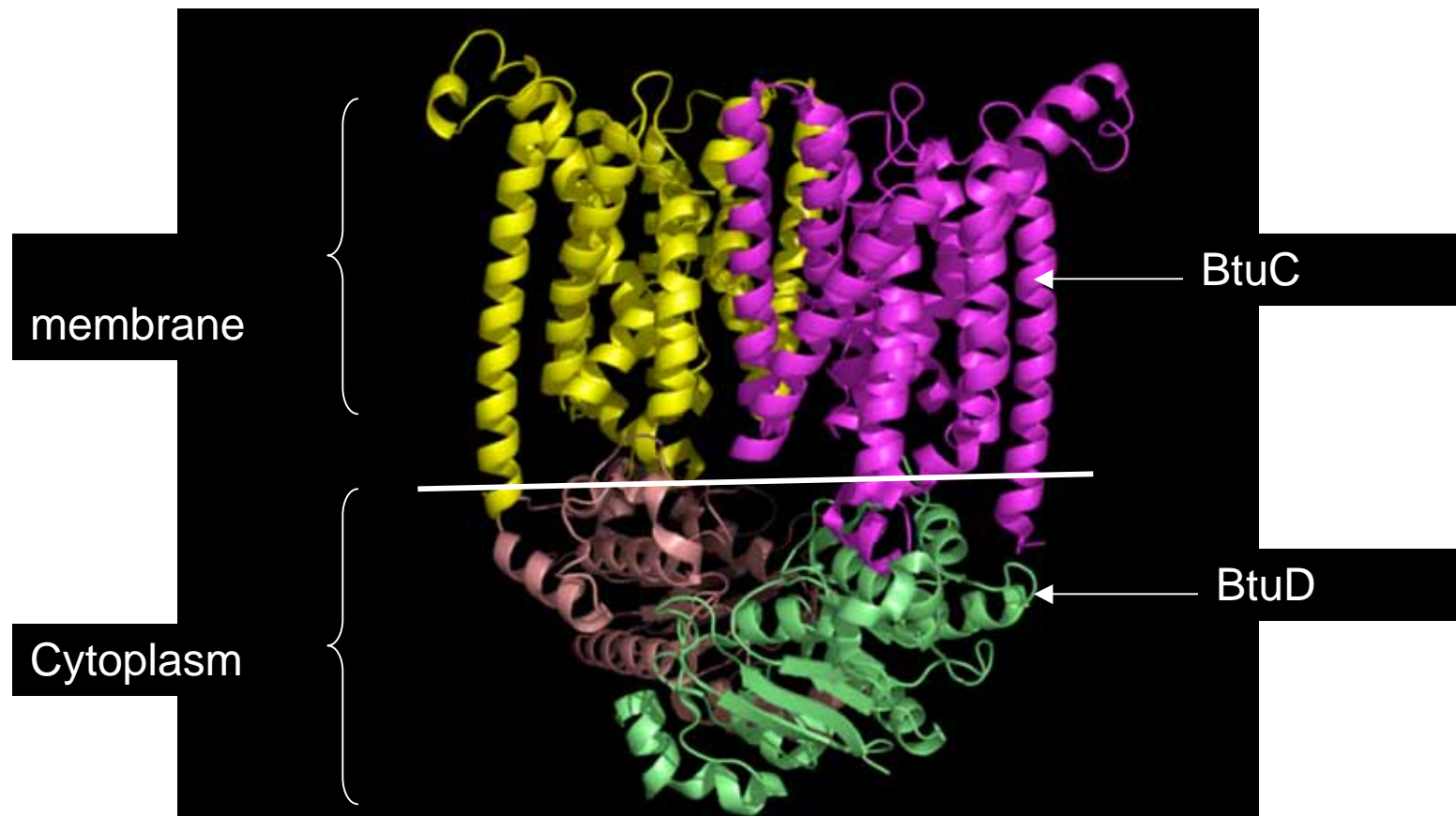
- Same function due to conserved motifs



The *E. coli* BtuCD protein

Pymol
part 2
F1-4

- ABC transporter mediating vitamin B₁₂ uptake.
- Consists of two ATP binding cassettes (BtuD) and two membrane-spanning subunits (BtuC). Both are in close contact with each other.

