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Understanding Biological Hydrogen Transfer Through the Lens of Temperature Dependent Kinetic Isotope Effects

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Conspectus:

Hydrogen atom transfer (HAT) is a salient feature of many enzymatic C-H cleavage mechanisms. In systems where kinetic isolation of HAT is achieved, selective labelling of substrate with hydrogen isotopes, such as deuterium, enables the determination of intrinsic kinetic isotope effects (KIEs). While the magnitude of the KIE is itself informative, ultimately the size of the temperature dependence of the KIE, $E_a = E_a(\text{D}) - E_a(\text{H})$, serves as a critical, and often misinterpreted (or even ignored) descriptor of the reaction coordinate. As will be highlighted in this Accounts, E_a is one of the most robust parameters to emerge from studies of enzyme catalyzed hydrogen transfer.

Kinetic parameters for C-H reactions via HAT can appear consistent with either classical “over-the-barrier” or “Bell-like tunneling correction” models. However, neither of these models is able to explain the observation of near-zero E_a values with many native enzymes that increase upon extrinsic or intrinsic perturbations to function. Instead, a full tunneling model has been developed that can account for the aggregate trends in the temperature dependence of the KIE. This model is reminiscent of Marcus-like theory for electron tunneling, with the additional incorporation of an H-atom donor-acceptor distance (DAD) sampling term for effective wavefunction overlap; the role of the latter term is manifested in the experimentally determined E_a .

Three enzyme systems from this laboratory that illustrate different aspects of HAT are presented: taurine dioxygenase, the dual copper β -monooxygenases, and soybean lipoxygenase (SLO). The latter provides a particularly compelling system for understanding the properties of hydrogen tunneling, showing systematic increases in E_a upon reduction in the size of hydrophobic residues both proximal and distal from the active site iron cofactor. Of note, recent ENDOR-based studies of enzyme-substrate complexes with SLO indicate an *increase* in DAD for mutants with increased E_a , observations that are inconsistent with “Bell-like correction” models.

Overall, the surmounting kinetic and biophysical evidence corroborates a multi-dimensional approach for understanding HAT, offering a robust mechanistic explanation for the magnitude and trends of the KIE and E_a . Recent DFT and QM/MM computations on SLO are compared to the developed nonadiabatic analytical constructs, providing considerable insight into ground state structures and reactivity. However, QM/MM is unable to readily reproduce the small E_a values

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characteristic of native enzymes. Future theoretical developments to capture these experimental observations may necessitate a parsing of protein motions for local, substrate deuteration-sensitive modes from the larger conformational landscape, in the process providing deeper understanding of how native enzymes have evolved to transiently optimize their active site configurations.

Graphical Abstract

Introduction:

For decades, this laboratory has been focused on the study of enzymatic C-H bond cleavage reactions that occur by quantum-mechanical tunneling mechanisms. These processes have unique kinetic features that provide insight into the complete shape, not just the height of the barrier for catalysis. The ability to stably label C-H bonds with hydrogen isotopes enables the magnitude of the kinetic isotope effect (KIE) to be determined and related to perturbations of the reaction coordinate arising from changes in e.g., temperature, pressure or protein mutation.^{1–10} Despite years of experimental findings that have led to the current prevailing theoretical constructs for hydrogen transfer from carbon, common misconceptions frequently still arise from non-specialists, including the elimination of tunneling mechanisms based on the magnitude of experimental KIEs. As is highlighted herein, one of the most robust kinetic parameters to emerge from such studies of hydrogen transfer is the *difference* between the enthalpic barriers for protium and deuterium wavefunction overlap, (i.e. the temperature dependence of the kinetic isotope effect, $E_a = E_a(D) - E_a(H)$). Because of the greater mass of deuterium and its less diffuse wavefunction, the magnitude of $E_a(D)$ provides a measure of an enzyme's ability to sample the short H-donor-acceptor distances (2.7 Å) conducive to tunneling. This mini-review will further emphasize the catalytic role of hydrophobic side chains in enzymes whose mutational impacts can, in certain instances, only be evident from the E_a parameter.

