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Supporting information for article:

Structural and functional roles of dynamically correlated residues in thymidylate kinase

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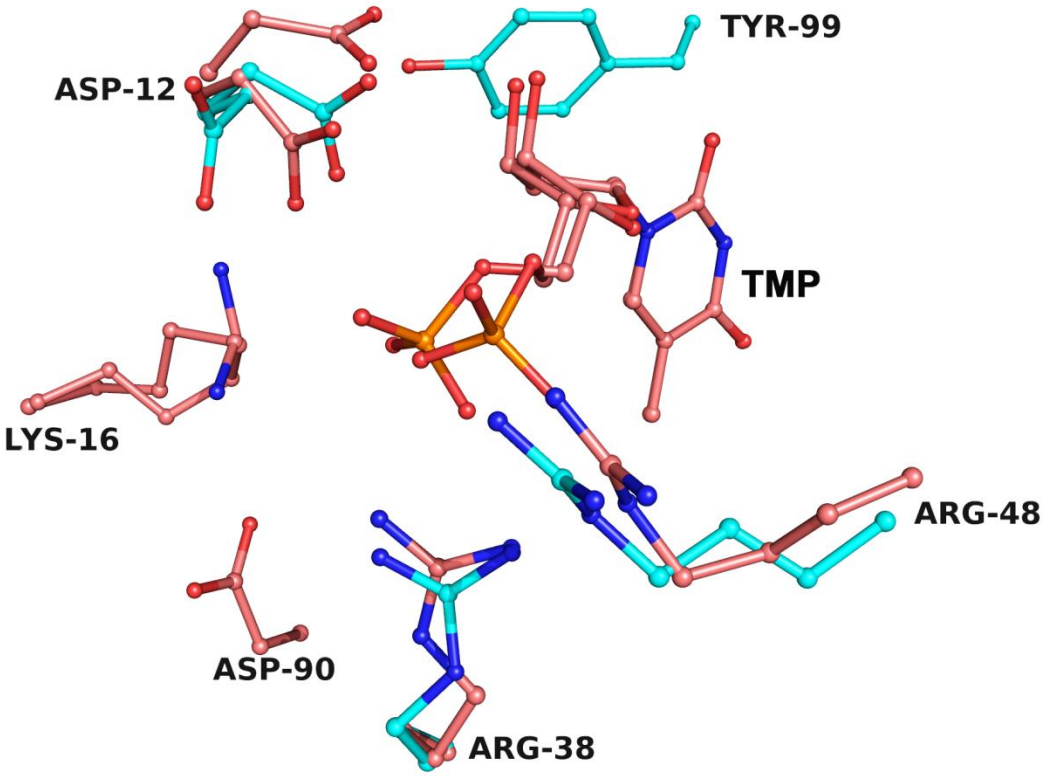


Figure S1 Multiple conformations of residues in ttTMPK-TMP complex. Different conformations of Asp12, Lys16, Arg38 , Arg48 and TMP is observed by the superposition of both protomers in TMP complex. The conformations of Asp12 (salmon) hydrogen bonded with Tyr99 and the conformation of TMP towards the reaction center (or Lys16) is regarded as catalytically competent conformation.

