The Use Of Kinetic Isotope Effects To Study Enzyme Mechanisms
Kinetic Isotope Effect (KIE): ratio of rate constants describing a reaction, with that associated with the reaction of the lighter isotope in the numerator
I. Theoretical basis of a $^2$H-KIE

A. In elementary steps of chemical reactions in which bonds to hydrogen ($m=1$) are broken, substitution with the heavier deuterium ($m=2$) slows down that step and often the entire reaction.

B. In general, bonds to the heavier of two isotopes ($^{12/13}$C, $^{14/15}$N, $^{16/18}$O) are cleaved more slowly, but the effects are by far the largest and most useful for hydrogen ($^1$H or H) and its isotopes deuterium ($^2$H or D) and tritium ($^3$H or T).

C. The magnitude of the KIE, its dependence on temperature, and its modulation by perturbation of the enzyme (e.g., by mutagenesis) can reveal the nature of the transition state of the elementary step in which the bond is cleaved.
I. Theoretical basis of a $^2$H-KIE

Old school explanation for $^2$H-KIE: zero-point energy differences

Vibrational energies ($E_n$) are dependent on the frequency of the bond stretch ($\nu$), which is dependent on the reduced mass ($\mu$) of the two connected atoms ($\mu$)

\[
\nu = \frac{1}{2\pi c} \sqrt{\frac{k}{\mu}}
\]

\[
\mu = \frac{m_1 m_2}{m_1 + m_2}
\]

\[
E_n = (n + 1) \hbar \nu
\]

Internuclear Distance ($r$)

Knowles, R. 2005 *Kinetic Isotope Effects in Organic Chemistry*
I. Theoretical basis of a $^2$H-KIE

Treat the homolytic cleavage of C-H/D bond where the bond is considered to be fully broken at the transition state. Reaction progress followed by observing the C-H/D bond stretch.

<table>
<thead>
<tr>
<th>frequency (cm$^{-1}$)</th>
<th>ZPE (kcal/mol)</th>
<th>rel. rate (300 K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C - H stretch</td>
<td>2900</td>
<td>4.15</td>
</tr>
<tr>
<td>C - D stretch</td>
<td>2100</td>
<td>3.00</td>
</tr>
</tbody>
</table>

$\Delta G = \Delta ZPE$

C-H/D bond breaks at the transition state; stretch becomes a translation; no new stretch in TS that corresponds to the stretch of ground state bond. For this mechanism, the isotope effect is entirely controlled by the difference in the ground state ZPE's.

Knowles, R. **2005** *Kinetic Isotope Effects in Organic Chemistry*
I. Theoretical basis of a $^2\text{H}$-KIE

**Caveat:** the dominant effect giving rise to most, if not all $^2/3\text{H}$-KIEs is the difference in tunneling efficiency, and thus in reality these KIEs can be much larger
I. Theoretical basis of a $^2$H-KIE

Heavy atom KIEs can be explained by the same physical formalism

1. In these cases, it is closer to physical reality because tunneling is not important for heavy atoms

2. Much smaller for heavy atom effects due to much less difference between reduced masses of light and heavy isotopes and absence of significant tunneling

3. Measurable only by competition methods
II. Vocabulary for enzymologists

1. kinetic isotope effect (KIE) – ratio of rate constants with that for the light isotope $k$ in the numerator

\[ ^2\text{H-KIE (D-KIE)} \] by convention is $k_H/k_D$

2. equilibrium isotope effect (EIE) – ratio of $K_{eq}$s with that for the light isotope rate constant in the numerator
II. Vocabulary for enzymologists

3. primary KIE–

a. KIE on reaction (or elementary step thereof) in which bond to isotopic pair is cleaved or undergoes change in bond order

b. Always *normal*, meaning $k_{\text{light}}/k_{\text{heavy}} > 1$

c. For $^2\text{H}$ and $^3\text{H}$ can be very large (up to 100 or more for $^2\text{H}$ and 1000 for $^3\text{H}$)

d. Smaller for heavier atoms (e.g., <1.05 for $^{13}\text{C}$, $^{18}\text{O}$)
II. Vocabulary for enzymologists

4. secondary KIE
   a. KIE on reaction (or elementary step thereof) in which bond to isotopic pair *is not* cleaved and does not undergo change in bond order
   b. Can be normal or *inverse*, meaning $k_{\text{light}}/k_{\text{heavy}} < 1$
   c. Usually small (e.g., ≤ 1.8 for $^2\text{H}$)

5. intrinsic KIE
   a. the actual $k_{\text{light}}/k_{\text{heavy}}$ on an elementary chemical step
   b. distinguished from an observed effect, as explained below
   c. has all the useful chemical mechanistic information, which Professor Hammes-Schiffer will discuss how to extract
II. Vocabulary for enzymologists

