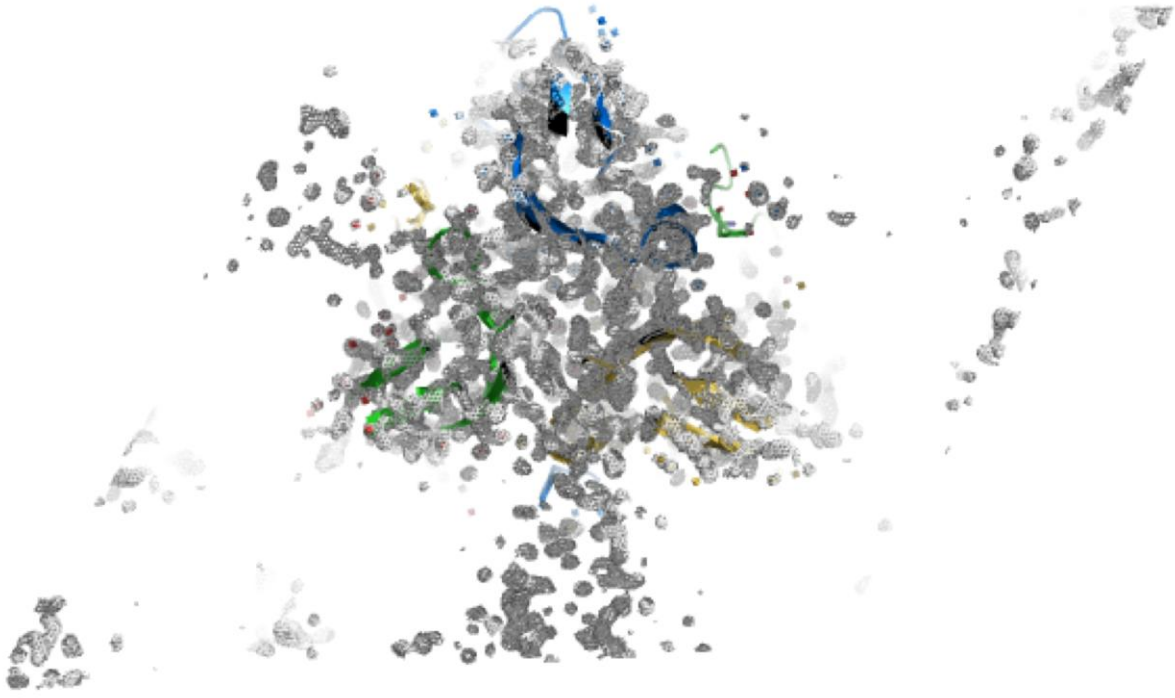


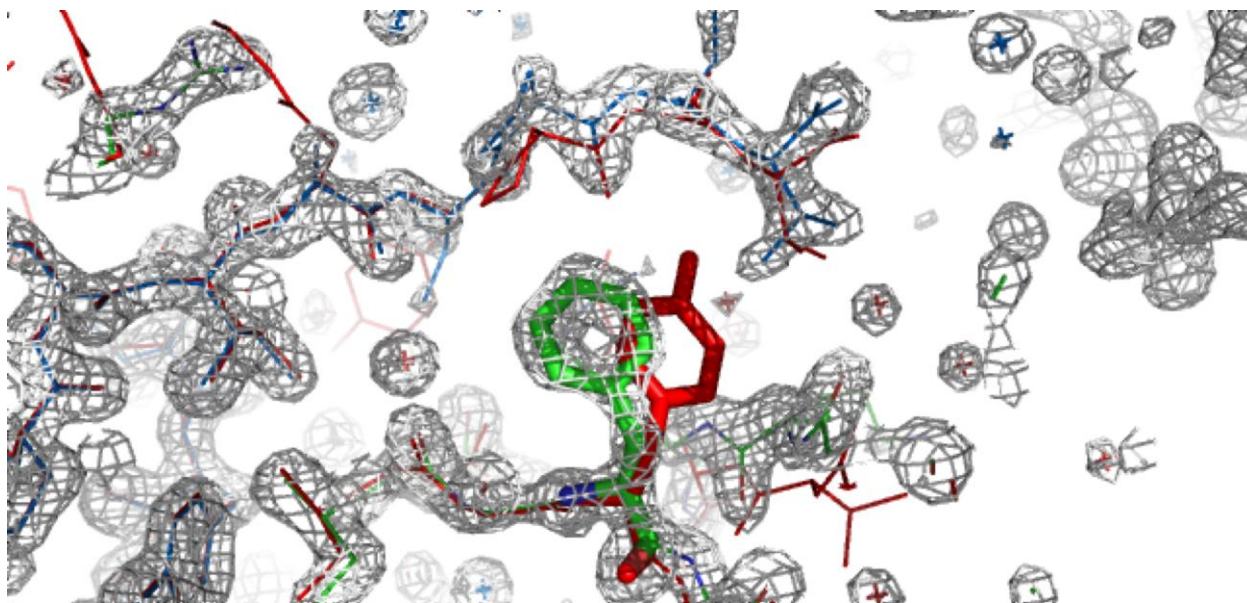
Motif			Motif 1	Motif 2
Human			21-----30-----40-----50-----60-----70-----80-----90	
Human	3EHW	(21)	VGGMQLRFARLSEHATAPTRGSARAAGYDLYSAYDYTIIPMEKAVVKTDIQIALPSGCYGRVAPRSGLAA	
Chlorella			1-----10-----20-----30-----40-----50-----60-----70-	
Chlorella	3SO2	(1)	LLREALRVHLLNEHAVLPKRGSSAGAAGFDLASCEDTEVPARGRAVVKTGLQIAIPPGTYARVAPRSGLAV	
Plant	2PC5	(21)	GIHGDSSPSPPFKVKLSEKAVIPTRGSPLSAGYDLSAVDSKVPARGKALIPDLSIAVPEGTYARIAPRSGLAW	
Yeast	3P48	(1)	MTATSDKVLKIQLRASATVPTKGSATAAGYDIYASQDITIPAMQGMVSTDISFTVPVGTYGRIAPRSGLAV	
Virus IL-3A	3C2T	(1)	MSSLLVKKLVESATTPMRGSEGAAGYDISSVEDVVVPAMGRIAVSTGISIRVDPGTYGRIAPRSGLAY	
Virus PBCV-1		(1)	MSSLLVKKLVESATTPMRGSEGAAGYDISSVEDVVVPAMGRIAVSTGISIRVDPGTYGRIAPRSGLAY	

Motif			Motif 3	Motif 4	Motif 5
