Electronic Supplementary Information

Theoretical investigation on the reaction mechanism of UTP cyclohydrolase

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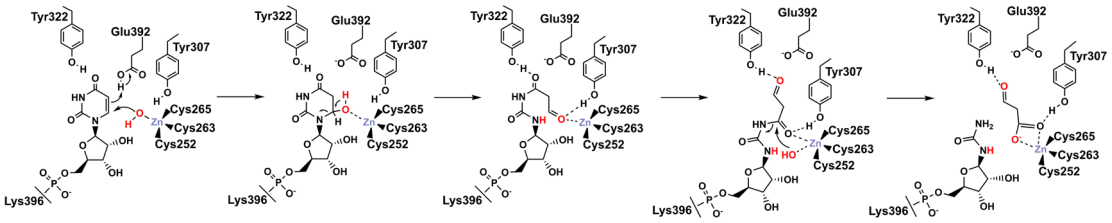
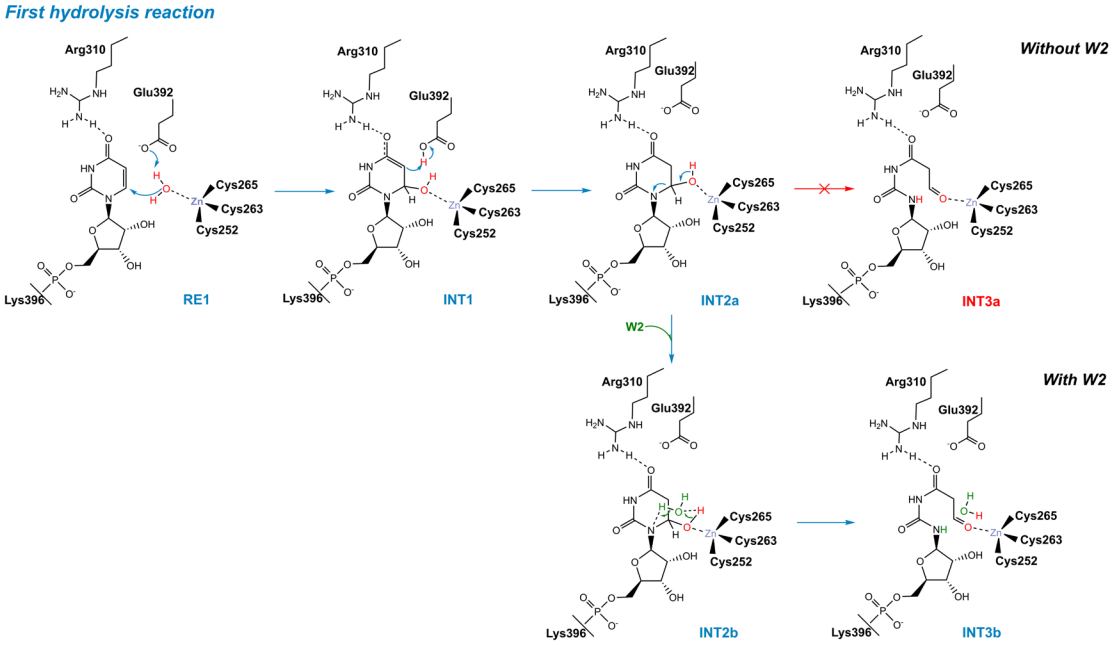
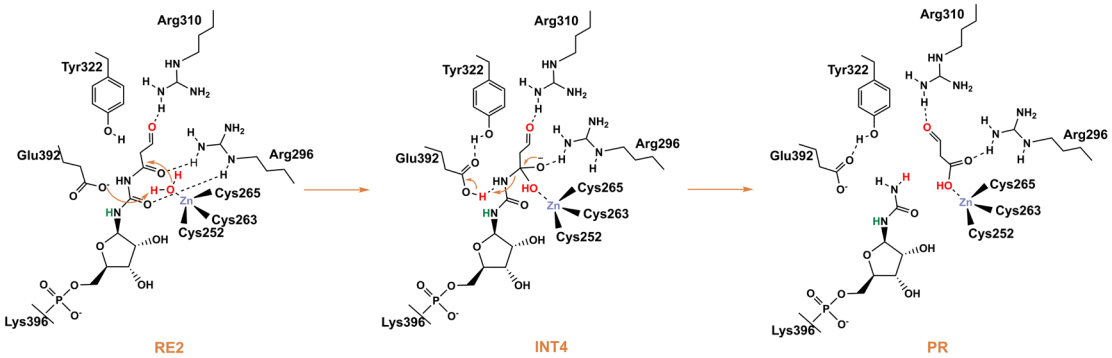
1 Schemes



Scheme S1 A possible mechanism of UrcA-catalyzed hydrolysis of UTP proposed by Zhang et al.