6. “masking” of intrinsic KIEs
   a. diminution of the observed KIE relative to the Intrinsic KIE

b. KIEs are measured on an observed rate constant ($k_{\text{intrinsic}}, k_{\text{cat}}, k_{\text{cat}}/K_M$)
   
i) $k_{\text{cat}}$ – number of cycles each enzyme molecule completes per unit time with saturating substrate(s)
   
   ii) $k_{\text{cat}}/K_M$ – equivalent to the apparent 2\textsuperscript{nd} order $k$ for productive encounter of substrate with the enzyme

c. even large intrinsic KIEs can fail to be “expressed” (can be masked) in $k_{\text{cat}}, k_{\text{cat}}/K_M$, or both as a result of the multi-step nature of enzyme reactions and unfavorable alignment of individual rate constants
III. Theoretical Examples

Simple kinetic scheme:

\[
E + S \xrightleftharpoons[k_1][k_{-1}] E\cdot S \xrightarrow{k_2} E\cdot P \xrightarrow{k_3} E + P
\]

Equations:

\[
k_{\text{cat}} = \frac{k_2k_3}{k_2 + k_3}
\]

\[
K_M = \frac{k_{-1}k_3 + k_2k_3}{k_1(k_2 + k_3)}
\]

\[
k_{\text{cat}}/K_M = \frac{k_1(k_2k_3)}{k_{-1}k_3 + k_2k_3}
\]

Chemistry occurs
III. Theoretical Examples

Scenario 1: “sticky” substrate, rate-determining chemistry, fast product release

1. Intrinsic KIE expressed in $k_{\text{cat}}$
2. Intrinsic KIE masked in $k_{\text{cat}}/K_M$
### Scenario 1:

\[
\begin{align*}
    k_1 &= 10^8 \text{ M}^{-1}\text{s}^{-1}, \quad k_{-1} = 1 \text{ s}^{-1} \\
    k_2 &= 100 \text{ s}^{-1} \text{ (H)}; \quad 10 \text{ s}^{-1} \text{ (D)} \\
    k_3 &= 10^4 \text{ s}^{-1} \\
    K_d &= 10 \text{ nM}
\end{align*}
\]

\[
\begin{align*}
    k_{\text{cat}, \text{H}} &= \frac{10^6}{1.01 \times 10^4} = 99 \text{ s}^{-1} \\
    k_{\text{cat}, \text{D}} &= \frac{10^5}{1.001 \times 10^4} = 9.99 \text{ s}^{-1} \\
    \frac{k_{\text{cat}, \text{H}}}{k_{\text{cat}, \text{D}}} &= \frac{99}{9.99} = 9.9 \\
    \frac{k_{\text{cat}/K_M, \text{H}}}{k_{\text{cat}/K_M, \text{D}}} &= \frac{10^{14}}{10^4+10^6} = 9.9 \times 10^7 \text{ M}^{-1}\text{s}^{-1} \\
    \frac{k_{\text{cat}/K_M, \text{D}}}{k_{\text{cat}/K_M, \text{H}}} &= \frac{10^{13}}{10^4+10^5} = 9.1 \times 10^7 \text{ M}^{-1}\text{s}^{-1} \\
\end{align*}
\]

Expressed effect on $k_{\text{cat}}$; no expressed effect on $k_{\text{cat}/K_M}$.
III. Theoretical Examples

Scenario 1:

\[ k_1 = 10^8 \text{M}^{-1}\text{s}^{-1}, \quad k_{-1} = 1 \text{s}^{-1} \]

\[ k_2 = 100 \text{s}^{-1} \text{ (H); } 10 \text{s}^{-1} \text{ (D)} \]

\[ k_3 = 10^4 \text{s}^{-1} \]

\[ K_d = 10 \text{nM} \]

Expressed effect on \( k_{cat} \); no expressed effect on \( k_{cat}/K_M \)

\[ E + S \xrightleftharpoons[k_{-1}]{k_1} E \cdot S \xrightarrow[k_2]{k_{-2}} E \cdot P \xrightarrow[k_3]{k_{-3}} E + P \]

\[ [S] = 20, 1, 0.4, 0.1 \mu\text{M} \]

- \( k_2 = 100 \text{s}^{-1} \)
- \( k_2 = 10 \text{s}^{-1} \)

\[ \text{P} \]

\[ \text{Time (s)} \]

Expressed effect on \( k_{cat} \); no expressed effect on \( k_{cat}/K_M \)
III. Theoretical Examples

Scenario 2: rapid-equilibrium substrate binding (not sticky), slow chemistry, rate-determining product release

