Supplemental Information

"Strain and near attack conformers in enzymic thiamin catalysis: X-ray crystallographic snapshots of bacterial transketolase in covalent complex with donor ketoses xylulose 5-phosphate and fructose 6-phosphate, and in noncovalent complex with acceptor aldose ribose 5-phosphate"

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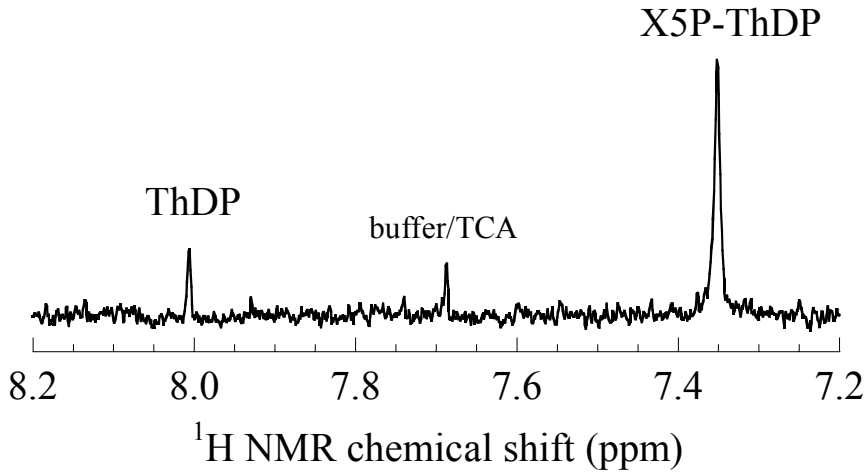
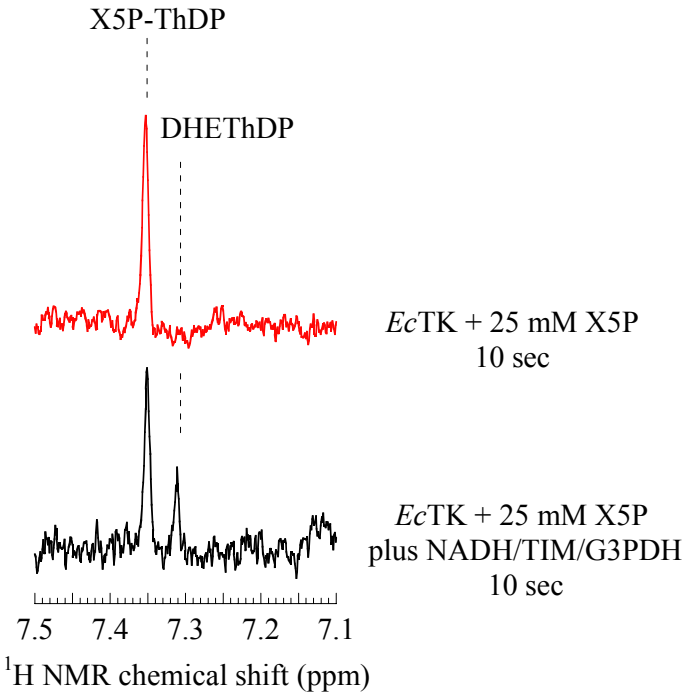


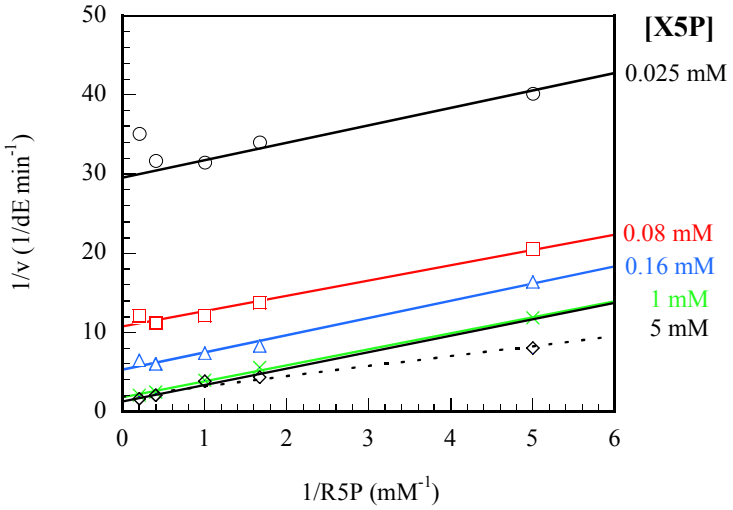
SUPPLEMENTAL FIGURE S1.

