

**Coupling of Protein Motions and Hydrogen Transfer during Catalysis by
Escherichia coli Dihydrofolate Reductase**

Richard S. Swanwick,* Giovanni Maglia,[†] Lai-hock Tey* & Rudolf K. Allemann*

* School of Chemistry

Cardiff University

Main Building

Park Place

Cardiff, CF10 3AT, UK

and

[†] School of Chemistry

University of Birmingham

Edgbaston

Birmingham, B15 2TT, UK

SUPPLEMENTARY MATERIAL

Table 1 Temperature dependence of the pre-steady state kinetic parameters at pH 7.0 for the reaction of NADPH and H₂F catalysed by DHFR from *E. coli*. All data points are the average of at least 6 measurements. The errors are standard errors of the mean (for one sigma).

T (°C)	k_H (s ⁻¹)	k_D (s ⁻¹)	KIE
5	81.9 ± 1.7	26.9 ± 1.9	3.0 ± 0.2
10	106.3 ± 3.1	34.1 ± 3.4	3.1 ± 0.3
15	135.3 ± 5.6	44.6 ± 3.7	3.0 ± 0.3
20	159.8 ± 7.9	60.2 ± 4.1	2.7 ± 0.2
25	203.7 ± 7.4	75.1 ± 5.4	2.7 ± 0.2
30	235.4 ± 13.8	98.5 ± 2.8	2.4 ± 0.2
35	287.9 ± 12.0	125.9 ± 3.5	2.3 ± 0.1
40	362.7 ± 7.5	166.9 ± 6.4	2.2 ± 0.1

Table 2 Temperature dependence of the steady state rates and the KIEs for the reaction between NADPH/NADPD and H₂F catalyzed by DHFR at pH 9.5. All data points are the average of at least 6 measurements. The errors are standard errors of the mean (for one sigma).

T (°C)	Rate (NADPH) (s ⁻¹)	Rate (NADPD) (s ⁻¹)	KIE
5	0.349 ± 0.005	0.175 ± 0.003	1.995 ± 0.045
10	0.484 ± 0.004	0.197 ± 0.002	2.451 ± 0.032
15	0.600 ± 0.008	0.208 ± 0.001	2.885 ± 0.041
20	0.722 ± 0.009	0.252 ± 0.004	2.865 ± 0.058
25	0.927 ± 0.012	0.341 ± 0.008	2.718 ± 0.073
30	0.994 ± 0.005	0.359 ± 0.006	2.769 ± 0.048
35	1.090 ± 0.007	0.385 ± 0.006	2.831 ± 0.048

Table 3 Temperature dependence of the pre-steady state kinetic parameters at pH 7.0 for the reaction of NADPH and H₂F catalysed by G121V-DHFR. All data points are the average of at least 6 measurements. The errors are standard errors of the mean (for one sigma).

T (°C)	k_H (s ⁻¹)	k_D (s ⁻¹)	KIE
5	0.086 ± 0.001	0.038 ± 0.002	2.263 ± 0.022
10	0.104 ± 0.001	0.051 ± 0.001	2.040 ± 0.045
15	0.122 ± 0.003	0.071 ± 0.004	1.723 ± 0.016
20	0.147 ± 0.002	0.092 ± 0.001	1.598 ± 0.028
25	0.171 ± 0.004	0.118 ± 0.002	1.450 ± 0.042
30	0.196 ± 0.006	0.150 ± 0.003	1.307 ± 0.047
35	0.235 ± 0.008	0.201 ± 0.005	1.170 ± 0.049