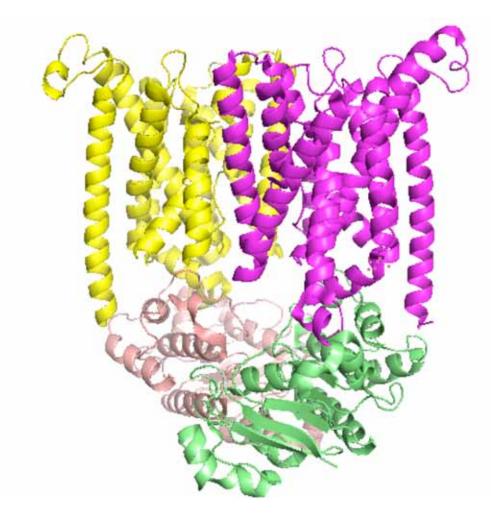
#### BtuCD – An ABC Transporter



**Biomolecular Structures** 

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# 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 polypetides. 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, archea, 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 cassesttes (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.

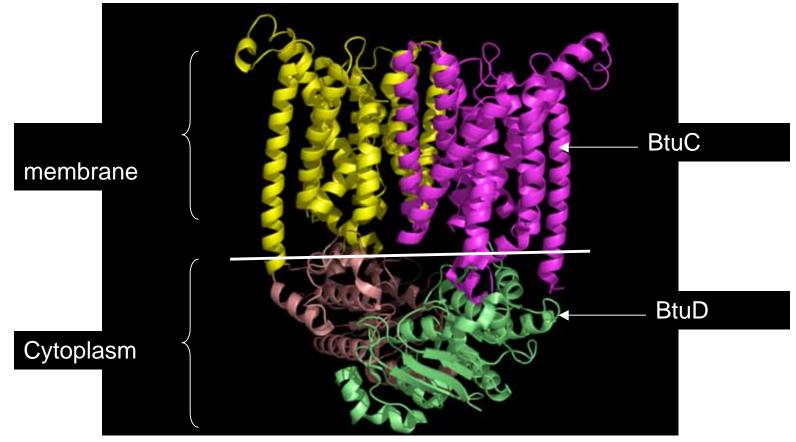
• ABC cassette has many highly conserved motifs, some of which are involved in the binding and hydrolysis of ATP, such as the P loop or Walker-A motif, the Walker-B motif, a glutamine residue in the Q loop, a histidine residue in the Switch region, and a D loop. The ABC signature sequence, a short polypeptide stretch, (LSGGE).

