Supplementary Information

Crystal structure of a polyphosphate kinase and its implications for polyphosphate synthesis

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Figure Legends:

Supplementary Figure 1S. Overall structure of the unliganded PPK tetramer. *E. coli* PPK forms a homotetramer of 80 kDa subunits in solution, and becomes a homodimer upon the binding of AMPPNP. It is unclear why unliganded PPK forms tetramer. Here, the four PPK molecules are colored in red, green, yellow and blue, respectively. The tetramer is formed by a 2-fold crystallographic symmetry operation between two dimers along the C-axis. Since there is no clear hydrophobic surface patch in the PPK dimer and tetramer structures, it is not clear why PPK is predominantly bound to cell membranes.

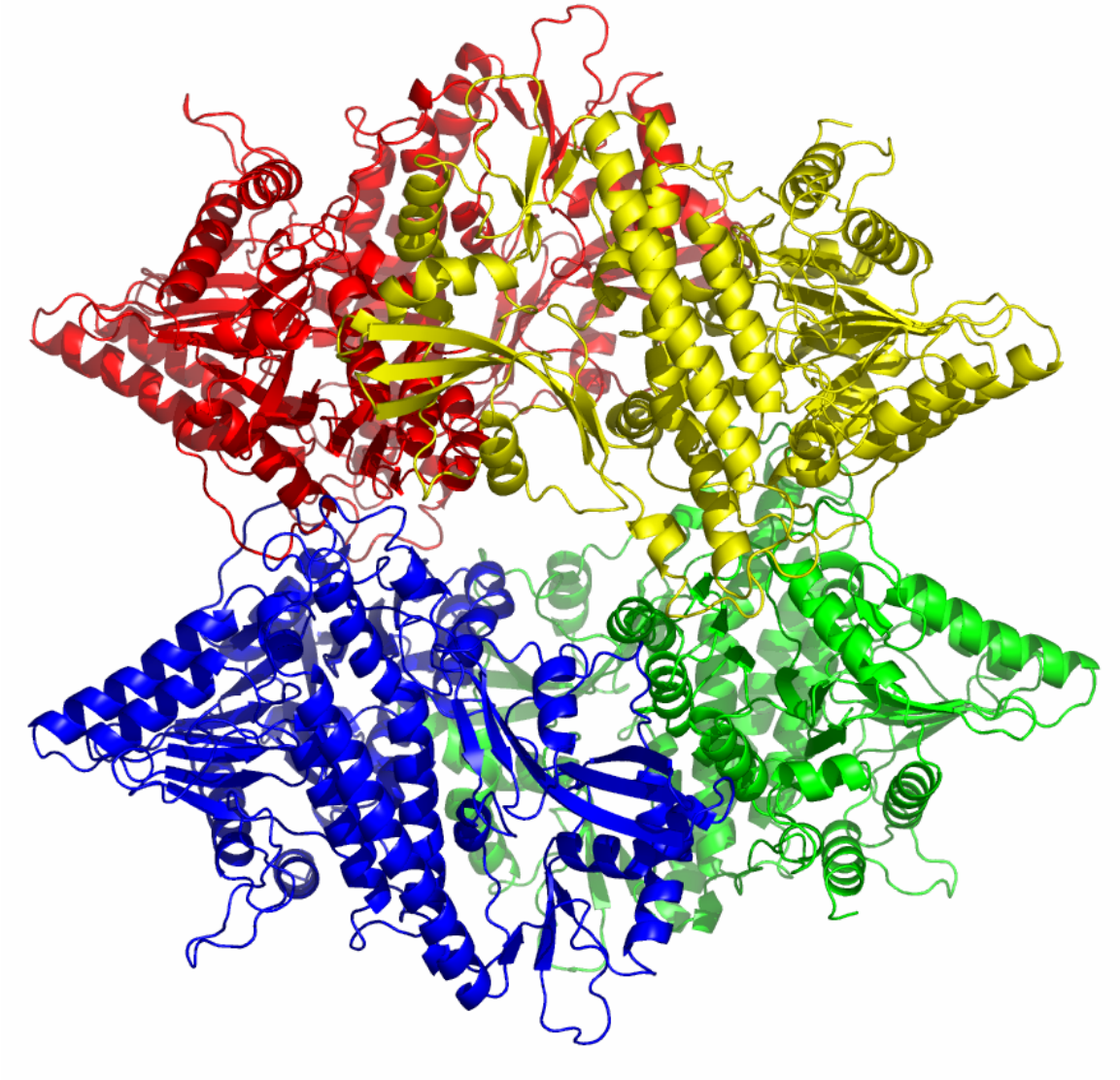
Supplementary Figure 2S. Stereo view of the tunnel exit. The tunnel is roughly parallel to the three long helices in the N-terminal domain and perpendicular to the paper plane here. The AMPPNP is colored by element (red, pink, blue). Several positively charged residues, Lys 10, Arg 53, Arg 60, Arg 375, Arg 405, Lys 433, Arg 616 and Arg 621, lie along the tunnel between the active site and the postulated polyP exit. All these residues, except Arg 60, are conserved in the PPK family. The carbon atoms of these side chains are shown in yellow, and nitrogen atoms in blue.

