Supporting Information

Nocek *et al.* 10.1073/pnas.0807563105

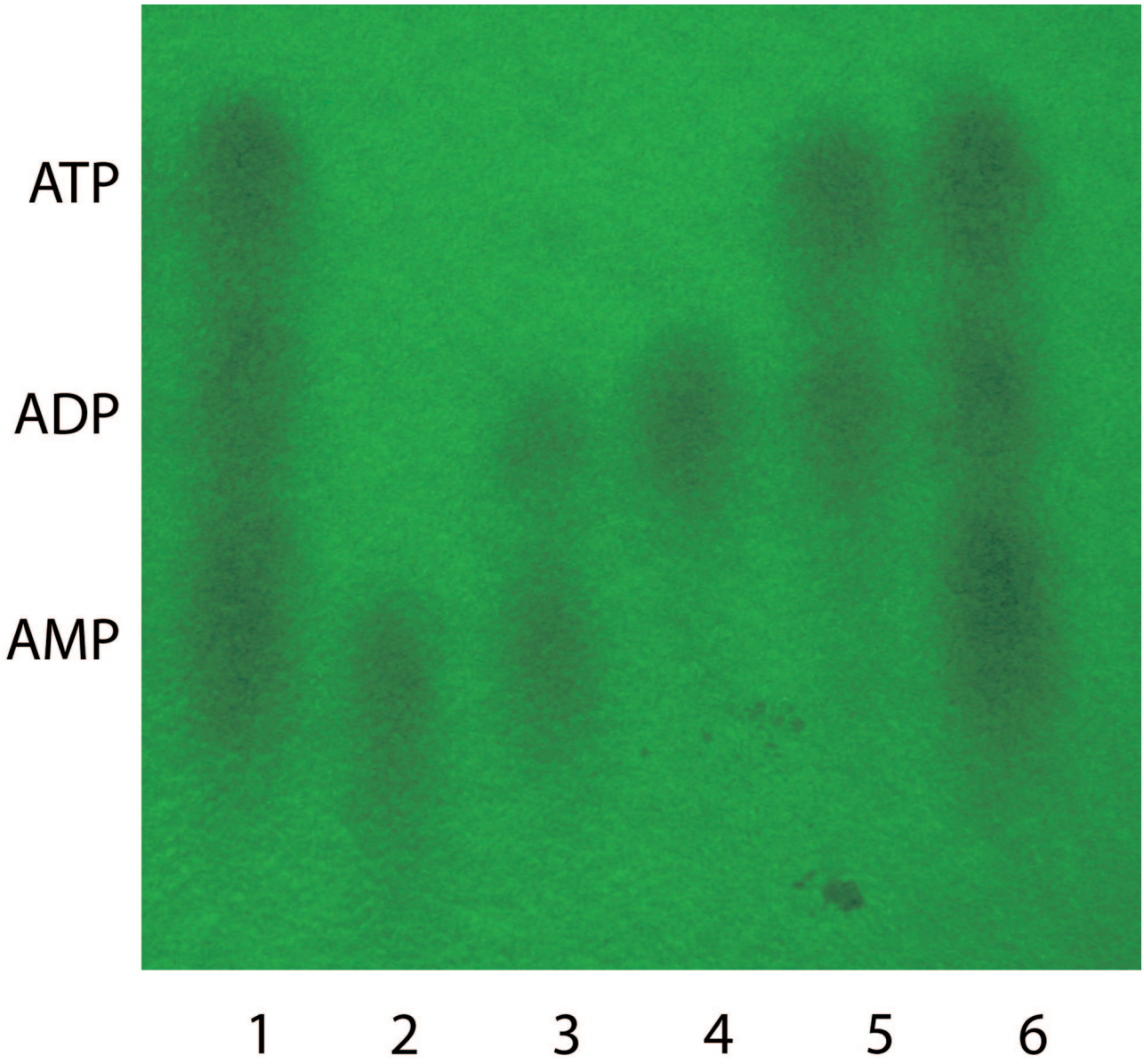

Fig. S1. Cellulose TLC plate (UV-visualized) showing the reaction products of the long (PA3455) and short (SMc02148) PPK2 proteins. Samples were: lanes 1 and 6, AMP, ADP, and ATP standards; lane 2, reaction mixture with AMP without PA3455; lane 3, reaction mixture with AMP and PA3455 showing the formation of ADP; lane 4, reaction mixture with ADP without SMc02148; and lane 5, reaction mixture with ADP and SMc02148 showing the formation of ATP. Experimental conditions were as described in *Materials and Methods*.

