Supporting Information:

Capturing the catalytic proton of dihydrofolate reductase: implications for general acid-base catalysis

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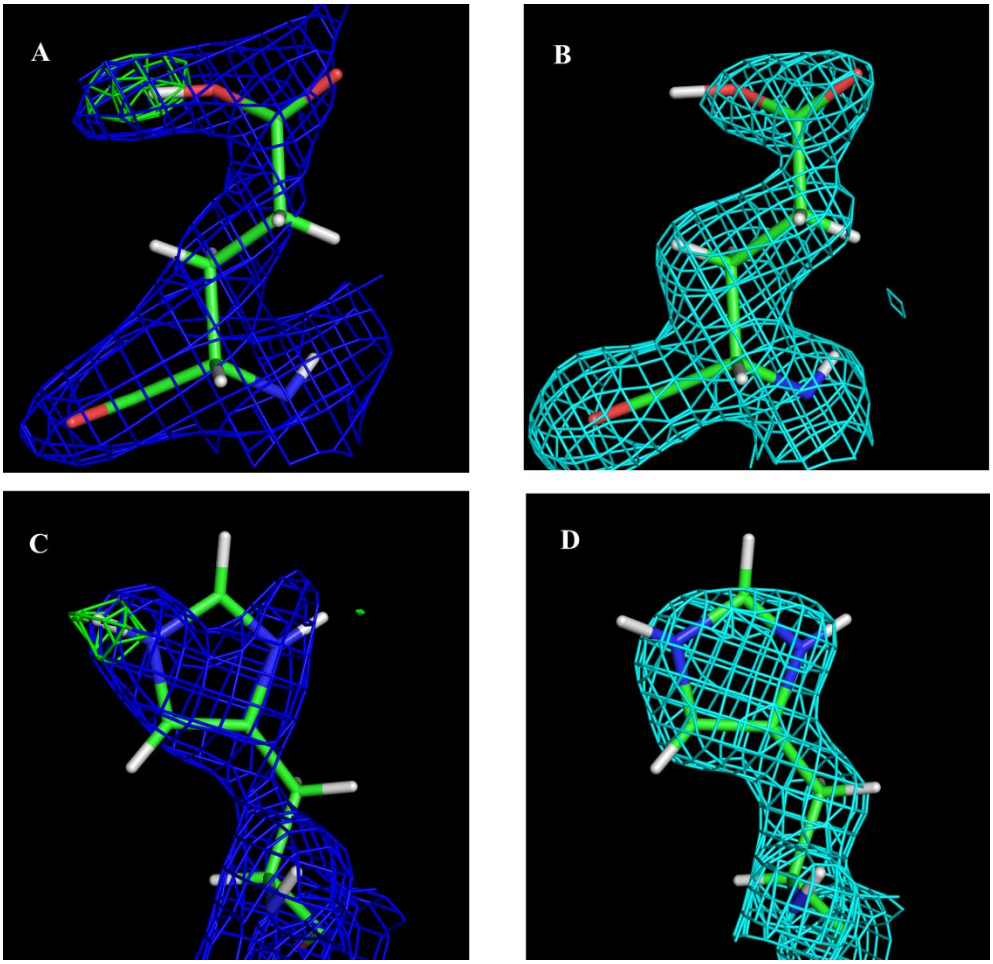

Fig. S1. Glu101 and His45 are protonated at pH4.5. (A) 2Fo-Fc nuclear density map (contour level: 1.0 σ) and Fo-Fc nuclear density omit map (contour level: 3.0 σ) around Glu101. The omit map was created without the deuterium contribution for neutron scattering. (B) 2Fo-Fc electron density map (contour level: 1.0 σ) around Glu101. (C) 2Fo-Fc nuclear density map (contour level: 1.0 σ) and Fo-Fc nuclear density omit map (contour level: 3.0 σ) around His45. (D) 2Fo-Fc electron density map (contour level: 1.0 σ) around His45.

