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

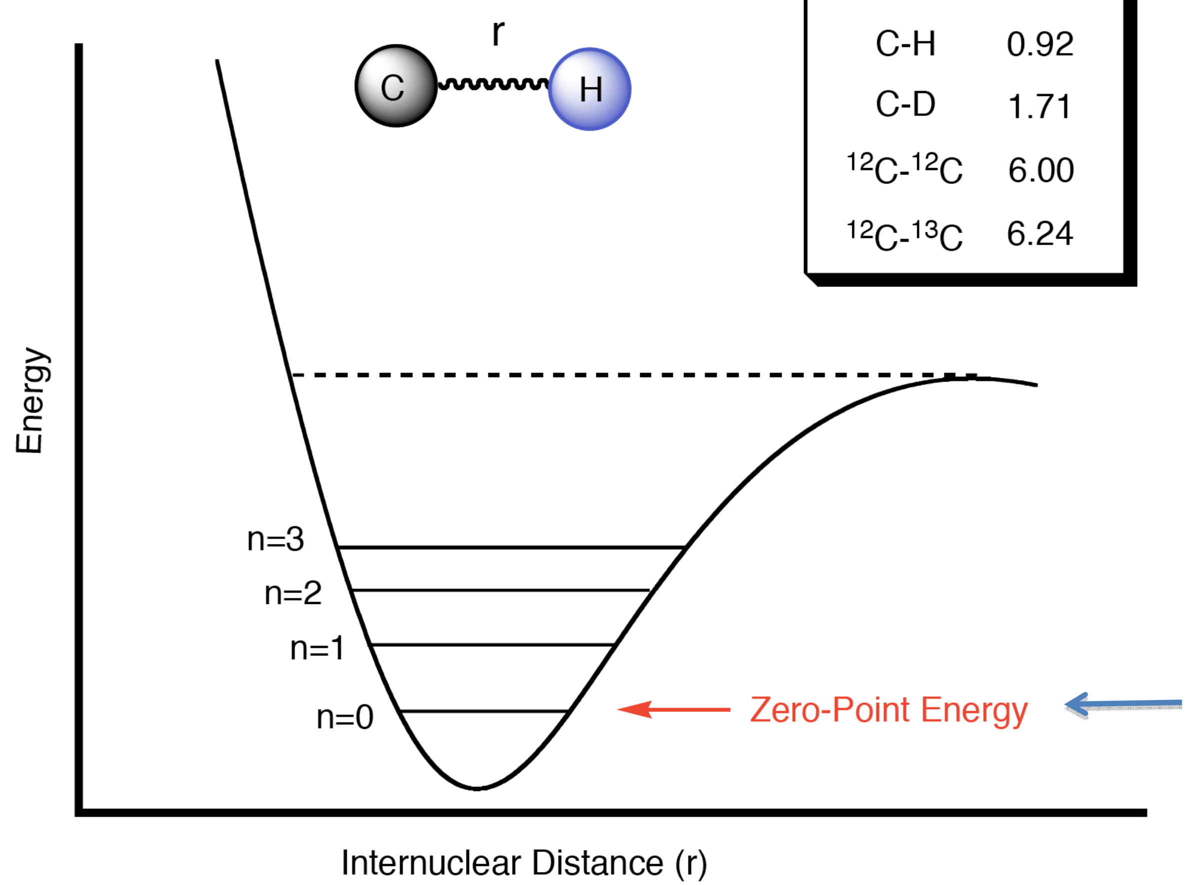
Vibrational energies (E_n) are dependent on the frequency of the bond stretch (ν), which is dependent on the reduced mass (m) of the two connected atoms (μ)

bond	μ
C-H	0.92
C-D	1.71
^{12}C-^{12}C	6.00
^{12}C-^{13}C	6.24

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

$$\mu = \frac{m_1 m_2}{m_1 + m_2}$$

$$E_n = (n + 1)\, h\nu$$



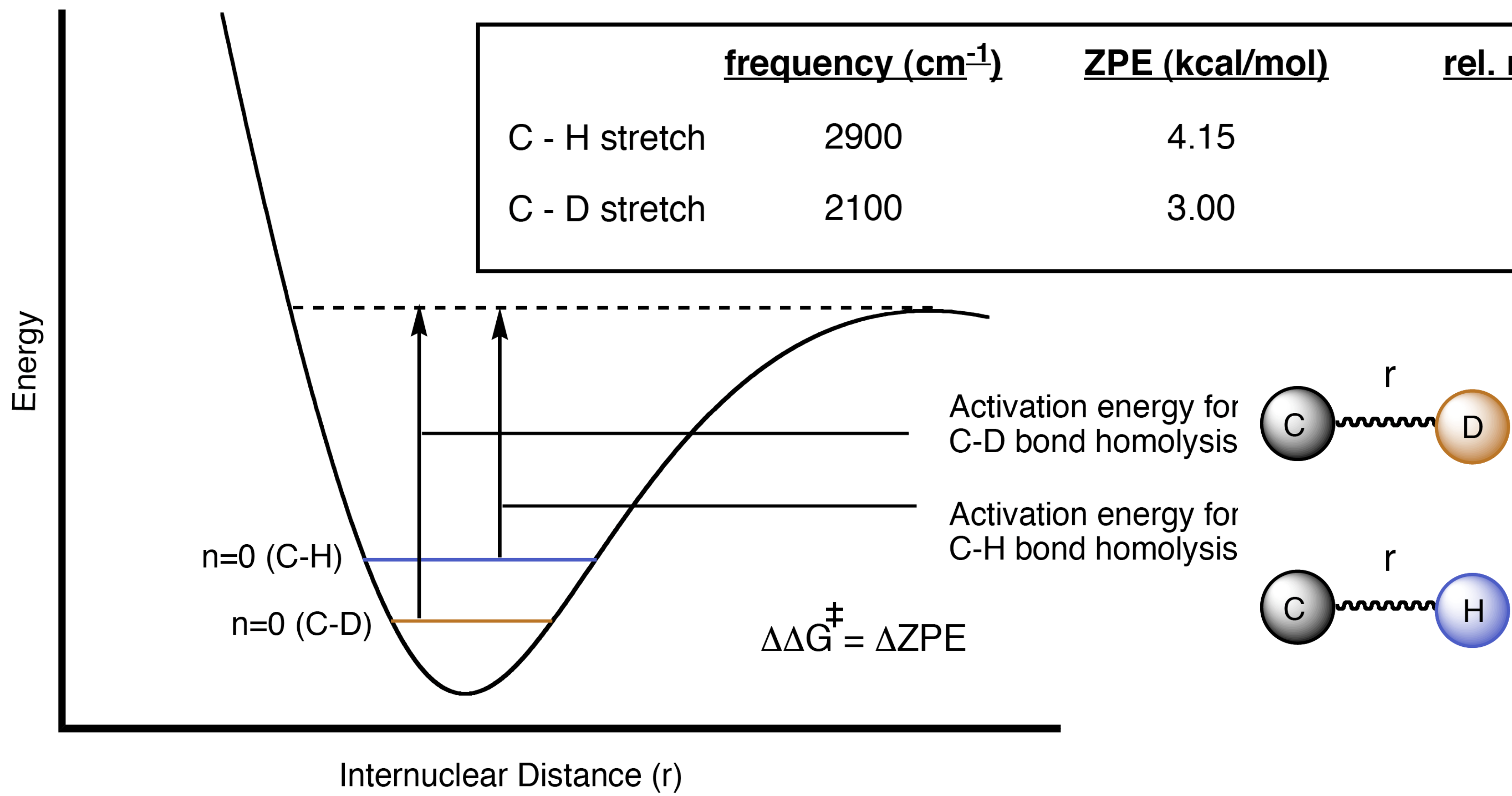
energy the molecule possesses in the ground vibrational state

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.

	frequency (cm^{-1})	ZPE (kcal/mol)	rel. rate (300 K)
C - H stretch	2900	4.15	~6.9
C - D stretch	2100	3.00	1.0



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}H-KIE

Caveat: the dominant effect giving rise to most, if not all $^{2/3}$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}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 $k_{light}/k_{heavy} > 1$

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

   d. Smaller for heavier atoms (e.g., <1.05 for ^{13}C, ^{18}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 $k_{light}/k_{heavy} < 1$

   c. Usually small (e.g., ≤ 1.8 for ^{2}H)

5. intrinsic KIE

   a. the actual k_{light}/k_{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 ($k_{intrinsic}$, k_{cat}, k_{cat}/K_M)

      i) k_{cat} – number of cycles each enzyme molecule completes per unit time with saturating substrate(s)

      ii) k_{cat}/K_M – equivalent to the apparent 2nd 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_{cat}, k_{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:

$$\boxed{E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E{\bullet}S \xrightarrow[\bigstar]{k_2} E{\bullet}P \xrightarrow{k_3} E + P}$$

Equations:

$$k_{cat} = \frac{k_2 k_3}{k_2 + k_3} \qquad K_M = \frac{k_{-1}k_3 + k_2 k_3}{k_1(k_2 + k_3)}$$

$$k_{cat}/K_M = \frac{k_1(k_2 k_3)}{k_{-1}k_3 + k_2 k_3}$$

★ Chemistry occurs

III. Theoretical Examples

<u>Scenario 1:</u> "sticky" substrate, rate-determining chemistry, fast product release

1. Intrinsic KIE expressed in k_{cat}
2. Intrinsic KIE masked in k_{cat}/K_M

III. Theoretical Examples

Scenario 1:
$k_1 = 10^8\ M^{-1}s^{-1}$, $k_{-1} = 1\ s^{-1}$
$k_2 = 100\ s^{-1}$ (H); $10\ s^{-1}$ (D)
$k_3 = 10^4\ s^{-1}$

$K_d = 10\ nM$

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E{\bullet}S \xrightarrow{k_2} E{\bullet}P \xrightarrow{k_3} E + P$$

$$k_{cat,\,H} = \frac{10^6}{1.01x10^4} = 99\ s^{-1} \qquad k_{cat}/K_{M,\,H} = \frac{10^{14}}{10^4+10^6} = 9.9x10^7\ M^{-1}s^{-1}$$

$$k_{cat,\,D} = \frac{10^5}{1.001x10^4} = 9.99\ s^{-1} \qquad k_{cat}/K_{M,\,D} = \frac{10^{13}}{10^4+10^5} = 9.1x10^7\ M^{-1}s^{-1}$$

$$\boxed{\frac{k_{cat,\,H}}{k_{cat,\,D}} = \frac{99}{9.99} = 9.9} \qquad \boxed{\frac{k_{cat}/K_{M,\,H}}{k_{cat}/K_{M,\,D}} = 1.09}$$

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

III. Theoretical Examples

Scenario 1:
$k_1 = 10^8$ M^{-1}s^{-1}, $k_{-1} = 1$ s^{-1}
$k_2 = 100$ s^{-1} (H); 10 s^{-1} (D)
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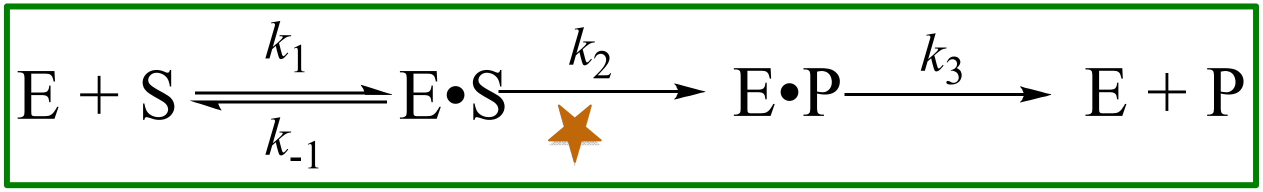
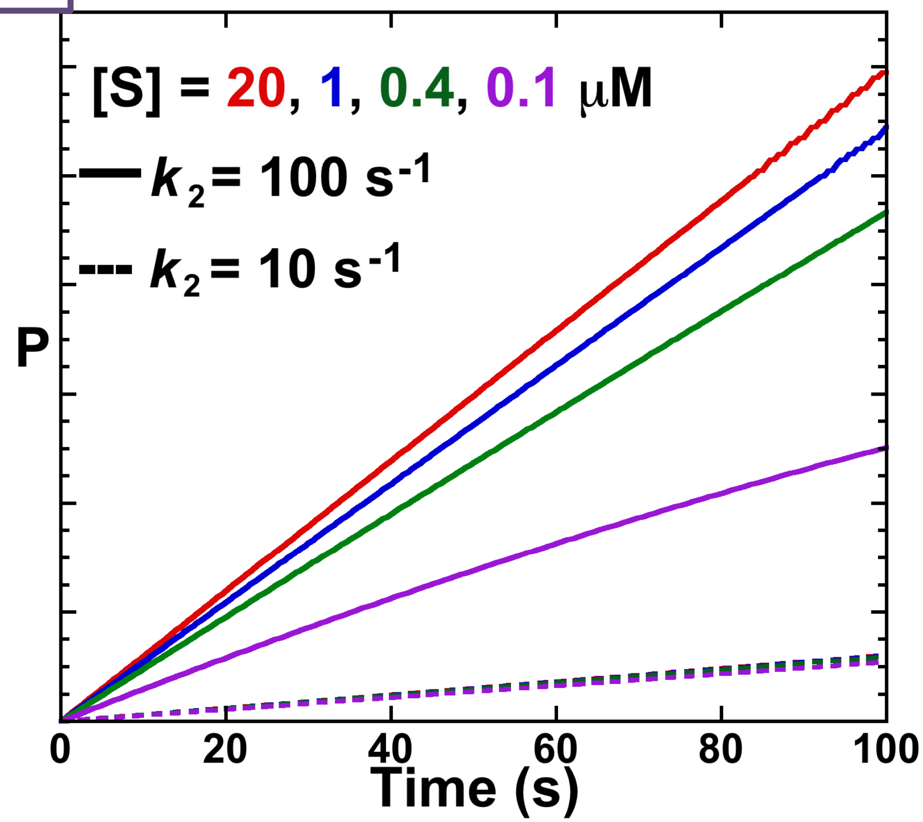




Expressed effect on k_{cat}; no expressed effect on k_{cat}/K_M

III. Theoretical Examples

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

1. Intrinsic KIE masked in k_{cat}
2. Intrinsic KIE expressed in k_{cat}/K_M

III. Theoretical Examples

Scenario 2:

$k_1 = 10^8\ \mathrm{M^{-1}s^{-1}}$, $k_{-1} = 10^5\ \mathrm{s^{-1}}$

$k_2 = 100\ \mathrm{s^{-1}}$ (H); $10\ \mathrm{s^{-1}}$ (D)

$k_3 = 1\ \mathrm{s^{-1}}$

$K_d = 1\ \mathrm{mM}$

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E\bullet S \xrightarrow{k_2} E\bullet P \xrightarrow{k_3} E + P$$

$$k_{cat,\,H} = \frac{100}{101} = 0.99\ \mathrm{s^{-1}}$$

$$k_{cat}/K_{M,\,H} = \frac{10^{10}}{10^5+100} = 9.99\mathrm{x}10^4\ \mathrm{M^{-1}s^{-1}}$$

$$k_{cat,\,D} = \frac{10}{11} = 0.91\ \mathrm{s^{-1}}$$

$$k_{cat}/K_{M,\,D} = \frac{10^{9}}{10^5+10} = 9.999\mathrm{x}10^3\ \mathrm{M^{-1}s^{-1}}$$

$$\boxed{\frac{k_{cat,\,H}}{k_{cat,\,D}} = \frac{0.99}{0.91} = 1.09}$$

$$\boxed{\frac{k_{cat}/K_{M,\,H}}{k_{cat}/K_{M,\,D}} = 9.99}$$

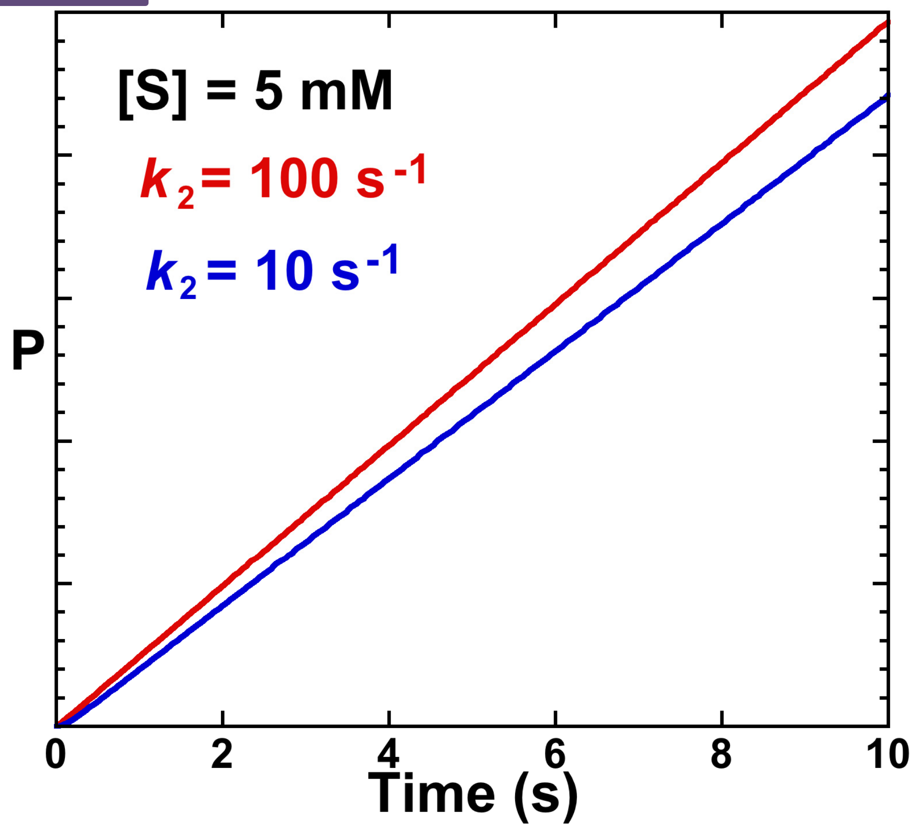
No expressed effect on k_{cat}; expressed effect on k_{cat}/K_M

III. Theoretical Examples

Scenario 2:
$k_1 = 10^8\ \mathrm{M^{-1}s^{-1}}$, $k_{-1} = 10^5\ \mathrm{s^{-1}}$
$k_2 = 100\ \mathrm{s^{-1}}$ (H); $10\ \mathrm{s^{-1}}$ (D)
$k_3 = 1\ \mathrm{s^{-1}}$

$K_d = 1$ mM

$$\mathrm{E + S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{E{\bullet}S} \xrightarrow{k_2} \mathrm{E{\bullet}P} \xrightarrow{k_3} \mathrm{E + P}$$



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?