1. Intrinsic KIE masked in $k_{\text{cat}}$
2. Intrinsic KIE expressed in $k_{\text{cat}}/K_M$
III. Theoretical Examples

Scenario 2:

\[ k_1 = 10^8 \text{ M}^{-1}\text{s}^{-1}, \quad k_{-1} = 10^5 \text{ s}^{-1} \]
\[ k_2 = 100 \text{ s}^{-1} \text{ (H)}; 10 \text{ s}^{-1} \text{ (D)} \]
\[ k_3 = 1 \text{ s}^{-1} \]
\[ K_d = 1 \text{ mM} \]

\[ E + S \xrightleftharpoons[k_{-1}]{k_1} E\cdot S \xrightarrow{k_2} E\cdot P \xrightarrow{k_3} E + P \]

\[ k_{\text{cat}, \text{H}} = \frac{100}{101} = 0.99 \text{ s}^{-1} \]
\[ k_{\text{cat}, \text{D}} = \frac{10}{11} = 0.91 \text{ s}^{-1} \]

\[ \frac{k_{\text{cat}, \text{H}}}{k_{\text{cat}, \text{D}}} = \frac{0.99}{0.91} = 1.09 \]

\[ \frac{k_{\text{cat}}/K_M, \text{H}}{k_{\text{cat}}/K_M, \text{D}} = \frac{9.99\times10^4 \text{ M}^{-1}\text{s}^{-1}}{9.999\times10^3 \text{ M}^{-1}\text{s}^{-1}} = 9.99 \]

*No expressed effect on* \( k_{\text{cat}}; \text{ expressed effect on} \ k_{\text{cat}}/K_M \)
III. Theoretical Examples

Scenario 2:
\[ k_1 = 10^8 \text{ M}^{-1}\text{s}^{-1}, \quad k_{-1} = 10^5 \text{ s}^{-1} \]
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\[ k_3 = 1 \text{ s}^{-1} \]
\[ K_d = 1 \text{ mM} \]

No expressed effect on \( k_{cat} \); expressed effect on \( k_{cat}/K_M \)
III. Theoretical Examples

Substrate competition: does the enzyme select for one isotope over the other? If so, which one? How can you measure?
III. Theoretical Examples

Scenario 2:

$\begin{align*}
  k_1 &= 10^8 \text{ M}^{-1}\text{s}^{-1}, \ k_{-1} = 10^5 \text{ s}^{-1} \\
  k_2 &= 100 \text{ s}^{-1} (\text{H}); \ 10 \text{ s}^{-1} (\text{D}) \\
  k_3 &= 1 \text{ s}^{-1} \\
  K_d &= 1 \text{ mM}
\end{align*}$

No expressed effect on $k_{cat}$; expressed effect on $k_{cat}/K_M$
III. Theoretical Examples

Scenario 3: “sticky” substrate, fast chemistry, rate-determining product release

1. Intrinsic KIE masked in $k_{cat}$
2. Intrinsic KIE masked in $k_{cat}/K_M$
III. Theoretical Examples

Scenario 3:
\[ k_1 = 10^7 \text{ M}^{-1}\text{s}^{-1}, \quad k_{-1} = 0.1 \text{ s}^{-1} \]
\[ k_2 = 100 \text{ s}^{-1} \text{ (H); } 10 \text{ s}^{-1} \text{ (D)} \]
\[ k_3 = 1 \text{ s}^{-1} \]
\[ K_d = 10 \text{ nM} \]

\[ k_{\text{cat}}, \text{H} = \frac{100}{101} = 0.99 \text{ s}^{-1} \]
\[ k_{\text{cat}}, \text{D} = \frac{10}{11} = 0.91 \text{ s}^{-1} \]

\[ \frac{k_{\text{cat}}, \text{H}}{k_{\text{cat}}, \text{D}} = \frac{0.99}{0.91} = 1.09 \]

\[ k_{\text{cat}}/K_M, \text{H} = \frac{10^9}{0.1+100} = 9.999 \times 10^6 \text{ M}^{-1}\text{s}^{-1} \]
\[ k_{\text{cat}}/K_M, \text{D} = \frac{10^8}{0.1+10} = 9.9 \times 10^6 \text{ M}^{-1}\text{s}^{-1} \]

No expressed effect on \( k_{\text{cat}} \) and on \( k_{\text{cat}}/K_M \)
III. Theoretical Examples

Scenario 4: rapid-equilibrium substrate binding, rate-determining chemistry, fast product release

1. Intrinsic KIE expressed in $k_{\text{cat}}$
2. Intrinsic KIE expressed in $k_{\text{cat}}/K_M$
Scenario 4:

\[ k_1 = 10^8 \text{ M}^{-1}\text{s}^{-1}, \quad k_{-1} = 10^5 \text{ s}^{-1} \]
\[ k_2 = 100 \text{ s}^{-1} \text{ (H); } 10 \text{ s}^{-1} \text{ (D)} \]
\[ k_3 = 10^4 \text{ s}^{-1} \]

\[ K_d = 1 \text{ mM} \]

\[ k_{\text{cat}, \text{H}} = \frac{1 \times 10^6}{1.01 \times 10^4} = 99 \text{ s}^{-1} \]
\[ k_{\text{cat}/K_M, \text{H}} = \frac{1 \times 10^{14}}{1 \times 10^9 + 1 \times 10^6} = 9.99 \times 10^4 \text{ M}^{-1}\text{s}^{-1} \]

\[ k_{\text{cat}, \text{D}} = \frac{1 \times 10^5}{1.001 \times 10^4} = 9.99 \text{ s}^{-1} \]
\[ k_{\text{cat}/K_M, \text{D}} = \frac{1 \times 10^{13}}{1 \times 10^9 + 1 \times 10^5} = 9.99 \times 10^3 \text{ M}^{-1}\text{s}^{-1} \]

\[ \frac{k_{\text{cat}, \text{H}}}{k_{\text{cat}, \text{D}}} = \frac{99}{9.99} = 9.9 \]

\[ \frac{k_{\text{cat}/K_M, \text{H}}}{k_{\text{cat}/K_M, \text{D}}} = 10 \]

