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Supporting Online Material for

Micelles Protect Membrane Complexes from Solution to Vacuum

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Published 12 June 2008 on *Science Express*

DOI: 10.1126/science.1159292

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Materials and Methods

E. coli cell pellets containing BtuC₂D₂ were a kind gift of K. Locher. The BtuC₂D₂ construct was prepared by subcloning the *btuC* and *btuD* genes from *E. coli* into the IPTG-inducible pET-19b vector, attaching an N-terminal decahistidine tag to BtuC and the protein expressed in *E. coli*. BtuC₂D₂ was purified as described previously (1) with some modifications. Briefly, LDAO was used to extract BtuC₂D₂ from the cell pellets according to and following cell lysis and centrifugation, the cytosolic and LDAO-solubilised membrane fractions were applied to a Ni-NTA affinity column where the detergent was exchanged from LDAO to DDM. After the removal of impurities with 100 mM imidazole BtuC₂D₂ was eluted with 500 mM imidazole. Further purification was carried out using gel filtration chromatography and the final buffer used to purify BtuC₂D₂ contained 500 mM NaCl, 50 mM Tris pH 7.5, 0.1 % DDM.

BtuD was expressed in *E. coli* as a soluble protein using a construct containing the *btuD* gene from *E. coli* subcloned into the IPTG-inducible pET-19b vector. The attachment of an N-terminal decahistidine tag allowed purification of the expressed protein by Ni-NTA affinity chromatography which was followed by gel filtration chromatography. Purified BtuD was found to be particularly prone to precipitation and an ATP-bound conformation was needed to maintain its solubility. The final buffer, used to purify BtuD, contained 300 mM NaCl, 50 mM Tris pH 7.5, 0.1 mM EDTA, 1 mM DTT and 4 mM ATP.

The modified molybdate assay (2) was used to determine the ATPase activity of purified BtuD. BtuD at a concentration of 1 μM and in a buffer containing 150 mM NaCl, 50 mM Tris pH 7.5, 2 mM ATP displayed an intrinsic rate of ATP hydrolysis upon the addition of 10 mM MgCl₂ of ~250 nmol min⁻¹ mg⁻¹ BtuD. BtuD loses some activity in 1 M urea (66 nmol min⁻¹ mg⁻¹ BtuD) and in 3 M urea all activity is lost (2.3 nmol min⁻¹ mg⁻¹ BtuD), suggesting monomer formation and some level of unfolding is occurring with these concentrations of urea.

BtuC in DDM was prepared by removing the BtuD component from purified BtuC₂D₂ bound to a Ni-NTA affinity column with 5 M urea. The BtuC subunits remaining bound to the column were washed in a buffer with urea omitted before being eluting with 500 mM imidazole and dialysed into a final buffer containing 500 mM NaCl, 25 mM Tris pH 7.5 and 0.1 % DDM. Circular dichroism studies on the BtuC subunits were carried out in a buffer containing 150 mM NaCl, 25 mM Tris pH 7.5 and 0.1 % DDM. No significant changes in far UV spectra, and thus secondary structure, were observed until ≥ 5 M urea.

Solution conditions for mass spectrometry

An aliquot (30 μl) of a BtuC₂D₂ solution (approx. 10 mg/ml) with 1.95 mM DDM, (~10 fold higher than the cmc (0.18 mM) and with an aggregation number of 110-140 (3)) in 0.5 M NaCl, 25 mM Tris and pH 7.5 was buffer-exchanged once only on a membrane filter and diluted 10-fold with 200 mM AmAc. Differential absorption of both protein and DDM on the filter complicates the final concentration estimate. To ensure that we were

above the cmc we carried out control experiments in which we added DDM stock solutions (4mM or 2mM DDM in 200mM ammonium acetate) to the working BtuC₂D₂/DDM solution to give final DDM concentrations of greater than 0.2 mM and 2 mM. For the >2mM DDM solution 5μL of the working BtuC₂D₂ solution was co-incubated with 5μL of the 4mM DDM stock for 15min prior to nano-electrospray. For >0.2mM DDM, 9μL of the working BtuCD solution was co-incubated with 1μL of the 2mM DDM stock.. Stock solutions of 1mM of ATP, ADP and AMPCPP were prepared in milliQ water. The working BtuC₂D₂ solution was co-incubated with an aliquot of the nucleotide stock (5% or less of the final co-incubation volume) for 15 min prior to nano-electrospray.