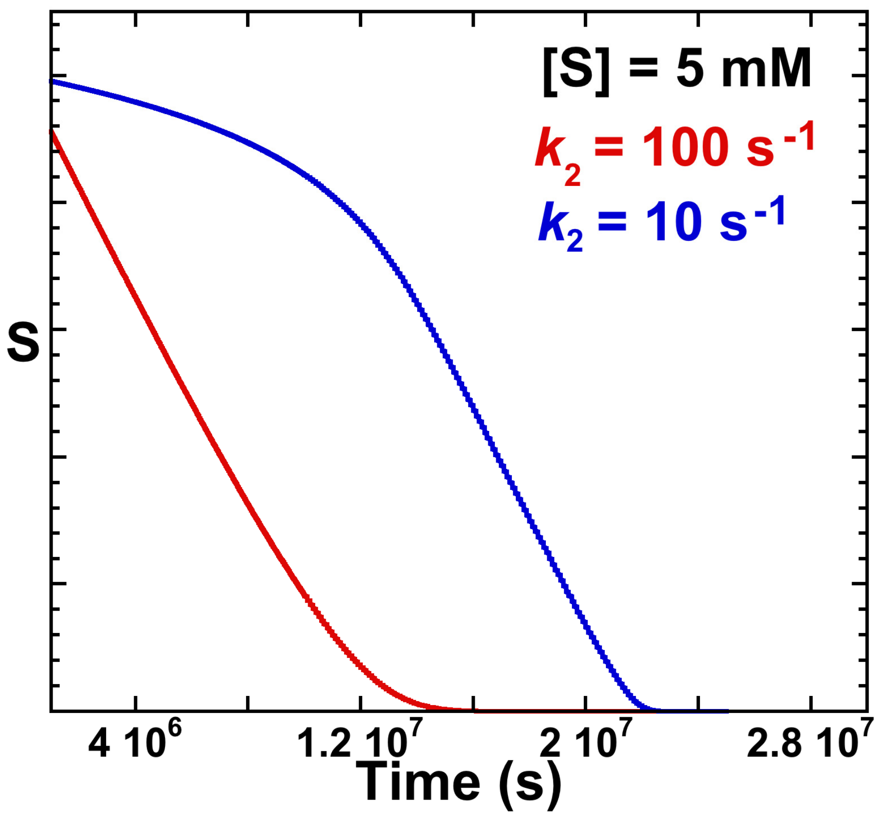
III. Theoretical Examples

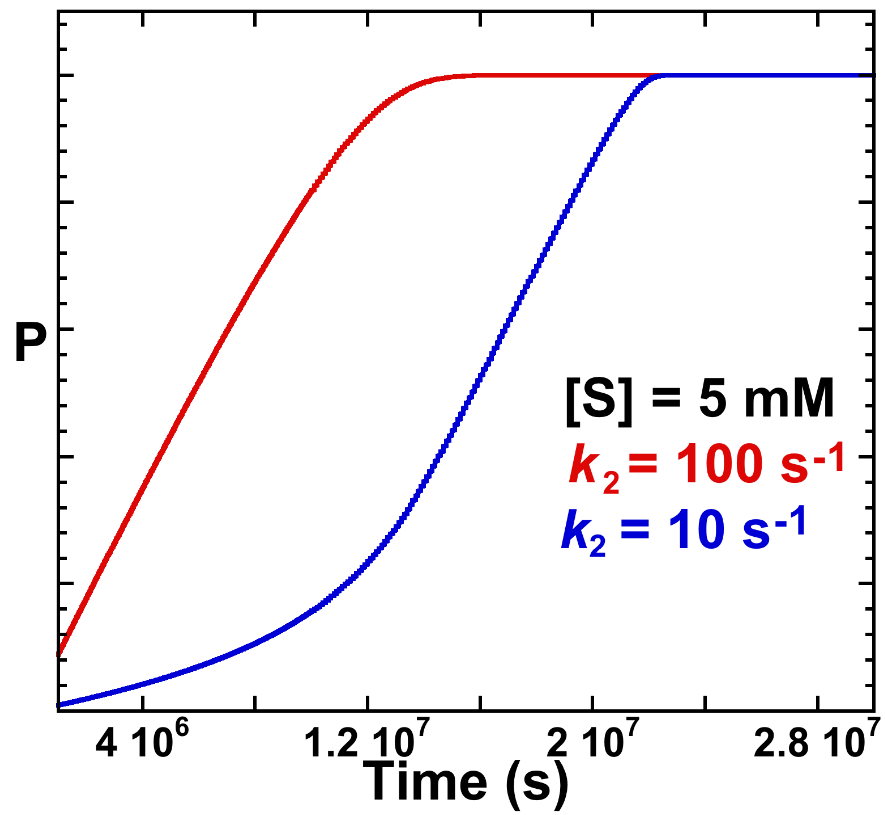
Scenario 2:
$k_1 = 10^8\ M^{-1}s^{-1}$, $k_{-1} = 10^5\ s^{-1}$
$k_2 = 100\ s^{-1}$ (H); $10\ s^{-1}$ (D)
$k_3 = 1\ s^{-1}$

$K_d = 1$ mM

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E{\bullet}S \xrightarrow{k_{2,\,H}} E{\bullet}P \xrightarrow{k_3} E + P$$

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E{\bullet}S \xrightarrow{k_{2,\,D}} E{\bullet}P \xrightarrow{k_3} E + P$$





No expressed effect on k_{cat}; expressed effect on k_{cat}/K_M

III. Theoretical Examples

<u>*Scenario 3:*</u> *"sitcky" 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:
$k_1 = 10^7\ \mathrm{M^{-1}s^{-1}}$, $k_{-1} = 0.1\ \mathrm{s^{-1}}$
$k_2 = 100\ \mathrm{s^{-1}}$ (H); $10\ \mathrm{s^{-1}}$ (D)
$k_3 = 1\ \mathrm{s^{-1}}$

$K_\mathrm{d} = 10\ \mathrm{nM}$

$$\mathrm{E + S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{E{\bullet}S} \xrightarrow{k_2} \mathrm{E{\bullet}P} \xrightarrow{k_3} \mathrm{E + P}$$

$$k_{\mathrm{cat,\ H}} = \frac{100}{101} = 0.99\ \mathrm{s^{-1}}$$

$$k_{\mathrm{cat}}/K_{M,\ \mathrm{H}} = \frac{10^9}{0.1+100} = 9.999\mathrm{x}10^6\ \mathrm{M^{-1}s^{-1}}$$

$$k_{\mathrm{cat,\ D}} = \frac{10}{11} = 0.91\ \mathrm{s^{-1}}$$

$$k_{\mathrm{cat}}/K_{M,\ \mathrm{D}} = \frac{10^8}{0.1+10} = 9.9\mathrm{x}10^6\ \mathrm{M^{-1}s^{-1}}$$

$$\boxed{\frac{\mathrm{k_{cat,\ H}}}{\mathrm{k_{cat,\ D}}} = \frac{0.99}{0.91} = 1.09}$$

$$\boxed{\frac{k_{\mathrm{cat}}/K_{M,\ \mathrm{H}}}{k_{\mathrm{cat}}/K_{M,\ \mathrm{D}}} = 1.01}$$

No expressed effect on k_{cat} and on k_{cat}/K_M

III. Theoretical Examples

<u>Scenario 4:</u> rapid-equilibrium substrate binding, rate-determining chemistry, fast product release

1. Intrinsic KIE expressed in k_{cat}
2. Intrinsic KIE expressed in k_{cat}/K_M

III. Theoretical Examples

Scenario 4:
$k_1 = 10^8\ M^{-1}s^{-1}$, $k_{-1} = 10^5\ s^{-1}$
$k_2 = 100\ s^{-1}$ (H); $10\ s^{-1}$ (D)
$k_3 = 10^4\ s^{-1}$

$K_d = 1$ mM

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E\bullet S \xrightarrow{k_2} E\bullet P \xrightarrow{k_3} E + P$$

$$k_{cat,\ H} = \frac{1\text{x}10^6}{1.01\text{x}10^4} = 99\ s^{-1} \qquad k_{cat}/K_{M,\ H} = \frac{1\text{x}10^{14}}{1\text{x}10^9 + 1\text{x}10^6} = 9.99\text{x}10^4\ M^{-1}s^{-1}$$

$$k_{cat,\ D} = \frac{1\text{x}10^5}{1.001\text{x}10^4} = 9.99\ s^{-1} \qquad k_{cat}/K_{M,\ D} = \frac{1\text{x}10^{13}}{1\text{x}10^9 + 1\text{x}10^5} = 9.99\text{x}10^3\ M^{-1}s^{-1}$$

$$\boxed{\frac{k_{cat,\ H}}{k_{cat,\ D}} = \frac{99}{9.99} = 9.9} \qquad \boxed{\frac{k_{cat}/K_{M,\ H}}{k_{cat}/K_{M,\ D}} = 10}$$

Expressed effect both on k_{cat} and on k_{cat}/K_M

III. Theoretical Examples

Scenario 4:
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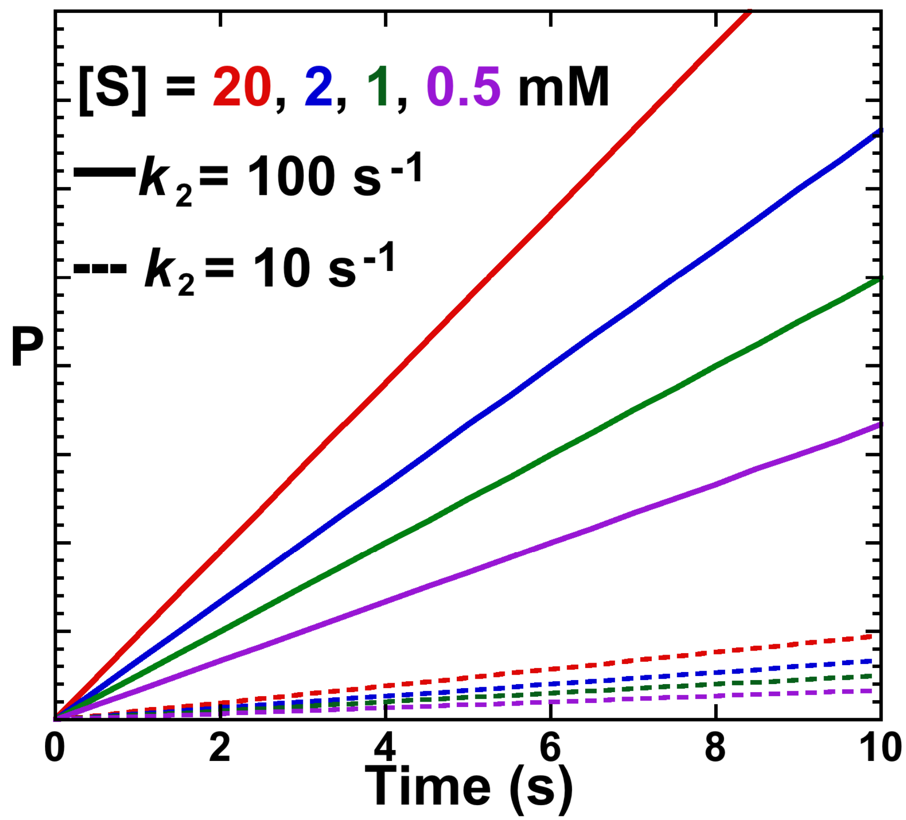
$K_d = 1$ mM

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E{\bullet}S \xrightarrow{k_2} E{\bullet}P \xrightarrow{k_3} E + P$$

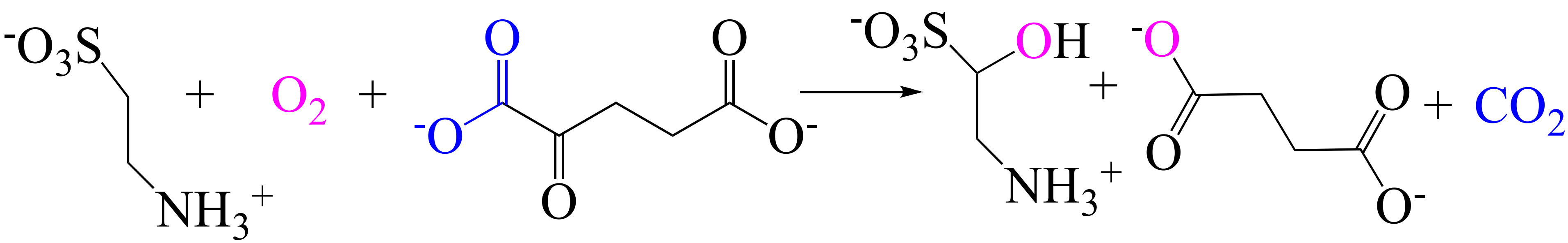


Expressed effect both on k_{cat} and on k_{cat}/K_M

IV. Case study: TauD

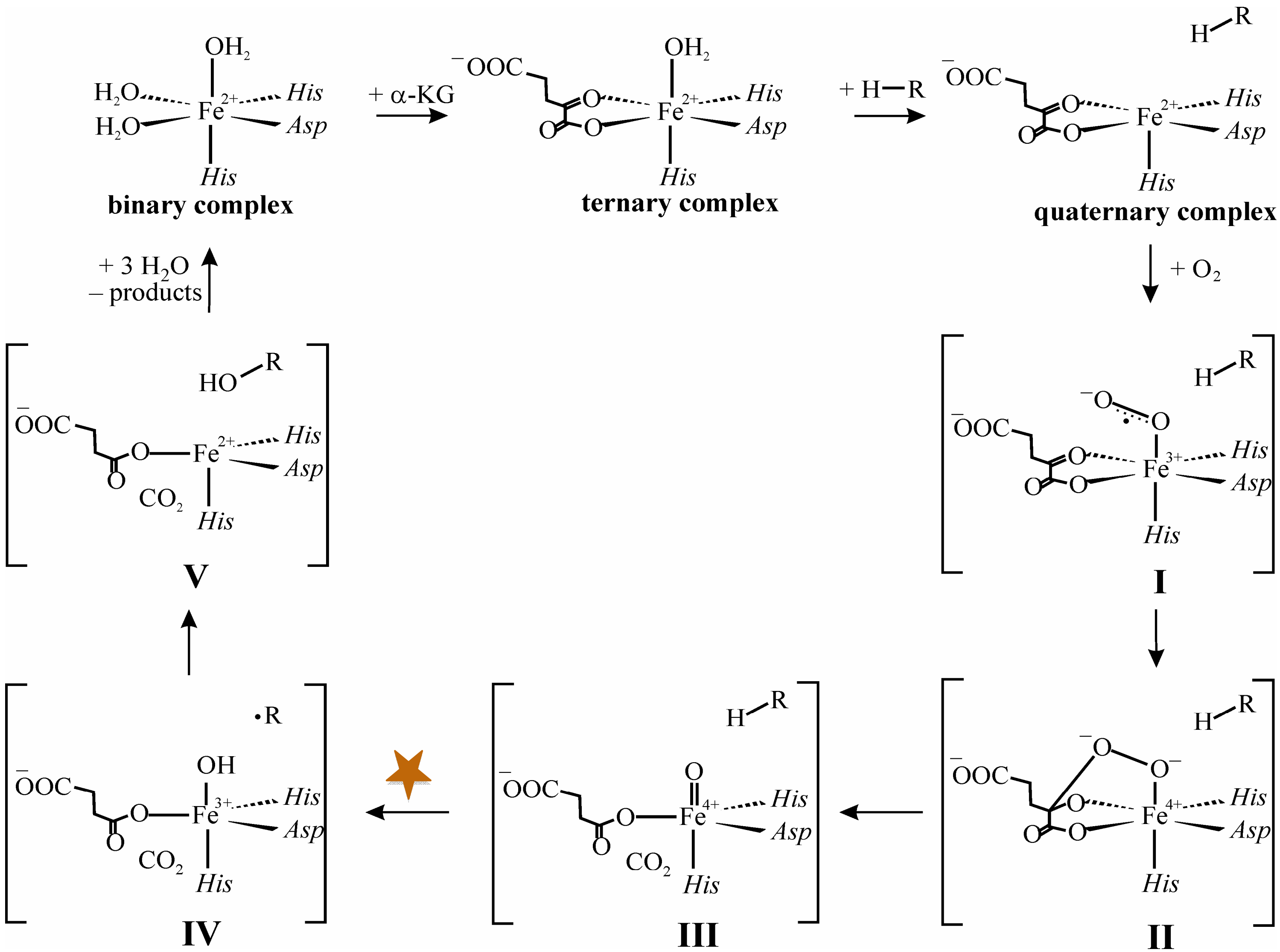
TauD: Taurine α-ketoglutarate dioxygenase

A. Reaction:



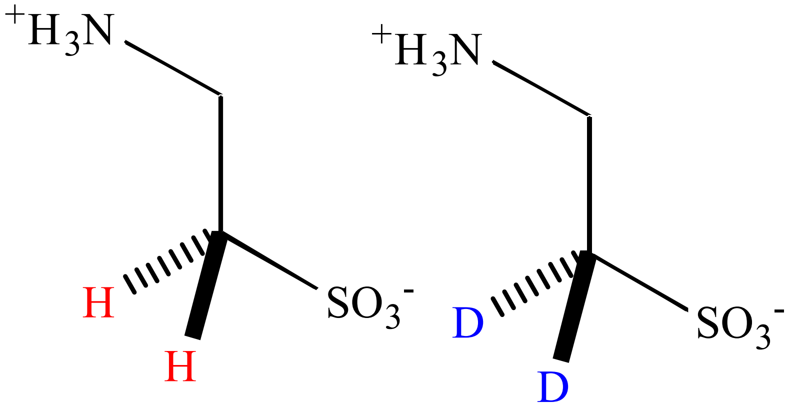
IV. Case study: TauD

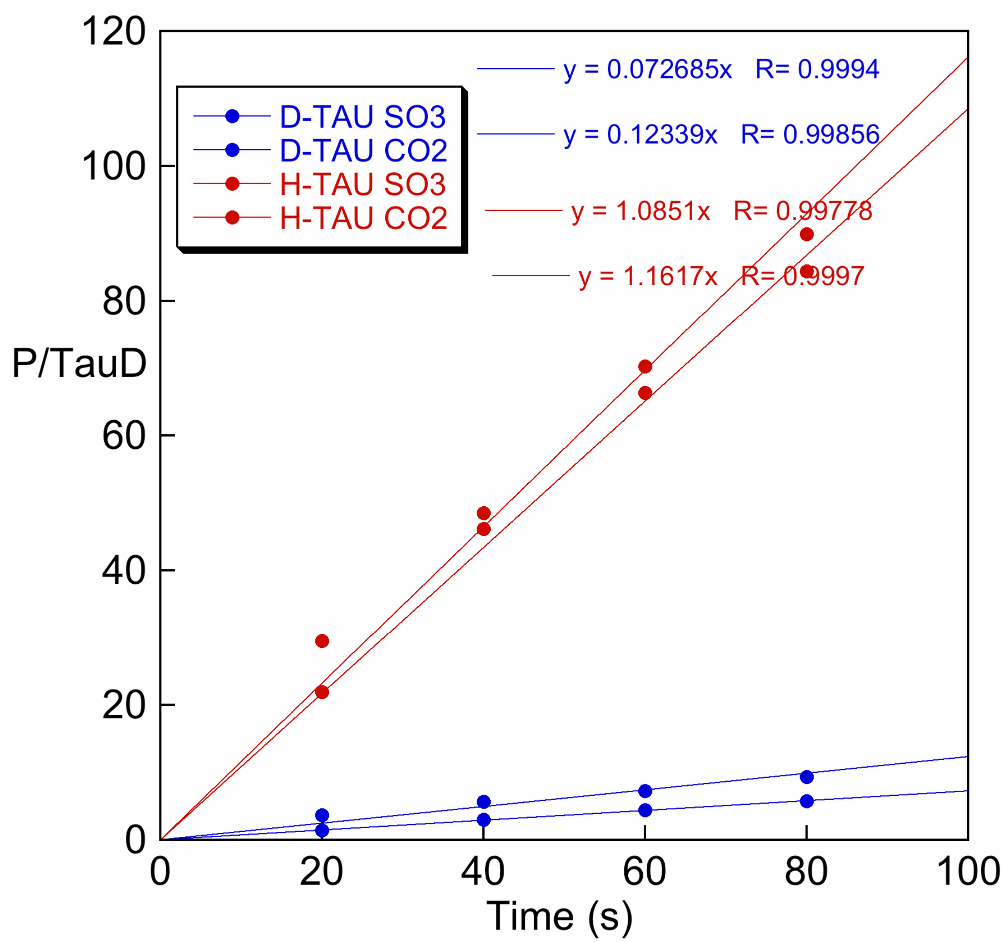
B. Mechanism:



IV. Case study: TauD

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





Reported:

