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Supporting information for article:

Crystal structure of recombinant phosphoribosylpyrophosphate synthetase-2 from *Thermus thermophilus* HB27 complexed with ADP and sulfate ions

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S1. Cloning, expression and purification of 5-phospho-D-ribosyl □-1-diphosphate (PRPP) synthetase from *T. thermophiles* HB27

The recombinant pET-23d+ plasmid harboring the genomic PRPPS2 (EC 2.7.6.1) from *T. thermophilus* HB27 was transformed into E. coli C3029/pGTf2 cells (New England Biolabs, Takara Bio Inc). The resulting producer strain was cultivated in LB medium containing 50 μg/mL ampicillin, 20 μg/mL chloramphenicol, and 1 ng/mL tetracycline. At density of OD600 = 0.8 and culture was supplemented with isopropyl-β-*D*-1-thiogalactopyranoside to final concentration 0.4 mM and grown for another 5 h at 37°C. The cells were harvested by centrifugation, resuspended in buffer 50 mM Tris HCl, pH 8.0, 500 mM NaCl, 5 mM MgCl2, 1 mM phenylmethylsulphonyl fluoride and disrupted by sonication. The cell debris was pelleted by centrifugation. The target protein was enriched by the immobilized metal ion affinity chromatography using a XK 16/20 column (GE Healthcare) packed with nickel (II)-iminodiacetic acid beads (Ni-IDA; Sigma-Aldrich). Ballast proteins were eluted with buffer containing 50 mM imidazole and desired enzyme was eluted 200 mM imidazole. The fractions containing the protein were pooled, concentrated by ultrafiltration (Amicon ultrafiltration cups with ultrafiltration polysulfone membrane PBGC 10 kDa, Millipore, USA) and loaded onto a column HiLoad 16/60 with Superdex 200 pg (GE Healthcare) equilibrated with buffer 20 mM Tris•HCl, pH 8.5; 1 mM ATP, 1 mM MgCl2, 5% glycerol, 0.04% NaN3. The fractions containing the protein were pooled and concentrated by ultrafiltration (Amicon ultrafiltration cups with ultrafiltration polysulfone membrane PBGC 10 kDa, Millipore, USA) to the final concentration 12.3 mg/mL that was stored in refrigerator at -80 ºC. The *Tth*PRPPS2 preparation was ca. 96.5% pure as evaluated by the Bio-Rad Molecular Imager Gel documentation system (Bio-Rad, Image Lab 5.0).

S2. Enzymatic activity assay

The *Tth*PRPPS2 activity was determined in a reaction mixture containing a 1 mM disodium ATP salt, a 1 mM disodium salt of *D*-ribose-5 phosphate, 5 mM $MgCl_2$, 10 mM KH_2PO_4, 20 mM Tris-HCl, pH 8.0, at 75 °C. *Tth*PRPPS2 (0.75 μg) was added to 0.5 mL of the mixture. Substrate and product concentrations were determined by HPLC under isocratic elution with 0.1 M KH_2PO_4 (water, pH 6.0, flow rate of 0.5 mL/min) with detection at 254 nm (Waters 2489 UV detector; Supelcosil LC-18-T column, 5 μm, 150 × 4.6 mm).

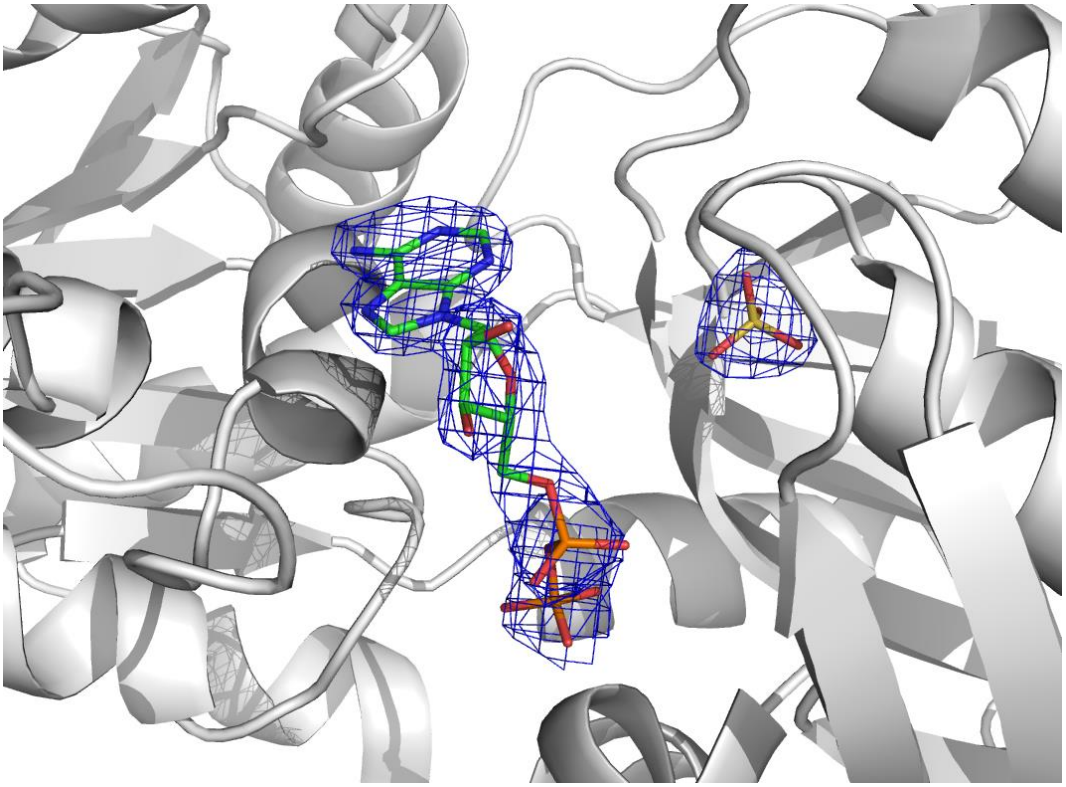

Figure S1 ADP and sulfate ions in the active site of B subunit of *Tth2*PRPP synthetase. The electron density is calculated with |Fo| -|Fc| coefficients at the 2.0 σ level. The ligands were excluded in the calculation of the electron-density map.