Mass spectrometry

Experiments were performed on a Q-toF2 mass spectrometer (Waters) modified for transmission and detection of ions at high m/z ratios (4). Aliquots of BtuC₂D₂ solution (2 μL) were introduced via gold coated nanoflow electrospray capillaries, prepared in house (5). The following instrument parameters were used to record mass spectra of BtuC₂D₂: needle voltage 1.5 kV, MCP 2350 V, cone voltage 197 V and collision voltage 200 V. The pressure in the collision cell is measured from the analyzer readback as lower: 6-8 × 10⁻⁶ mbar, middle: 4.5 × 10⁻⁵ mbar, upper: 7.0 × 10⁻⁵ mbar for fig. 1 and 9.0 × 10⁻⁵ mbar for fig. 2.

Calculation of the accessible surface area

The accessible surface area (ASA) of the BtuC₂D₂ was calculated according to Mark Gerstein's algorithm (6). A value of 47677 Å² was calculated from the PDB file (1L7V) without entering the cyclotetranadate coordinates. Using the same PDB file, the area of the protein complex encompassed by the micelle, as well as the hydrophobic thickness of the micelle, was obtained from the Orientations of Proteins in Membranes database (7). A value of 19863 Å² and 29.1 Å were used for area encompassed by the micelle and the depth of the hydrophobic layer respectively. Since the structure of MacB is unknown, we have estimated the ASA using the following assumptions. MacB is part membrane and part soluble (one cytoplasmic, one membrane and one periplasmic domain). The protein was therefore approximated to three spheres, according to secondary structure predictions (8) and the surface area of each domain was estimated.

