Electronic Supplementary Material (ESI) for Faraday Discussions.

Supporting Information:

What are the signatures of tunnelling in enzyme-catalysed reactions?

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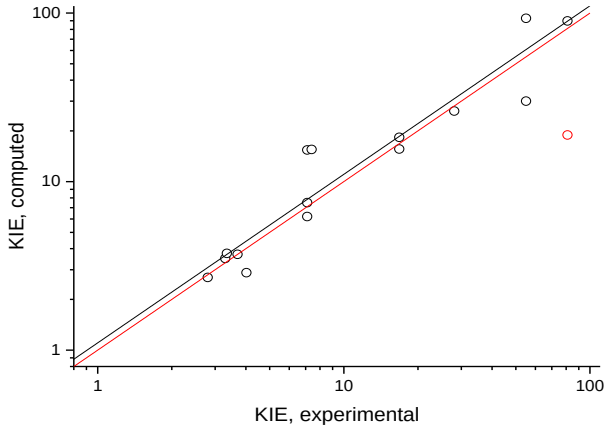

Figure S1. Comparison of computed and experimental KIE values taken from Table 1. The solid black line is a linear fit to these data, which has a slope of 0.83 ± 0.13 (fixed *y* intercept) and R^2 = 0.83. The red point is considered to be an outlier as its inclusion reduces R^2 to 0.52. The solid red line is KIE, computed = KIE, experimental (*i.e*. *y* = *x*).

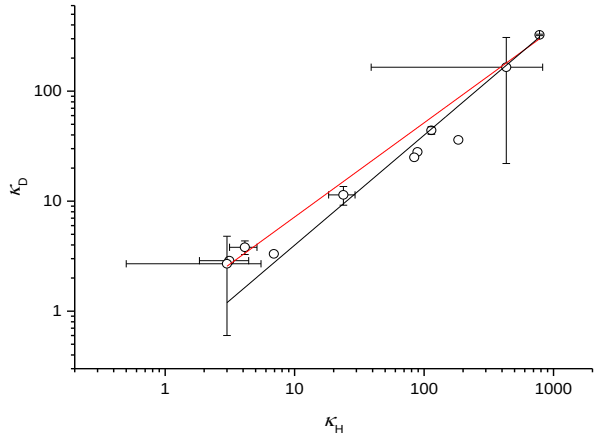

Figure S2. Computed κ_H *vs*. κ_D values taken from Table 1. These data are fitted to both a linear (black line) and power (red line) function.

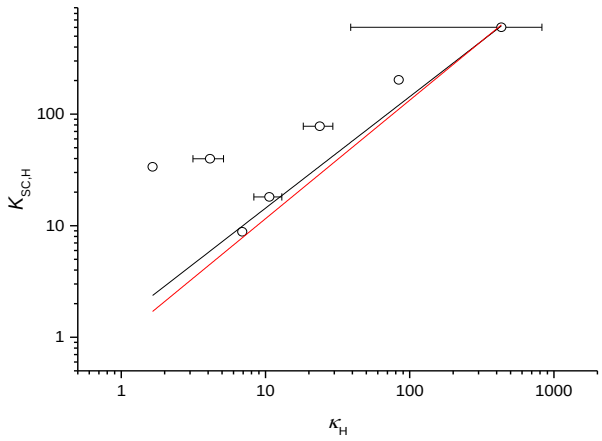

Figure S3. Computed K_{SC} and κ_H values taken from **Table 1**. These data are fitted to both a linear (black line; $y = (1.4\pm0.1)x$) and power (red line; $y = x^{(1.06\pm0.01)}$) function.

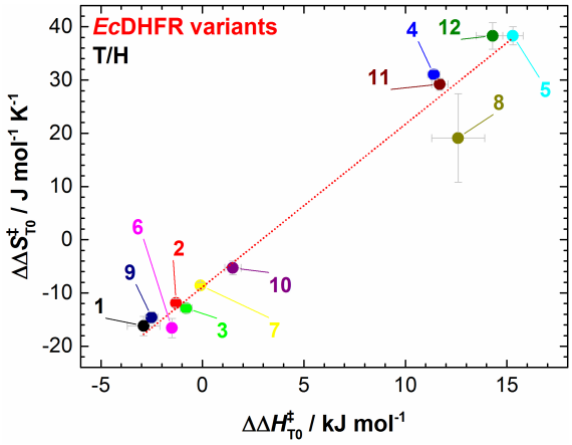

Figure S4. Additional data to accompany **Figure 3**. The H/T data for *Ec*DHFR taken from **Table S6**.

Table S1. Thermodynamic parameters for variants from OYE family presented in **Figure 3**.