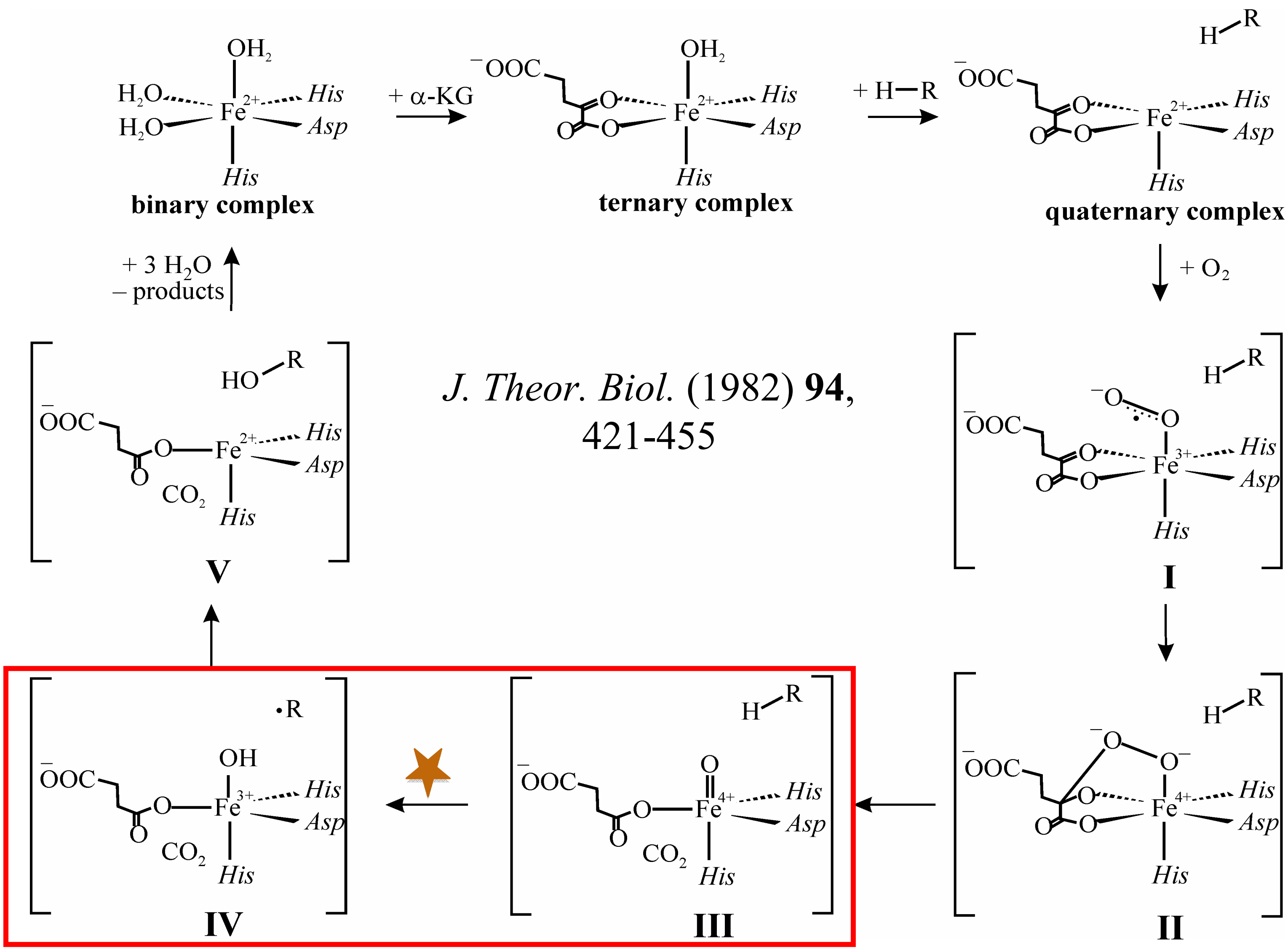
$k_{cat,\,H} = 1.3\ s^{-1}$

$k_{cat,\,D} = 0.11\ s^{-1}$

$k_{cat,\,H}/k_{cat,\,D} \sim 12$

IV. Case study: TauD

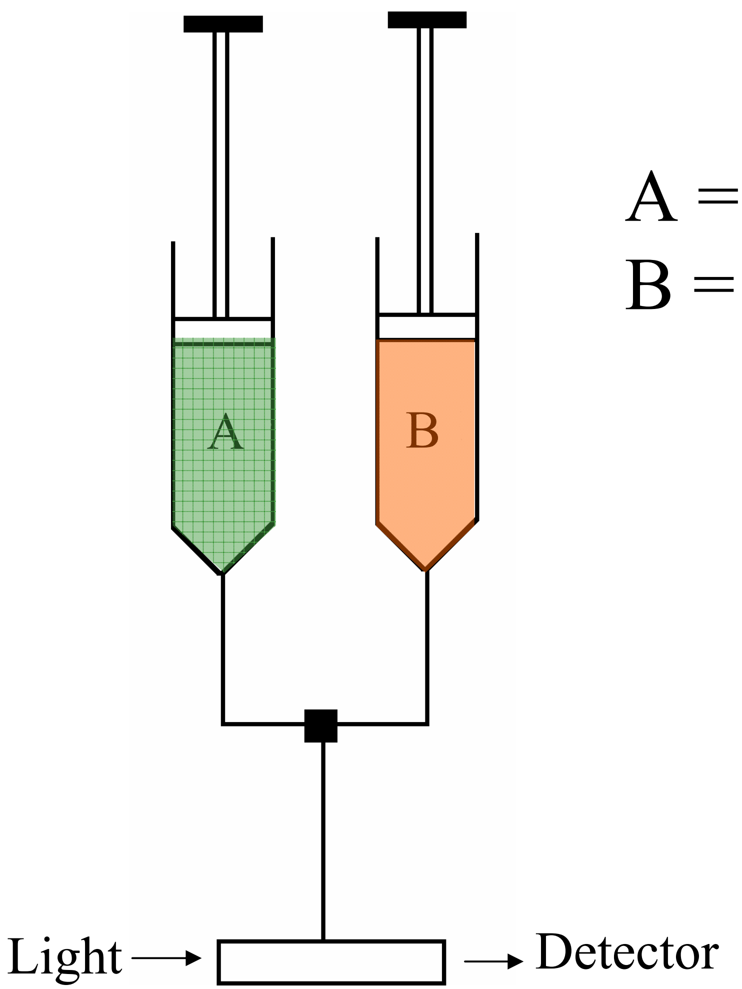
D. Direct measurement of k_{H} and k_{D} for H• abstraction by ferryl complex



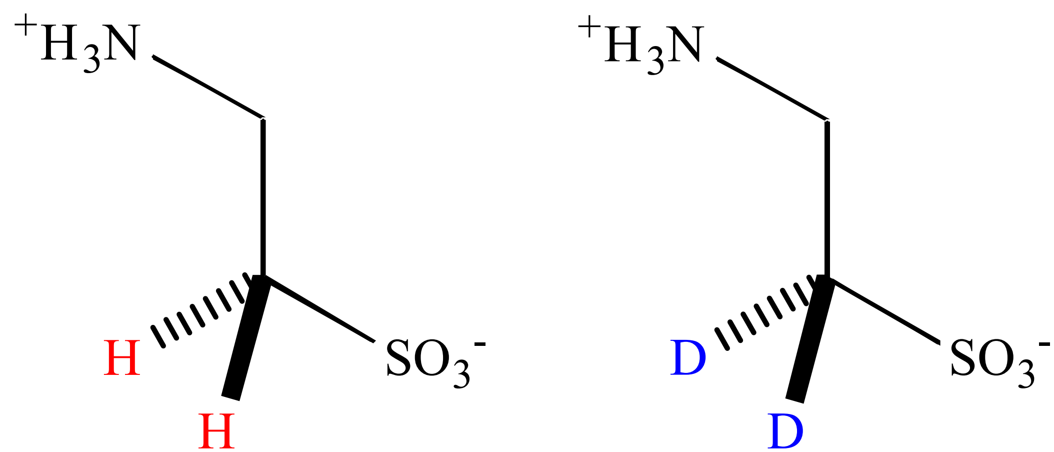
J. Theor. Biol. (1982) **94**, 421-455

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



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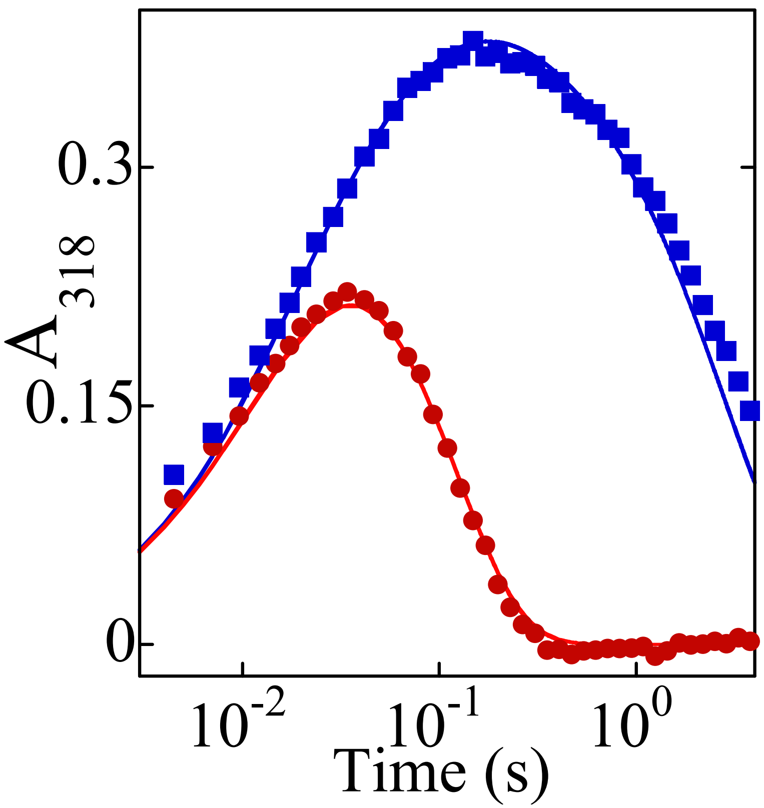

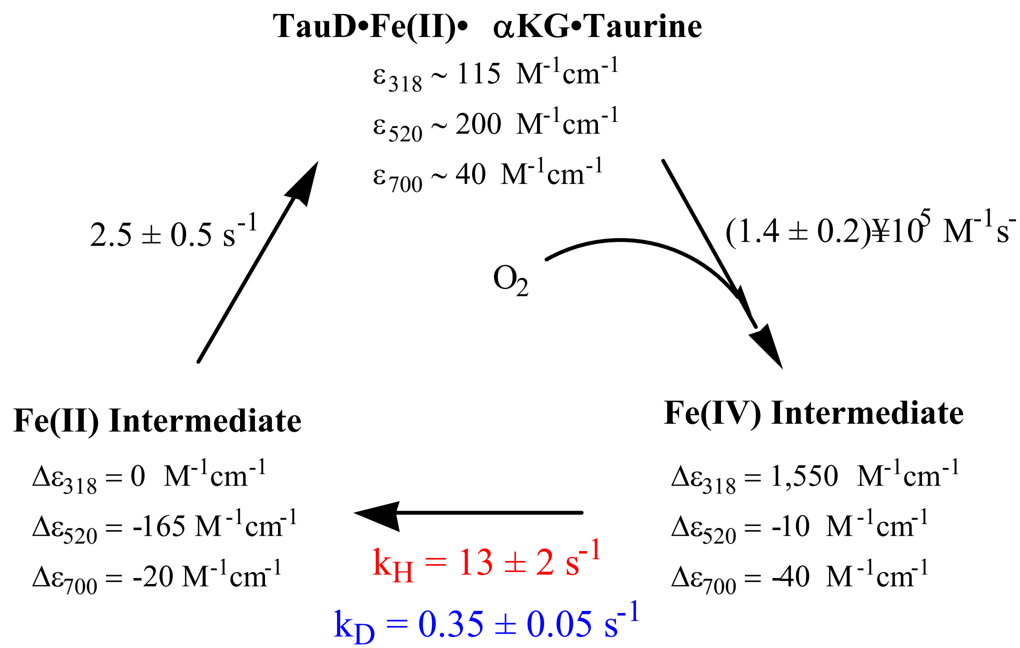

$k_H/k_D = 37$

Intrinsic KIE? Not quite!

Price, et al. (2003) *Biochemistry, 42*, 7497-7508

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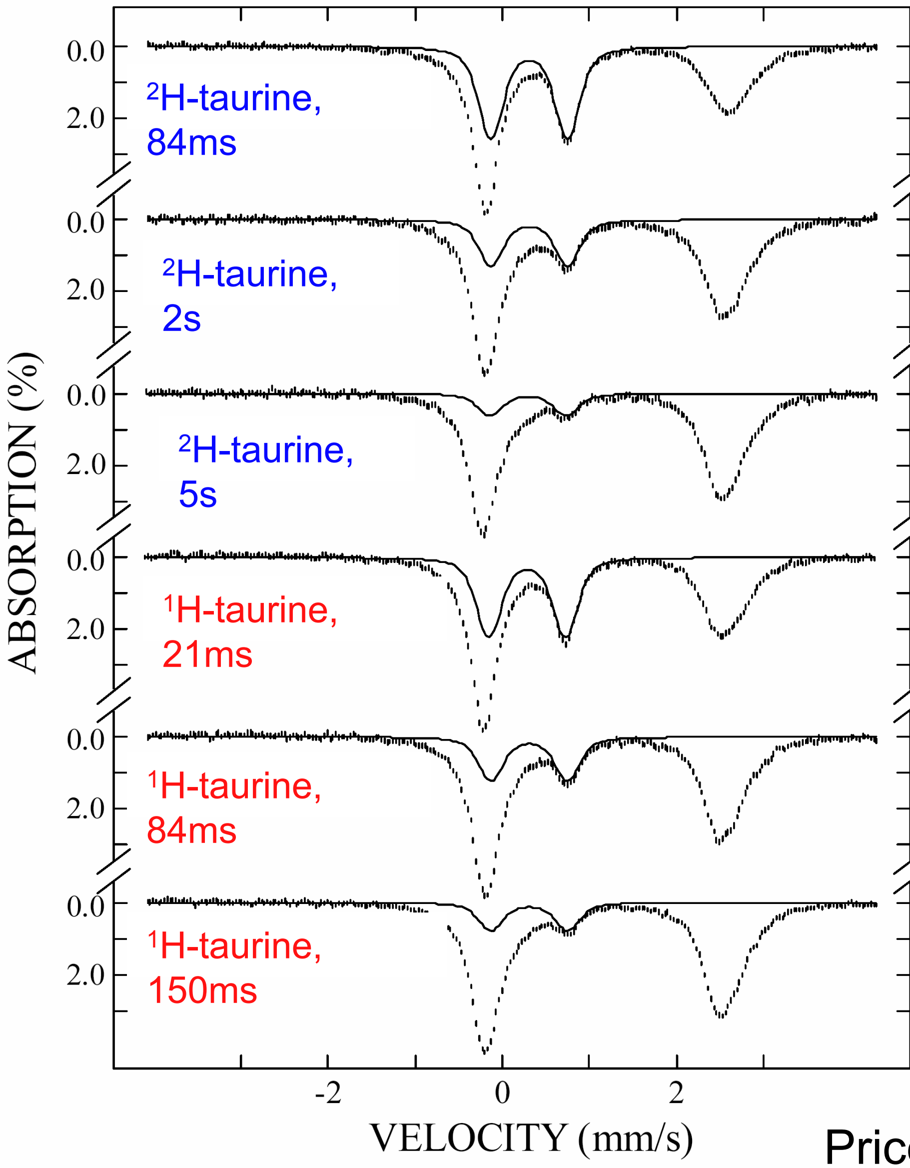
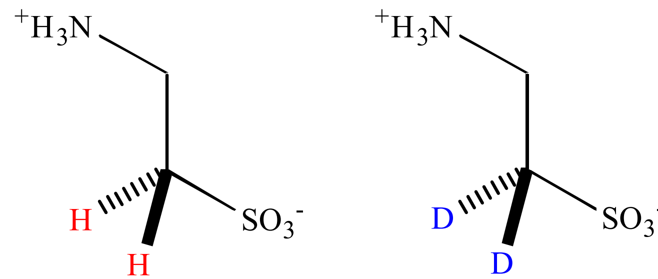
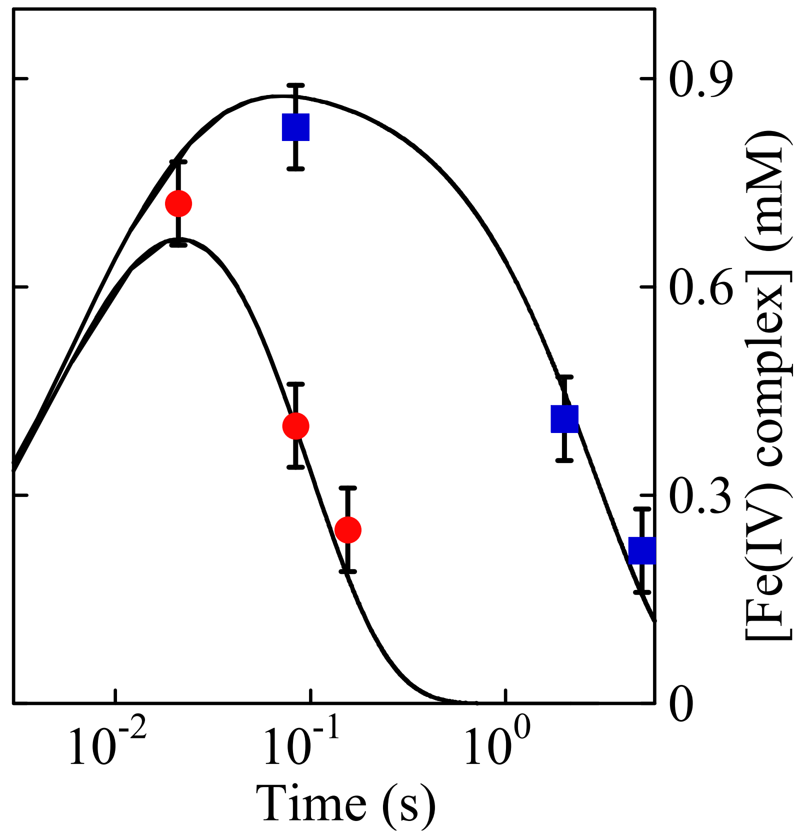


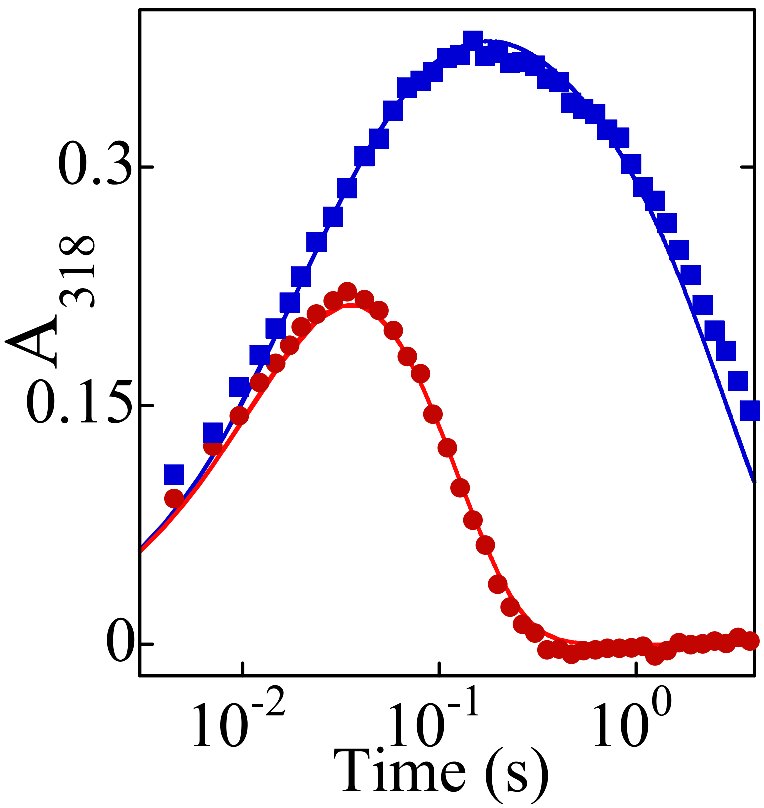


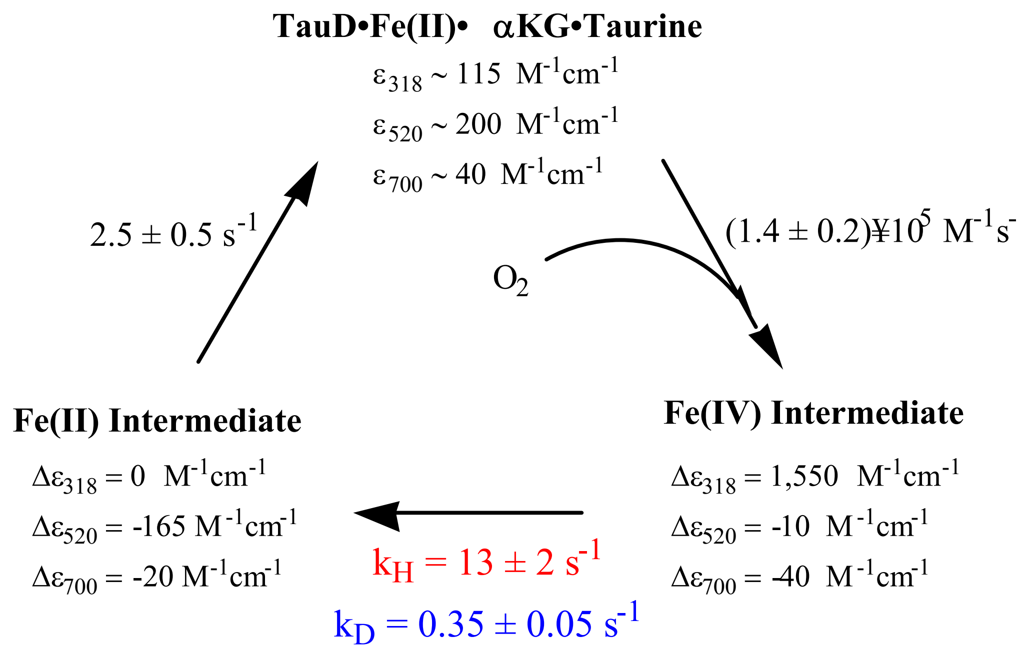


Price, et al. (2003) *JACS, 125*, 13008-13009.

