Structural basis for ligand binding modes of CTP synthase

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SUPPLEMENTAL INFORMATION

SUPPLEMENTAL TABLE S1 AND FIGURES S1-S12

SUPPLEMENTAL MOVIE S1

	dmCTPS+Sub	dmCTPS+Pro			
	(EMD-30810, PDB 7DPT)	(EMD-30811, PDB 7DPW)			
Data collection					
EM equipment	Titan Krios	Titan Krios			
Detector	K3 camera	K3 camera			
Magnification	22,500x	22,500x			
Voltage (kV)	300	300			
Electron exposure ((e–/Å ²))	60	60			
Defocus range(µm)	-1.0 to -2.3	-1.0 to -2.3			
Pixel size(Å)	0.53	0.53			
Symmetry imposed	D2	D2			
Number of collected movies	2878	2259			
Initial particle images (no.)	424195	1563553			
Final particle images (no.)	107556	344869			
Map resolution (Å)	2.5	2.7			
FSC threshold	0.143	0.143			
Map resolution range (Å)	2.4 - 3.8	2.5 - 4.7			
Refinement					
Initial model used (PDB code)	6L6Z	6LFG			
Map sharpening B-factor(Å ²)	-77	-90			
Model composition					
Non-hydrogen atoms	18036	17812			
Protein residues	2224	2224			
Ligands	UTP,ATP,GTP,DON	CTP			
Waters	76	32			
lons	12	8			
B factors($Å^2$)					
Protein	97	68			
Ligand	88	32			
Water	87	38			
R.m.s. deviations					
Bond lengths (Å)	0.006	0.008			
Bond angles (°)	0.663	0.81			
Validation					
MolProbity score	1.69	2.56			
Clashscore	3.24	7.84			
Poor rotamers (%)	1.44	8.02			
Ramachandran plot					
Favored (%)	92.78	93.14			
Allowed (%)	7.04	6.68			
Disallowed (%)	0.18	0.18			

Table S1. Cryo-EM data collection and model refinement



Figure S1. Image processing work flow of substrate-bound dmCTPS tetramer. In the reconstruction of the dmCTPS^{+Sub} tetramer, we collected 2461 micrographs and 253,332 particles were kept after 2D classification. After re-extraction of particles of dmCTPS^{+Sub} tetramer, different reconstructions were performed to generate the final map and model.



Figure S2. Statistics of the final density maps of the dmCTPS^{+Sub} tetramer. a, A representative cryo-EM micrograph of dmCTPS^{+Sub} tetramer/polymer. b, Representative 2D class averages of different views of the porcine dmCTPS^{+Sub} tetramer. c, Particle orientation distributions of the dmCTPS^{+Sub} tetramer in the last iteration of the 3D auto-refinement. d, Local resolution map of the dmCTPS^{+Sub} tetramer final 3D density map. e, Gold-standard FSC curve of the final density maps of the dmCTPS^{+Sub} tetramer.



Figure S3. Image processing work flow of product-bound dmCTPS tetramer. In the reconstruction of the dmCTPS^{+Pro} tetramer, we collected 1520 micrographs and 607,762 particles were kept after 2D classification. After re-extraction of particles of dmCTPS^{+Pro} tetramer, different reconstructions were performed to generate the final map and model.



Figure S4. Statistics of the final density maps of the dmCTPS^{+Pro} tetramer. **a**, A representative cryo-EM micrograph of dmCTPS^{+Pro} tetramer/polymer. **b**, Representative 2D class averages of different views of the porcine dmCTPS^{+Pro} tetramer. **c**, Particle orientation distributions of the dmCTPS^{+Pro} tetramer in the last iteration of the 3D auto-refinement. **d**, Local resolution map of the dmCTPS^{+Pro} tetramer final 3D density map. **e**, Gold-standard FSC curve of the final density maps of the dmCTPS^{+Pro} tetramer.



Figure S5. Representative electron density map of individual regions of dmCTPS^{+Sub} model.