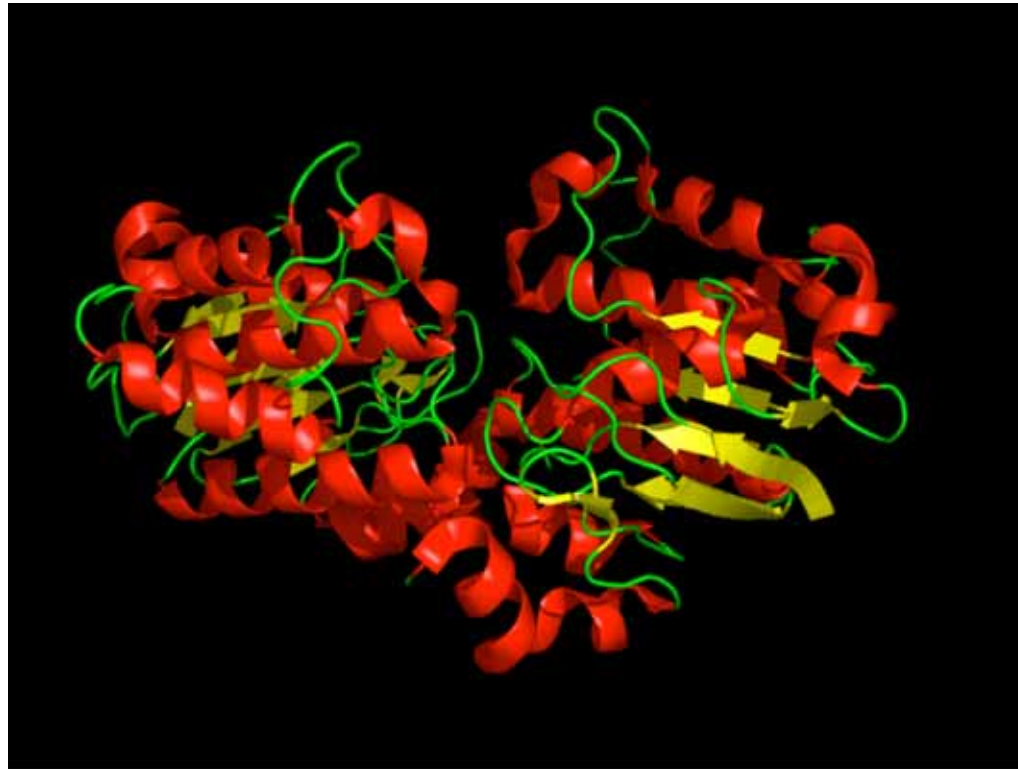


Structural information

- 3.2 Å resolution crystal structure
- R value – 0.262
- Free r value - 0.286
- amino acids - 8378
- Regions of the data where the occupancy and the b factors merge