Sequence Alignment						
		P-loop/Walker A				
E.coli_BtuD S.typHisP L.lacLmrA H.sapMDR1 H.sapMDR1 H.sapTAP1	1 337 386 1029 497	MSIVMQLQDVAESTRLGPLSGEVRAGEILHLVGENGAGKSTLLARMAG TS-GKGSIQFAGQ MMSENKLHVIDLHKRYGGHEVLKGVSLQARAGDVISIIGSSGSGKSTFLRCINFLEKPSEGAIIVNGG DLEGKTLSARHVDFAYDDSEQILRDISFEAQPNSIIAFAGPSGGGKSTIFSLLERFYQPTAGEITIDGQ DNIKGNLEFRNVHFSYPSRKEVKILKGLNLKVQSGQTVALVGNSGCGKSTTVQLMQRLYDPTEGMVSVDGQ NTLEGNVTFGEVVFNYPTRPDIPVLQGLSLEVKKGQTLALVGSSGCGKSTVVQLLERFYDPLAGKVLLDGK LHLEGLVQFQDVSFAYPNRPDVLVLQGLTFTLRPGEVTALVGPNGSGKSTVAALLQNLYQPTGGQLLLDGK	<ul> <li>30% or more amino acid</li> </ul>			
H.sapTAP2 H.sap. CFTR	462 417	TTLQGVVKFQDVSFAYPNRPDRPVLKGLTFTLRPGEVTALVGPNGSGKSTVAALLQNLYQPTGGQVLLDEK NNRKTSNGDDSLFFSNFSLLGTPVLKDINFKIERGQLLAVAGSTGAGKTSLLMMIMGELEPSEGKIKHSG-	identity			
H.sap. CFTR P.fur.Rad50	1203	IWPSGGQMTVKDLTAKYTEGGNAILENISFSISPGQRVGLLGRTGSGKSTLLSAFLRL-LNTEGEIQIDGV	•			
P.101.Ra050	1	MKLERVTVKNFRSHSDTVVEFKEG-INLIIGONGSGKSSLLDAI-4371-IDLIF-75	between ABC			
		Q-loop h2 s5 h3 h4	transporters			
		000000 10000	transporters			
E.coli_BtuD S.typHisP L.lacLmrA H.sap. MDR1	62 69 406 457	PLEAWS KL LH A L OO TP TFV LTOHD TR EL NDVAGALALDDK N-/-NQLRLLRTRLTMVFOHFNLWSHMTVLENVMEA/-SKHDARERALKYLAKVGIDERAQ PIDNISLENWRSQIGFVSDSAIM-AGTIRENLTYGLEGDYTD-EDLWQVLDLAFARSFVENMPDQLNTEV DIRTINVRFLREIIGVVSOEPVL-FATTIAENIRYGRENVTMDEIEKAVKEANAYDFIMKLPHKFDTLV				
H.sapMDR1	1100	EIKRLNVQWLRAHLGIVSOEPIL-FDCSIAENIAYGDNSRVVSQEEIVRAAKEANIHAFIESLPNKYSTKV				
H.sapTAP1 H.sap. TAP2	568 533	PLPQYEHRYLHRQVAAVGQEPQV-FGRSLQENIAYGL-TQKPTMEEITAAAVKSGAHSFISGLPQGYDTEV PISQYEHCYLHSQVVSVGQEPVL-FSGSVRNNIAYGLQSCEDDKVMAAAQAAHADDFIQEMEHGIYTDV	<ul> <li>Different</li> </ul>			
H.sapCFTR	487 1273	RISFCSOFSWIM-PGTIKENIIFGVSYDEYRYRSVIKACQLEEDISKFAEKDNIVL	folds			
H.sapCFTR P.fur.Rad50	136	SWDSITLQQWRKAFGVIPOKVFI-FSGTFRKNLDPYEQWSDQEIWKVADEVGLRSVIEQFPGKLDFVL 748-IGELASEIFAEFT-760	10105			
		ABC signature h5 Walker B D-loop h6 s7				
E.coli_BtuD	121	GRSTNQLSGGEWQRVRLAAVVL ITPQANPAGQLLLLDEPMNSL DVAQQSALDKILSALCQQ-GLAIVMSS				
S.typHisP L.lac. LmrA	148	GKYPVHLSGGQQQRVSIARALAMEPDVLLFDEPTSALDPELVGEVLRIMQQLAEE-GKTMVVVT GERGVKISGGQRORLAIARAFLRNPKILMLDEATASLDSESESMVQKALDSLMKGRTTLVIA	•Same			
H.sap. MDR1 H.sap. MDR1	525 1170	GERGAQLSGGQKQRIAIARALVRNPKILLLDEATSALDTESEAVVQVALDKARKGRTTIVIA				
H.sapTAP1	637	GDKGTQLSGGQKQRIAIARALVRQPHILLLDEATSALDTESEKVVQEALDKAREGRTCIVIA DEAGSQLSGGQRQAVALARALIRKPCVLILDDATSALDANSQLQVEQLLYESPERYSRSVLLIT	function due			
H.sapTAP2 H.sap. CFTR	601 542	GEKGSQLAAGQKQRLAIARALVRDPRVLILDEATSALDVQCEQALQDWNSRGDRTVLVIA GEGGITLSGGQRARISLARAVYKDADLYLLDSPFGYLDVLTEKEIFESCVCKLMA-NKTRILVT	to conserved			
H.sap. CFTR	1340	VDGGCVLSHGHKQLMCLARSVLSKAKILLLDEPSAHLDPVTYQIIRRTLKQAFADCTVILCE				
P.fur.Rad50	789	ERPLTFLSGGERIALGLAFRLAMSLYLAGE-ISLLILDEPTPYLDEERRRKLITIMERYLKK-IPQVILVS	motifs			
	5	Switch h7 s8 s9 h8 h9				
E.coli_BtuD S.typHisP L.lacLmrA H.sapMDR1 H.sapMDR1 H.sapTAP1 H.sapTAP2 H.sapCFTR H.sapCFTR	191 211 537 587 1232 701 661 605 1402	NM       S8       S9       NO       NS         HELMHTLRHAHRAWLLKGGKMLASGRREEVLTPPNLAQANGGNFRRLDIEGHRMLISTI       249         HEMGFARHVSSHVIFLHQGKIEEEGDPEQVFGNPQSPRLQOFLKGSLK       258         HRLSTIVDAD-KIYFIEKGQITGSGKHNELVATHPLYAKYVSEQLTVGQ       584         HRLSTIVDAD-KIYFIEKGQITGSGKHNELVATHPLYAKYVSEQLTVGQ       584         HRLSTURNAD-VIAGFDDGVIVEKGNHDELMKEKGIYFKLVTMQTAGNEVELENAADESKSEIDA       650         HRLSTURAD-LIVVFQNGRVKEHGTHQQLLAQKGIYFSMVSVQAGTKRQ       1280         OHLSLVEQAD-HILFLEGGAIREGGTHQQLMEKKGCYWAMVQAPADAPE       748         HRLQAVQRAH-QILVLQEGKLQKLAQLQEGQDLYSRLVQQRLMD       703         SKMEHLKKAD-KILILHEGSSYFYGTFSELQNLQPDFSSKLMGCDSFDQFSAERRNSILTETLHR       668         HRIEAMLECQ-QFLVIEENKVRQYDSIQKLLNERSLFRQAISPSDRVKLFPHRNSSKCKSKPQIA       1465				

