

Supporting Information for Publication

Extension and limits of the network of coupled motions correlated to hydride transfer in dihydrofolate reductase

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Figures

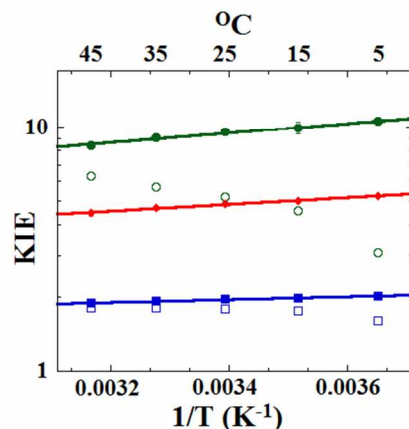


Figure S1. Arrhenius plot of observed (open structures) and intrinsic (closed structures) primary KIEs for F125M-ecDHFR. H/T KIEs are in green, H/D KIEs are in red and D/T KIEs are in blue. The solid lines represent a fit of the intrinsic KIEs to Arrhenius Equation.

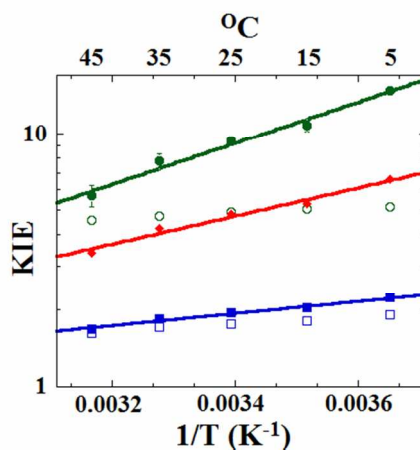


Figure S2. Arrhenius plot of observed (open structures) and intrinsic (closed structures) primary KIEs for M42W-F125M-ecDHFR. H/T KIEs are in green, H/D KIEs are in red and D/T KIEs are in blue. The solid lines represent a fit of the intrinsic KIEs to Arrhenius Equation.

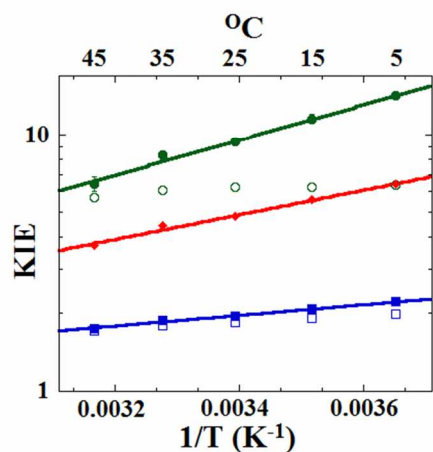


Figure S3. Arrhenius plot of observed (open structures) and intrinsic (closed structures) primary KIEs for G121V-F125M-*ecDHFR*. H/T KIEs are in green, H/D KIEs are in red and D/T KIEs are in blue. The solid lines represent a fit of the intrinsic KIEs to Arrhenius Equation.

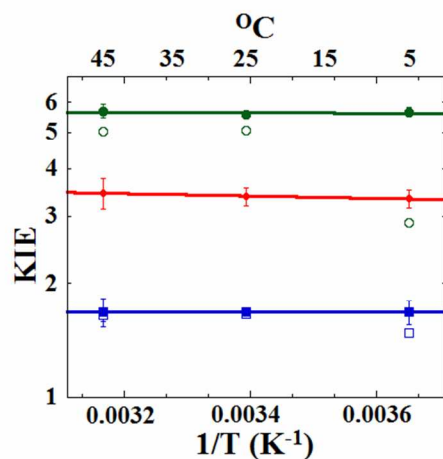


Figure S4. Arrhenius plot of observed (open structures) and intrinsic (closed structures) primary KIEs for W133F-*ecDHFR*. H/T KIEs are in green, H/D KIEs are in red and D/T KIEs are in blue. The solid lines represent a fit of the intrinsic KIEs to Arrhenius Equation.

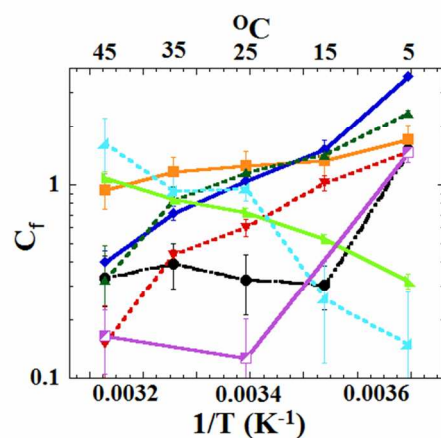


Figure S5. Comparison of the Arrhenius plots of the commitment to catalysis (C_f – Eq 4) on k_{cat}/K_M for H-transfer for the WT (black) and distal DHFR mutants: W133F (magenta), M42W (orange), G121V (light green), F125M (blue), M42W-F125M (green), G121V-F125M (red), M42W-G121V (light blue). Dashed line is used for WT, solid lines for single mutants and dotted lines for the double mutants, which accords with Figure 1 in the main text.