**Expressed effect both on** \( k_{\text{cat}} \) **and on** \( k_{\text{cat}/K_M} \)**
Scenario 4:

\[ k_1 = 10^8 \text{ M}^{-1}\text{s}^{-1}, \quad k_{-1} = 10^5 \text{ s}^{-1} \]

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\[ K_d = 1 \text{ mM} \]

III. Theoretical Examples

**Expressed effect both on** \( k_{\text{cat}} \) **and on** \( k_{\text{cat}}/K_M \)**
IV. Case study: TauD

TauD: Taurine \(\alpha\)-ketoglutarate dioxygenase

A. Reaction:

\[
\text{-O}_3\text{S}^- + \text{O}_2 + \text{NH}_3^+ + \text{CO}_2 \rightarrow \text{NH}_3^+ + \text{OH}^- + \text{CO}_2 + \text{H}_2\text{O} + \text{R}\text{-CO}_2\text{OH}^- + \text{-O}_3\text{S}^-
\]
IV. Case study: TauD

B. Mechanism:
IV. Case study: TauD

C. Expression of intrinsic KIE in steady-state kinetic parameters

Reported:

\[ k_{\text{cat, } H} = 1.3 \text{ s}^{-1} \]
\[ k_{\text{cat, } D} = 0.11 \text{ s}^{-1} \]
\[ k_{\text{cat, } H}/k_{\text{cat, } D} \sim 12 \]
IV. Case study: TauD

D. Direct measurement of $k_H$ and $k_D$ for H• abstraction by ferryl complex

IV. Case study: TauD

D. Direct measurement of $k_H$ and $k_D$ for H• abstraction by ferryl complex: transient state techniques

A = TauD•Fe(II)•αKG•taurine (O$_2$-free)
B = O$_2$-saturated buffer
IV. Case study: TauD

D. Direct measurement of $k_H$ and $k_D$ for H• abstraction by ferryl complex: transient state techniques

$k_H = 13 \pm 2 \text{ s}^{-1}$

$k_D = 0.35 \pm 0.05 \text{ s}^{-1}$

$k_H/k_D = 37$

Intrinsic KIE? Not quite!

IV. Case study: TauD

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IV. Case study: TauD

E. Effect of uncoupling

1. Dramatic retardation of $\mathbf{D}^\bullet$ abstraction by ferryl exposes unproductive pathway for decay
2. ~ 30% uncoupled decay indicates that unproductive pathway has $k = 0.1 \text{ s}^{-1}$ and $\mathbf{D}^\bullet$ abstraction has $k = 0.25 \text{ s}^{-1}$
IV. Case study: TauD

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IV. Case study: TauD

E. Effect of uncoupling

\[ \frac{k_H}{k_D} = 52 \]
IV. Case study: TauD

F. What about $^3$H?

Swain-Schaad Equation

\[ \frac{k_H}{k_T} = \left( \frac{k_H}{k_D} \right)^{1.44} = 50^{1.44} = 280!! \]

\[ k_T = 13 \text{ s}^{-1}/280 = 0.0546 \]

Uncoupling $\sim 70\%$!

Predicted $k_{\text{cat}}/K_M > 3$
IV. Case study: TauD

F. What about $^3$H?

Sharon Hammes-Schiffer

Lipoxygenase $k_H/k_D = 80$; $k_H/k_T \sim 1000 \sim 2(k_H/k_D)^{1.44}$

Predicted $k_{\text{cat}}/K_M > 5$

Not a conventional selection effect, but a result of failures in events involving heavy isotope
IV. Case study: TauD

Apparent selection effect occurring \textit{after the first irreversible step} (ferryl formation)

Goes against enzymology dogma
V. Conclusion

A. Intrinsic KIEs potentially provide detailed insight into enzyme mechanisms

B. Meaningful extraction of the required intrinsic effects is very challenging and fraught with peril
Theory of Proton-Coupled Electron Transfer for Bioinorganic Chemists

Sharon Hammes-Schiffer
Pennsylvania State University

Note: More information on PCET theory is available in the following JPC Feature Article:
Hammes-Schiffer and Soudackov, JPC B 112, 14108 (2008)

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General Definition of PCET

- Electron and proton transfer reactions are coupled
- Electron and proton donors/acceptors can be the same or different
- Electron and proton can transfer in same direction or different directions
- Concerted vs. sequential PCET
- Concerted PCET is also denoted EPT, CPET, CEPT
- Hydrogen atom transfer (HAT) is a subset of PCET
- Distinction between EPT and HAT defined in terms of nonadiabaticity
  (not universally accepted definition)
Classification Scheme for PCET


Electrons respond instantaneously to nuclei; remains in electronic ground state.

