

Supplementary Information

Protein Mass-modulated Effects in the Catalytic Mechanism of Dihydrofolate

Reductase: Beyond Promoting Vibrations

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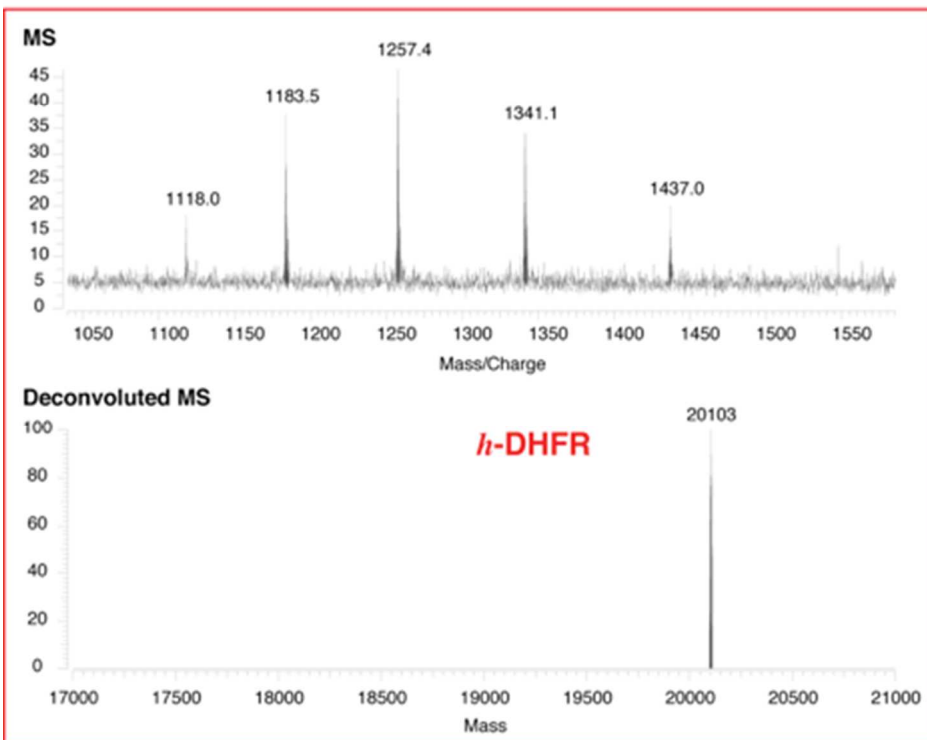
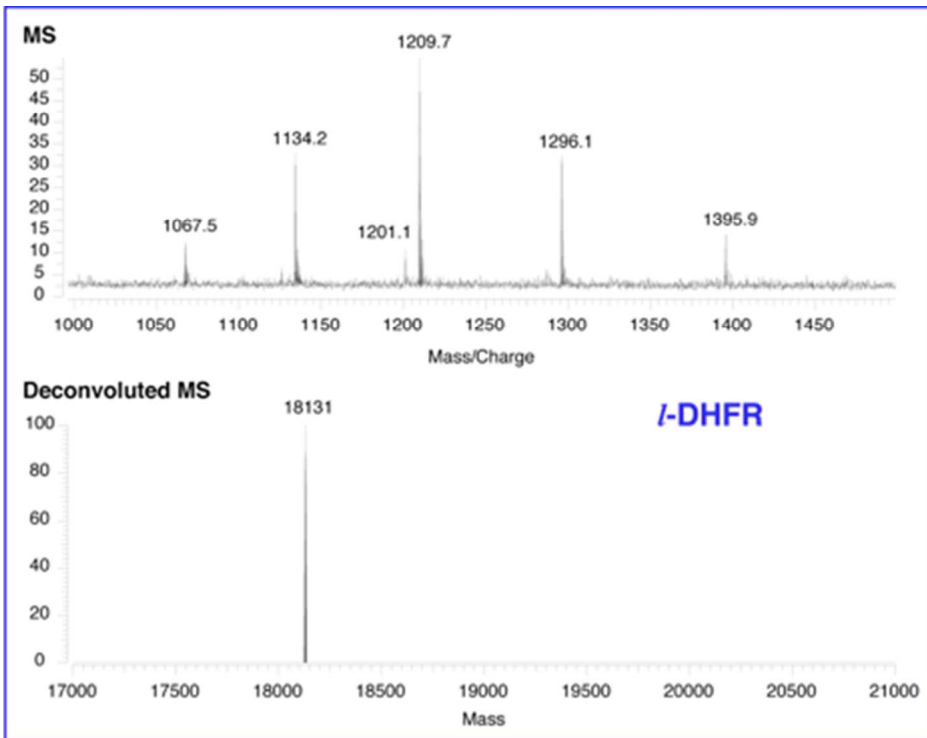