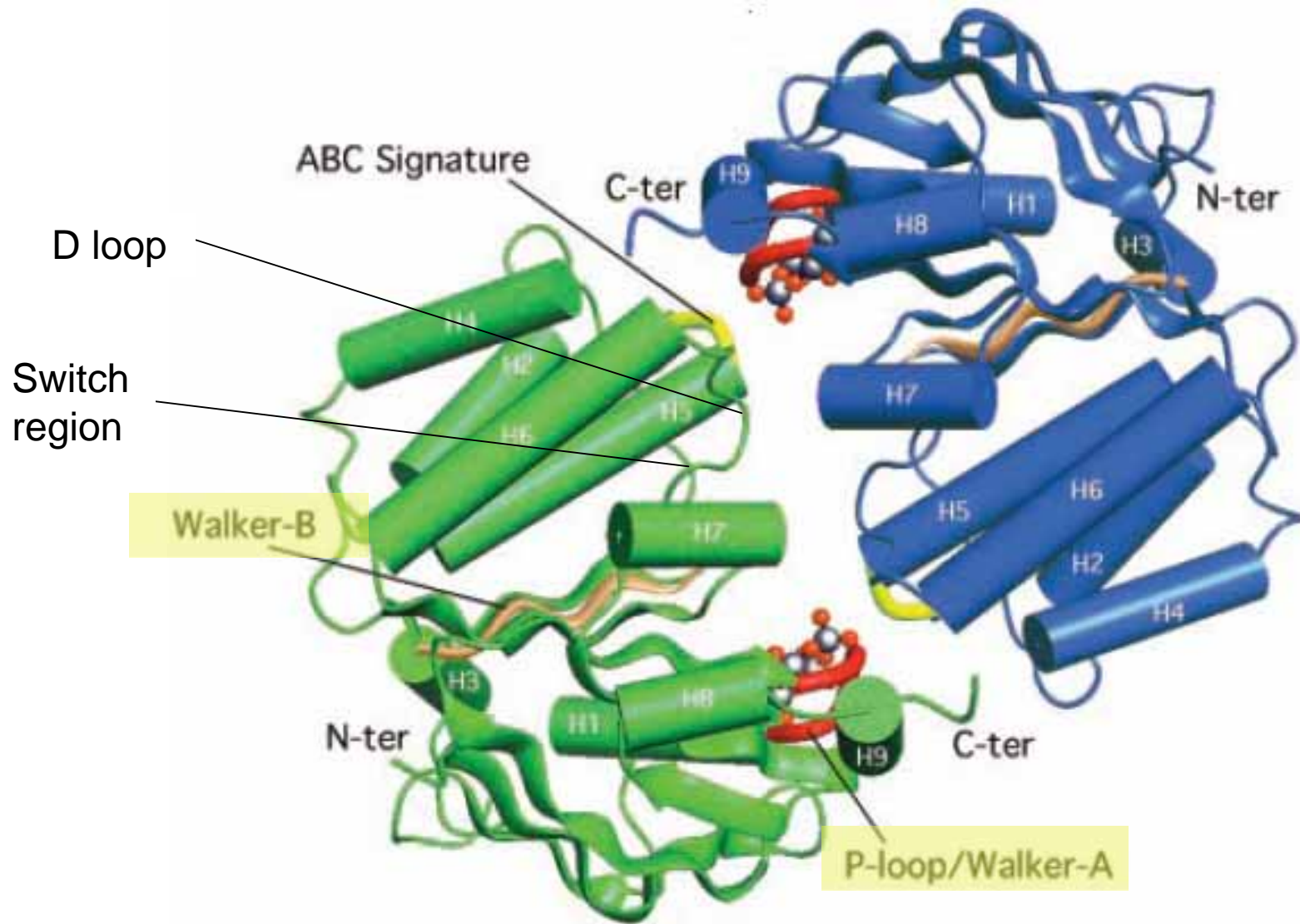
ATOM	70	CB	GLN	A	9	66.613	88.435	72.114	1.00123.41	C
ATOM	71	CG	GLN	A	9	66.511	89.258	73.384	1.00126.37	C
ATOM	72	CD	GLN	A	9	67.849	89.801	73.834	1.00128.76	C
ATOM	73	OE1	GLN	A	9	68.606	90.350	73.036	1.00130.35	O
ATOM	74	NE2	GLN	A	9	68.142	89.660	75.121	1.00130.55	N
ATOM	75	N	ARG	A	10	63.901	89.651	71.080	1.00 99.24	N