## The E. coli BtuCD protein

• ABC transporter mediating vitamin B<sub>12</sub> uptake.

• Consists of two ATP binding cassettes (BtuD) and two membrane- spanning subunits (BtuC). Both are in close contact with each other.

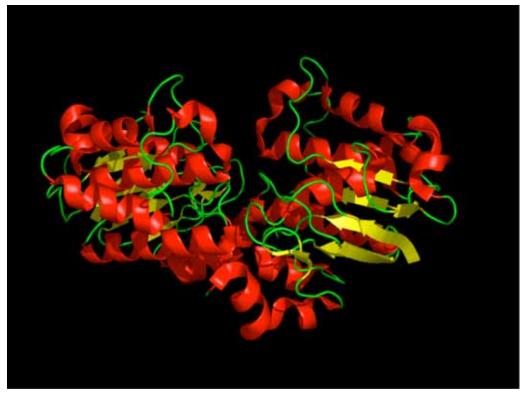


Pymol part 2 F1-4

## Structural information

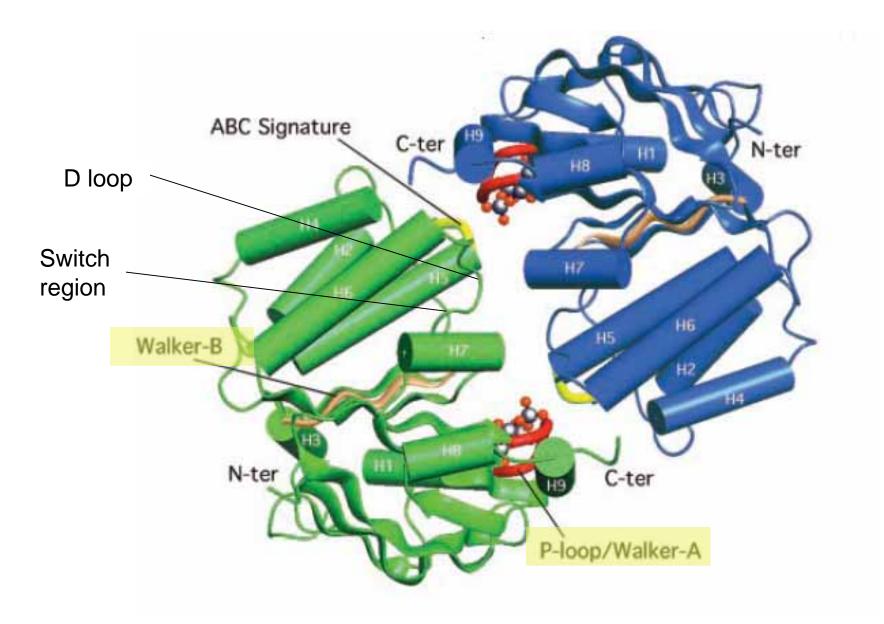
- 3.2 Å resolution crystal structure
- R value 0.262
- •Free r value 0.286
- amino acids 8378
- Regions of the data where the occupany and the b factors merge