There is a rich history regarding mechanistic interpretations of E_a in condensed phase, beginning with the original expectation that the Arrhenius plot of rates for H vs D transfer would converge on the y-axis, $A_H/A_D = 1$, if a reaction proceeds classically, and that the KIE would arise predominantly from differences in isotopically sensitive zero-point energies (cf. Figure 1A).¹¹ As introduced by Bell,¹² room temperature through-the-barrier tunneling can also occur near the top of the classical barrier, leading to $A_H < A_D$ (Figure 1B). Lastly, in the context of enzyme-catalyzed hydrogen tunneling, another property has become apparent for native enzymes, in which E_a values are close to zero ($A_H/A_D = \text{KIE} \gg 1$) across the experimentally accessible temperature range (Figure 1C).

Importantly, as revealed from decades of study of protein enzyme catalysis, a distinctive pattern for the temperature dependence of the KIE emerges when a native enzyme is subjected to either intrinsic or extrinsic perturbations (Figure 2). Beginning with the most active (wild-type, WT) form of enzyme, E_a is small, often close to zero (Figure 2A). Subsequently, as the enzyme system is impaired, the kinetic behavior can appear to “mimic” semi-classical behaviors where $A_H/A_D = 1$ (Figure 2B). And finally, when perturbation to the enzyme becomes extreme, E_a will greatly exceed the semi-classical limit, resembling tunneling correction models where $A_H/A_D \ll 1$ (Figure 2C). In select instances, these trends in E_a are accompanied by nearly constant KIE values under physiological conditions, raising the compelling question of how to arrive at a predictable physical model that incorporates the range of observed kinetic properties. From the perspective of traditional, semi-classical theory it is, simply put, impossible to explain the behavior of native enzymes (Figure 2A). Also, while the patterns in Figure 2 could suggest a progression to semi-classical behavior (Figure 2B), that is followed by a role for significant tunneling (Figure 2C), this would be in direct conflict with structural evidence of increased hydrogen donor-acceptor distances following increased enzyme impairment.^{13,14}

Significantly, nonadiabatic theoretical models for tunneling mechanisms (Figure 3) are able to explain both the native behavior of enzyme (Figure 2A) as well as the changes that arise when a system undergoes perturbation (Figure 2B-C).^{2,5,10,15–18} On the surface, these theoretical constructs are extensions of Marcus models for electron transfer, that describe the reaction barrier in terms of the environmental reorganization energy, λ , and reaction free energy, G^0 (Figure 3B); this coordinate controls the achievement of transient, reactant-product degeneracy (Figure 3C), a pre-requisite for wavefunction overlap.¹⁹ In addition, another aspect uniquely emerges in hydrogen tunneling reactions, as a result of the heightened sensitivity of nuclear wavefunction overlap to small changes in DAD. Illustrated in Figure 3D, local dynamical sampling over an ensemble of donor-acceptor distances (DADs) can become important when the distance between the H-donor and acceptor is outside the regime of a dominant tunneling distance, R_{dom} , of 2.7 Å.^{2,20,21} The experimental manifestation of the behavior in Figure 3D is E_a . In the context of the model, E_a values of zero in native enzyme systems indicate a tunneling ready state (TRS) where the DAD has been reduced from R_{eq} to a sufficiently short distance (R_0) to promote efficient wavefunction overlap of both protium and deuterium, leading to a minimal contribution of local DAD sampling to the reaction barrier (Figure 3D, **top**). The introduction of protein packing defects through, for example, the strategic mutagenesis of hydrophobic side chains causes E_a values to become enlarged (Figure 3D, **bottom**), at first appearing to approximate semi-classical “over the barrier” catalytic behavior and then, with increasing impairment, appearing to approximate “tunneling correction” models. *The beauty of this model is its ability to link regular increases in E_a to structurally-induced increases in DADs.* In almost all instances this can be compensated by local dynamical sampling that enhances wavefunction overlap. The latter plays a disproportionate role for D-transfer, arising from an isotope-dependent change in wavefunction overlap that is completely distinct from the zero point energy-based, semi-classical origin of primary hydrogen KIEs.¹¹

In the context of the behavior illustrated in Figure 2 and the theoretical model in Figure 3, we now introduce three different HAT systems that have yielded a combination of KIEs and/or E_a parameters. These include taurine/ α -ketoglutarate dioxygenase, the dual copper β -monooxygenases and soybean lipoxygenase. Each system has been chosen to illustrate different aspects of the experimental challenges that have been encountered in the course of mechanistic studies and the resulting insights that lead to our current perspective. The reactions catalyzed by each enzyme system are illustrated in Scheme 1, while Figure 4 presents representative X-ray structures.

