Supporting Information

Caillat *et al.* 10.1073/pnas.0804525105

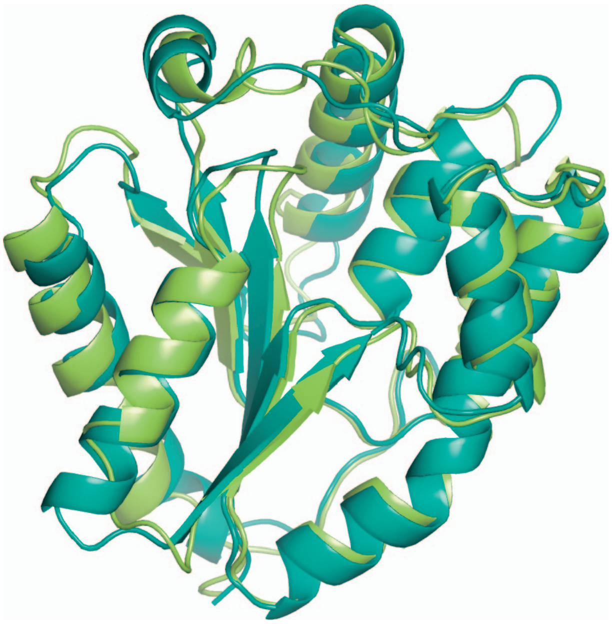

Fig. S1. Superimposition of the structures of the vaccinia virus and the human enzyme monomers in cartoon representation. The human enzyme is shown in cyan and the vaccinia virus monomer in green.

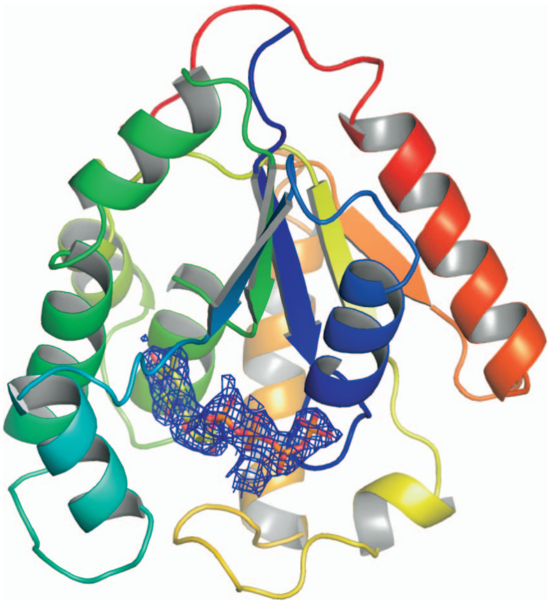

Fig. S2. The TDP and PPi (atom-type colored sticks) and a magnesium ion (gray sphere) are bound to the acceptor site of the vaccinia virus TMP kinase monomer B (rainbow-colored cartoon). The $2F_{\mathrm{obs}} - F_{\mathrm{calc}}$ composite annealed omit map contoured at 1 σ is shown (blue mesh) around TDP, PPi, and Mg^{2+}.