IV. Case study: TauD

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





$$k_H/k_D = 37$$

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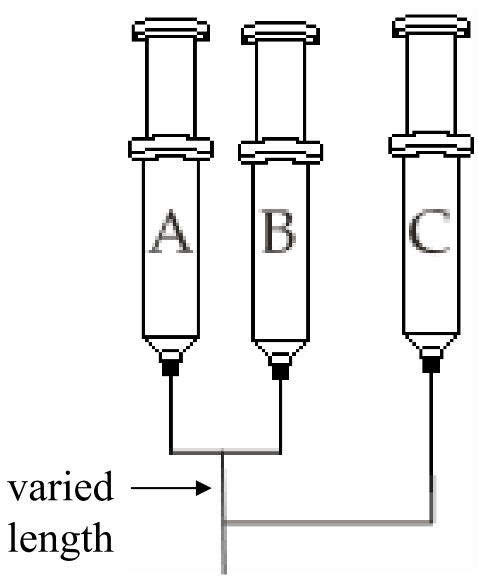
IV. Case study: TauD

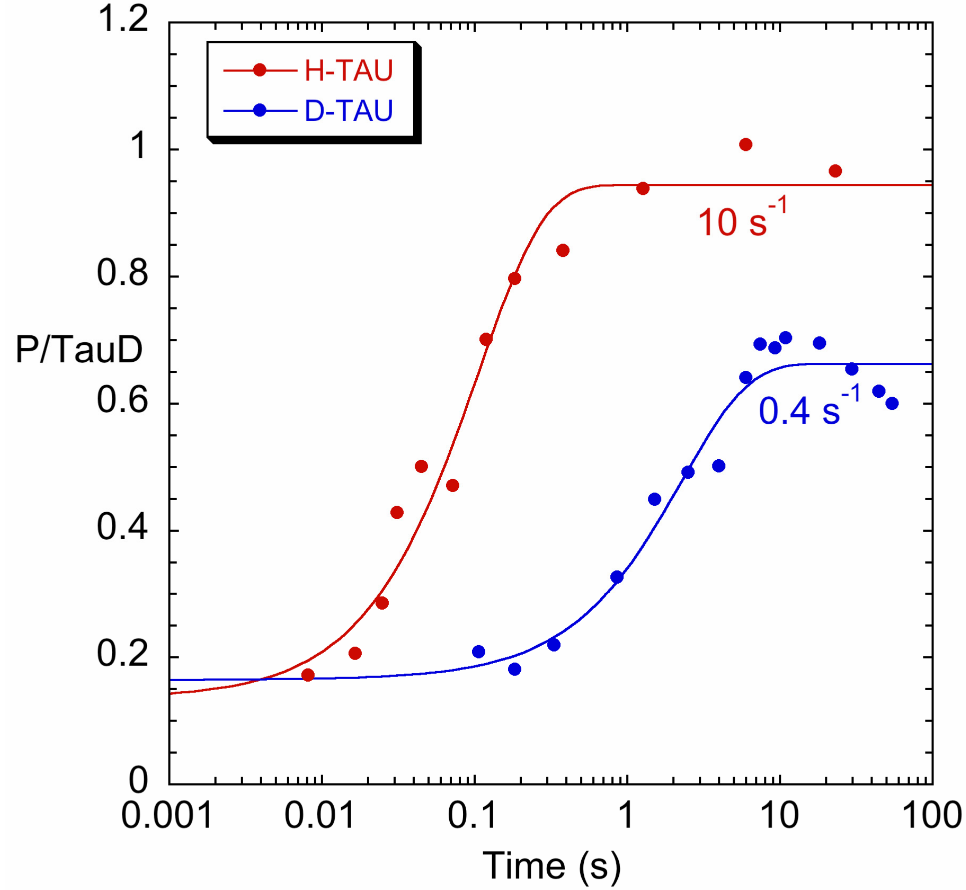
E. Effect of uncoupling

1. Dramatic retardation of D• abstraction by ferryl exposes unproductive pathway for decay

2. ~ 30% uncoupled decay indicates that unproductive pathway has $k = 0.1\ s^{-1}$ and D• abstraction has $k = 0.25\ s^{-1}$



A: TauD•Fe(II)•αKG•taurine
B: air-saturated buffer
C: acid or base "quench" solution



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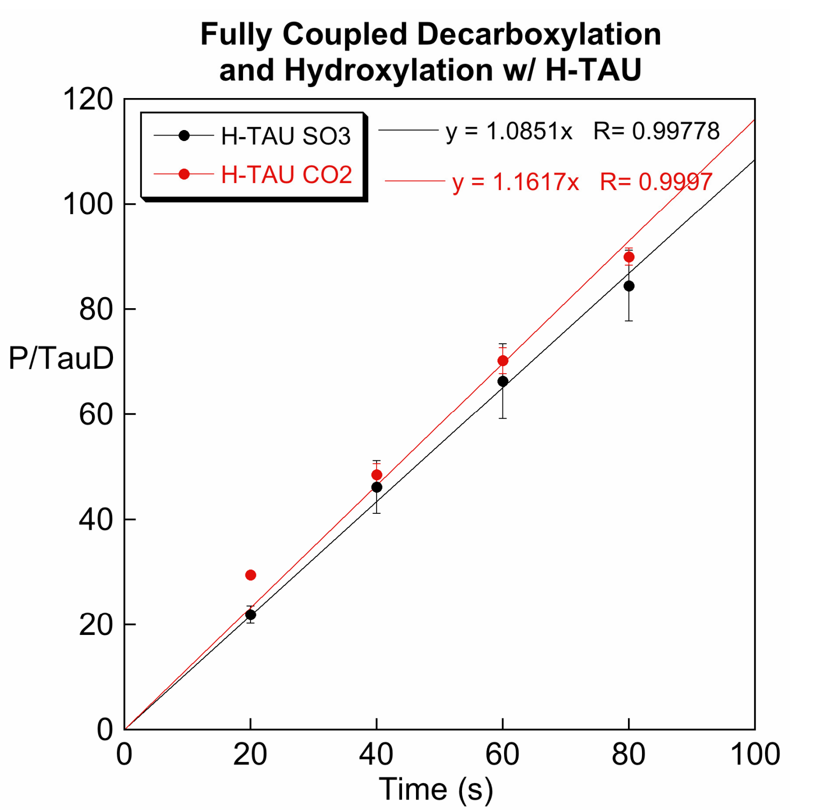

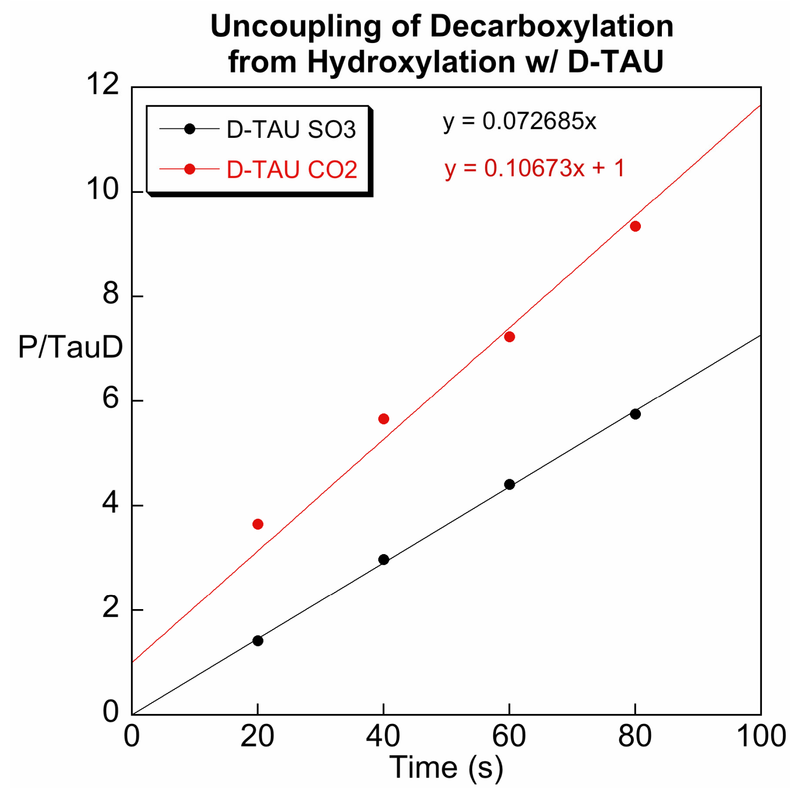

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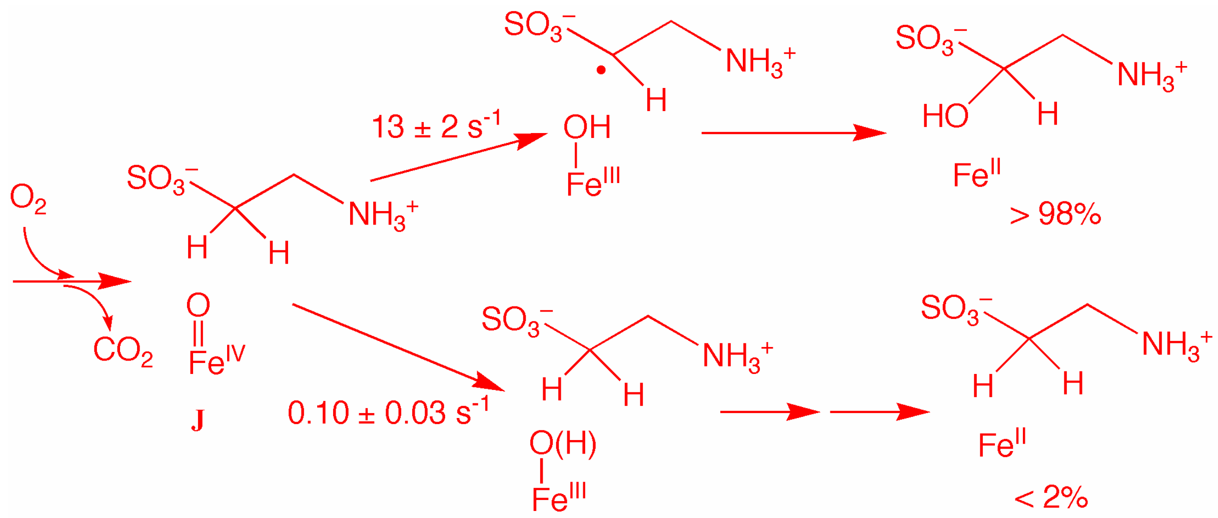
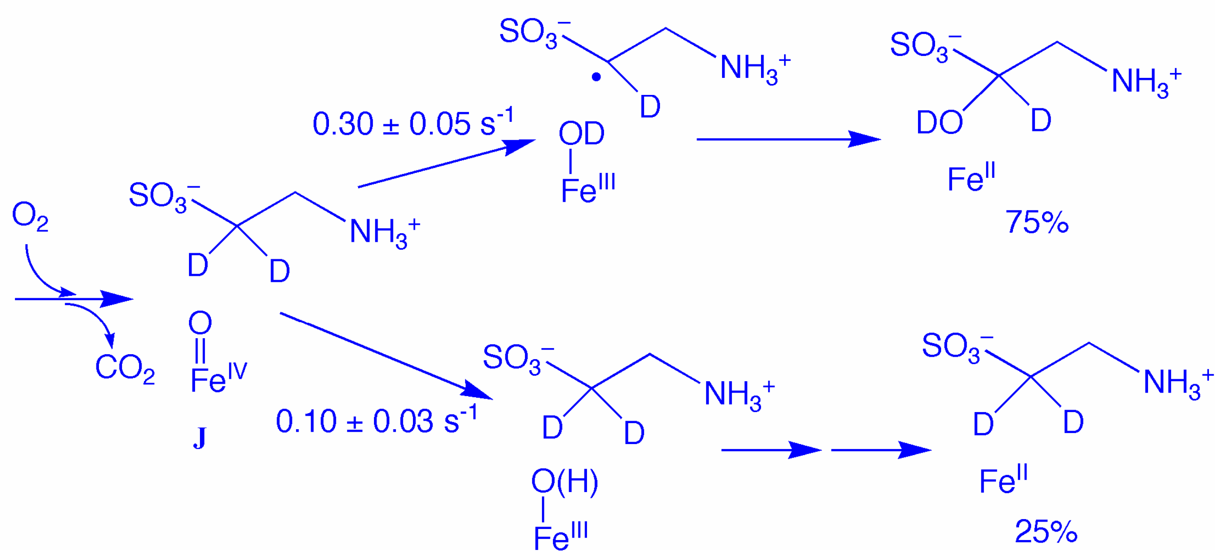




Intrinsic k_H/k_D =52!!

IV. Case study: TauD

F. What about ^{3}H?

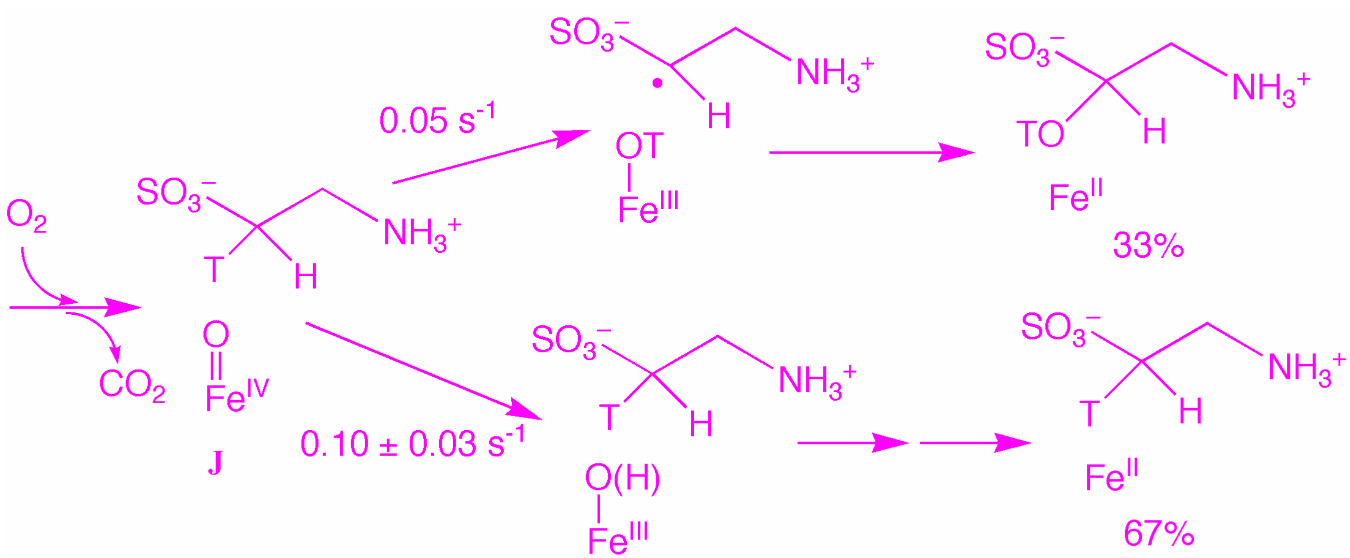
Swain-Schaad Equation

$k_H/k_T = (k_H/k_D)^{1.44} = 50^{1.44} = 280!!$

$k_T = 13\ s^{-1}/280 = 0.0546$

Uncoupling ~ 70%!

Predicted $k_{cat}/K_M > 3$



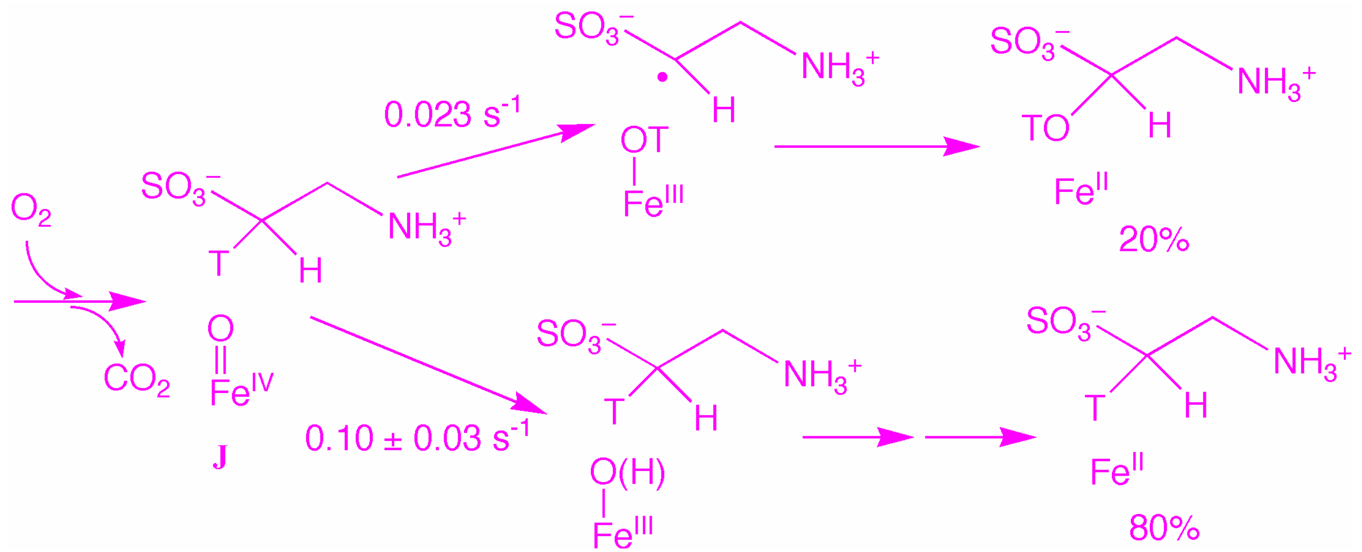
IV. Case study: TauD

F. What about ^{3}H?

<u>Sharon Hammes-Schiffer</u>

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

Predicted $k_{cat}/K_M > 5$



Not a conventional selection effect, but a result of failures in events involving heavy isotope

IV. Case study: TauD

Apparent selection effect occurring ***after*** *the first irreversible step* (ferryl formation)

