



STRUCTURAL BIOLOGY  
COMMUNICATIONS

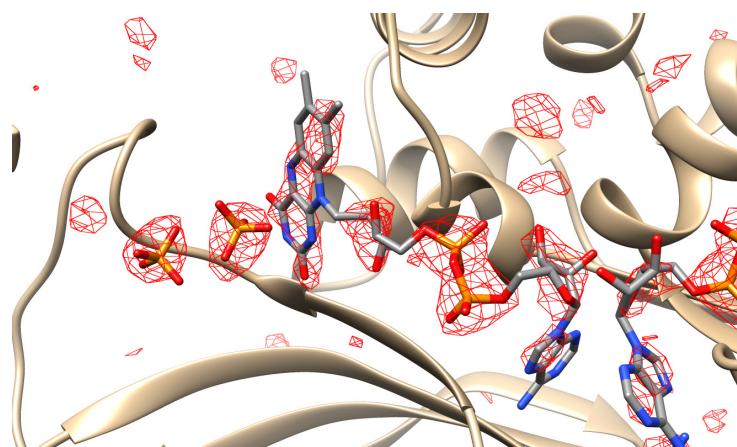
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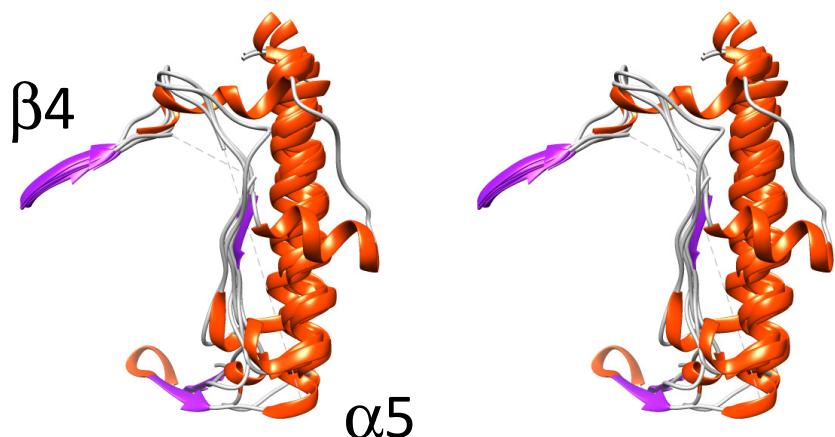
**Crystal structure of the flavin-dependent thymidylate synthase Thy1 from *Thermus thermophilus* with an extra C-terminal domain**

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Supplemental Fig. S1  $F_o$ - $F_c$  omit map for FAD and phosphate ions  
(Ogawa *et al.*)



Supplemental Fig. S2 Stereo view of a superposition among six Thy1 structures  
Regions from  $\beta 4$  to  $\alpha 5$  are shown. PDB IDs for *TmThy1*, *MtbThy1*, *CvThy1*,  
*CgThy1*, *HpThy1* and *ScThy1* are 4GT9, 2AF6, 4FZB, 3FNN, 3AH5 and 4P5A,  
respectively  
(Ogawa *et al.*)



Supplemental Fig. S3 Sequence alignment among Thy1 proteins based on the crystal structures

Secondary structures are indicated by colour. Residues contacting ligands are indicated by boxes; solid, dotted and broken lines indicate FAD, dUMP and MEF, respectively. Residues disordered in crystal structures were indicated by small characters.  
 (Ogawa et al.)

