**Structure *15***

# Supplemental Data

# Quaternary Structure Change

# as a Mechanism for the Regulation

# of Thymidine Kinase 1-Like Enzymes

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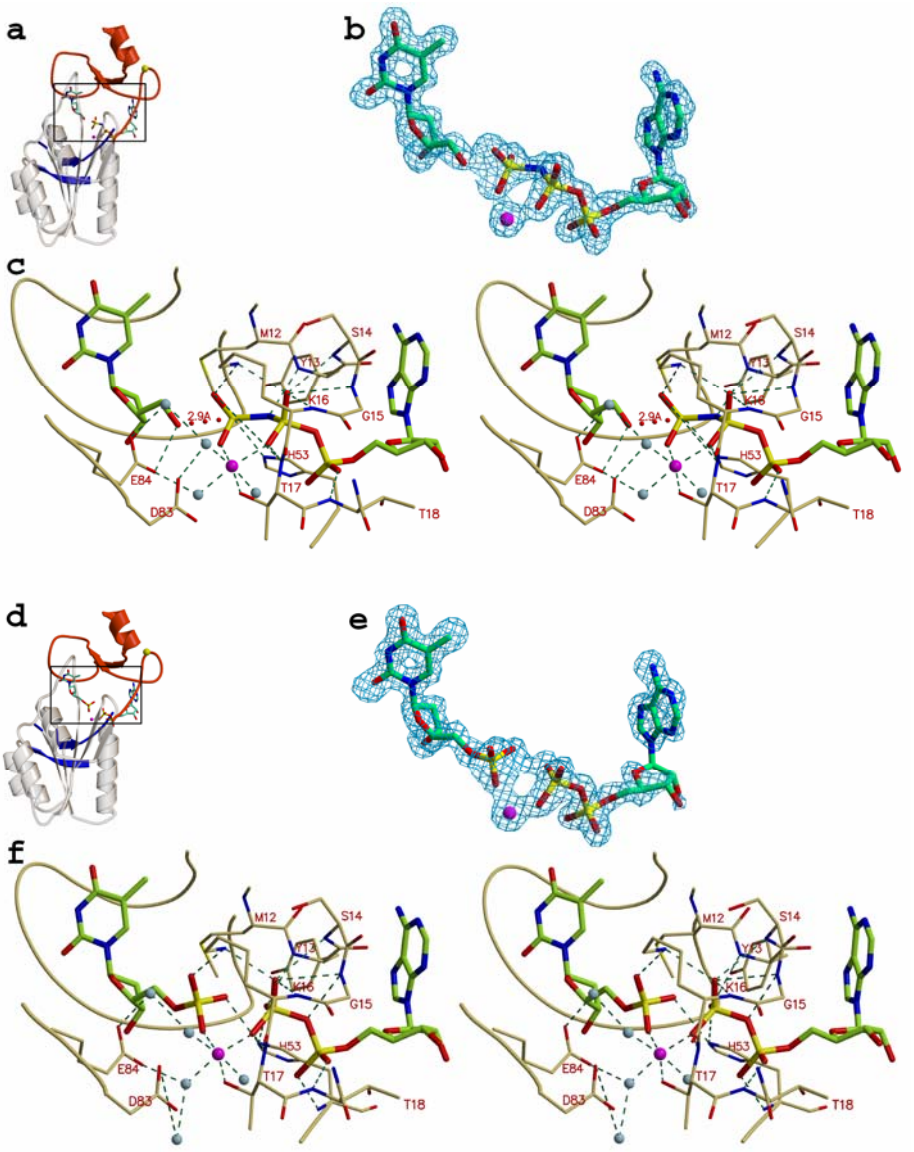
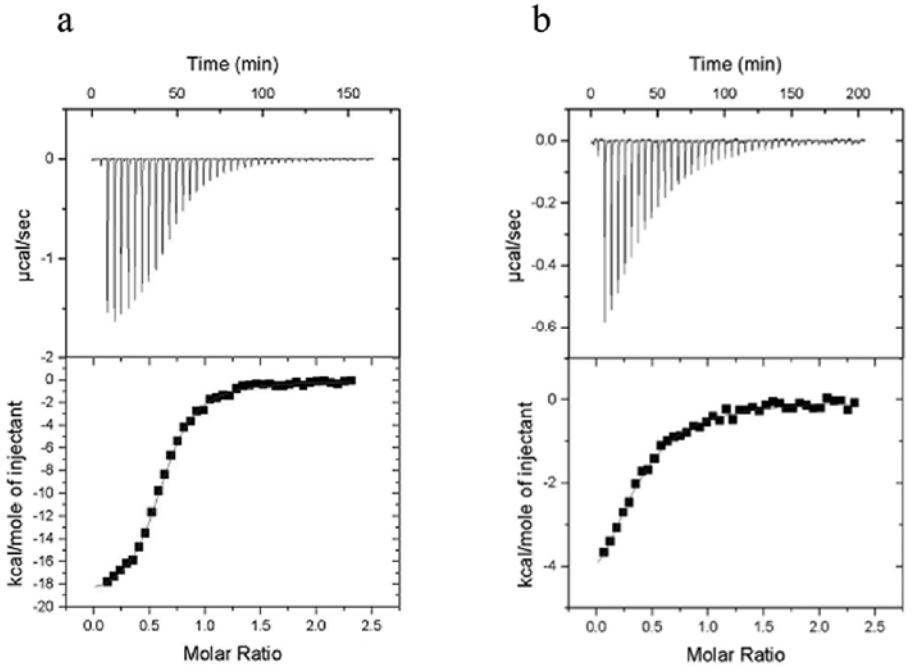