Human			91-----100-----110-----120-----130-----140-----150-----160----		
Human	3EHW	(91)	KHFIDVGA GVIDEDYR GVVDEYRGNVGVVLFNFGKEKFEVKKGDRIAGLICERIFYPEIEEVQALDDTE		RGSGGFGSTGKN
Chlorella			81-----80-----90-----100-----110-----120-----130-----140----		
Chlorella	3SO2	(81)	KHFIDTGA GVVDEYR GVVDEYRGEVGVVLFNHNGETPPQVRRGDRVAGLILERIATPEVVEVESLDETT		RGTGGYGSTGVAS
Plant	2PC5	(97)	KHSIDVGA GVIDADYR GVVDEYRFPVGVILFNHSDADFEVVKFGDRIAGLILIEKIVTPDVVEVDDLDET		RGDGGFGSTGV
Yeast	3P48	(74)	KNGIQTGA GVVDRDYT GVVDRDYTEVVKVLFNHSQRDFAIKKGDRIAGLILEKIVDDAQIVVDSLEESARG		GGGFGSTGN
Virus IL-3A	3C2T	(70)	KYGIDVLA GVIDEDYT GVVDEYTG EVK VILYNTTERDYIIKKGDRIAGLILEQIVTPGVAVVDLSDTA		RGSGGFGSTGI
Virus PBCV-1		(70)	KYGIDVLA GVIDSDYR GVVDEYR ELK AILYNTTERDYIIKKGDRIAGLILEQIVTPDVAVVELEDTA		RGSGGFGSTGI

(a)

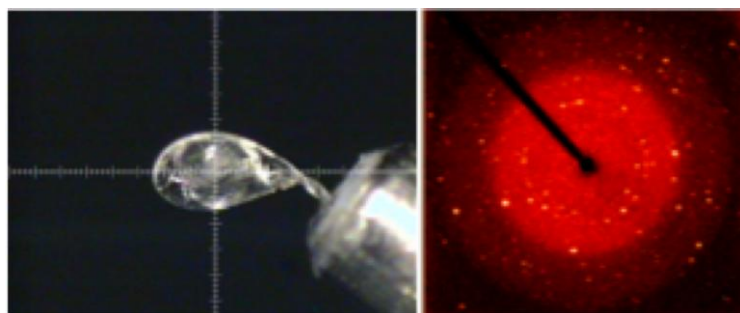


(b)



