SUPPLEMENTARY MATERIAL

X-ray structures of isopentenyl phosphate kinase

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Gene amplification and cloning. The THA IPK gene was amplified from genomic DNA (ATCC: 25905D) by PCR using *Pfu*Ultra II DNA polymerase (Stratagene) and the forward and reverse primers 5'–CACCATGATGATACTGAAGATAGG–3' and 5'–TCATCTTATCACCGTACCTATGAATG–3', respectively. The forward primer contains the sequence CACC, which anneals with the overhang sequence of the topoisomerase-charged cloning vector, pET151/D-TOPO (Invitrogen). The double stranded PCR product was directionally cloned into the pET151/D-TOPO vector to contain an N-terminal hexahistidine tag, the V5 epitope, and the TEV Prprotease cleavage sequence upstream of the start codon. The resulting construct was used to transform *E. coli* DH5α cells, which were then grown overnight in LB plates containing ampicillin. Single colonies were picked for purification of plasmids containing the THA IPK gene. Plasmids containing the correct insert were used to transform *E. coli* BL21 (DE3)-CodonPlus-RIL cells (Stratagene) for repeatable and high-yield expression.

The amplification and cloning of the MTH IPK gene is described in reference *(1)*.

Protein expression and purification. Selenomethionine-substituted THA IPK was overexpressed in *E. coli* BL21 (DE3)-CodonPlus-RIL (Stratagene) using the auto-induction method *(24)*. A starter culture was grown overnight in PA-0.5G media containing chloramphenicol and ampicillin, and then diluted 1000-fold in PASM-5052 media containing 25 mg ml^{-1} selenomethionine, 1x trace metals mix, 100 μM vitamin B_{12} and 17 amino acids (each at 10 mg mL^{-1}) excluding cysteine, tyrosine and methionine. The culture was incubated at 37 ^{o}C for 8 h followed by incubation at 23 ^{o}C until OD_{600} > 8.0. Cells were harvested by centrifugation, resuspended and lysed in binding buffer containing 50 mM Na_2HPO_4, pH 8.0, 150 mM NaCl, 10 mM imidazole and 1 mM β-mercaptoethanol. DNAse (1 μg mL^{-1}) and lysozyme (1 mg mL^{-1}) were added to the cell suspension and incubated for 30 min. The cell suspension was sonicated for three min, and cell debris were removed by centrifugation (20,000 x g for 25 min at 4 ^{o}C). The supernatant was

heat-treated for 15 min at 50 ^{o}C before another centrifugation step. The clarified supernatant was incubated at 4 ^{o}C with Ni-NTA resin (Qiagen) pre-equilibrated with binding buffer. Bound proteins were eluted with buffer containing 50 mM Na_2HPO_4, pH 8.0, 150 mM NaCl, 250 mM imidazole and 1 mM β-mercaptoethanol. Eluted proteins were pooled and the hexahistidine tag and V5 epitope were removed by adding recombinant TEV protease at 1 mM with dialysis against a buffer solution containing 50mM Tris-HCl, 150mM NaCl and 1mM DTT at 4 ^{o}C for 24 h. The dialysate was applied to Ni-NTA and the flow through containing the cleaved THA IPK was subjected to a final purification step by gel filtration using Superdex 200 (GE Healthcare) equilibrated with 50 mM HEPES pH 7.5, 150 mM NaCl, 1mM β-mercaptoethanol. Using the extinction coefficient 17,880 M^{-1} cm^{-1} at 280 nm calculated using the ProtParam tool in the ExPASy Proteomics server *(2)*, THA IPK concentration was estimated and protein was concentrated to 0.30 mM for subsequent crystallization. Selenomethione incorporation of cleaved THA IPK was confirmed by mass spectrometry.

Native THA IPK was obtained by growing transformed BL21 (DE3)-CodonPlus-RIL cells (Stratagene) in ZY-5052 autoinduction media and using the purification procedure above.

The expression and purification of MTH IPK is described in *(1)*. For crystallization, the purified protein was concentrated to 5 mg ml^{-1} in a stabilization buffer of 25 mM HEPES, pH 7.5, 75 mM NaCl, 0.5 mM β-mercaptoethanol.

