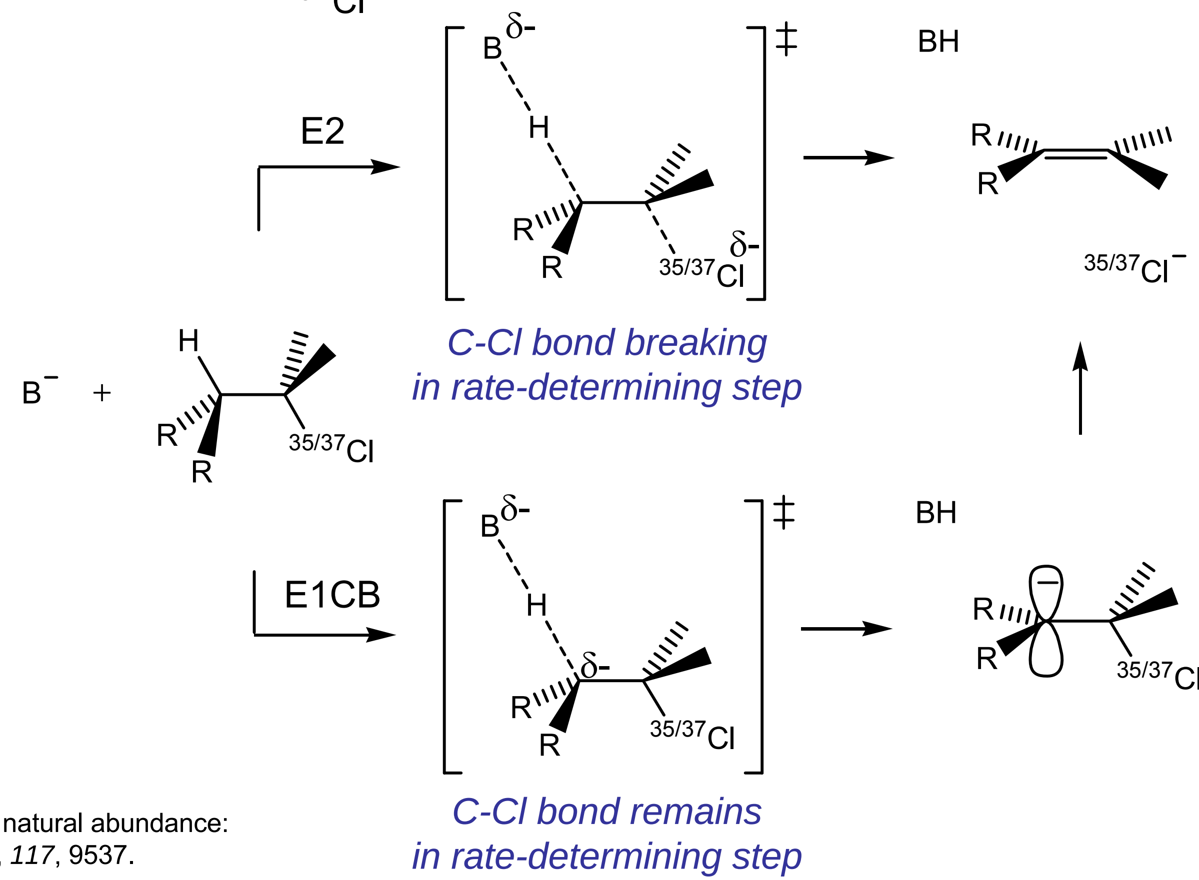
Heavy-Atom Isotope Effects

k_H/k_D provides large change in reduced mass,
but other isotope effects also studied.

isotope effect	*typical maximum value*
$\frac{k_{^{12}C}}{k_{^{13}C}}$	~ 1.04
$\frac{k_{^{12}C}}{k_{^{14}C}}$	~ 1.07
$\frac{k_{^{14}N}}{k_{^{15}N}}$	~ 1.03
$\frac{k_{^{16}O}}{k_{^{18}O}}$	~ 1.03

Example: $\frac{k_{^{35}Cl}}{k_{^{37}Cl}}$



Method for measuring heavy isotope effects at natural abundance:
Singleton, D. A. et al. *J. Am. Chem. Soc.* **1995**, *117*, 9537.

Heavy-Atom Isotope Effects

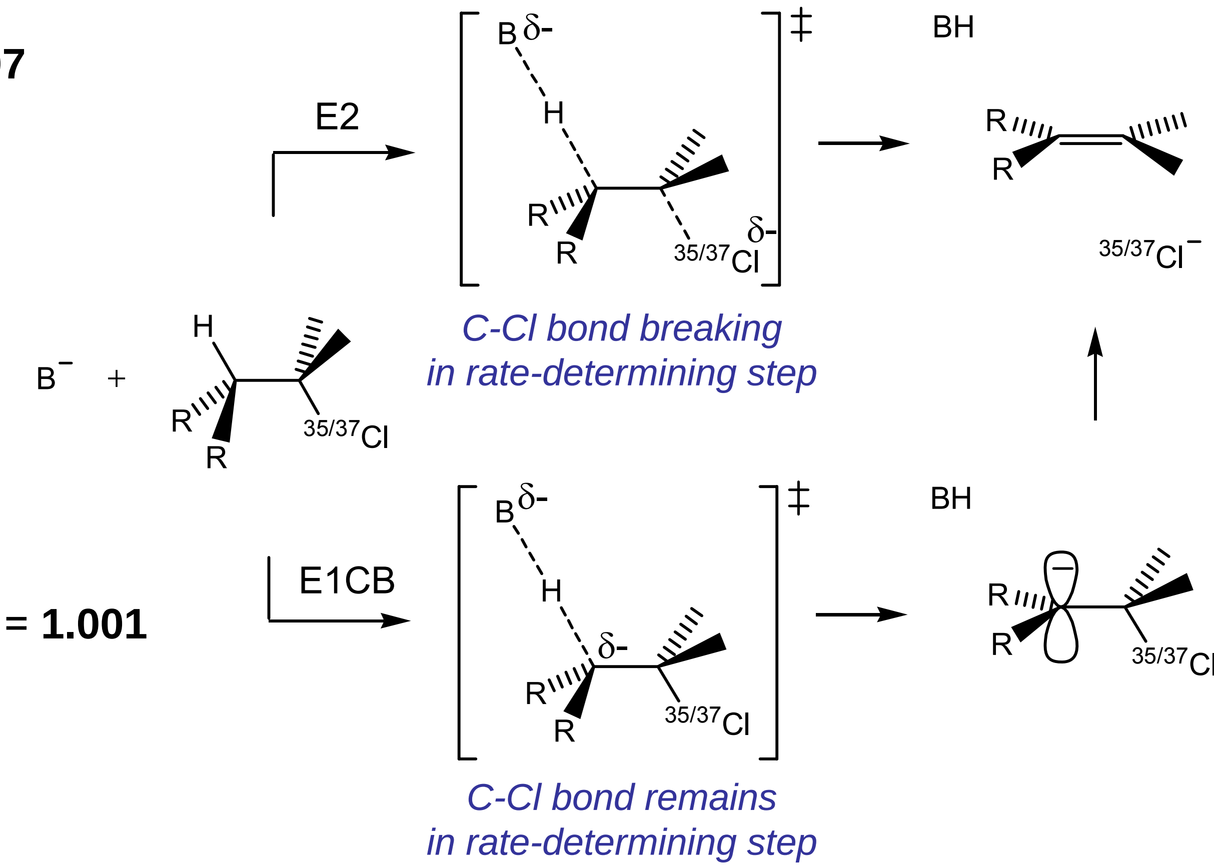
k_H/k_D provides large change in reduced mass,
but other isotope effects also studied.