OYE variant	Label	$\Delta\Delta H_{T0}^{\ddagger}$ / kJ mol^{-1}	SD	$\Delta\Delta S_{T0}^{\ddagger}$ / J mol^{-1} K^{-1}	SD	Conditions/substrates/etc.	Reference
PETNR							
WT	1	6.4	0.4	5.8	1.5	5-40 °C, NADPH	1
L25A	2	8.6	1.3	13.3	5.6		
L25I	3	6.3	0.7	5.4	2.5		
I107A	4	1.2	2.3	-12.3	7.7		
I107L	5	4.2	0.8	-1.3	2.8		
WT	6	4.9	0.2	0.1	0.7	5-40 °C, NADH	
L25A	7	6.6	1.2	6.4	4.2		
L25I	8	2.3	1.8	-9.2	6.0		
I107A	9	7.1	2.0	6.8	6.7		
I107L	10	6.7	0.4	6.1	1.2		
WT	11	12.2	1.2	23.9	4.0	5-40 °C, mNH_2	2
WT	12	11.1	1.3	20.4	4.5	5-40 °C, mBu	
TOYE							
WT	13	3.3	2.8	-6.0	9.6	5-40 °C, NADPH	2
	14	3.4	0.5	-5.4	1.6	5-40 °C, NADH	
	15	4.7	1.9	-0.9	6.8	5-40 °C, mNH2	
	16	13.8	2.2	28.4	7.4	5-40 °C, mBu	
XenA							
WT	17	6.0	2.5	-7.7	8.8	5-40 °C, NADPH	2
	18	4.8	3.3	3.2	11.3	5-40 °C, NADH	
	19	12.1	2.7	22.8	9.2	5-40 °C, mNH2	
	20	17.9	1.4	42.7	4.8	5-40 °C, mBu	
MR							
WT	21	8.3	0.8	11.7	2.8	5-40 °C, NADH	3

Table S2. Thermodynamic parameters for SLO-1 variants presented in **Figure 3**.

SLO-1 variant	Label	$\Delta\Delta H_{T0}^{\ddagger}$ / kJ mol^{-1}	SD	$\Delta\Delta S_{T0}^{\ddagger}$ / J mol^{-1} K^{-1}	SD	Conditions/substrates/etc.	Reference
WT	1	1.3	0.8	-24.0	-2.3	5-50 °C, LA	4
L546A	2	5.5	2.5	-11.5	-8.3		
L754A	3	5.9	2.1	-9.1	-8.3		
I553A	4	14.3	1.3	17.6	-4.2		
I553V	5	8.4	2.1	10.0	-5.5	10-50 °C, LA	5
I553L	6	11.7	2.5	10.0	-11.1		
I553G	7	19.7	2.9	30.0	-10.5		
L546A/I553A	8	9.2	1.7	-0.4	-3.6	15-50 °C, LA	6

Table S3. Thermodynamic parameters for AADH variants presented in **Figure 3**.

AADH variant	Label	$\Delta\Delta H_{T0}^{\ddagger}$ / kJ mol^{-1}	SD	$\Delta\Delta S_{T0}^{\ddagger}$ / J mol^{-1} K^{-1}	SD	Conditions/substrates/etc.	Reference
WT	1	-3.8	3.6	-48.3	-0.1	tryptamine	7
	2	0.7	1.7	-24.7	-0.2	PEA-H	8
	3	6.3	1.7	1.3	-0.5	PEA-OH	
	4	11.2	1.6	13.0	-0.4	PEA-CH3	
	5	11.9	1.9	17.0	-0.6	PEA-OCH3	
	6	5.5	3.1	-6.2	-0.5	PEA-NO2	
	7	7.0	2.0	0.3	-0.3	PEA-F	
	8	10.8	1.5	11.2	-0.3	PEA-Cl	
	9	9.5	1.8	7.0	-0.2	PEA-Br	

Table S4. Thermodynamic parameters for *Bs*DHFR variants presented in **Figure 3**.

*Bs*DHFR variant	Label	$\Delta\Delta H_{T0}^{\ddagger}$ / kJ mol^{-1}	SD	$\Delta\Delta S_{T0}^{\ddagger}$ / J mol^{-1} K^{-1}	SD	Conditions/substrates/etc.	Reference
WT	1	0.8	1.0	1.2	-0.2	5-35(55) °C	9
L20M	2	-0.2	1.8	-0.8	-0.4		
A104Q	3	3.3	1.0	14.3	-0.5		
L20M/A104Q	4	1.4	1.0	8.0	0.0		
P122E	5	0.8	1.9	5.4	-0.3		
P129D	6	1.6	2.0	8.0	-0.4		
P122E/P129D	7	2.4	1.0	11.2	-0.3		

Table S5. Thermodynamic parameters for *Tm*DHFR variants presented in **Figure 3**.