Metal ion requirement. The metal ion dependence of IP kinase was determined by the radioactivity assay using IP and [^{32}P]ATP (American Radiolabeled Chemicals) as substrates *(1)*. Reaction mixtures contained 100 mM HEPES, pH 7.5, 10 mM β-mercaptoethanol, 0.1% (w/v) BSA, 400 μM IP, 400 μM [^{32}P]ATP and 10 mM of $MgCl_2$, $MnCl_2$, $ZnCl_2$, $CuCl_2$, $NiCl_2$, $CdCl_2$ or $CoCl_2$. The reactions were initiated by adding purified THA IPK to a final volume of 50 μL followed by incubation at 37 ^{o}C for 10 min. Reactions were quenched by adding 113 μL of methanol/750 mM EDTA (100:13, v/v). Samples (6 μL) of the

quenched reaction mixtures were spotted on silica TLC plates and developed with $CHCl_3$/pyridine/formic acid/H_2O (30:70:16:10, v/v). The TLC plates were imaged for 24 h using a storage phosphor imager autoradiography cassette and visualized by a Typhoon 8600 variable mode imager.

REFERENCES

1. Chen, M., and Poulter, C.D. (2010) Characterization of thermophilic and archaeal isopentenyl phosphate kinases, *Biochemistry 49*, 207–217.

2. Gasteiger E., Hoogland C., Gattiker A., Duvaud S., Wilkins M.R., Appel R.D., and Bairoch A. (2005) Protein Identification and Analysis Tools on the ExPASy Server, in *The Proteomics Protocols Handbook* (Walker, J., Ed.), pp 571–607.

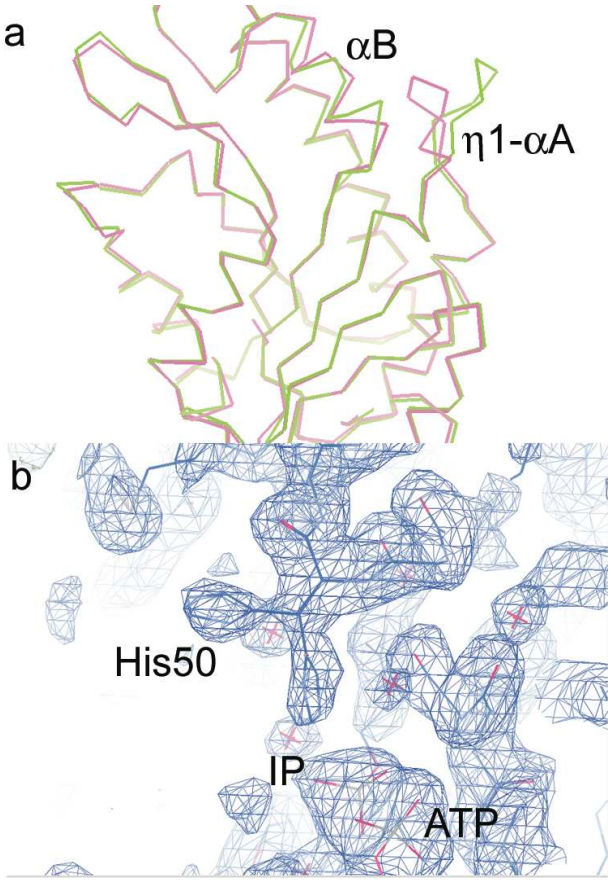

Figure S1 Unique molecules of IPK•IPP•ADP in the asymmetric unit (a) The loop connecting η1 and αA has two different conformations, causing Tyr17 in one conformation (pink ribbon) to make crystal contacts with neighboring molecules. This loop movement also results in the displacement of Lys14 from the optimal position for catalysis. The movement of αB is also shown in (a). (b) Two conformations of His50 in the active site of molecule D in the asymmetric unit. The two conformations are also seen in molecule C.

