Supplemental Material for:

Crystal structures of the Cid1 poly (U) polymerase reveal the mechanism for UTP selectivity

Bradley M. Lunde[1], Iris Magler[1] & Anton Meinhart[1*]

[1]Department of Biomolecular Mechanisms, Max-Planck-Institute for Medical Research, Jahnstrasse 29, 69120 Heidelberg, Germany

* To whom correspondence should be addressed. Tel: +49 6221 486 505; Fax: +49 6221 486 585; Email: anton.meinhart@mpimf-heidelberg.mpg.de

Supplementary figure legends

Supplemental Figure 1: Sequence alignment of *S. pombe* Cid1 with other Cid1 family members. Coloring of the alignment is based on conservation with green being highly conserved to yellow being less conserved. The Cid1 secondary structure is shown above the alignment. The catalytic aspartates are indicated by black boxes. Residues from the Cid1-UTP structure that are important for triphosphate recognition are highlighted with green boxes and residues involved in ribose base recognition are shown by yellow stars. The nucleotide recognition motif is surrounded by a black box and indicated by NRM on the figure. Abbreviations are same as in Figure 4B.

Supplemental Figure 2: UTP recognition by the *Trypanosomal brucei* TUTases. Key residues involved with UTP-specific recognition from each of the TbTUTase structures are shown for (A) *Tb*RET2-UTP (PDB ID 2B51), (B) *Tb*MEAT1-UTP (PDB ID 3HJ1), and (C) *Tb*TUT4-UTP (PDB ID 2IKF). Coloring is the same as in figure 2.

Supplemental Figure 3: Electron density maps for Cid1-NTP structures. 2Fo-Fc electron density maps (blue mesh) contoured at 1 σ are shown for each of the bound NTPs in the (A) Cid1-3'dATP, (B) Cid1-GTP, and (C) Cid1-CTP structures.

Supplemental Figure 4: Determination of the kinetic parameters of ATP and UTP addition of Cid1 and the H336N variant. (A) Michaelis-Menten kinetics of ATP incorporation by Cid1 (filled circles) and the H336N mutant (open circles). (B)

Michaelis-Menten kinetics of UTP-incorporation by Cid1 (filled circles) and H336N mutant (open circles).

Supplementary tables

Supplemental Table 1: DNA primers for cloning and mutagenesis used in this work.

cid1_fl_ndeI	5′-CGATCACATATGAGCTACCAAAAGGTCCCTAATTCG-3′
cid1_dN31_ndeI	5′-CGATCACATATGAGCTACCAAAAGGTCCCTAATTCG-3′
cid1_xhoI	5′-CGATCTCGAGTCACTCAGAATTGTCACCATCGG-3′
cid1_378stop_f	5′-GATTCATTATTTGAGGAGGCCTAGATTCCGCCTCGTCGCCAGAAA-3′
cid1_378stop_r	5′-TTTCTGGCGACGAGGCGGAATCTAGGCCTCCTCAAATAATGAATC-3′
cid1_h336n_f	5′-GAAGATCCTTTCGAGATTTCAAATAATGTGGGTAGGACAGTTA-3′
cid1_h336n_r	5′-TAACTGTCCTACCCACATTATTTGAAATCTCGAAAGGATCTTC-3′
cid1_d160a_f	5′-TGGGGCTTCGTTTCAATGTGCTATTGGATTTAACAATCGTC-3′
cid1_d160a_r	5′-GACGATTGTTAAATCCAATAGCACATTGAAACGAAGCCCCA-3′

Supplemental Table 2: Data collection and refinement statistics for Cid1 (H336N) crystals