Scheme S2 The calculated mechanism of UrcA-catalyzed first hydrolysis reaction of UTP.



Scheme S3 The calculated mechanism of UrcA-catalyzed second hydrolysis reaction of UTP.

2 Figures

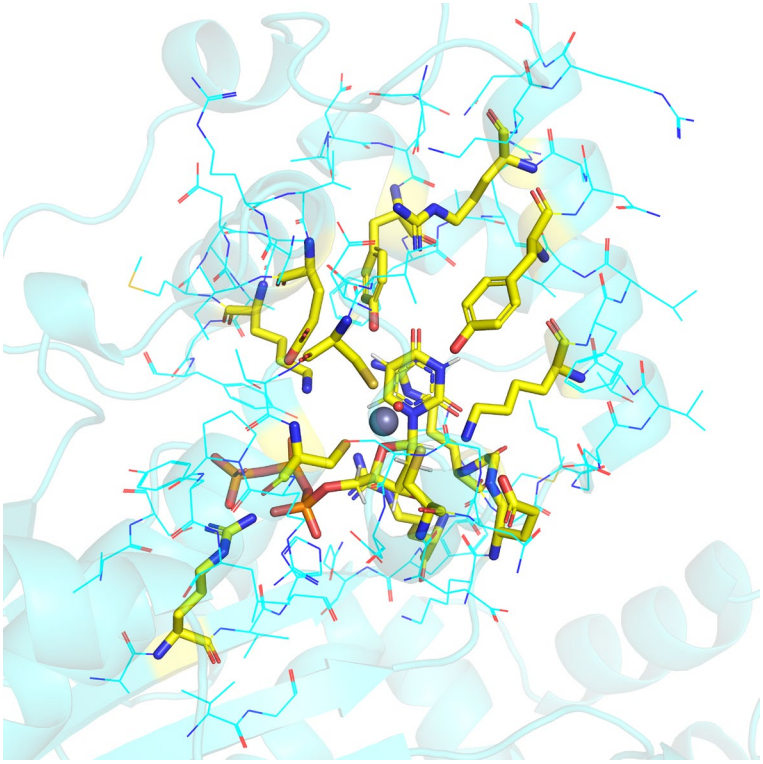


Figure S1 The residues included in the cluster models for DFT mechanism investigation (sticks), and the surrounding residues within 5 Å of them (lines), as well as the protein environment (cartoon).