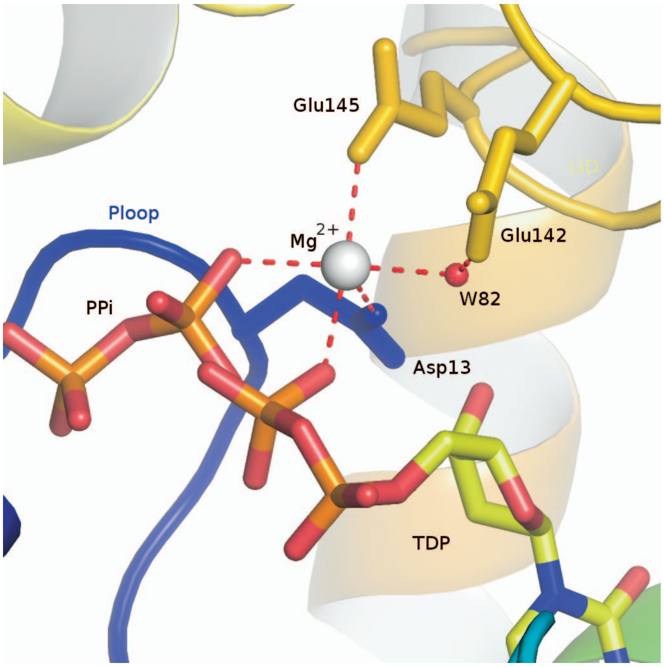

Fig. S3. The magnesium coordination is octahedral with a missing apical ligand. It involves oxygen atoms from the phosphates of TDP and PPi and the side-chain oxygen of Asp-14 in the P loop and Glu 154 from the LID. The fourth ligand is a water molecule held in place by Glu-142 from the LID. Magnesium was the only divalent cation present in millimolar amounts in the crystallization solution.

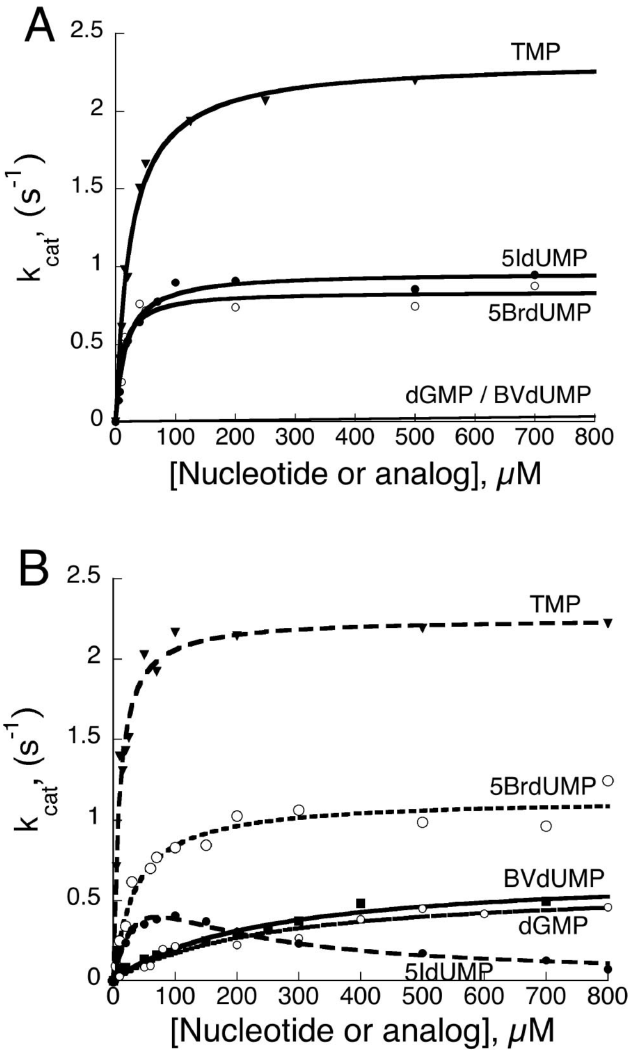

Fig. S4. Substrate specificity of vaccinia and human TMPKs. Saturation curves of hTMPK (A) and Vacc-TMPK (B) with TMP and analogs: TMP (filled inverted triangle), BVdU-MP (filled squares), dGMP (small open circles), 5-Br-dUMP (large open circles) and 5-I-dUMP (filled circles). Dashed curves are for published data [Topalis D, *et al.* (2005) Substrate specificity of vaccinia virus thymidylate kinase. *FEBS J* 272:6254–6265].