Figure S6. Representative electron density map of individual regions of dmCTPS^{+Pro} model.



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Figure S7. The comparisons of electron density maps of dmCTPS with reported CTPS models. a, The electron density maps of Phe373 in dmCTPS^{+Sub}, dmCTPS^{+Pro}, and of Phe365 in *Thermus thermophilus* CTPS in the apo state (1VCM) and the glutamine-bound state (1VCO). Phe373 in dmCTPS and Phe365 in ttCTPS are indicated by arrows. **b**, B factors are shown on monomers of dmCTPS^{+Sub}, dmCTPS^{+Pro} and substrate-bound hCTPS2 (6PK4) models. The region of the ammonia tunnel is highlighted.



Figure S8. Analysis for generation of CTP by mutant dmCTPS (F50A and L444A) in condition with 2 mM of GTP.



Figure S9. The structure comparison indicates the difference of distance between His55 and UTP in the open and closed CTPS conformations. The dmCTPS^{+Sub} model is shown in pink, the models of *Mycobacterium tuberculosis* CTPS (4ZDK, bound with UTP, ACP and DON) and (4ZDJ, bound with UTP) are shown in green and yellow, respectively.



Figure S10. The electron density map of ligands at the AL domain active site of substrate-bound state. a, The general map of ligands at the AL domain when the map level was set as 10σ . In the map the α -phosphate and β -phosphate of the ATP match the density very well. However, the γ -phosphate of the ATP is not seen with the same setting. Meanwhile, a large density appears to be connected with the uracil of the UTP. **b**, **c**, There are two other density groups are located at both sides of the β -phosphate of ADP (indicated by arrow and arrowhead). Although the density indicated by the arrow

is connected with the β -phosphate, its size is too small to be a phosphate. In addition, it is also connected with Asp70, Glu145 and the additional density on the uracil, making it unlikely a phosphate. **d**, The density indicated by the arrowhead displays a density extension under lower map level (7.2 σ), implying the presence of six bound water molecules. According to the catalytic process, we suggest the additional density on the uracil is the γ -phosphate of the ATP transferred to the 4-oxygen atom of the uracil base and the arrow and arrowhead indicate a water and a Mg²⁺ ion, respectively. **e**, The distance from the β -phosphate of ADP to the phosphate on the uracil is about 4.09 Å, far larger than the ordinary distance between β - and γ -phosphate. Meanwhile, the Mg²⁺ is slightly merged with the phosphate on the uracil. These suggest that transfer of γ phosphate which is catalyzed by the Mg²⁺ is captured in our model.



Figure S11. The comparison of dmCTPS and hCTPS2 at ATP/ADP, UTP and CTP binding sites. a, The structural comparison of dmCTPS^{+Sub} and substrate-bound hCTPS2 (6PK4) shows similar binding modes of ATP/ADP and UTP. **b** and **c**, The structural comparison of dmCTPS^{+Pro} and product-bound hCTPS2 (6PK7) shows the binding modes of CTP at UTP binding site (**b**) and ADP binding site (**c**).