Supplementary figures

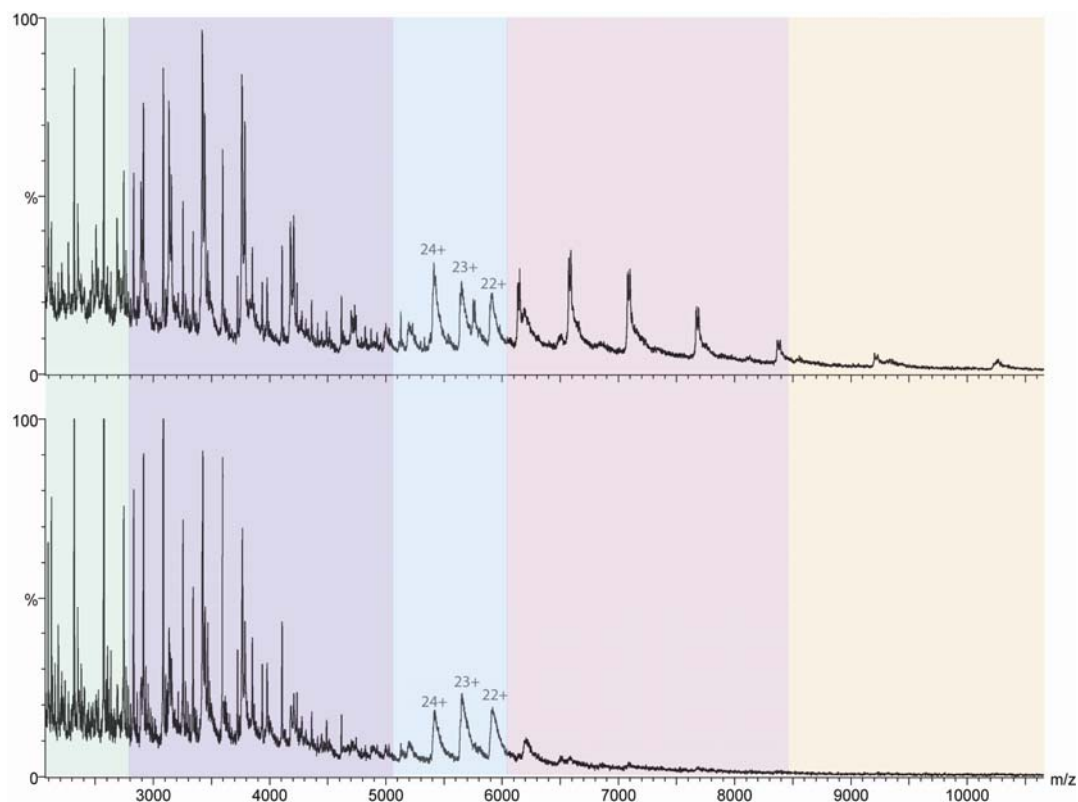


Figure S1 spectra recorded under identical MS conditions to those shown in figure 1A middle and top panels but from a solution with $\geq 2\text{mM}$ DDM concentration, ~ 10 fold higher than the cmc (3). The mass spectra of the intact complex and the dissociation products are closely similar to those shown in Fig. 1 with the exception that greater numbers of peaks assigned to DDM clusters are observed from the solution conditions used here. Similar results were obtained for spectra recorded in 0.2mM DDM solutions. Regions of the spectrum are shaded as follows: blue charge states of the intact complex, pink BtuCD₂, orange BtuC₂D, purple BtuC and green DDM clusters and BtuD subunits.