Goes against enzymology dogma

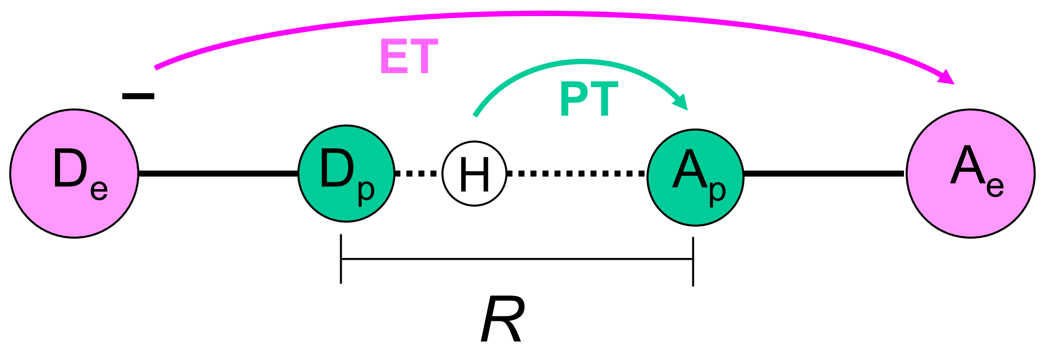
V. Conclusion

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

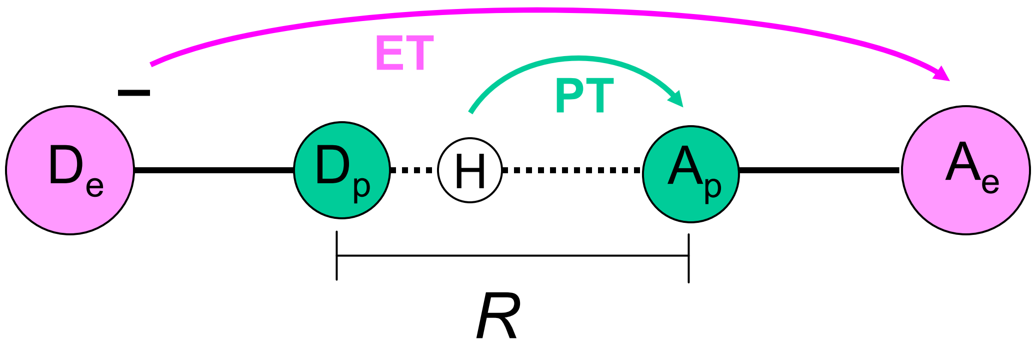
Theory of Proton-Coupled Electron Transfer for Bioinorganic Chemists

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)

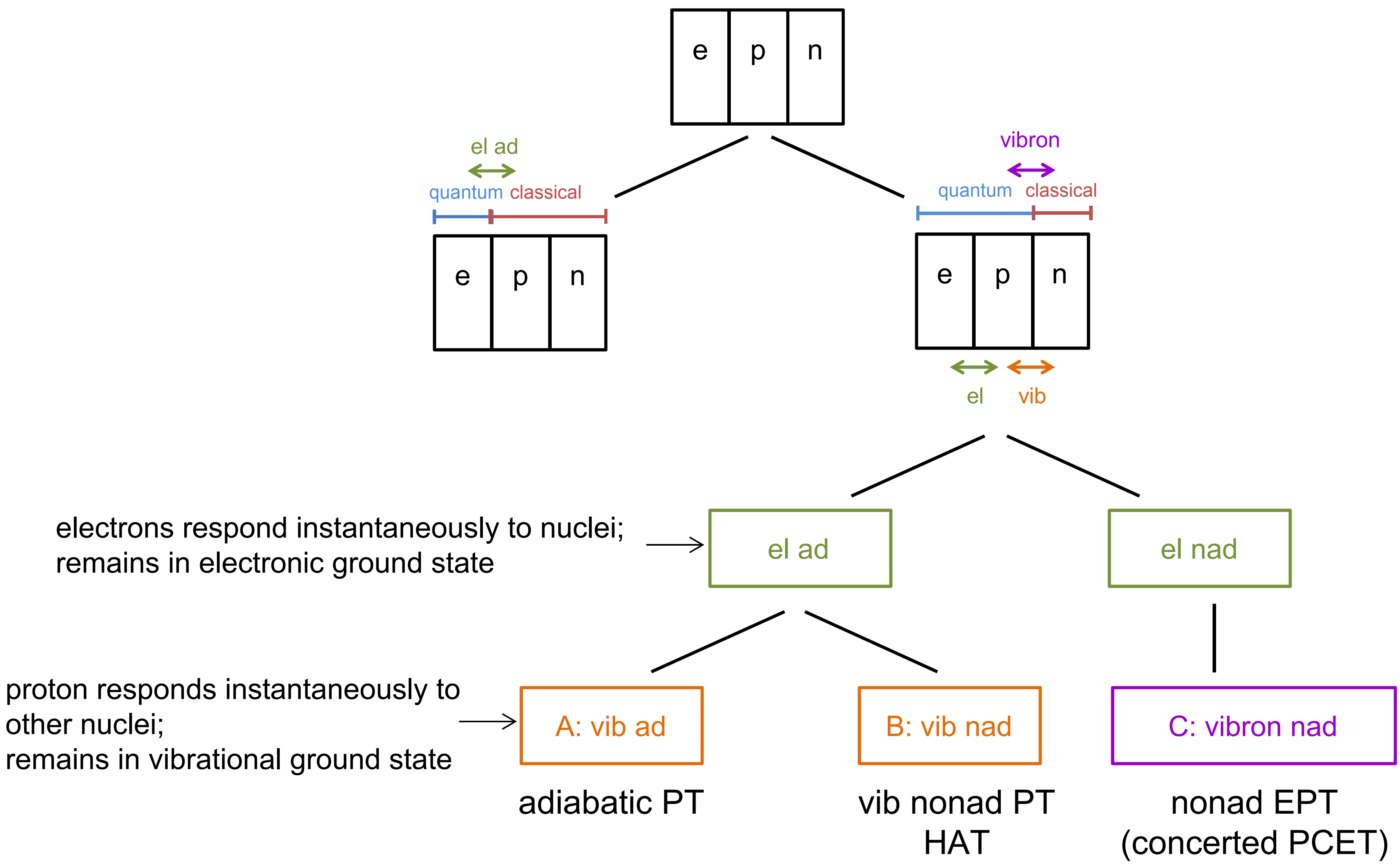
General Definition of PCET



- 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

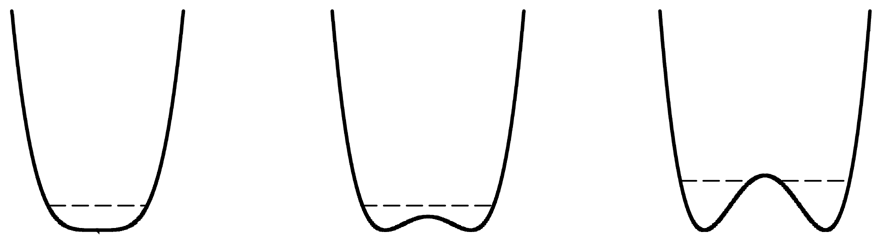
S. Hammes-Schiffer, Energy and Environ. Sci. 2012



Classification Scheme: Proton Potentials

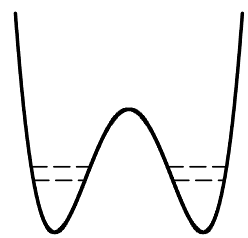
Case A: electronically and vibrationally adiabatic

adiabatic PT



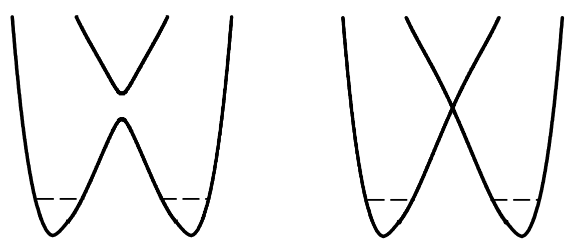
Case B: electronically adiabatic, vibrationally nonadiabatic

vib nonadiabatic PT, HAT



Case C: electronically and vibronically nonadiabatic

nonadiabatic EPT



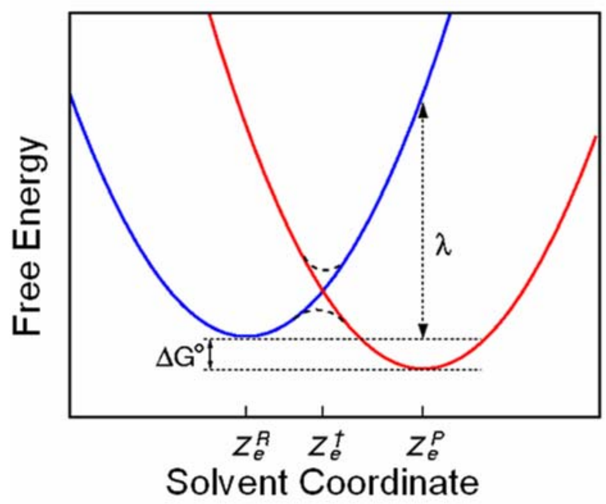
adiabatic states   diabatic states

Single Electron Transfer

Diabatic states:

(1) $D_e^- \quad A_e$

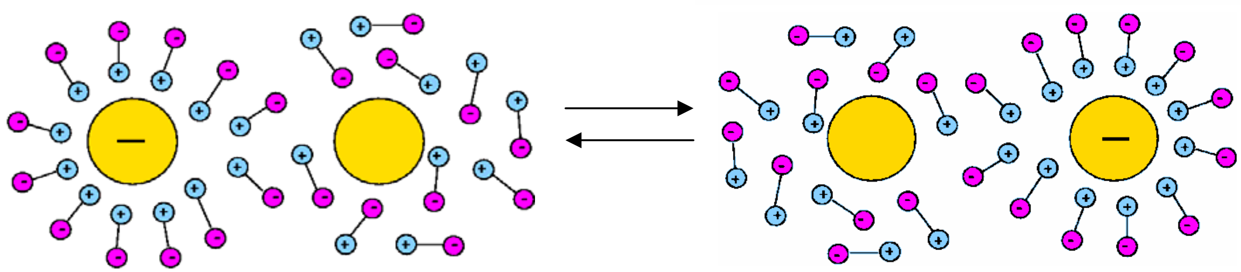
(2) $D_e \quad A_e^-$



Marcus theory

Solvent coordinate

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



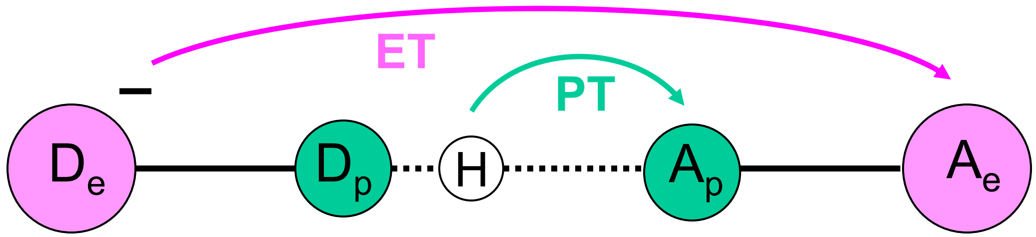
Nonadiabatic ET rate: $k = \frac{2\pi}{\hbar} V_{12}^2 (4\pi\lambda k_B T)^{-1/2} \exp\left[-\Delta G^\dagger / (k_B T)\right]$

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

V_{12} :coupling between diabatic states

Proton-Coupled Electron Transfer Theory

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



- Four diabatic states:

$$(1a)\ \mathrm{D_e^-}\text{—}{}^{+}\mathrm{D_pH\,L\ L\ \ A_p}\text{—}\mathrm{A_e}$$

$$(1b)\ \mathrm{D_e^-}\text{—}\ \ \mathrm{D_p\,L\ L\ \ HA_p^+}\text{—}\mathrm{A_e}$$

$$(2a)\ \mathrm{D_e}\text{—}{}^{+}\mathrm{D_pH\,L\ L\ \ A_p}\text{—}\mathrm{A_e^-}$$

$$(2b)\ \mathrm{D_e}\text{—}\ \ \mathrm{D_p\,L\ L\ \ HA_p^+}\text{—}\mathrm{A_e^-}$$

- Free energy surfaces depend on 2 collective solvent coordinates

$$\mathrm{PT}\quad (1a)\rightarrow(1b):\quad z_p = \int d\mathbf{r}(\rho_{1b}-\rho_{1a})\Phi_{\mathrm{in}}(\mathbf{r})$$

$$\mathrm{ET}\quad (1a)\rightarrow(2a):\quad z_e = \int d\mathbf{r}(\rho_{2a}-\rho_{1a})\Phi_{\mathrm{in}}(\mathbf{r})$$

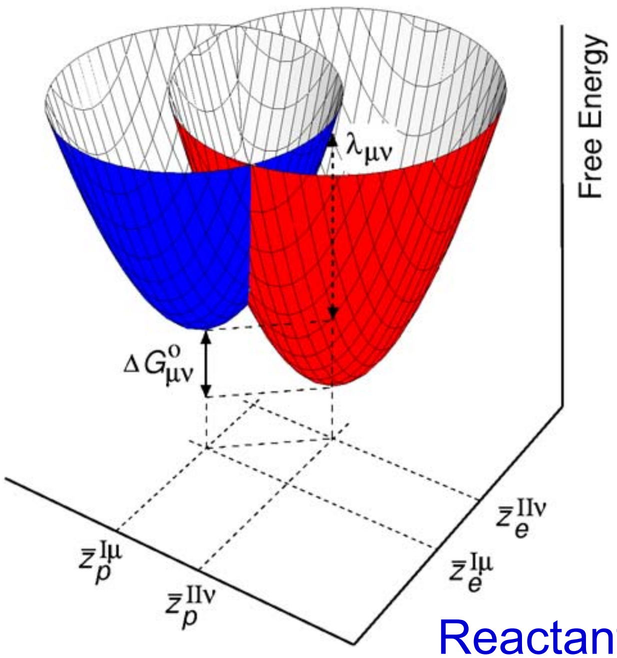
- Hydrogen nucleus: quantum mechanical wavefunction

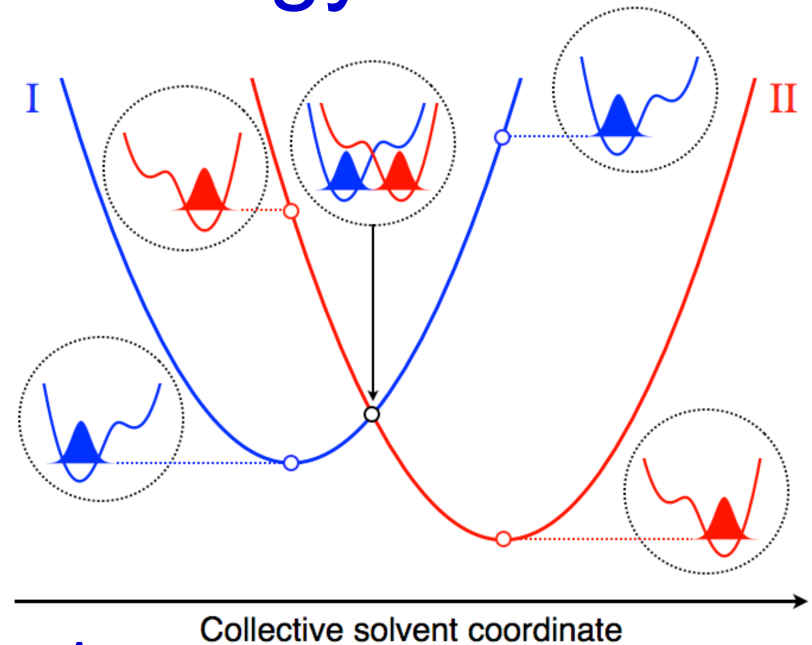
Sequential vs. Concerted PCET

$$
\begin{tikzcd}[column sep=large, row sep=large]
(1a)\ \mathrm{D_e^-}\text{—}{}^{+}\mathrm{D_pH\,L\,L\,\,A_p}\text{—}\mathrm{A_e} \arrow[r, "\text{PT}"] \arrow[d, "\text{ET}"'] \arrow[dr, "\text{EPT}"] & (1b)\ \mathrm{D_e^-}\text{—}\ \mathrm{D_p\,L\,L\,\,HA_p^+}\text{—}\mathrm{A_e} \arrow[d, "\text{ET}"] \\
(2a)\ \mathrm{D_e}\text{—}{}^{+}\mathrm{D_pH\,L\,L\,\,A_p}\text{—}\mathrm{A_e^-} \arrow[r, "\text{PT}"'] & (2b)\ \mathrm{D_e}\text{—}\ \mathrm{D_p\,L\,L\,\,HA_p^+}\text{—}\mathrm{A_e^-}
\end{tikzcd}
$$

- Sequential: ET-PT or PT-ET
- Concerted: EPT
- Mechanism determined by relative energies and couplings
- $1b$ and $2a$ much higher in energy → concerted EPT, reduces to two-state model: $(\mathbf{1}\boldsymbol{a}/1b)$ → $(2a/\mathbf{2}\boldsymbol{b})$

2D Vibronic Free Energy Surfaces





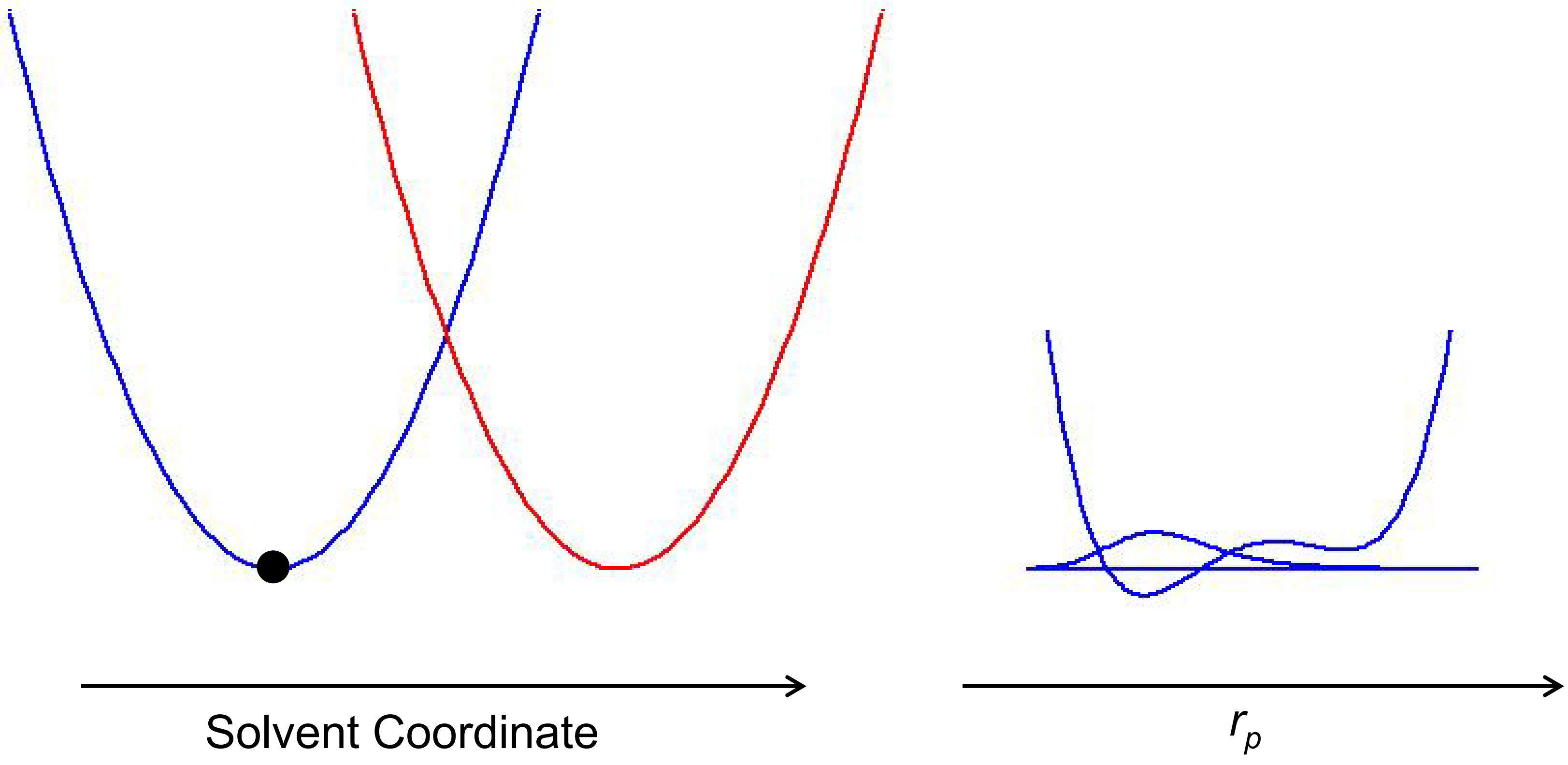
Reactant (**1*a***/1*b*) D⁻ A

Product (2*a*/**2*b***) D A⁻

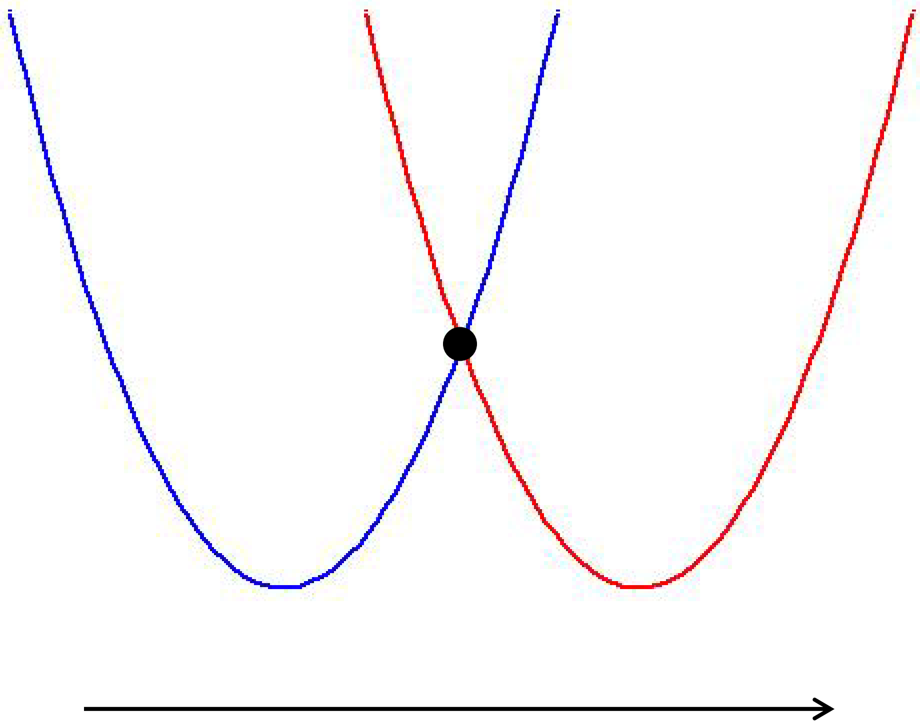
- Free energy surfaces depend on 2 collective solvent coordinates: z_p (PT) and z_e (ET)
- Electron-proton vibronic surfaces corresponding to different proton vibrational states for each electronic state
- Vibronic coupling between reactant/product states

$$V_{\mu\nu} = \left\langle \Phi^{\mathrm{I}}\left(\mathbf{r}_e, \mathbf{r}_p\right) \middle| \hat{H} \middle| \Phi^{\mathrm{II}}\left(\mathbf{r}_e, \mathbf{r}_p\right) \right\rangle \approx V^{\mathrm{el}} S_{\mu\nu}$$