	MgATP
Data collection	
Wavelength (Å)	1.00
Space group	$P\ 2_12_12_1$
Cell dimensions	
a, *b*, *c* (Å)	53.72, 62.47, 111.83
Resolution (Å)[a]	2.7 (2.77-2.70)
R_{meas}[a, b]	10.6 (74.3)
$I / \sigma I$[a]	11.41 (2.24)
Completeness (%)[a]	98.5 (97.4)
Redundancy[a]	3.5 (3.4)
Refinement	
Resolution (Å)	41.7-2.7
No. reflections	10,674
R_{work}[c] / R_{free}[d]	20.8/28.0
No. atoms	
Protein	2620
NTP	31
Ions (Mg^{+2}, Ca^{+2})	1
No. water molecules	39
Avg B-factors ($Å^2$)	
Protein	46.95
NTP	85.58.
Ion (Mg^{+2}, Ca^{+2})	71.9
Water	44.08
R.m.s. deviations[e]	
Bond lengths (Å)	0.0096
Bond angles (°)	1.183
Ramachandran Favored (%)	97.18
Ramachandran Disallowed (%)	0
Molprobity score [percentile]	2.04 [97th]

[a] Values in parentheses are for highest-resolution shell.

[b] $R_{meas} = \sum_h [n_h/(n_h-1)]^{1/2} \sum_i |I_{hl} - I_h| / \sum_h \sum_i I_{h,i,}$ where I_h is the mean intensity of symmetry-equivalent reflections and n_h is the redundancy.

[c] $R_{work} = \sum ||F_o| - |F_c|| / \sum |F_o|$

[d] R_{free} is the same as R_{work}, but calculated based on 5% of the data excluded from refinement