BtuD

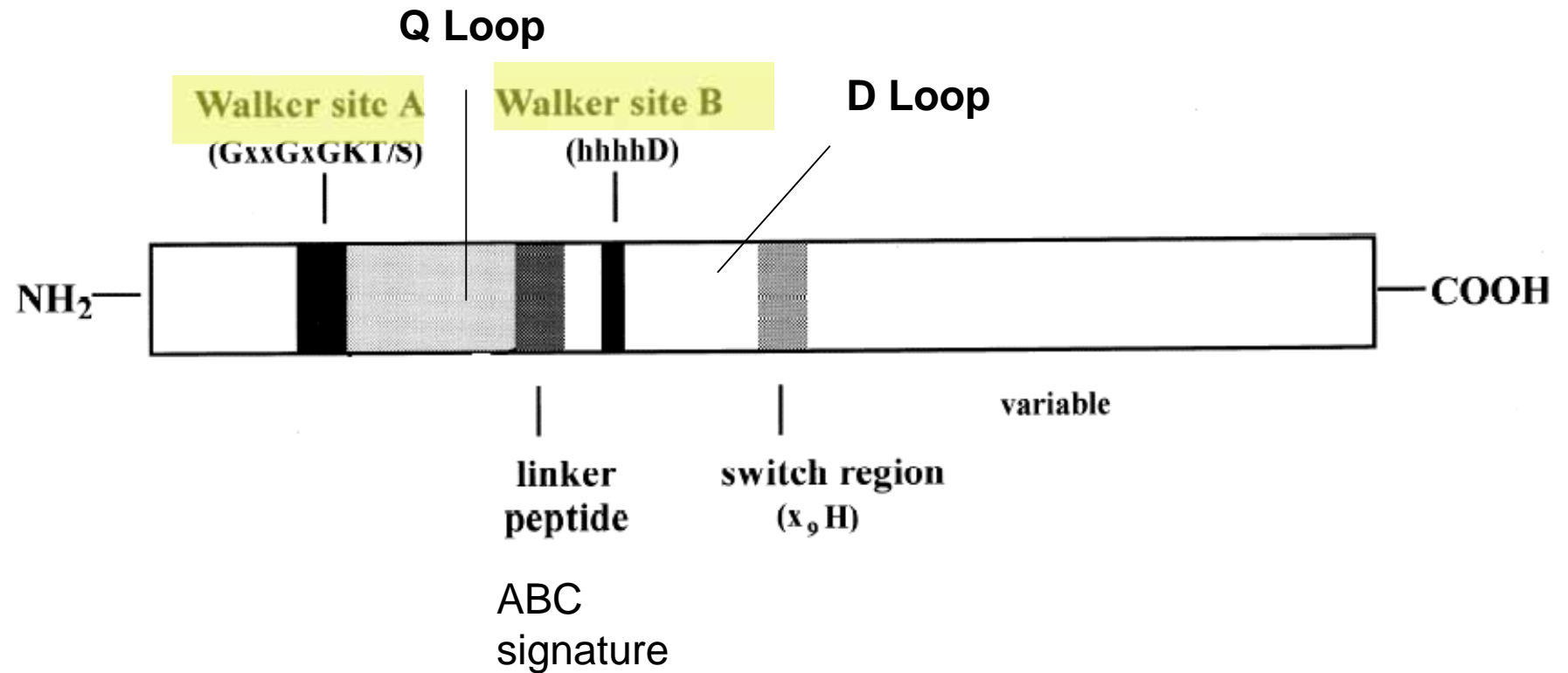


- Consists of six-stranded β -sheet in the core surrounded by nine α -helices and a peripheral, three-stranded β -sheet.
- Powers the transport of vitamin B12 through ATP hydrolysis.
- Conserved motifs: The Walker A motif or P loop, the Walker B motif, a glutamine residue in the Q loop, a histidine residue in the Switch region, a D loop and a short polypeptide sequence (LSGGE) referred to as the “ABC signature sequence.”

BtuD



BtuD



Schneider, E.; Hunke, S., ATP-binding-cassette (ABC) transport systems: Functional and structural aspects of the ATP-hydrolyzing subunits/domains. *Fems Microbiology Reviews* **1998**, 22, (1), 1-20.