		_	β1	α1	α2	β2	
SP (29V0L1) P1RG_DROME SP (29V0L1) P1RG_DROME SP (29V0L1) P1RG1_HUMAN SP (29NRF8) P1RG2_HUMAN SP (05L5) P1RG_ECCL1 SP (05L5) P1RG_ECCL1 SP (05L8) P1RG_HET8	1 1 1 1	• • • • • • • • • • • • • • • • • • • •	XYILVTGGVIS XYILVTGGVIS XYILVTGGVIS YYIFVTGGVVS XYVFITGGVVS	GVGKGVIAS GIGKGIIAS GIGKGIIAS SLGKGIAAA SLGKGILTS	SFGTLLKSCGL SVGTILKSCGL SIGTILKSCGL SLAAILEARGL SLGALLEARGL	DVTSIKIDPYI HVTSIKIDPYI RVTSIKIDPYI NVTAIKIDPYI RVTAIKIDPYI	NIDAGT NIDAGT NIDAGT NVDPGT NVDAGT
SP P9WHK7 PYRG_MYCTU TR A0A2A9ISY4 A0A2A9ISY4_9LACT	1	MRKHPQTATE	(HLFVS <mark>GG</mark> VAS (YIFVT <mark>GG</mark> GTS	SL <mark>GKG</mark> LTAS SM <mark>GKGIVAA</mark>	SLGQLLTARGL SLGRLLKNRGL	HVTMQKLDPYL KVTVQKFDPYL	NVDPGT NIDPGT
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SDIOSVIII 1 I DVDC DDOME		0.0.0.0	α5	TT	β4	α	6
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SP P9WHK7 PYRG_MYCTU TR AOA2A9ISY4 AOA2A9ISY4_9LACT	118 112	TVQVIPHI <mark>T</mark> DEIK TVQMVPHVTNMLK	KRILAM <mark>A</mark> QPD EKIKRA <mark>A</mark> T	ADGNRPD TTDAD	VVITEI <mark>GGT</mark> VG IIITEVGGTVG	DIESQPFLEAA DMESLPFIEAL	<mark>RQ</mark> VRHY <mark>RQ</mark> MKAE
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SP Q9NRF8 PYRG2_HUMAN SP POATES PYRG_ECOLI SP Q5SAB PYRG_THET8 SP P9WHK7 PYRG_MYCTU TR A0A2A9ISY4 A0A2A9ISY4_9LACT	170 164 175 176 166	AKRENFCNIHVSI IGREHTLFMHLTI EGEGNTLYLHLTI LGREDVFFLHVSI VGADNVMYI <mark>H</mark> TVF	LVPQLSATGEQ JVPYMAASGEV JVPYLETSEEF JVPYLAPSGEI PILHLRAAGEI	KTKPIQNSV KTKPTQHSV KTKPTQHSV KTKPTQHSV KTKPTQHSV KTKIAQNAT	RALRGIGLSPD KELLSIGIQPD ATLRGVGIQPD AALRSIGITPD KTLREYGIQAN	LIVCRSSTPIE ILICRSDRAVP ILVLRSARPVP ALILRCDRDVP MLVLRSEVPIT	MAVKEK ANERAK EEVRRK EALKNK TEMRDK
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SP 105SIA8 PYRG_THET8 SP P9WHK7 PYRG_MYCTU TR A0A2A9ISY4 A0A2A9ISY4_9LACT	235 236 226	VALFINVRPGHVF IALMCDVDIDGVI IAMFCDVAPEAVI	SSPTVEHLYE STPDAPSIYD QSLDVEHLYC	VPLLLEEQG IPKVLHREE IPLNLQAQN	LGRAVERALGI LDAFVVRLNI MDQIVCDHLKI	E AVIPNL PFRDVDW E APKADM	SFWQEA TEWDDL TEWSAM
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SP P9WHK7 PYRG_MYCTU TR AOA2A9ISY4 AOA2A9ISY4_9LACT	292 282	LRRVHEPHETVR VDHVMNLKKKVK	ALV <mark>GKY</mark> VELS ALV <mark>GKY</mark> VELP	DAYLSVAEA DAYISVTEA	LRAGGFKHRA <mark>K</mark> LK <u>H</u> AG <u>Y</u> SSDAE	VEICWVASDGC VDINWVNANDV	ETTSGA TDE.NV
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SP P9WHK7 PYRG_MYCTU TR A0A2A9ISY4 A0A2A9ISY4_9LACT	352 341	ADL	GDVHGVLIPG GDAAGIIVPG	GFGIRGIEG GFGHRGTEG	KIGAIÂY <mark>AR</mark> AR KIAAIKY <mark>AR</mark> EN	GLPVLGLCLGL DVPMLGICLGM	QCIVIE QLTAVE