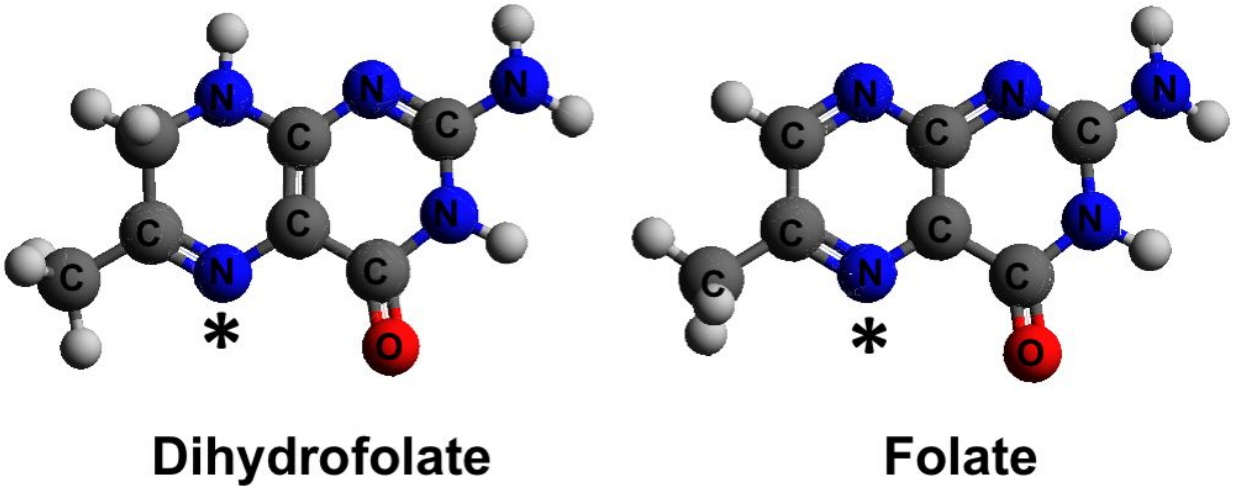

Fig. S2. Structures of geometry-optimized dihydrofolate and folate moieties used to calculate the proton affinity at N5. The star symbol shows the location of N5 that is protonated in order to calculate the proton affinity. The estimated proton affinity at N5 for dihydrofolate is 24.0 kcal/mol greater than that of folate. See Table S2 for calculations.