[e] R.m.s. deviations from target geometries

Figure S1

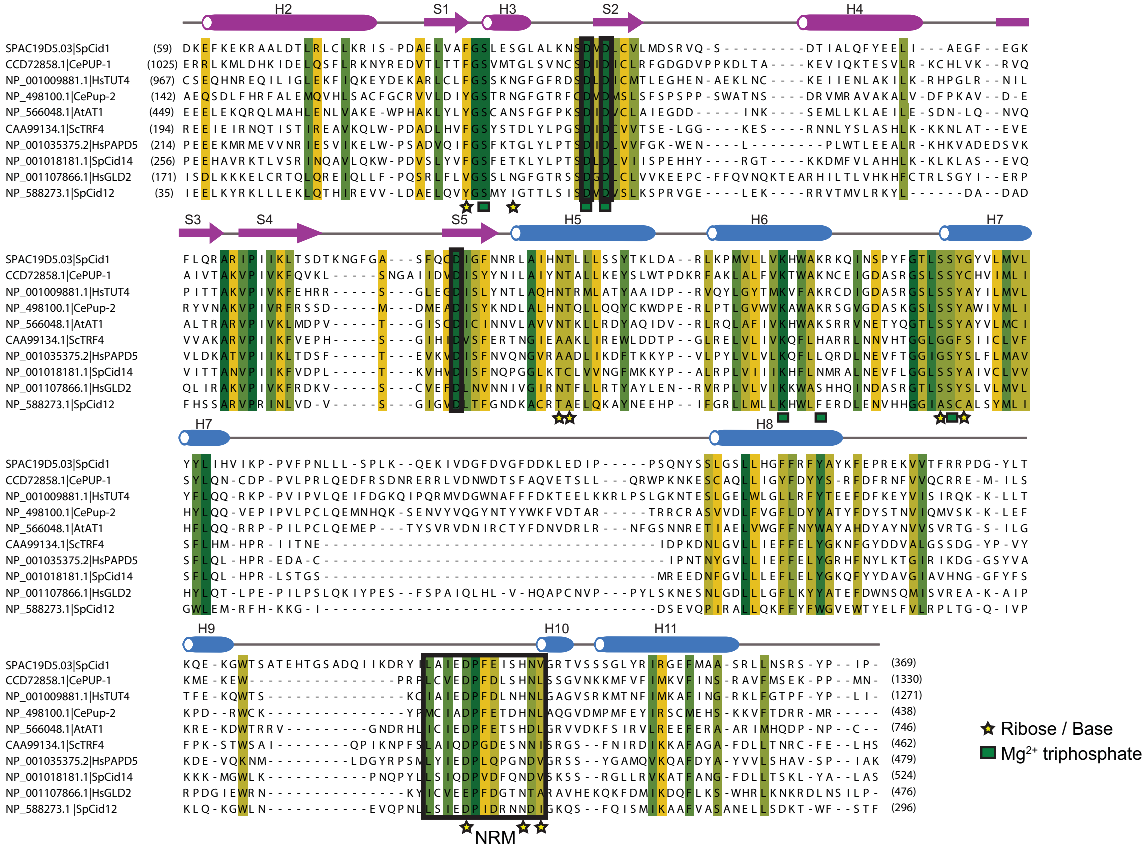

H2
S1
H3
S2
H4
S3
S4
S5
H5
H6
H7
H8
H9
H10
H11
SPAC19D5.03|SpCid1
CCD72858.1|CePUP-1
NP_001009881.1|HsTUT4
NP_498100.1|CePup-2
NP_566048.1|AtAT1
CAA99134.1|ScTRF4
NP_001035375.2|HsPAPD5
NP_001018181.1|SpCid14
NP_001107866.1|HsGLD2
NP_588273.1|SpCid12
(59)
(1025)
(967)
(142)
(449)
(194)
(214)
(256)
(171)
(35)
(369)
(1330)
(1271)
(438)
(746)
(462)
(479)
(524)
(476)
(296)
NRM
☆ Ribose / Base
🟩 Mg²⁺ triphosphate