R = CH_3 $\quad \dfrac{k_{^{35}Cl}}{k_{^{37}Cl}} = \mathbf{1.007}$

(follows E2)

R = (p-NO_2)Ph $\quad \dfrac{k_{^{35}Cl}}{k_{^{37}Cl}} = \mathbf{1.001}$

*(follows E1CB;
carbanion stabilized
by Ph groups)*



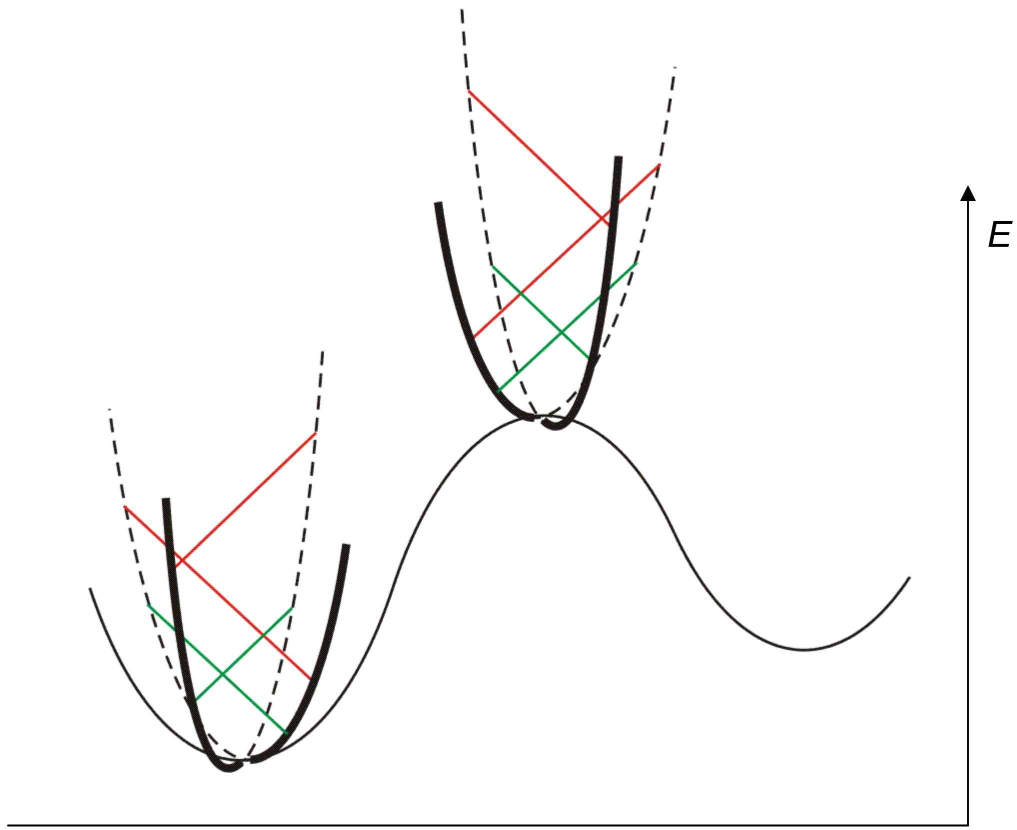
Calculating Isotope Effects

$$\left(\frac{k_H}{k_D}\right)_{\substack{\text{overall,}\\ \text{for } n \text{ modes}}} = \left(\frac{k_H}{k_D}\right)_{\nu_1}\left(\frac{k_H}{k_D}\right)_{\nu_2}\cdots\left(\frac{k_H}{k_D}\right)_{\nu_n}$$

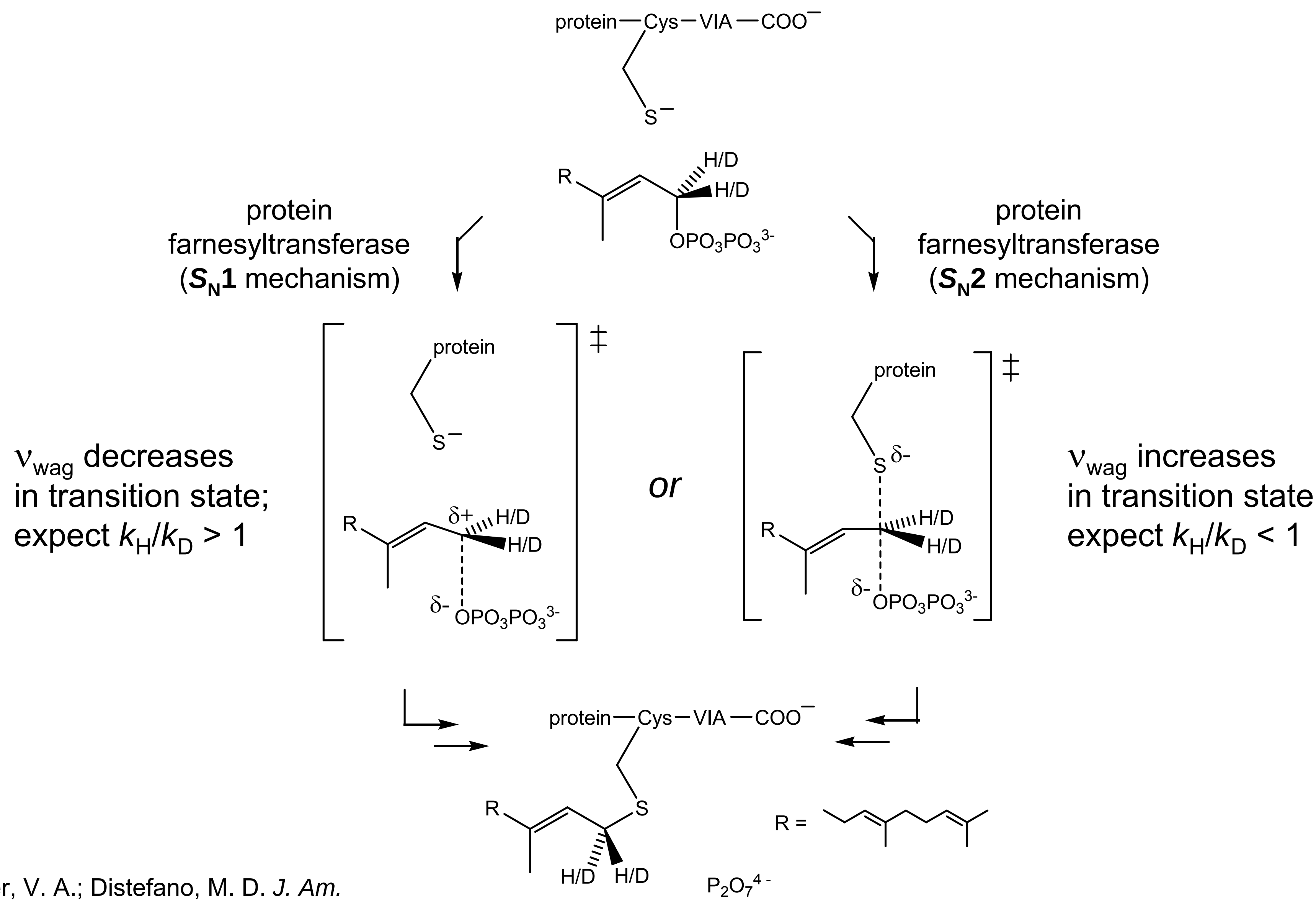
For simplicity, we have tried to identify one major component to isotope effect;