Distribution of catalytic intermediates after reaction of *Ec*TK with 25 mM X5P in the absence of acceptor for 60 sec at 8 °C. Further reaction conditions are detailed in the Materials and Methods section of the main manuscript.



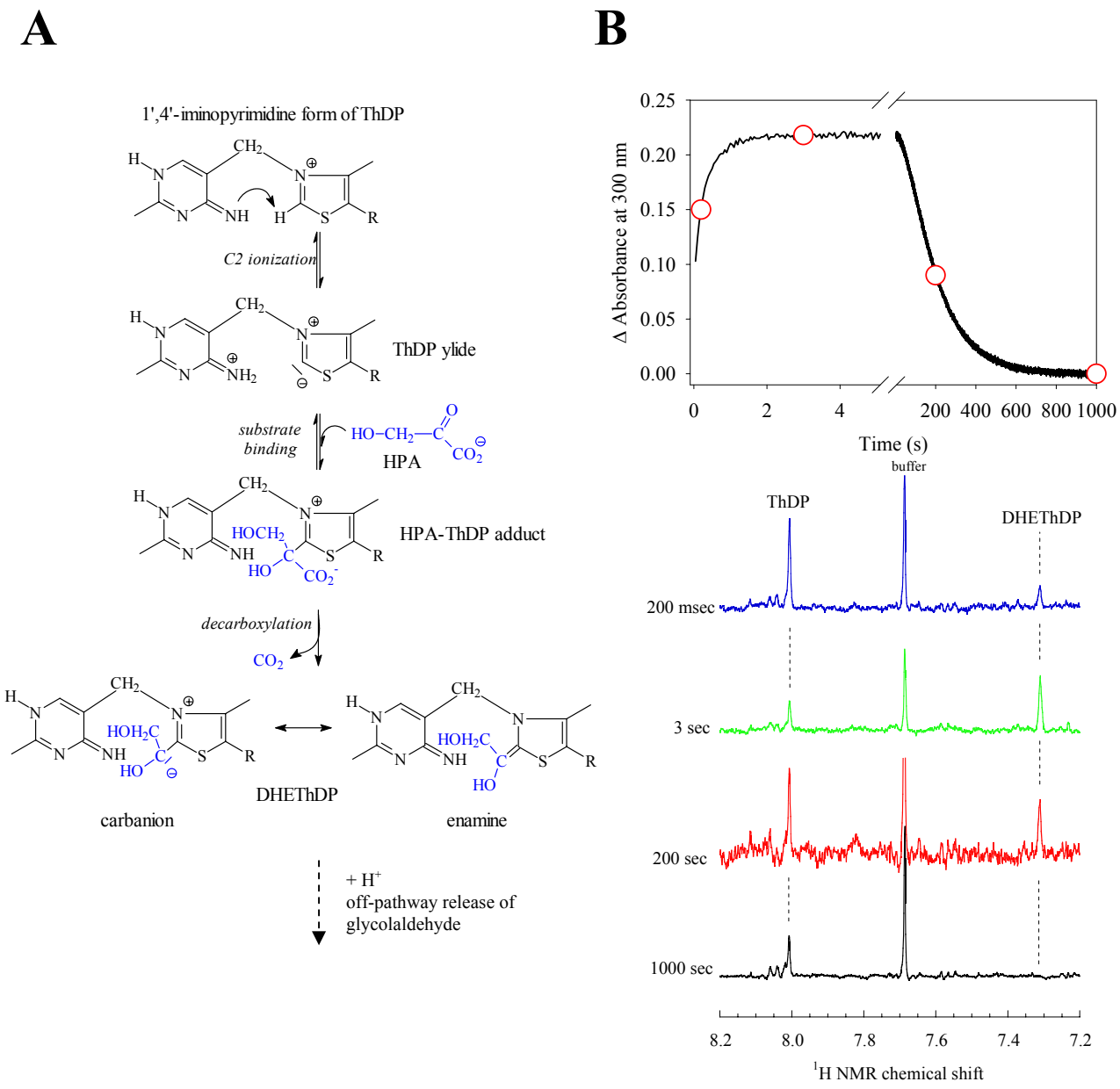
SUPPLEMENTAL FIGURE S2.

