The role of quantum effects in proton transfer reactions in enzymes: quantum tunneling in a noisy environment?

To cite this article: Jacques P Bothma et al 2010 New J. Phys. 12 055002

View the article online for updates and enhancements.

Related content
- Theoretical investigations of proton and hydrogen atom transfer in the condensed phase
  Mikhail V Basilevsky and Mikhail V Vener
- Molecular photophysics
  G Beddard
- Electrostatics and dynamics of proteins
  Thomas Simonson

Recent citations
- Mixed quantum-classical simulation of the hydride transfer reaction catalyzed by dihydrofolate reductase based on a mapped system-harmonic bath model
  Yang Xu et al
- Isotopic fractionation in proteins as a measure of hydrogen bond length
  Ross H, McKenzie et al
- Factors Affecting Hydrogen-Tunneling Contribution in Hydroxylation Reactions Promoted by Oxoiron(IV) Porphyrin - Cation Radical Complexes
  Zhiqi Cong et al
The role of quantum effects in proton transfer reactions in enzymes: quantum tunneling in a noisy environment?

Jacques P Bothma¹, Joel B Gilmore² and Ross H McKenzie³

School of Mathematics and Physics, The University of Queensland, Brisbane 4072, Australia
E-mail: r.mckenzie@uq.edu.au

Received 21 January 2010
Published 21 May 2010
Online at http://www.njp.org/
doi:10.1088/1367-2630/12/5/055002

Abstract. We consider the role of quantum effects in the transfer of hydrogen-like species in enzyme-catalyzed reactions. This review is stimulated by claims that the observed magnitude and temperature dependence of kinetic isotope effects (KIEs) implies that quantum tunneling below the energy barrier associated with the transition state significantly enhances the reaction rate in many enzymes. We review the path integral approach and the Caldeira–Leggett model, which provides a general framework to describe and understand tunneling in a quantum system that interacts with a noisy environment at nonzero temperature. Here the quantum system is the active site of the enzyme, and the environment is the surrounding protein and water. Tunneling well below the barrier only occurs for temperatures less than a temperature $T_0$, which is determined by the curvature of the potential energy surface near the top of the barrier. We argue that for most enzymes this temperature is less than room temperature. We review typical values for the parameters in the Caldeira–Leggett Hamiltonian, including the frequency-dependent friction and noise due to the environment. For physically reasonable parameters, we show that quantum transition state theory gives a quantitative description of the temperature dependence and magnitude of KIEs for two classes of enzymes that have been claimed to exhibit signatures of quantum tunneling. The only quantum effects

¹ Current address: Biophysics Graduate Group, University of California, Berkeley, CA 94720-3200, USA.
² Current address: ROAM consulting, 49 Sherwood Road, Toowong, Queensland 4066, Australia.
³ Author to whom any correspondence should be addressed.
are those associated with the transition state, both reflection at the barrier top and tunneling just below the barrier. We establish that the friction and noise due to the environment are weak and only slightly modify the reaction rate. Furthermore, at room temperature and for typical energy barriers environmental fluctuations with frequencies much less than 1000 cm\(^{-1}\) do not have a significant effect on quantum corrections to the reaction rate. This is essentially because the time scales associated with the dynamics of proton transfer are faster than much of the low-frequency noise associated with the protein and solvent.

Contents

1. Introduction 3
2. Background 5
   2.1. KIEs 5
   2.2. Semi-classical transition state theory 5
   2.3. Heuristic justification for quantum transition state theory 5
   2.4. Arrenhius parameters for enzymes are inconsistent with semi-classical transition state theory 7
3. Rate theory 7
   3.1. The Caldeira–Leggett model Hamiltonian describes a quantum system interacting with its environment 7
   3.2. The spectral density describes the frequency-dependent friction due to the environment 9
   3.3. Classical Kramers Theory defines an effective barrier frequency 11
   3.4. Path integral representation of quantum Kramers theory 11
   3.5. The bounce solution describes quantum tunneling, which only occurs below a temperature, \(T_0\) 12
4. Quantum correction factor: \(T > T_0\) 13
   4.1. The weak friction limit reduces to Bell’s expression 13
   4.2. Apparent Arrhenius parameters in the weak friction limit 14
   4.3. Effect of the environment on the crossover temperature 15
   4.4. Non-parabolicity of the barrier only matters at temperatures close to \(T_0\) 15
5. Estimates of model parameter values for enzymes 16
   5.1. Frequency of oscillations in the reactant well (\(\omega_0\)) 16
   5.2. Barrier frequency (\(\omega_b\)) 17
   5.3. Frequency-dependent friction due to the solvent–protein environment 17
6. Quantum transition state theory describes the experimental data 21
7. Conclusions 23
Acknowledgments 24
References 24
1. Introduction

The possible existence and importance of quantum effects in biomolecular systems are intriguing and controversial. Whether quantum effects such as superposition, interference, tunneling or entanglement are crucial to the function of specific biomolecules is receiving increasing attention [1]–[14]. One might expect most quantum effects to be destroyed by decoherence [15]–[17] because biomolecules interact strongly with their ‘hot and wet’ environment, i.e. they function at room temperature in a highly polar solvent, water. Arguably, the most well-established case of a quantum effect being crucial for biomolecular function is electron tunneling in proteins [18]. Furthermore, it has been argued that by evolution, electron transfer proteins vary and are selected based on tunneling parameters [18]. The role of tunneling in other biomolecular systems has also been examined [19]. For example, in myoglobin it has been found that the reaction rate for binding of carbon monoxide becomes independent of temperature below 80 K, due to the presence of quantum tunneling [20, 21].

Over the past two decades, the possibility of quantum tunneling of protons in enzymes has attracted considerable attention [1, 11], [22]–[26]. The large kinetic isotope effects and their temperature dependence are inconsistent with semi-classical transition state theory, whereby the chemical reaction occurs via thermal activation over an energy barrier. These discrepancies have been interpreted as evidence for the presence of tunneling [11], [27]–[40]. However, it should be stressed that this evidence is rather indirect, being based on the values of fitting parameters for Arrhenius plots for the temperature dependence of the reaction rate, where the absolute temperature only varies by about 10%. In contrast, for chemical reactions involving much simpler organic molecules, such as benzoic acid [41] or hydroxymethylene [42] much more definitive signatures of proton tunneling have been observed. These include a temperature-independent rate at low temperatures and tunnel splitting of the ground state energy [43, 44].

Key questions that need to be answered include the following.

- Are there definitive experimental signatures of tunneling?
- Can some of the experimental results be explained without invoking tunneling?
- To what extent is it necessary to go beyond semi-classical transition state theory to explain the observed kinetic isotope effects (KIEs)?
- If tunneling does occur, is it important for the function of the enzyme?
- Have enzymes evolved in a manner that enhances the contribution of tunneling?

There are currently a wide range of views on the answers to these questions. For example, a review in science states that ‘the entire and sole source of the catalytic power of enzymes is due to the lowering of the free energy of activation and any increase in the generalized transmission co-efficient, as compared to that of the uncatalyzed reaction’ [45]. Villa and Warshel state that ‘the most important contribution to catalysis comes from the reduction of the activation free energy by electrostatic effects . . . the popular proposal that enzymes catalyze reactions by special dynamical effects is not supported by a consistent simulation study . . . the interpretation of recent experiments as evidence for dynamical contributions to catalysis is unjustified’ [46]. In contrast, Klinman et al state that ‘our present findings on hydrogen transfer under physiological conditions cannot be explained without invoking both quantum mechanics and enzyme dynamics’ [35]. Furthermore, Klinman and Kohen proposed that ‘the optimization of enzyme catalysis may entail the evolutionary implementation of chemical strategies that increase the probability of tunneling and thereby accelerate the reaction rate’ [47].
Doll and Finke [48] and Doll et al [49] synthesized artificial catalysts that performed the same chemistry in solution (i.e. in the absence of the enzyme) and exhibited the same kinetic isotopic effects. Hence, they conclude that enzymes have not evolved to enhance tunneling. In a paper that focused on simulations, Schwartz and co-workers [50] express a similar view to Klinman’s, ‘the action of the enzyme in speeding the chemical reaction, however, is postulated to be intimately connected to the directed vibrational motion identified in this paper. Thus, it appears that evolution has designed the protein matrix of an enzyme not just to hold substrates or stabilize transition state formation, but rather to channel energy in a specific chemically relevant direction’.