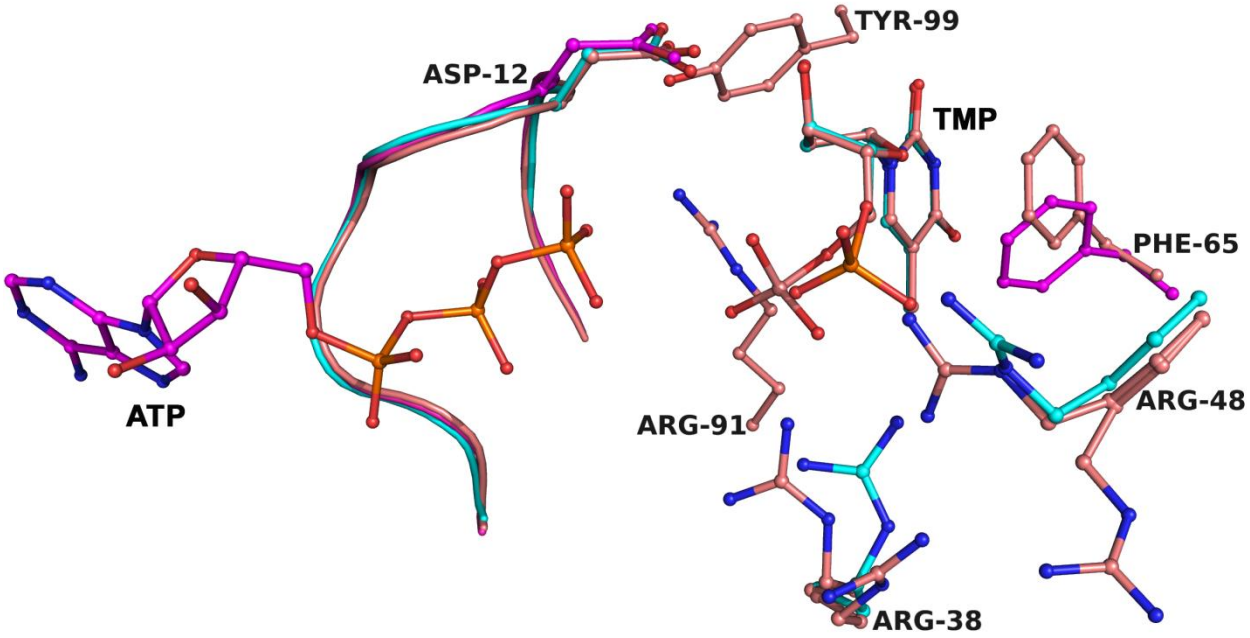


Figure S2 Superposition of ttTMPK-ATP-Mg^{2+}, ATP-Mg^{2+}-TMP and AMPPCP-Mg^{2+}-TMP complexes. In ttTMPK-ATP-Mg^{2+} complex (magenta), the P-loop is open and Asp12 is present in catalytically competent conformation. Since TMP is absent, Phe65 is in catalytically incompetent conformation. In ttTMPK-ATP-Mg^{2+}-TMP complex (salmon), all the residues and TMP are in catalytically competent conformation. Two alternate conformations of Arg38 and Arg48 is also seen in the complex, one catalytically competent (towards TMP) and other catalytically incompetent (away from TMP). The ttTMPK-AMPPCP-Mg^{2+}-TMP complex (cyan), Asp12 is in catalytically competent conformation but the TMP is away from the active site (catalytically incompetent) and therefore the residues Arg38 and Arg48 are also away.

