Supplementary Information

Hydrogen Donor-Acceptor Fluctuations from Kinetic Isotope Effects: A Phenomenological Model

Daniel Roston, Christopher M. Cheatum, and Amnon Kohen*

Department of Chemistry, University of Iowa, Iowa City, IA 52242

Additional discussion of the limits of a single population

As mentioned in the main text, models with a single population along the DAD coordinate could not fit the data from the systems with the most steeply temperature-dependent KIEs. As an example of the failure of a single population, we show the resultant fits to the KIEs from I14G DHFR (ΔE_a=3.3 kcal/mol) using both the present model and a previous model for nonadiabatic tunneling (Figure S1).(*1*) At 298 K, the best fit using the present model has a ΔE_a=1.2 kcal/mol and the fit with the previous model has a ΔE_a=1.5 kcal/mol, which appear to be the upper limits for the temperature dependence that can be modeled with a single population. Note that these limits refer only to H/T KIEs: the limits for H/D KIEs will be different. Furthermore, the precise limit for ΔE_a will depend on the actual size of the KIEs in question. We have not provided an exhaustive list of the limits of various models of this type, but given that tunneling probability as a function of DAD is qualitatively very similar among these models,(*1-4*) we do not expect any of them to fit KIEs like those from I14G DHFR or the systems found to have even steeper temperature dependence.

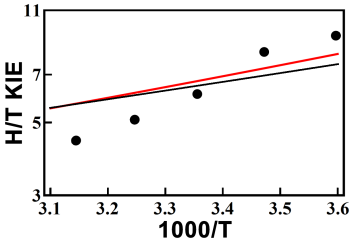


Figure S1: Best fits to the KIEs from I14G DHFR using a single population along the DAD coordinate in the model described in the main text (black) and a model for nonadiabatic H-transfer (red). Figure 8 in the main text presents the good fitting of the new model to the same set of data.

Additional discussion of the overall behavior of the model

The overall behavior of the two models, that is, the KIE and its temperature dependence as a function of the two fitting parameters, is not, in general, simple to describe. Figures S2 and S3 show contour plots that demonstrate the complex behavior of the one- and two-population models, respectively, within relevant ranges of the fitting parameters. While trends exist within certain sectors of these figures, we hesitate to make any broad statements about the effects of any given fitting parameter.

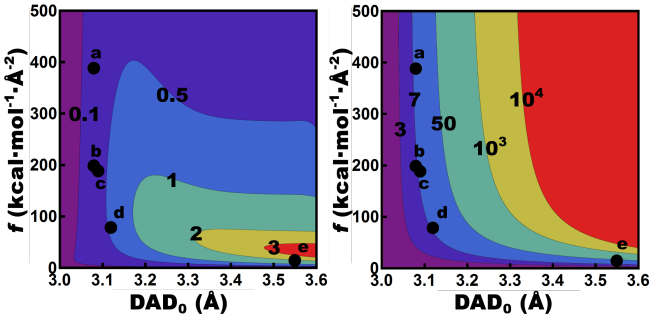


Figure S2: Contour plots of ΔE_a in kcal/mol (left) and H/T KIE (right) as a function of average DAD (DAD_0) and force constant (f), using the one population model for systems with little or no temperature dependence in their KIEs. The black dots represent the places where some of the systems re-analyzed in the present study fall on these surfaces and are labeled as follows: a, G121V DHFR; b, I14V DHFR; c, I14A DHFR; d, M42W DHFR; e, [1,5] sigmatropic rearrangement of pentadiene.