Walker A motif

- Nucleotide binding site, binds to the β and γ phosphates
- Glycine rich sequence

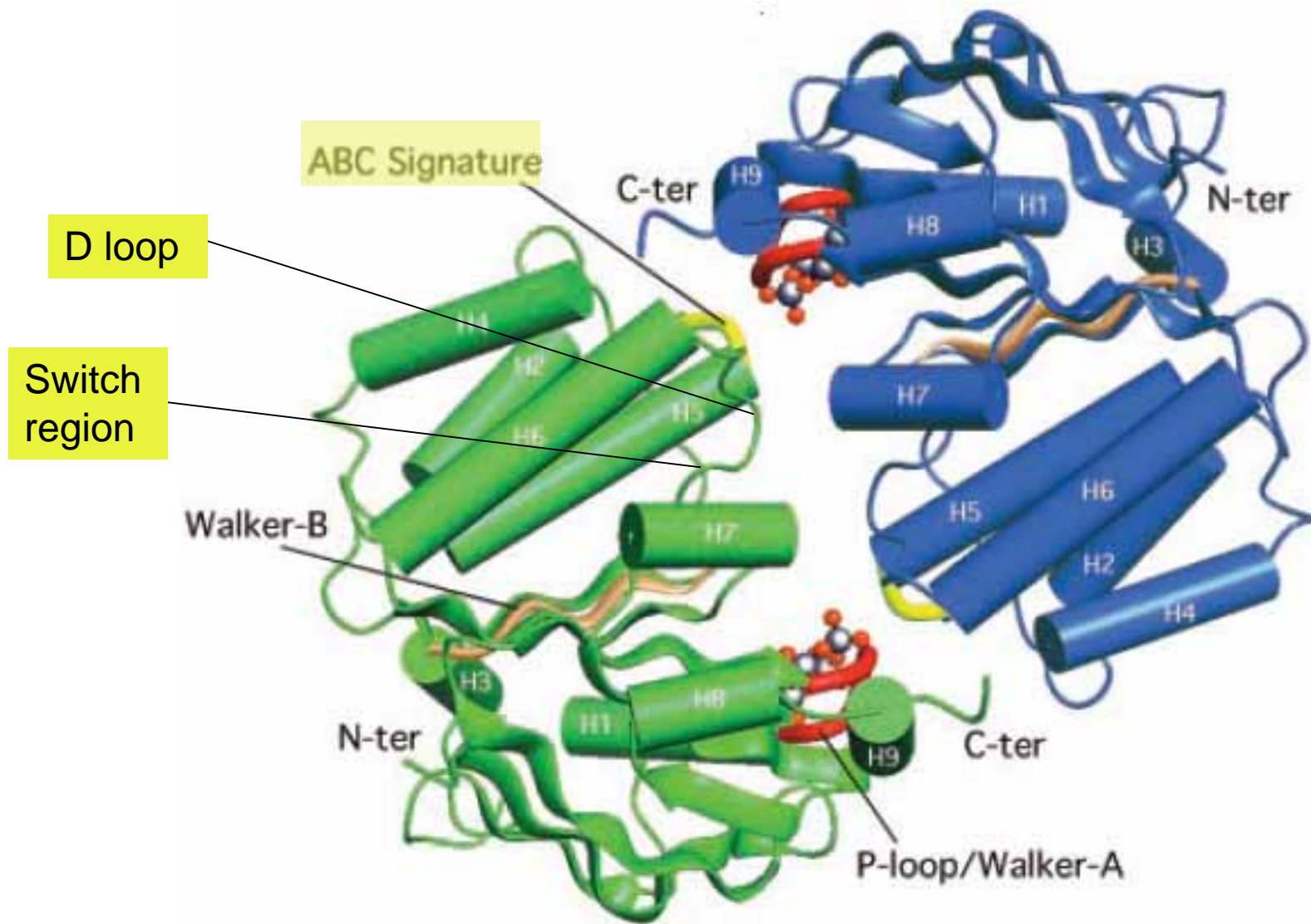
Pymol part 1 F2 &3

Walker B motif

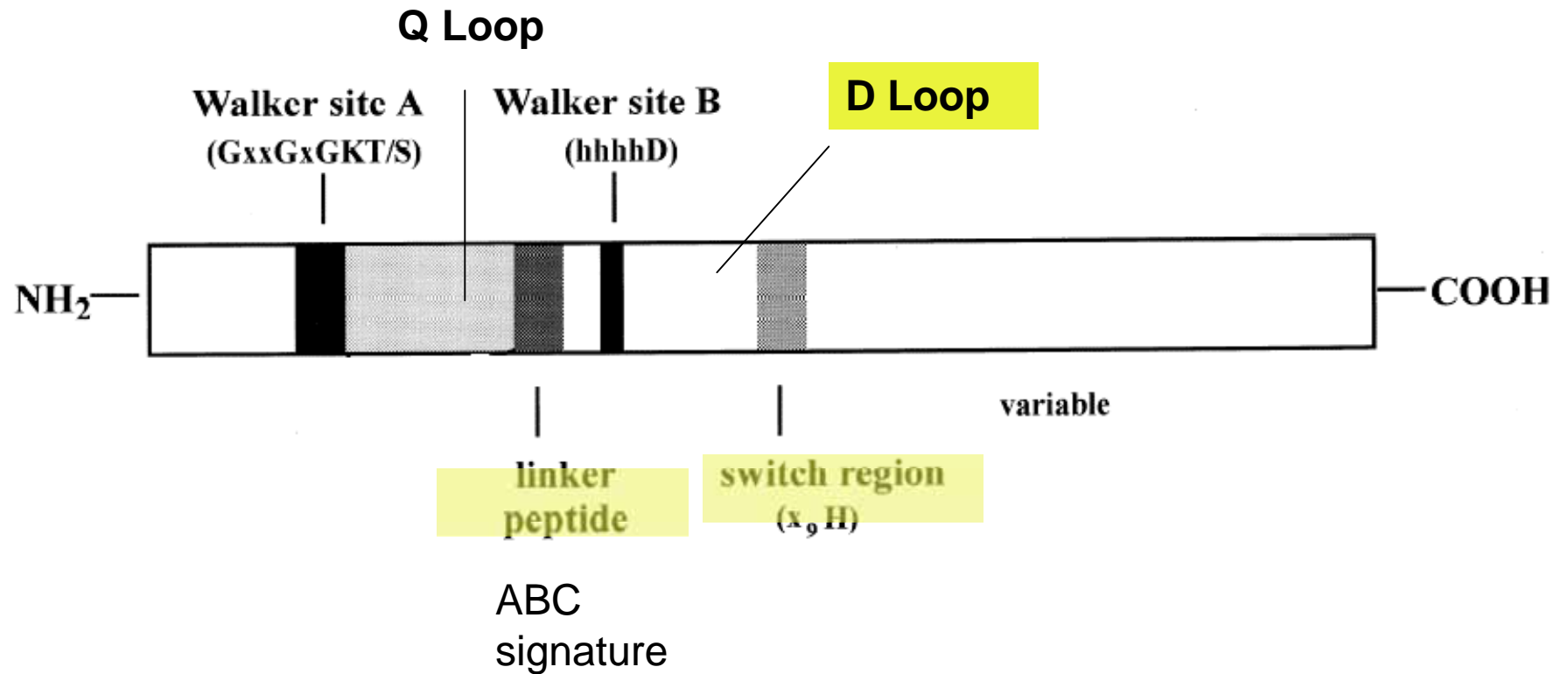
- Binds to the attacking water molecule and Mg^{2+} ion as well as the gamma phosphate of ATP.

Pymol part 1 F4

BtuD



BtuD



Schneider, E.; Hunke, S., ATP-binding-cassette (ABC) transport systems: Functional and structural aspects of the ATP-hydrolyzing subunits/domains. *Fems Microbiology Reviews* **1998**, 22, (1), 1-20.

Signature Motif and the D Loop

Pymol
part 1
F5 &6

- The ABC family signature motif (LSGGE) completes the ATP-binding site of the opposing cassette such that ATP molecules are buried in the dimer interface.
- Responsible for linking ATP hydrolysis to conformational changes in the ATP-binding cassette.
- Binds to an ATP γ -phosphate via Ser-109 sidechain and a mainchain N on Gly111.
- The activation of ATP hydrolysis is thought to be associated with the movement of the signature motif contact with the phosphates of ATP. Likewise the release of ADP and Pi may require the withdrawal of the signature motif.
- D loop is thought to be responsible for linking ATP hydrolysis in one dimer to ATP hydrolysis in the other.