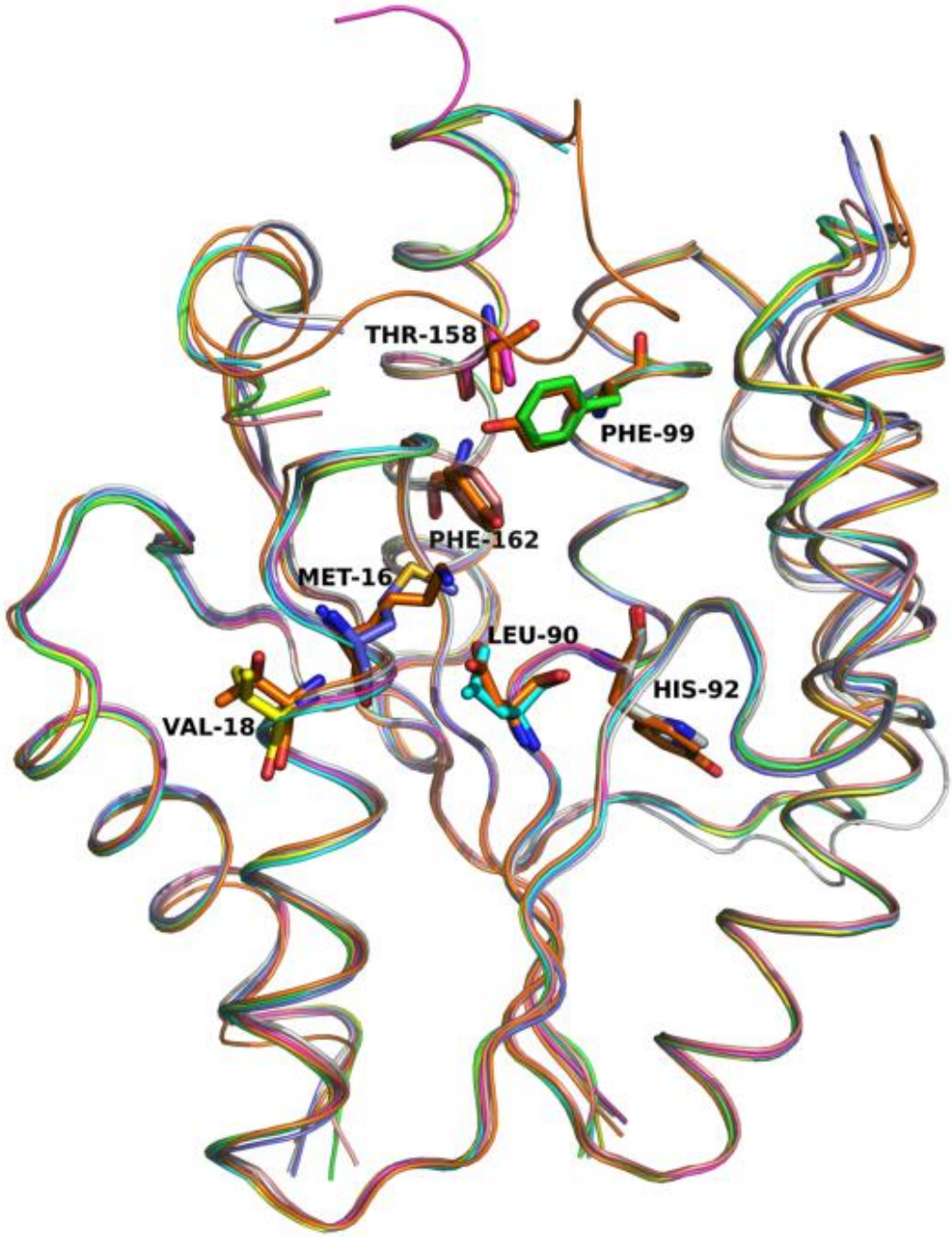


Figure S3 Superposition of the wild type and mutant ttTMPK structures. The backbones of the wild type ttTMPK (orange), D90L (cyan), K16M (blue), Y99F (green), Y92H (white), Y162F (salmon), T18V (yellow) and V158T (pink) structures superpose well with each other. The mutated residues are shown in stick representation along with its wild type counterpart.