But, there may be multiple components.

Computational methods for calculating isotope effects for all vibrational modes are available.

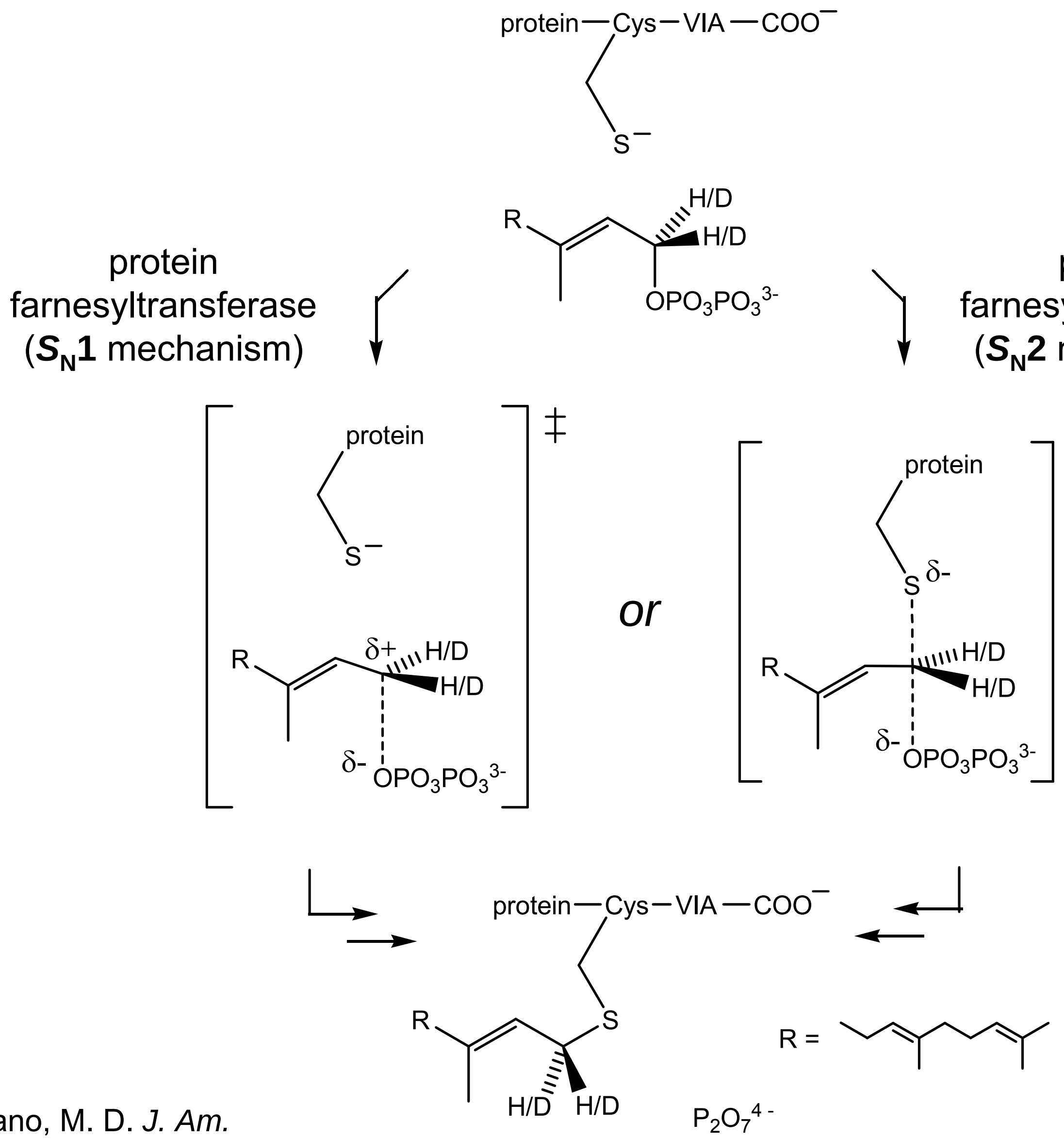


Isotope Effects and Enzyme Mechanisms



Weller, V. A.; Distefano, M. D. *J. Am. Chem. Soc.* **1998**, *120*, 7975-7976.