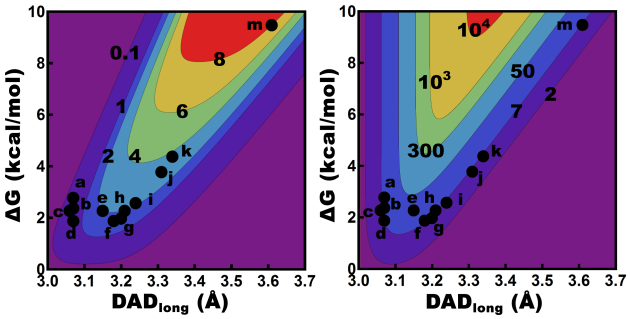


Figure S3: Contour plots of ΔE_a in kcal/mol (left) and H/T KIE (right) for the enzymes with steeply temperature dependent KIEs using two distinct populations along the DAD coordinate. This model is a function of the DAD of the long population (DAD_L) and the difference in free energy between the two populations (ΔG). The black dots represent the places where some of the systems examined in the present study fall on these surfaces and are labeled as follows: a, G121V DHFR; b, I14A DHFR; c, I14V DHFR; d, M42W DHFR; e, [1,5] sigmatropic rearrangement of pentadiene; f, PETNR/NADPH; g, bsADH < 30˚ C; h, wt MR; i, V108L MR; j, I14G DHFR; k, G121V-M42W DHFR; m, TSase H^+-transfer.

Details and discussion of examples addressing environmental and alternative-substrate effects

From all the examples presented in Table 1, only the active site and the remote mutations of dihydrofolate reductase (DHFR, Figure 8) are discussed in the main text. Here we present a similar, though more concise, discussion of the other examples, for which reported KIEs' temperature dependence was fitted (Table 1).

***Formate dehydrogenase (FDH)*:** The intrinsic KIEs for the wild type FDH(*5*) also presented temperature independent KIEs (Figure S4), suggesting a single narrow population of DADs.

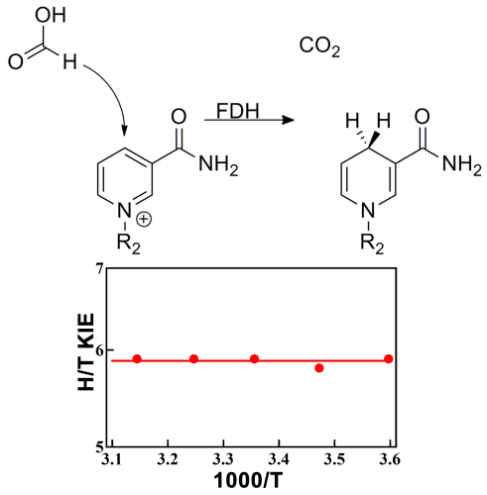


Figure S4: The reaction catalyzed by FDH and the fit to KIEs for the reaction. The fit corresponds to the two-population model. Experimental data are from ref. (*5*).

***Morphinone reductase (MR)*:** The differences resulting from specific mutations and their effects on conformational sampling along the DAD coordinate has also been seen for MR.(*6*) Interestingly, the wild type enzyme and one of the mutants (V108L) presented temperature dependent KIEs while another mutant (W106A) presented temperature independent KIEs (Figure S5). The example of MR is somewhat similar to that of the effect of mutations on DHFR (Figure 8).