Distribution of catalytic intermediates after reaction of *Ec*TK with 25 mM X5P (upper panel), and after reaction with 25 mM X5P in presence of NADH plus the auxiliary enzymes triosephosphate isomerase (TIM) and *sn*-glycerol-3-phosphate : NAD^+ 2-oxido-reductase (G3PDH) (lower panel) for 10 sec at 8°C.



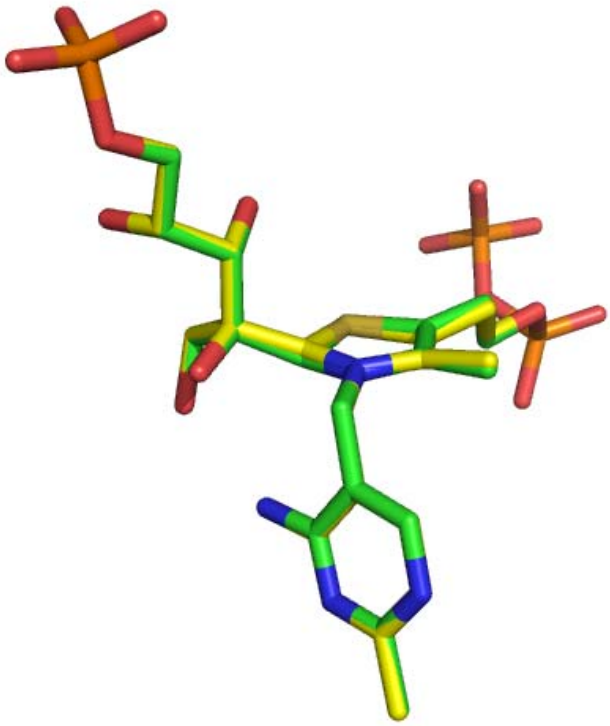
SUPPLEMENTAL FIGURE S3.

Analysis of the kinetic mechanism of *Ec*TK-catalyzed conversion of donor X5P and acceptor R5P yielding G3P and S7P. The formation of G3P was detected in a continuous spectrophotometric assay employing TIM (4.5 U) and G3PDH (1.55 U) as auxiliary enzymes. The concomitant oxidation of NADH (0.25 mM) was monitored at 340 nm and 25 °C in 50 mM glycyl-glycine pH 7.6 supplemented with 0.3 mM ThDP and 2.5 mM Ca^{2+}. Steady-state rates were determined for different fixed concentrations of X5P (0.025 – 5 mM) and R5P (0.2 – 5 mM) at constant concentration of *Ec*TK (3 µg/mL). Exemplary, the double-reciprocal plot 1/v versus 1/R5P is shown being consistent with a Ping Pong mechanism. There is evidence for a kinetic competition between donor and acceptor at high concentrations of either substrate (5 mM) being most apparent at low concentrations of the corresponding substrate. For regression analysis (solid lines), the data obtained at 5 mM R5P/0.025 mM X5P and 0.2 mM R5P/5 mM X5P were not considered. For the latter case, regression analysis including high concentrations is shown as a dotted line. A likewise competition between donor and acceptor has been described for TK from rat liver (*30*).