Isotope Effects and Enzyme Mechanisms

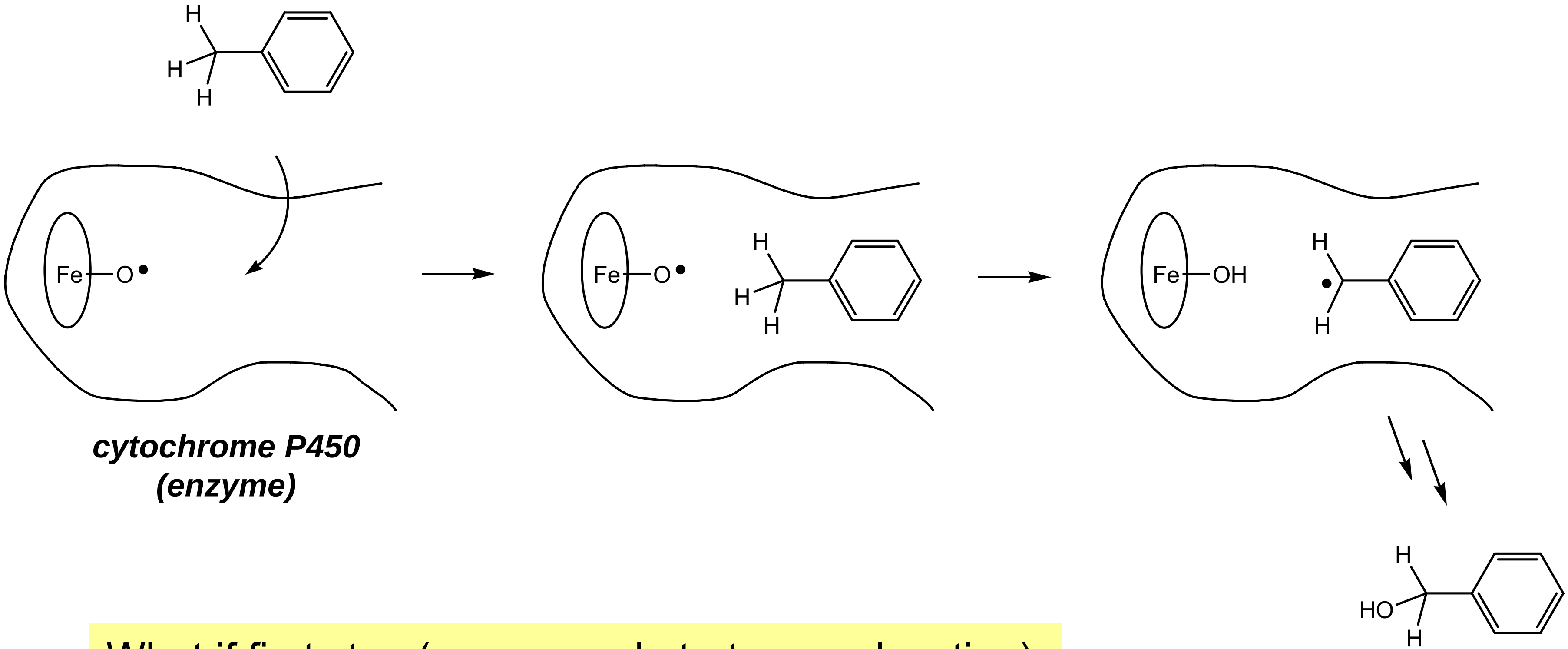


$$\frac{k_H}{k_D} = \mathbf{0.977}$$

Enyme facilitates
S_N2 mechanism

Weller, V. A.; Distefano, M. D. *J. Am. Chem. Soc.* **1998**, *120*, 7975-7976.

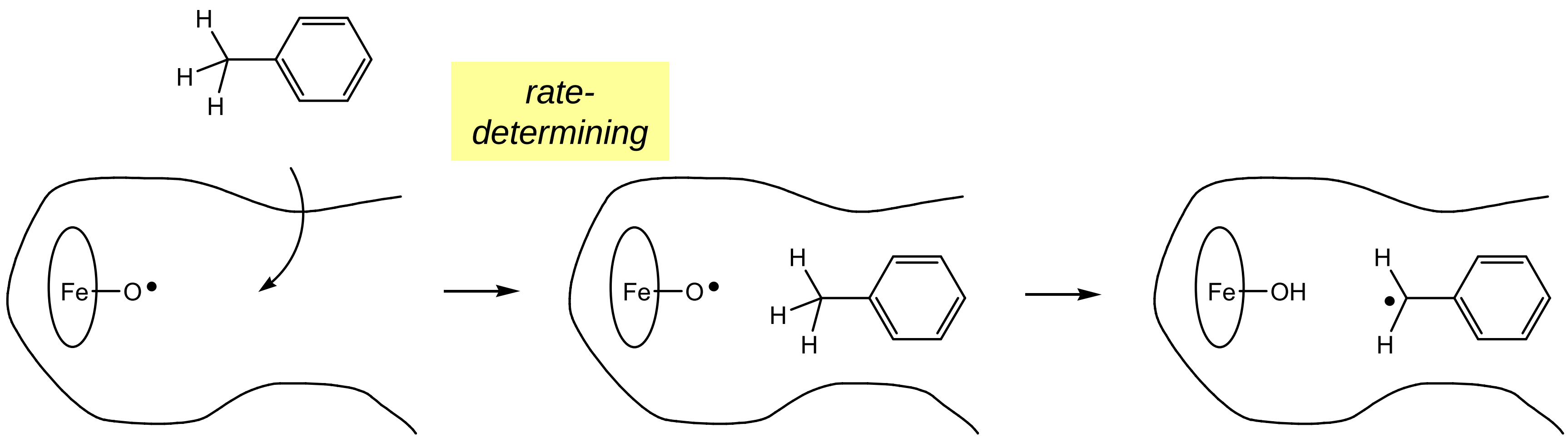
Intra- vs. Inter-molecular Isotope Effects: Probing Non-Rate-Determining Steps



cytochrome P450 (enzyme)

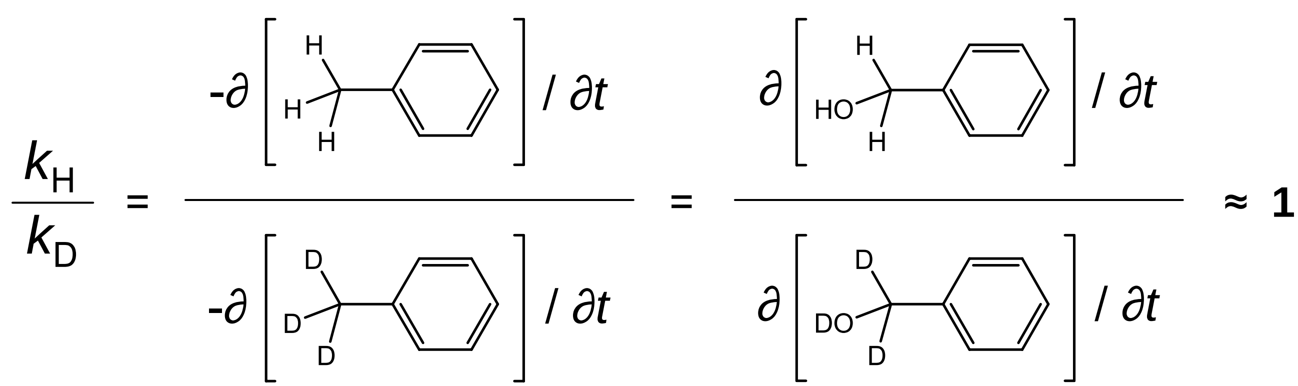
What if first step (enzyme-substrate complexation) is rate-determining?
How do we find out about mechanism of subsequent steps?