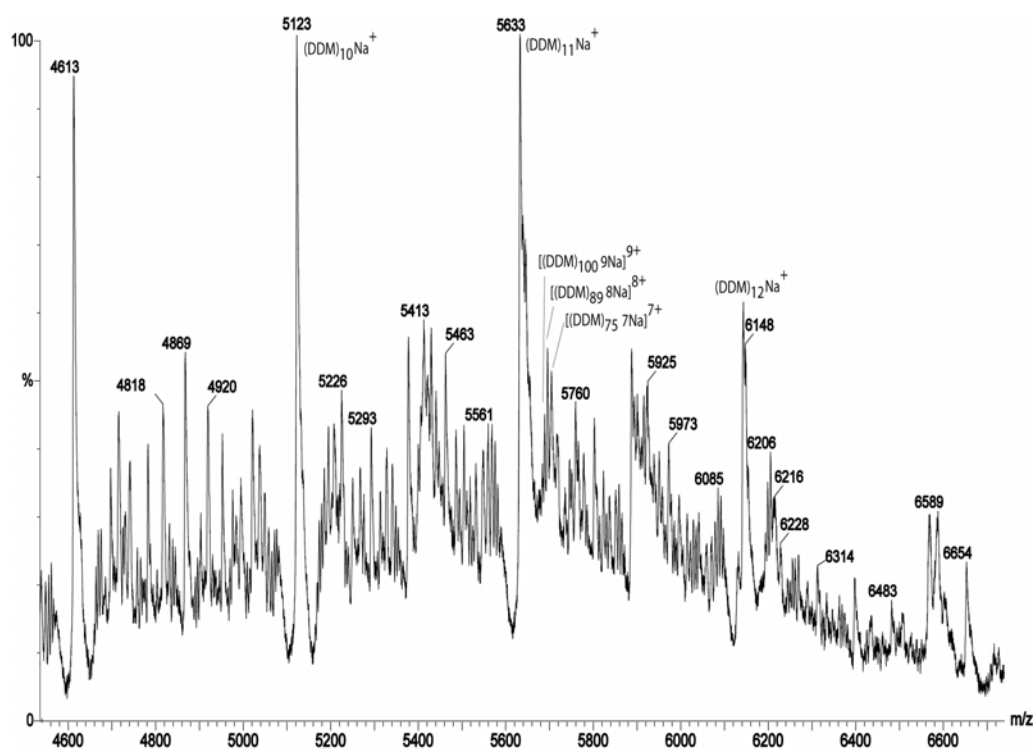


Figure S2 Mass spectrum of a region of the BtuC₂D₂ DDM solution showing the predominance of DDM clusters with up to 100 molecules remaining intact in the gas phase. Many of the charge states overlap. In cases where multiple species coincide, the smallest possible oligomer is indicated in each case. All possible combinations of DDM molecules with the appropriate number of Na⁺ ions were calculated and the spectrum was assigned in full. For clarity only one series has been labeled with unique composition and charge state assignments.