Proton responds instantaneously to other nuclei; remains in vibrational ground state.

A: vib ad
Adiabatic PT

B: vib nad
Vib nonad PT HAT

C: vibron nad
Nonad EPT (concerted PCET)
Classification Scheme: Proton Potentials

Case A: electronically and vibrationally adiabatic

adiabatic PT

Case B: electronically adiabatic, vibrationally nonadiabatic

vib nonadiabatic PT, HAT

Case C: electronically and vibronically nonadiabatic

nonadiabatic EPT

adiabatic states

diabatic states
Diabatic states:

(1) $D_e^-$ $A_e$

(2) $D_e^- A_e$

Solvent coordinate

$$z_e = \int d\mathbf{r} (\rho_2 - \rho_1) \Phi_{\text{in}}(\mathbf{r})$$

Nonadiabatic ET rate:

$$k = \frac{2\pi}{h} V_{12}^2 (4\pi k_B T)^{-1/2} \exp\left[-\frac{\Delta G^\dagger}{k_B T}\right]$$

$$\Delta G^\dagger = \left(\Delta G^\circ + \lambda\right)^2 / (4\lambda)$$

$V_{12}$: coupling between diabatic states
Proton-Coupled Electron Transfer Theory

Soudackov and Hammes-Schiffer, JCP 111, 4672 (1999)

- Four diabatic states: 
  
  1a) \( D_e^- \rightarrow D_p^+ H L A_p \rightarrow A_e \)
  
  1b) \( D_e^- \rightarrow D_p L L HA_p^+ \rightarrow A_e \)
  
  2a) \( D_e^- \rightarrow D_p^+ H L A_p \rightarrow A_e^- \)
  
  2b) \( D_e^- \rightarrow D_p L L HA_p^+ \rightarrow A_e^- \)

- Free energy surfaces depend on 2 collective solvent coordinates

  \[
  \text{PT } (1a) \rightarrow (1b): \quad z_p = \int dr (\rho_{1b} - \rho_{1a}) \Phi_{\text{in}}(r)
  \]

  \[
  \text{ET } (1a) \rightarrow (2a): \quad z_e = \int dr (\rho_{2a} - \rho_{1a}) \Phi_{\text{in}}(r)
  \]

- Hydrogen nucleus: quantum mechanical wavefunction
Sequential vs. Concerted PCET

- Sequential: ET-PT or PT-ET
- Concerted: EPT
- Mechanism determined by relative energies and couplings
- $1b$ and $2a$ much higher in energy $\Rightarrow$ concerted EPT, reduces to two-state model: $(1a/1b) \Rightarrow (2a/2b)$
2D Vibronic Free Energy Surfaces

- Free energy surfaces depend on 2 collective solvent coordinates: $z_p$ (PT) and $z_e$ (ET)
- Electron-proton vibronic surfaces corresponding to different proton vibrational states for each electronic state
- Vibronic coupling between reactant/product states

$$V_{\mu\nu} = \left\langle \Phi^I(r_e, r_p) \right| \hat{H} \left| \Phi^II(r_e, r_p) \right\rangle \approx V^\text{el} S_{\mu\nu}$$
Fundamental Mechanism for PCET
Fundamental Mechanism for PCET

Solvent Coordinate

$r_p$
Fundamental Mechanism for PCET

Solvent Coordinate

$r_p$
**PCET Rate Constant Expressions**

*Soudackov and SHS, JCP 2000; Soudackov, Hatcher, SHS, JCP 2005*

- Typically PCET reactions nonadiabatic due to small vibronic coupling
- Use Golden Rule to derive rate constant expressions

Reactant $D^{-}$ $A$
Product $D$ $A^{-}$

\[
k = \frac{2\pi}{\hbar} \sum_{\mu} P_{\mu}^{\dagger} \sum_{\nu} (4\pi\lambda k_B T)^{-1/2} \left(V^\text{el} S_{\mu\nu}\right)^2 \exp\left[-\frac{\Delta G^\dagger_{\mu\nu}}{(k_B T)}\right]
\]

\[
\Delta G^\dagger_{\mu\nu} = \left(\Delta G^0_{\mu\nu} + \lambda\right)^2 / (4\lambda)
\]
Role of H Wavefunction Overlap

- Rate decreases as overlap decreases (as $R$ increases)
  \[ k_H \propto (H \text{ overlap})^2 \]

- KIE increases as overlap decreases (as $R$ increases)
  \[ \frac{k_H}{k_D} \propto \frac{(H \text{ overlap})^2}{(D \text{ overlap})^2} \] (for a pair of vibronic states)
Ruthenium Polypyrindyl Complexes