Intra- vs. Inter-molecular Isotope Effects: Probing Non-Rate-Determining Steps

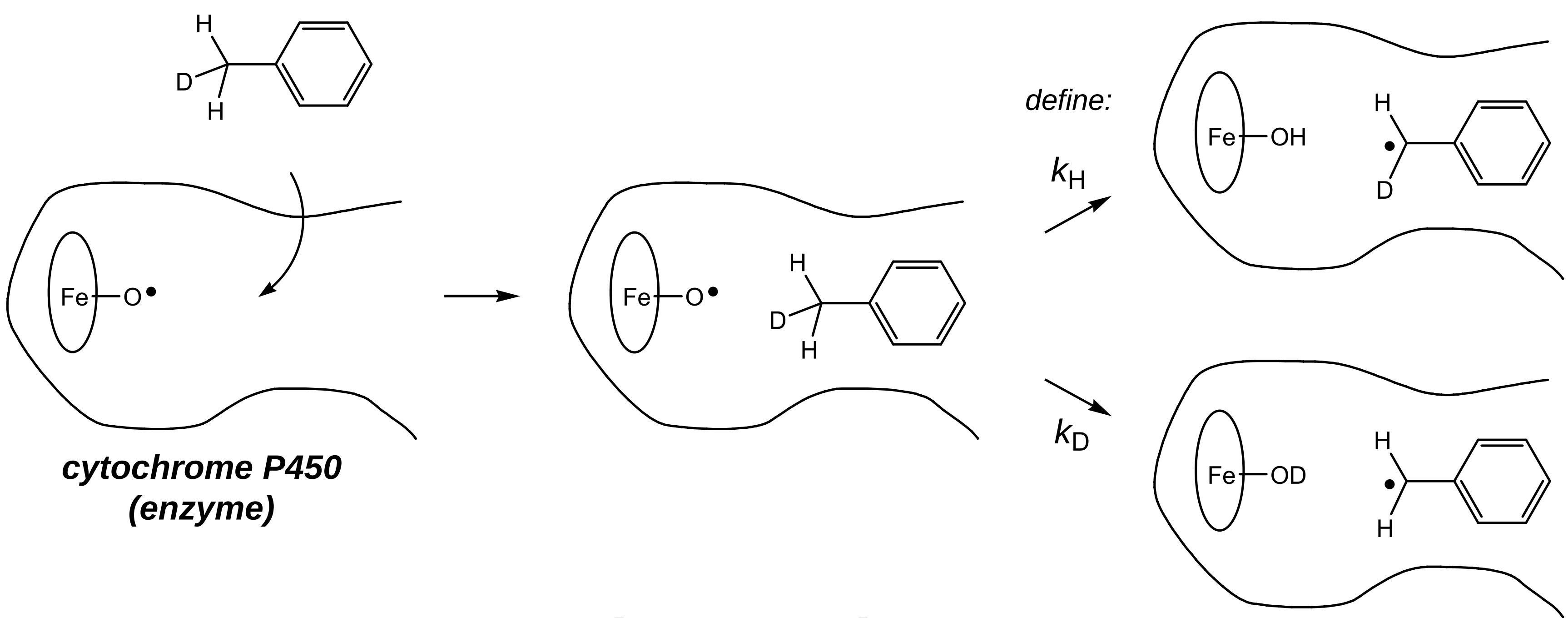


cytochrome P450
(enzyme)