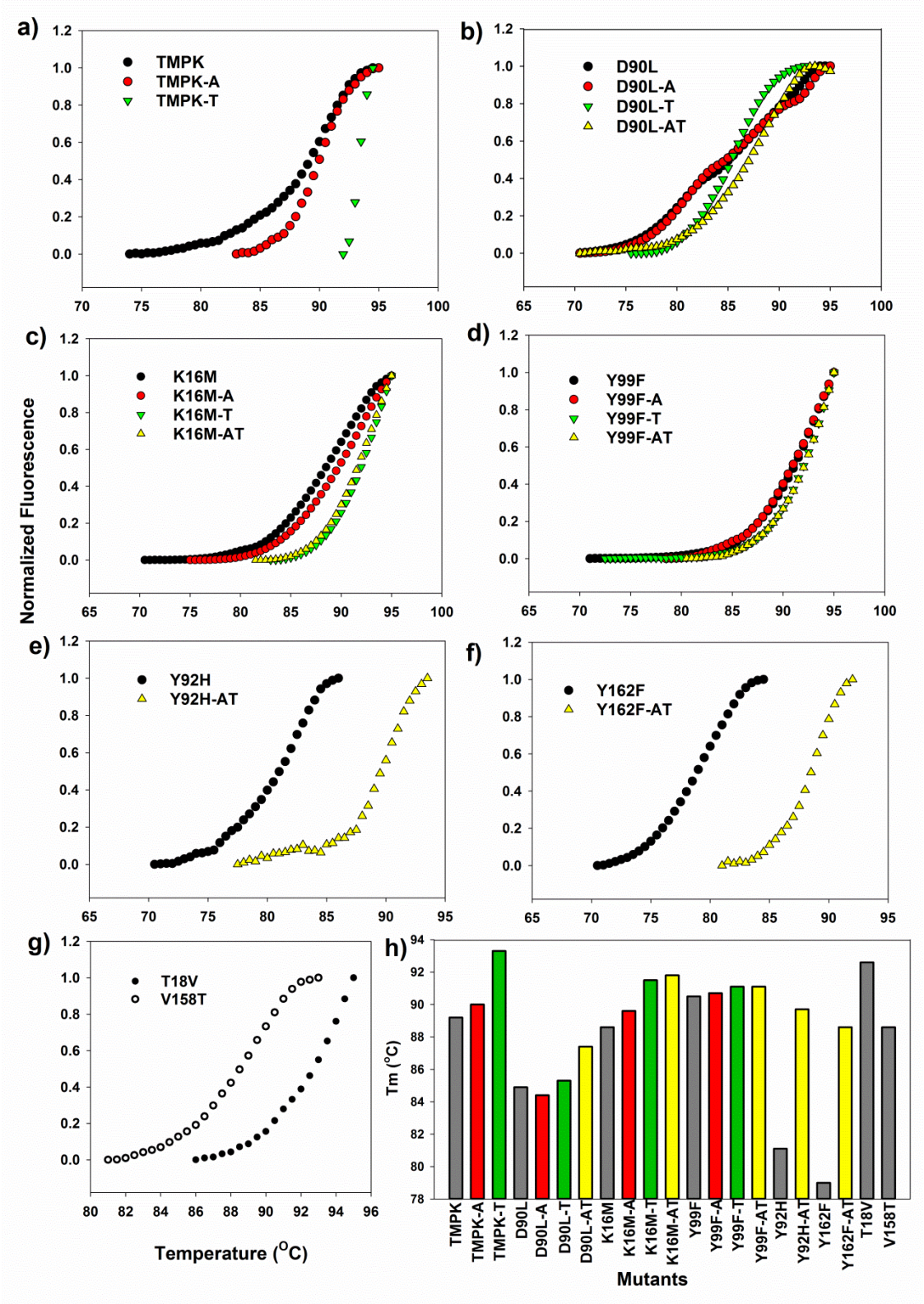


Figure S4 Thermal shift assay to measure the changes in thermal stabilty upon mutations in ttTMPK. Thermal shift assay has been performed on the wild type and mutant ttTMPKs in the presence and in

absence of the substrates (ATP, TMP and ATP-TMP). Changes in fluorescence for the wild type and mutant ttTMPKs in the presence of the substrate are depicted in different colors. Black shows apo state (TMPK, D90L, K16M, Y99F, Y162F, Y92F, T18V and V158T), red shows thermal shift in the presence of ATP (TMPK-A, D90L-A, K16M-A and Y99F-A), green shows thermal shift in the presence of TMP (TMPK-T, D90L-T, K16M-T and Y99F-T) and yellow shows thermal shift in the presence of both ATP and TMP (D90L-AT, K16M-AT, Y99F-AT, Y92F-AT and Y162F-AT). The thermal shift curves were fitted to a four parameter sigmoidal equation. The experiments were performed in triplicates and the error is within 10%. (a) Wild type ttTMPK shows Tm of 89.2 °C, which increases in the presence of ATP and TMP and it goes beyond 95 °C in the presence of both ATP-TMP, hence not indicated in the plot. (b) The T_m of K16M mutant is comparable to ttTMPK. ATP binding has very little effect on T_m but TMP and ATP-TMP binding has significant effect on thermal stability. (c) The Tm of D90L mutant is slightly less than the wild type ttTMPK. Binding of the ATP has negligible effect on the T_m but the binding of TMP and ATP-TMP stabilizes the protein. (d) Y99F mutant is slightly more stable than the wild type ttTMPK and the binding of substrates has almost negligible effect on the thermal stability. (e and f) Y162F and Y92H mutants has reduced thermal stability but in the presence of substrates ATP and TMP, their thermal stability becomes comparable to that of wild type ttTMPK. (g) T18V is more thermally stable than the wild type ttTMPK, as the curve did not stabilize at the plateau. V158T has comparable thermal stability as that of the wild type ttTMPK. After substrates (ATP and TMP) binding, both mutants showed an increased thermal stability which was beyond the detectable limit of the instrument. (h) A bar plot showing a comparative T_m values of the wild type ttTMPK and its mutants in the substrate bound and unbound states.