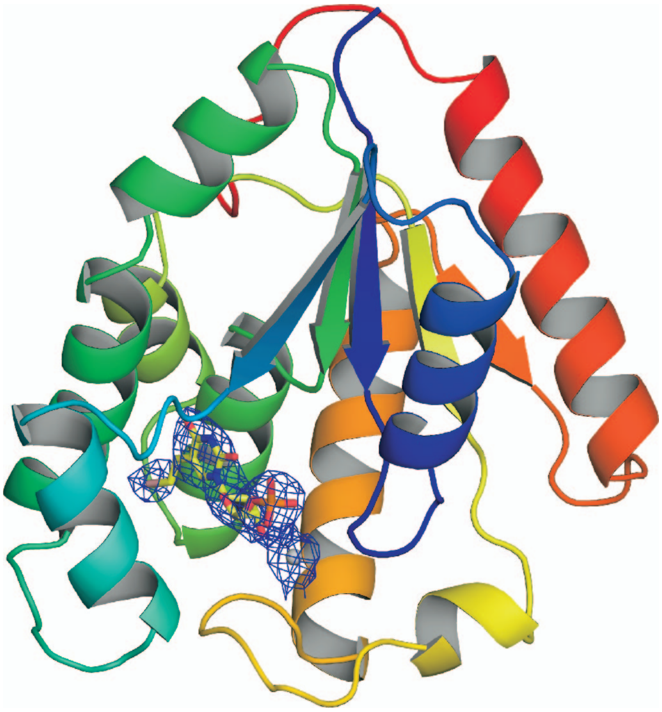

Fig. S5. The brivudin monophosphate (atom type colored sticks) and a magnesium ion (gray sphere) are bound to the acceptor site of the vaccinia virus TMP kinase monomer (rainbow-colored cartoon). The composite annealed omit map contoured at 1 σ is shown (blue mesh) around BVdU-MP and Mg^{2+}.

Table S1. Data collection and refinement statistics

Data collection	dTDP	BVdU-MP
Space group	$P2_12_12_1$	$P2_12_12_1$
Unit cell, Å		
a	56.77	58.37
b	55.59	78.35
c	161.16	95.04
Resolution, Å	30–2.40	45–2.9
Observed reflections	79,520	69,977
Unique reflections	20,187	9,967
Completeness (%)	97.2 (93.6)[*]	99.4 (95.7)[*]
$I/\sigma(I)$	12.3 (2.25)	9.9 (2.3)
R_{sym}[†], %	9.7 (59.2)	11.2 (52.4)
Refinement statistics		
R_{cryst}[‡], %	21.8	22.7
R_{free}[§], %	27.9	29.1
No. of water molecules	107	6
RMSD		
Bond lengths, Å	0.0076	0.0088
Bond angles, °	1.4322	1.5519
Ramachandran statistics[¶], %		
Most-favored regions	87.8	89.1
Additional allowed regions	12.1	10.7
Disallowed regions	0.1	0.2

[*] Numbers in parentheses represent values in the highest-resolution shell (last of 10 shells).

[†] $R_{sym} = \Sigma_h \Sigma_i |I(h,i) - \langle I(h) \rangle| / \Sigma_h \Sigma_i I(h,i)$, where $I(h,i)$ is the intensity value of the ith measurement of h and $\langle I(h) \rangle$ is the corresponding mean value of $I(h)$ for all I measurements.

[‡] $R_{cryst} = \Sigma ||F_{obs}| - |F_{calc}|| / \Sigma |F_{obs}|$, where $|F_{obs}|$ and $|F_{calc}|$ are the observed and calculated structure factor amplitudes, respectively.

[§] R_{free} is the same as R_{cryst} but calculated with a 10% subset of all reflections that was never used in crystallographic refinement.

[¶] As evaluated by PROCHECK [Laskowski RA, *et al.* (1993) PROCHECK: A program to check the stereochemical quality of protein structures. *J Appl Crystallogr* 26:283–291].

Table S2. Thermal denaturation monitored by microcalorimetry

TMP kinase	Tm, °C	+ Natural ligand 1 mM	+ dGMP 1 mM	+ BVdUMP 4 mM
Human TMPK	52.2	59.9 (+TMP)	53.3	53.1
Vacc-TMPk	42.5	47.4 (+TMP)	44.8	45.3
HSV-TK	43.4*	52.6* (+T)	–	–

Conditions: 1.5 mg/ml; 50 mM Tris·HCl (pH7.4); denaturation between 10 °C and 80 °C.

*Data are from ref. 1

1. Wurth C, *et al.* (2001) The effect of substrate binding on the conformation and structural stability of Herpes simplex virus type 1 thymidine kinase. *Protein Sci* 10:63–73.