For ***intermolecular*** comparison, would expect

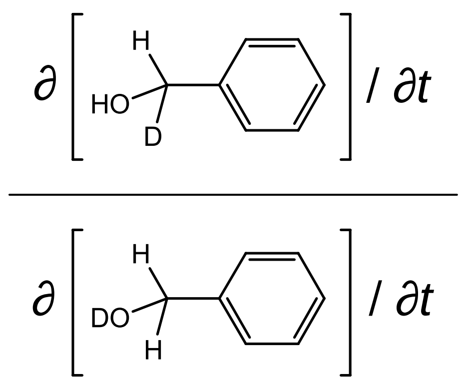


Intra- vs. Inter-molecular Isotope Effects: Probing Non-Rate-Determining Steps



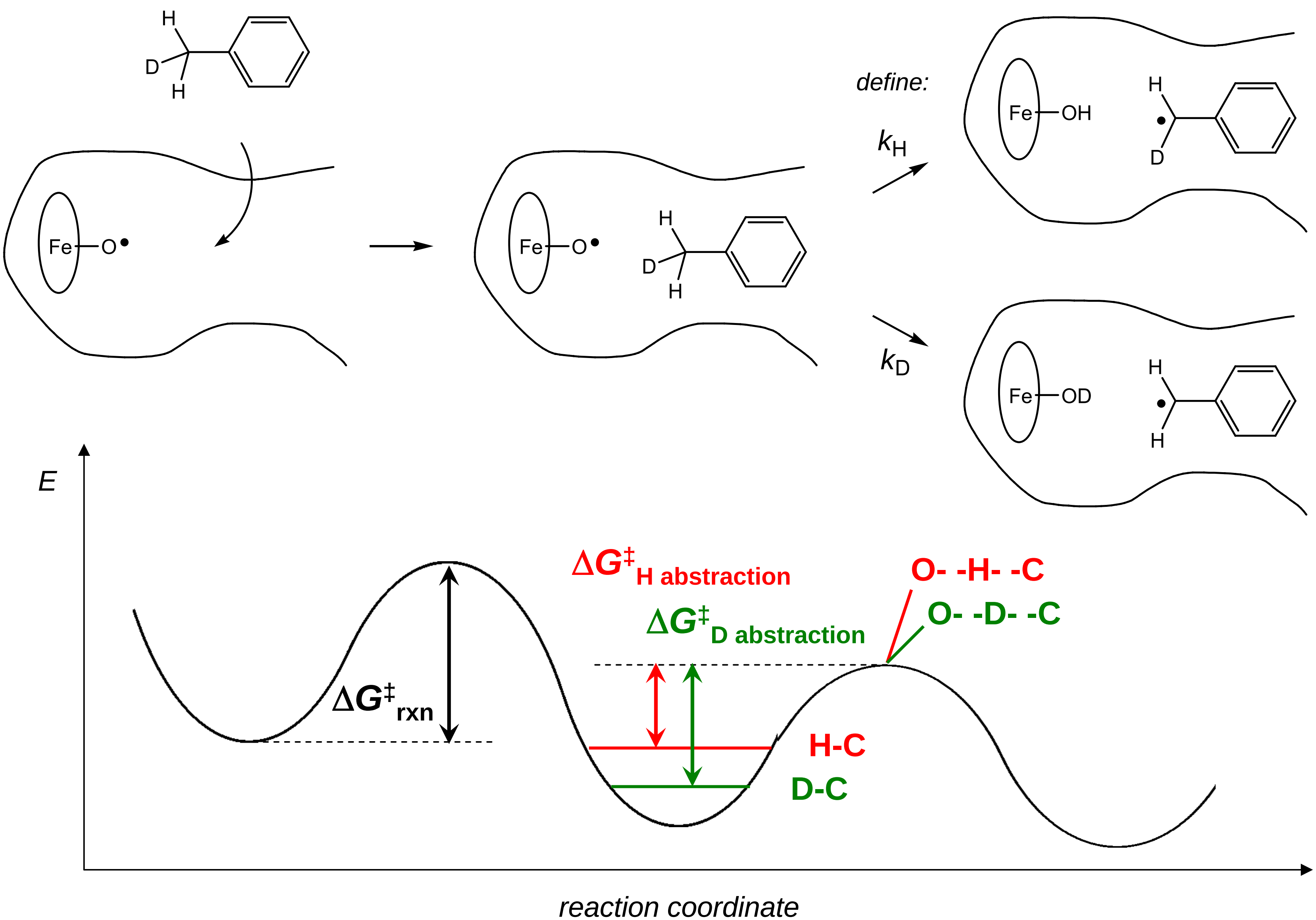
For ***intramolecular*** comparison, what would we expect for

$\frac{k_H}{k_D}$ =

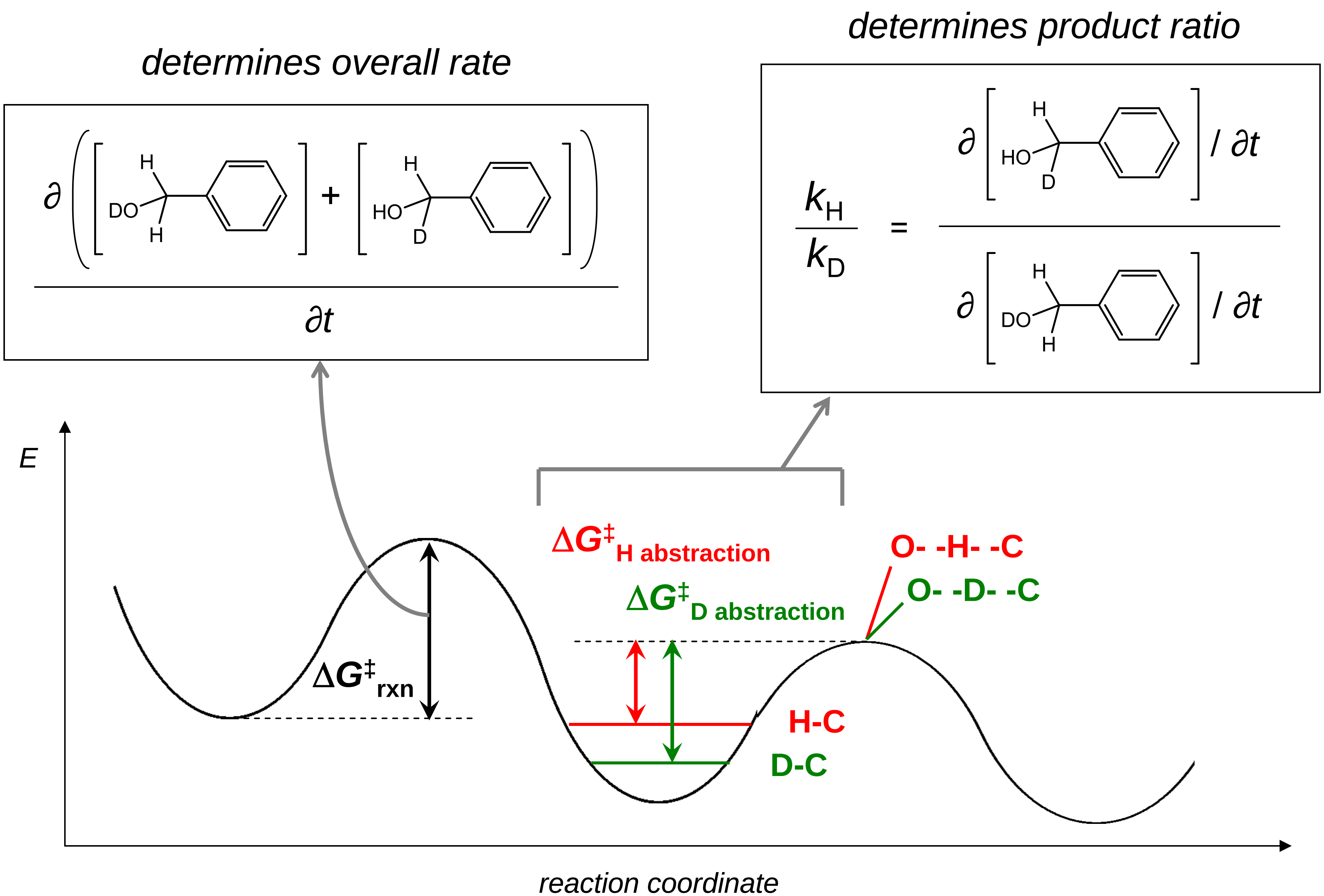


??