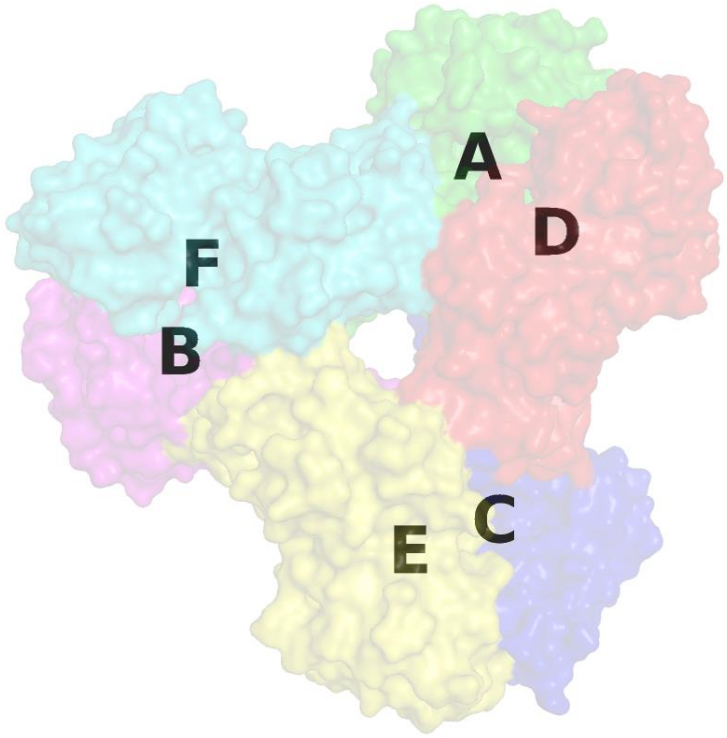

Figure S2 Biological assembly of *Tth2*PRPP synthetase. A, B and C are independent subunits, and D, E and F are symmetric subunits.

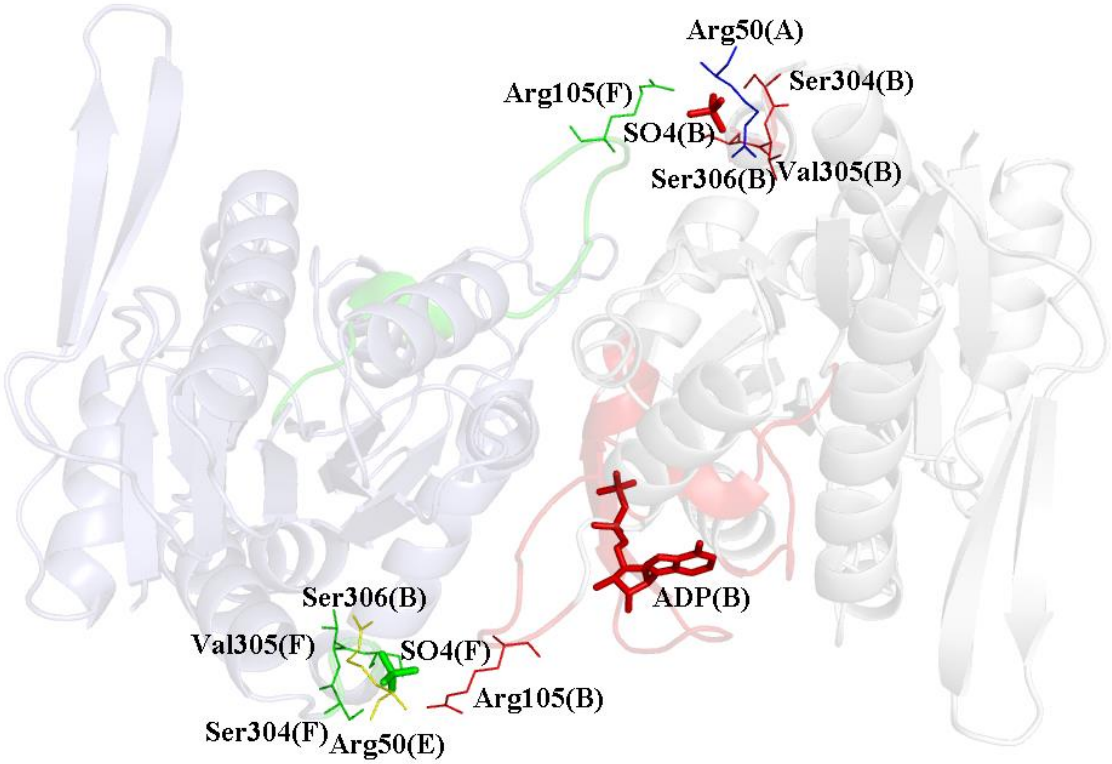

Figure S3 The allosteric sites in the subunits B and F of *Tth2* PRPP synthetase.