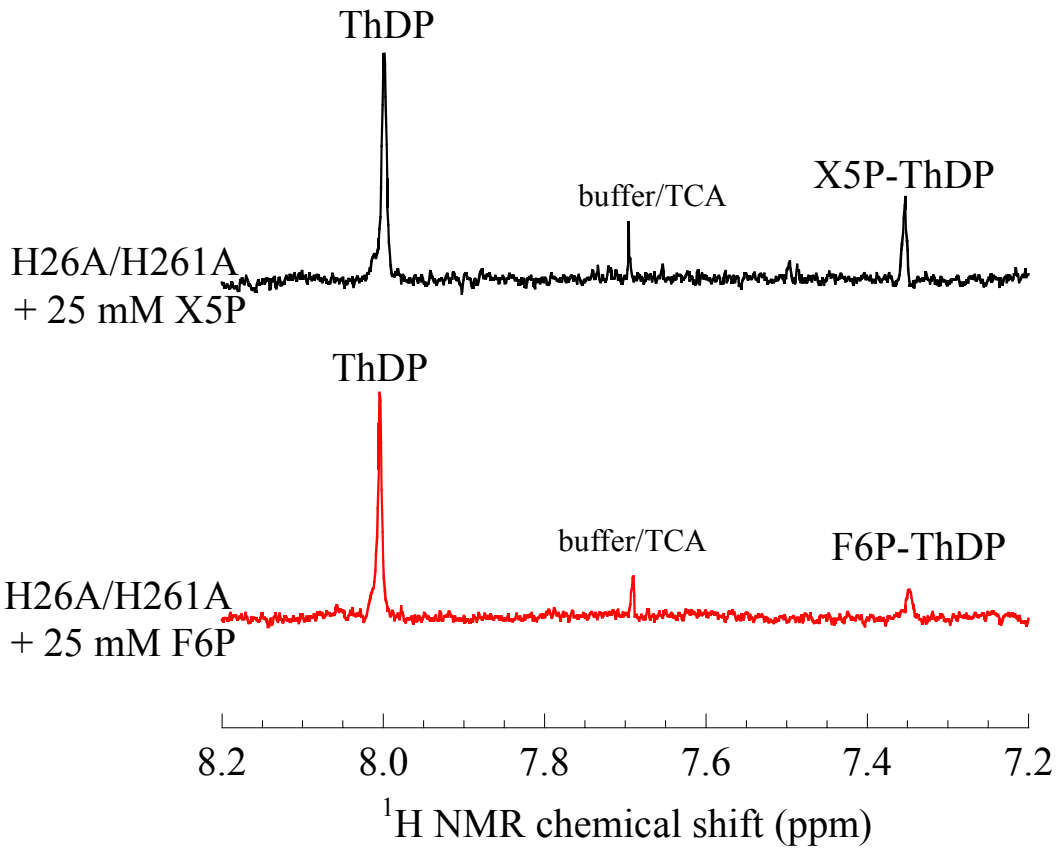
SUPPLEMENTAL FIGURE S4.

Kinetic analysis of the reaction of *Ec*TK with the artificial donor HPA (**A**) under single turnover conditions and in the absence of acceptor by stopped-flow kinetics (**B**, upper panel) and chemical quenched-flow/^{1}H NMR spectroscopy (**B**, lower panel). 15 mg/mL holo-*Ec*TK (205 µM active site concentration) in 50 mM glycyl-glycine buffer, pH 7.6 were mixed with 205 µM HPA (in the same buffer) in a 1+1 mixing ratio at 25 °C. Stopped-flow transients of enamine formation (**B**, upper panel) were recorded at 300 nm and an optical path length of 10 mm. The distribution of covalent ThDP intermediates after different reaction times (red circles in **B**, upper panel) was analyzed by quenched-flow/NMR (**B**, lower panel) as detailed in ref *20*.



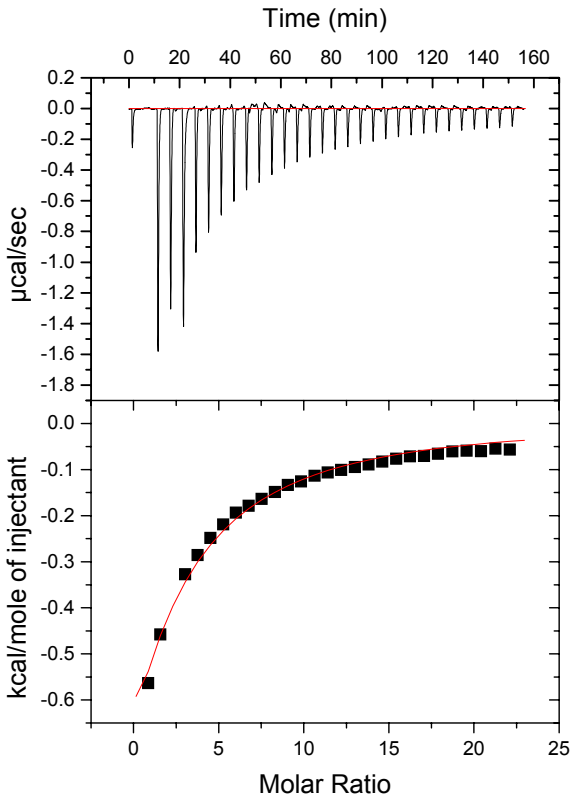
SUPPLEMENTAL FIGURE S5.

Refined structures of X5P-ThDP trapped in the active site of *Ec*TK while employing either in-plane restraints for the thiazolium ring atoms (green), and alternatively, without any restraints for the torsion angles of the thiazolium bonds (yellow). Note that the thiazolium C2 ring atom is slightly out of the ring plane (yellow structure) by a few degrees when no in-plane restraints are imposed for refinement.