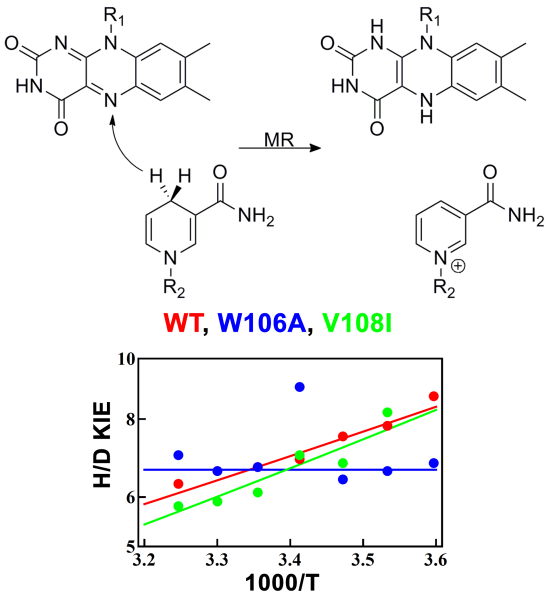


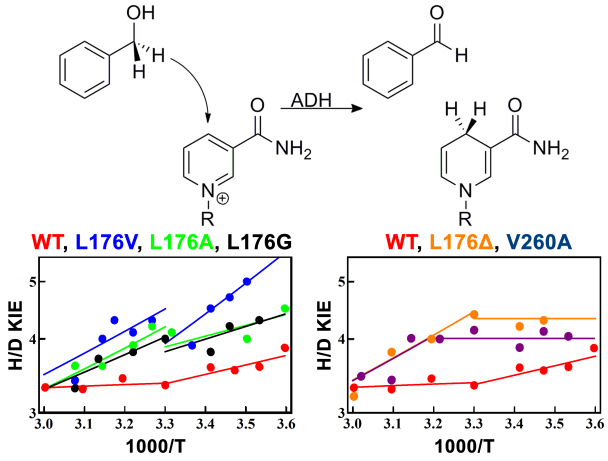
Figure S5: The reductive half-reaction catalyzed by MR and the fits to KIEs of the enzyme and a series of its mutants. All fits correspond to the two-population model. Experimental data are from ref. (*6*).

***Thermophilic alcohol dehydrogenase from B. stearothermophilus (bsADH)*:** In some cases it has been observed that a wild-type enzyme can exhibit temperature-independent KIEs under one set of conditions but give steeply temperature-dependent KIEs under a different set of conditions. The earliest example of this kind of behavior came from a study of a thermophilic ADH from *B. stearothermophilus* (bsADH)(*7*) over a large temperature range (Figure S6). The study found that above 30° C, the KIEs were temperature independent, but below that (outside the physiological range of the enzyme), the KIEs were temperature dependent. The authors posited that thermal motions dictating the DAD sampling at the TRS were "frozen" below 30° C, amounting to a sort of phase transition. Indeed, the present model could not achieve a good fit to the full range of KIEs, but we found that by fitting the KIEs above and below 30° C separately, the best-fit DAD distribution is substantially altered outside the physiological temperature range. From the new fitting (Table 1), the system appears to exhibit a single narrow distribution of DADs within the physiological range (above 30°C), but at temperatures below 30° C, rigidified portions of the protein seem to alter the available conformations, leaving most of the reactive ensemble in a state with a longer DAD and only a small population in a state with a short DAD (ΔG of 1.1 kcal/mol between the two states, i.e., 15% at the short DAD). This result is consistent with the recent implication of multiple states to explain anomalous Arrhenius pre-exponential factors in the mutants of this enzyme.(*8*) We note that it is possible to fit the entire range of KIEs using a high-order polynomial as the PES dictating the DAD distribution, but the interpretability of the model declines with each additional fitting parameter so we prefer to fit the two ranges separately.

Recently, additional work on this enzyme showed that some of its mutants exhibit precisely the opposite effect of the wild type.(*9*) That is, some of the mutants show temperature dependent KIEs within the normal physiological range, but temperature independent KIEs at lower temperatures (Figure S6). Since many of the KIEs from that study were steeply temperature dependent and could not be fitted to a single population using our model, we cannot directly compare all of our fits to the qualitative interpretation described in ref. (*9*), which assumed a single population along the DAD coordinate. We do note, however, that among the three systems that had temperature independent KIEs (wild type > 30 ˚C, L176Δ < 30 ˚C, and V260A < 40 ˚C) and could thus be fitted to a single population, the trend in DAD concurs with the conclusions of ref. (*9*). That is, longer DADs in the mutants lead to larger KIEs. That said, since the best fit for the wild-type is slightly temperature dependent, its best fit force constant in the one population model is somewhat lower than those of the two mutants (despite the lower limit of error being the same), meaning that the same DAD gives smaller KIEs in the wild-type. A shorter DAD for the wild type >30 ˚C (leading to smaller KIE) is only observed for the two populations model (Figure S3 and Table 1). We note that as in ref. (*4*), the size of temperature independent KIEs is very sensitive to the DAD (Figure S2). Thus, only very small changes in structure are needed to change the KIE from 3 (wild type > 30 ˚C) to 4 (L176Δ < 30 ˚C and V260A < 40 ˚C). The similarity of fitting parameters for these systems resembles the similarity of fitting parameters found in ref. (*4*) for the wild type, L546A, and L754A mutants of soybean lipoxygenase, where within a reasonable level of precision, the fitting parameters were identical, despite some differences in the size of the KIEs. Thus, a general feature emerges: according to

