

## BEAMLINE

X4A

## PUBLICATION

Locher, K. P., Lee, A. T., Rees, D. C. (2002)  
 "The *E. coli* BtuCD Structure: A Framework for ABC Transporter Architecture and Mechanism," *Science* **296** 1091-1098.

## FUNDING

Howard Hughes Medical Institute

## FOR MORE INFORMATION

Kaspar P. Locher, Division of Chemistry and Howard Hughes Medical Institute, California Institute of Technology, Pasadena, Email: locher@caltech.edu  
 Home page: <http://www.its.caltech.edu/~locher/>

## Structure of a Bacterial ATP-Binding Cassette Transporter

Kaspar P. Locher, Allen T. Lee, and Douglas C. Rees

Howard Hughes Medical Institute and Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena

*Adenosine triphosphate (ATP)-binding cassette (ABC) transporters are ubiquitous membrane proteins that couple ATP hydrolysis to the transport of diverse substrates across cell membranes. Clinically relevant examples are associated with cystic fibrosis and with multidrug resistance of pathogenic bacteria and cancer cells. Using x-rays produced at the National Synchrotron Light Source at Brookhaven National Laboratory, and other light sources, scientists at the California Institute of Technology in Pasadena have determined the crystal structure at 3.2 angstrom resolution of the Escherichia coli BtuCD protein, an ABC transporter mediating vitamin B<sub>12</sub> uptake.*

To survive, cells import nutrients from the surrounding environment and pump toxic substances out of the cytoplasm. These functions are carried out by transport proteins, called transporters, embedded in cell membranes. The largest family of these proteins, called ATP-binding cassette (ABC) transporters, is ubiquitous in all branches of life. (ATP, or adenosine triphosphate, is the primary source of energy in all living cells.) ABC transporters power the transport of substrates across membranes by using the energy released by the hydrolysis, or water-induced decomposition, of ATP into ADP (adenosine diphosphate) and inorganic phosphate.

Several human ABC transporters are medically relevant. For example, mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) protein cause cystic fibrosis. Other ABC transporters are associated with multidrug resistance of tumor cells against cytotoxic substances used in chemotherapy.

ers are predominantly involved in nutrient uptake, although they also participate in the export of bacterial toxins and harmful substances, contributing to bacterial multidrug resistance.

Despite the immense amount of biochemical studies, and recent advances in the visualization of ABC transporters, their transport mechanisms have remained elusive. To understand better how these transporters operate, the crystal structure of the *Escherichia coli* vitamin B<sub>12</sub> importer BtuCD protein has been determined, with

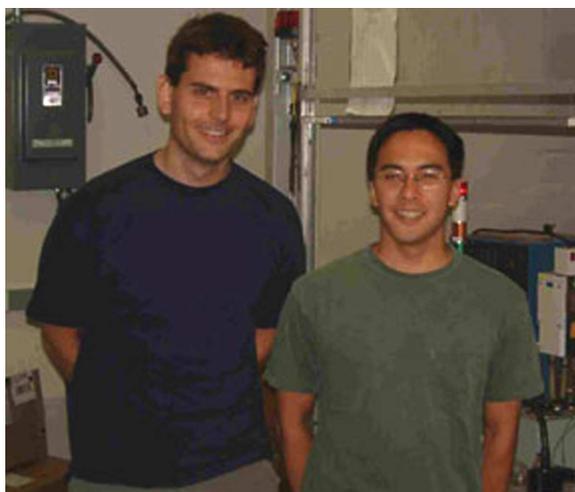
all critical parts ordered and resolved.

To solve the structure of BtuCD, close to one thousand crystals were screened and data were collected at various synchrotron light sources, including the National Synchrotron Light Source at Brookhaven National Laboratory.

The structure of BtuCD has revealed three key elements to the transport of vitamin B<sub>12</sub> into the cytoplasm (see figure):

(1) A transport pathway through the membrane-spanning BtuC subunits. In the absence of ATP, this pathway is accessible from the outside, but is sealed to the cytoplasm by a gate region.

(2) The ATP-binding cassettes (ABCs): Located beneath the BtuC subunits, these proteins present binding sites for two ATP molecules at the interface between the ABCs. As the ABCs bind and hydrolyze ATP, mechanical energy is generated and transmitted to the membrane-spanning domains BtuC, where it induces rearrangements that



Kasper Locher (left) and Allen Lee

In bacteria, ABC transport-

open the BtuC gate and allow the substrate to cross the membrane.