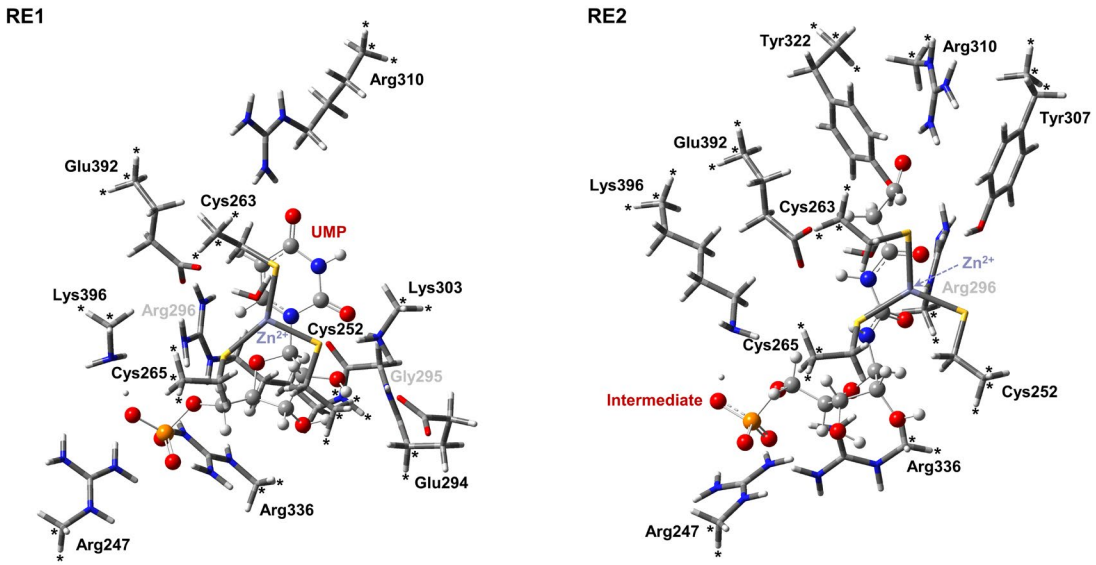


Figure S2 The optimized cluster models for investigation of two hydrolysis reactions in UrcA (RE1 (left) and RE2 (right)). The fixed atoms are marked with asterisks.

Figure S3 displays the intermediate INT2b^2 and TS3b^2 obtained from this small cluster model. Without the assistance from the protein environment, the corresponding free energy barrier of this step is as high as 36.8 kcal/mol, which clearly indicated that the enzyme plays critical role in catalyzing this reaction step. Compared to that in INT2b, in the optimized INT2b^2, the conjugated bond of the uracil ring in the intermediate is stronger so that it presents a more planar conformation. Especially, C6-N1 bond is 1.51 Å in INT2b, which shortens to 1.47 Å in INT2b^2. By comparing INT2b^2 with INT2b, it is believed that the hydrogen bond between Arg310 and C4=O carbonyl of uracil ring weakens the conjugation system of the uracil ring and activates the C6-N1 bond in this step. In addition, in INT2b^2, the bridge water is no longer restrained by the hydrogen bond formed between it and Lys303, so that it does not point its O-H bond towards N1 but turns to form a hydrogen bond with the 2'-OH group on the glycosyl ring. Hence, to reach the transition state TS3b^2, it costs extra energy to break down the hydrogen bond between the lytic water and 2'-OH group so that the bridge water is able to point its O-H bond towards N1 to deliver proton. It also infers that the residue Lys303 of the enzyme plays an indispensable role in the reaction.

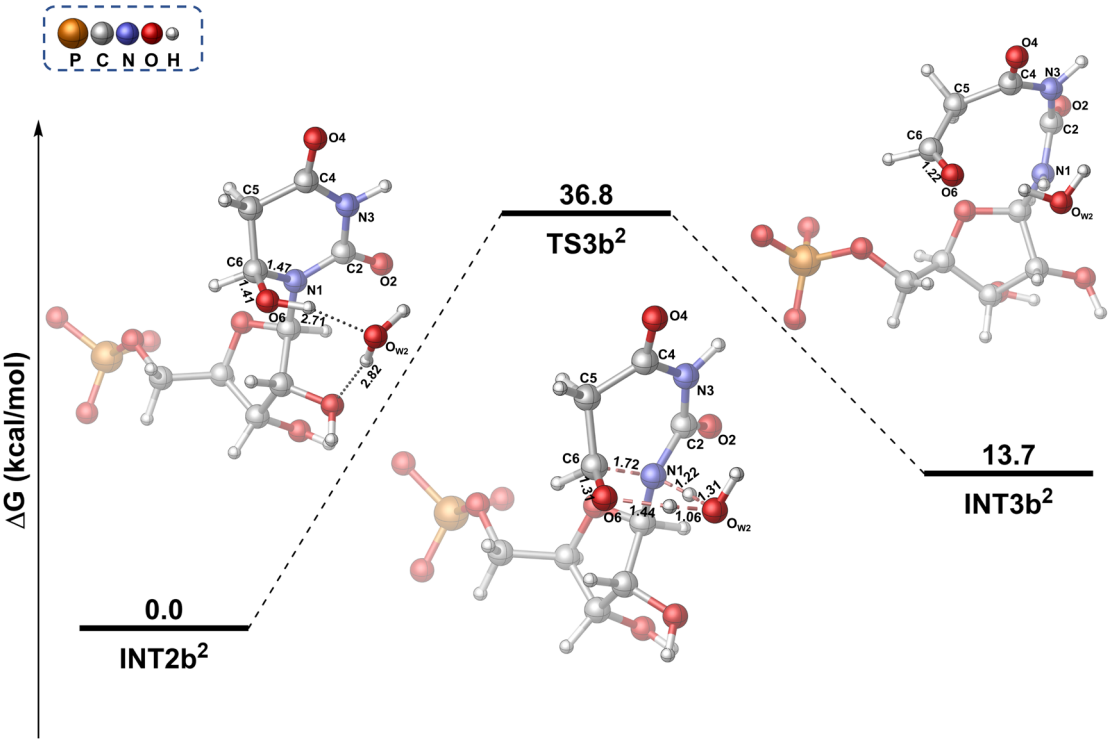


Figure S3 Free energy profile of the C6-N1 bond cleavage step of UTP which is calculated for the model without assistance from the enzyme. Reaction coordinates and hydrogen bonds are represented by coral and black dashed lines. The relative energies are labeled in kcal/mol.

