Supporting Information

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Fig. S1. ATP stability at 30 °C. The stability of ATP after incubation at 30 °C for 15 min was assessed by HPLC, as described in *Materials and Methods*. (A) ATP sample after incubation. (B) ATP control (no incubation). (C) ADP control (no incubation), contains significant amounts of AMP.



Fig. S2. ATPase activity of *E. coli* GlnK, using standard protocol. Analysis of ATP hydrolysis by TLC, using the standard protocol, as described in *Materials and Methods*. Reactions were initiated by the addition of ATP (0.8 μ L of 100 mM ATP plus 1 μ 1 [γ^{32} P]-ATP; 3000 Ci mmol, Amersham Biosciences). The final ATP concentration in the assay was 4 mM.



Fig. S3. ATPase activity of *E. coli* GInK. Analysis of ATP hydrolysis, using $[\alpha^{32}P]$ -ATP as described in *Materials and Methods*. Components of the assay are listed below the figure. Hydrolysis to ADP is shown in lanes 2–4.



Fig. S4. pH dependence of ATP hydrolysis by GlnK. Analysis of ATP hydrolysis by TLC at a range of pH values from 4.0 to 8.0. All assays contained GlnK (100 μ M); Mg²⁺ (6 mM). (A) using [γ^{32} P]-ATP showing release of free Pi. (B) using [α^{32} P]-ATP showing production of ADP.