Figure S1. The raw (x-axis: Mass/Charge) and deconvoluted (x-axis: Mass) mass spectra of light ecDHFR (*l*-DHFR) and heavy ecDHFR (*h*-DHFR). The *h*-DHFR is 11% heavier than *l*-DHFR.

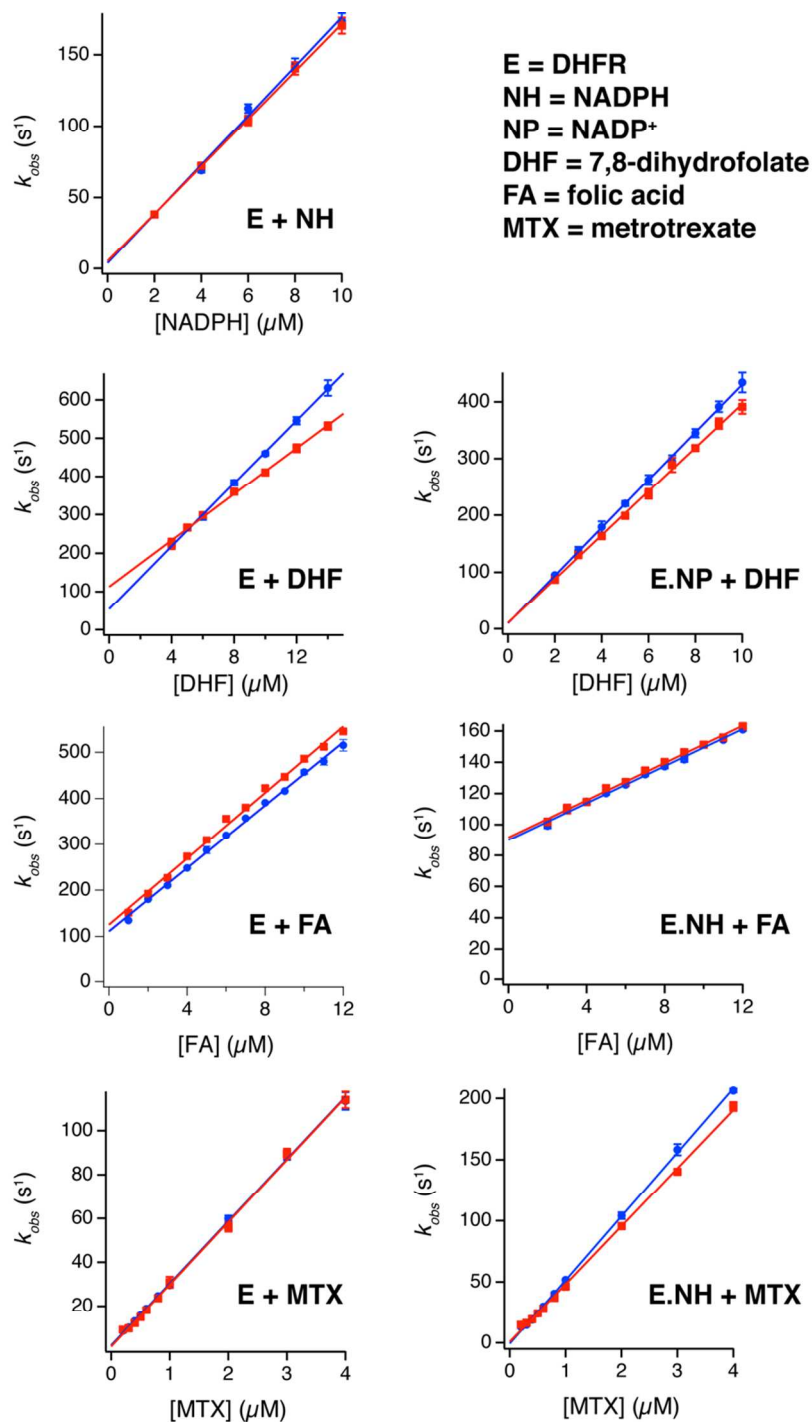


Figure S2. The observed rate constants (k_{obs}) of the ligand-dependent exponential phase in formation of binary and ternary complexes of *l*-DHFR (blue) and *h*-DHFR (red) with various ligands (L), measured at 25 °C, pH 7. The association and dissociation rate constants (k_{on} and k_{off} , respectively) of each ligand can be solved from: $k_{obs} = k_{on} \cdot [L] + k_{off}$.¹ The values of k_{on} and k_{off} are summarized in Table 4 in the main text.

Table S1. The observed H/T and D/T KIEs on k_{cat}/K_M^{NADPH} ($^T(V/K)$ and $^T(V/K)_D$, respectively), intrinsic H/T and H/D KIEs on the hydride transfer ($^T k_{hyd}$ and $^D k_{hyd}$, respectively), and forward commitment factor (C_f) of *l*-DHFR (numbers in blue) and *h*-DHFR (numbers in red) determined by the competitive KIE experiments at pH 9 (Figure 4 in main text).

Temperature (°C)	$^T(V/K)$	$^T(V/K)_D$	$^T k_{hyd}$	$^D k_{hyd}$	C_f
5	3.1 ± 0.1	1.51 ± 0.02	6.2 ± 0.4	3.5 ± 0.2	1.5 ± 0.2
	2.40 ± 0.05	1.50 ± 0.02	11.7 ± 0.5	5.6 ± 0.2	6.6 ± 0.4
10	3.69 ± 0.03	1.57 ± 0.03	5.9 ± 0.5	3.7 ± 0.2	0.8 ± 0.2
15	4.76 ± 0.04	1.63 ± 0.01	5.9 ± 0.3	3.5 ± 0.1	0.30 ± 0.08
	3.29 ± 0.04	1.61 ± 0.05	9.2 ± 0.7	4.7 ± 0.3	2.6 ± 0.3
25	4.85 ± 0.09	1.66 ± 0.03	6.1 ± 0.4	3.6 ± 0.2	0.3 ± 0.1
	4.10 ± 0.03	1.64 ± 0.04	6.9 ± 0.1	3.88 ± 0.05	0.91 ± 0.03
35	4.8 ± 0.1	1.66 ± 0.01	6.3 ± 0.4	3.6 ± 0.2	0.4 ± 0.1
	4.91 ± 0.04	1.69 ± 0.02	6.9 ± 0.2	3.9 ± 0.1	0.52 ± 0.04
45	4.8 ± 0.1	1.65 ± 0.02	6.1 ± 0.4	3.6 ± 0.2	0.3 ± 0.1
	5.05 ± 0.06	1.70 ± 0.07	6.9 ± 0.4	3.88 ± 0.03	0.5 ± 0.1

(1) Fierke, C. A.; Johnson, K. A.; Benkovic, S. J. *Biochemistry* **1987**, *26*, 4085-4092.