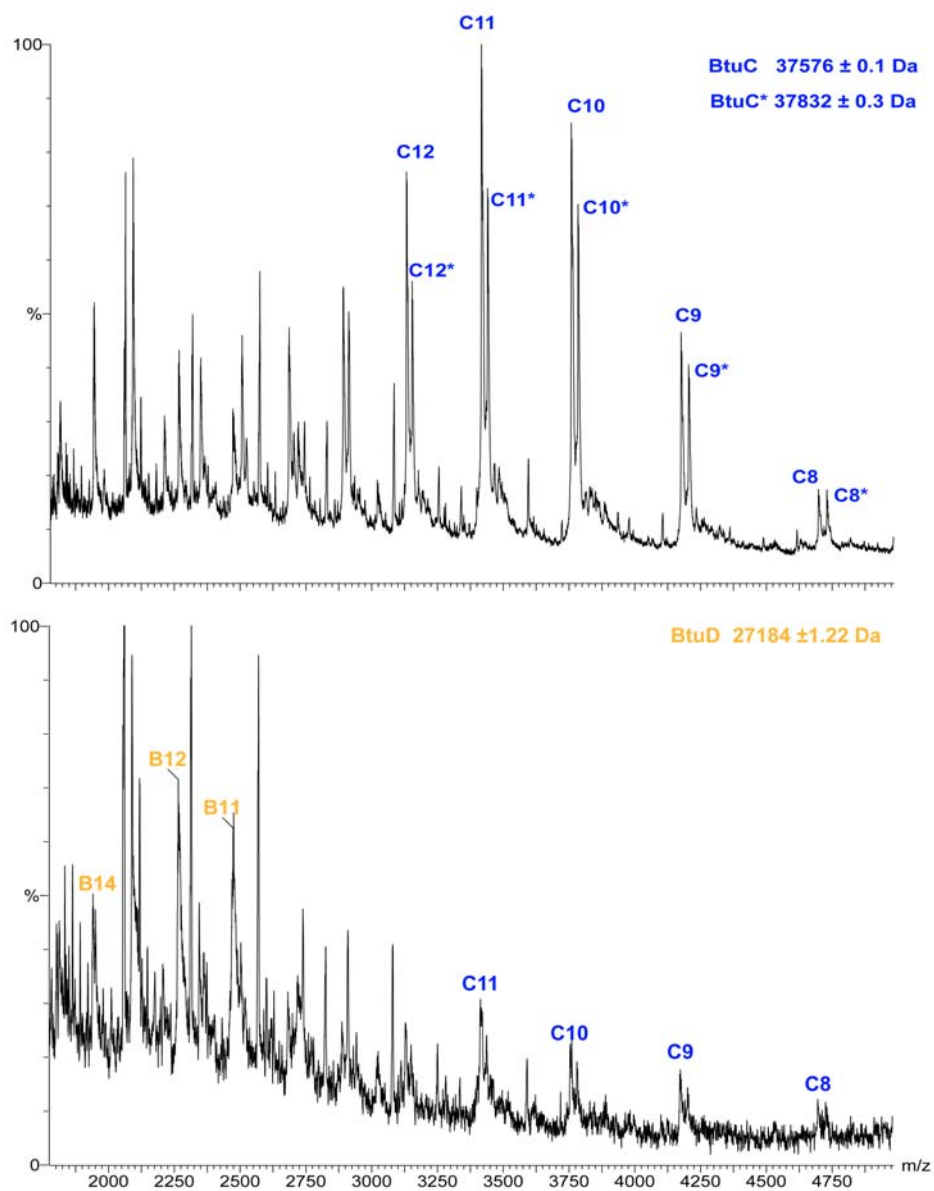


Figure S3 Under conditions where a large number of DDM molecules adhere to the membrane protein complex (lower panel) the dominant dissociation product is BtuD. By contrast when the complex is largely devoid of detergent molecules, both dissociation products are formed but ions of the BtuC dissociation product dominate the spectra. Unlabelled peaks in the spectra are assigned to DDM clusters.

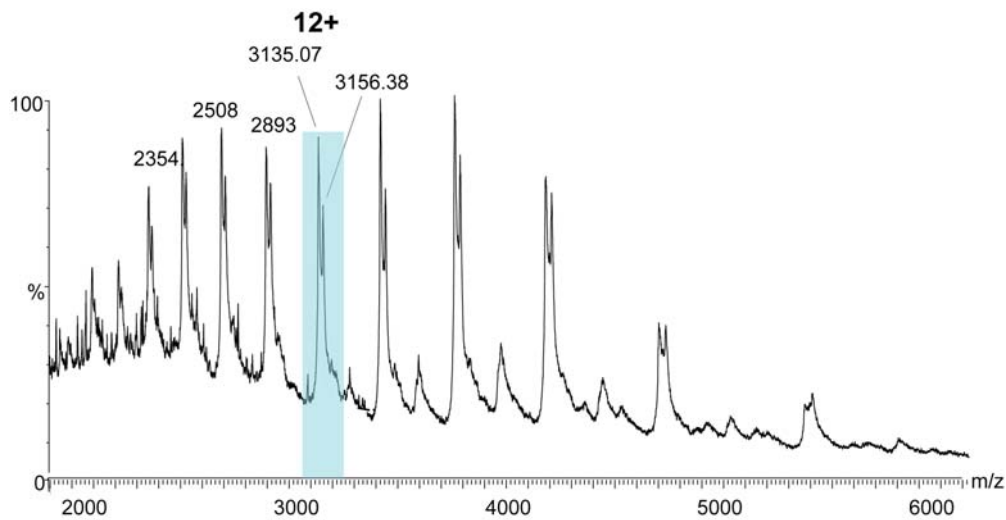


Figure S4 Mass spectrum of the denatured complex recorded after addition of a denaturing solution of 50% acetonitrile 1% formic acid. The doublet of peaks persists even after heating to 90°C. Since non covalent binding, for example to small molecules or lipids, would be disrupted under these harsh conditions, the spectra are consistent with a covalent modification. A molecular mass of 256 Da for this modification was calculated from the mass difference in the two 12+ charge states. From databases of known post translational modifications only α -N-6-Phosphogluconoylation of a His tagged protein (258 Da) is within an acceptable error limit (+/- 2 Da).

