Supporting Information

Deprotonations in the Reaction of Flavin-Dependent Thymidylate Synthase

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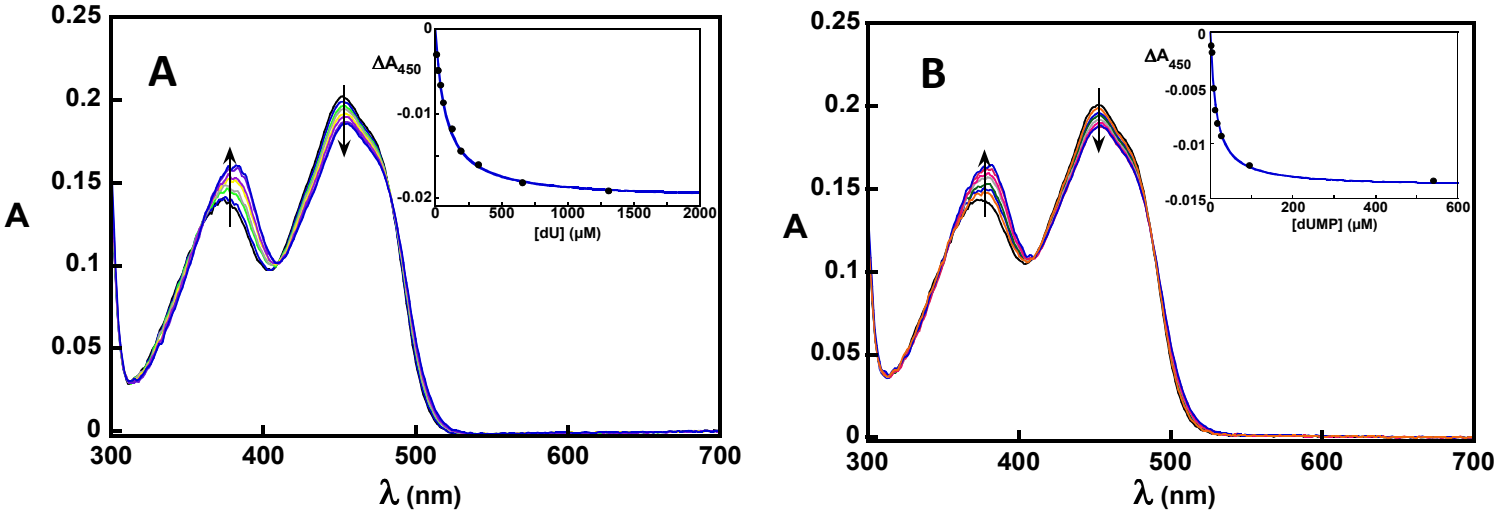
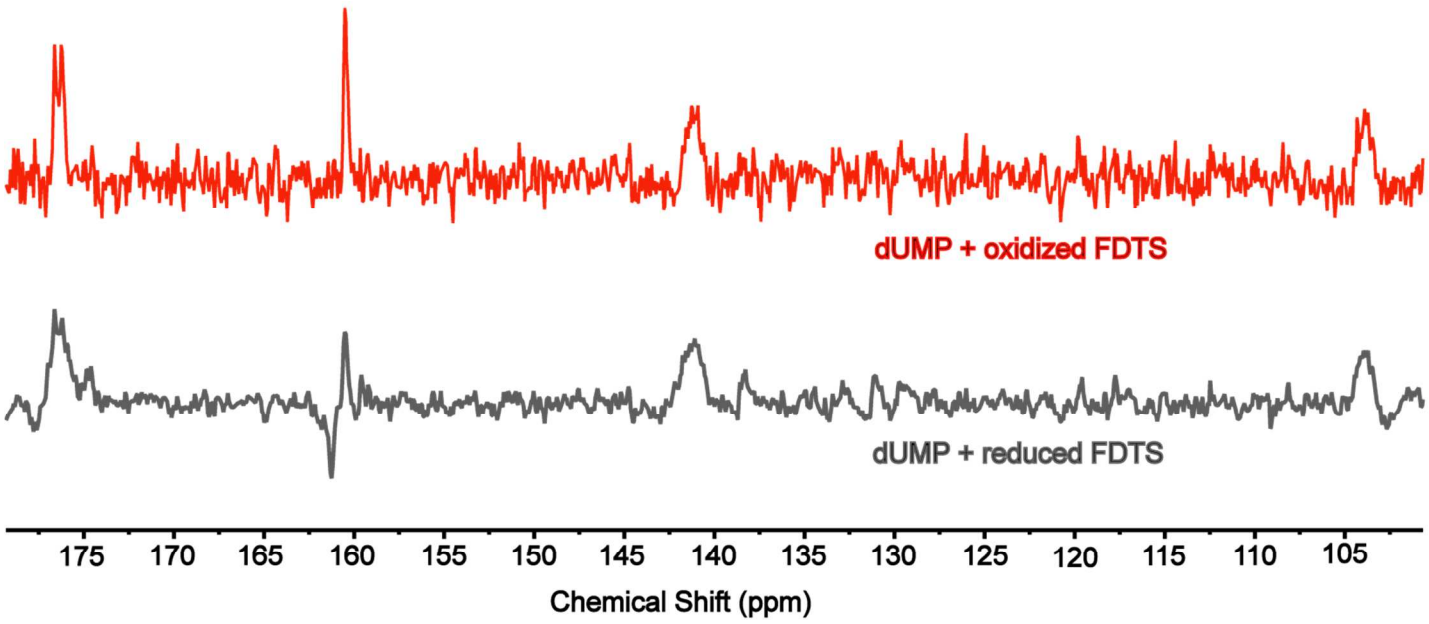
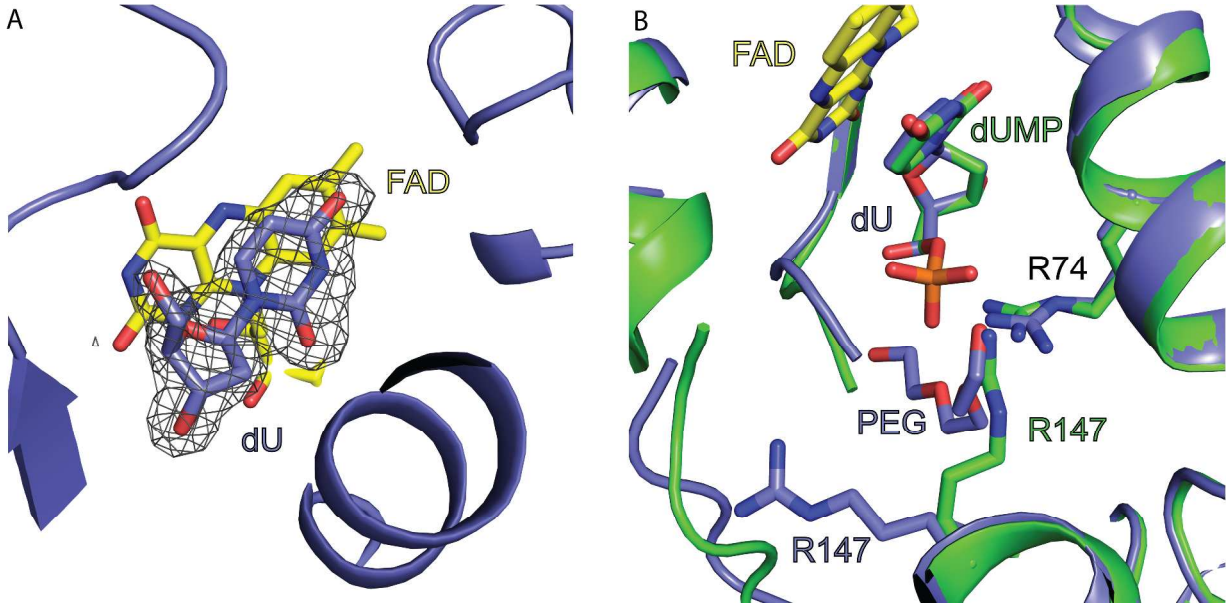