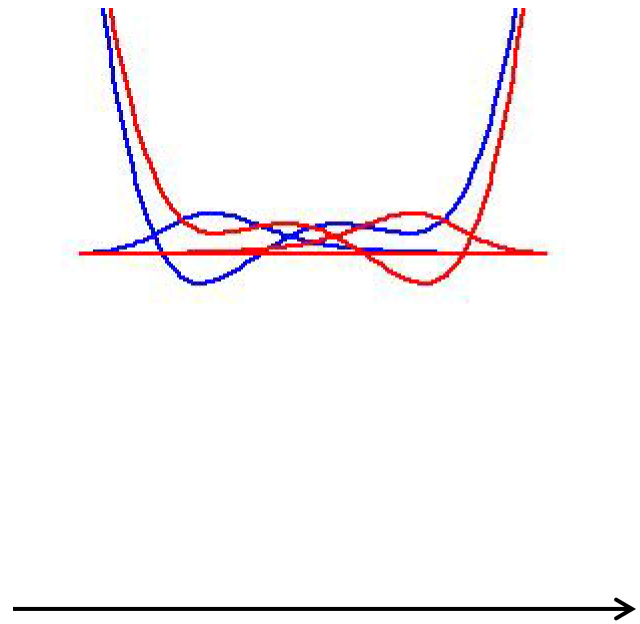
Fundamental Mechanism for PCET



Fundamental Mechanism for PCET

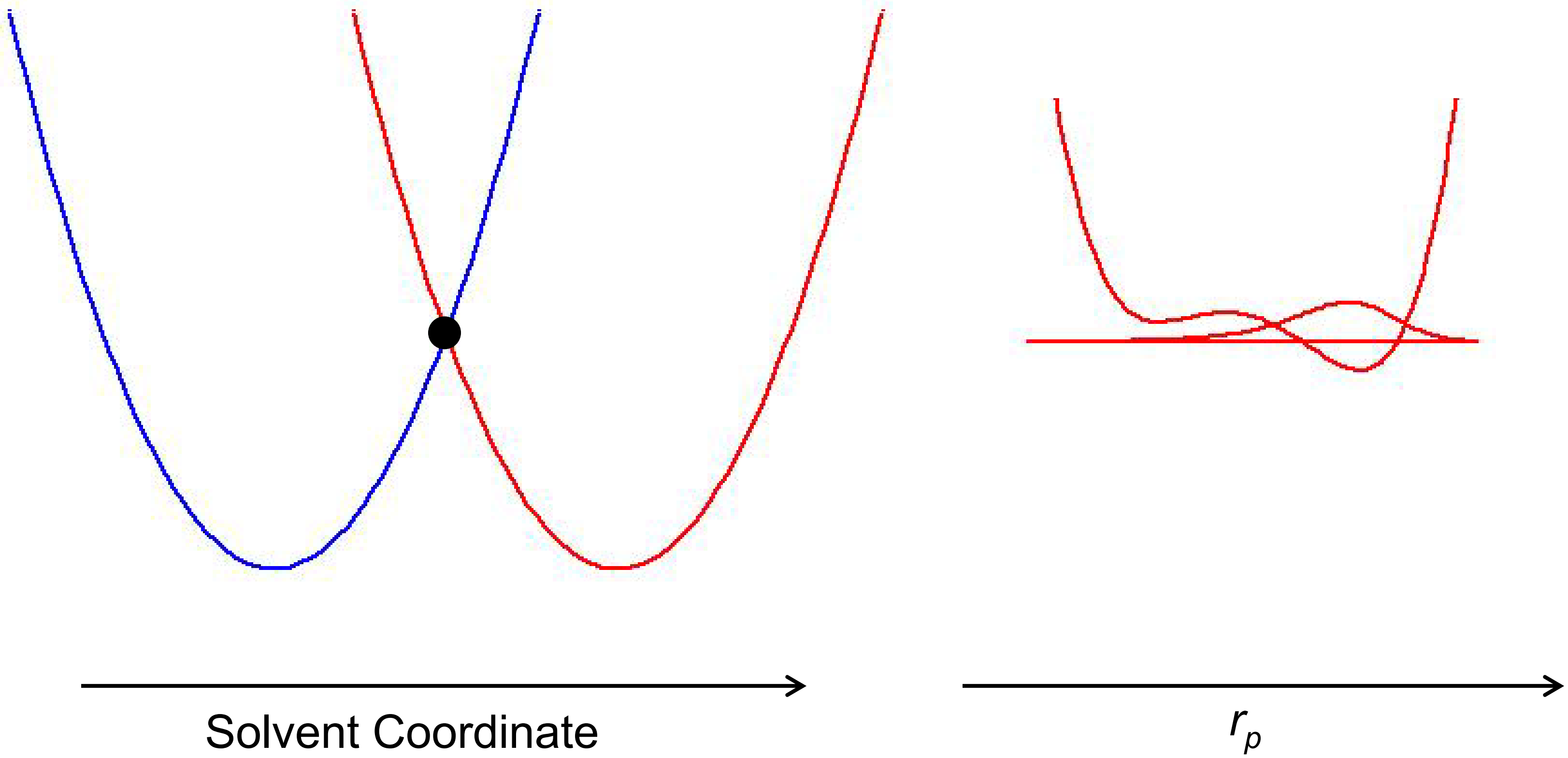


Solvent Coordinate



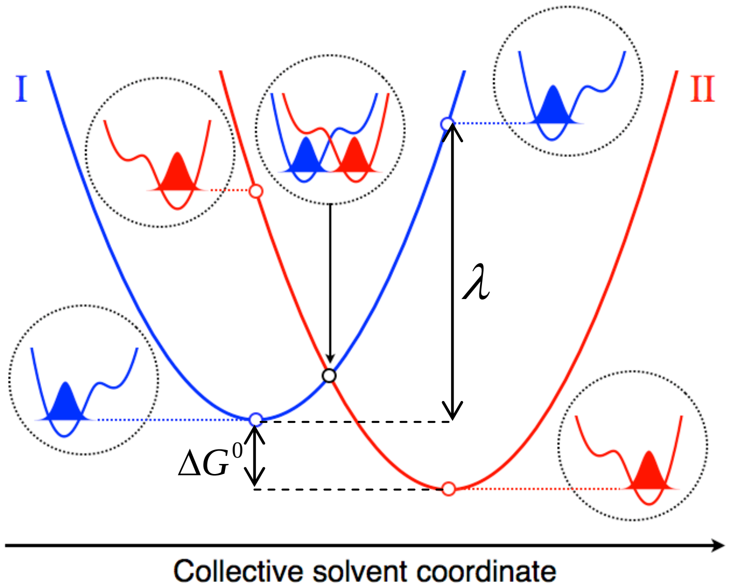
r_p

Fundamental Mechanism for PCET



PCET Rate Constant Expressions

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

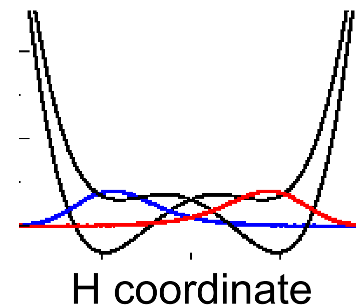


- Typically PCET reactions nonadiabatic due to small vibronic coupling
- Use Golden Rule to derive rate constant expressions

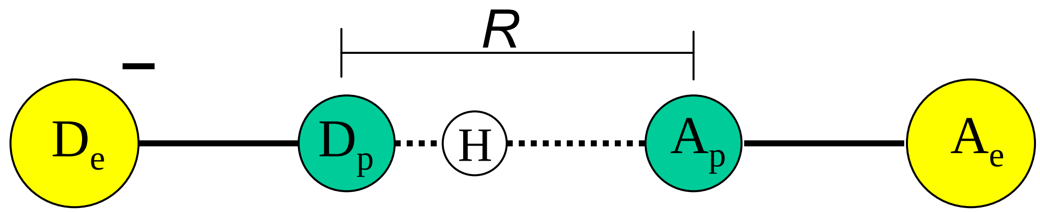
Reactant D⁻ A
Product D A⁻

$$k = \frac{2\pi}{\mathrm{h}} \sum_{\mu} P_{\mu}^{\mathrm{I}} \sum_{\nu} \left(4\pi\lambda k_B T\right)^{-1/2} \left(V^{\mathrm{el}} S_{\mu\nu}\right)^2 \exp\left[-\Delta G_{\mu\nu}^{\dagger} / (k_B T)\right]$$

$$\Delta G_{\mu\nu}^{\dagger} = \left(\Delta G_{\mu\nu}^{0} + \lambda\right)^2 / (4\lambda)$$



Role of H Wavefunction Overlap



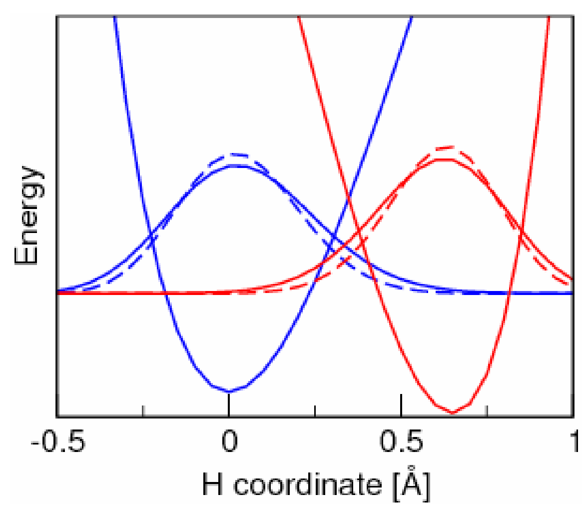
- Rate decreases as overlap decreases (as *R* increases)

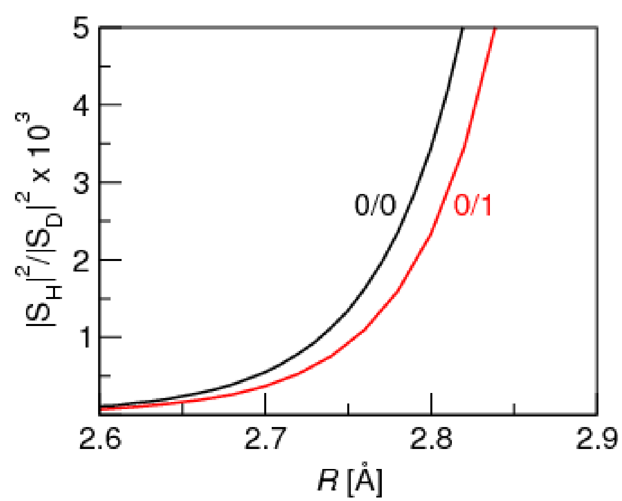
$$k_H \propto (H \text{ overlap})^2$$

- 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)



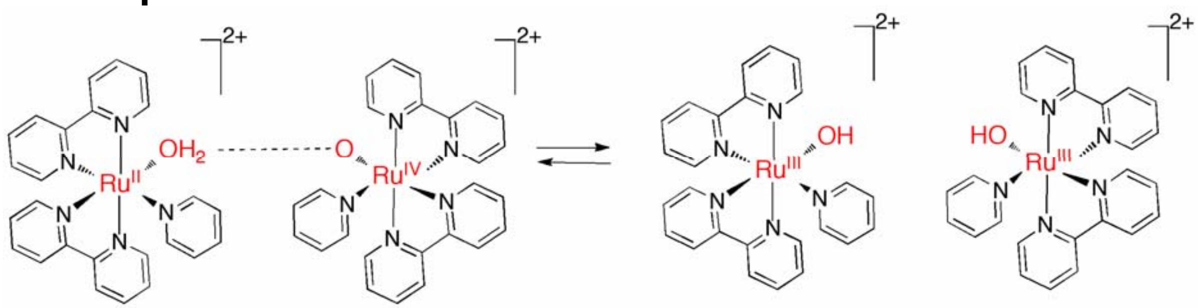


solid: H
dashed: D

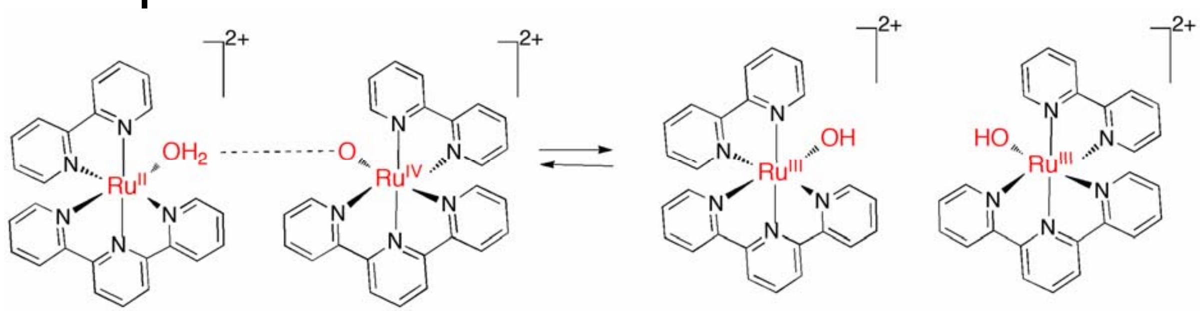
Ruthenium Polypyridyl Complexes

Iordanova and Hammes-Schiffer, JACS 2002

CompA



CompB



	KIE	Rate
CompA	16.1 ± 0.4	1.0
CompB	11.3 ± 1.3	9.6

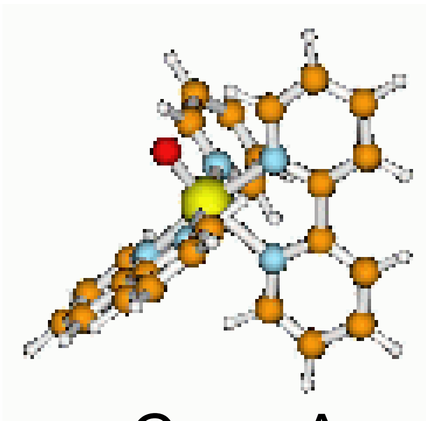
Experiments: *Binstead and Meyer, JACS 1987;*
Farrer and Thorp, Inorg. Chem. 1999

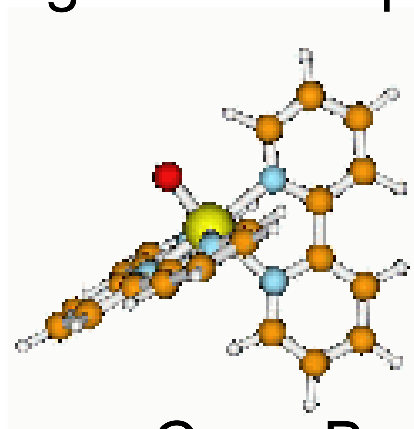
Donor-Acceptor Distances

$$Ru\text{-}OH_2\text{------}O\text{-}Ru$$

$$\xleftrightarrow{\quad R_{OO} \quad}$$

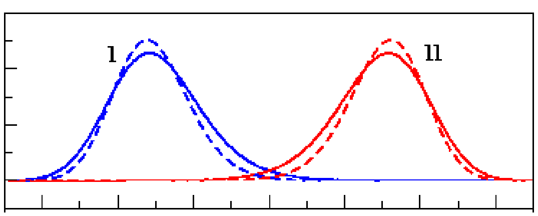
R_{oo} decreases as steric crowding near acceptor O decreases

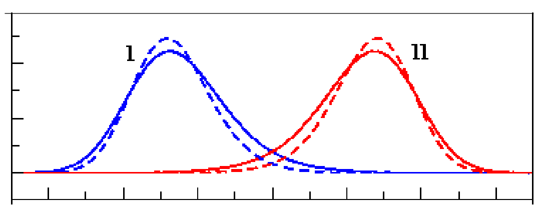




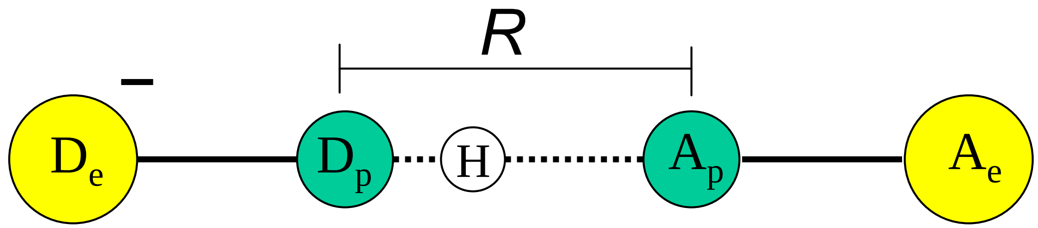
<u>CompA</u>
Rate = 1
KIE = 16.1
R_{OO} = 2.70 Å

<u>CompB</u>
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*

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

V^{el}: electronic coupling
$S_{\mu\nu}^{0}$: proton wavefunction overlap at R_{eq}
R_{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