Intramolecular Isotope Effects

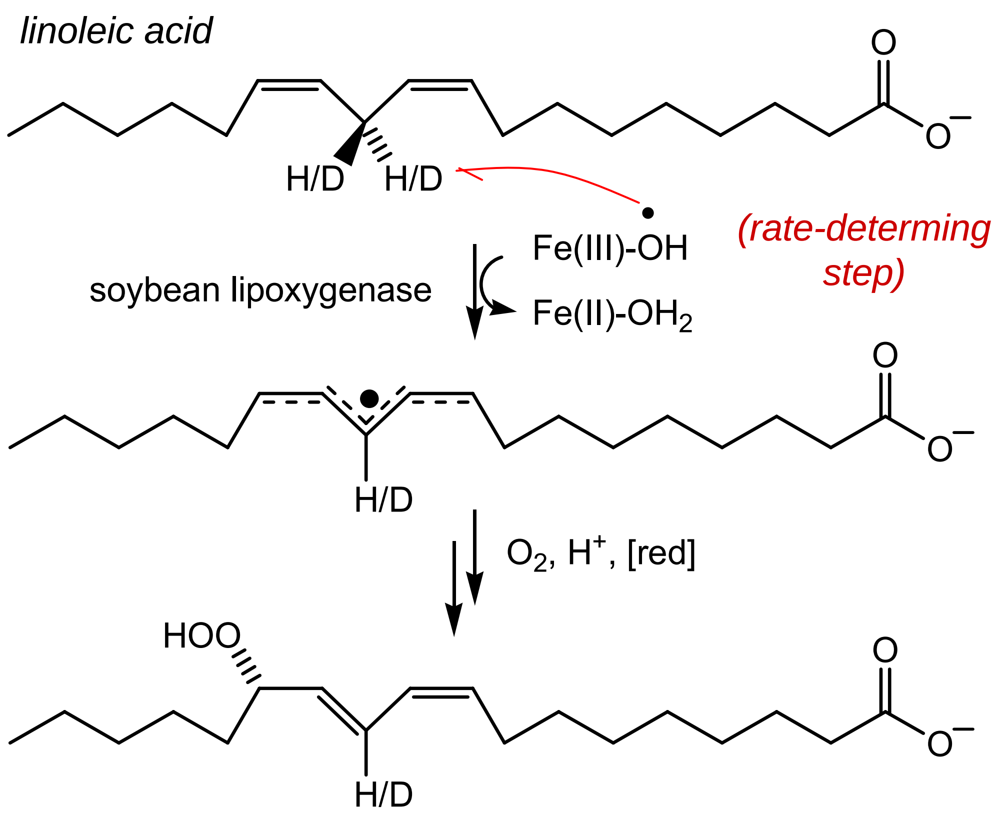


Intramolecular Isotope Effects



Isotope Effects and Tunneling

Occasionally, isotope effects are too large to explain with vibrational mode analysis.



$$\frac{k_H}{k_D} = \mathbf{81\ (!)}$$

Explanation:

Isotope effect from differing abilities of H & D to “tunnel” through kinetic reaction barrier.

See: Kohen, A.; Klinman, J. P. *Chem. Biol.* **1999**, *6*, R191-R198; Kohen, A.; Klinman, J. P. *Acc. Chem. Res.* **1998**, *31*, 397 -404.

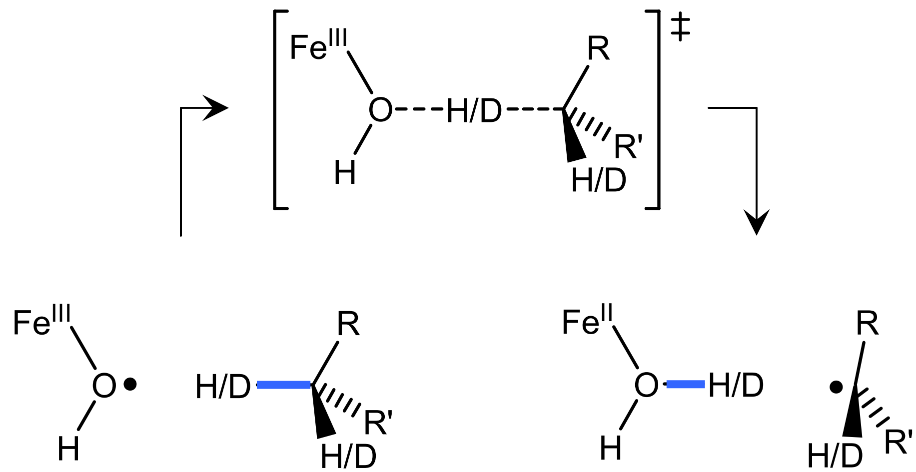
Isotope Effects and Tunneling

de Broglie Wavelength:

$$\lambda = \frac{h}{mv} = \frac{h}{\sqrt{2mkT}}$$

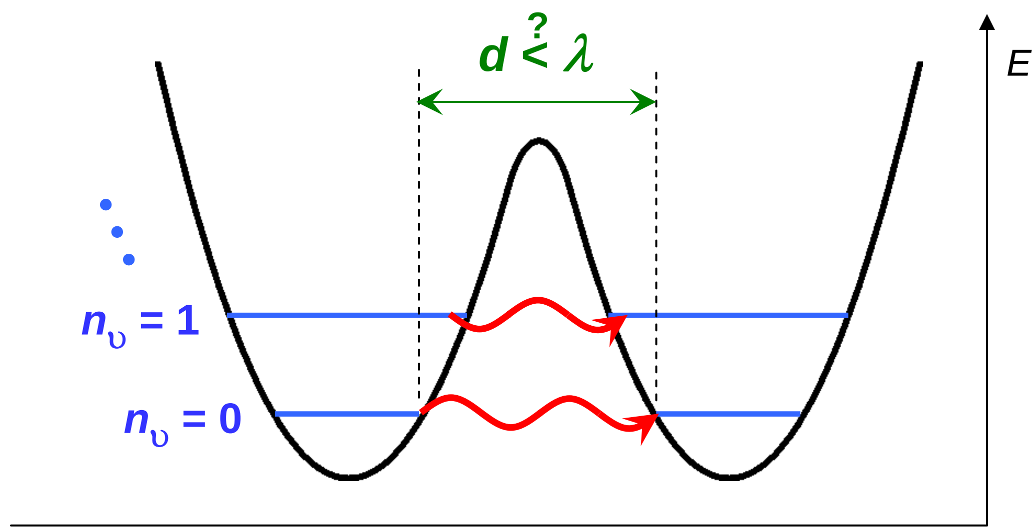
λ(H) = 1.8 Å (at 298 K)