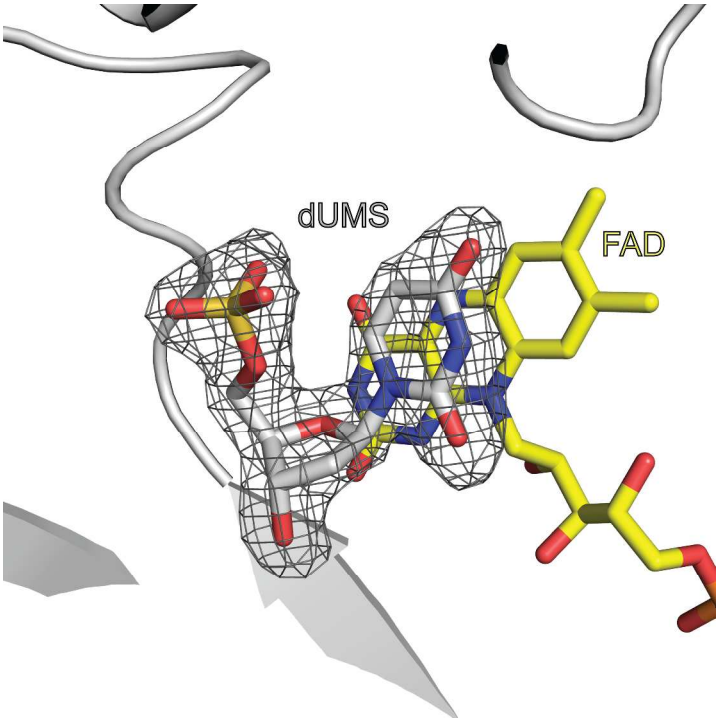
SI Figure 1. Spectrophotometric titrations of WT FDTS with dU (a) and R90A FDTS with dUMP (b) in 0.1 M Tris-HCl, pH 8 at 25 °C. The insets show the change in absorbance as a function of ligand concentration. Fitting to a square hyperbola gives a K_d of 78 μM for dU binding to WT FDTS and 14 μM for dUMP binding to R90A FDTS.