TauD illustrates the impact of isotope- and mutation-dependent reaction uncoupling during C-H oxidation.

TauD, responsible for the hydroxylation of taurine, is a member of the α -ketoglutarate (α KG) dependent mono- and dioxygenases. The diverse reactions in this family are initiated by the decarboxylation of α KG leading to the production of a non-heme oxyferryl intermediate that catalyzes the cleavage of substrate C-H bonds (Scheme 1A).²⁵ TauD had been reported to exhibit a large, intrinsic deuterium KIE, $k_{(H)}/k_{(D)} = {}^Dk_{cat} \cong 60$ under the partial reaction of hydrogen atom transfer from C1 of taurine to the oxygen acceptor on $Fe^{IV}=O$, and has served as a model system for extensive spectroscopic and structural characterization of iron intermediates within this family of enzymes.^{26,27} The X-ray structure of substrate-bound tauD (Figure 4A) displays a metal-bound α KG and includes substrate taurine sandwiched approximately equidistant between iron (ferrous state) and F159. Following the initial report of the large isotope effects suggestive of tunneling behavior,²⁶ our group turned to an investigation of the possible role of sidechain bulk at position 159 in tuning tunneling efficiency. The expectation was that replacement of F159 by smaller hydrophobic sidechains would concomitantly expand the active size cavity and reduce the ability of protein to promote hydrogenic wavefunction overlap.

As shown in Table 1, substitution of F159 by L results in modest changes to rate constants, with a significantly greater impact under pre-steady-state conditions.²⁸ Importantly the intrinsic KIE, as reflected in the pre-steady-state ${}^Dk(\text{prod})$, Table 1, is nearly the same (within error) for WT and F159L. The fact that the KIEs are within error, while the rate of hydrogen atom abstraction is reduced *ca* 20-fold, suggested a successful recovery for F159L to the native donor-acceptor distance via the distance sampling term represented in Figure 3D. Proceeding through the series to the less bulky sidechain, F159V, the fact that a 50-fold reduction in the pre-steady-state $k(\text{prod})$ leads to an elevation of ${}^Dk(\text{prod})$ to *ca.* 200, further suggested that F159V had crossed a threshold whereby DAD sampling was no longer capable of recapturing the close DAD of WT.²⁸

Reaction uncoupling precludes a determination of E_a .

In principle, the predicted trends in the DAD sampling modes for the F159X could have been tested by measurement of the corresponding values for E_a .²⁸ However, while WT tau D catalyzes a tightly coupled reaction, in which O_2 uptake and product formation occur in a 1:1 ratio, perturbations via substrate deuteration and/or mutation lead to increasingly large degrees of reaction uncoupling, Figure 5. As a result, our ability to determine precise

intrinsic rate constants became limited to the conditions of Table 1 with estimates of $^Dk(\text{prod})$ not even attempted for the Ala and Gly mutants.²⁹ TauD thus illustrates some of the potential complications that can arise in experimental efforts to estimate E_a values. As shown in this case, pre-steady state conditions may be required to separate the C-H cleavage step from other rate determining steps (Table 1) while reaction uncoupling (Figure 5) may compromise the ability to obtain sufficiently precise intrinsic rate constants for a reliable determination of trends in the E_a parameter.

PHM illustrates estimation of intrinsic deuterium KIEs and their temperature dependence via precise measurement of tritium KIEs.

PHM is a member of a family of eukaryotic monooxygenases that include dopamine β -monooxygenase (D β M) and tyramine β -monooxygenase (T β M) and contain two protein-bound, uncoupled copper atoms,³⁰ separated by a ~ 10 Å solvent cleft (Figure 4B).³¹ Despite differences in substrate/product structures, these reactions occur by a highly conserved chemical mechanism that is initiated via hydrogen atom abstraction at an activated oxygen within the Cu_M site (Figure 4B).³ In contrast to tauD, and of importance for characterization of HAT in this family of enzymes, *substrate and oxygen activation occur in a fully coupled manner*.^{3,32} However, similar to the tauD reaction, steady-state rate constants are largely rate limited by substrate binding and product release. In the case of tauD, pre-steady state decay rates determined from the spectral signal of Fe^{IV}=O enabled direct determination of the intrinsic values for $k(\text{prod})$ and $^Dk(\text{prod})$. The lack of an amenable spectral handle for active site chemistry in the copper monooxygenases required an alternate kinetic strategy. Isolation of the intrinsic isotope effects on C-H bond cleavage was made possible by developed methods for the estimation of highly precise competitive kinetic measurements of primary tritium isotope effects, k_H/k_T and k_D/k_T that allow calculation of the magnitude of k_H/k_D .^{1,33}

