Supplementary Information

Title: Examination of Enzymatic H-Tunneling through Kinetics and Dynamics.

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Running title: Enzyme Kinetics and Dynamics

Footnotes:

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Table S1. HPLC method for separation of NAD^+ and NADH

Time (min)	%A	%B	%C	Flow Rate (ml/min)	Max. Pressure (bar)
0	100	0	0	0.8	400
4.9	100	0	0	0.8	400
5	99	1	0	0.8	400
15	90	10	0	0.8	400
24.9	90	10	0	0.8	400
25	0	0	100	0.8	400
35	0	0	100	0.8	400

Note: Here Buffer A is 0.1 M phosphate at pH 6.0. Buffer B is a 60:40 mixture of A and methanol, and C is methanol.

Table S2. Observed V/K KIEs (with the standard deviation).

Temperature ˚C	Observed H/T KIE	Observed D/T KIE
5	3.69±0.09	1.57±0.02
15	3.82±0.05	1.57±0.05
25	3.80±0.09	1.58±0.03
35	3.78±0.05	1.57±0.03
45	3.79±0.13	1.57±0.02

The table contains observed H/T and D/T KIEs obtained from the competitive experiments as describe in the text.

Table S3. Intrinsic KIEs presented in Figure 4 in the main text.

Temperature ˚ C	Intrinsic KIE (H/T)	Intrinsic KIE (H/D)	Intrinsic KIE (D/T)
5	5.89±0.79	3.14±0.31	1.70±0.07
15	5.80±0.70	3.38±0.28	1.69±0.06
25	5.93±0.73	3.43±0.30	1.70±0.06
35	5.88±0.75	3.41±0.30	1.70±0.06
45	5.88±0.62	3.41±0.25	1.70±0.05

The table presents the intrinsic H/T, H/D and D/T KIEs, calculated using the Northrop's method as described in the text. Reported errors are from all combinations of H/T and D/T observed KIEs for each temperature point. Errors were calculated at the 95% confidence interval.