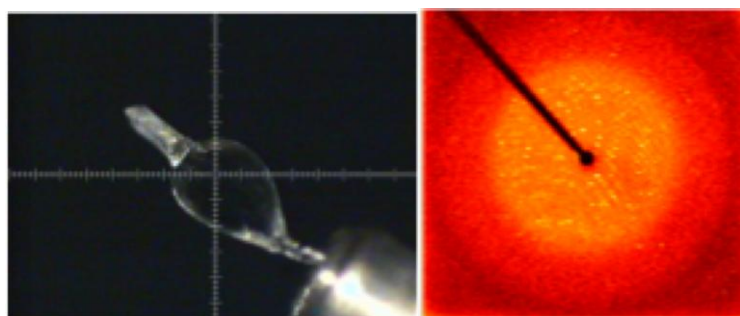
(c)

Supplementary Fig. S1. (a) A comparison of amino acid sequences of dUTPases. The aligned sequences were from human (PDB ID, 3ehw), *Chlorella* (*Chlorella variabilis* NC64A, 3so2), plant (*Arabidopsis thaliana*, 2pc5), yeast (*Saccharomyces cerevisiae*, 3p4b), chlorellavirus IL-3A (3c2t), and *Paramecium bursaria Chlorella virus 1* (PBCV-1; UniProt ID, O41033; yet to be crystallized). The five conserved motifs are highlighted in green, blue, magenta, red, and yellow, respectively. Key residues in the formation of the reaction intermediates are underlined in the human dUTPase sequence. The glycyserine, indicated by khaki, is added due to the construction (a residual thrombin site). The potential sites of compensatory mutations are underlined. (b) A composite 2Fo-Fc map level at 2σ shows a typical dUTPase triangle. Cartoon models of symmetrically related subunits are embedded in xy-plane projected electron density (green, blue, and yellow). (c) Current structural model and corresponding electron density of 29F of *Chlorella* dUTPase (green; subunit A). The side chain of 29F was pointing to the hydrophobic P121. A super-imposed structure of 48Y of human dUTPase is shown in red. The structure of subunit B is shown in blue.



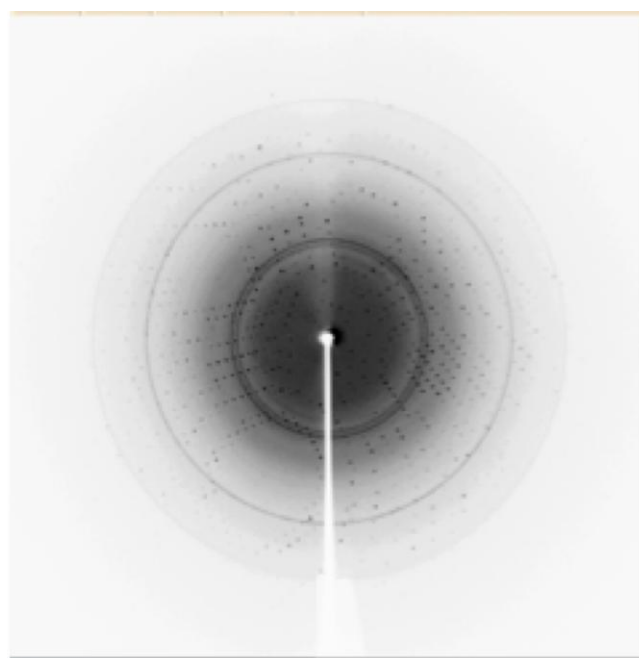
(a)

(b)



(c)

(d)



(e)

Supplementary Fig. S2. (a) A type 2 crystal of core *Chlorella* dUTPase mounted in a loop. (b) A diffraction image of the *Chlorella* dUTPase by a sealed tube. (c) A crystal of plant dUTPase

grown as described (Bajaj and Moriyama, 2007) but with added dUMP/NPP. (d) A diffraction image of plant dUTPase as a positive control purpose. (e) A diffraction image of *Chlorella* dUTPase on a rotating anode.