Using this approach, PHM has been shown to display an intrinsic primary isotope effect, ~ 11 ,¹ elevated from the semi-classical limit (*ca.* 7), that compares well to values estimated for D β M³³ and T β M³⁴ (Table 2). Extension of this intrinsic primary isotope effect measurement to a range of physiological temperatures (Figure 6),¹ indicates a relatively flat dependence with an isotope effect on the Arrhenius prefactor, A_H/A_D , that is well above the semi-classical limit of unity and an accompanying value for E_a that is essentially zero (Table 2). We note that while the size of the intrinsic isotope effects for the hydrogen atom transfer catalyzed by PHM and the related copper dependent monooxygenases are near the Bell tunneling correction limit ($\text{KIE} \sim 10$),¹² it was only after interrogation of the E_a (~ 0 kcal/mol) that such behavior could be ruled out. It was shown possible to model this behavior of PHM using the analytical equations described in greater detail for SLO below.
1,2,10,15,18,22

SLO provides insight into role of hydrophobic sidechain bulk on active site precision through mutational impact to KIE and E_a

SLO oxidizes its substrate, linoleic acid (LA), via an initial, rate-limiting abstraction of a hydrogen atom from position C11 of the substrate by an active site ferric-hydroxide, in

which a proton is transferred to the iron-bound hydroxide and an electron reduces the ferric center to ferrous (Scheme 1C). The very large Dk_{cat} (*ca.* 80) near room temperature that is weakly temperature dependent ($E_a = 0.9 \pm 0.2$ kcal/mol) has enabled comparative measurement of KIEs under a wide variety of conditions. Because the active site is deeply buried and surrounded by bulky hydrophobic residues, SLO has further established itself as an especially attractive model to probe the contributions of individual amino acids in mediating active site tunneling-appropriate DADs.

Single alanine “scanning” was initially applied to hydrophobic sidechains, including two proximal, conserved leucines, L546 and L754 and a more distal isoleucine, I553.² The reduction in sidechain volume at either 546 or 754 results in expanded active site cavities and altered kinetic features, such as decreased k_{cat} values with concomitant increases to the activation energy parameters, E_a and E_a (Table 3).

For I553A alone, the k_{cat} and E_a are comparable to WT with the only parameter impacted being the E_a (4.0 kcal/mol), whereas the double mutant I553A/L546A results in a value for E_a that is midway between WT and I553A, with an “apparent” semi-classical pattern for A_H/A_D of unity. This combination of results can be readily explained by mutationally-produced changes in DAD that are accompanied by enhanced local dynamic sampling to restore catalytic proficiency (Figure 3D).²

Subsequently, the mutational analysis of I553A was followed by preparation of a series of aliphatic mutants of varying sidechain volume at this position, Table 4. Extensive X-ray analyses of this series in the absence of substrate have ruled out significant structural changes with decreasing sidechain volume, while kinetic analyses highlight a remarkable trend in the size of E_a .⁵ According to developed analytical expressions for the temperature dependence of the KIE,^{2,15,18,22} both a pre-tunneling donor-acceptor (R_0) and a frequency for the DAD sampling coordinate (Ω_s) can be estimated, illustrating the significant impact of hydrophobic side chain mutations on trends in both the R_0 and Ω_s .

The modest E_a value for WT SLO supports a highly evolved, compacted active site. In contrast, the E_a data for I553X indicate a DAD sampling coordinate that begins to contribute significantly when packing defects both alter/enlarge the initial DAD and decrease the force constant of the local DAD sampling mode.

Modulation of enzyme-substrate ground state alignment by active site mutations.

The I553X variants offer a unique opportunity to investigate the relationship between dominant ground state enzyme-substrate structures and the kinetic detection of changes in active site compaction.