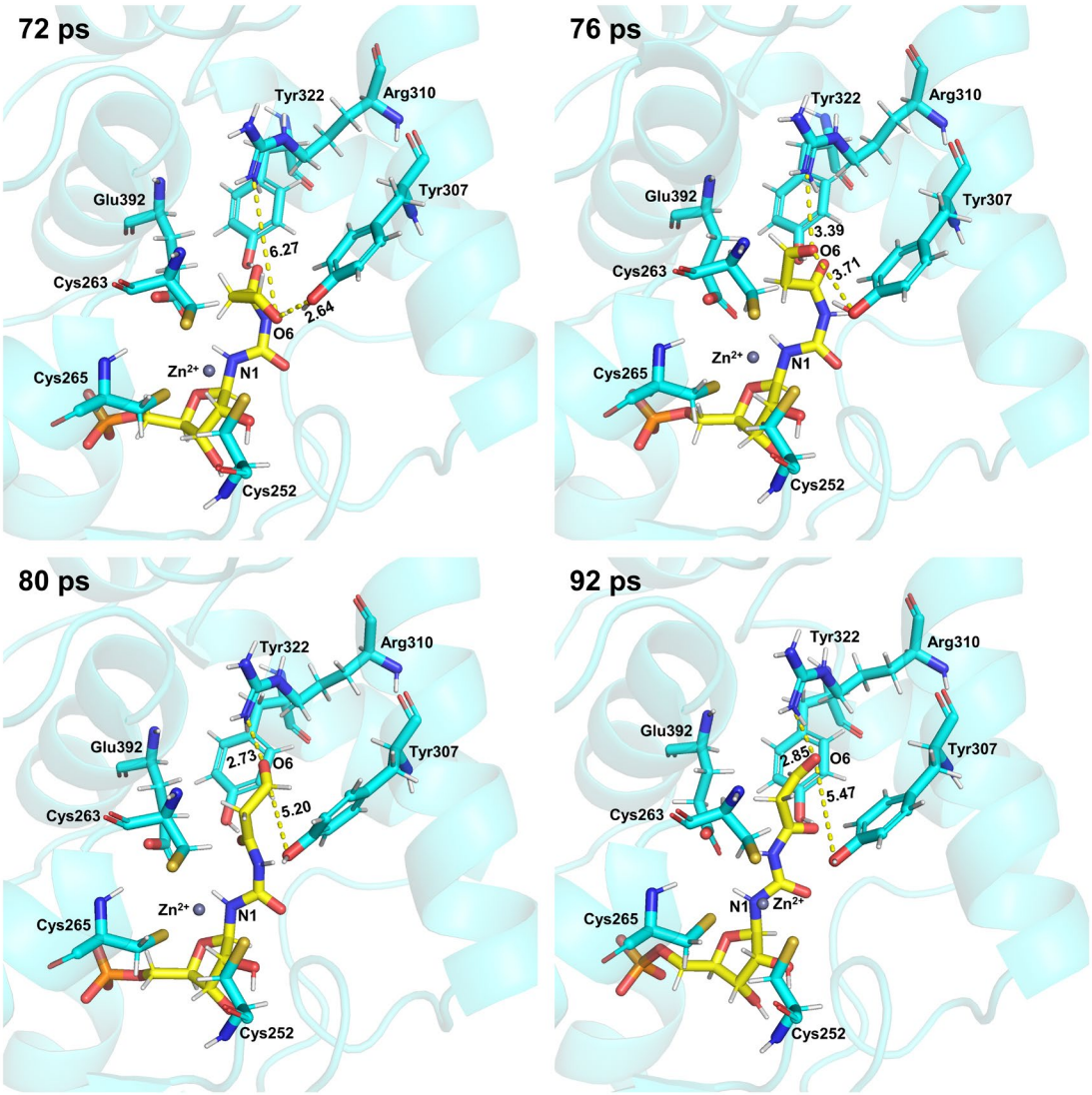


Figure S4 Representative structures of the conformational transition of the intermediate along the 120 ps heating stage of wild-type UrcA in complex with the intermediate. The key residues in the active site are represented as sticks. Zinc, carbon, oxygen, sulfur, phosphorus and nitrogen atoms are colored in slate, cyan, red, straw, orange and blue, respectively. The hydrogen bonds are represented as yellow dashed lines. Bond lengths are given in Å.