Figure S2

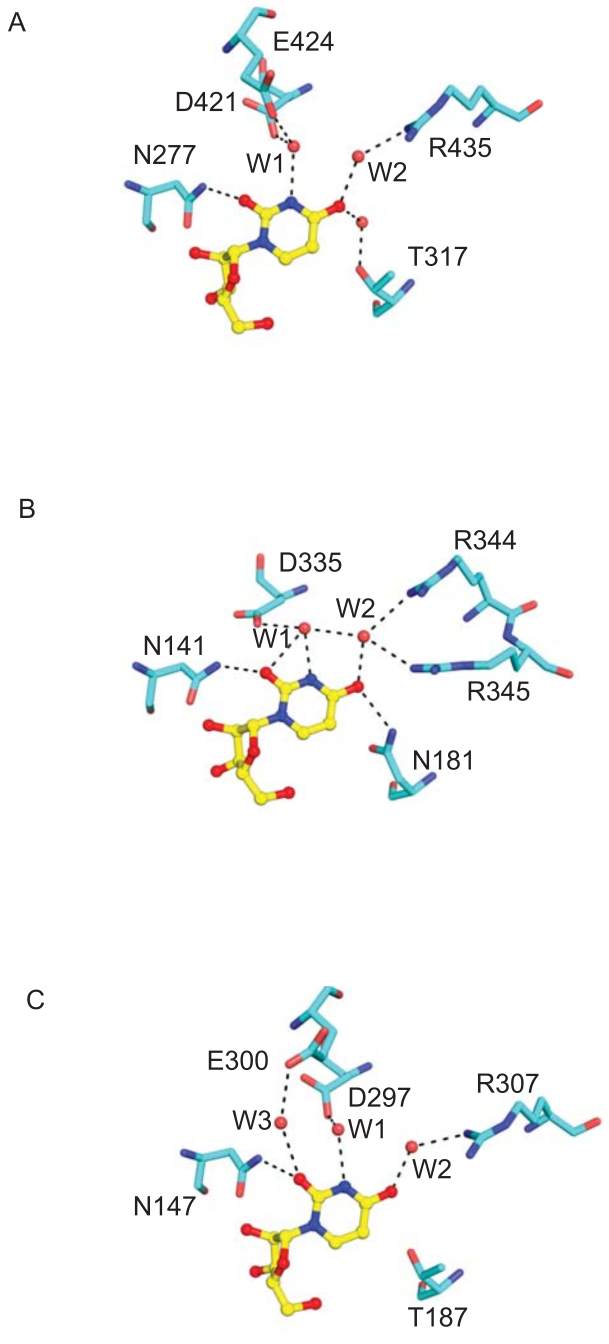

A
E424
D421
N277
W1
W2
R435
T317
B
D335
R344
W2
W1
N141
R345
N181
C
E300
D297
R307
W3
W1
W2
N147
T187

Figure S3

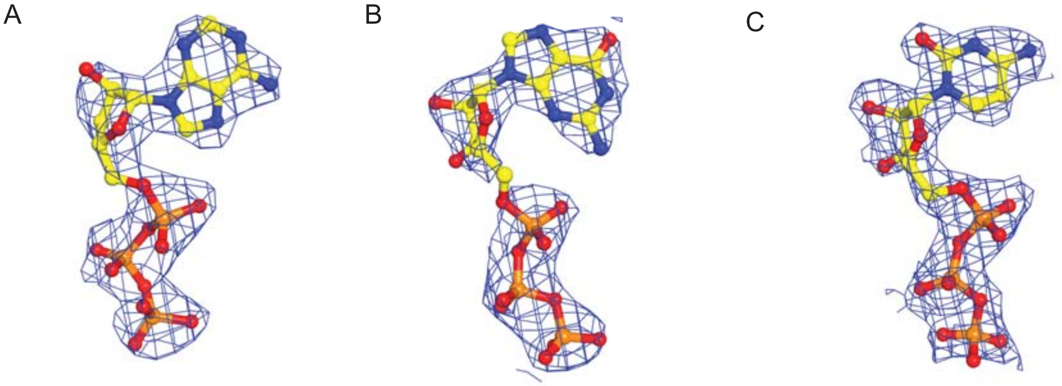
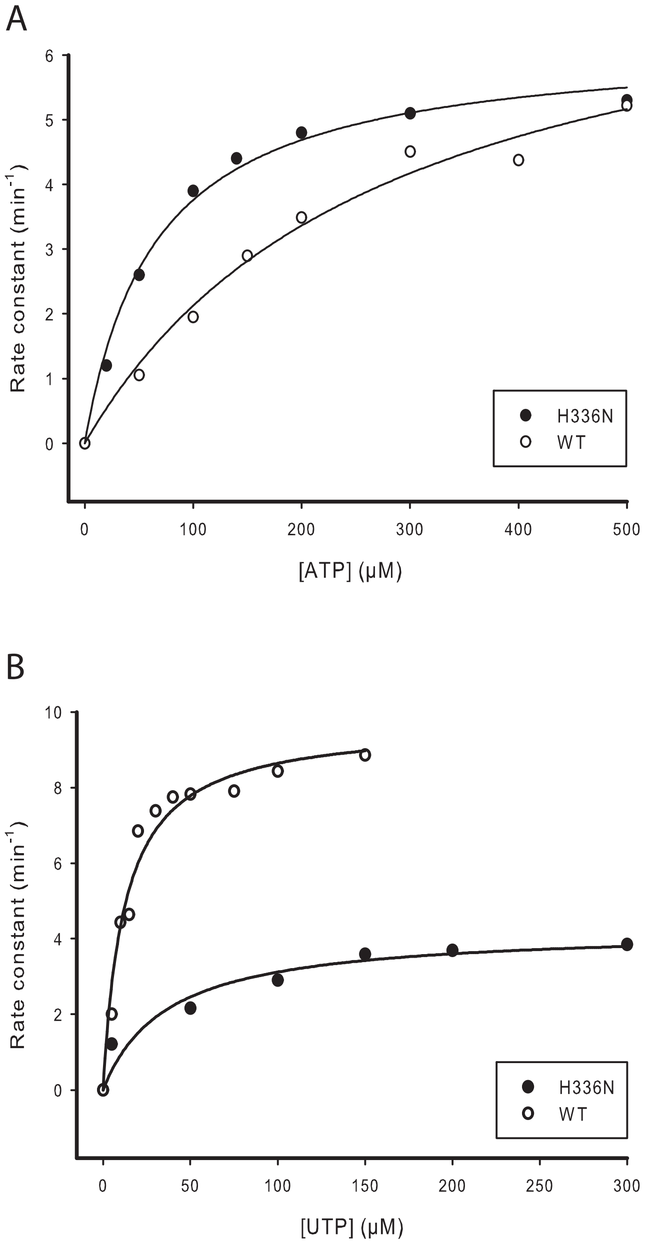


Figure S4


A
Rate constant (min^{-1})
0
1
2
3
4
5
6
0
100
200
300
400
500
[ATP] (µM)
● H336N
○ WT
B
Rate constant (min^{-1})
0
2
4
6
8
10
0
50
100
150
200
250
300
[UTP] (µM)
● H336N
○ WT