However, there has been the perennial inability to capture co-crystal structures of SLO with substrate, leading to a search for alternative methods to examine enzyme-substrate complexes. ENDOR is often regarded as “EPR-detected NMR”, because of its ability to measure interactions of nuclei, such as ^1H , ^2H , ^{13}C and ^{15}N , within close proximity to unpaired electron spins.^{37,38} Application of ENDOR to SLO leads not only towards the

depiction of the ground state structure of the native enzyme (Figure 7A), but also towards a sensitive assay for structural impacts from seemingly modest active site mutations.¹⁴

The desired structure of the WT SLO-LA complex was generated using a structurally-faithful, manganese ($S = 5/2$) substituted enzyme samples and selectively ^{13}C -labeled substrates (at the reactive carbon C11 as well as the adjacent carbon, C10 as a reference point). With a focus on C11, the ^{13}C ENDOR response of SLO is summarized in Figure 7.¹⁴ The data (Figure 7A) indicate two conformations, with the larger shifted feature showing a shoulder at ~ 0.4 MHz representing the “active” *a* substrate conformation that places the hydrogen donor atom (C11) within van der Waals distance of the acceptor oxygen. The second *b* conformation reflects a substrate conformation positioned nearly 1 Å further from the hydrogen acceptor oxygen and is anticipated to be non-catalytic (cf. Figure 7B). We expanded the investigation of WT to include the impact of site specific mutations, with studies of the most extreme I553 mutant, I553G, leading to a complete loss of the *a* conformer (Figure 7B). In short, the I553 variant with the largest E_a value (Table 4) has reduced the population of the near, active site enzyme-substrate conformer to a point where it can no longer be detected experimentally. While an observation of $E_a = 5.3 \pm 0.7$ for I553G could, on the surface, have been compatible with tunneling correction models as originally introduced by Bell, it seems inconceivable that a mutant that *increases* the effective DAD distance would be capable of increased tunneling relative to WT. In the context of the critical dependence of tunneling on a close approach of the H-atom donor and acceptor, this combination of kinetic data and biophysical probes for SLO provides some of the strongest validation of the originally proposed multi-dimensional analytical model for HAT illustrated in Figure 3.

Comparison of analytical constructs for nonadiabatic HAT to recent quantum computations.

The remarkable simplification of the analysis of the tunneling process using isotopically-labeled substrates, when combined with various biophysical probes, can provide great insight into catalysis, as illustrated above for the I553X series in SLO. While the majority of other single site mutations in enzymes behave like I553X, showing a combination of largely unchanged KIE values to WT with elevated E_a values, a new double mutant of SLO (DM), constructed from simultaneous mutation of the highly conserved leucine active site residues, L546A and L754A, has emerged as an outlier.^{7,10} This variant is characterized by a significantly reduced rate (k_{cat} down 10^4 -fold), an enormous KIE of 661 ± 27 across five temperatures, and a E_a that is essentially zero ($E_a = 0.3 \pm 0.7$ kcal/mol).^{7,10} With these extreme kinetic parameters, the DM variant provides a benchmark to examine the limits of the nonadiabatic tunneling construct. Impressively, statistical analysis of the composite data for this enzyme has shown that the combination of an elongated DAD at the tunneling-ready state and a modified DAD sampling mode that prevents restoration of tunneling efficient configurations, is sufficient to explain all of the experimental parameters.¹⁰

Two recent quantum calculations^{39,40} extend the nonadiabatic analytical theory developed for SLO in new directions by introducing anharmonicity into the potential surface defining

the DAD sampling mode. Both studies deal with theoretical aspects of rate, isotope effects and their temperature dependencies that rely on non-adiabatic models, differing in their primary focus on gas phase DFT³⁹ or QM/MM⁴⁰ computations. The former has a primary goal of assessing the physical parameters relevant to the observed PCET, whereas the latter is targeted toward an atomistic picture of the protein's role in catalysis. Despite the different strategies, an important agreement between the works is the elongation of the ground state equilibrium DAD from 3.2–3.3 Å in WT to 3.6–3.8 Å in DM, values that approach ENDOR-determined values of 3.1 Å (WT) and 3.9 Å (DM).¹⁴