Supplementary Figure 3S. Stereo view of the adenine ring binding pocket. The adenine ring of AMPPNP is sandwiched between PPK residues Leu 589 (in the “bottom”) and Ile 41 (on “top” of the paper, not shown here). The N7 of AMPPNP forms a hydrogen bond,

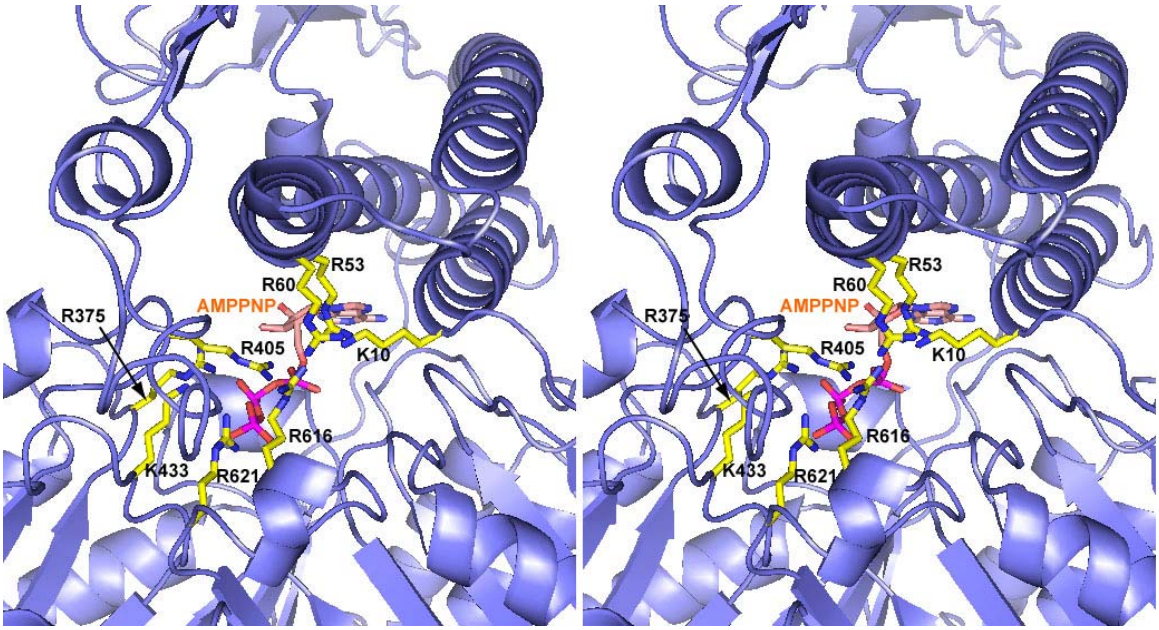
shown with a bold dash, with the side chain of Asn 45. The distance between N6 and the carbonyl of N587 is too long for a hydrogen bond (4.2 Å, shown in fine dash), but may contribute to the PPK substrate selectivity for ATP over GTP.

Supplementary Figure 4S. Comparison of the C1- and C2-domains of PPK and the catalytic domain of phospholipase D. The C1- and C2-domains of PPK fold similarly to PLD, whereas the elements of secondary structure are topologically arranged different from that of PLD (PLD, PDB entry 1F0I).