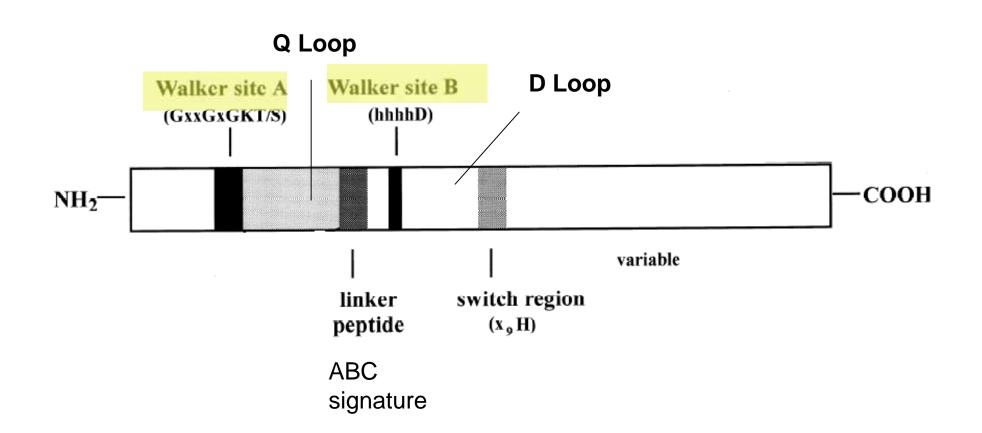
ATOM	70 CB GLN A 9	66.613 88.435 72.114 1.00123.41	С
ATOM	71 CG GLNA 9	66.511 89.258 73.384 1.00126.37	С
ATOM	72 CD GLN A 9	67.849 89.801 73.834 1.00128.76	С
ATOM	73 OE1 GLN A 9	68.606 90.350 73.036 1.00130.35	0
ATOM	74 NE2 GLN A 9	68.142 89.660 75.121 1.00130.55	Ν
ATOM	75 N ARGA 10	63.901 89.651 71.080 1.00 99.24	Ν



- Consists of six-stranded  $\beta$ -sheet in the core surrounded by nine  $\alpha$ -helices and a peripheral, three-stranded  $\beta$ -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."





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

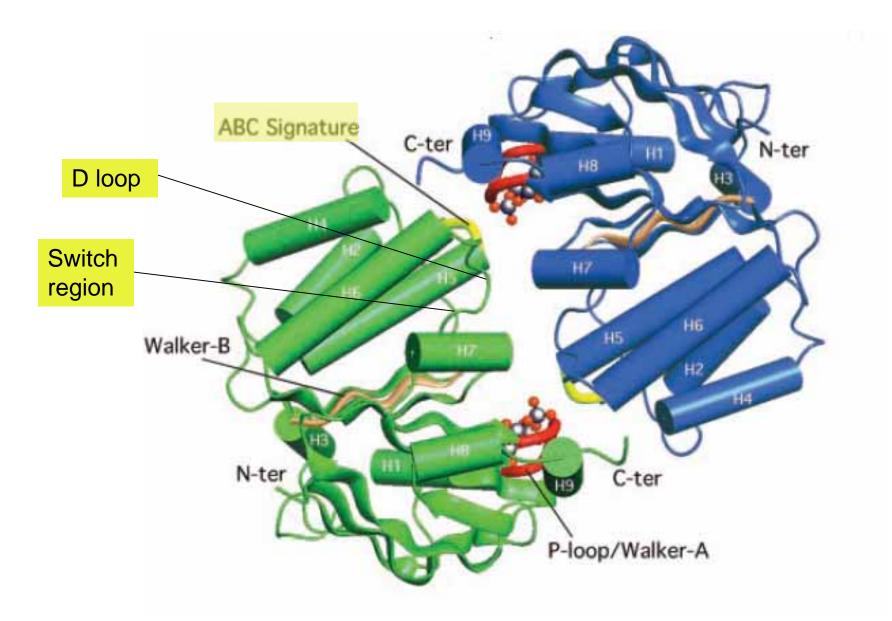
- Nucleotide binding site, binds to the  $\beta$  and  $\gamma$  phosphates
- •Glycine rich sequence