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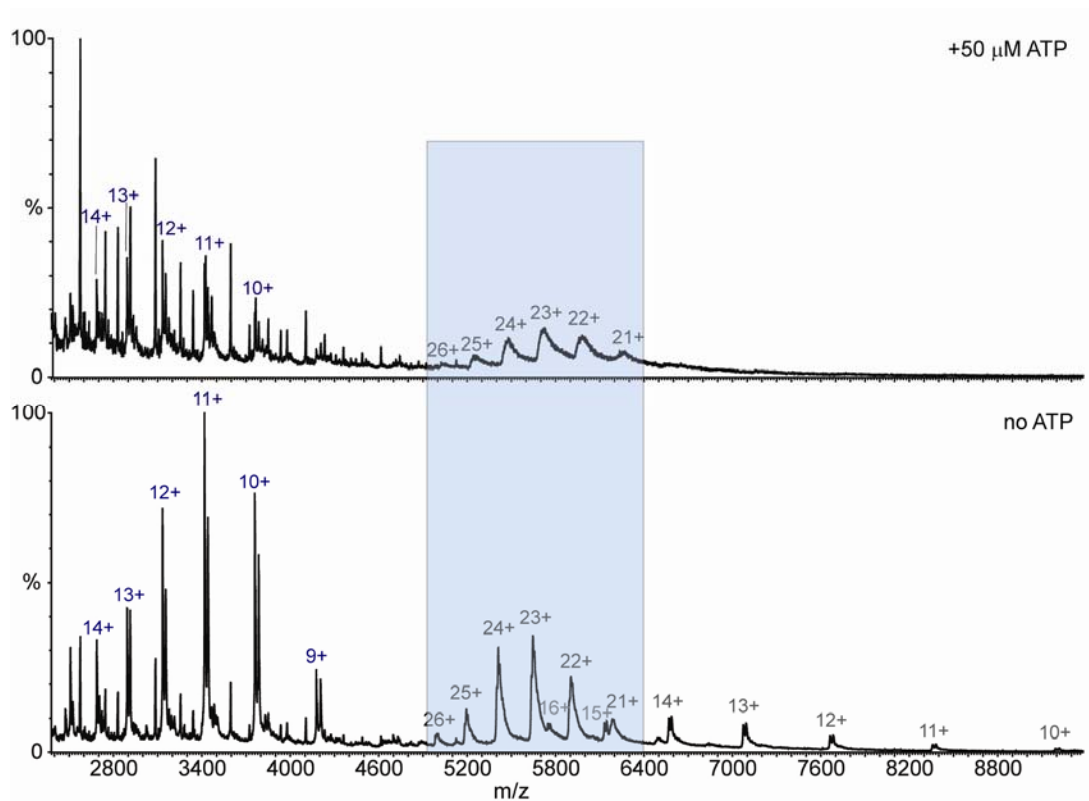


Figure S5 Mass spectra of BtuC₂D₂, with and without ATP Na⁺ recorded under identical mass spectrometry conditions: cone 197 V, collision cell 200 V and collision pressure 5×10^{-5} mbar. The charge states assigned to the intact complex 21-25⁺ (highlighted in blue) undergo dissociation to form stripped trimers in the absence of nucleotides while in the presence of ATP Na⁺, AMPCPP or ADP (not shown) much less dissociation is observed, the intact tetramer predominates at m/z values >5000. The predominant peaks at low m/z are assigned to BtuC and clusters of DDM. This demonstrates the increased stability of the complex in the presence of nucleotides.