***Tm*DHFR variant**	**Label**	**$\Delta\Delta H_{T0}^{\ddagger}$ / kJ mol^{-1}**	**SD**	**$\Delta\Delta S_{T0}^{\ddagger}$ / J mol^{-1} K^{-1}**	**SD**	**Conditions/substrates/etc.**	**Reference**
WT	1	16.8	4.1	51.7	-4.2	6-25 °C	10
	2	0.0	0.9	-3.7	-2.5	25-65 °C	
	3	0.0	1.0	-3.7	-2.5	17% glycerol, 25-50 °C	11
	4	-0.4	1.7	-1.7	-5.6	33% glycerol, 25-50 °C	
	5	0.2	1.5	2.2	-4.9	50% glycerol, 25-50 °C	
	6	-0.6	1.7	-4.3	-5.5	17% sucrose, 25-50 °C	
	7	-1.3	1.6	-5.8	-5.4	30% sucrose, 25-50 °C	
	8	18.9	4.0	63.1	-0.6	17% glycerol, 6-25 °C	
	9	27.1	5.8	93.7	-1.0	30% glycerol, 6-25 °C	
	10	37.9	5.0	133.9	-0.7	50% glycerol, 6-25 °C	
K129E	11	0.3	0.7	-0.8	-2.3	5-65 °C	12
E136K	12	-2.2	0.4	-8.6	-1.2		
E138K	13	-1.4	0.4	-5.8	-1.2		
E136K/E138K	14	-2.6	0.6	-1.5	-1.4		
WT	15	12.4	7.2	57.4	-8.3	0.25% CHAPS, 5-25 °C	13
	16	1.7	2.5	2.1	-0.4	0.25% CHAPS, 25-55 °C	
V126E	17	21.3	5.8	72.6	-1.0	5-25 °C	
	18	-7.8	3.6	-26.0	-0.8	25-55 °C	
V11D	19	-1.1	3.3	-5.3	-0.6	5-40 °	
	20	6.0	2.2	23.4	-0.4	0.25% CHAPS, 5-50 °C	
WT	21	7.9	1.2	22.1	-2.4	folate, 6-25 °C	14
	22	-1.5	1.4	-9.0	-2.0	folate, 25-60 °C	

Table S6. Thermodynamic parameters for *Ec*DHFR variants presented in **Figure 3** and **Figure S4**.

*Ec*DHFR variant	Label	$\Delta\Delta H_{T0}^{\ddagger}$ / kJ mol^{-1}	SD	$\Delta\Delta S_{T0}^{\ddagger}$ / J mol^{-1} K^{-1}	SD	Conditions/substrates/etc.	Reference
D/H							
WT	1	5.3	0.8	18.5	-3.0	pH 7	15
G121V	2	13.2	0.7	49.1	-2.2		
WT	3	7.0	0.7	23.8	-1.0	17% methanol, pH 7	16
	4	8.6	2.1	29.4	-3.4	33% methanol, pH 7	
	5	4.5	6.8	15.6	-6.9	50% methanol, pH 7	
	6	7.9	4.6	27.2	-0.7	17% glycerol, pH 7	
	7	7.3	3.6	24.9	-0.5	33% glycerol, pH 7	
	8	4.4	3.8	15.4	-0.6	50% glycerol, pH 7	
	9	0.0	0.9	-1.2	-0.1	17% sucrose, pH 7	
	10	-1.3	3.2	-6.2	-0.7	30% sucrose, pH 7	
	11	5.4	0.9	18.4	-1.5	pH 9	17
N23P/S248A	12	5.2	1.5	18.4	-3.0	pH 7	
	13	-2.1	1.7	-7.3	-0.2	pH 9	
S148P	14	-0.5	7.4	5.8	-1.7	pH 7	18
WT	15	-2.4	0.2	-8.6	-1.9	pH7	19
	16	-2.5	1.0	-10.7	-2.0	pH9	
Y100F	17	-1.5	0.1	-10.1	-0.3	pH 7	
D27S	18	0.7	0.2	-2.9	-0.5	pH 7	
D27S/Y100F	19	6.9	0.4	19.1	-0.8	pH 7	
T/H							
WT	1	-2.9	0.8	-16.2	-1.8	tritium/hydrogen	20
I14V	2	-1.3	0.2	-11.9	-0.8		21
I14A	3	-0.8	0.3	-12.9	-0.9		
I14G	4	11.4	0.3	31.0	-1.0		
I14A/G121V	5	15.3	0.5	38.3	-1.7		22
G121V	6	-1.5	0.1	-16.6	-1.8		23
M42W	7	-0.1	0.2	-8.6	-0.6		
G121V/M42W	8	12.6	1.3	19.1	-8.3		24
W133F	9	-2.5	0.3	-14.6	-1.1		25
F125M	10	1.5	0.4	-5.3	-1.3		
G121V/F125M	11	11.7	0.4	29.2	-1.1		
M42W/F125M	12	14.3	0.8	38.3	-2.5		

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