Over the past 5 years, several reviews of different theoretical approaches to this problem have appeared [11, 22], [51]–[53]. Most of the theoretical works make two particular assumptions that may be debatable: (i) that the proton transfer process is adiabatic and (ii) that a single reaction coordinate is adequate. For a detailed discussion of these issues, we refer to a recent review by Marcus [22]. We also note that for non-enzymatic reactions, the first assumption has been brought into question and an alternative non-adiabatic picture (analogous to electron transfer) due to Borgis and Hynes [54] has been proposed [55] and is found to give a good description of the molecule HONO [56]. The non-adiabatic proton transfer theory has been applied to enzymes [57]–[60]. Then, the only way for the proton to move from the reactant to the product state is via tunneling. The activation energy is then associated with the reorganization of the environment rather than that of the transition state. Siebrand and Smedarchina [61] considered such an approach to explain how some enzymes have a large KIE that is weakly temperature dependent.

One approach to examine the role of quantum effects in complex biological molecules is to use quantum mechanical molecular mechanics (QM-MM) simulations. In this approach, atoms that are directly involved in the reaction are treated quantum mechanically, while the rest of the enzyme is treated classically. This approach has been applied to a number of different enzymes [26], [62]–[71]. Schwartz and co-workers have as their starting point a Hamiltonian similar to the one used here [50, 52, 72]. They used classical molecular dynamic techniques to simulate specific reactions and extract the spectral density.

Using a low-energy effective Hamiltonian model such as the Caldeira–Leggett Hamiltonian [73] to capture the essential physics of the relevant process offers a complementary approach to QM-MM simulations. It has the advantage that quantum effects and the role of the environment can be treated more rigorously, via path integral methods [15, 74]. Furthermore, the dependence of behavior on the key physical parameters such as the curvature of the potential energy surface near the transition state can be elucidated.

In this review, we make the following points using a path integral approach. (i) Tunneling well below the barrier only occurs for temperatures less than a temperature $T_0$, which is largely determined by the curvature of the top of the barrier. We argue that for most enzymes this temperature is less than room temperature. (ii) For physically reasonable parameters, quantum transition state theory gives a quantitative description of the temperature dependence and magnitude of KIEs for two classes of enzymes that have been claimed to exhibit signatures of quantum tunneling. The only quantum effects are those associated with the transition state, both reflection at the barrier top and tunneling just below the barrier. (iii) The friction on the proton due to the environment is weak and only slightly modifies the reaction rate. (iv) At room temperature, environmental degrees of freedom with frequencies much less than 1000 cm$^{-1}$ do not have a significant effect on quantum corrections to the reaction rate.

2. Background

2.1. KIEs

The rate coefficient \( k_L \) for a chemical reaction involving a species \( L \) at temperature \( T \) can be written in the Arrhenius form

\[
    k_L = A_L \exp\left(-\frac{E_L}{k_B T}\right),
\]

where \( E_L \) denotes the activation energy for the reaction and \( A_L \) is the prefactor. The two quantities \( A_L \) and \( E_L \) are generally referred to as the Arrhenius parameters.

All the reactions we will be interested in involve breaking or forming bonds that contain hydrogen species (protons, deuterium, tritium, hydrogen atoms and hydrogen anions). We will only be considering the primary KIEs for systems where the hydrogen transfer step is rate limiting. The kinetic isotope effect (KIE) is generally expressed as the ratio of rate constants \( k_H / k_T \) or \( k_H / k_D \), where the subscripts H, D and T denote the reactions in which a proton, deuterium and tritium are being transferred, respectively.

2.2. Semi-classical transition state theory

Consider the one-dimensional potential energy shown in figure 1. A reaction from \( A \) to \( C \) proceeds via a transition state at \( B \). The following expression for the rate coefficient is widely used [75, 76]:

\[
    k = \kappa \left(\frac{k_B T}{\hbar}\right) \frac{Z^\dagger}{Z_A} \exp\left(-\frac{E_b}{k_B T}\right) \equiv \kappa k_{TST},
\]

where \( Z_A \) is the quantum partition function of the metastable state \( A \) and \( Z^\dagger \) is the quantum partition function of the activated complex \( B \). The parameter \( \kappa \) was originally introduced to account for the fact that some trajectories may re-cross the transition state and return to the reactant state and is also the set used to include the effects of quantum tunneling. In an ad-hoc semi-classical transition state theory, the activation energy is replaced by \( E_b - \hbar \omega_0 / 2 \), which corrects for the effect of the quantum-zero point motion in the reactant well. This leads to KIEs because \( \omega_0 \) depends on the mass of the hydrogenic species being transferred.

Kim and Kreevoy [77] gave three criteria that are widely considered to be signatures of quantum tunneling in hydrogen-transfer reactions: (i) a deuterium KIE \( k_H / k_D \) significantly larger than 6.4 at 20°C (or 8.9 if secondary isotope effects are included); (ii) an activation energy difference \( E_H - E_D \) larger than 5.0 kJ mol\(^{-1}\); and (iii) a ratio of prefactors \( A_H / A_D \) less than 0.7. However, it should be noted that Kim and Kreevoy also stated that ‘it appears that completely unambiguous experimental proof that tunneling occurs at \( \sim 300 \text{K} \) would be impossible to obtain, although tunneling clearly becomes evident at much lower temperatures’.

Following Bell [78] (p 89), Kohen and Limbach [79] state that if tunneling is not significant, prefactor ratios should fall within the range of \( 0.3 \leq A_H / A_T \leq 1.7 \) and \( 0.5 \leq A_D / A_T \leq 1.4 \). These bounds are obtained by considering the range of possible values of \( Z^\dagger / Z_A \) from the contribution of the vibrational modes transverse to the reaction coordinate.

2.3. Heuristic justification for quantum transition state theory

Here we reproduce the simple arguments described by Weiss [15]. A particle in thermodynamic equilibrium in the reactant well \( A \) is in a metastable state, and each quantum microstate
Figure 1. Potential energy as a function of the reaction coordinate, \( x \), with the metastable reaction state at A, the transition state at B and the final product state at C (after [75]). Escape occurs via the forward rate \( k \) and \( E_b \) is the corresponding activation energy. The angular frequency of oscillations about the reactant state is \( \omega_0 \), which depends on the curvature of the potential energy surface at the local minimum (\( x = x_a \)) and the mass of the particle. Similarly, the barrier frequency \( \omega_b \) depends on the curvature of the potential energy at the local maximum (\( x = x_b \)) and the mass of the particle. This paper addresses the question to what extent the reaction \( A \rightarrow C \) can proceed via quantum tunneling below the barrier for enzyme-catalyzed hydrogen transfer reactions.

associated with A can be viewed as contributing a term to the system partition function \( Z \) with an imaginary part, i.e. \( \epsilon_n = E_n + i\hbar\Gamma_n \), where \( E_n \gg \hbar\Gamma_n \), and

\[
Z = \sum_n \exp(-\epsilon_n/k_B T) = Z_1 + i Z_2 \simeq \sum_n \exp(-E_n/k_B T) - i \sum_n \frac{\hbar \Gamma_n}{k_B T} \exp(-E_n/k_B T). \tag{3}
\]

The total decay rate out of the reactant well is then

\[
k = \frac{1}{Z_1} \sum_n \Gamma_n \exp(-\epsilon_n/k_B T) = \frac{k_B T}{\hbar} \frac{Z_2}{Z_1}. \tag{4}
\]

If the motion in the reactant well A is described by a single harmonic oscillator in thermal equilibrium, with frequency \( \omega_0 \), the partition function is

\[
Z_A = \frac{1}{\sinh (\hbar \omega_0/2k_B T)}. \tag{5}
\]

If the barrier is an inverted parabola, then in this partition function we can replace \( \omega_0 \) with \( i \omega_b \) to obtain

\[
Z^2 = \frac{i}{\sin (\hbar \omega_b/2k_B T)} \exp(-E_b/k_B T). \tag{6}
\]

Assuming no quantum coherence between the bottom and the top of the barrier, the total partition function is then \( Z = Z_A + i Z^2 \). Substituting this into (4) gives

\[
k = \frac{\omega_b \sinh (\hbar \omega_b/2k_B T)}{4\pi} \frac{1}{\sin (\hbar \omega_b/2k_B T)} \exp(-E_b/k_B T). \tag{7}
\]

An important limitation of this expression is that it is only well defined for temperatures \( T > T_0 \) where

\[
T_0 = \frac{\hbar \omega_b}{2\pi k_B}. \tag{8}
\]
Expression (7) was first derived by Bell [80] using an expression for the energy dependence of the transmission probability through a parabolic barrier. Bell referred to this as the ‘tunnel correction’ because it allows for tunneling below the barrier. However, this nomenclature is misleading because it actually comes largely from the fact that in quantum mechanics a particle with energy $E > E_b$ has less than unit probability of transmission (i.e. the above barrier reflection occurs). Bell’s derivation required that $T \gg T_0$ and $2\pi E_b \gg \hbar \omega_b$ [78].