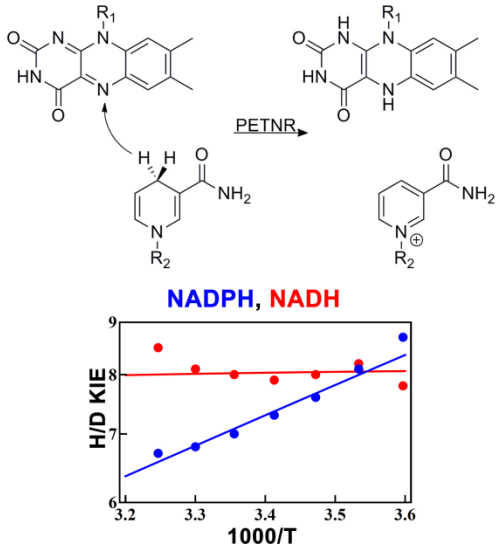
fits of KIEs to Marcus-like models, the KIE is so sensitive to small changes in DAD that small differences in the size of KIEs only reflect very subtle changes in average DAD. Nevertheless, observed differences in the temperature dependence of the KIEs (ΔE_a) do reflect significant differences in the distribution of DADs (*f* or ΔG).

Figure S6: The reaction catalyzed by bsADH and fits to the KIEs of the wildtype and a series of mutants in the physiological temperature range (>30°) and below that range (<30°). The fits all belong to the same series of mutants and are only shown in two separate plots for clarity. All fits correspond to the two-population model. Note that the V260A has its break at 40° and above that temperature the best fit is nearly identical to the L176Δ. Experimental data are from refs (*7, 9*).



***Pentaerythritol tetranitrate reductase (PETNR)*: Another** intriguing case is the situation of PETNR,(*10*) the KIEs of which were measured using both NADH and NADPH as substrates (Figure S7). Despite being faster by a factor of 15 at 25° C, the reaction with NADPH exhibited temperature-dependent KIEs, giving two populations at the TRS instead of the single population for NADH. The large change in conformational sampling for the alternate substrate is especially surprising given how far away the additional phosphate group is from the transferred H (15 Å in the crystal structure, PDB ID 3KFT). This finding, like the results for distal mutants of DHFR, supports the theory that motions involved in shaping the conformational distribution at the active site involve residues throughout the protein. The fact that the more reactive substrate shows a less optimal distribution may indicate that the enzyme can use both substrates under physiological conditions and experienced more evolutionary pressure to "fine tune" the slower reaction, as also seen in the example below (TSase).