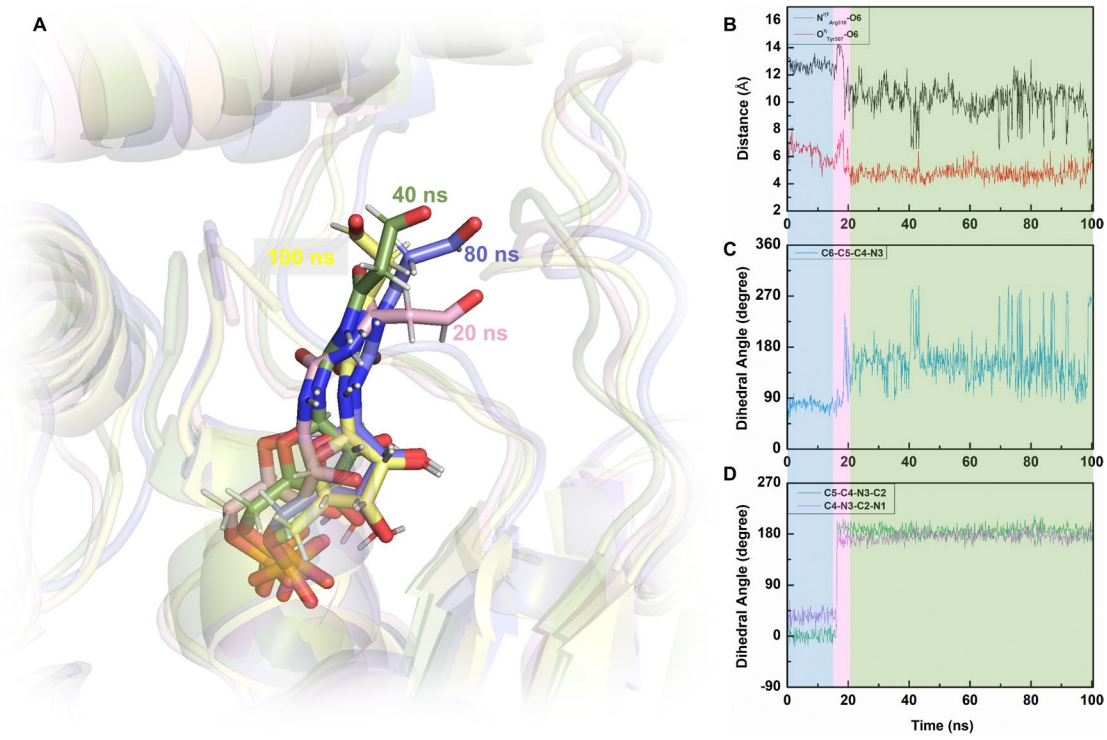


Figure S5 During MD simulation, (A) the cleaved uracil moiety of the intermediate (chain from N1 to C6) keeps swinging at the active site, taking snapshot overlays at 20ns, 40ns, 80ns and 100ns, (B) distance variations of the hydrogen bonds between the intermediate, Arg310 and Tyr307, (C) time dependences for C6-C5-C4-N3 dihedral angle of the intermediate, (D) time dependences for C5-C4-N3-C2 and C4-N3-C2-N1 dihedral angles of the intermediate.