The QM study by Champion has investigated the impact on reactivity of an increasingly negative charge on the iron-bound hydroxide ion that can be modulated by hydrogen bonding to the C-terminal ligand to iron (I839). This analysis is simplified by use of an active site structure represented by iron, the ligands to the iron (without backbone), and methane as a model for the reactive carbon of the substrate LA. The computations begin with either “conformer *a*” for WT or “conformer *b*” for DM, using ENDOR-derived distances as a guide. These ground state configurations are the starting point to reach the tunneling ready states, via the application of a relatively large (pN) force that is “extrinsic” to the QM region and primarily arises from an electric field. As discussed extensively in other publications, the process of reaching the reduced distances within the TRS involves a series of stochastic conformational fluctuations that can originate far from the active site. The detailed analysis by Champion and co-workers is fundamentally similar to a model first put forth by Kuznetsov and Ullstrup,²⁰ further developed in our Berkeley laboratory,² and subsequently elaborated in numerous publications.^{5,10,15–18,36}

A caveat to the above described work is that the quantum treatment of substrate was limited to methane, an issue addressed by Hammes-Schiffer and co-workers, who have extended the DFT computation to a 2,5-heptadiene moiety and to linoleic acid itself in their QM/MM analysis. One surprising conclusion is that the electrostatic force field needed to reach the R_{dom} (Figure 3D) is relatively local rather than global and depends largely on the double bonds flanking the reactive carbon of substrate. This conclusion arises in both their simplified DFT treatment of the reaction coordinate as well as the full QM/MM analysis, raising the question of what aspects of the reaction trajectory have been influenced by the full protein structure. In fact, the MM portion of the protein is shown to uncover an altered substrate binding mode and a reaction driving force in the double mutant enzyme; however, the QM/MM approach has thus far been unable to capture an experimentally determined long-range dynamical network that has recently been shown to communicate thermal activation between the protein-water interface and the active site of WT SLO (cf. Ref ²³).

From the perspective of this Account, it is also notable that the new QM/MM study of SLO is unable to readily reproduce the experimental trends in E_a : while this analysis of SLO is able to match computed to experimental KIEs at select temperatures, via the introduction of small corrections to the computed potentials of mean force, it remains unable to reproduce well the weakly temperature dependent KIEs for WT and the DM variant. The extensively characterized system, for which analytical models allow a precise fit of both KIE and E_a , thus may provide valuable perspective into the long recognized and persistent difficulty in fitting temperature independent KIEs by way of QM/MM computations.⁴¹ In the recent

analysis by Li *et al.*,⁴⁰ the productive E-S complex in SLO is the starting point for movement along an all atom-generated potential energy surface. This is accomplished using a non-adiabatic approximation, in which all protein motions have been combined together into a collective work term that is both anharmonic in nature and expected to be comprised of a hierarchy of modes that spans global and local motions; the latter is the primary determinant of the E_a term, Figure 3D. The net result is a single, aggregate protein sampling mode that will have a softer potential than anticipated for an isolated, local DAD sampling process. As shown repeatedly using non-adiabatic analytical approaches (*cf.* Table 4), *reduced force constants for DAD sampling produce more temperature-dependent KIEs.* Thus, as it now stands, the QM/MM analysis is unable to easily rationalize or reasonably model the very small E_a values routinely observed for native enzymes. It will be of great interest to see if continued refinement of all atom computations can solve this problem, perhaps via methodology that permits resolution of the hierarchy of protein motions that is expected to be transiently populated as the potential energy surface progresses from ground state to tunneling-ready state.

Conclusions.

Kinetic isotope effects have played an important role in our analysis of hydrogen transfer processes. In the context of enzyme reactions, this review highlights the unique information that can be gleaned from an interrogation of the temperature dependence of the KIE. Once appropriate controls have been set in place, to rule out complications such as multiple rate limiting steps and reaction uncoupling, the magnitude of E_a provides key information regarding the catalytic role of reduced barrier width and its dependence on environmentally-controlled active site alignment between the hydrogen donor and acceptor atoms.

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Biographical Information

Adam R. Offenbacher received his B.S. in biochemistry from Ohio Northern University and his Ph.D. at Georgia Institute of Technology. Adam's postdoctoral studies in Prof. Klinman's group focused on elucidating the fundamentals of hydrogen tunneling in SLO. He is now an Assistant Professor in Chemistry at East Carolina University (ECU), where he is studying protein allostery and biological proton-coupled electron transfer.

