

**Supplementary figure 4.** Titrations of the wild type and His462Gln mutant CopA-PN with AMPPCP. The initial concentrations of the wild type (**A, B**) and His462Gln mutant (**C, D**) CopA-PN in the ITC cell were, respectively, 0.26 mM (~4 mg ml<sup>-1</sup> protein) and 0.79 mM (~12 mg ml<sup>-1</sup> protein). The concentrations of AMPPCP in the injection syringe were 6 mM (wild type) and 24 mM (mutant). CopA-PN was titrated with AMPPCP in 50 mM imidazole-HCl, pH 7.0 (**A** and **C**). The normalized data were fitted to a model for a single class of binding (red solid lines in **B** and **D**). The fits yield the  $K_d$  values of 0.099 mM (wild type) and 2.2 mM (mutant). Higher concentrations of protein and AMPPCP were used with the mutant because the mutation causes a ~20-fold reduction in affinity.