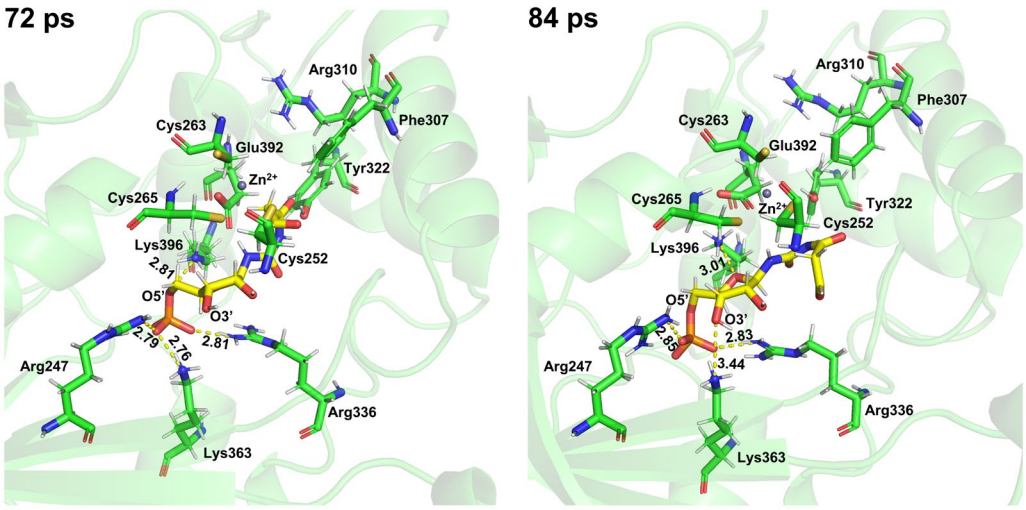


Figure S6 Representative structures of the position shift of the intermediate in 120 ps heating process of the Y307F mutant in complex with the intermediate. The key residues in the active site are represented as sticks. Zinc, carbon, oxygen, sulfur, phosphorus and nitrogen atoms are colored in slate, green, red, straw, orange and blue, respectively. The salt bridges and hydrogen bonds are represented as yellow dashed lines. Bond lengths are given in Å.

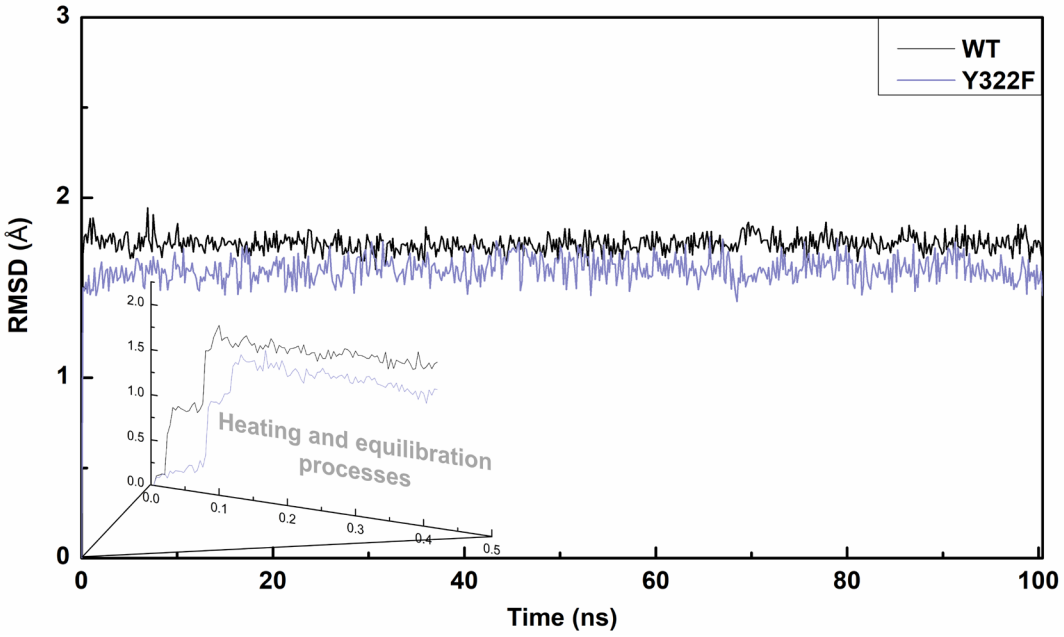


Figure S7 The root mean square deviation (RMSD) values of intermediates during 100ns simulations of the intermediate complexed of wild type UrcA (black) and Y322F mutant (purple), respectively, including initial heating and equilibration processes.