Tables S1 – S4 summarize the observed and intrinsic primary H/T and D/T isotope effects and their standard deviations over a temperature range from 5 °C to 45 °C, measured for mutants of *ecDHFR* as described in ref.¹ The intrinsic KIEs were calculated from the observed KIEs and the errors were propagated as described in ref.¹⁻⁵

Table S1: Observed and intrinsic *V/K* KIEs for F125M

Temp. °C	Observed H/T KIE	Observed D/T KIE	Intrinsic H/T KIE	Intrinsic H/D KIE	Intrinsic D/T KIE
5	3.07 ± 0.02	1.61 ± 0.01	10.60 ± 0.28	5.23 ± 0.07	2.03 ± 0.05
15	4.55 ± 0.04	1.76 ± 0.01	9.97 ± 0.55	5.01 ± 0.03	1.99 ± 0.02
25	5.20 ± 0.09	1.80 ± 0.01	9.59 ± 0.30	4.87 ± 0.02	1.97 ± 0.02
35	5.71 ± 0.02	1.81 ± 0.02	9.06 ± 0.28	4.68 ± 0.09	1.93 ± 0.01
45	6.28 ± 0.03	1.82 ± 0.01	8.41 ± 0.28	4.44 ± 0.08	1.89 ± 0.02

Table S2: Observed and intrinsic *V/K* KIEs for M42W-F125M

Temp. °C	Observed H/T KIE	Observed D/T KIE	Intrinsic H/T KIE	Intrinsic H/D KIE	Intrinsic D/T KIE
5	5.12 ± 0.01	1.91 ± 0.02	14.77 ± 0.34	6.60 ± 0.10	2.24 ± 0.02
15	5.06 ± 0.05	1.82 ± 0.02	10.81 ± 0.65	5.30 ± 0.04	2.04 ± 0.03
25	4.90 ± 0.02	1.77 ± 0.01	9.39 ± 0.22	4.80 ± 0.06	1.96 ± 0.01
35	4.72 ± 0.03	1.71 ± 0.01	7.83 ± 0.51	4.23 ± 0.02	1.85 ± 0.02
45	4.54 ± 0.03	1.62 ± 0.03	5.68 ± 0.57	3.38 ± 0.02	1.68 ± 0.06

Table S3: Observed and intrinsic V/K KIEs for G121V-F125M

Temp. °C	Observed	Observed	Intrinsic	Intrinsic	Intrinsic
	H/T KIE	D/T KIE	H/T KIE	H/D KIE	D/T KIE
5	6.33 ± 0.03	1.99 ± 0.01	14.26 ± 0.35	6.43 ± 0.03	2.22 ± 0.01
15	6.23 ± 0.04	1.92 ± 0.01	11.57 ± 0.49	5.58 ± 0.03	2.08 ± 0.02
25	6.22 ± 0.04	1.85 ± 0.01	9.36 ± 0.30	4.79 ± 0.03	1.95 ± 0.01
35	6.08 ± 0.04	1.80 ± 0.003	8.29 ± 0.13	4.41 ± 0.01	1.88 ± 0.01
45	5.71 ± 0.05	1.72 ± 0.03	6.42 ± 0.40	3.69 ± 0.02	1.75 ± 0.01

Table S4: Observed and intrinsic V/K KIEs for W133F

Temp. °C	Observed	Observed	Intrinsic	Intrinsic	Intrinsic
	H/T KIE	D/T KIE	H/T KIE	H/D KIE	D/T KIE
5	2.89 ± 0.05	1.48 ± 0.03	5.64 ± 0.17	3.34 ± 0.18	1.68 ± 0.13
25	5.04 ± 0.03	1.65 ± 0.02	5.56 ± 0.10	3.38 ± 0.18	1.68 ± 0.04
45	5.02 ± 0.07	1.65 ± 0.07	5.68 ± 0.23	3.46 ± 0.32	1.68 ± 0.14

References

- (1) Wang, L.; Goodey, N. M.; Benkovic, S. J.; Kohen, A. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 15753-15758.
- (2) Stojković, V.; Perissinotti, L. L.; Willmer, D.; Benkovic, S. J.; Kohen, A. *J. Am. Chem. Soc.* **2012**, *134*, 1738-1745.
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