Supplemental Information

Site-specific Tryptophan Labels Reveal Local Millisecond Motions of Dihydrofolate Reductase

Morgan B. Vaughn, Chloe Biren, Qun Li, Ashwin Ragupathi, R. Brian Dyer

Table S1. Relative activity of the midW mutants compared to wildtype DHFR

Enzyme	wildtype	midW22	midW30	midW47	midW74	midW133
Relative Activity	100%	90%	33%	20%	20%	22%

Isothermal Calorimetry

The dissociation constant and thermodynamic parameters of folate binding to the midW mutants at 25°C were determined using isothermal calorimetry (ITC) on a MicroCal Auto-iTC200 from Malvern Panalytical (United Kingdom). The samples contained 350 μL of 100 μM enzyme and were injected with 2.4 μL of 1 mM folate (buffer: 50 mM sodium phosphate, 100 mM NaCl, pH 7). The system was allowed to equilibrate for 150 seconds between injections. Representative ITC data are shown in **Figure S1**. The ITC data were analyzed using the MicroCal ITC-ORIGIN Analysis Software. The resulting K_d, ΔH, and ΔS of ligand binding (**Table S2)** were used to calculate the sum of free concentrations, which were used to the determine the concentration dependence of the temperature jump relaxation rates.

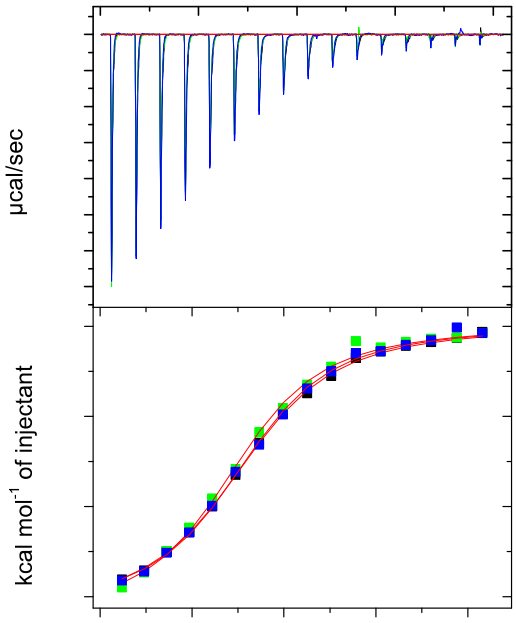

Figure S1 Raw ITC data for folate binding to midW74 in triplicate (top). Processed ITC data for folate binding to midW74 in triplicate (bottom). The curves represent fits using the OneSite model in the MicroCal ITC-ORIGIN Analysis Software.

Table S2 Dissociation and thermodynamic constants for the binding of folate to the five midW mutants.

midW mutant	K_d (μM)	ΔH (kcal/mol)	ΔS (cal/mol/K)
midW22	3.7	-7.78	-1.26
midW30	4.5	-5.04	7.55
midW47	12	-7.03	-1.16
midW74	8.4	-6.34	1.98
midW133	9.4	-5.70	3.85

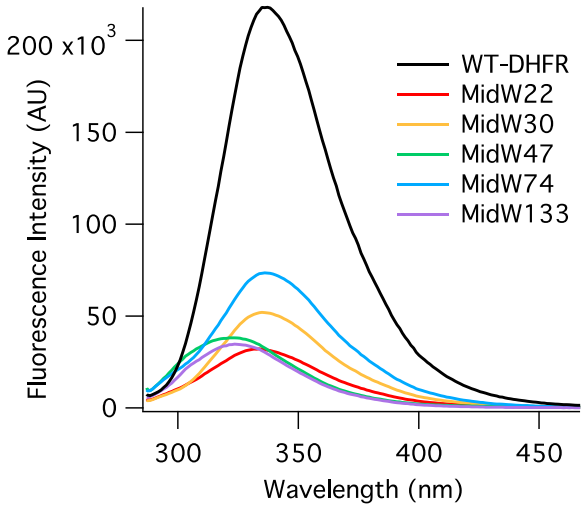

Figure S2 Equilibrium fluorescence of the apoenzyme wt-DHFR and all of the midW mutants. To adjust for the different integration times, (0.5 s for wt-DHFR and 1.5 s for the midW mutants) the wt-DHFR spectra has been scaled by a factor of three.