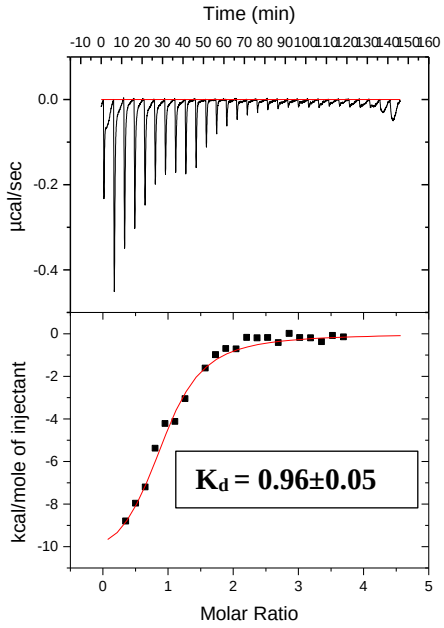
Table S1 Primer sequences used for mutation.

Primer names	Primer sequences
D90L_FWD	CTGCGCTACCTGGACTCCAG
D90L_REV	GGAGATCACCACCTTCCCC
K16M_FWD	ATGACCACCCAGGCGAGGC
K16M_REV	GCCGCTGCCGTCCAGGC
Y162F_FWD	TTCCTGGCCCTCGCCCGGGC
Y162F_REV	CCCCTCCCGCACCCTGCGGAA
V158T_FWD	ACGCGGGAGGGGTACCTGGCCC
V158T_REV	CCTGCGGAAGAACTCCAGGCCG
T18V_FWD	GTCCAGGCGAGGCGGCTCG
T18V_REV	GGTCTTGCCGCTGCCGTCCAG
Y92H_FWD	CACCTGGACTCCAGCCTGGCC
Y92H_REV	GCGGTCGGAGATCACCACCTTC
Y99F_FWD	TTCCAAGGCTATGGCCGGGG
Y99F_REV	GGCCAGGCTGGAGTCCAGG

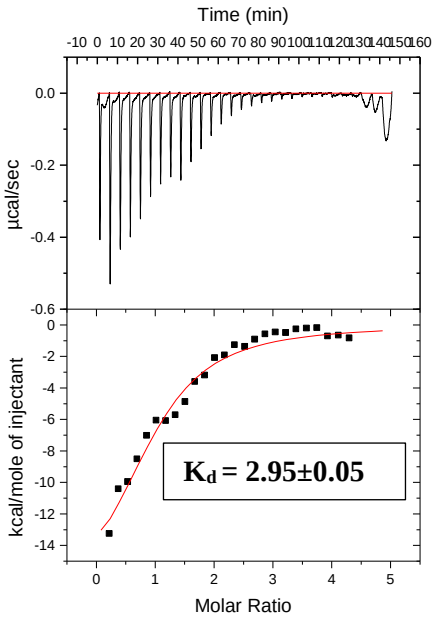
Table S2 Crystallization conditions of the wild type, ligand complexes and mutants of ttTMPK.