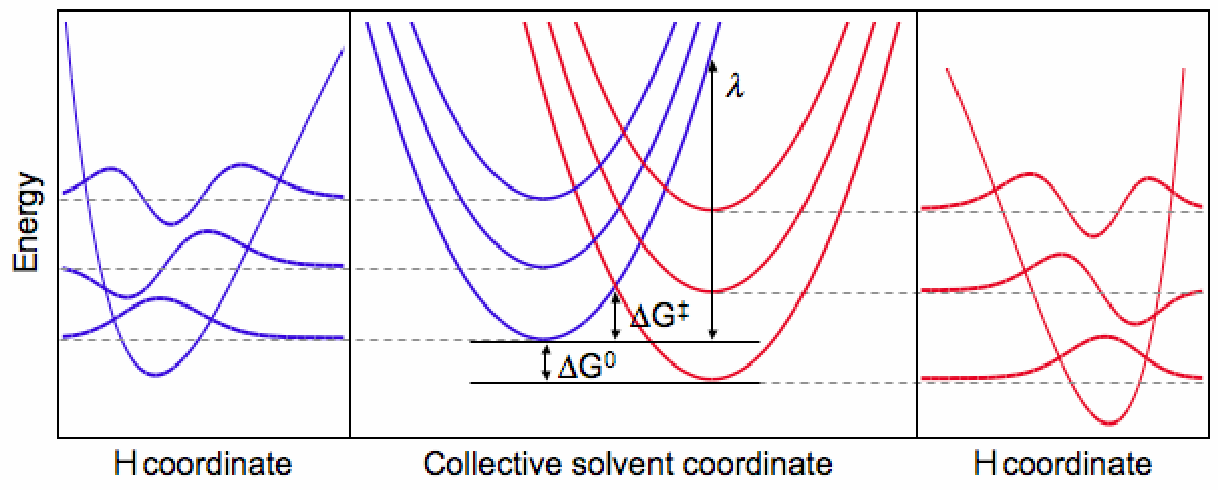
Analytical derivations: Soudackov, Hatcher, SHS, JCP 2005

Excited Vibronic States

$$k = \frac{2\pi}{\hbar} \sum_{\mu} P_{\mu}^{\mathrm{I}} \sum_{\nu} \left(4\pi\lambda_{\mu\nu} k_B T\right)^{-1/2} \left|V_{\mu\nu}\right|^2 \exp\left[-\Delta G_{\mu\nu}^{\dagger} / (k_B T)\right]$$

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 (λ)
  - 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) (ΔG^0)
  - QM calculations or estimate from pK_a's and redox potentials
- *R*-mode mass, frequency (M, Ω) 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 Ω; 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:

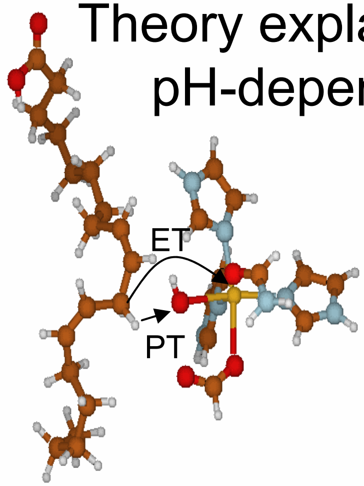
  Low-frequency R-mode expression predicts KIE decreases with T

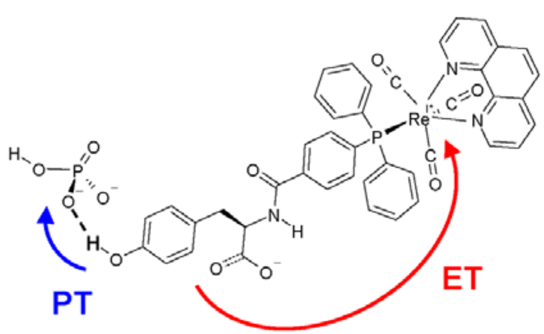
  Fixed-*R* and high-frequency R-mode expressions can lead to either increase or decrease of KIE with T

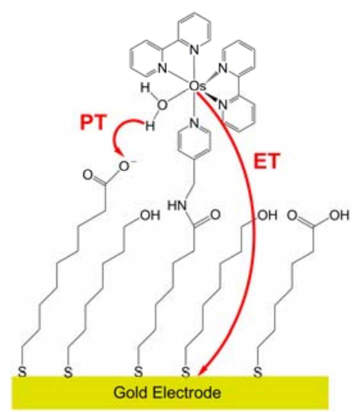
Applications to PCET Reactions

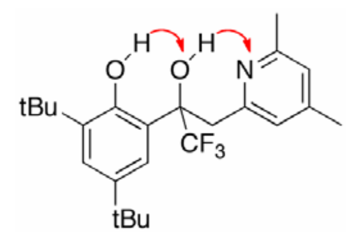
- Amidinium-carboxylate salt bridges (Nocera), *JACS* 1999
- Iron bi-imidazoline complexes (Mayer/Roth), *JACS* 2001
- Ruthenium polypyridyl complexes (Meyer/Thorp), *JACS* 2002
- DNA-acrylamide complexes (Sevilla), *JPCB* 2002
- Ruthenium-tyrosine complex (Hammarström), *JACS* 2003
- Soybean lipoxygenase 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









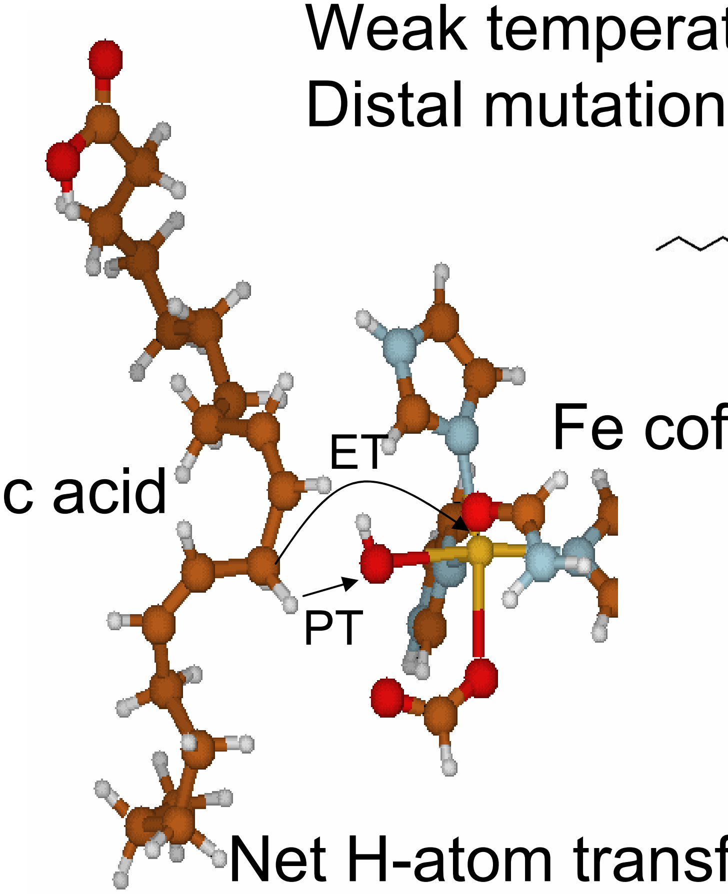
Soybean Lipoxygenase

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

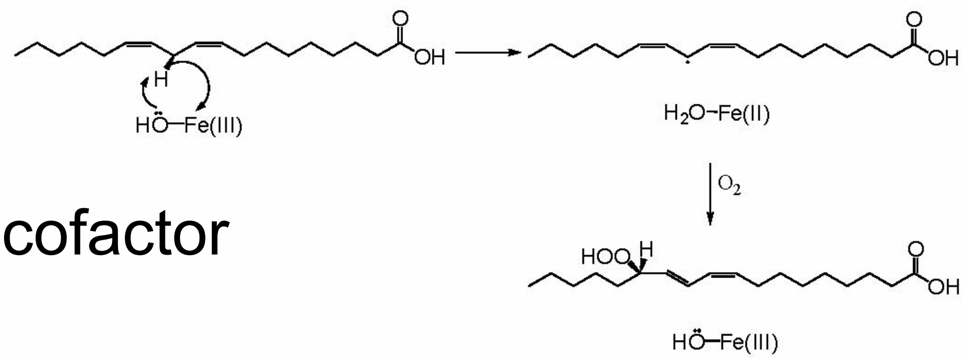
- Catalyzes oxidation of unsaturated fatty acids
- Experiment: KIE ≈81 at room temperature

Weak temperature dependence of rates and KIE
Distal mutations impact KIE

linoleic acid



Fe cofactor



Net H-atom transfer
Proton-coupled electron transfer (PCET) mechanism

Low-Frequency *R*-mode

$$\Omega << k_B T$$

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

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

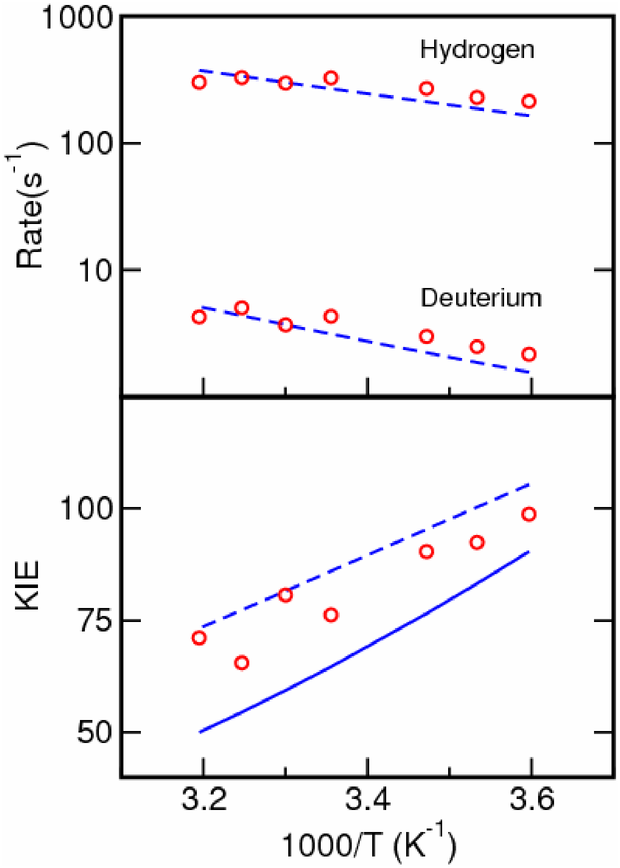
M, Ω: mass and frequency of *R*-mode
α: exponential *R*-dependence of vibronic coupling

Approximate KIE
(only ground states)

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

- T-dependence of KIE determined mainly by α and Ω
- Magnitude of KIE determined also by ratio of overlaps: smaller overlap $\rightarrow$ larger KIE

T-Dependence of Rates and KIE



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

Hatcher, Soudackov, SHS, JACS 2004 and 2007

Red circles: Experimental data

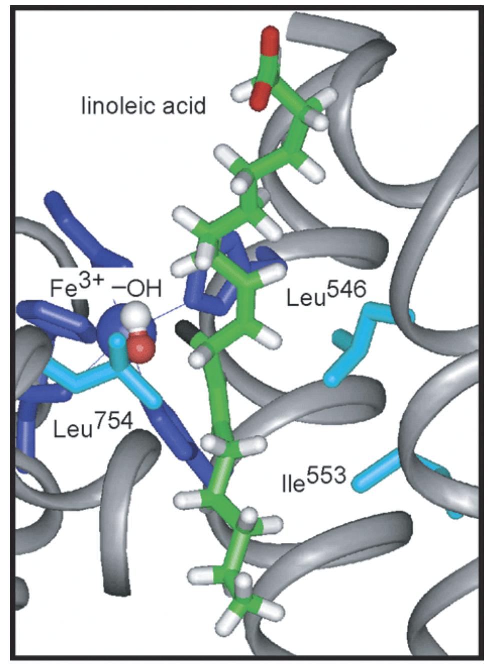
Knapp, Rickert, Klinman, JACS 124, 3865 (2002)

- High KIE: small overlap and dominance of lowest energy states
- Weak T-dependence of KIE: local *R*-mode (Ω not too low)

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

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

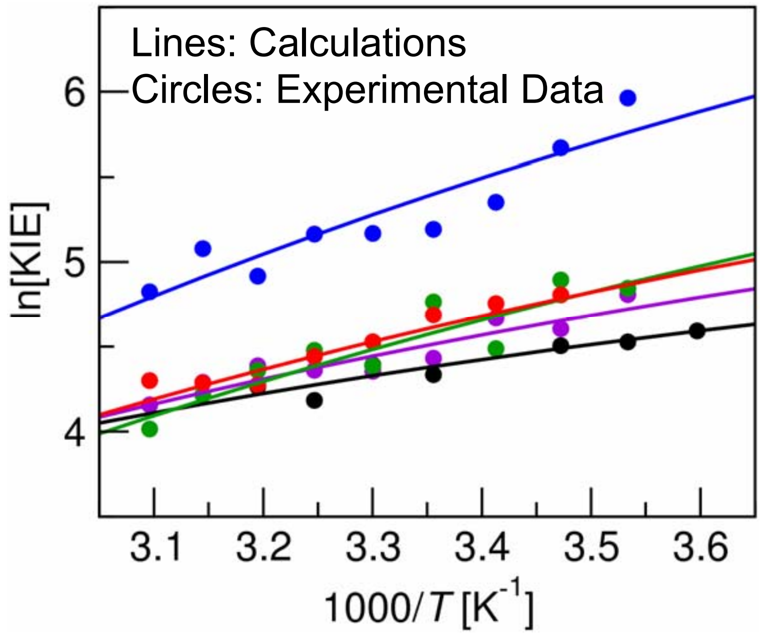


Experiments by Klinman group:
Mutation of Ile553, ~15 Å from iron →
Magnitude and T-dependence of KIE increase as residue 553 less bulky

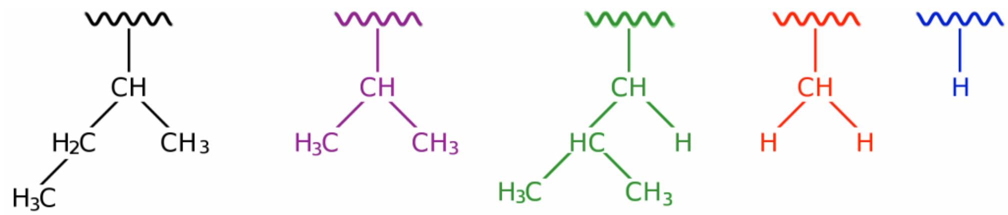
Knapp, Rickert, Klinman, JACS 124, 3865 (2002)

Calculations on Mutants

Edwards, Soudackov, and SHS, JPC B 2010



	R_{eq} (Å)	Ω (cm^{-1})
WT-SLO	2.72	174
$Ile^{553} \rightarrow$ Val	2.78	152
$Ile^{553} \rightarrow$ Leu	2.87	131
$Ile^{553} \rightarrow$ Ala	2.83	140
$Ile^{553} \rightarrow$ Gly	2.96	127



WT I553V I553L I553A I553G

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

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

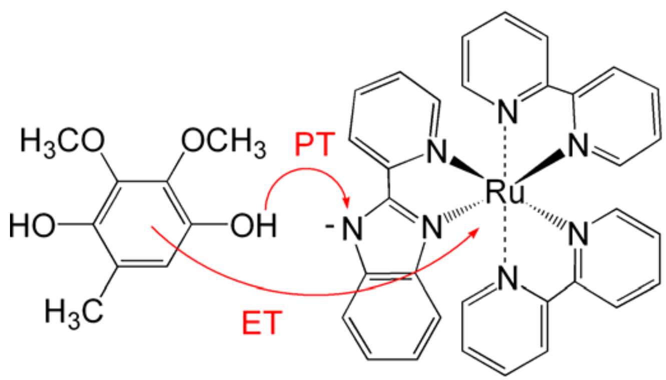
Calculated KIE from full expression including excited vibronic states

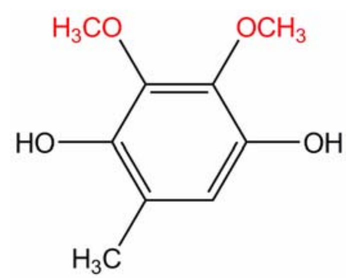
Expts: Knapp, Rickert, Klinman, JACS 2002; Meyer, Tomchick, Klinman, PNAS 2008

Quinol Oxidation

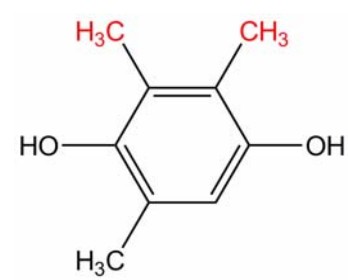
Experiments: *Cape, Bowman, Kramer, JACS 127, 4208 (2005)*

- 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





UQH_2



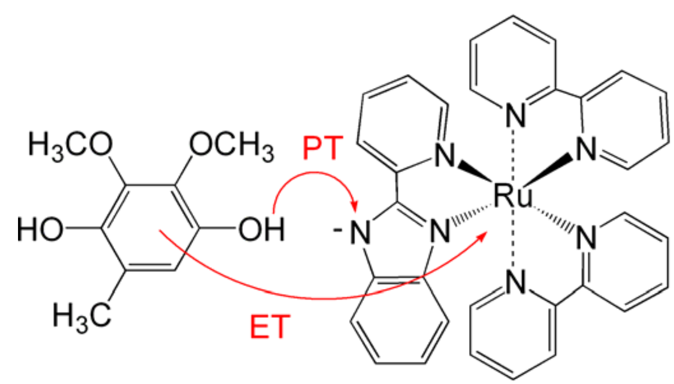
PQH_2

Calculations on Quinol Oxidation

Ludlow, Soudackov, SHS, JACS 2009

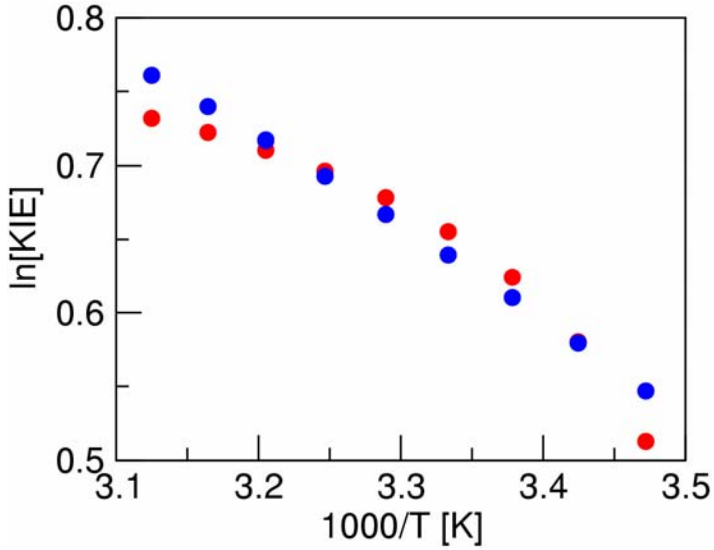
- DFT on complex
  $\Omega \approx 1000\ \mathrm{cm}^{-1}$, $M = 7.2$ amu for O–N motion in both quinols
  $R_{eq} \approx 2.65$ Å for both quinols
  Stiff H-bond, high-frequency R-mode
- Calculated solvent reorganization energies:
  $\lambda \approx 6.3$ kcal/mol for both quinols
  Net ET from N to Ru over 4.2 Å in acetonitrile
- Estimated driving forces from redox potentials, $\mathrm{p}K_a$s
  $\Delta G^0 \approx -6.0, -4.5$ kcal/mol for UQH_2, PQH_2
- Similarity of λ and ΔG^0 magnitudes indicates close to inverted Marcus region, $\lambda < -\Delta G^0$

QOH ··· ⁻NRu(III) $\rightarrow$ QO• ··· HNRu(II)

