

ALLOSTERIC COMMUNICATION BETWEEN THE NUCLEOTIDE BINDING DOMAINS OF ClpB

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SUPPLEMENTAL DATA

Fig. S1. Oligomerization of wt ClpB and protein variants at different salt concentrations. (A) Elution profiles of ClpB variants (wt, T1T2, T1N2, N1T2) in 50 mM Tris, pH 7.5, 20 mM MgCl₂, 50 mM KCl. The elution positions of protein standards thyroglobulin (669 kDa), γ -globulin (159 kDa), ovoalbumin (44 kDa), and myoglobin (17 kDa) are also shown. (B) Elution profiles of the same proteins in the above buffer containing 500 mM KCl.

Fig. S2. Interaction of MANT-nucleotides with wt ClpB and protein variants. (A) Hydrolysis of MANT-ATP (filled symbols) and ATP (open symbols) by wt ClpB. Protein (5 μ M) and nucleotide (20 μ M) were dissolved in buffer 50 mM Tris-HCl, pH 7.5, 20 mM MgCl₂, containing 15 (circles), 50 (squares) or 500 (triangles) mM KCl. Inorganic phosphate released as a function of time was quantified by the malachite green method. (B) Titrations of monomeric ClpB variants with MANT-ATP. Proteins (5 μ M) in 50 mM Tris-HCl, pH 7.5, 20 mM MgCl₂, 500 mM KCl were incubated with increasing amounts of MANT-ATP at 25 °C, and the emission spectra recorded with excitation at 356 nm. The K_d values estimated from these titrations are shown in Table 3.

Fig. S3. Nucleotide binding to NBD1 as seen by partial proteolysis. (A) Limited proteolysis of wt ClpB and selected protein variants with thermolysin. ClpB and protease concentration were 4 μ M and 2.8 μ g/ml, respectively. Digestion was carried out in 50 mM Tris, pH 7.5, 20 mM MgCl₂, 150 mM KCl, 2 mM ATP at 30 °C for 60 minutes. Cleavage products were analyzed by SDS-PAGE (15 %) and Coomassie blue Staining. (B) Limited proteolysis of wt ClpB at different KCl concentrations. Proteolysis was performed as in A, at 50, 150 or 300 mM KCl.

Figure S1

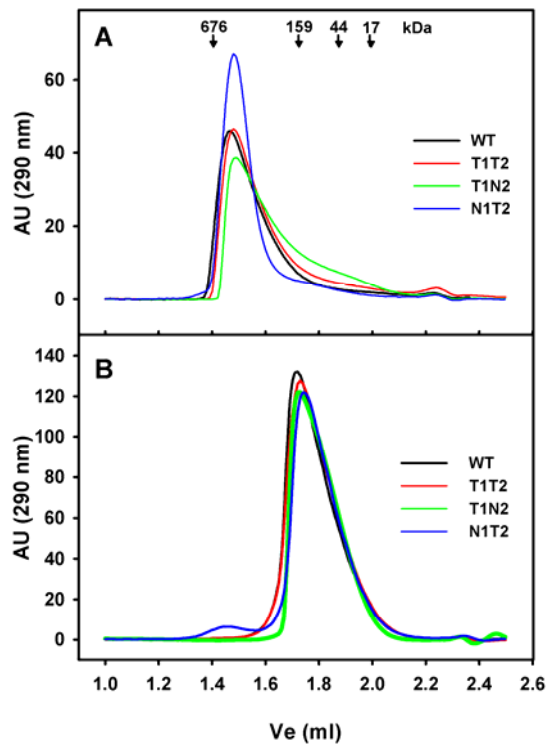
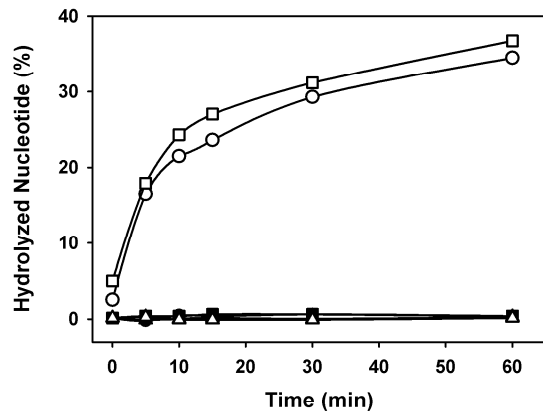


Figure S2

A



B

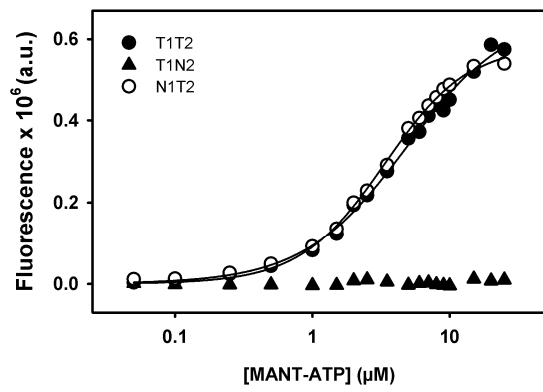


Figure S3