The switch region

- Located approximately 25 residues down stream of the Walker B motif.
- Contains a well conserved histidine residue. Mutations of this residue in some prokaryotic transporters abolished transport and function.

Pymol part 1 F7

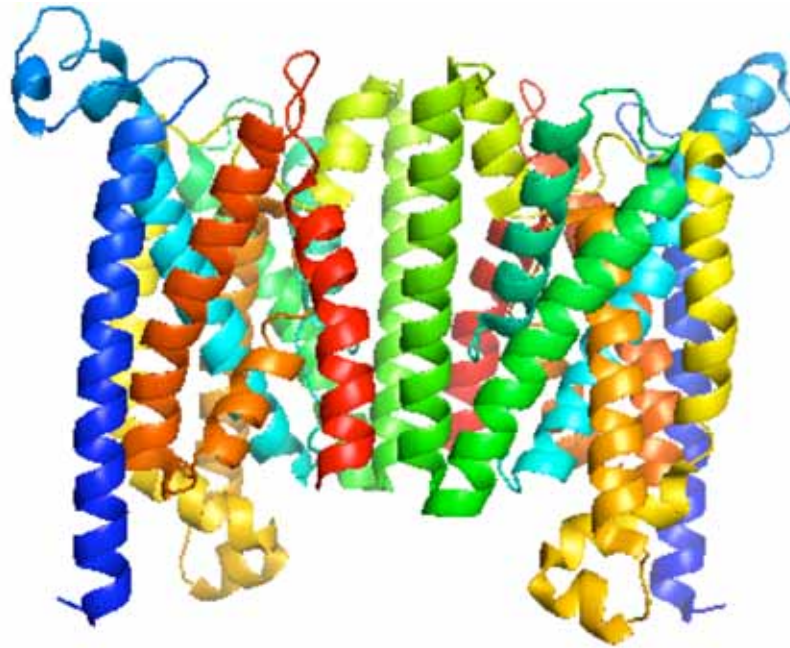
The Q loop

- Forms structural contacts with the membrane spanning domain.
- Glutamine residue binds to the Mg^{2+} ion and the attacking water molecule.