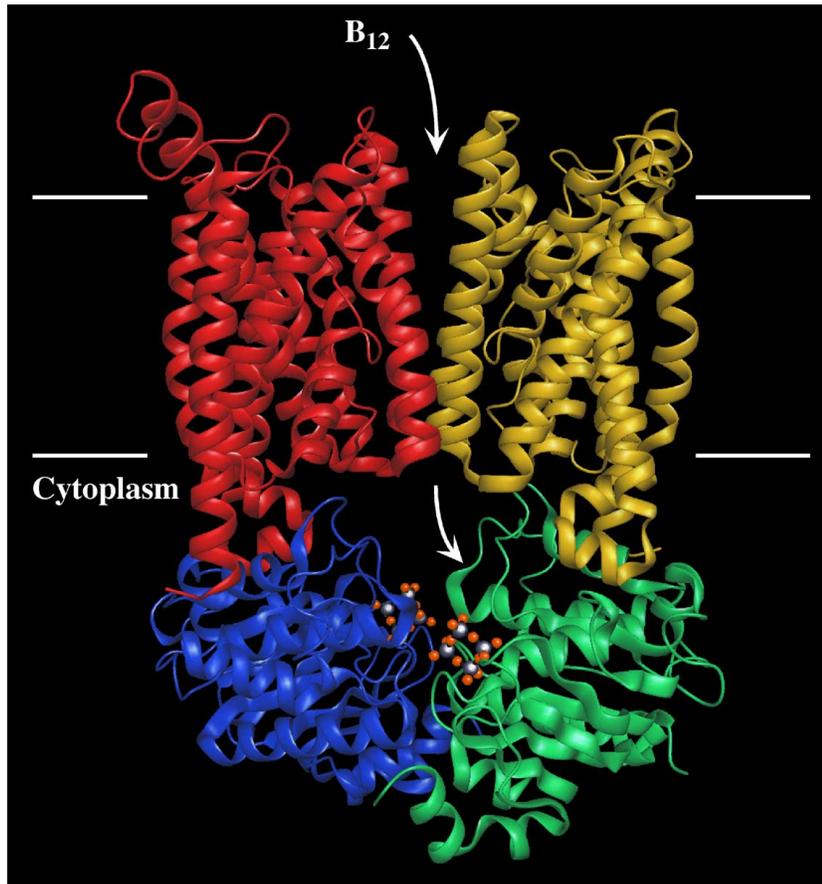
(3) A cytoplasmic loop of BtuC makes extensive contact with BtuD. Mutations in these critical interface residues severely affect the function and assembly of ABC transporters. For example, 70% of cystic fibrosis patients have a single residue deleted at a position that corresponds to this contact region of BtuD.

The present structure suggests the design of further biochemical studies that could probe the conformational changes of BtuCD during vitamin B<sub>12</sub> transport. This structure also provides a framework for understanding the structure and mechanism of other ABC transporters.

Additional contact information:  
Allen T. Lee, Division of Chemistry and Howard Hughes Medical Insti-

tute, California Institute of Technology, Pasadena  
Email: atlee@its.caltech.edu  
<http://www.its.caltech.edu/~atlee/>

Douglas C. Rees, Division of Chemistry and Howard Hughes Medical Institute, California Institute of Technology, Pasadena  
Email: dcree@caltech.edu  
<http://www.its.caltech.edu/~reesgrp/>



**Figure caption:** Ribbon diagram of the BtuCD protein structure. The ATP-binding cassette (ABC) transporter is assembled from two membrane-spanning domains (BtuC, red and yellow) and two ABCs (BtuD, green and blue). At the ATP binding sites, cyclotetranadate molecules are bound to the transporter (ball and stick models at the BtuD interface). Vitamin B<sub>12</sub> is delivered to the transporter by a binding protein (not shown), likely transported through a pathway provided at the interface of the two membrane-spanning BtuC subunits, and then released into the cytoplasm at the large gap between the four subunits (arrows). B<sub>12</sub> transport is powered by the hydrolysis of ATP by BtuD.