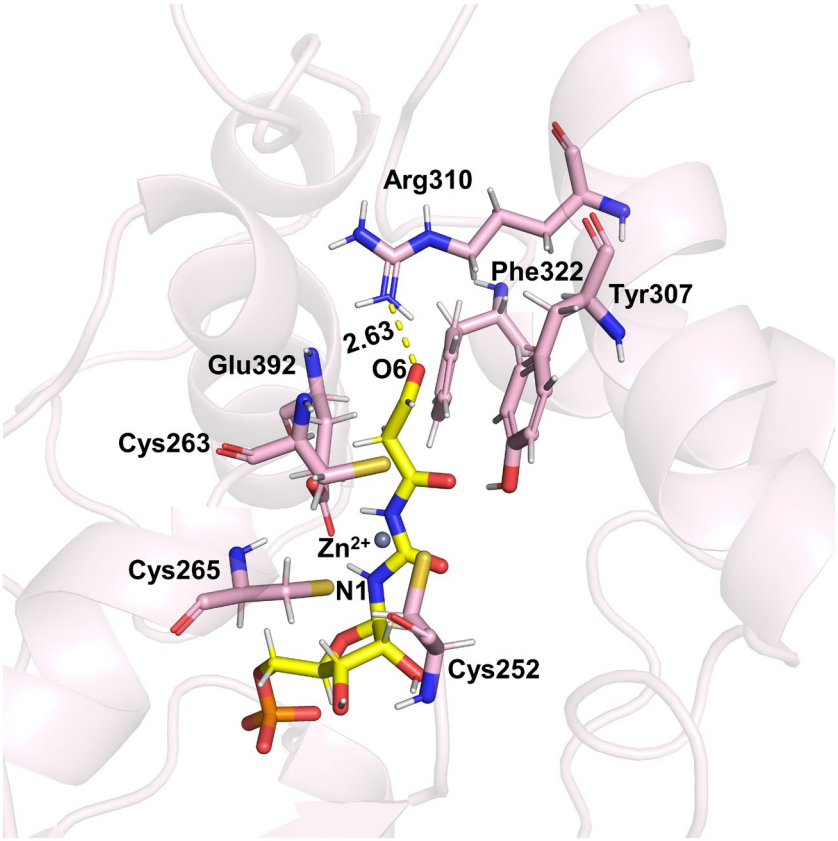
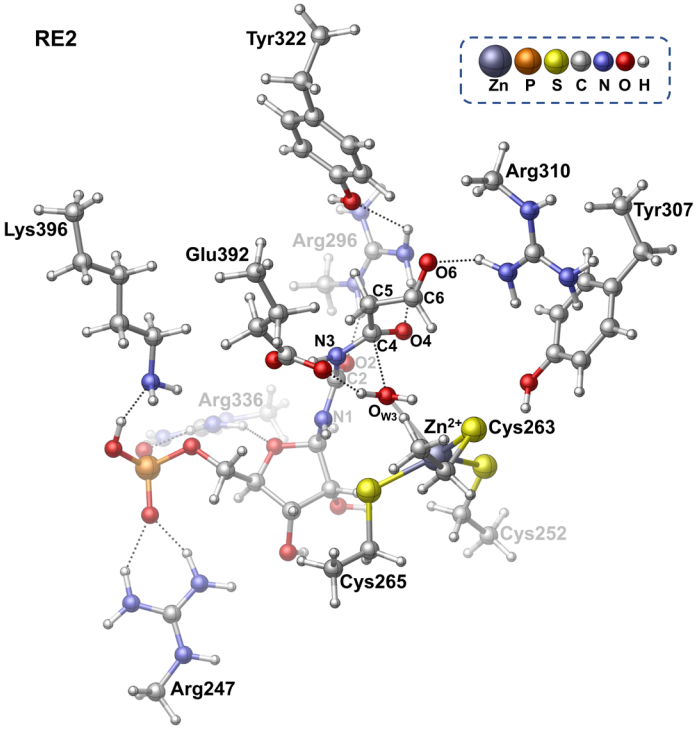


Figure S8 The conformation of the Y322F mutant in complex with the intermediate after 100 ns simulation. The key residues in the active site are represented as sticks. Water molecules not shown for clarity. Zinc, oxygen, sulfur, phosphorus and nitrogen atoms are colored in slate, red, straw, orange and blue, respectively. The carbon atoms are shown in pink and yellow in Y322F mutant and intermediate, respectively. The hydrogen bonds is represented as yellow dashed lines. Bond lengths are given in Å.