Intrinsic Temperature Dependence of Tryptophan Fluorescence

The temperature dependent equilibrium fluorescence intensity of the midW mutants is corrected for the intrinsic change in tryptophan fluorescence with increasing temperature. The fluorescence spectra of free tryptophan were collected from 12ºC to 45ºC in increments of 3ºC using the same method described for the midW mutants. The fluorescence peak was integrated between 327 nm and 353 nm and normalized to the lowest temperature. The normalized fluorescence intensity vs temperature is shown in **Figure S3**. As the temperature increases, the fluorescence intensity of tryptophan decreases. The normalized fluorescence intensities of free tryptophan are then subtracted from the normalized fluorescence intensities of the midW mutants to correct for the effects of the intrinsic temperature dependence of tryptophan fluorescence.

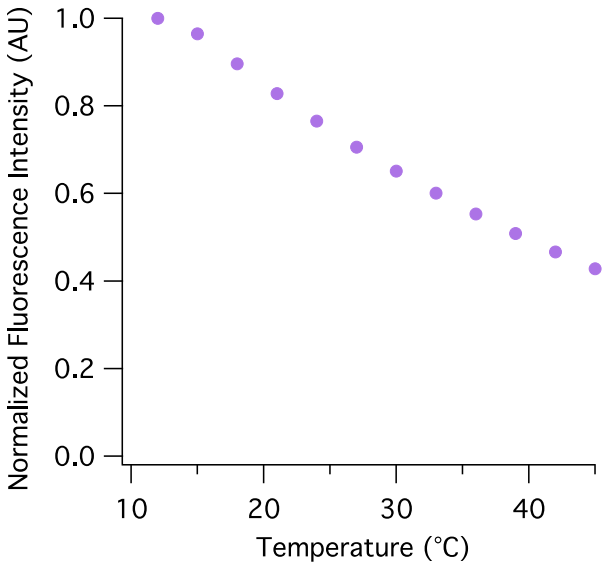

Figure S3 Normalized fluorescence intensity of free tryptophan versus temperature.