Note that in the limit $\hbar \omega_b \ll 2k_B T \ll \hbar \omega_b$, equation (7) reduces to the semi-classical expression

$$k = \frac{k_B T}{\hbar} \exp \left( -\frac{E_b - \hbar \omega_b/2}{k_B T} \right).$$

In section 3.5, we use path integral methods to give a rigorous derivation of Wigner’s expression, which will also elucidate its range of validity and the physical significance of the temperature scale $T_0$. In section 6, we show that this expression can be used to give a quantitative description of the magnitude and temperature dependence of KIEs in several important classes of enzymes. In section 4.4, we will see the fact that the rate diverges as $T$ approaches $T_0$ is an artifact of treating the potential barrier as parabolic.

2.4. Arrenhius parameters for enzymes are inconsistent with semi-classical transition state theory

Table 1 summarizes the experimentally determined kinetic parameters for a number of enzymes. The evidence for tunneling generally comes from examining the prefactor ratios and also the difference in activation energy for different isotopes. Specifically, when these quantities lie outside the bounds proposed by Bell [78], it is usually claimed that tunneling occurs. As a reference, the activation energy for the hydrogen reactions $E_H$ has also been included. Note that for some of the enzymes the condition $E_H \gg k_B T \sim 2.4 \text{ kJ mol}^{-1}$ is only weakly satisfied.

The table also includes some data for some non-enzymes reactions that have kinetic parameters that are inconsistent with semi-classical transition state theory.

The enzymes listed are not catalysts for the same chemistry. Although all the listed enzymes involve breaking a C–H bond, in some cases the proton is transferred to a nitrogen or oxygen atom, rather than another carbon atom. In some cases proton transfer is coupled to an electron transfer (i.e. there is a net transfer of a hydrogen atom or even a hydride ion) and then the question arises as to whether the transfers are sequential or concerted. For example, morphinone reductase catalyzes the biologically important hydride transfer reaction from NADH to FMN, where the proton is transferred from a carbon atom on NADH to a nitrogen atom on FMN [81].

3. Rate theory

3.1. The Caldeira–Leggett model Hamiltonian describes a quantum system interacting with its environment

Consider a system that consists of a single particle of mass $M$ described by one degree of freedom and coupled to a large environment, which can be represented by a bath of harmonic oscillators. This is equivalent to representing some arbitrary environment in terms of its normal modes. The interaction of the degree of freedom with each of the bath modes is inversely
Table 1. Deviations of the Arrhenius parameters for the kinetic isotopic effects of hydrogen transfer reactions in a range of enzymes from the predictions of semi-classical transition state theory. A number of hydrogen transfer reactions involving small organic molecules also exhibit parameters that fall outside the semi-classical limits.

<table>
<thead>
<tr>
<th>Enzyme, deuterium</th>
<th>$k_H/k_D$ (300 K)</th>
<th>$A_H/A_D$</th>
<th>$E_D - E_H$ (kJ mol$^{-1}$)</th>
<th>$E_H$ (kJ mol$^{-1}$)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Semi-classical limits</td>
<td>$\leq 5$</td>
<td>0.5–1.4</td>
<td>$\leq 3.1$</td>
<td>—</td>
<td>[79]</td>
</tr>
<tr>
<td>(assuming $\omega_0 \sim 3000$ cm$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methylmalonyl-CoA mutase</td>
<td>35.6 ± 2.4</td>
<td>0.082 ± 0.028</td>
<td>14.3 ± 0.3</td>
<td>79 ± 3</td>
<td>[48]</td>
</tr>
<tr>
<td>Ethanolamine ammonia lyase</td>
<td>$\sim 30$</td>
<td>0.038 ± 2</td>
<td>13 ± 4</td>
<td>45 ± 4</td>
<td>[49]</td>
</tr>
<tr>
<td>Soybean lipoxygenase (wild type)</td>
<td>81</td>
<td>18 ± 5</td>
<td>3.8 ± 0.8</td>
<td>8.4 ± 0.8</td>
<td>[57]</td>
</tr>
<tr>
<td>Soybean lipoxygenase, 553V mutant</td>
<td>82 ± 6</td>
<td>0.3 ± 0.2</td>
<td>10 ± 2</td>
<td>10 ± 2</td>
<td>[82]</td>
</tr>
<tr>
<td>Soybean lipoxygenase, 553L mutant</td>
<td>116 ± 10</td>
<td>0.3 ± 0.4</td>
<td>13 ± 3</td>
<td>1.6 ± 3</td>
<td>[82]</td>
</tr>
<tr>
<td>Soybean lipoxygenase, 553A mutant</td>
<td>93</td>
<td>0.12 ± 0.06</td>
<td>16.8 ± 1.2</td>
<td>8.1 ± 0.8</td>
<td>[57]</td>
</tr>
<tr>
<td>Soybean lipoxygenase, 553G mutant</td>
<td>182 ± 8</td>
<td>0.027 ± 0.034</td>
<td>20 ± 3</td>
<td>0.1 ± 0.1</td>
<td>[82]</td>
</tr>
<tr>
<td>Methylenedehydrogenase (MADH)</td>
<td>16.8</td>
<td>13.3</td>
<td>0.4 ± 1.0</td>
<td>44.6 ± 0.5</td>
<td>[34]</td>
</tr>
<tr>
<td>MADH (substrate: ethanolamine)</td>
<td>14.7</td>
<td>13</td>
<td>8.4 ± 1.7</td>
<td>43.5 ± 0.6</td>
<td>[83]</td>
</tr>
<tr>
<td>Aromatic amine dehydrogenase</td>
<td>12.9</td>
<td>9.4</td>
<td>0.7 ± 0.7</td>
<td>50.9 ± 0.7</td>
<td>[83]</td>
</tr>
<tr>
<td>(substrate: dopamine)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aromatic amine dehydrogenase</td>
<td>4.8</td>
<td>3.7</td>
<td>1.0 ± 2.3</td>
<td>68.1 ± 1.4</td>
<td>[83]</td>
</tr>
<tr>
<td>(substrate: benzyalnine)</td>
<td>55 ± 6</td>
<td>8.3 ± 4.6</td>
<td>57.3 ± 3.4</td>
<td></td>
<td>[70]</td>
</tr>
<tr>
<td>Trimethylamine dehydrogenase</td>
<td>4.6 ± 0.4</td>
<td>7.8 ± 1</td>
<td>0.5 ± 5.2</td>
<td>41</td>
<td>[84]</td>
</tr>
<tr>
<td>Acyl CoA desaturase</td>
<td>22.9 ± 2.8</td>
<td>19.8 ± 2.9</td>
<td>1.2</td>
<td>18.5</td>
<td>[38]</td>
</tr>
<tr>
<td>MADH</td>
<td>14.7</td>
<td>0.57</td>
<td>8.4 ± 1.1</td>
<td>43.5 ± 0.6</td>
<td>[83]</td>
</tr>
<tr>
<td>Peptidylglycine $\alpha$-hydroxylating monoxygenase</td>
<td>10.4 ± 0.3</td>
<td>5.9 ± 0.3</td>
<td>1.7 ± 1.1</td>
<td>9</td>
<td>[85]</td>
</tr>
<tr>
<td>Sarcosine oxidase</td>
<td>7.3</td>
<td>5 ± 3</td>
<td>0.6 ± 2.1</td>
<td>39.4 ± 0.9</td>
<td>[37]</td>
</tr>
<tr>
<td>Morphinone reductase</td>
<td>4.6 ± ?</td>
<td>?±</td>
<td>8.8 ± 1.6</td>
<td>? ± ?</td>
<td>[86]</td>
</tr>
<tr>
<td>E. coli dihydrofolate reductase</td>
<td>4.6 ± 0.2</td>
<td>4.0 ± 1.5</td>
<td>-0.3 ± 1</td>
<td>12 ± 1</td>
<td>[87]</td>
</tr>
<tr>
<td>Thermotoga maritima dihydrofolate reductase (25–65 °C)</td>
<td>3.3</td>
<td>1.56 ± 0.47</td>
<td>2.5 ± 0.8</td>
<td>53.5 ± 0.4</td>
<td>[88]</td>
</tr>
<tr>
<td>Thermotoga maritima dihydrofolate reductase (&lt;25 °C)</td>
<td>5.4</td>
<td>0.002 ± 0.001</td>
<td>19 ± 5</td>
<td>49.9 ± 1.7</td>
<td>[88]</td>
</tr>
<tr>
<td>Protochlorophyllide oxidoreductase</td>
<td>$\sim 3$</td>
<td>0.041 ± 0.016</td>
<td>8.8 ± 1.6</td>
<td>74.8 ± 0.8</td>
<td>[89]</td>
</tr>
</tbody>
</table>