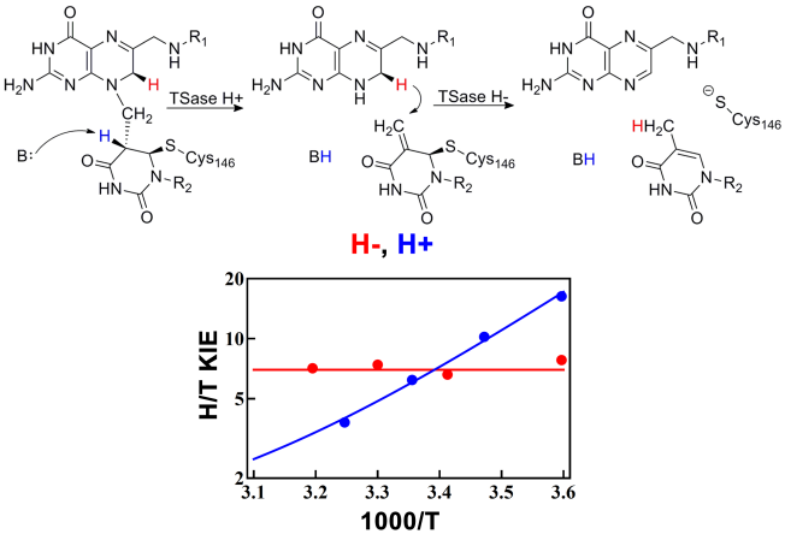
Figure S7: The reductive half-reaction catalyzed by PETNR and fits to KIEs measured with two different cofactors. Both fits correspond to the two-population model. Experimental data are from ref. (*10*).



***Thymidylate synthase from E. coli (TSase)*:** Other interesting examples are the two different C-H bonds activated by TSase, for which KIEs on both the H^+- and H^-- transfers were measured (refs. (*11, 12*), Figure S8). These studies found temperature-independent KIEs for the rate-

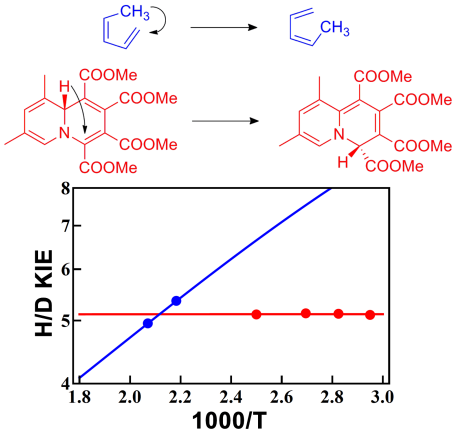
limiting H^--transfer, which yield a single narrow population of DADs,(*11*) while the H^+-transfer showed very steeply temperature-dependent KIEs. These findings may again indicate that the enzyme has experienced more evolutionary pressure to optimize the slower, rate-limiting step. Despite the fact that the fitting method was parameterized for C-H-C rather than C-H-O transfers, we suggest that the fit for the H^+-transfer, which gives two very distinctive populations, is at least qualitatively meaningful.

Figure S8: The H^+-transfer and H^--transfer catalyzed by TSase and fits to the KIEs for the two steps. Both fits correspond to the two-population model. Experimental data are from refs. (*11*) and (*12*).



***Non-enzymatic intra-molecular H-transfer*:** We also analyzed the KIEs for two examples of non-enzymatic [1,5] sigmatropic rearrangements (refs. (*13, 14*), Figure S9). In these cases, the rearrangement of pentadiene yielded temperature-dependent KIEs suggesting two populations at the TRS, or alternatively, a single very loosely bound population. The KIEs measured for the more rigid reaction of quinolizine, on the other hand, were temperature independent, indicating a single narrow distribution of DADs. This corresponds well with the notion that the fused ring system of quinolizine severely hinders conformational flexibility, while the pentadiene has much more freedom at the TRS.

Figure S9: KIEs of the [1,5] sigmatropic rearrangements of quinolizine (red, ref. (*13*)) and pentadiene (blue, ref. (*14*)) and fits to the KIEs for the two reactions. Both fits correspond to the two-population model. Since the rates for H- and D-transfer in pentadiene were measured at different temperatures (though the same overall range of temperatures), the points shown represent the KIEs computed with the Arrhenius equation (eq. 12) from the difference in the Arrhenius parameters for the H- and D-transfer. The fitted line is then a fit to those two points using the computational model presented in the main text.