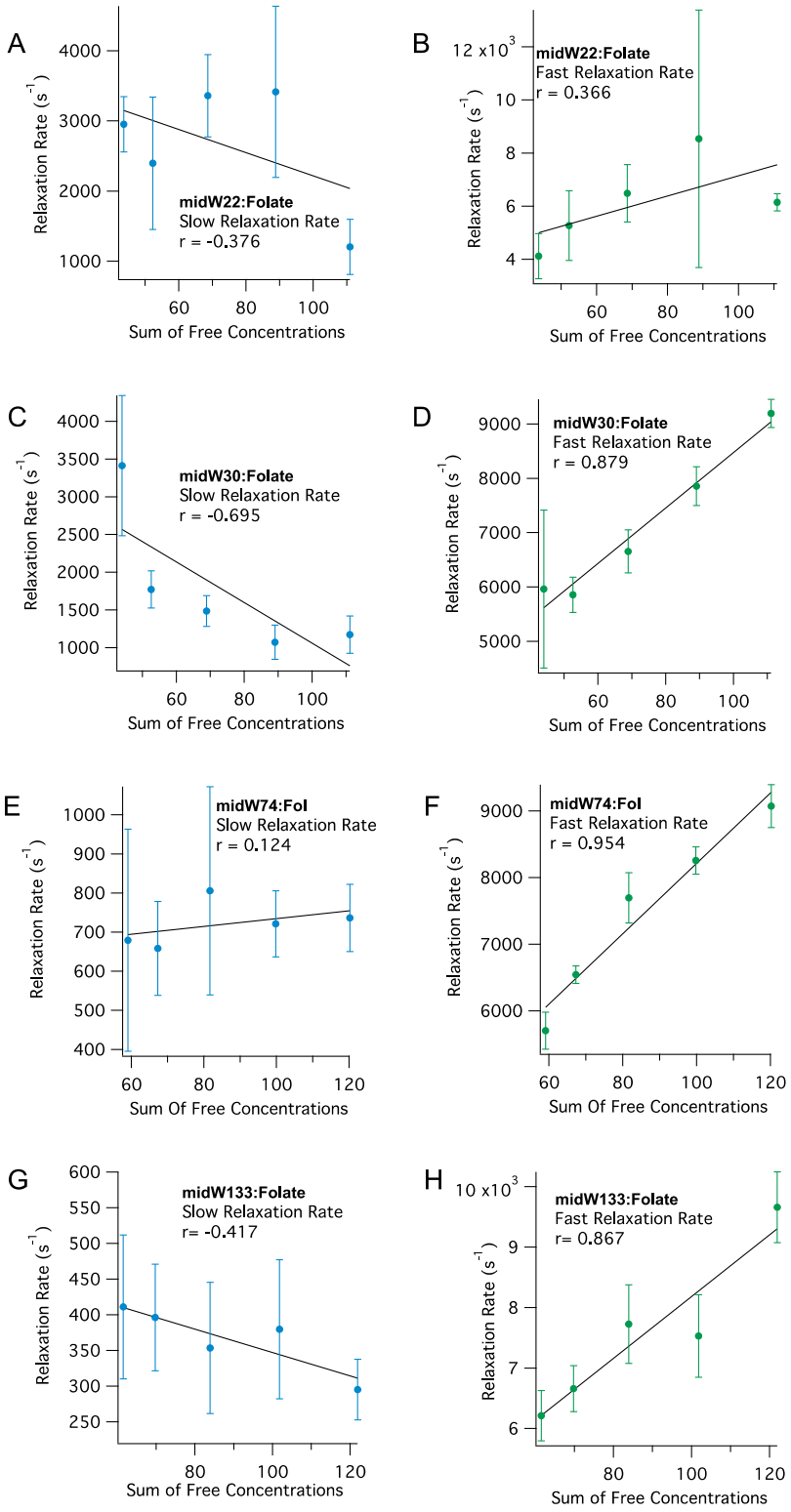

Figure S4 Concentration dependence of the slow (A, C, E, G) and fast (B, D, F, G) relaxation rates for midW22, midW30, midW74, and midW133, respectively.

Table S3 Average (n = 5) slow relaxation rates and amplitudes from double exponential fits of T-jump transients for each midW mutant sample