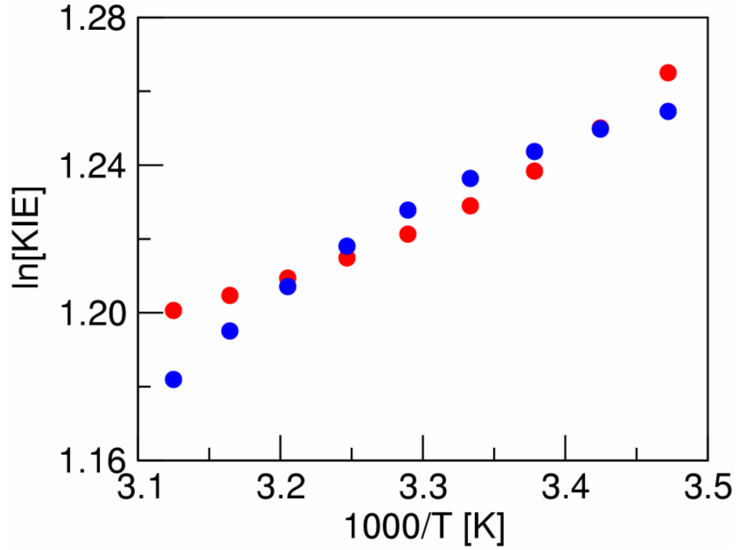


Inverse T-Dependence of KIE

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



UQH_2

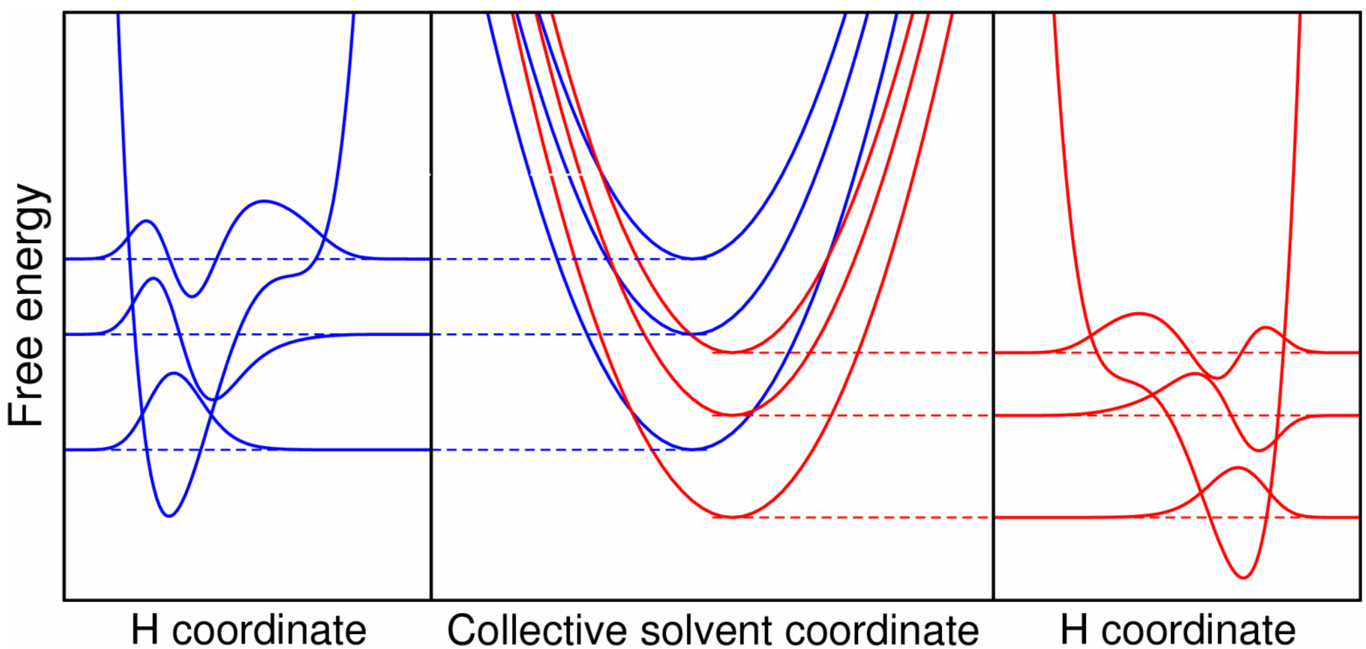


PQH_2

Blue: Theory
Red: Experiment

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

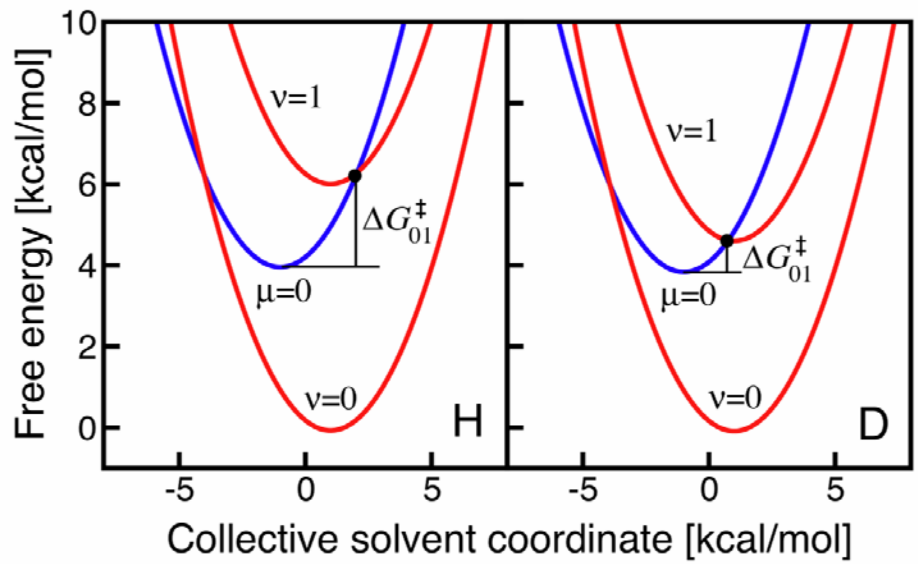
- Stiff H-bond (high-frequency *R*-mode)
- Small reorganization energy
- 0/0 transition in inverted region, 0/1 in normal region
- 0/1 is dominant contributor to overall rate

$$\text{KIE} \propto \exp\left[-\left(\Delta G^{\ddagger}_{01}(\text{H}) - \Delta G^{\ddagger}_{01}(\text{D})\right)\Big/ k_{\text{B}}T\right]$$

- Vibronic energy level splittings smaller for D than H

$$\Delta G^{\ddagger}_{01}(\text{H}) > \Delta G^{\ddagger}_{01}(\text{D})$$

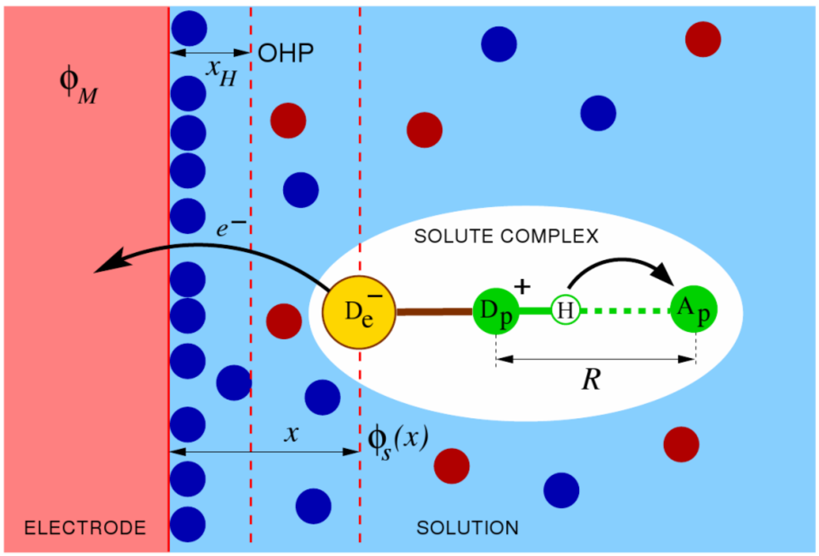
KIE increases as T increases

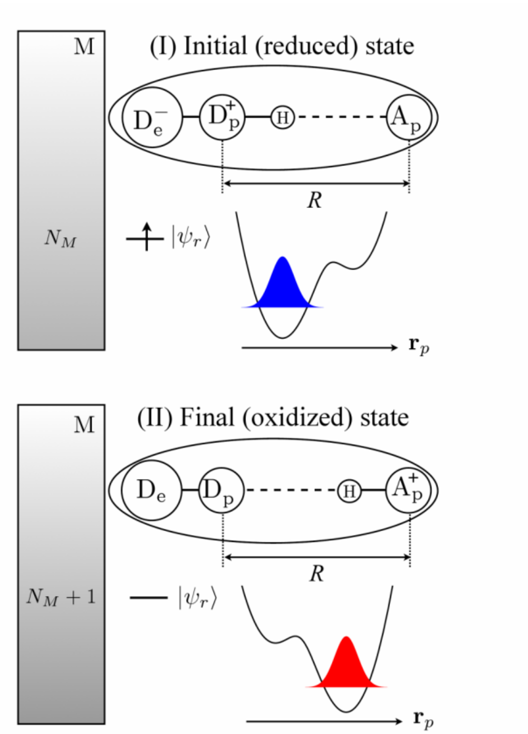


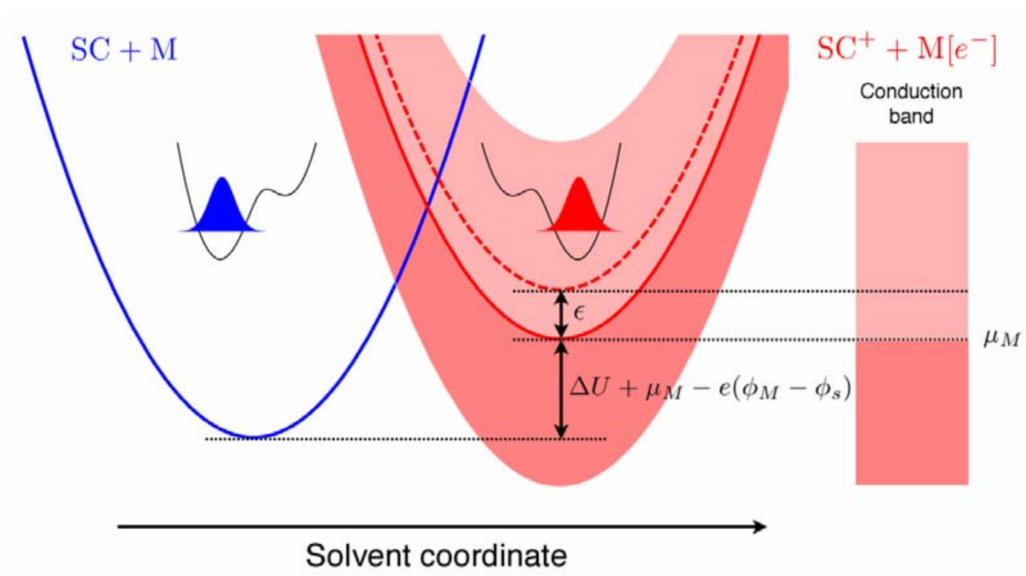
$$\Delta G^{\ddagger}_{01}(\text{H}) = 2.89 \text{ kcal/mol}$$

$$\Delta G^{\ddagger}_{01}(\text{D}) = 1.01 \text{ kcal/mol}$$

Electrochemical PCET Theory







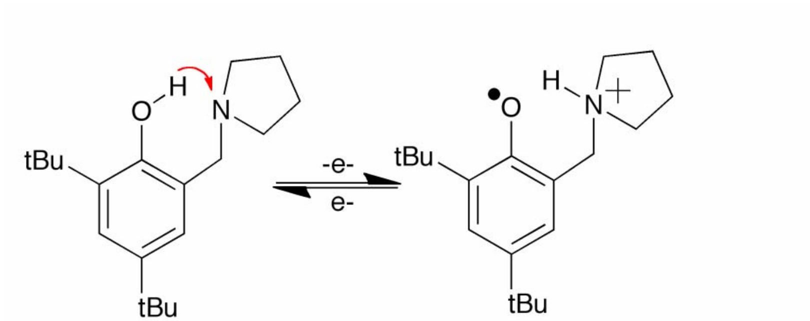
Derived expressions for electrochemical rate constants and current densities

Venkataraman, Soudackov, SHS, JPC C 2008

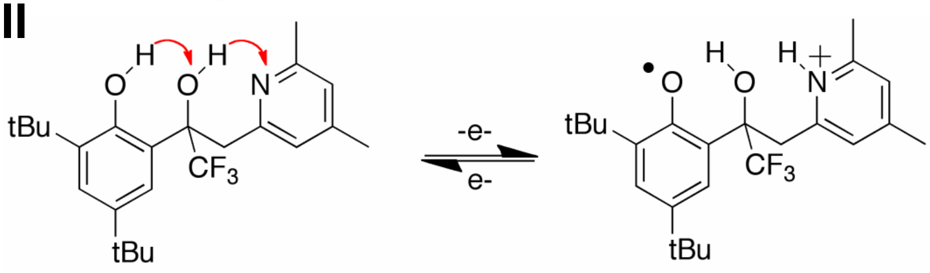
Proton Relay System: Proton Transport

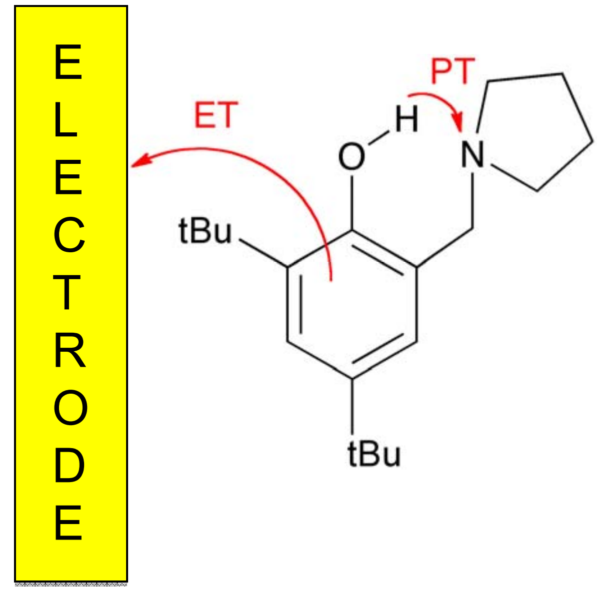
Costentin, Robert, Savéant, Tard, Angew. Chem. Int. Ed. 2010, 49, 1-5

System I



System II





	System I KIE	System II KIE	$k_{\mathrm{I}}/k_{\mathrm{II}}$
Experiment	1.7	2.4	16
Calculations	1.9	2.2	15

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

Nonadiabatic PCET treatment → System II slower because of smaller overlap between two-dimensional proton vibrational wavefunctions

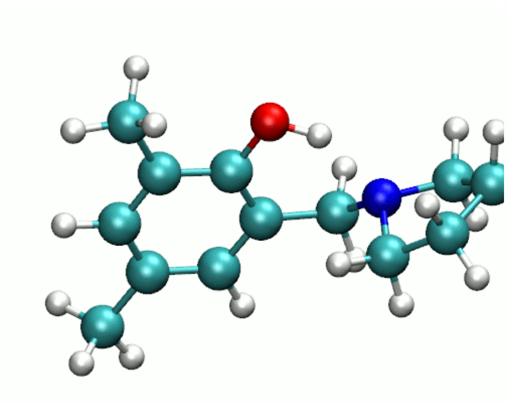
Calculation of Standard Rate Constants

Auer, Fernandez, SHS, JACS 2011

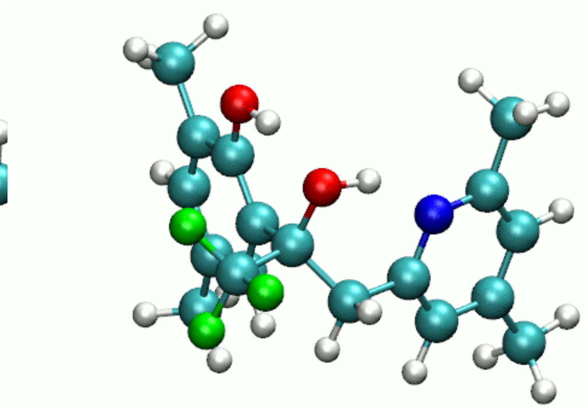
- Include proton donor-acceptor motion by thermal averaging over *R* with probability distribution *P*(*R*)

$$k(\eta) = \int_0^\infty {\color{red}k(\eta;R)}\,{\color{blue}P(R)}\,dR$$

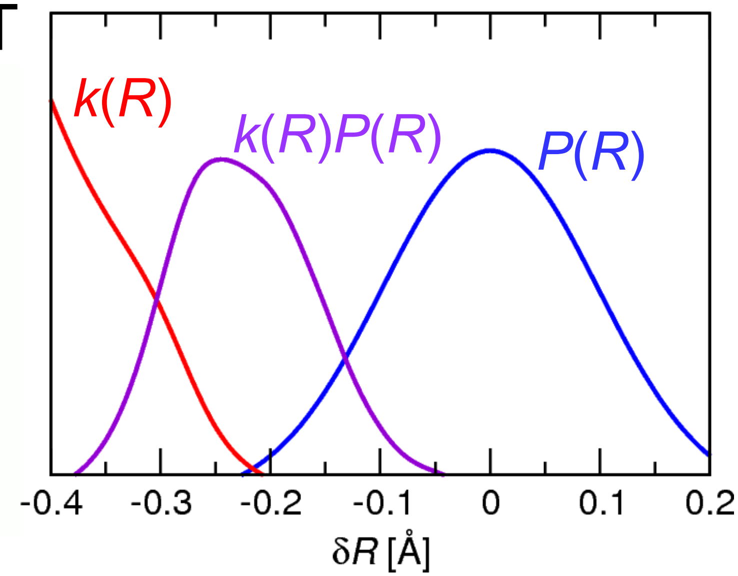
- Two-proton system: include only symmetric motion where both distances decrease and increase concurrently
- Optimize reduced and oxidized structures with constrained *R* and calculate proton potentials w/ DFT



System I

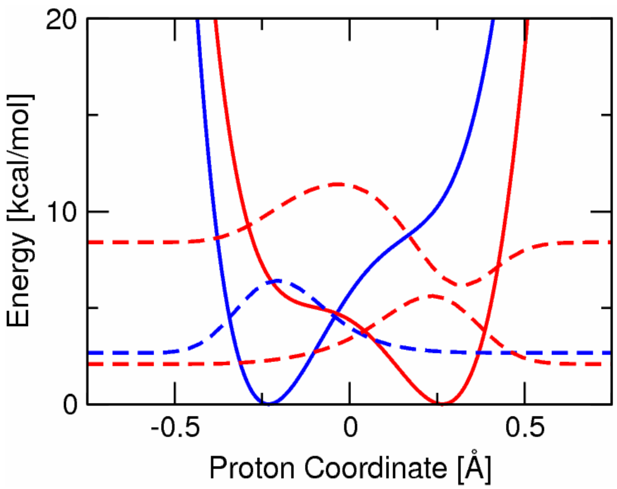


System II



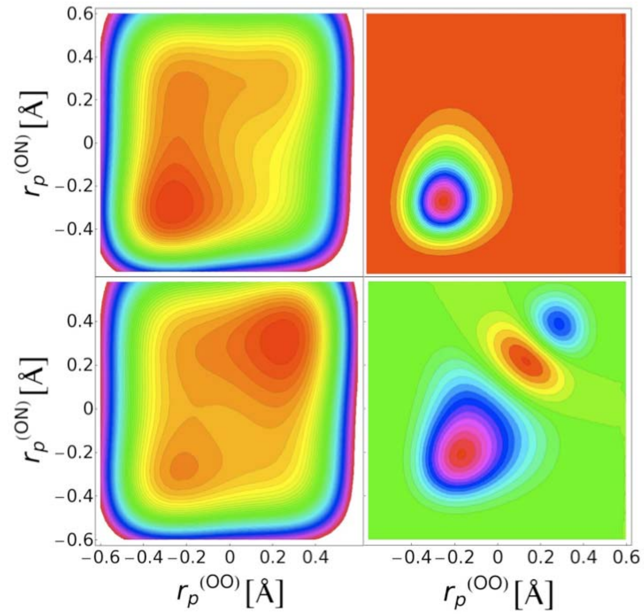
Analysis of Results

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 → KIE(I) = 1.7, KIE(II) = 2.4, k_I/k_{II} = 16

At equilibrium *R*-distances including only ground states → KIE(I) ≈ 2.4 ×10^3, KIE(II) ≈ 2.7 ×10^6, k_I/k_{II} ≈ 2.5 ×10^5,

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 → 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