Non-enzyme Reaction

| NpCbl                             | 35.2 ± 1.8        | 0.14 ± 0.07 | 12.9 ± 1.3                   | —                    | [48]      |
| AdoCbl                            | 29.3              | 0.16 ± 0.07  | 12.9 ± 1.3                   | —                    | [49]      |
| 8-MeOAdoCbl                       | 21.8              | 0.5 ± 0.4    | 8.8 ± 2.5                     | —                    | [49]      |
| H$^+$ + c-C$_6$H$_{12}$ → H$_2$ + c-C$_6$H$_{11}^+$ | 9.5 | 0.43 ± 0.03 | 9.67 ± 0.25 | — | [90] |
| H$^+$ + neo-C$_6$H$_{12}$ → H$_2$ + neo-C$_6$H$_{11}^+$ | 11 | 0.32 ± 0.04 | 11.00 ± 0.46 | — | [90] |
| H$^+$ + c-C$_6$H$_{12}$ → H$_2$ + c-C$_6$H$_{11}^+$ | 9.5 | 0.43 ± 0.03 | 9.67 ± 0.25 | — | [90] |
| H$^+$ + n-C$_{10}$H$_{22}$ → H$_2$ + n-C$_{10}$H$_{21}^+$ | 11 | 0.47 ± 0.03 | 9.41 ± 0.21 | 30 | [91] |
| Proton transfer in porphyrin      | 11.4              | 0.13         | 11.3                          | 37.2                 | [92]      |
Table 1. Continued.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>$k_H/k_D$ (300 K)</th>
<th>$A_H/A_D$</th>
<th>$E_D - E_H$ (kJ mol$^{-1}$)</th>
<th>$E_H$ (kJ mol$^{-1}$)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proton transfer in porphyrin anion</td>
<td>16.5</td>
<td>3 × 10$^{-4}$</td>
<td>25.3</td>
<td>17.7</td>
<td>[92]</td>
</tr>
<tr>
<td>4-Nitrophenylnitromethane with tetramethylguanidine</td>
<td>45 ± 2</td>
<td>0.03 ± 0.01</td>
<td>18 ± 1</td>
<td>17.5 ± 0.5</td>
<td>[93]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Enzyme, tritium</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Semi-classical limits (assuming $ω_0$ of 3000 cm$^{-1}$)</td>
<td>⩽ 100</td>
<td>0.3-1.7</td>
<td>⩽ 10</td>
<td>—</td>
<td>[79]</td>
</tr>
<tr>
<td>Flavoenzyme monoamine oxidase</td>
<td>22 ± 1</td>
<td>0.13 ± 0.03</td>
<td>13</td>
<td>54</td>
<td>[30]</td>
</tr>
<tr>
<td>E. coli dihydrofolate reductase</td>
<td>4.81 ± 0.06</td>
<td>7.4 ± 0.4</td>
<td>−0.4 ± 1</td>
<td>12 ± 1</td>
<td>[87]</td>
</tr>
<tr>
<td>Thymidylate synthase</td>
<td>7</td>
<td>6.8 ± 2.8</td>
<td>0.02 ± 0.25</td>
<td>16 ± 0.4</td>
<td>[40]</td>
</tr>
<tr>
<td>Bovine serum amine oxidase</td>
<td>35</td>
<td>0.12 ± 0.04</td>
<td>14.2 ± 0.7</td>
<td>58</td>
<td>[28]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Non-enzyme reaction</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Porphyrin</td>
<td>39</td>
<td>8 × 10$^{-3}$</td>
<td>14.3</td>
<td>37.2</td>
<td>[92]</td>
</tr>
<tr>
<td>Porphyrin anion</td>
<td>49.6</td>
<td>1 × 10$^{-5}$</td>
<td>37.3</td>
<td>17.7</td>
<td>[92]</td>
</tr>
</tbody>
</table>

proportional to the volume of the bath. Hence, for a macroscopic environment, the coupling to each of the individual modes will be weak [15]. The environment is modeled by $N$ harmonic oscillators, where $m_α$ and $ω_α$ are the mass and frequency of the $α$th oscillator. It is assumed that the interaction of the system with the environment is separable and linear in both the reaction coordinate $x$ and each of the oscillator coordinates. This means that the friction is the same at all points along the reaction coordinate. In that case the complete Hamiltonian is the Caldeira–Leggett Hamiltonian [73]:

$$\mathcal{H} = \frac{p^2}{2M} + V(x) + \frac{1}{2} \sum_{α=1}^{N} \left[ \frac{p_{α}^2}{m_α} + m_αω_α^2 \left( q_α - \frac{C_α}{m_αω_α^2} x \right)^2 \right] - \frac{K_e}{2} x^2,$$

(10)

where we have introduced an effective curvature induced by the environment and defined by

$$K_e = \sum_{α=1}^{N} \frac{C_α^2}{m_αω_α^2}.$$

(11)

This counterterm cancels the renormalization of the potential by the environment and so we also define the effective potential

$$U(x) = V(x) - \frac{K_e}{2} x^2.$$

(12)

3.2. The spectral density describes the frequency-dependent friction due to the environment

The role the environment plays can be embodied in a single function. It depends on how the coupling strength to each oscillator mode changes with the frequency of the oscillator. This can

be expressed in terms of the memory friction kernel, $\gamma(t)$, defined as [15]

$$
\gamma(t) = \frac{1}{M} \sum_{a=1}^{N} \frac{C_a^2}{m_a \omega_a^2} \cos(\omega_a t).
$$

(13)

The Laplace transform of the memory friction kernel, $\hat{\gamma}(z)$, is what determines the effect of the environment on the reaction rate,

$$
\hat{\gamma}(z) = \frac{1}{M} \sum_{a=1}^{N} \frac{C_a^2}{m_a \omega_a^2} \left[ \frac{z}{z^2 + \omega_a^2} \right].
$$

(14)

The spectral density $J(\omega)$ is defined as

$$
J(\omega) \equiv \pi \sum_{\alpha} \frac{C_\alpha^2 m_\alpha \omega_\alpha}{1 + \omega_\alpha^2} \delta(\omega - \omega_\alpha).
$$

(15)

and is an alternative means of characterizing the coupling to the environment. The spectral density and the Laplace transform of the memory friction kernel are related by the identity [15]

$$
\hat{\gamma}(z) = \frac{2}{\pi M \int_0^\infty d\omega \frac{J(\omega)}{\omega^2 + z^2}}.
$$

(16)

From this we can obtain an upper bound for the friction kernel

$$
\hat{\gamma}(z) \leq \frac{2}{\pi M z} J(\omega) \int_0^\infty d\omega \frac{J(\omega)}{\omega^2 + z^2} = \frac{2K_e}{\pi M z},
$$

(17)

where $K_e$ is the curvature (11). This expression will be used in section 5 to estimate the magnitude of the friction.

The simplest kind of dissipation is memoryless friction, $\hat{\gamma}(z) = \gamma$ or $J(\omega) = M \gamma \omega$. However, there is always some microscopic memory that sets the timescale on which inertial effects in the bath are significant. The simplest form of damping kernel that captures this is the Drude regularization [15]

$$
\hat{\gamma}(z) = \frac{\gamma}{1 + z/\omega_D},
$$

$$
J(\omega) = \frac{M \gamma \omega}{1 + \omega^2/\omega_D^2}.
$$

(18)

We can model a peak in the spectral density at a frequency $\omega_r$ by

$$
\text{Re} \gamma(\omega) = \frac{J(\omega)}{M \omega} = \frac{\gamma_r (\omega \Gamma)^2}{(\omega^2 - \omega_r^2)^2 + (\omega \Gamma)^2},
$$

(19)

which has a value of $\gamma_r$ at the peak that has a width $\Gamma$. The corresponding friction kernel is

$$
\hat{\gamma}(z) = \frac{\gamma_r z \Gamma}{z^2 + \omega_r^2 + z \Gamma}.
$$

(20)
3.3. Classical Kramers Theory defines an effective barrier frequency

The classical rate that incorporates a dissipative interaction with the environment is [75]

\[ k_{cl} = \frac{\mu}{\omega_b} \frac{\omega_0}{2\pi} \exp(-E_b/k_BT) \quad (21) \]

and here the effective barrier frequency \( \mu \) is the solution of the equation,

\[ \mu = \sqrt{\frac{\gamma^2(\mu)}{4} + \omega_b^2} - \frac{\gamma(\mu)}{2} \quad (22) \]

In this framework, the activation energy has no mass dependence and hence remains unchanged by an isotopic substitution. The only quantities that are altered are \( \omega_0, \omega_b \) and \( \mu \), which all appear in the prefactor. The particle mass term appearing in \( \omega_0 \) and \( \omega_b \) cancel each other out so that the only mass dependence lies in the effective barrier frequency. The entire KIE comes from the effective barrier frequency. Equation (22) then gives the bounds on the KIE. In the case where one is comparing the rate of a reaction where protium is transferred with a reaction where tritium is transferred, this predicts that \( 1 \leq k_H/k_T \leq 1.7 \). Similarly for protium and deuterium \( 1 \leq k_H/k_D \leq 1.4 \). Experimentally the KIE does often depend on the temperature and in the systems of interest often falls outside of the former bounds (see table 1). The inconsistency between the KIEs predicted by classical Kramers theory and those measured by experiment shows that this classical description does not capture the relevant physics.