SPICATITII PYPC DOME		0.0.0			β1	2					β13
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SP Q9VUL1 PYRG_DROME SP Q9VUL1 PYRG_DROME SP P17812 PYRG1_HUMAN SP Q9NRF8 PYRG2_HUMAN SP Q9NRF8 PYRG2_HUMAN SP P0NF5 PYRG_ECOLI SP S5SITA8 PYRG_THET8 SP P9WHR7 PYRG_WTCTU TR A0A2A9ISY4 A0A2A9ISY4_9LACT	458 458 458 449 451 456 442	SDGPSVI QTKNSVM KTENSIL VD.DSLV KP.GTLL EP.DSVV KN.G <u>SRA</u>	RQLYG RKLYG RKLYG HRLYG AQAYC KAAYN	β14 SNPKSVQ SDADYLE SDVPFIE NAPTI.V SKEEV.I 2TTQV.S IDAEVVQ	ß1 ERHRHRY ERHRHRF ERHRHRY ERHRHRY ERHRHRY RHRHRY	5 EVNPKY EVNPW EVNPW EVNNML EVNNALY EVNNAY EFNNKF	η5 VHLLEE KKCLEE KKCLEE KQIED VDGLER RDKIAE REDFEK	β16 • QGMRFV QGLKFV NDLSFV AGLRVA AGLVVS SGLRFS SGLRFS	β17 GDVDI GQDVEG GQDVDG GRSGDI ATTPGI GTSPDG GVSPDI	KTI 3EI 3DI 0QI MRGRGAGI 3HI NRI	AMEI. AMEI. AMEI. LVEI. LVEI. LVEI.
<i>SP</i> (<i>Q9VUL1</i> (<i>PYRG_DROME</i> <i>SP</i> (<i>99VUL1</i> (<i>PYRG_DROME</i> <i>SP</i> (<i>917812</i>) (<i>PYRG_HUMAN</i> <i>SP</i> (<i>99NF8</i>) (<i>PYRG_EUMAN</i> <i>SP</i> (<i>99NF8</i>) (<i>PYRG_ECOL1</i> <i>SP</i> (<i>95NF8</i>) (<i>PYRG_HET8</i> <i>SP</i> (<i>99WBK7</i>) (<i>PYRG_MYCTU</i> <i>TR</i> (<i>A0A2A9ISY4</i> (<i>A0A2A9ISY4_9LACT</i>	512 512 501 508 509 495	β18 · IELS GH · VELEDH · IELANH · IELANH · IELKDH EYPPDRH · VELSDK	β19 PYFVA PFFVA PYFVA PYFVA PFFLC PFVVC KFFVA	ATQYHPE GVQYHPE GVQFHPE GVQFHPE GTQAHPE GTQAHPE ACQYHPE	TT YLSRPIK FLSRPIK FTSTPRD FKSRPMR LKSRPNR LQSRPNR	<u>SPPFL</u> PSPPYL PSPPYL GHPLFA PSPPFV PHPLFV PHPLFV PHPLFV PEELYT	X17 SLILAS SLLLAS SLLLAS SFVKAA SFVKAA SFVKAA SFVGAA AFVGAA SFIRVA	α	18 200 YLQKGO YLQQGO QAK ELLPVI 	C.RLSPR(C.RLSPRI C.KLSSSI C.KLSSSI EIPEIPEI	2LSD. DTYS. DRYS. HTPNG
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SP Q9VUL1 PYRG_DROME SP Q9VUL1 PYRG_DROME SP P17812 PYRG1_HUMAN SP Q9NRP8 PYRG2_HUMAN SP P0ATE5 PYRG_ECCL1 SP Q5STA8 PYRG_THET8 SP P9WHK7 PYRG_MYCTU TR A02A291SY4 A02A91SY4 9LACT	625	GHK 									

Figure S12. Comparison of CTPS sequences of various organisms.

Legends for SI Appendix videos

SUPPLEMENTAL MOVIE S1. Animation of CTPS morphing from the CTP-state to DON-state tetramer conformations. The movie displays the dmCTPS structure morphed from product-bound to substrate-bound state. Colored by monomer.