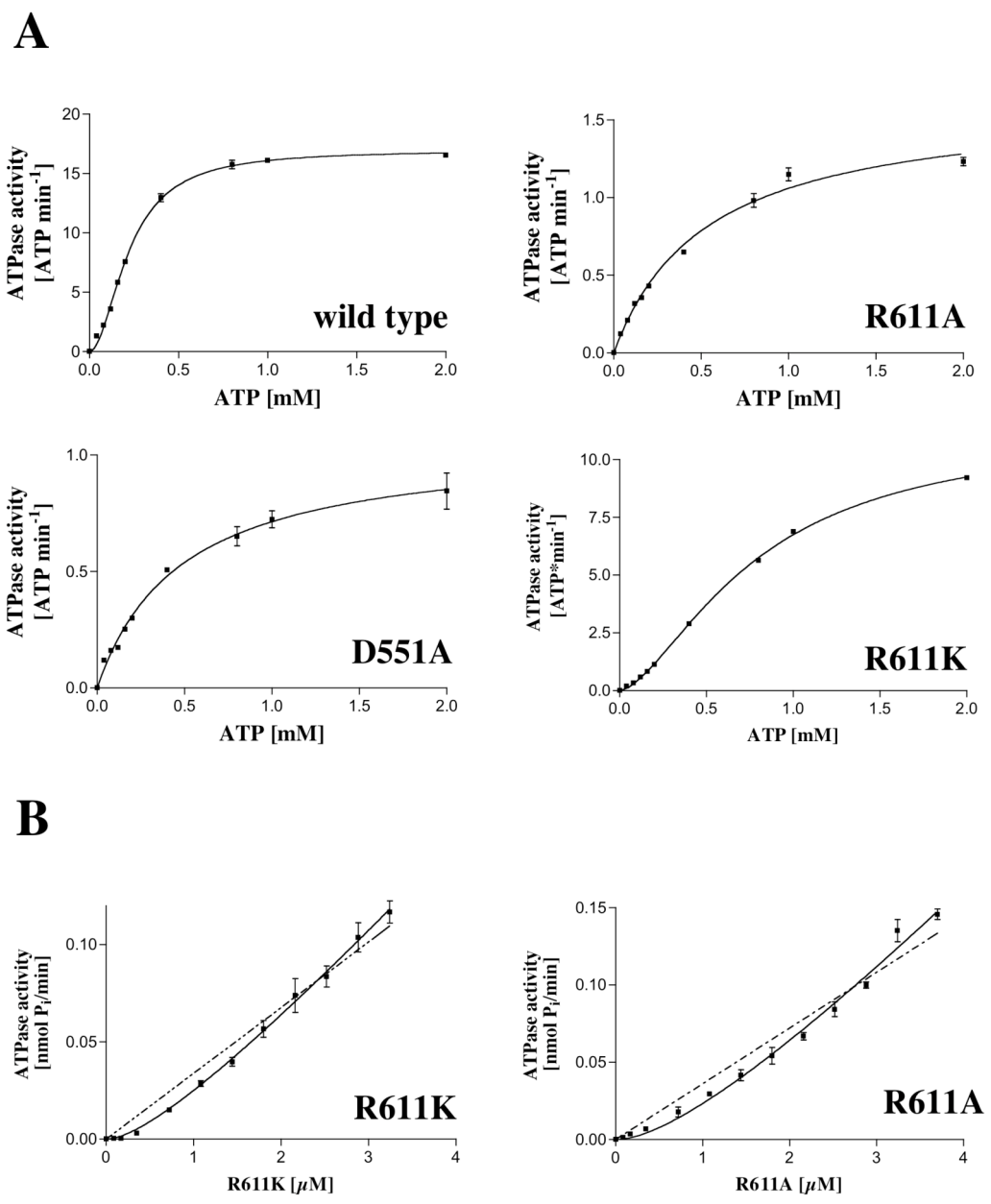
Supplementary material

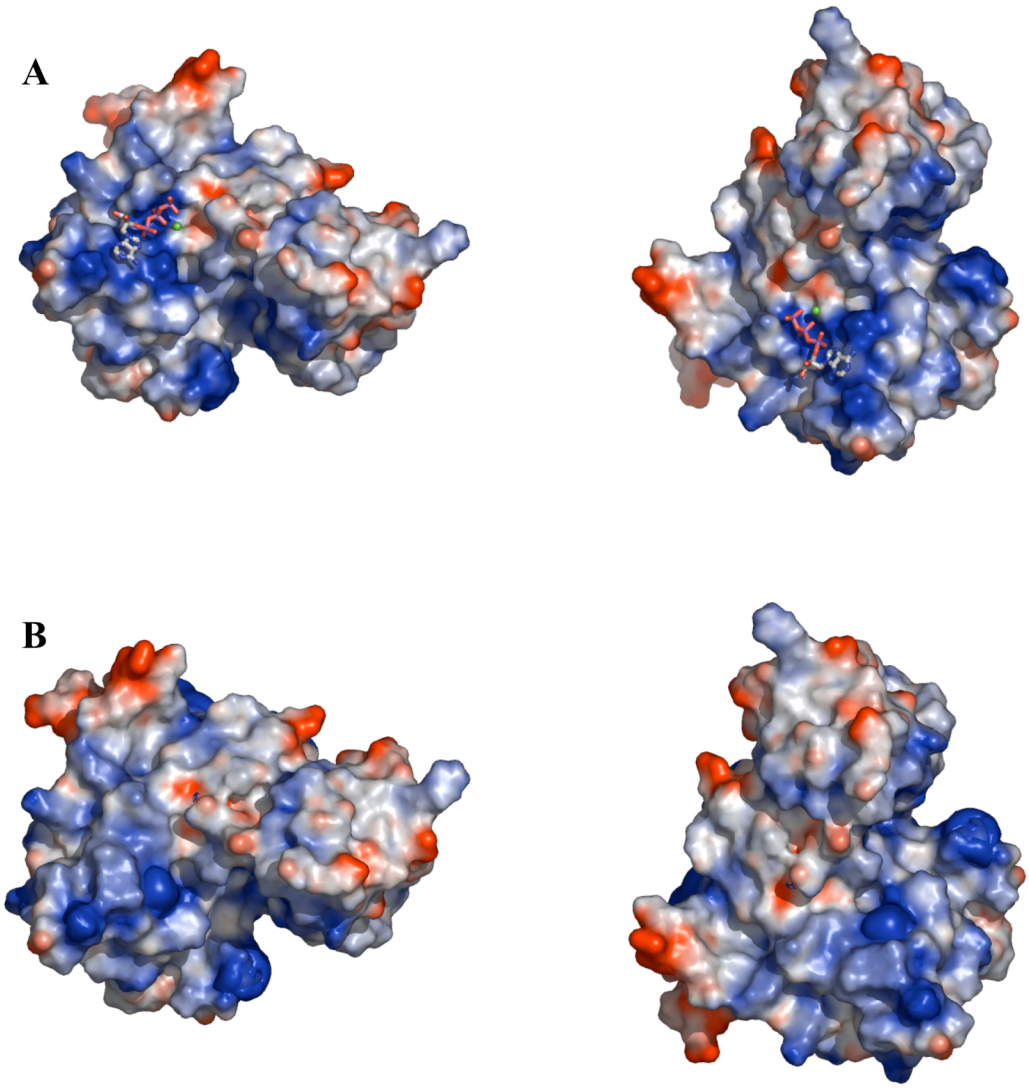
Figure S1:



ATPase activity of wild type HlyB-NBD and the R611A, D551A and R611K mutants. (A) Steady state ATPase activity was determined at 22 ± 1°C as described in detail in Zaitseva et al. (Zaitseva, J., *et al*. (2005) Functional characterization and ATP-induced dimerization of the isolated ABC-domain of the haemolysin B transporter *Biochemistry* **44** 9680- 9690). Final protein concentration used in the assay was 0.06 mg/ml (2.2 μM, wild type, upper left panel), 0.05 mg/ml (2 μM, R611A, upper right panel), 0.04 mg/ml (1.6 μM, D551A, lower left panel)