λ(D) = 1.2 Å

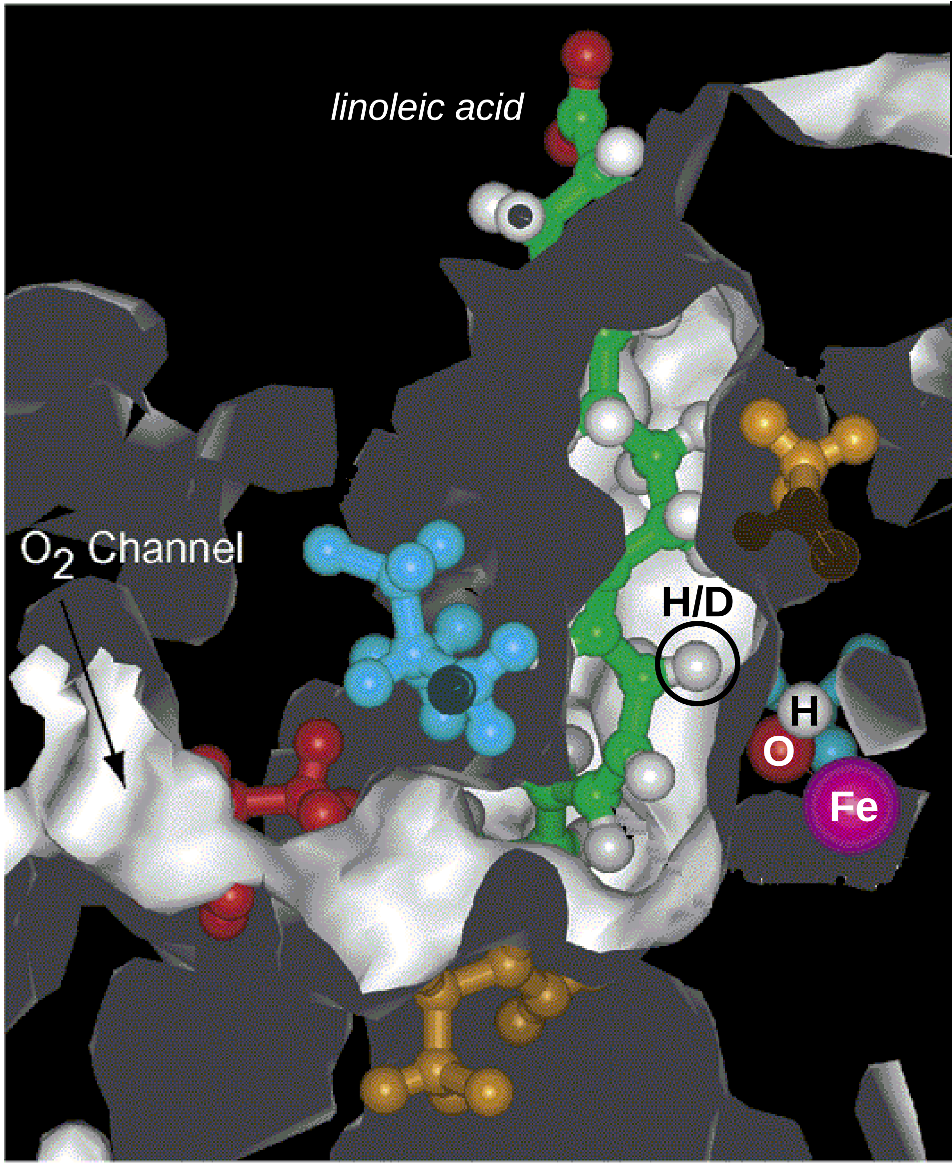


Heisenberg uncertainty results in larger displacements for H than D.

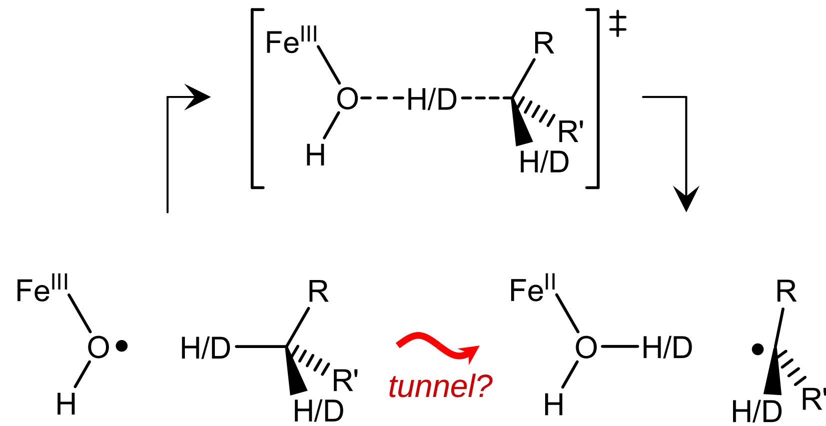
So, H "tunnels" through barriers better than D.



Isotope Effects and Tunneling



Crystal structure, soybean lipoxygenase w/ linoleic acid



Hypothesis:

Enzyme operates by forcing H(D) into tunneling distance (< λ).

Knapp, M. J.; Seebeck, F. P.; Klinman, J. P. *J. Am. Chem. Soc.* **2001**, *123*, 2931-2932.

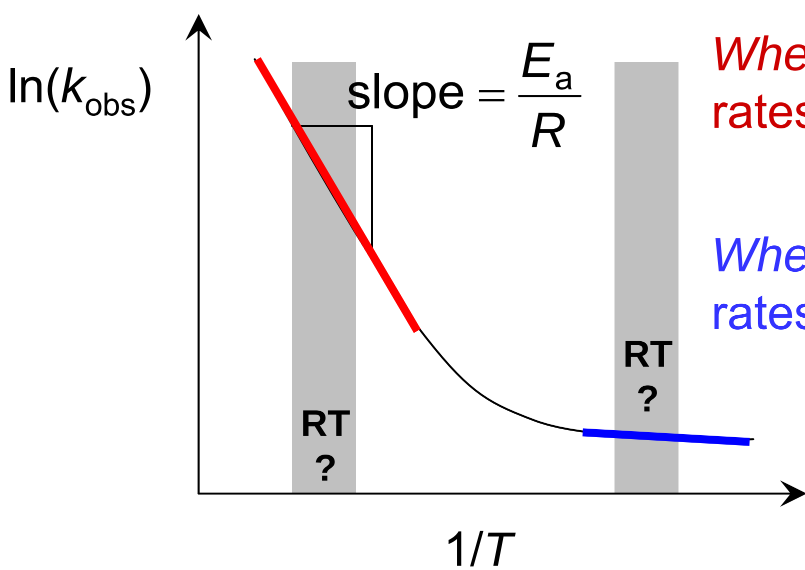
Isotope Effects and Tunneling

Important feature of tunneling:

de Broglie wavelength λ much less sensitive to temperature than rate constants k.

So, if

$$k_{obs} = k_{thermal} + k_{tunneling} \,,$$



When $k_{thermal}$ dominates,
rates vary with temperature in usual way.

When $k_{tunneling}$ dominates,
rates vary little with temperature. ("E_a" ≈ 0)

Question is, which regime is exhibited at room temperature?