	$\beta_1$	$\beta_2$	$\alpha_1$	$\alpha_2$	$\alpha_3$								
<i>T. thermophilus</i>	megPLTIPVLDKGFVRLVDQM	G	DDRAIVQAARVSYGEGTKT	VREDAAIIDYLMRH	RITSPFEMV	64							
<i>T. maritima</i>	---	MKIDILDKGFVELVDVM	G	NDLSAVRAARVSFDMGlkd	EERDRHIIIEYLMKH	GIETPFEHI	60						
<i>S. cacaoi</i>	-mesPRIELRSDITVELVDSSA	-	-SDLAVVKAARVSTAGEDandelydg	GSTRGIIIRYLMRS	RHGSPFEHN	68							
Pbcv1	-----MSAKLISVTKP	VVEGVN	-----TAEELIAYAARVSNPENQINNK	-----TASGLLKYCIRH	KHW SIFET	A 60							
<i>H. pylori</i>	-----MEVICKHYT	-----	-----PLDIASQAIRT	CWQSFEYSDDG	GCKDKEIIHRVGNI	FREISSTLEHL 78							
<i>C. glutamicum</i>	-----maEQVKLSVELIACSSF	TPPADVEWSTDVE	GAEALVEFAGRA	CYETFDKPNPRTA	SNAAYLRHIMEV	GHTALLEHA 76							
<i>M. tuberculosis</i>	-----maETAPLRVQLIAKTD	F	LAPPDVPWTTDADGGPALVEFAGRA	CYQSWSKPNPKTA	-----TNAGI	LRHIIIDV-GTF SVLEHA 76							
	$\beta_3$	$\alpha_4$	$\beta_4$										
<i>T. thermophilus</i>	VFKFHVK-AP IFVARQWF	RHRTA	SVNEISGRYSILKE	-----EFYEPEAFRKQakrnkqasegall	-	124							
<i>T. maritima</i>	VFTFHVK-AP IFVARQWF	RHRIAS	SYNELSGRYSKLSY	-----EFYIIPS PERLEGYKTTIP	-	114							
<i>S. cacaoi</i>	SMTFLVR-APIFTVRHLMR	RHRTWSFNE	ESARYREVGA	-----AFYVPDATRLLRQEG	KPGDYRYVGG	129							
Pbcv1	FMTLELK-TS	RGIAAQVILRHR	HFQEFSQRYASVme	-----tpphpQARFQDHKNRQN	SLDTVPE	120							
<i>H. pylori</i>	YYNFEIKGLS	RGALQELSRHRIAS	LSVKS	-----NETNLERA	KEFLVFVD	140							
<i>C. glutamicum</i>	NATMYIRGIS	RSATHELV	RHRHF	-----SEVVVP TLIDED	-	125							
<i>M. tuberculosis</i>	SVSFYITGIS	RSCTHELI	RHRHF	-----SRVVVP PGMEDD	-	125							
	$\alpha_5$ (to be checked)			$\alpha_6$	$\beta_5$	$\alpha_7$							
<i>T. thermophilus</i>	----deeala	LLRKVQQEAYGAYRALLEKG	V	-----AREMARMV	PLNLTYTEFYWKQDLHNLFHFLKIRLAPEA	-	189						
<i>T. maritima</i>	----PERVTEKISEIV	DKAYRTYLELIESG	V	-----PREVARIVL	PLNLTYTRFFWTVN	ARSLMNFLNLRADSHA	-	179					
<i>S. cacaoi</i>	STDDHQVVR	SATRAYEVAFEEYQRLLDSG	I	-----AREIARLVL	PVSTYSVLYATCN	ARALMHFLSIP	THR PDAAYVSHP	205					
Pbcv1	----DDQTWWATEQEKLYA	QSMELYNKALEKG	I	-----AKECARFIL	PLSTPTT	YIMSGTIIRDWIHYIELRT	TSNGT	187					
<i>H. pylori</i>	----NEKVNAMS	VLALENLRILLSE	HNI	-----KNDLAKY	YAMPESYKTHLAYSIN	ARSLQNF	LTLRS	202					
<i>C. glutamicum</i>	----PQLREL	FMHAMDESRAF	NELLNALEEK	lgdepnall	RKKQARQAARAVLPNATE	ESRIVVSGN	FRTWRHFIGM	204					
<i>M. tuberculosis</i>	----ADLRHIL	TEAADARATYSELLAK	LEAKF	-----ADQPAILRR	QARQAARAVLPNATE	ETRIVVTGN	YRAWRHFIA	204					
	$\alpha_8$	$\alpha_9$	$\beta_6$	$\alpha_{10}$	$\alpha_{11}$	$\alpha_{12}$							
<i>T. thermophilus</i>	QWEIRQYARAI	AEIVKER	VPLAWAA	FEHLL	LEGAF	LSRTTELRALRGL	LT	PEVYEKAL	SSLGLG	GSRLKEA	LEKVFG	gpgeal	270
<i>T. maritima</i>	QWEIQQYALAI	ARI	KEKCPWT	FEAFLKYAY	KGDIL	KEVQV	-	-	-	-	-	-	220
<i>S. cacaoi</i>	QREIEMVAE	QMETAWAKI	MPV	THEAFTA	FGFV	SP	-	-	-	-	-	-	239
Pbcv1	QREHIDL	ANACKEIFIKE	FPSI	AKALD	WV	-	-	-	-	-	-	-	216
<i>H. pylori</i>	LKEMQDL	AKALFDAL	PGE	HQYL	FEDCL	KH	-	-	-	-	-	-	231
<i>C. glutamicum</i>	DVEIREV	AVECLRKLQVA	APTVFG	D	FEIET	LADGS	QM	ATSPYVMDF	-	-	-	-	250
<i>M. tuberculosis</i>	DVEIRR	LAIECLRQLAAV	APAVFA	DF	EVTT	LADGT	EVAT	SPLATEA	-	-	-	-	250