Crystal	Crystallization conditions
ttTMPK_Mg^{2+}	0.2M $MgCl_2.6H_2O$, 0.1M Tris hydrochloride, 30% PEG 4,000
ttTMPK_Cs^{+}	0.05M CsCl, 0.1M MES Monohydrate pH 6.5, 30% v/v Jeffamine M-600
ttTMPK + ligands (ATP-Mg^{2+}, TMP, ATP-Mg^{2+}-TMP, AMPPCP-Mg^{2+}-TMP)	0.2M $MgCl_2.6H_2O$, 0.1M Tris hydrochloride, 30% PEG 4,000, 40mM ligand
D90L, K16M, Y99F, T18V, Y162F	0.1M Magnesium formate dehydrate, 20% PEG 3350
V158T, Y92H	0.1M Sodium Malonate, 12% PEG 3350

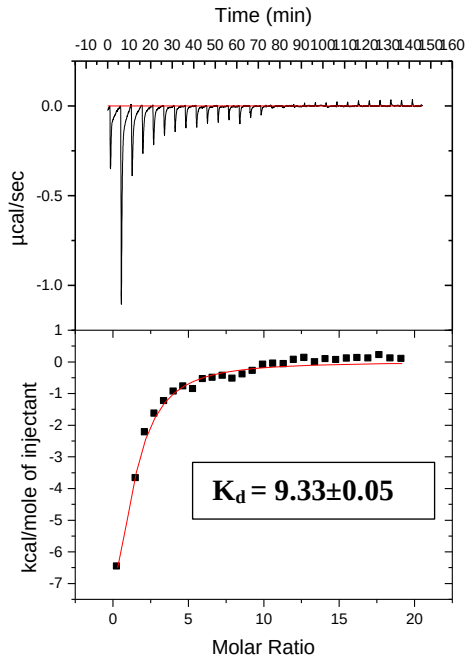
A) ttTMPK + TMP



B) mutant K16M + TMP



C) ttTMPK + ATP



D) mutant K16M + ATP

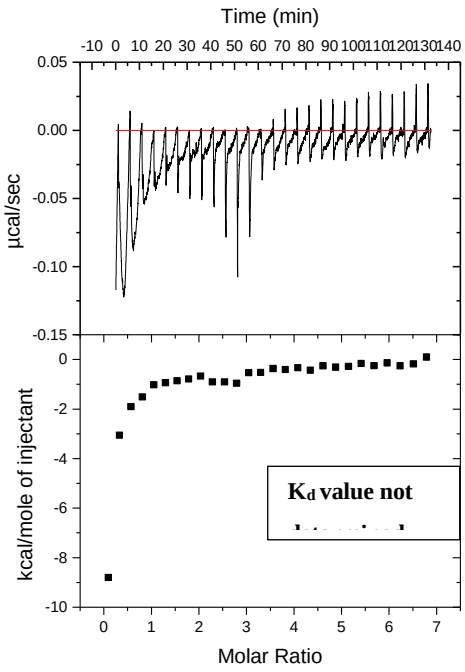


Figure S5 Isothermal Titration Calorimetry (ITC). Calorimetric experiments were carried out using VP-ITC from MicroCal, Inc. (Northampton, MA). The protein (15uM) was titrated with ATP (500uM)

and TMP (150uM) in a buffer containing 25mM Tris-Cl (pH 7.5), 150mM NaCl and 5mM $MgCl_2$. The experiment was performed at 25°C and the stirring speed was set to 300rpm. The control experiments were performed to determine the heat of dilution by injecting nucleotides in to the buffer. This dilution correction was incorporated before the data analysis. The data was analyzed using Origin software package and fitted to single site model. ITC profile of the binding of TMP and ATP molecules with the wild type ttTMPK protein and its mutant K16M are provided in their respective figures (Figs A, B, C and D). The top panel in each figure (A, B, C and D) represents the heat change produced by successive injections and the lower panel represents the integrated binding isotherms as a function of ligand (ATP or TMP) molar ratio. The data points are shown in solid circles and the fit is shown in solid red line. K_d values represent dissociation constant.