Pymol part 1 F8

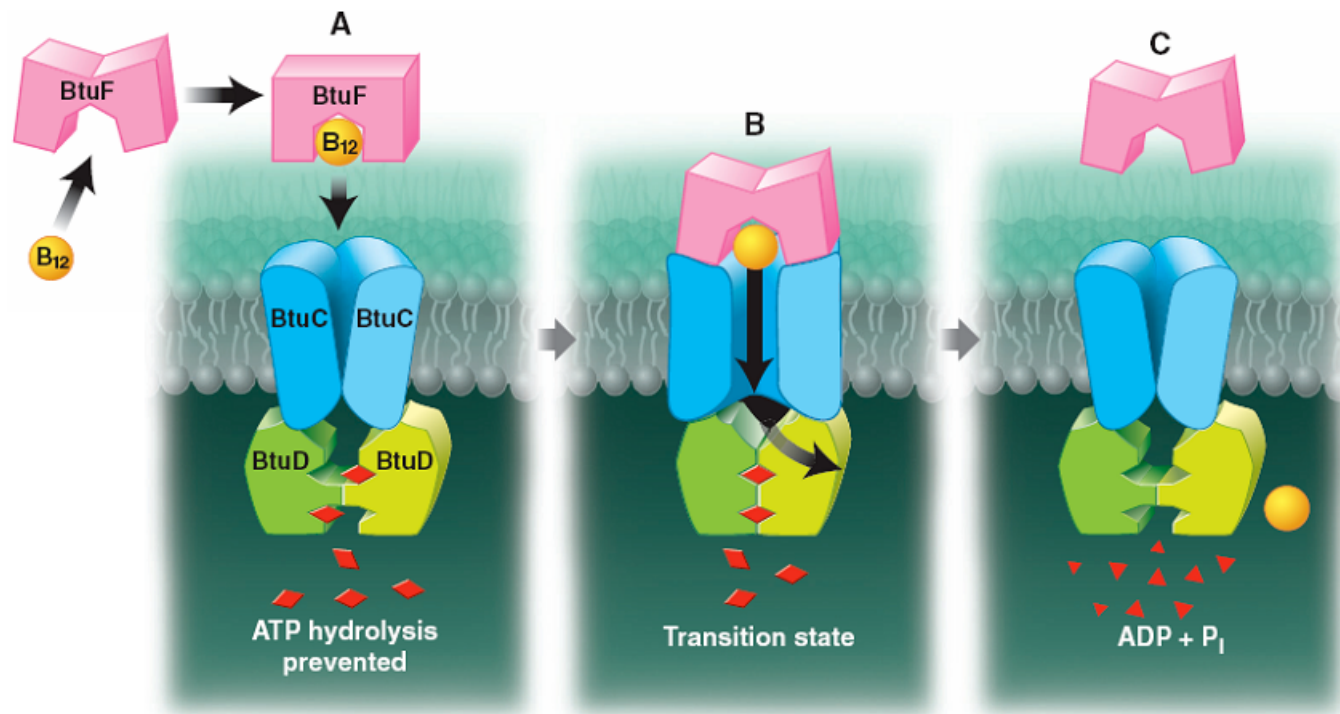
BtuC

Pymol
part 1
F9 -11



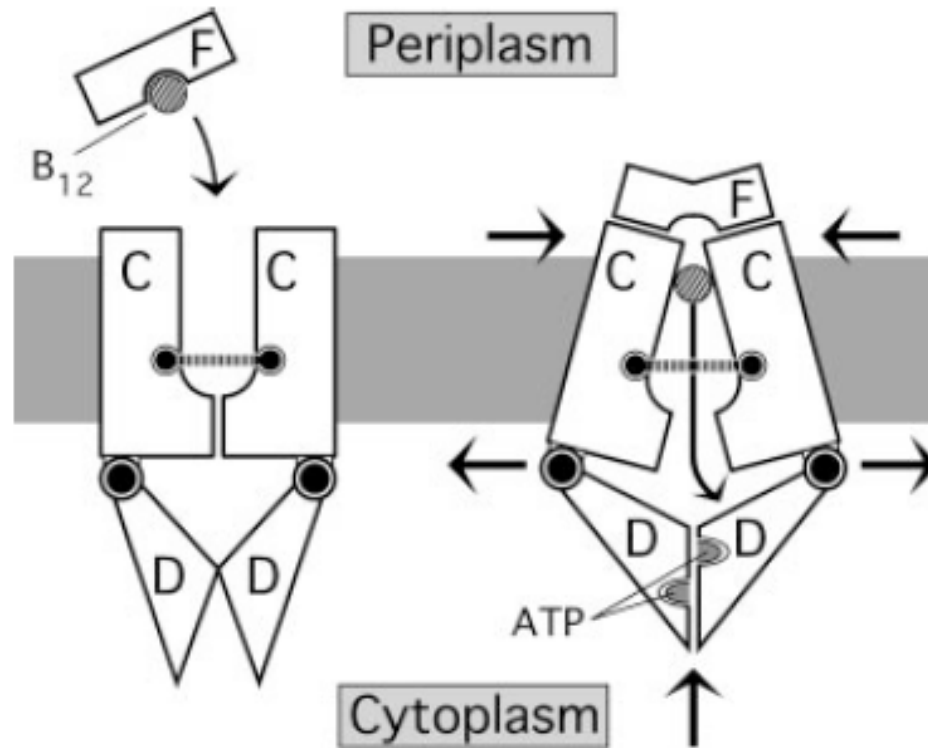
- Consists of 20 transmembrane helices.
- Cytoplasmic loop, L loop (conserved motif) forms the contact region with the ATP-binding cassette, BtuD.

Model - The ABC of transport



Davidson, A. L., Structural biology. Not just another ABC transporter. *Science* **2002**, 296, (5570), 1038-40

Model - The ABC of transport



Locher, K. P.; Lee, A. T.; Rees, D. C., The E. coli BtuCD structure: a framework for ABC transporter architecture and mechanism. *Science* **2002**, 296, (5570), 1091-8.

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