**Figure S1.** Phosphoryl Transfer in the Ternary Complex
(a) Ribbon representation of the first monomer present in the asymmetric unit in the substrate complex with AppNHp (ATP analog) and thymidine. The active site is enclosed

in the square. (b) A composite omit map at the 2-σ level for the substrate complex. (c) Stereoview of the active site showing the substrates thymidine and AppNHp. Amino acid side chains in the active site are shown as sticks with labels while the magnesium (purple) and bound water molecules (grey) are displayed as spheres. The P-loop region (amino acid 12-17) and H53 of the β-hairpin loop form extensive contacts with the β and γ-phosphate group of phosphoryl donor. On the phosphoryl acceptor side, D83 and E84 assist in positioning the magnesium ion. In addition, E84 functions as a general base on the 5'-OH of thymidine which was confirmed by mutagenesis of E84A, resulting in a drop of catalytic activity by three orders of magnitude (Lichter & Lutz, unpublished data).
(d) A ribbon representation of the second monomer present in the asymmetric unit with the product complex (TMP and ADP), indicating that phosphoryl transfer took place under the crystallization conditions. (e) A composite omit map at the 2-σ level for the product complex. (f) Stereoview of the active site showing the reaction products TMP and ADP. Components are labeled as described above. A comparison of the substrate and product complexes showed no significant conformational changes among the amino acid side chains or reactants in the active site.




γ-S ATP/TmTK

$K_D$ = 1.11 µM
ΔG = -8.5 kcal/mol
ΔH = -19.4 kcal/mol
TΔS = -11.0 kcal/mol

thymidine/TmTK

$K_D$ = 12.3 µM
ΔG = -6.7 kcal/mol
ΔH = -6.2 kcal/mol
TΔS = +0.5 kcal/mol

**Figure S2.** ATP and Thymidine-Binding Study by Isothermal Titration Calorimetry (ITC)
(a) Binding of the ATP-analog γS-ATP to *Tm*TK is accompanied by a strong exotermic reaction and a dramatic decrease in entropy. The significant change in entropy is consistent with substantial conformational changes as observed in the crystal structure of the ternary complex. (b) Titration of *Tm*TK with thymidine. The phosphoryl acceptor site shows about ten-fold lower substrate affinity than the donor site. As suggeted by the crystal structures and our fluorescence experiments, thymidine binding does not induce significant structural changes in the enzyme which is reflected in the small entropy contribution. In both graphs, raw data are shown in the top panel while data, fitted to a one-binding site model, are shown in the lower panel. Data fitting was performed in Origin software (OriginLab, Northhampton, MA).

The ITC results suggest that both substrates can bind to *Tm*TK independently. These findings for our type-II TK differ from previous reports of an ordered binding mode for the TK from Herpes Simplex Virus (HSV-tk), a member of the type-I subfamily [28]. In HSV-tk, ATP binding only takes place after thymidine occupies the phosphoryl acceptor site. In contrast, *Tm*TK likely binds ATP first, due to its low $K_D$ and the millimolar ATP concentration in situ, followed by recruiting the phosphoryl acceptor. In summary, the two enzymes might catalyze the same reaction, yet their subfamilies do not

share structural homology and, as shown by our ITC data, follow different reaction pathways.

The ITC experiments were performed with a Microcal VP isothermal titration calorimeter (MicroCal). The protein *Tm*TK was extensively dialyzed in the same buffer used to prepare the ligands solution, ITC buffer containing 150 mM NaCl, 25 mM Tris HCl (pH 7.5) and 2 mM $MgCl_2$. All solutions were degassed. The ligand concentration in the injection syringe was 20 times higher that the concentration of *Tm*TK in the sample cell. The experiments were carried out at 37 $^{o}$C. The raw data was corrected for the ligand heat of dilution, integrated and fitted to the one set site model, using the program Microcal Origin.