3.4. Path integral representation of quantum Kramers theory

The general problem of quantum tunneling at nonzero temperature in the presence of an environment can be treated using complex-time path integrals [15, 74, 75]. Consider the partition function \( Z = \text{Tr}\{\exp(-\mathcal{H}/k_BT)\} \), where \( \mathcal{H} \) denotes the full Hamiltonian operator corresponding to the system plus environment. This quantity can be expressed in the form of a functional path integral over the tunneling coordinate \( x(\tau) \) [75]

\[ Z = \int \mathcal{D}x(\tau) \exp\{-S_E[x(\tau)]/\hbar\}, \quad (23) \]

where \( \tau = it \) is a real variable. This integral sums over all paths \( x(\tau) \) that satisfy the periodic boundary condition

\[ x(\tau = -\theta/2) = x(\tau = \theta/2) \quad (24) \]

with period \( \theta \) determined by the temperature,

\[ \theta = \hbar/(k_BT). \quad (25) \]

After integrating over the bath modes the effective Euclidean action takes the form

\[ S_E[x] = \int_{-\theta/2}^{\theta/2} d\tau \left\{ \frac{M}{2} \dot{x}^2(\tau) + U[x(\tau)] \right\} + \frac{1}{2} \int_{-\theta/2}^{\theta/2} d\tau \int_{-\theta/2}^{\theta/2} d\tau' \zeta(\tau - \tau') x(\tau) x(\tau'). \quad (26) \]

The influence of kernel \( \zeta(\tau) \) is periodic in imaginary time with period \( \theta \). It is related to \( \hat{\gamma}(z) \), the Laplace transform of the memory friction (see equation (14)) and can be represented in terms of a Fourier series as [94]

\[ \zeta(\tau) = \frac{M}{\theta} \sum_{n=-\infty}^{\infty} |v_n| \hat{\gamma}(|v_n|) \exp(iv_n\tau), \quad (27) \]
where $\nu_n$ are the Matsubara frequencies for bosons,

$$\nu_n = n2\pi k_B T / \hbar.$$  

(28)

For a metastable potential, the partition function has an imaginary part that can be related to the escape rate from the potential [15]. The dominant contributions to the partition function, and indirectly the rate expression, come from the vicinity of paths in which the action (26) is stationary. These paths, $x_e(\tau)$, satisfy the equation of motion

$$M\ddot{x_e}(\tau) - \frac{\partial U[x_e(\tau)]}{\partial x_e(\tau)} - \int_{-\theta/2}^{\theta/2} d\tau' \zeta(\tau - \tau')x_e(\tau') = 0$$  

(29)

and the periodic boundary condition (24). In the absence of dissipation, i.e. $\zeta(\tau) = 0$, the evolution of $x_e(\tau)$ in imaginary time corresponds to real-time motion in the metastable inverted potential $-U(x)$ [95, 96].

Equation (29) has two trivial but physically important solutions. The first where the particle remains at the bottom of the reactant well ($x_e(\tau) = x_a$) and the other where it sits on top of the barrier ($x_e(\tau) = x_b$). The latter corresponds to thermal activation over the barrier top.

3.5. The bounce solution describes quantum tunneling, which only occurs below a temperature, $T_0$

A nontrivial solution to equation (29) which satisfies the periodic boundary condition (24) (which has been dubbed the bounce or instanton solution) describes quantum tunneling below the barrier [95, 97]. This solution exists only below a temperature $T_0$ [75]. Basically, if the temperature is too high, the required period $\theta$ is smaller than the natural oscillation frequency of the inverted potential. In the absence of dissipation, an analytic solution has been found for an inverted parabola, an Eckart potential [96] and a cubic potential [98]. In the presence of Ohmic dissipation, an analytic solution for a cubic potential has been found for specific values of the dissipation [99].

For temperatures $T > T_0$, the period of the $\theta$-periodic orbit is not of sufficient length to admit an oscillation of the particle in the classically forbidden regime. For the case of zero temperature, the bounce solution gives a rate that agrees with that calculated from the tunneling probability calculated in the WKB approximation [97].

For temperatures $T > T_0$, the bounce solution does not exist and the only contribution to the path integral comes from the constant solution ($x_e(\tau) = x_b$) where the particle sits at the barrier top. Figure 40 in [75] depicts the different kinetic regimes that occur for different temperatures.

We now focus on the case of a parabolic barrier. In the absence of any dissipation ($\gamma = 0$), the crossover temperature has the value given by (8). In the presence of dissipation, the crossover temperature $T_0$ is given by [15, 75]

$$T_0 \equiv \frac{\hbar \mu}{2\pi k_B} = 0.23 \text{ K} \cdot \frac{\mu}{\text{cm}^{-1}},$$  

(30)

where $\mu$ is the effective barrier frequency defined in equation (22). This means that tunneling can only occur at room temperature if $\mu > 1300 \text{ cm}^{-1}$. In section 4.3 is shown how for a Lorentzian spectral density $\mu$ is reduced by friction, and the extent of the reduction increases as $\omega_b/\omega_D$ decreases.

4. Quantum correction factor: $T > T_0$

In the high-temperature regime for a parabolic barrier, one can obtain an analytic expression for the rate constant [100, 101],

$$k(T) = k_{\text{cl}} c_{\text{qm}} \equiv \left[ \frac{\mu}{\omega_b} \left( \frac{\omega_0}{2\pi} \right) \exp\left( -\frac{E_b}{k_B T} \right) \right] \left\{ \prod_{n=1}^{\infty} \frac{\omega_0^2 + n^2 \nu^2 + n \nu \hat{\gamma}(n \nu)}{-\omega_b^2 + n^2 \nu^2 + n \nu \hat{\gamma}(n \nu)} \right\}. $$ \hspace{1cm} (31)

The first term in the square brackets denotes the classical Kramers rate for memory friction (equation (21)). Here $\nu$ is the smallest Matsubara frequency,

$$\nu = 2\pi k_B T / \hbar,$$ \hspace{1cm} (32)

and we must have $\nu > \mu$, where $\mu$ is the effective barrier frequency given by (22). We note that (31) is proportional to $Z^\perp / Z_A$, where the corresponding partition functions are for damped harmonic oscillators [15].

The quantum correction to the rate expression, $c_{\text{qm}}$, is encapsulated by the term inside the curly brackets. For $T \gg T_0$, this correction factor approaches unity. Moreover, it always exceeds unity, which implies that quantum effects always enhance the classical rate.

4.1. The weak friction limit reduces to Bell’s expression

This limit has to be treated with some care, as when the friction is exceptionally weak, thermal equilibrium no longer prevails in the reactant well. It has been shown that so long as the following condition is satisfied the assumption can be made that the reactant system is always in thermal equilibrium [75]:

$$\frac{\hat{\gamma}(\mu)}{\omega_b} > \frac{k_B T}{E_b}.$$ \hspace{1cm} (33)

However, we note that for some of the enzymes shown in table 1 the activation energy is sufficiently small that this assumption may not be justified.

In the limit where $\gamma \to 0$, equation (31) can be simplified such that the correction factor can be written as

$$c_{\text{qm}} = \frac{\omega_0 \sinh \left( \hbar \omega_0 / 2k_B T \right)}{\omega_0 \sin \left( \hbar \omega_0 / 2k_B T \right)}. $$ \hspace{1cm} (34)

This expression will be a reasonable approximation provided that for all $n = 1, 2, \ldots$

$$\frac{\hat{\gamma}(n \nu)}{n \nu} \ll 1.$$ \hspace{1cm} (35)

Since $\nu \sim 1300 \text{ cm}^{-1}$ at room temperature this means that any friction associated with environmental modes of much lower frequency may have little effect on the quantum correction factor.