Iordanova and Hammes-Schiffer, JACS 2002

<table>
<thead>
<tr>
<th>CompA</th>
<th>KIE</th>
<th>Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>16.1 ± 0.4</td>
<td>1.0</td>
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</table>

<table>
<thead>
<tr>
<th>CompB</th>
<th>KIE</th>
<th>Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>11.3 ± 1.3</td>
<td>9.6</td>
</tr>
</tbody>
</table>

Experiments: Binstead and Meyer, JACS 1987;
Farrer and Thorp, Inorg. Chem. 1999
Donor-Acceptor Distances

Ru-OH₂----O-Ru

$R_{OO}$ decreases as steric crowding near acceptor O decreases

CompA
Rate = 1
KIE = 16.1
$R_{OO} = 2.70$ Å

CompB
Rate = 9.6
KIE = 11.4
$R_{OO} = 2.64$ Å
Include Proton Donor-Acceptor Motion

- Vibronic coupling (overlap) depends strongly on $R$
- Derived analytical rate constant expressions in various regimes, approximating overlap as decreasing exponentially with $R$

$$V_{\mu\nu}(R) \approx V^{\text{el}} S^0_{\mu\nu} \exp\left[-\alpha_{\mu\nu} (R - R_{\text{eq}})\right]$$

$V^{\text{el}}$: electronic coupling
$S^0_{\mu\nu}$: proton wavefunction overlap at $R_{\text{eq}}$
$R_{\text{eq}}$: equilibrium $R$ value

- Thermal averaging over $R$ using probability distribution $P(R)$

$$k = \int_0^{\infty} k(R)P(R) dR$$

*These two approaches become equivalent in certain regimes*

*Analytical derivations: Soudackov, Hatcher, SHS, JCP 2005*
Excited Vibronic States

\[ k = \frac{2\pi}{h} \sum_{\mu} P_{\mu}^{1} \sum_{\nu} \left( 4\pi \lambda_{\mu\nu} k_{B} T \right)^{-1/2} |V_{\mu\nu}|^2 \exp\left[ -\Delta G^{\dagger}_{\mu\nu} / (k_{B} T) \right] \]

Relative contributions from excited vibronic states determined from balance of factors (different for H and D, depends on T)
- Boltzmann probability of reactant state
- Free energy barrier
- Vibronic couplings (overlaps)
Input Quantities

• Reorganization energies ($\lambda$)
  - outer-sphere (solvent): dielectric continuum model or MD
  - inner-sphere (solute modes): QM calculations of solute

• Free energy of reaction for ground states (driving force) ($\Delta G^0$)
  - QM calculations or estimate from pK$_a$’s and redox potentials

• $R$-mode mass, frequency ($M$, $\Omega$) or probability distribution ($P(R)$)
  - QM calculation of normal modes or MD
  - $R$-mode is dominant mode that changes proton donor-acceptor distance

• Proton vibrational wavefunction overlaps ($S_{\mu\nu}$, $\alpha_{\mu\nu}$)
  - approximate proton potentials with harmonic/Morse potentials
    or generate with QM methods
  - numerically calculate H vibrational wavefunctions w/ Fourier grid methods

• Electronic coupling ($V^{el}$)
  - QM calculations of electronic matrix element or splitting

Note: this is a multiplicative factor that cancels for KIE calculations
Warnings about Prediction of Trends

*Edwards, Soudackov, SHS, JPC A113, 2117 (2009)*

- Experimentally challenging to change only a single parameter
  
  Examples:
  
  Increasing $R$ often decreases $\Omega$; may impact KIE in opposite way
  Changing driving force by altering $pK_a$ can also impact $R$

- Relative contributions from pairs of vibronic states are sensitive to parameters, H vs. D, and temperature
  
  Must perform full calculation (converging number of reactant and product vibronic states) to predict trend

- Rate constants are qualitatively different in distinct regimes
  
  Example:
  
  Low-frequency R-mode expression predicts KIE decreases with $T$
  Fixed-$R$ and high-frequency R-mode expressions can lead to either increase or decrease of KIE with $T
Applications to PCET Reactions

- Amidinium-carboxylate salt bridges (Nocera), JACS 1999
- Iron bi-imidazoline complexes (Mayer/Roth), JACS 2001
- Ruthenium polypyridyl complexes (Meyer/Thorp), JACS 2002
- DNA-acrylamide complexes (Sevilla), JPCB 2002
- Ruthenium-tyrosine complex (Hammarström), JACS 2003
- Soybean lipoxygenase enzyme (Klinman), JACS 2004, 2007
- Rhenium-tyrosine complex (Nocera), JACS 2007
- Quinol oxidation (Kramer), JACS 2009
- Osmium complex attached to gold electrode (Finklea), JACS 2010
- Proton relays in electrochemical PCET (Savéant), JACS 2011

Theory explained experimental trends in rates, KIEs, T-dependence, pH-dependence
Soybean Lipoxygenase