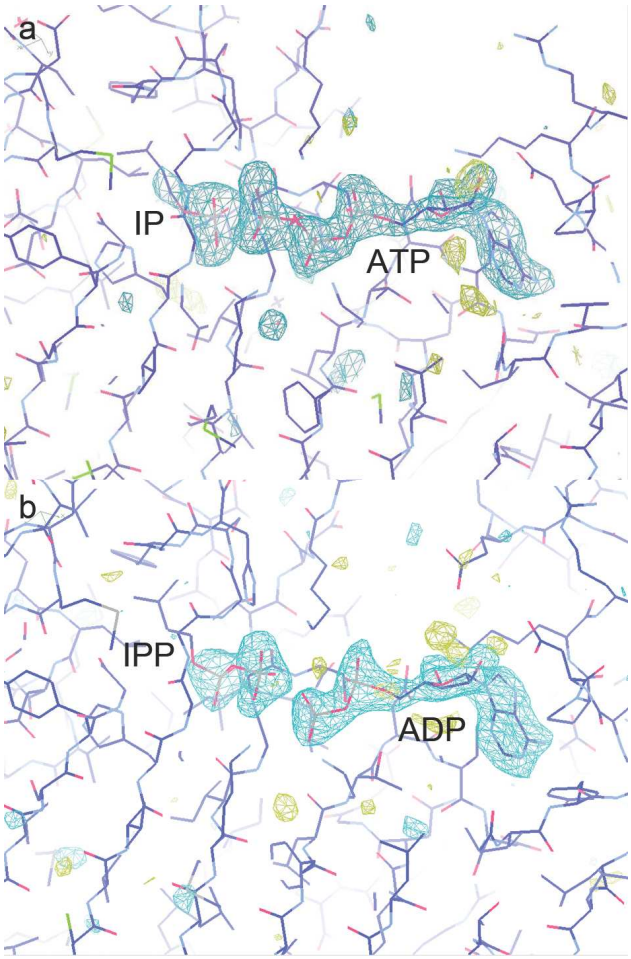

Figure S2 F_o-F_c ligand omit maps contoured at 1.0 RMSD. (a) Ligand omit map for IP and ATP. Green density shows the substrates before phosphoryl transfer. (b) Ligand omit map for IPP and ADP, showing bound products in the THA IPK active site.

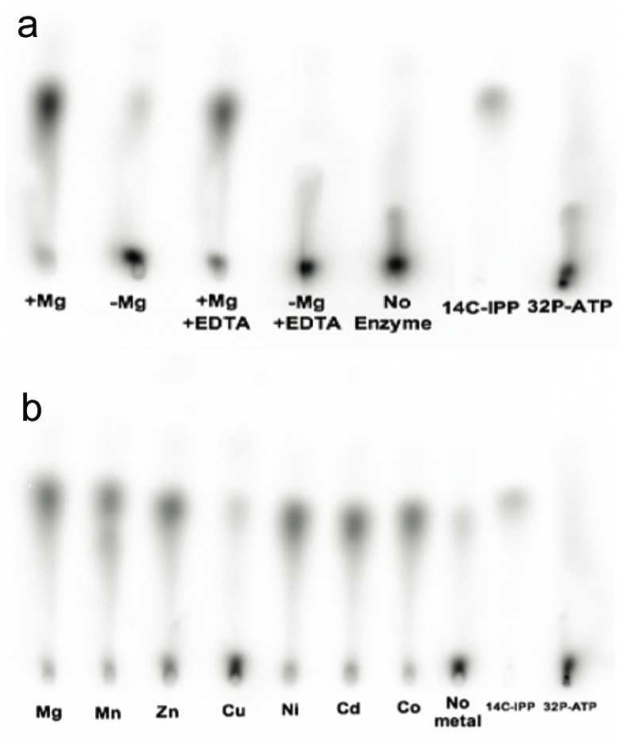

Figure S3 Autoradiogram showing dependence of THA IPK activity on divalent metals. (a) The Mg^{2+} ion enhances the turnover of [^{32}P]IPP by THA IPK, while EDTA (100 mM) abolishes product formation. (b) Different divalent metals can efficiently substitute for Mg^{2+}, with Cu^{2+} showing the least enhancement of THA IPK activity.