

**Structural and Biochemical Characterization  
of the Vitamin B<sub>12</sub> ABC Transporter, BtuCD-F**

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I dedicate this work to my teachers

In life and in school

Who have led me

By words and example

To this moment in time

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## Abstract

BtuCD-F is a binding protein dependent ABC transporter system that uses the power of ATP hydrolysis to pump vitamin B<sub>12</sub> into the cytoplasm of *E. coli*. The crystal structure of BtuF, the protein that binds vitamin B<sub>12</sub> and delivers it to the transporter, BtuCD, has been solved by x-ray crystallography. BtuF is a bi-lobed protein and B<sub>12</sub> is bound in a deep cleft formed at the interface between the two lobes. A stable complex between BtuF and BtuCD is demonstrated to form *in vitro* and was modeled using the individual crystal structures. Two conserved surface glutamates from BtuF may interact with conserved arginine residues on the periplasmic surface of the BtuCD transporter, playing a role in docking and the transmission of conformational changes.

BtuCD has also been reconstituted *in vitro* into proteoliposomes. In the presence of ATP, BtuCD proteoliposomes can mediate uptake of vitamin B<sub>12</sub> in a BtuF dependent fashion. In the absence of ATP, B<sub>12</sub> appears to become sequestered between BtuF and BtuCD. The ATPase activity of BtuCD was examined in proteoliposomes as well as in detergent solution. BtuCD has a significant basal rate of hydrolysis under all conditions tested, and B<sub>12</sub>-bound and apo-BtuF can stimulate that rate. Interestingly, the rate of ATP hydrolysis, as well as the effect of BtuF, vitamin B<sub>12</sub> and sodium ortho-vanadate on that rate, is different in each detergent and lipid environment. These results indicate that ABC transporters are highly sensitive to their environment and underline the importance of detergent or lipid choice in functional reconstitution and membrane protein crystallization experiments. Our results lead us to propose a revised model of the ABC transport cycle.

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## Abbreviations

ABC	ATP-binding cassette
ADP	adenosine diphosphate
ARS	ATP-regenerating system
ATP	adenosine triphosphate
BtuCD	<i>Escherichia coli</i> vitamin B <sub>12</sub> ABC transporter
BtuCD-F	<i>Escherichia coli</i> vitamin B <sub>12</sub> ABC transport system
BtuF	<i>Escherichia coli</i> vitamin B <sub>12</sub> binding protein
DDM	dodecyl maltoside
DMB	dimethylbenzimidazole
FOS12	Fos-choline 12
LDAO	lauryl dimethyl-amine oxide
LS	liposome
MAD	multiwavelength anomalous diffraction
MSD	membrane spanning domain
NCS	non-crystallographic symmetry
Pi	inorganic phosphate ion
PLS	proteoliposome
rmsd	root mean square deviation
SDS	sodium dodecyl sulfate
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
TX-100	Triton X-100