Knapp, Rickert, Klinman, JACS 124, 3865 (2002)

- Catalyzes oxidation of unsaturated fatty acids
- Experiment: KIE \( \approx 81 \) at room temperature
  - Weak temperature dependence of rates and KIE
  - Distal mutations impact KIE

Net H-atom transfer
Proton-coupled electron transfer (PCET) mechanism
Low-Frequency $R$-mode

$\Omega << k_B T$

$$k = \sum_{\mu} \sum_{\nu} P_{\mu} \left| V^{cl} S_{\mu\nu}^0 \right|^2 \frac{2k_B T \alpha_{\mu\nu}^2}{M \Omega^2} \exp \left[ \frac{\pi}{\left( \lambda + \lambda_\alpha \right) k_B T} \right] \exp \left[ -\frac{\left( \Delta G_{\mu\nu}^0 + \lambda + \lambda_\alpha \right)^2}{4 \left( \lambda + \lambda_\alpha \right) k_B T} \right]$$

$$\lambda_\alpha = \frac{\hbar^2 \alpha_{\mu\nu}^2}{2M}$$

$M, \Omega$: mass and frequency of $R$-mode

$\alpha$: exponential $R$-dependence of vibronic coupling

Approximate KIE

(only ground states)

$$\text{KIE} \approx \frac{\left| S_H \right|^2}{\left| S_D \right|^2} \exp \left\{ \frac{-2k_B T}{M \Omega^2} \left( \alpha_D^2 - \alpha_H^2 \right) \right\}$$

• $T$-dependence of KIE determined mainly by $\alpha$ and $\Omega$
• Magnitude of KIE determined also by ratio of overlaps: smaller overlap $\rightarrow$ larger KIE
High KIE: small overlap and dominance of lowest energy states
Weak T-dependence of KIE: local R-mode ($\Omega$ not too low)

Blue lines: Calculated results
- Dashed: Multistate continuum theory; fit two parameters to kinetic data
- Solid: Molecular dynamics with explicit protein/solvent; no fitting to kinetic data

Red circles: Experimental data

Hatcher, Soudackov, SHS, JACS 2004 and 2007
Predictions for Lipoxygenase

• Magnitude of KIE will increase as equilibrium C–O distance increases
• Temperature dependence of KIE will increase as frequency of C–O motion decreases

\[
\text{KIE} \approx \left| \frac{S_H}{S_D} \right|^2 \exp \left\{ -\frac{2k_B T}{M \Omega^2} \left( \alpha_D^2 - \alpha_H^2 \right) \right\}
\]

Experiments by Klinman group:
Mutation of Ile553, \(\sim 15\) Å from iron →
Magnitude and T-dependence of KIE increase as residue 553 less bulky

\[\text{Knapp, Rickert, Klinman, JACS 124, 3865 (2002)}\]
Calculations on Mutants

*Edwards, Soudackov, and SHS, JPC B 2010*

- As residues get less bulky, $R_{eq}$ increases and $\Omega$ decreases
- As $R_{eq}$ increases, magnitude of KIE increases
- As $\Omega$ decreases, T-dependence of KIE increases

\[
\text{KIE} \approx \frac{|S_H|^2}{|S_D|^2} \exp \left\{ \frac{-2k_BT}{M\Omega^2} \left( \alpha_D^2 - \alpha_H^2 \right) \right\}
\]

Calculated KIE from full expression including excited vibronic states

Quinol Oxidation

Experiments: Cape, Bowman, Kramer, JACS 127, 4208 (2005)
• Photoexcited Ru complex to MLCT state in acetonitrile
• KIE at 296K is 1.87 and 3.45 for UQH$_2$ and PQH$_2$
• KIE increases with T for UQH$_2$ and decreases with T for PQH$_2$
• Similar behavior observed in cyt $bc1$ complex (Cape, Kramer) and oxidation of quinol by tocopherol in ethanol (Nagaoka)
• Most theories predict that KIE decreases as T increases: more tunneling at low T, pre-exponential term for low frequency $R$-mode
Calculations on Quinol Oxidation

Ludlow, Soudackov, SHS, JACS 2009

• DFT on complex
  \( \Omega \approx 1000 \text{ cm}^{-1}, M = 7.2 \text{ amu} \) for O–N motion in both quinols
  \( R_{\text{eq}} \approx 2.65 \text{ Å} \) for both quinols
  Stiff H-bond, high-frequency \( R \)-mode

• Calculated solvent reorganization energies:
  \( \lambda \approx 6.3 \text{ kcal/mol} \) for both quinols
  Net ET from N to Ru over 4.2 Å in acetonitrile

• Estimated driving forces from redox potentials, \( \text{pK}_a \)s
  \( \Delta G^0 \approx -6.0, -4.5 \text{ kcal/mol} \) for UQH\(_2\), PQH\(_2\)