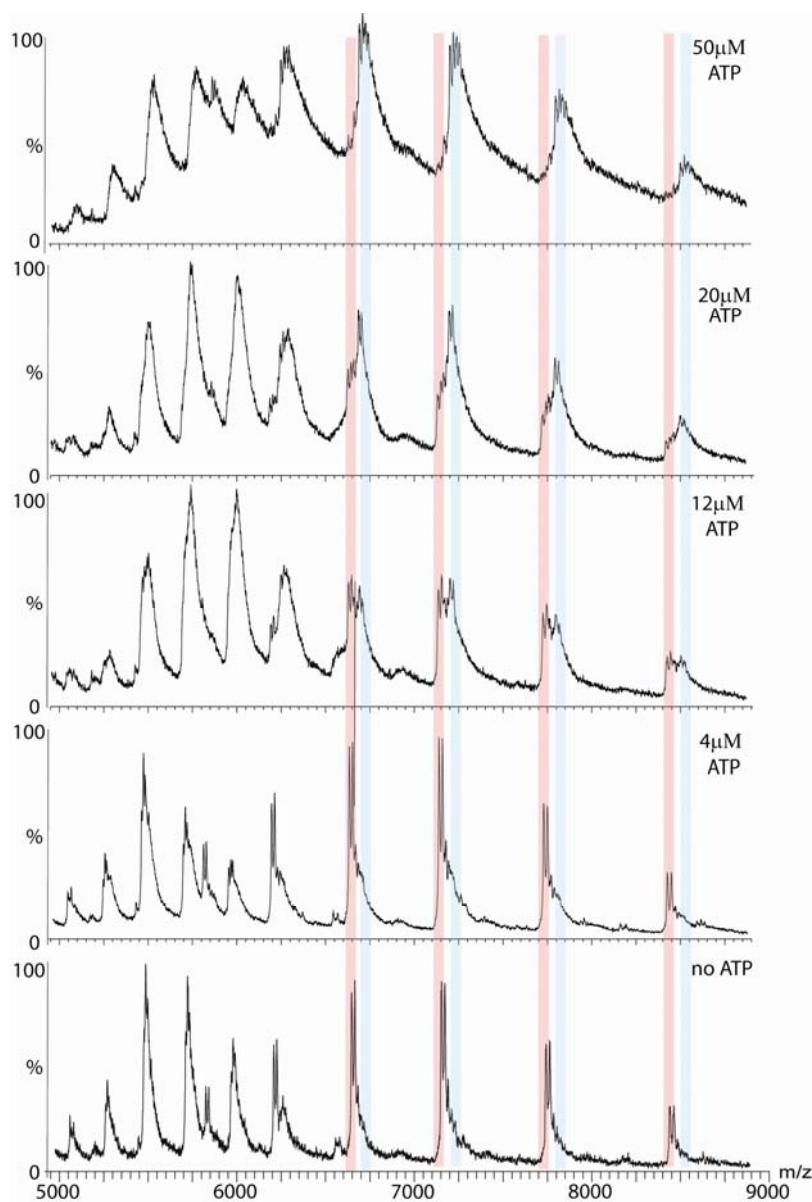


Figure S6 Cooperativity of ATP binding. ATP/Mg²⁺ was added to solutions of BtuC₂D₂ in DDM and spectra recorded under similar conditions. ATP binding was monitored using the BtuCD₂ dissociation product to enable sufficient resolution to distinguish the different ATP bound states. The *apo* state (pink) persists with sub-stoichiometric quantities of ATP (4 μM) while the 2-ATP bound form (blue) is clearly observed in the presence of 12 μM ATP. The absence of species corresponding to 1-ATP bound to BtuCD₂ demonstrates cooperativity in ATP binding.

Table S1 Table of measured and calculated masses for BtuC₂D₂ with and without ATP together with the gas phase dissociation products.

Protein species	Calculated mass (Da)	Measured mass (Da)
BtuC ^{1,2}	37716	37576±0.1
BtuC* ¹		37832±0.3
BtuD	27111	27184±1.2
BtuC ₂ D ₂ tetramer	129520 ³	129642±20
BtuC ₂ *D ₂ tetramer		129820±7
BtuC ₂ **D ₂ tetramer		130063±17
BtuC ₂ D	102336 ³	102546±9
BtuCD ₂	91944 ³	91975±7
BtuC*D ₂	92200	92224±5
From spectrum containing 50 μM ATP		
BtuCD ₂ + 2 ATP	92966	92983±14
BtuC*D ₂ + 2 ATP	93222	93240±17
BtuC ₂ D ₂ + 2 ATP	130542	130782±10

Footnotes

1. Consistent with the removal of the N-terminal methionine residue.
2. Including the N-terminal decahistidine tag.
3. calculated from the measured masses of the subunits
4. Mass differences between the dissociated BtuC subunits, with and without modification, give a mass of 256 ± 2 Da across the 10-13⁺ charge states.
5. Peaks assigned to alpha-N-6-phosphogluconoylation of one* and two ** BtuC subunits.

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