Judith P. Klinman received her A.B. and Ph. D. from the University of Pennsylvania, followed by postdoctoral research with Dr. David Samuel at the Weizmann Institute of Science, Israel, and Dr. Irwin Rose at the Institute for Cancer Research, Philadelphia. She has been at the University of California at Berkeley since 1978, and is currently a Professor of the Graduate School in the Departments of Chemistry and of Molecular and Cell Biology. She is a member of the National Academy of Sciences, the American Philosophical Society, and the American Academy of Arts and Sciences, among others, and was awarded the National Medal of Science in 2014. Over many decades, the work in her laboratory has been focused on understanding the fundamental properties that underlie enzyme catalysis.

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Scheme 1.

Reactions of (A) taurine dioxxygenase (tauD), (B) peptidylglycine α -amidating monooxygenase (PHM), a representative of the dual copper β -monooxygenase family, and (C) soybean lipoxygenase (SLO).

A. Semi-classical

B. Tunneling correction

C. Full tunneling

Figure 1.

Energy barrier diagrams (left) and Arrhenius plots (right) for the cleavage of C-L bonds where L refers to H (red), D (cyan), or T (purple). The classical transition state is defined by “‡”. The isotopic parameters derived from these Arrhenius plots are the ratio of the Arrhenius prefactors and the difference in the enthalpic barriers, A_H/A_D and $E_a(D) - E_a(H) = E_a$, respectively for protium vs. deuterium transfer.

A. Native enzyme

B. Modestly-impaired enzyme

C. Greatly-impaired enzyme

Figure 2.

Frequently observed trends in Arrhenius plots for enzyme catalyzed C-H cleavage reactions under physiological temperature conditions. As discussed in the text, the experimental trend in the magnitude of $E_a(D) - E_a(H) = E_a$ is incompatible with previous interpretations in the context of either semi-classical behavior or tunneling correction models.

Figure 3.

Rate constants for enzyme catalyzed nonadiabatic hydrogen transfer can be formalized as $k = K_{eq} \times k_{tun}$.^{10,22,23} (A) $K_{eq} (\ll 1)$ represents a stochastic ground state search across the conformational landscape for catalytically active E-S complexes (R_{eq} , blue) and subsequently, thermally activated, pre-tunneling states that further reduce the distance between the H-donor and acceptor (R_0 , green). (B–D) illustrate the tunneling portion of the reaction from R_0 as represented by k_{tun} : (B) Heavy atom protein motions transiently produce tunneling ready states (TRS) of degenerate energies for the reactant and product wells, a prerequisite for wave function overlap (illustrated in C). (D) The effective potential of the TRS (purple) along the DAD sampling coordinate can produce either $E_a \cong 0$ for native enzyme (bottom) or $E_a > 0$ when the DAD is elongated following perturbation of the system (top). For native systems (bottom), both H and D wave function overlap are expected with minimal sampling (D wave function not shown). The poor overlap for D in top case (hatched purple wave functions) necessitates further DAD sampling. Adapted with permission from ref. ²⁴. Copyright 2013, Annual Reviews.

Figure 4.

Structures of tauD (2.5 Å; PDB: 1OS7), where the individual subunits are shown in wheat, magenta, green and blue (A); PHM (2.1 Å; PDB: 1OPM) (B); and SLO (1.4 Å; PDB: 3PZW) where wheat and blue represent the catalytic and lipid binding domains (C). Active sites of these enzymes are highlighted on their global structures by a red rectangle. Relevant sidechains and substrates are labeled for reference. The hydrogens of the reactive carbon (H-donor) are shown for each substrate as white sticks. The iron atoms in A and C are shown as orange spheres; acceptor oxygen in (C) is shown as red sphere. Substrate in (C) was modeled.¹⁴

Figure 5.

Ratios for formation of product sulfite to oxygen uptake for WT tauD and a series of F159 mutants. This shows the high sensitivity of reaction coupling to substrate deuteration and site-specific mutagenesis. The dark vs. light shaded bars represent protiated and dideuterated substrate, respectively. Reproduced with permission from ref. ²⁹. Copyright 2009, PNAS Publications.

Figure 6.
Temperature dependence of the primary intrinsic primary deuterium isotope effect for PHM.
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Figure 7.