Note that the expression (34) diverges as $T \to T_0^+$. This divergence turns out to be an artifact from treating the potential near the transition state as a perfect inverted parabola. Below we will show how a more rigorous treatment shows that in this temperature regime the correction factor is always finite. Furthermore, for realistic parameters, (31) is a good approximation, except relatively close to $T_0$. 

4.2. Apparent Arrhenius parameters in the weak friction limit

Most kinetic experiments are performed over a narrow temperature range. The temperature dependence appears to be activated (i.e. a plot of $\ln(k_L/k_T)$ versus $1/T$ is linear over the measured temperature range) and so it is natural to determine the Arrhenius parameters. If the full quantum transition state theory (QTST) rate expression is expanded about room temperature, one can obtain an expression for the KIE that has a simple activated temperature dependence. The subscripts $L$ and $L^\dagger$ denote possible combinations of the three different isotopes of hydrogen. This is typically what is done in experiments where the heavier isotopes are used as a reference. Combining the results from equations (31) and (34) gives an expression for the rate constant. The KIE is given by

$$
\frac{k_L}{k_{L^\dagger}} = \sqrt{\frac{m_{L^\dagger}}{m_L}} \frac{\sinh \left( \frac{\hbar \omega_0}{\sqrt{4m_Lk_B}T} \right)}{\sin \left( \frac{\hbar \omega_0}{\sqrt{m_{L^\dagger}k_B}T} \right)} \left( \frac{\sinh \left( \frac{\hbar \omega_b}{\sqrt{4m_Lk_B}T} \right)}{\sin \left( \frac{\hbar \omega_b}{\sqrt{4m_{L^\dagger}k_B}T} \right)} \right),
$$

(36)

where $m_L$ is the unitless mass number of the $L$ isotope and $\omega_0$ and $\omega_b$ are the ground state oscillation frequency and barrier frequency, respectively, for hydrogen. For a typical C–H stretch frequency, $\hbar \omega_0 \gg 2k_B T$, at room temperature so the hyperbolic sine terms can be approximated as exponential functions. Over the biologically relevant temperature range, $T$ only varies by less than 10% and so one can expand the other temperature-dependent parts of the expression up to linear terms in $1/T$. This gives an expression for the KIE that has a simple activated temperature dependence with the following apparent Arrhenius parameters:

$$
\frac{A_L}{A_{L^\dagger}} = \sqrt{\frac{m_{L^\dagger}}{m_L}} \frac{\sin(h\omega_b/2k_B T\sqrt{m_{L^\dagger}})}{\sin(h\omega_b/2k_B T\sqrt{m_L})} \times \exp \left[ -\frac{h\omega_b}{2k_B T_\text{R}} \left( \cot(h\omega_b/2k_B T R \sqrt{4m_{L^\dagger}}) / \sqrt{m_{L^\dagger}} - \frac{\cot(h\omega_b/2k_B T R \sqrt{4m_L})}{\sqrt{m_L}} \right) \right].
$$

(37)

$$
E_{L^\dagger} - E_L = \frac{h\omega_0}{2} \left( \frac{1}{\sqrt{m_L}} - \frac{1}{\sqrt{m_{L^\dagger}}} \right) + \frac{h\omega_b}{2} \left( \frac{\cot(h\omega_b/2k_B T R \sqrt{m_{L^\dagger}})}{\sqrt{m_{L^\dagger}}} - \frac{\cot(h\omega_b/2k_B T R \sqrt{m_L})}{\sqrt{m_L}} \right),
$$

where $T_\text{R}$ is the temperature around which the expansion is performed. From these expressions, it is also possible to place bounds on the apparent Arrhenius parameters. When $T_\text{R} \gg T_0$ and $m_L < m_{L^\dagger}$, it can be shown that the Arrhenius parameters are monotonic functions of $\omega_b$.

On their own the expressions in equation (37) may not seem to shed much light. However, evaluating these expressions for typical parameter values shows that typical values for $\omega_0$ and $\omega_b$ give kinetic parameter values that are inconsistent with the predictions of standard semi-classical rate theory. Moreover, table 2 shows that the parameter trends are consistent with experimentally determined Arrhenius parameters for a number of systems. In a number of systems where tunneling has been invoked, the difference in apparent activation for the different isotopes is greater than would be predicted by semi-classical theories. The effective activation energies derived from QTST are quantitatively similar to a number of the experimental values and significantly exceed the semi-classical values. The prefactor values that have been observed experimentally are smaller than would be expected from semi-classical theories.
Table 2. The effective Arrhenius parameters calculated for the expanded QTST expression are compared with experimentally determined values. Both the calculated and experimentally determined parameters are inconsistent with a standard semi-classical analysis. This shows that there are a subset of enzymatic systems where the anomalous kinetics can be explained by quantum transition state theory.

<table>
<thead>
<tr>
<th>Parameter values/ experimental system</th>
<th>$E_T - E_H$ (kJ mol$^{-1}$)</th>
<th>$A_H/A_T$</th>
<th>$E_T - E_D$ (kJ mol$^{-1}$)</th>
<th>$A_D/A_T$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Semiclassical limits</td>
<td>$\leq 10.1$</td>
<td>0.3–1.7</td>
<td>$\leq 3.1$</td>
<td>0.5–1.4</td>
</tr>
<tr>
<td>$\omega_0 = 3000$ cm$^{-1}$, $\omega_b = 1000$ cm$^{-1}$, $T_R = 288$ K</td>
<td>16</td>
<td>0.08</td>
<td>3.6</td>
<td>0.7</td>
</tr>
<tr>
<td>Bovine serum amine oxidase [28]</td>
<td>14.2 ± 0.7</td>
<td>0.12 ± 0.04</td>
<td>4.51 ± 0.48</td>
<td>0.51 ± 0.1</td>
</tr>
<tr>
<td>Flavoenzyme monoamine oxidase B [30]</td>
<td>13</td>
<td>0.13</td>
<td>4.1</td>
<td>0.51</td>
</tr>
</tbody>
</table>

4.3. Effect of the environment on the crossover temperature

In the case of a Lorentzian spectral density, the equation for $\mu$ takes the form

$$\mu^2 - \omega_0^2 + \frac{\mu \omega_D \gamma}{\omega_0 + \mu} = 0.$$  \hspace{1cm} (38)

$T_0$ varies as a function of the friction strength for different bath response frequencies, $\omega_D$. Figure 2 shows how the positive root of equation (38) changes as a function of the scaled friction and bath response frequency. For all values of $\omega_D$ an increase in the strength of the damping, $\gamma$, reduces the effective barrier frequency and hence the crossover temperature. However, as the bare barrier frequency $\omega_b$ increases to values comparable to the bath response frequency, the environment is less effective in reducing the crossover temperature. This underscores the importance of knowing the relative size of the three different frequency scales $\omega_b$, $\gamma$, and $\omega_D$. Physically reasonable values for proton transfer in enzymes will be discussed in the next section.

4.4. Non-parabolicity of the barrier only matters at temperatures close to $T_0$

In the regime where $T \sim T_0$, the action associated with the bounce solution and the trivial solution at the top of the barrier becomes comparable (see section 3.5). In the limit where $T \rightarrow T_0$, the expression for the quantum correction given in equation (31) diverges. This divergence is a consequence of the fact that the saddle point approximation of the imaginary time functional integral employed in evaluating the expressions presented so far breaks down in the vicinity of $T_0$. In order to obtain an expression that is valid around the crossover temperature the analysis needs to be extended to include the effects of a non-parabolic barrier [94] or equivalently a finite value of $E_b/\hbar \omega_b$ [78]. The issue is also reviewed in detail in chapter 7 of Ankerhold’s book [74]. Essentially, the temperature region where the parabolic approximation breaks down is determined by a dimensionless parameter $\kappa$ that is of the order of $\sqrt{(E_b/\hbar \omega_b)} \gg 1$ [94]. However, caution is in order because for $E_b \sim 50$ kJ mol$^{-1}$ $\sim 4000$ cm$^{-1}$ this condition is only
Figure 2. The crossover temperature for a parabolic barrier as a function of the friction strength for a Lorentzian spectral density. The friction strength is the dimensionless parameter $\gamma / \omega_b$. The different plots show how the relative size of the response frequency of the bath, $\omega_D$, and the barrier frequency, $\omega_b$, change the influence that friction has on the crossover temperature. These show that when $\omega_D \gg \omega_b$, the crossover temperature becomes most sensitive to the friction strength. Then the bath is able to respond on a timescale that is much faster than all of the other relevant timescales. When the response frequency of the bath is much less than the barrier frequency, the crossover temperature is only weakly suppressed as the friction strength increases.

weakly satisfied. Also, quantum chemistry calculations discussed in section 5.2 show highly non-parabolic barriers.