Parameters	RE2
Bond length (Å)	
O6-$N^{\eta1}_{Arg310}$	2.85
O4-$N^{\eta1}_{Arg296}$	2.73
O2-N^{ε}_{Arg296}	2.68
O1A-N^{ζ}_{Lys396}	2.89
O2A-$N^{\eta1}_{Arg247}$	2.71
O2A-$N^{\eta2}_{Arg247}$	2.89
O3A-$N^{\eta1}_{Arg336}$	2.70
O5'-$N^{\eta1}_{Arg336}$	2.91
O_{W3}-Zn	2.65
O_{W3}-$O^{\varepsilon2}_{Glu392}$	2.69
O_{W3}-C4	2.54
O^{η}_{Tyr322}-$N^{\eta1}_{Arg296}$	2.93
Atomic charge (a.u.)	
N1	-0.15
C2	0.25
O2	-0.34
N3	-0.15
C4	0.12
O4	-0.32
C5	0.08
C6	0.15
O6	-0.27

Figure S9 The cluster model structure for studying the second hydrolysis reaction, exemplified by the optimized structure of the initial reactant (RE2). The data presented in the table summarize the important bond lengths and atomic charges of RE2. The hydrogen bonds are represented by black dashed lines.

3 Tables

Table S1 Residues within 5 Å of the corresponding residues in the cluster models.

Residues within 5 Å of the cluster models										
Cys252	Glu251	Glu253	Gly254	Ser255	Asp256					
Cys263	Ser255	Asp261	Ile262	Thr264	Cys265	Ala389				
Cys265	Cys263	Thr264	Arg266	Pro267	Tyr268	Leu269				
Glu392	Cys263	Thr264	Ala389	Arg390	Val391	Ile393	Asp394	Ala395	Lys396	
Lys396	Ile393	Asp394	Ala395	Met397	Ala398	Ala399	Gly400	Tyr401		
Arg336	Arg296	Ala297	Asp334	Met335	Phe337	Gln338				
Arg247	Ala246	Val248	His249	Try268	Leu269	Val288	Ala289	Val357	Ser358	
Arg296	Glu294	Gly295	Ala297	Leu298	Asp334	Met335	Arg336			
Arg310	Tyr307	Asn308	Ala309	Lys311	Arg312	Gln313	Asp317	Val391		
Lys303	Cys252	Gly295	Glu300	Val301	Thr302	Phe304	Leu305	Val306	Tyr307	
Glu294	Glu251	Cys252	Asn253	Lys293	Gly295	Arg296	Ala297	Leu298	Gly299	Glu300
Gly295	Glu294	Arg296	Ala297	Leu298	Gly299					
Tyr307	Phe304	Leu305	Val306	Asn308	Ala309	Arg310	Lys311			
Tyr322	Ala319	Asp320	Gln321	Phe323	Ala324	Arg325	Glu392			

Table S2 Mulliken charge population of important atoms of uracil at the stationary points of UrcA-catalyzed first hydrolysis pathway of UTP.

	N1	**C2**	**O2**	**N3**	**C4**	**O4**	**C5**	**C6**
RE1	-0.25	0.26	-0.27	-0.12	0.21	-0.31	-0.14	0.18
TS1	-0.29	0.26	-0.31	-0.12	0.22	-0.38	-0.25	0.30
INT1	-0.34	0.24	-0.35	-0.11	0.24	-0.43	-0.30	0.38
TS2	-0.30	0.24	-0.33	-0.13	0.22	-0.40	-0.34	0.30
INT2a	-0.32	0.25	-0.28	-0.10	0.08	-0.27	0.05	0.24
INT2b	-0.43	0.22	-0.22	-0.13	0.13	-0.22	0.05	0.24
TS3a	-0.35	0.23	-0.20	-0.08	0.12	-0.24	0.11	0.21
TS3b	-0.33	0.21	-0.18	-0.12	0.12	-0.21	0.08	0.23
INT3b	-0.19	0.18	-0.23	-0.11	0.10	-0.28	0.02	0.17

Table S3 Mulliken charge population of important atoms of uracil at the stationary points of UrcA-catalyzed second hydrolysis pathway of UTP.

	N1	C2	O2	N3	C4	O4	C5	C6
RE2	-0.15	0.25	-0.34	-0.15	0.12	-0.32	0.08	0.15
TS4	-0.15	0.23	-0.34	-0.18	0.16	-0.39	0.11	0.16
INT4	-0.16	0.17	-0.28	-0.27	0.22	-0.28	-0.01	0.15
TS5	-0.15	0.21	-0.36	-0.33	0.22	-0.36	0.02	0.15
PR	-0.20	0.17	-0.36	-0.11	0.22	-0.27	-0.03	0.13