^{13}C ENDOR spectra and model of the active site structure of the SLO-LA complex.¹⁴ (A) ^{13}C ENDOR for the C11 of substrate shows two conformers for WT. The active *a* conformer is lost in the mutant, I553G. (B) Structural models of *a* and *b* conformers. Adapted with permission from ref. ¹⁴. Copyright 2017, American Chemical Society.

Table 1.Steady-state and pre-steady-state kinetic parameters for WT tauD and F159 variants.^a

tauD	Steady state, 30°C		Pre-steady state, 10°C	
	k_{cat} (s ⁻¹)	$D k_{\text{cat}}$ ^b	$k(\text{prod})$ (s ⁻¹) ^c	$D k(\text{prod})$ ^c
WT	5.0 ± 0.2	11.5 ± 0.7	21 ± 1	60 ± 11
F159L	1.52 ± 0.07	39 ± 4	1.31 ± 0.09	44 ± 4
F159V	1.19 ± 0.03	120 ± 20	0.41 ± 0.03	200 ± 50
F159A	0.52 ± 0.01	70 ^d	N.D. ^e	N.D. ^e

^aData from reference 28.^bSteady-state rate constants are derived from measurement of product formation, which is less than O₂ consumption due to reaction uncoupling (see Figure 5 and accompanying discussion).^cThe reported pre-steady-state parameters are obtained from the observed decay rates corrected for the degree of reaction uncoupling.^dThe $D k_{\text{cat}}$ of >70 for F159A represents a lower estimate, with the expectation of an intrinsic value in excess of F159V.^eNot determined.

Table 2.Kinetic parameters for PHM, D β M and T β M

Enzyme	$k_{\text{C-H}}$ (s ⁻¹), 35–37 °C	Dk , 35–37 °C	$A_{\text{H}}/A_{\text{D}}$	E_{a} (kcal/mol)
PHM ^a	870 (60)	10.4 (0.3)	5.9 (3.2)	0.37 (0.33)
D β M ^b	~1200	10.9 (1.9)	N.D. ^c	N.D. ^c
T β M ^d	100 (40)	~12	N.D. ^c	N.D. ^c

^aFrom ref 1.^bFrom ref 33.^cNot determined.^dFrom ref 34. Dk estimated for Y216A, where $D(k_{\text{cat}}/K_{\text{M}})$ approaches intrinsic KIE.

Table 3.Kinetic parameters of WT SLO and variants.^a

	k_{cat} , s ⁻¹	^D k_{cat} , 30 °C	E _a , kcal/mol	A _H /A _D	E _a , kcal/mol
WT ^b	297 (12)	81 (5)	2.1 (0.2)	18 (5)	0.9 (0.2)
L546A ^b	4.8 (0.6)	93 (9)	4.1 (0.4)	4 (4)	1.9 (0.6)
L754A ^b	0.31 (0.02)	112 (3)	4.1 (0.3)	3 (3)	2.0 (0.5)
I553A ^b	280 (10)	93 (4)	1.9 (0.2)	0.12 (0.06)	4.0 (0.3)
I553A/L546A ^c	2.21 (0.09)	128 (3)	3.8 (0.4)	1.05 (0.45)	2.8 (0.4)

^aKinetics determined with linoleic acid, 0.1 M borate, pH 9.0, 30°C.^bFrom reference ².^cFrom reference ³⁵.

Table 4.

Temperature dependence of Dk_{cat} within the I553X series of SLO variants, together with estimated pre-tunneling distances (R_0) and distance sampling frequencies (Ω).^a

	E_a , kcal/mol	R_0 , Å	Ω , cm ⁻¹
WT	0.9 (0.2)	2.85	320
I553V	2.6 (0.5)	2.97	271
I553L	3.4 (0.6)	3.07	243
I553A	4.0 (0.3)	3.04	252
I553G	5.3 (0.7)	3.32	218

^aDistances R_0 and frequencies (Ω) for the DAD sampling term were determined by fitting the experimental Dk_{cat} and E_a values to an expanded analytical rate constant expression with quadratic terms.^{10,18} Though the absolute magnitude of Ω will be overestimated due to the treatment of the DAD sampling as a harmonic oscillator in this instance,³⁶ the trends in both the E_a and computed parameters are readily apparent.