5. Estimates of model parameter values for enzymes

The results reviewed above show that the reaction rate and its isotope dependence are determined by the following parameters: the frequency of oscillations in the reactant well, $\omega_0$, the barrier frequency, $\omega_b$, and the frequency-dependent friction kernel, $\tilde{\gamma}(\omega_n)$. We now review reasonable estimates of these parameters for proton transfer reactions in biomolecules.

5.1. Frequency of oscillations in the reactant well ($\omega_0$)

The parameter $\omega_0$ is defined as $\sqrt{U''(x_0)/M}$, where $M$ is the effective mass of the hydrogen isotope being transferred and $U''(x_0)$ is the curvature of the potential at $x = x_0$. Using IR spectroscopy the oscillation frequencies of the different chemical bond stretches can be measured [102]. Results from molecular dynamics simulation show that the value of $\omega_0$ is similar to the value obtained from IR spectroscopy. For example, in [65] it was established that the oscillation frequency of the C–H bond that participates in the reaction for LADH, $\omega_0$, is 2900 cm$^{-1}$. A typical IR spectrum of an organic molecule would contain peaks corresponding to C–H stretch frequencies in the range 2700–3300 cm$^{-1}$ [102].
The value of $\omega_0$ may be influenced by interactions with atoms in the active site. This interaction can decrease the stiffness of the bond [103]. As a result it is possible that in some of the systems that we are studying, the binding of the substrate could bring about a change in the ground state oscillation frequency, but based on what happens with hydrogen bonds, it is unlikely that this could ever be a reduction larger than a factor of two.

5.2. Barrier frequency ($\omega_b$)

The barrier frequency, $\omega_b$, strongly influences to what extent quantum effects affect the kinetics of the hydrogen transfer process. It depends on both the curvature of the potential at the barrier and the mass of the hydrogen species being transferred. It is a parameter that would be difficult to obtain directly from experiment. Generally one needs to resort to quantum chemistry calculations to obtain an estimate of $\omega_b$. For our purposes $\omega_b$ is a crucial parameter. Firstly, it sets the upper limit of the crossover temperature, $T_0$, around which the kinetics becomes more classical in nature. Secondly, in the intermediate- and high-temperature regime, it strongly influences exactly how much the rate gets modified due to quantum corrections.

Bell [78] discusses values of $\omega_b$ for non-enzyme reactions. Following Christov he gives a heuristic argument that $\omega_b > \sqrt{2}\omega_t$, where the latter frequency is that of the bending mode that is perpendicular to the reaction coordinate. In table 5.5 of his book [78], Bell gives values of $\omega_b$ for different reactions in a range of solvents. These values are deduced from experimental estimates of the quantum correction factor, and lie in the range 600–1500 cm$^{-1}$.

Table 3 shows the values of the barrier frequency that have been calculated using quantum chemistry for a number of different enzymes.

From these calculated values of $\omega_b$ it is clear that in a number of the cases considered, the barrier frequency is of the order of 1000 cm$^{-1}$, which corresponds to a maximum crossover temperature of around $-40$ $^\circ$C. Of the enzymes that appear in the table both MADH and soybean lipoxygenase have a large KIE, associated with a prefactor ratio that is much larger than one (see table 1).

A recent study [107] performed a QM-MM study of the hydrogen transfer reaction in methylmalonyl-CoA mutase, treating 44 atoms near the active site at the AM1 level. They emphasize the role of corner cutting but do not give a value for the curvature of the barrier. The ‘representative tunneling energy’ (the energy at which the product of the transmission coefficient and the Boltzmann factor is a maximum) is about 1400 cm$^{-1}$ below the top of the barrier.

These values for the barrier curvature need to be taken with some caution. Most of the values listed in table 3 were determined using hybrid QM/MM calculations. These techniques encounter some methodological problems that may influence the calculated barrier frequencies [108]. Obtaining a reliable value from computational chemistry represents a major challenge. We note how the Table shows that the values obtained depend on the level of theory. Other factors to consider are the role of anharmonicity, dependence of the results on possible errors in the active site geometry, and the fact that in a dynamic environment (protein plus water) there are actually many reaction paths.

5.3. Frequency-dependent friction due to the solvent–protein environment

The analysis in the previous sections shows that the magnitude and frequency dependence of friction $\gamma(\omega)$, and its Laplace transform, the memory friction kernel $\gamma(z)$, can lead to not just
Table 3. Barrier frequency values for several enzymes estimated from quantum chemistry calculations of potential energy surfaces for the hydrogen transfer reaction. The maximum crossover temperature $T_0$ is related to $\omega_b$ by equation (8). Friction will tend to reduce these values. The corresponding temperatures for deuterium and tritium transfer will be about 30 and 50% lower, respectively. SCC-DFTB denotes self-consistent charge-density functional tight binding. AM1 and PM3 are semi-empirical methods, and SRP denotes a specific reaction parameterization, against a DFT calculation using the B3LYP functional and the 6-31G* basis set.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Level of theory</th>
<th>$\omega_b$ (cm$^{-1}$)</th>
<th>Max–$T_0$ (K)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triosephosphate isomerase (TIM) model (gas phase)</td>
<td>AM1-SRP</td>
<td>1365</td>
<td>315</td>
<td>[62]</td>
</tr>
<tr>
<td>TIM model (in water)</td>
<td>AM1-SRP</td>
<td>591</td>
<td>140</td>
<td>[62]</td>
</tr>
<tr>
<td>TIM model (in protein model)</td>
<td>AM1-SRP</td>
<td>798</td>
<td>190</td>
<td>[62]</td>
</tr>
<tr>
<td>Liver alcohol dehydrogenase (LADH)</td>
<td>SCC-DFTB</td>
<td>783</td>
<td>180</td>
<td>[64]</td>
</tr>
<tr>
<td></td>
<td>AM1</td>
<td>1046</td>
<td>240</td>
<td>[65]</td>
</tr>
<tr>
<td></td>
<td>AM1</td>
<td>1229</td>
<td>240</td>
<td>[69]</td>
</tr>
<tr>
<td>Monoamine oxidase</td>
<td>B3LYP/6-31G*</td>
<td>1054</td>
<td>240</td>
<td>[104]</td>
</tr>
<tr>
<td></td>
<td>PM3</td>
<td>1782</td>
<td>410</td>
<td>[104]</td>
</tr>
<tr>
<td>MADH</td>
<td>PM3</td>
<td>2000</td>
<td>460</td>
<td>[67]</td>
</tr>
<tr>
<td></td>
<td>AM1-SRP</td>
<td>2218</td>
<td>510</td>
<td>[69]</td>
</tr>
<tr>
<td></td>
<td>PM3</td>
<td>2000</td>
<td>460</td>
<td>[105]</td>
</tr>
<tr>
<td>Soybean lipoxygenase</td>
<td>PM3/d</td>
<td>2913</td>
<td>670</td>
<td>[69]</td>
</tr>
<tr>
<td>Aromatic amine dehydrogenase (tryptamine)</td>
<td>PM3-SRP</td>
<td>2057</td>
<td>450</td>
<td>[106]</td>
</tr>
</tbody>
</table>

quantitative but also qualitative differences in the reaction rate. For example, if $\hat{\gamma}(\omega_b) > \omega_b$, then the friction can substantially reduce the temperature $T_0$ (see figure 2) below which the ‘bounce’ solution exists, i.e. strong enough friction can prevent tunneling from the bottom of the well occurring. The friction also causes vibrational energy relaxation and dephasing of the vibrations in the reactant well [109]. The former is proportional to $\text{Re} \, \gamma(\omega_0)$. Hence, measurements of the relaxation and dephasing rates provide a means to determine the magnitude of the friction [110].