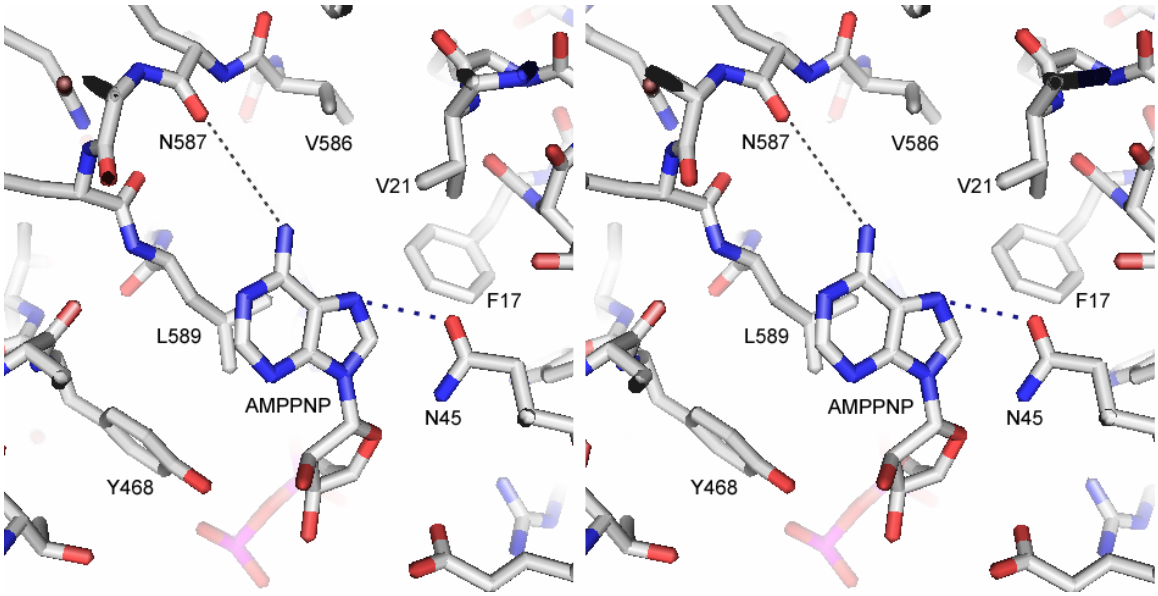
Supplementary Figure 5S. Comparison of the catalytic sites of PPK and PLD. PPK is shown in blue, while the AMPPNP in complex with PPK shown in colored sticks. PLD is shown in green, with the phosphate group observed in PLD crystal structure shown in red and pink. Hydrogen bonds are indicated by dash lines. The active site hydrogen bond network is partially conserved between PPK and PLD.