midW22	Slow Relaxation Rate (s-1)	Slow Amplitude
100 μM enzyme, 100 μM folate	2950 ± 390	2.79 ± 0.64
100 μM enzyme, 125 μM folate	2400 ± 950	5.5 ± 3.2
100 μM enzyme, 150 μM folate	3360 ± 590	9.3 ± 3.9
100 μM enzyme, 175 μM folate	3400 ± 1200	1.46 ± 0.86
100 μM enzyme, 200 μM folate	1200 ± 390	1.63 ± 0.21
midW30		
100 μM enzyme, 100 μM folate	3410 ± 930	4.5 ± 2.3
100 μM enzyme, 125 μM folate	1770 ± 250	2.31 ± 0.48
100 μM enzyme, 150 μM folate	1480 ± 200	2.28 ± 0.22
100 μM enzyme, 175 μM folate	1070 ± 230	2.77 ± 0.18
100 μM enzyme, 200 μM folate	1170 ± 250	2.354 ± 0.088
midW47		
100 μM enzyme, 100 μM folate	760 ± 130	7.00 ± 0.69
100 μM enzyme, 125 μM folate	767 ± 71	7.91 ± 0.38
100 μM enzyme, 150 μM folate	690 ± 130	8.56 ± 1.4
100 μM enzyme, 175 μM folate	746 ± 26	10.4 ± 1.0
100 μM enzyme, 200 μM folate	754 ± 46	10.0 ± 2.0
midW74		
100 μM enzyme, 100 μM folate	680 ± 280	2.15 ± 0.33
100 μM enzyme, 125 μM folate	660 ± 120	2.14 ± 0.25
100 μM enzyme, 150 μM folate	810 ± 270	2.73 ± 0.16
100 μM enzyme, 175 μM folate	721 ± 85	3.07 ± 0.17
100 μM enzyme, 200 μM folate	736 ± 86	3.65 ± 0.17
midW133		
100 μM enzyme, 100 μM folate	410 ± 100	1.00 ± 0.19
100 μM enzyme, 125 μM folate	396 ± 75	1.26 ± 0.26
100 μM enzyme, 150 μM folate	353 ± 92	1.52 ± 0.36
100 μM enzyme, 175 μM folate	380 ± 97	1.34 ± 0.37
100 μM enzyme, 200 μM folate	295 ± 42	1.87 ± 0.34

Table S4 Average (n = 5) fast relaxation rates and amplitudes from double exponential fits of T-jump transients for each midW mutant sample

midW22	Fast Relaxation Rate (s-1)	Fast Amplitude
100 µM enzyme, 100 µM folate	4120 ± 850	1.89 ± 0.41
100 µM enzyme, 125 µM folate	5300 ± 1200	5.4 ± 2.6
100 µM enzyme, 150 µM folate	6500 ± 1100	5.5 ± 2.3
100 µM enzyme, 175 µM folate	8500 ± 4800	1.4 ± 1.0
100 µM enzyme, 200 µM folate	6150 ± 320	6.62 ± 0.79
midW30		
100 µM enzyme, 100 µM folate	6000 ± 1500	4.7 ± 2.5
100 µM enzyme, 125 µM folate	5860 ± 330	9.81 ± 0.79
100 µM enzyme, 150 µM folate	6660 ± 400	9.75 ± 0.69
100 µM enzyme, 175 µM folate	7860 ± 360	9.7 ± 1.0
100 µM enzyme, 200 µM folate	9190 ± 260	8.2 ± 1.2
midW47		
100 µM enzyme, 100 µM folate	3430 ± 280	8.15 ± 0.64
100 µM enzyme, 125 µM folate	3820 ± 170	7.96 ± 1.1
100 µM enzyme, 150 µM folate	3850 ± 160	8.3 ± 1.9
100 µM enzyme, 175 µM folate	4630 ± 190	7.9 ± 0.7
100 µM enzyme, 200 µM folate	4640 ± 190	8.1 ± 1.5
midW74		
100 µM enzyme, 100 µM folate	5700 ± 280	10.77 ± 0.90
100 µM enzyme, 125 µM folate	6540 ± 130	10.37 ± 0.68
100 µM enzyme, 150 µM folate	7700 ± 380	10.83 ± 0.99
100 µM enzyme, 175 µM folate	8260 ± 200	10.73 ± 0.79
100 µM enzyme, 200 µM folate	9070 ± 320	10.83 ± 0.48
midW133		
100 µM enzyme, 100 µM folate	6220 ± 420	3.17 ± 0.26
100 µM enzyme, 125 µM folate	6660 ± 380	3.80 ± 0.18
100 µM enzyme, 150 µM folate	7730 ± 650	3.11 ± 0.14
100 µM enzyme, 175 µM folate	7530 ± 680	2.72 ± 0.22
100 µM enzyme, 200 µM folate	9660 ± 580	2.96 ± 0.19