References

1. Kuznetsov, A. M., and Ulstrup, J. (1999) Proton and hydrogen atom tunnelling in hydrolytic and redox enzyme catalysis, *Canadian Journal of Chemistry-Revue Canadienne De Chimie 77*, 1085-1096.

2. Hay, S., Johannissen, L. O., Sutcliffe, M. J., and Scrutton, N. S. (2010) Barrier Compression and Its Contribution to Both Classical and Quantum Mechanical Aspects of Enzyme Catalysis, *Biophysical Journal 98*, 121-128.

3. Meyer, M. P., and Klinman, J. P. (2005) Modeling temperature dependent kinetic isotope effects for hydrogen transfer in a series of soybean lipoxygenase mutants: The effect of anharmonicity upon transfer distance, *Chemical Physics 319*, 283-296.

4. Knapp, M. J., Rickert, K., and Klinman, J. P. (2002) Temperature-Dependent Isotope Effects in Soybean Lipoxygenase-1: Correlating Hydrogen Tunneling with Protein Dynamics, *J. Am. Chem. Soc. 124*, 3865-3874.

5. Bandaria, J. N., Cheatum, C. M., and Kohen, A. (2009) Examination of enzymatic H-tunneling through kinetics and dynamics, *J Am Chem Soc 131*, 10151-10155.

6. Pudney, C. R., Johannissen, L. O., Sutcliffe, M. J., Hay, S., and Scrutton, N. S. (2010) Direct Analysis of Donor Acceptor Distance and Relationship to Isotope Effects and the Force Constant for Barrier Compression in Enzymatic H-Tunneling Reactions, *J. Am. Chem. Soc. 132*, 11329-11335.

7. Kohen, A., Cannio, R., Bartolucci, S., and Klinman, J. P. (1999) Enzyme dynamics and hydrogen tunneling in a thermophilic alcohol dehydrogenase, *Nature 399*, 496-499.

8. Nagel, Z. D., Dong, M., Bahnson, B. J., and Klinman, J. P. (2011) Impaired protein conformational landscapes as revealed in anomalous Arrhenius prefactors, *Proceedings of the National Academy of Sciences of the United States of America 108*, 10520-10525.

9. Nagel, Z. D., Meadows, C. W., Dong, M., Bahnson, B. J., and Klinman, J. P. (2012) Active Site Hydrophobic Residues Impact Hydrogen Tunneling Differently in a Thermophilic Alcohol Dehydrogenase at Optimal versus Nonoptimal Temperatures, *Biochemistry 51*, 4147-4156.

10. Pudney, C. R., Hay, S., Levy, C., Pang, J. Y., Sutcliffe, M. J., Leys, D., and Scrutton, N. S. (2009) Evidence To Support the Hypothesis That Promoting Vibrations Enhance the Rate of an Enzyme Catalyzed H-Tunneling Reaction, *J. Am. Chem. Soc. 131*, 17072-17073.

11. Agrawal, N., Hong, B., Mihai, C., and Kohen, A. (2004) Vibrationally enhanced hydrogen tunneling in the *E. coli* thymidylate synthase catalyzed reaction, *Biochemistry 43*, 1998-2006.

12. Wang, Z., and Kohen, A. (2010) Thymidylate Synthase Catalyzed H-transfers: Two Chapters in One Tale, *J. Am. Chem. Soc. 132*, 9820–9825.

13. Kwart, H., Brechbiel, M. W., Acheson, R. M., and Ward, D. C. (1982) Observations on the Geometry of Hydrogen Transfer in [1,5]Sigmatropic Rearrangements, *J. Am. Chem. Soc. 104*, 4671-4672.

14. Roth, W. R., and Konig, J. (1966) Wasserstoffverschiebungen .4. Kinetischer Isotopeneffekt Der 1.5-Wasserstoffverschiebung Im Cis-Pentadien-(1.3), *Annalen Der Chemie-Justus Liebig 699*, 24-32.