SI Figure 2. The ^{13}C-NMR spectra of uracil carbons of dUMP bound to oxidized or reduced WT FDTS at 45 °C. The ^{13}C chemical shifts were nearly identical when the flavin was oxidized or reduced.



SI Figure 3. Deoxyuridine binding to FDTS. A. The 1.93 Å F_O-F_C omit map contoured at 3 σ shows clear density for the dU ligand. B. Overlay of dUMP-WT FDTS and dU-WT FDTS structures. R147 – which forms a salt bridge with the phosphate of dUMP – is in a different conformation in the dU-WT FDTS structure. A PEG molecule from the crystallization solution occupies the vacant space. The dUMP-WT FDTS complex is shown in green, dU-WT FDTS is shown in blue, and dUMP and dU are shown as sticks. A single copy of FAD is shown in yellow sticks.



SI Figure 4. dUMS binding to FDTS. The 1.95 Å F_O-F_C omit map contoured at 3 σ shows clear density for the dUMS ligand. The dUMS ligand is shown in grey sticks and FAD is shown in yellow sticks.

SI Table 1: Crystallographic summary

Crystal Contents	ThyX R90A-FAD dUMP	ThyX R174A-FAD dUMP	ThyX-FAD dU	ThyX-FAD dUMS
Diffraction Data				
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$I4_122$
Cell (Å)	54.1 116.2 141.0	54.1 115.9 141.1	54.2 116.1 141.9	109.7 109.7 119.9
d_{min} (Å)	1.9 (1.97-1.90)[1]	2.00 (2.11-2.00)	1.93 (2.02- 1.93)	1.95 (2.06-1.95)
Average I/σ_I	22.9 (2.3)	8.7 (2.4)	22.8 (2.5)	20.4 (2.6)
R_{sym}	0.073 (0.856)	0.115 (0.682)	0.094 (0.897)	0.118 (1.096)
$CC_{1/2}$				99.9 (82.5)
Multiplicity	6.6 (5.9)	6.0 (6.1)	7.6 (6.8)	14.6 (14.2)
Completeness	99.5 (99.6)	90.7 (86.0)	98.1 (96.9)	99.7 (98.1)
No. of unique reflections	70476	54863	67137	27043
Refinement				
Data Range (Å)	89.68 -1.90	89.54 - 2.00	89.86 - 1.93	80.92-1.95
Reflections	66829	52046	62459	25671
R_{work}/ R_{free}	0.178 / 0.211	0.183 / 0.217	0.170 / 0.202	0.162 / 0.200
RMSD bonds (Å)	0.010	0.010	0.010	0.009
RMSD angles (°)	1.48	1.49	1.46	1.37
Ramachandran (%)				
Allowed	100	100	100	100
Outliers	0	0	0	0
Average B-factors (Å^2)				
Protein	37.1	34.8	39.3	22.8
Ligand	25.9	25.6	36.8	32.5
Water	38.0	37.3	40.4	37.8

[1] Values in parentheses refer to the outermost shell of data.