• Similarity of \( \lambda \) and \( \Delta G^0 \) magnitudes indicates close to inverted Marcus region, \( \lambda < -\Delta G^0 \)

\[
\text{QOH} \cdots \cdot \cdot \cdot \text{NRu(III)} \rightarrow \text{QO}^\cdot \cdots \cdot \cdot \cdot \text{HNRu(II)}
\]
Inverse T-Dependence of KIE

- T-dependence of KIE very sensitive to interplay among driving force, reorganization energy, and vibronic coupling
- Generated proton potentials with DFT and fit $\lambda$ and $\Delta G^0$ to experimental data for fixed-$R$ and full dynamical rate constants

![Graphs showing ln(KIE) vs. 1000/T for UQH$_2$ and PQH$_2$. Blue: Theory, Red: Experiment.](image)
Contributions from Excited Vibronic States

- 0/0 transition in inverted Marcus region
- 0/0 and 0/1 transitions have similar free energy barriers
- Vibronic coupling (overlap) greater for 0/1 than 0/0
- 0/1 dominant contributor to overall rate
- 0/1 contributes 89% for UQH$_2$ and 60% for PQH$_2$: subtle differences in proton potentials and driving forces
Explanation for Inverse T-Dependence

- Stiff H-bond (high-frequency \( R \)-mode)
- Small reorganization energy
- 0/0 transition in inverted region, 0/1 in normal region
- 0/1 is dominant contributor to overall rate

\[
\text{KIE } \propto \exp \left[ -\left( \Delta G_{01}^{\dagger} (H) - \Delta G_{01}^{\dagger} (D) \right) / k_B T \right]
\]

- Vibronic energy level splittings smaller for D than H

\[
\Delta G_{01}^{\dagger} (H) > \Delta G_{01}^{\dagger} (D)
\]

- KIE increases as \( T \) increases

\[
\Delta G_{01}^{\dagger} (H) = 2.89 \text{ kcal/mol}
\]

\[
\Delta G_{01}^{\dagger} (D) = 1.01 \text{ kcal/mol}
\]
Electrochemical PCET Theory

Derived expressions for electrochemical rate constants and current densities
Venkataraman, Soudackov, SHS, JPC C 2008
**Proton Relay System: Proton Transport**

*Costentin, Robert, Savéant, Tard, Angew. Chem. Int. Ed. 2010, 49, 1-5*

<table>
<thead>
<tr>
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<th>System I KIE</th>
<th>System II KIE</th>
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<tr>
<td>Experiment</td>
<td>1.7</td>
<td>2.4</td>
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$k_1/k_{II}$

Exptl paper: adiabatic ET treatment $\rightarrow$ System II slower because of larger inner-sphere reorganization energy

Nonadiabatic PCET treatment $\rightarrow$ System II slower because of smaller overlap between two-dimensional proton vibrational wavefunctions
Calculation of Standard Rate Constants

* Auer, Fernandez, SHS, JACS 2011

- Include proton donor-acceptor motion by thermal averaging over $R$ with probability distribution $P(R)$

$$k(\eta) = \int_0^\infty k(\eta; R)P(R)dR$$

- Two-proton system: include only symmetric motion where both distances decrease and increase concurrently

- Optimize reduced and oxidized structures with constrained $R$ and calculate proton potentials w/ DFT

\[\text{System I} \quad \text{System II}\]

\[\text{Graph}\]
Analysis of Results

**System I**

0/0 and 0/1 pairs at dominant $R = 2.46$ Å for anodic process.

**System II**

2D proton potentials/wavefunctions

0/3 pair at dominant $R$-distances

Dominant $R$ much less than equilibrium value and excited states contribute $\Rightarrow$ KIE(I) = 1.7, KIE(II) = 2.4, $k_1/k_2 = 16$

At equilibrium $R$-distances including only ground states $\Rightarrow$ KIE(I) $\approx 2.4 \times 10^3$, KIE(II) $\approx 2.7 \times 10^6$, $k_1/k_2 \approx 2.5 \times 10^5$, 

**Diagram**

- Energy vs Proton Coordinate [Å]
- 2D proton potentials/wavefunctions
Insights into Proton Relays

- Expect multidimensional process to be slower with higher KIEs because of smaller ground state overlaps
- Decrease in proton donor-acceptor distances and contributions from excited vibronic states $\rightarrow$
double PT only slightly slower, moderate KIEs

Interpretation of experimental data: Smaller standard rate constant for double proton transfer due to smaller overlap between ground state wavefunctions, leading to greater participation of excited states with higher free energy barriers

Prediction: Enhance rate constant by decreasing equilibrium proton donor-acceptor distances or altering thermal motions to facilitate concurrent decrease of these distances
Summary of KIEs for PCET

- KIEs for PCET arise from complex balance of many factors
  - reorganization energy
  - driving force
  - overlaps between proton vibrational wavefunctions
  - proton donor-acceptor distance and frequency
- KIEs can be as small as ~1.6 and as large as >80
- KIEs can exhibit varying temperature dependence
  - strong or weak dependence
  - increase or decrease with temperature