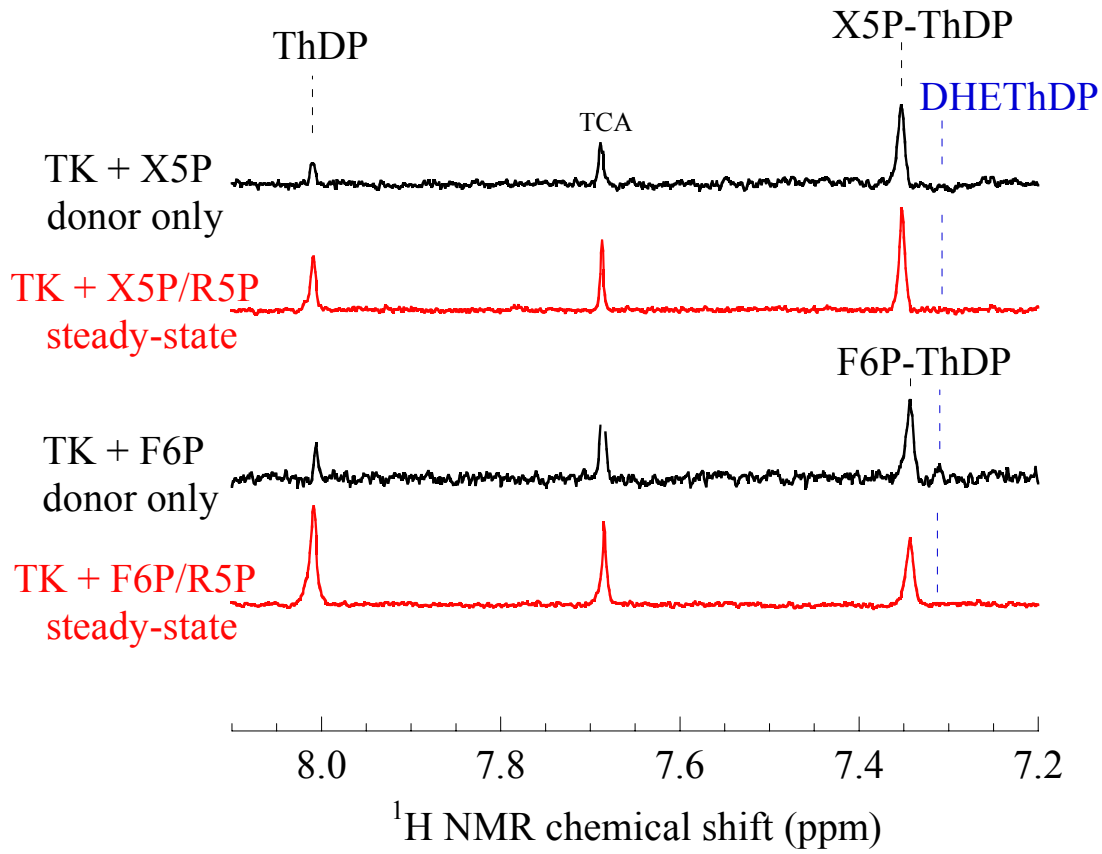
SUPPLEMENTAL FIGURE S6.

Distribution of covalent reaction intermediates after reaction of *Ec*TK double variant H26A/H261A with 25 mM X5P or 25 mM F6P in the absence of acceptor under equilibrium conditions at 8 °C. Reaction conditions were as detailed for the wild-type enzyme in the Materials and Methods section of the main manuscript. The variant exhibits a residual activity of less than 1 %, the apparent K_M values for donor and acceptor in steady-state assays are very similar to those of the wild-type enzyme (*19*).



SUPPLEMENTAL FIGURE S7.

Isothermal titration calorimetric analysis of the binding of the acceptor aldose R5P to bacterial TK in the absence of donor substrates. The data were fit (red solid line) to a one-site binding model yielding an apparent dissociation constant of $K_{app} = 714$ µM for R5P. Experimental details are given in the Materials and Methods section.



SUPPLEMENTAL FIGURE S8.

Quantitative NMR-based comparison of the intermediate distribution in bacterial TK for the donor half-reaction (black) and at the true steady-state (red), employing X5P and F6P as donor ketoses and R5P as acceptor aldose. The steady-state intermediate distribution suggests that formation and decay of the covalent donor-ThDP adducts are rate-limiting for TK catalysis. As neither DHEThDP nor S7P-ThDP are detectable, it may be concluded that carboligation (DHEThDP + acceptor) and product release are significantly faster than binding and cleavage of the donor-ThDP adduct (compare Figure 1A in the main text).