and 0.05 mg/ml (2 μM, R611K, lower right panel). The ATPase activity was analyzed according to the Hill equation. Kinetic parameters were determined as: wild type enzyme ($K_{0.5}$= 0. 23 ± 0.06 mM, k_{cat}= 17.3 ± 0.3 ATP min^{-1}, Hill coefficient= 1.8 ± 0.1), R611A (K_M= 0.39 ± 0.07 mM, k_{cat}= 1.4 ± 0.1 ATP min^{-1}), D551A (K_M= 0.49 ± 0.01 mM, k_{cat}= 1.1 ± 0.1 ATP min^{-1}), and R611K ($K_{0.5}$= 0.58 ± 0.03 mM, k_{cat}= 11.3 ± 0.3 ATP min^{-1}, Hill coefficient= 1.6 ± 0.1). Thus, these residues are crucial to the observed positive cooperativity of ATPase activity. (B) Protein concentration dependent ATPase activity of the R611K (left panel) and R611A mutants (right panel). Data points were analyzed according to Nikaido et al. (Nikaido, H., Liu, P.-Q. & Ferro-Luzzi Ames, G. (1997) Purification and Characterization of the of HisP, the ATP-binding subunit of the traffic ATPase (ABC transporter), the histidine permease of *Salmonella typhimurium J. Biol. Chem.* **272** 27745- 27752). The dashed line corresponds to a simulated protein concentration dependent ATPase activity in the absence of any dimer formation of the NBD, which assumes that ATPase activity arises only from the monomeric NBD. This analysis demonstrates that the mutants (R611K, R611A and D551A (data not shown) still dimerize although cooperativity is strongly dependent on the nature of the mutations.

Figure S2:



Electrostatic surface potential of a monomer of the ATP/Mg^{2+}-loaded dimer of the HlyB-NBD H662A.

The color-coding was set according to electrostatic potentials assuming an ionic strength of 100 mM. Red and blue are used to represent negative and positive potentials, with blue indicating a magnitude of the potential of > 4 kT and red indicating a magnitude of the potential of < - 4 kT. Here, k is the Boltzman constant and T the absolute temperature. Calculations were performed with PyMol using the APBS tool. The orientation of the NBD is identical to Figure 4. Right panels are rotated 90° perpendicular to the plane of the paper. (A) ATP/Mg^{2+} was not included in the calculations and is shown in sticks (ATP) and as a green sphere (Mg^{2+}). (B) The ATP/Mg^{2+} complex was included in the calculations.

Figure S3:

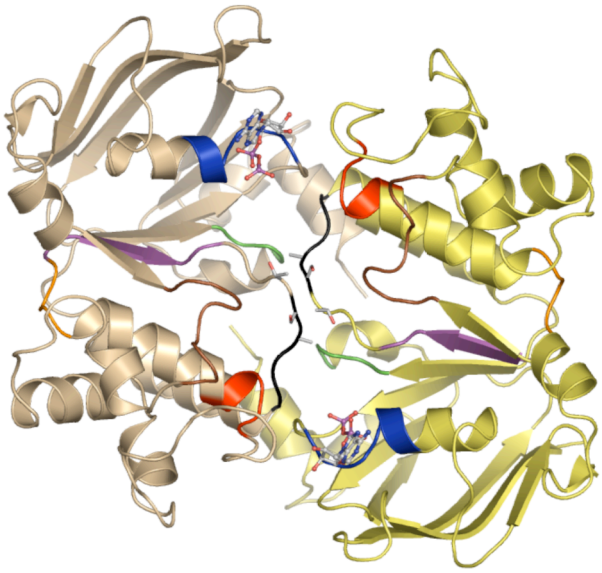


Figure S3: The hypothetical dimer of the HlyB-NBD in the ADP bound state.
The dimeric state was obtained by superimposition of two post catalysis, ADP-bound monomers of the HlyB-NBD and the crystal structure of BtuD (pdb entry 1L7V), which is structurally identical to the ADP-bound form (Locher, K.P. (2004) Structure and mechanism of ABC transporters. Curr Opin Struct Biol, 14, 426-431). For simplicity, the BtuD dimer is not shown. The rmsd of the individual ADP monomers of HlyB-NBD and BtuD was 1.98 Å over 231 Cα-atoms. Color-coding of the conserved motifs is identical to Figure 1, with one monomer shown in light yellow and the other in light tan. ADP is shown in ball-and-sticks representation. Immediately evident is the steric clash of the D-loops of the opposing HlyB monomers (shortest distance of 2.9 Å between the Cα atoms of S634 and A635).