

# Structure of the *E. coli* ABC Transporter BtuCD

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## Introduction

ABC transporters are ubiquitous membrane proteins that couple ATP hydrolysis to the translocation of diverse substrates across cell membranes. Clinically relevant examples are associated with cystic fibrosis and with multidrug resistance of pathogenic bacteria and cancer cells. We have solved the crystal structure at 3.2-Å resolution of the *E. coli* BtuCD protein, an ABC transporter that mediates vitamin B<sub>12</sub> uptake.

## Methods and Materials

Crystals were grown by mixing BtuCD protein (20 mg/mL) with an equal volume of reservoir solution (100 mM Tris pH 8.0, 300 mM magnesium nitrate, 21% polyethylene glycol 2000, 0.8% 2-methyl-2,4-pentanediol in D<sub>2</sub>O) in sitting drops. Crystals grew to a final size of 0.1 × 0.15 × 0.5 mm<sup>3</sup> in a week and were frozen in liquid N<sub>2</sub> prior to data collection. Crystals were monoclinic (P2<sub>1</sub> with a = 92.99 Å, b = 106.29 Å, c = 95.54 Å, β = 99.29°), with one complete transporter, consisting of two copies each of the ABC cassette BtuD and of the membrane-spanning BtuC subunit, in the asymmetric unit. Data from native crystals as well as from derivatives were collected at APS beamlines 19-BM and 19-ID.

## Results

Initial phases were obtained from two separate derivatives (Na<sub>3</sub>Au(S<sub>2</sub>O<sub>3</sub>)<sub>2</sub> and orthovanadate), which yielded the envelope of the molecule and the location of several of the transmembrane helices. These data sets were collected at APS and proved critical to locate 34 selenium sites per full transporter in a selenomethionine three-wavelength anomalous diffraction data set collected elsewhere.

## Discussion

The crystal structure of the B<sub>12</sub>-translocating BtuCD transporter revealed two ATP-binding cassettes (BtuD) and two membrane-spanning subunits (BtuC) in close contact with each other. The BtuC subunits provide a total of 20 transmembrane helices grouped around a translocation pathway that is closed to the cytoplasm by a gate region, while the interface between the BtuD subunits resembles that observed in the ATP-bound form of the Rad50 DNA repair enzyme. A prominent cytoplasmic loop of the membrane-spanning subunit

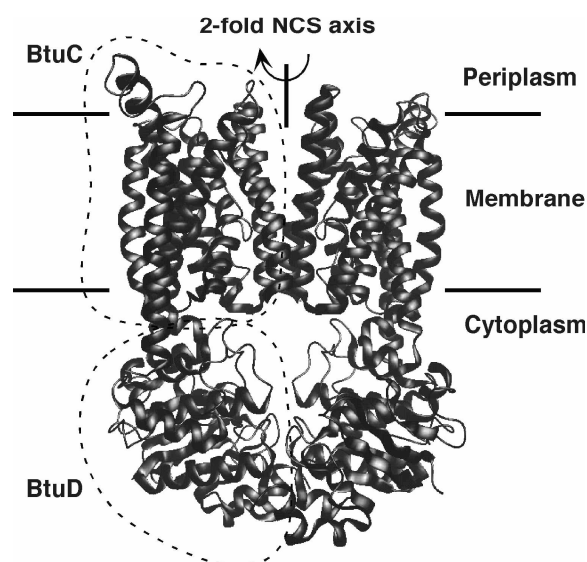


FIG. 1. Ribbon diagram of the B<sub>12</sub> transporter structure. The approximate boundaries of the lipid bilayer are indicated with horizontal lines. One molecule of BtuC and one of BtuD are circled by a dashed line.

forms the contact region with the ATP-binding cassette. This loop appears conserved throughout the protein family and is likely responsible for transmitting the power stroke during the ATP hydrolysis cycle. Our results reveal the structural context of all conserved sequence motifs of ABC transporters and provide a framework for elucidating their transport mechanism. (See Fig. 1.)

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## References

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