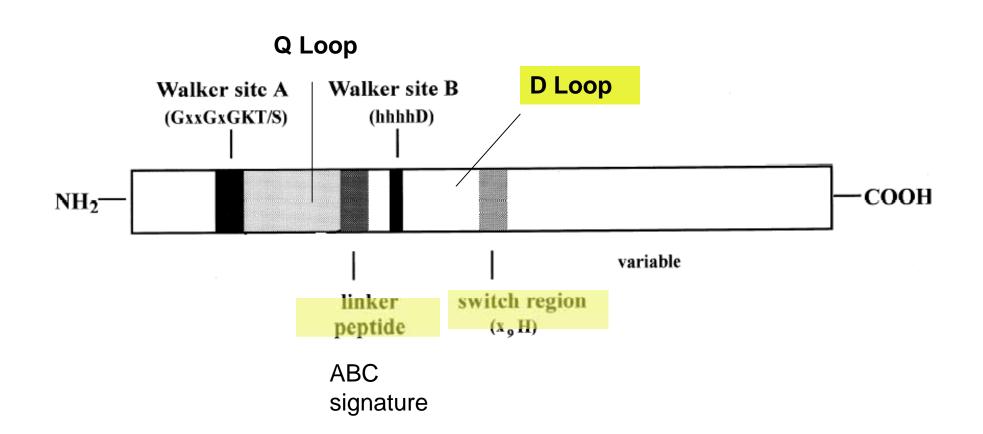
Pymol part 1 F2 &3

# Walker B motif

• Binds to the attacking water molecule and Mg<sup>2+</sup> ion as well as the gamma phosphate of ATP.

Pymol part 1 F4





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-phospate 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.

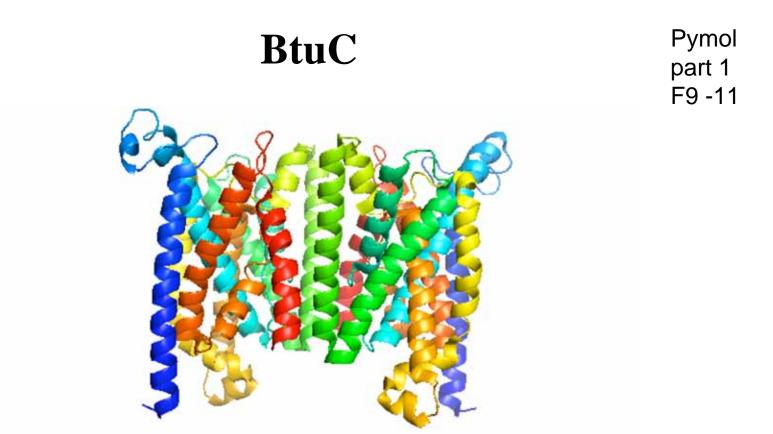
•Contains a well conserved histidine residue. Mutations of this residue in some prokaryotic transporters abolished transport and function.

Pymol part 1 F7

# The Q loop

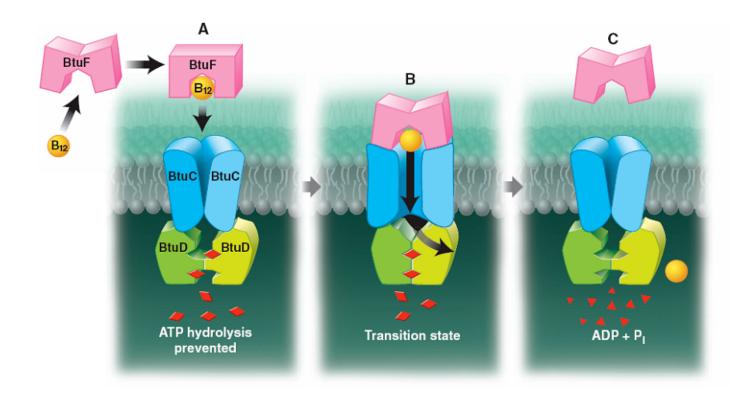
- Forms structural contacts with the membrane spanning domain.
- Glutamine residue binds to the Mg<sup>2+</sup> ion and the attacking water molecule.

Pymol part 1 F8



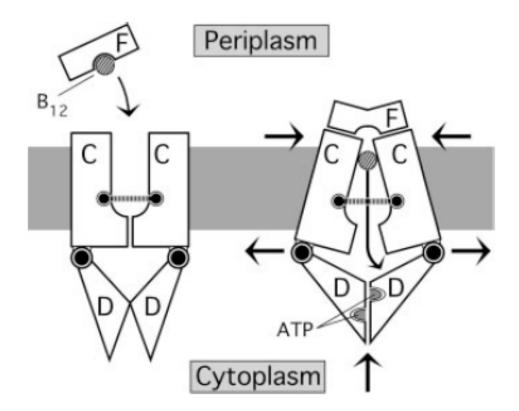
- Consists of 20 transmembrane helices.
- Cytoplasmic loop, L loop (conserved motif) forms the contact region with the ATP-binding cassette, BtuD.

#### 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.

# References

1. 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.

2. 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.

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