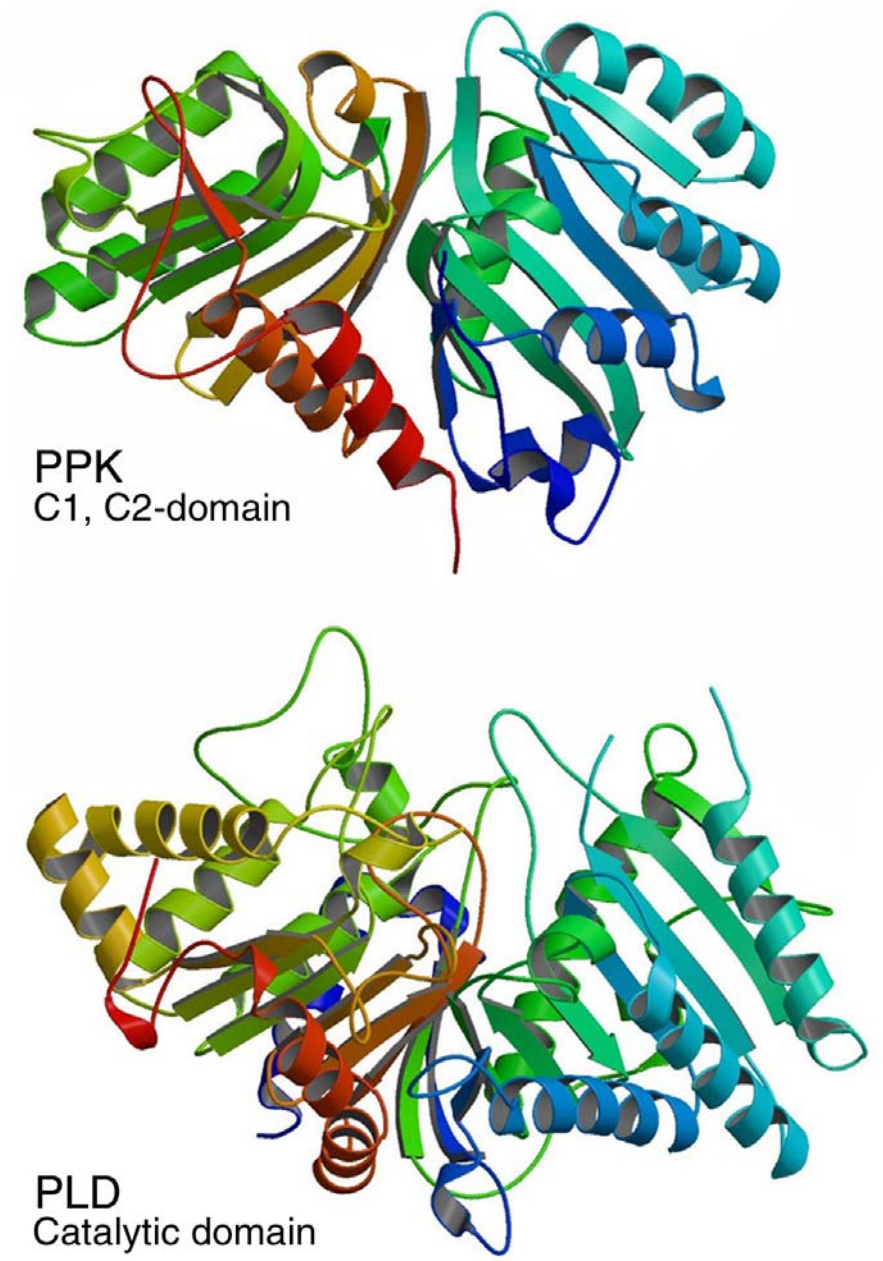
Supplementary Figure 1S



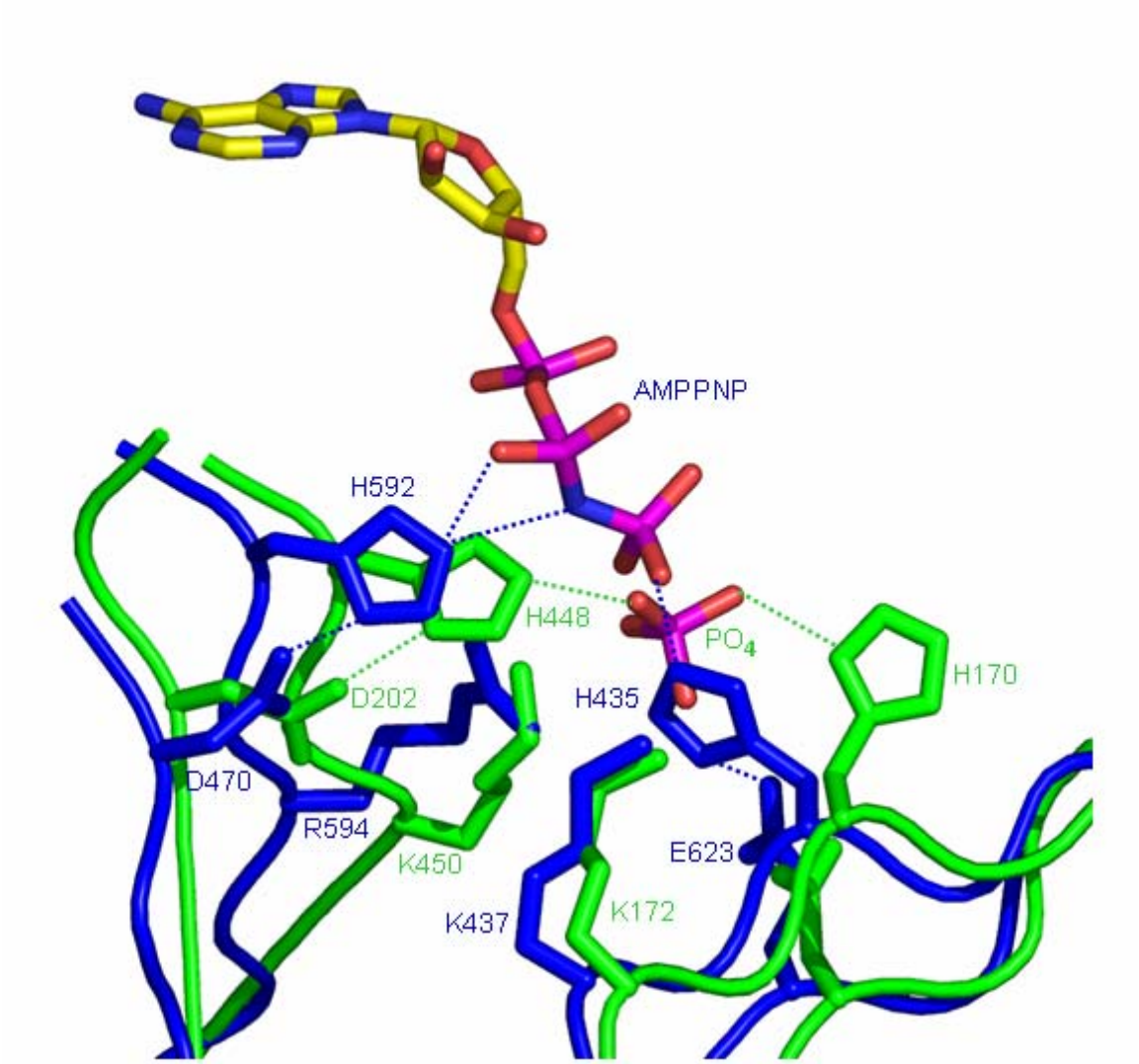
Supplementary Figure 2S



Supplementary Figure 3S



Supplementary Figure 4S



Supplementary Figure 5S

Supplementary Table 1. Summary of structure determination

Data set	SeMet MAD (PPK-AMPPNP)				
	Peak	Inflection	remote	PPK-AMPPNP	PPK
Wavelength (Å)	0.97929	0.979399	0.95372	0.97929	0.9729
Unique Reflections	105842	86379	81097	113237	37809
Resolution (Å)	20–2.8	20–2.8	20–2.8	20-2.5	20-3.0
redundancy	3.8	3.9	4.8	4.9	7.4
Completeness*	95.8 (86.8)	94.1 (86.1)	95.4(88.2)	97.4 (92.1)	89.5(80.3)
$I/\sigma(I)$*	7.4 (2.5)	6.9 (1.8)	9.6 (1.6)	9.1 (1.7)	18.2(4.5)
R_{merg} (%)*	9.0 (26.7)	13.0 (31.4)	10.5(32.5)	9.9 (33.7)	8.3(35.6)

Phasing from SeMet MAD data set

Resolution (Å)	20-3.0
Number of selenium sites found (expected)	21 (out of 30)
Anomalous phasing power from peak (acentric)	2.1
Anomalous phasing power from inflection (acentric)	2.0
Anomalous phasing power from remote (acentric)	2.2
Figure of merit (centric/acentric)	0.51/0.55

Refinement Statistics	PPK-AMPPNP‡	PPK
Resolution	20-2.5	20-3.0
No. of reflections (working/test)	58341/1802	32521/1007
No. of nonhydrogen atoms†	11407	11342
R_{free}/R_{cryst} (%)	27.4/24.8	27.3/25.5
Average B factor (Å^2)	19.2	21.5
Rmsd bond length (Å)	0.0081	0.0090
Rmsd bond angle (°)	1.45	1.42

*Numbers in parentheses refer to data in the highest resolution shell. †This number does not include atoms that cannot be observed in the electronic density. ‡Refinement against PPK-AMPPNP data set. Rmsd, root mean square deviation