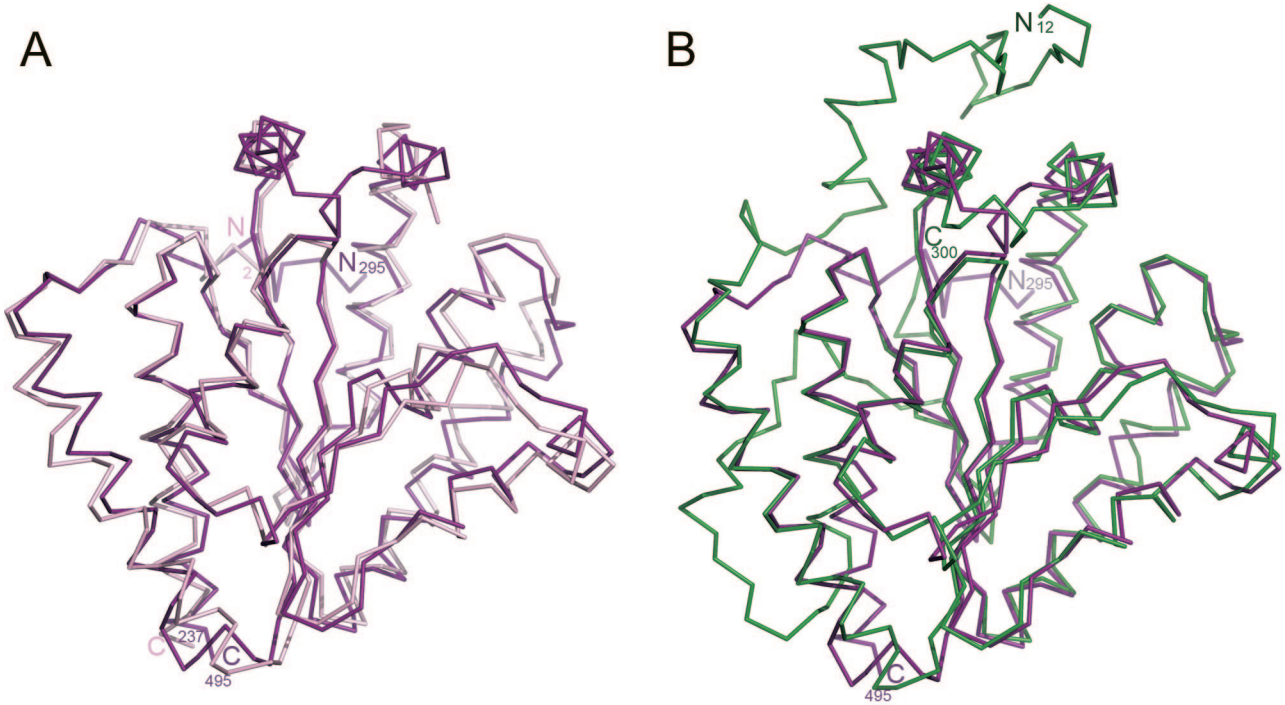

Fig. S2. Comparison of the structural folds of PPK2 domains. Superpositions of the Cα atoms of the structure models of the PA3455 C-terminal PPK2 domain (magenta) and the PA3455 N-terminal PPK2 domain (pink) (*A*) or the SMc02148 PPK2 domain (green) (*B*).