Supplemental Table S1 Purification of *TtThy1* protein

*TtThy1* protein was purified by 8 steps of column chromatography.

Method	Column	Buffer	Gradient
gel filtration	GE Healthcare UK Ltd., HiPrep 26/10 Desalting	20 mM Tris-HCl, pH 8.0	-
anion exchange	TOSOH CORPORATION, Super Q TOYOPEARL 650M	20 mM Tris-HCl, pH 8.0	buffer to buffer + 0.4 M NaCl
anion exchange	GE Healthcare UK Ltd., RESOURCE Q	20 mM Tris-HCl, pH 8.0	buffer to buffer + 0.4 M NaCl
anion exchange	GE Healthcare UK Ltd., RESOURCE Q	20 mM Tris-HCl, pH 8.0	buffer to buffer + 0.4 M NaCl
hydroxyapatite	Bio-Rad Laboratories, Bio Scale CHT20-I	10 mM NaPi, pH 7.0	Buffer to buffer + 0.125 M Na phosphate
gel filtration	GE Healthcare UK Ltd., HiLoad 16/60 Superdex 75 pg	20 mM Tris-HCl, 150 mM NaCl, pH 8.0	-
anion exchange	GE Healthcare UK Ltd., RESOURCE Q	20 mM Tris-HCl, pH 8.0	buffer to buffer + 0.4 M NaCl
hydrophobic interaction	GE Healthcare UK Ltd., RESOURCE PHE	50 mM NaPi, pH 7.0	buffer + 1.05 M (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> to buffer

Supplemental Table S2 Sequence similarity of *TtThy1* to Thy1 proteins whose structures are known.

Species	Identity (%)	Positives (%)	E-value	Coverage (%)
<i>Thermotoga maritima</i>	50	64	1.36129e-70	84
<i>Streptomyces cacaoi</i> subsp. <i>asoensis</i>	41	54	1.79198e-32	79
Paramecium bursaria Chlorella virus 1	31	48	1.44047e-18	68
<i>Helicobacter pylori</i>	28	51	0.000384036	54
<i>Corynebacterium glutamicum</i>	26	45	0.00934971	68
<i>Mycobacterium tuberculosis</i>	23	39	0.0094941	68