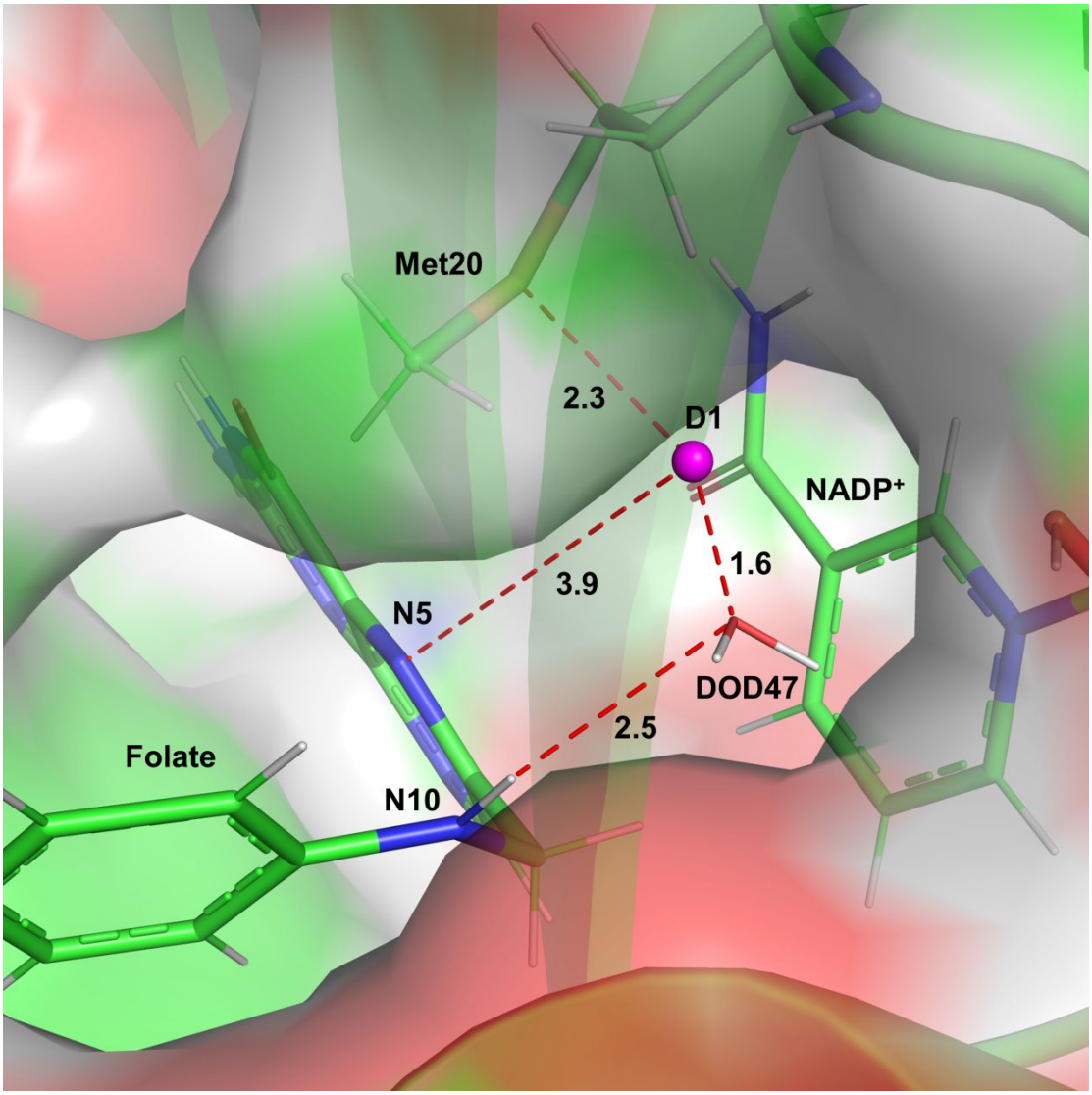

Fig. S3. Surface representation of the DHFR active site around the deuteron. The discrete deuteron (D1) is close to the surface of the Met20 side chain. This short, shallow "tunnel" cannot sterically accommodate a water molecule.

Table S1. Data collection and refinement			
Data collection	Neutron	X-ray	X-ray
Temperature (K)	291	291	291
pH	4.5	4.5	7.0
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions			
a, b, c (Å)	34.3, 45.6, 99.2	34.1, 45.6, 99.1	34.3, 45.6, 99.0
α, β, γ (°)		α=β=γ=90	
Resolution (Å)	2.1	1.65	1.60
Unique reflections	7031	18163	20751
Redundancy	2.3(2.0)[a]	3.9(3.3)[a]	4.1 (4.0)
Completeness (%)	72.4(61.7)[a]	93.9(77.8)[a]	97.6 (94.0)
R_{sym} [b]	0.093(0.303)[a]	0.040(0.422)[a]	0.097 (0.446)
I/σ(I)	6.6(2.3)[a]	24.9(2.5)[a]	13.7 (2.97)
refinement			
R_{work} [c]/R_{free} [d]	0.1766/ 0.2187	0.1522/ 0.1816	0.1767/0.1956
No. atoms			
Protein	2768	1268	1268
$NADP^+$	80	48	48
folate	54	32	32
Mn^{2+}	-	-	1
water	345	119	87
B factors			
Protein	24	16	21
$NADP^+$	20	13	15
folate	23	16	24
water	46	34	34
Deviation from			
Bond length (Å)	0.013	0.006	0.005
Bond angle (°)	2.943	1.047	0.991
Ramachandran plot			
Most favorable	99.36	99.36	99.36
allowed	0.64	0.64	0.64
disallowed	0	0	0
PDB ID	7D6G		
single structure	-	7D3Z	7D4L
ensemble structure	-	7D49	7D4X

[a] Numbers in parentheses represent values in the highest resolution shell.

[b] $R_{sym} = \sum(|I_i - < I >|)/\sum(I)$, where Ii is the measured intensity and <I> is the mean intensity of all measured observations equivalent to reflection Ii.

[c] $R_{work} = \sum||F_{obs}| - |F_{calc}||/\sum|F_{obs}|$, where |Fobs| is the observed diffraction amplitude, $|F_{calc}|$ is the corresponding calculated structure factor amplitude.

[d] R_{free} is defined by R_{work}, but involved 5% of the measured reflections not used in refinement and set aside for cross-validation purpose.

Table S2: SCS-MP2 /def2-SVPD Electronic Energies and Energy Differences in Proton Affinity between Dihydrofolate and Folate

	E (Hartrees)-neutral	E(Hartrees)-protonated	ΔE (kcal/mol)	ΔΔE (kcal/mol)
Dihydrofolate	-618.945825	-619.336145	244.9	24.0
Folate	-617.775131	-618.127115	220.9	