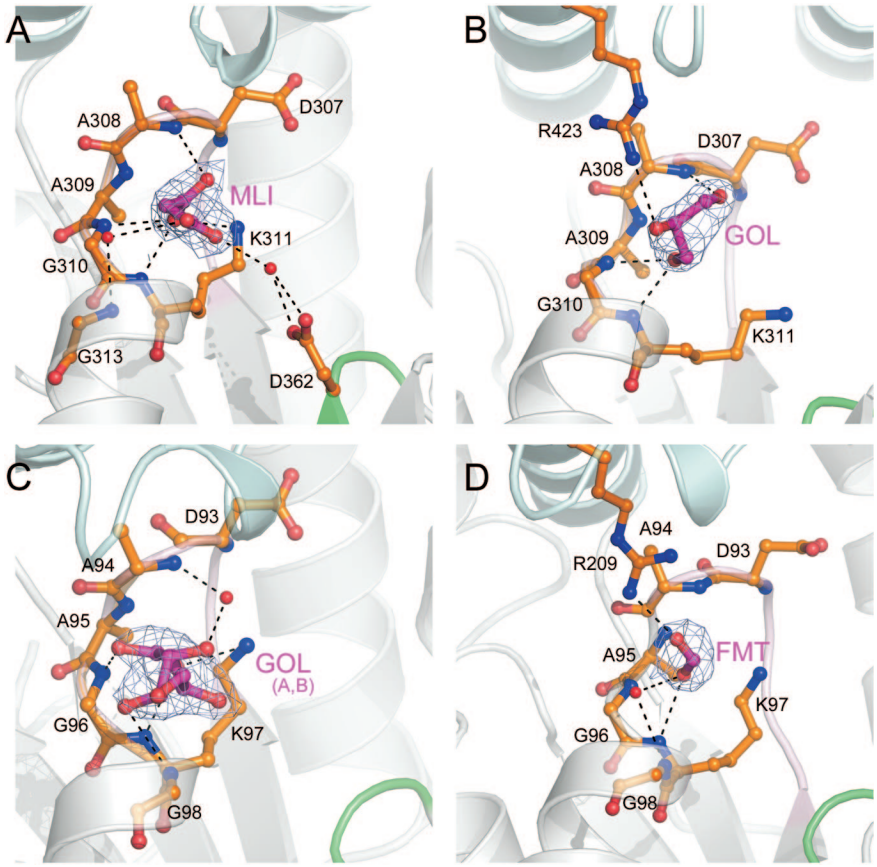

Fig. S3. Close-up view of the active sites of PA3455 (*A* and *B*) and SMc02148 (*C* and *D*) showing the bound ligands: malonic acid (*A*, MLI), glycerol (*B* and *C*, GOL), and formic acid (*D*, FMT). The ligands are shown as pink sticks covered by gray density ($F_0 - F_c$) contoured at 1.5 sigma. The residues comprising the Walker A motif are shown as orange sticks and labeled, the Walker B loop is shown in green, the lid helices are in light blue, and water molecules are red balls.

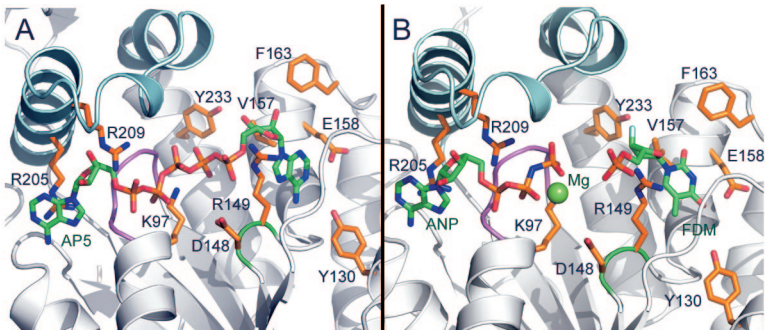

Fig. S4. SMc02148 structure overlay experiment. Close-up view showing the modeled position of the ligands from the structures of the *Bacillus stearothermophilus* adenylate kinase (PDB ID code 1ZIN; *A*) or human thymidylate kinase (PDB ID code 1NMY; *B*) in the SMc02148 active site. The structures of the adenylate kinase complex with bis(adenosine)-5′-pentaphosphate (AP5; PDB code 1ZIN) or thymidylate kinase complex with phosphoaminophosphonic acid-adenylate ester (ANP) and 3′-fluoro-3′-deoxythymidine monophosphate (FDM; PDB ID code 1NMY) were overlaid on the SMc02148 structure (PDB ID code 3CZQ) by using the SSM server. For clarity, only the secondary structure elements and residues of SMc02148 and ligands are shown. The ligands are displayed as sticks with the carbon atoms labeled in green, oxygen in red, phosphate in orange, and nitrogen in blue.

Table S1. Data collection and refinement statistics

Data collection	PA3455	SMc02148
Space group	$P2_12_12_1$	P1
Beamline	SBC-19id	SBC-19id
Cell *a*, *b*, *c*, Å	97, 101, 120	60, 72, 89
α, β, γ, °	90,90,90	76, 86, 65
Wavelength	0.9794	0.9792
Resolution, Å	40–2.0	34.7–2.3
R_{merge}*	0.079 (0.52)	0.066 (0.38)
$I/\sigma I$†	18 (2.1)	26.4 (1.99)
Completeness, %	95 (72.2)	89.4 (46.8)
Redundancy	4.6 (3.8)	3.6 (2.2)
No. of measurements	349,078	228,073
Unique reflections	76,187	63,353
Phasing (SAD)		
Resolution range, Å	40–2.70	34.7–2.23
No.of Se-Met	6	40
Phasing power	1.65	1.63
Refinement		
Resolution, Å	40–2.0	34.7–2.23
No. of reflections	76,108	53,711
R_{cryst}/R_{free}, %‡	17.35 (23.0)	18.7 (24.0)
No. of residues		
Protein	940	1147
Glycerol/malonic acid/acetate ion/ formate ion	4;1;5;0	1;0;0;2
Water	670	266
B factors		
Protein	31.6	56.4
Glycerol/malonic acid/acetate ion/formate ion	53.7/58/49/-	42.2/-/-/67.0
Water	45.1	49.2
rmsd		
Bond lengths, Å	0.020	0.015
Bond angles, °	1.58	1.43
Ramachandran plot, % favored/allowed/generously allowed/outliers	94.7/4.8/0.3/0.3	92.4/7.6/-/-
PDB ID code	3CZP	3CZQ

Values in parentheses are for highest-resolution shell.

*$R_{merge} = (\Sigma_{hkl} - <I>|)/\Sigma I_{hkl}$, where the average intensity $<I>$ is taken over all symmetry equivalent measurements and I_{hkl} is the measured intensity for any given reflection.

†I/σ is the mean reflection intensity divided by the average estimated error.

‡$R_{cryst} = |F_o| - |F_c|/|F_o|$, where F_o and F_c are the observed and calculated structure factor amplitudes, respectively. R_{free} is equivalent to R_{cryst} but is calculated for 5% of the reflections chosen at random and omitted from the refinement process.