There are believed to be several main sources of friction associated with bond deformation and breaking in biomolecules. The first source is the interaction of the dipole moment associated with displacement of the proton with the fluctuating electric field associated with fluctuations in its environment [111], the surrounding protein and solvent. Recent ultrafast infrared (IR) spectroscopy experiments of the OH stretch of HOD in liquid D$_2$O have shown that in bulk water this fluctuating electric field is the dominant source of vibrational dephasing [112]–[114]. The second source of friction is the interaction of oscillations of the reaction coordinate with a modulating low-frequency mode, which in turn is strongly damped by the environment. This is particularly important in hydrogen-bonded systems [110, 115]. For the case, A–H···B, the A–H stretch is modulated by the A···B oscillations associated with the hydrogen bond. A third possible source of friction is anharmonicities [116] and Fermi resonances with other vibrational modes in the biomolecule.
5.3.1. Comparison with the spectral density for biological chromophores. The dynamics of optically active molecules (chromophores) within proteins have been studied extensively, both theoretically and experimentally [6]. An optical transition from the ground to an excited electronic state is usually associated with a change in electric dipole moment, $\Delta \mu$, of the order of a few Debyes, which then couples to the electric field (reaction field $R(t)$) associated with the dielectric relaxation of the chromophores environment. The relaxation can usually be assigned to three components of the environment: the surrounding protein, water bound to the surface of the protein and the bulk water surrounding the protein. The corresponding timescales are, respectively, of the order of nanoseconds, 10–100 ps, and 0.1–1 ps. Consider a proton at the same location within the protein as the chromophore and described by a continuous position coordinate $x$, then the time-dependent interaction energy with the reaction field is $e x(t) R(t)$, where $e$ is the proton charge. For the chromophore, modeled as a two-level system, the interaction energy is $\Delta \mu R(t) (P_e - P_g)(t)$, where the last factor represents the relative occupation probability of the excited and ground states of the chromophore. Then the spectral density, $J(\omega) = \text{Re} \gamma(\omega)\omega$, relevant to the Caldeira–Leggett model can be related to the spectral density $J_c(\omega)$ associated with a spin boson model for the chromophore [6]

$$J(\omega) = \left( \frac{e}{\Delta \mu} \right)^2 J_c(\omega). \quad (39)$$

Comparison with ultra-fast laser spectroscopy experiments, with molecular dynamics simulations, and continuum dielectric models shows that [6] the high-frequency ($\omega > 10 \text{ cm}^{-1}$) part of the chromophore spectral density is dominated by the bulk water. It should be noted that the femtosecond laser spectroscopy experiments used to extract spectral densities do have limited time resolution ($\sim 10–100 \text{ fs}$) and so only give information about the spectral density for $\omega < 500 \text{ cm}^{-1}$. Furthermore, quantum Kramers theory requires a knowledge of $\gamma(\omega)$ and $\gamma(z)$ at frequencies of the order of $\omega_b$ and $\omega_h$.

Figure 1 of Lang et al. [117] shows the frequency dependence of a chromophore–water spectral density for frequencies up to about $\omega \sim 3000 \text{ cm}^{-1}$, calculated by the continuum dielectric method of Song and Chandler [118], which considers the chromophore in a cavity with the same geometry as the solvent accessible surface of the molecule. It has peaks of magnitude of the order of a few hundred cm$^{-1}$, at frequencies of about 180, 600, 1800 and 3200 cm$^{-1}$. There is a substantial contribution from the librational band of water centered at 600 cm$^{-1}$.

If we combine (17) with (39) we find that the curvature $K_e$ can be related to the reorganization energy $E_R$ associated with the excited state of the chromophore [6],

$$K_e = \frac{2}{\pi} \left( \frac{e}{\Delta \mu} \right)^2 E_R \quad (40)$$

and

$$\hat{\gamma}(z) \leqslant \frac{2}{\pi M z} \left( \frac{e}{\Delta \mu} \right)^2 E_R. \quad (41)$$

Given typical values of $E_R$ of $\sim 1000 \text{ cm}^{-1}$ [6] and $\Delta \mu \sim 5 \text{ D}$, for chromophores in proteins ($\frac{(\bar{h}e/\Delta \mu)^2}{M} \simeq 30 \text{ cm}^{-1}$ for the proton mass $M$ and $\Delta \mu = 1 \text{ D}$) equation (41) then gives an estimated upper bound

$$\frac{\hat{\gamma}(z)}{z} \leqslant \left( \frac{150 \text{ cm}^{-1}}{z} \right)^2. \quad (42)$$

Hence, we see that for typical protein and solvent environments, we expect to be in the weak friction limit for proton transfer reactions at room temperature, since according to the discussion around equation (35) only values of $z \sim \omega_b$ and larger are relevant. An alternative way of estimating an upper bound for $K_e$ is to note that we must have $K_e < M \omega_b$, because otherwise there will no longer be a single barrier.

5.3.2. IR spectroscopy. IR spectroscopy provides a means to measure the frequencies, damping and decoherence of vibrational modes in a molecule. Recent advances in femtosecond two-dimensional IR spectroscopy have yielded such information for several specific systems [119], including O–H stretches in water [120], N–H stretches in Watson–Crick base pairs [121]. It is generally found that hydrogen bonding leads to broad IR spectra [110, 120, 122].

5.3.3. Molecular dynamics simulations. Stimulated by recent time-resolved IR spectroscopy measurements of vibrational energy relaxation and dephasing, a number of molecular dynamics studies have been made for specific vibrations in biomolecules. The difficulties associated with extracting the vibrational energy relaxation rate, $1/T_1$, from molecular dynamics simulations have recently been summarized [123]. If the vibration frequency is in the classical regime ($\omega_0 < k_B T \simeq 200 \text{ cm}^{-1}$), then the Landau–Teller equation allows one to extract $T_1$ from the classical force–force correlation function. However, most modes of interest are not in this classical regime. Fujisaki and Straub [123] considered the specific case of a C–D vibration in cytochrome-c surrounded by water. The experimental value of $T_1$ for this mode is about 1 ps. They found that the value of $T_1$ found in the simulations could vary by as much as two orders of magnitude with only a 10% change in the bond force constant. Time-resolved IR spectroscopy experiments show that the C=O stretch of the peptide bond for a wide range of proteins has a relaxation time of about 1 ps. A recent molecular dynamics simulation [124] yielded values that were two orders of magnitude larger than this.

A recent combined molecular dynamics–quantum mechanics calculation [125] calculated the IR spectrum for the water networks in (the proton pump) bacteriorhodopsin. They found broad continuum bands, around 1800 and 2700 cm$^{-1}$, and associated them with the solvated Zundel complex ($\text{H}_3\text{O}_2^+$) and Eigen complex ($\text{H}_2\text{O}^+$). A time-resolved Fourier-transform IR spectroscopy experiment found that the precise arrangement of the water molecules within bacteriorhodopsin was crucial to proton transfer [126]. Given that many proton transfer reactions in enzymes also involve water molecules inside the protein, such hydrogen-bonding networks may also be a significant source of friction for proton transfer.

Moritsugu and Smith performed molecular dynamics simulations of myoglobin both in water and in vacuum. They used a Langevin model to describe the dynamics of the different vibrational models [127]. The frictional damping of different vibrational modes of the protein was found to be proportional to the accessible surface area of the mode, confirming the importance of the solvent that we have stressed here. At 300 K, they found that for modes with $\omega$ in the range 100–400 cm$^{-1}$, the friction could be fitted to

$$\text{Re } \gamma (\omega) = \Delta \gamma + A \omega,$$

with $\Delta \gamma \simeq 20 \text{ cm}^{-1}$, and $A \simeq 0.38$ and was temperature independent between 120 and 300 K.
5.3.4. Dielectric continuum models. Continuum models [6, 118, 128] allow one to express the spectral density in terms of the frequency dependent (complex) dielectric function $\epsilon_s(\omega)$ of the solvent. If the proton is at the center of a spherical cavity of radius $a$ inside the water, and undergoes displacements much less than the radius, then using (39) and results for chromophores,

$$\text{Re} \gamma(\omega) = \frac{e^2}{2\pi \varepsilon_0 a^3 M \omega} \text{Im} \left( \frac{\epsilon_s(\omega) - \epsilon_c}{2\epsilon_s(\omega) + \epsilon_c} \right)$$  \hspace{0.5cm} (44)

and $\epsilon_c$ is the (static) dielectric constant of the cavity, which can be approximated as the local dielectric constant of the protein environment surrounding the proton.

Measurements of the frequency-dependent dielectric constant of water $\epsilon_s(\omega)$ in the range 1–200 cm$^{-1}$, have been fitted to a form involving three Debye relaxation terms and one damped resonant term [129]

$$\epsilon_s(\omega) = \epsilon_\infty + \sum_{i=1}^{3} \frac{\Delta \epsilon_i}{1 + i\omega \tau_i} + \frac{\Delta \epsilon_4}{1 + i\omega \tau_4 - \omega^2/\omega_4^2},$$  \hspace{0.5cm} (45)

where $\tau_i$ is the Debye relaxation time of the relevant component. For water at 298 K, the coefficients are $\Delta \epsilon_i$ ($i = 1, 2, 3$ and 4) = 71.5, 2.8, 1.6 and 0.92, respectively. The corresponding relaxation times are $\tau_i$ = 8.3, 1.0, 0.1 and 0.025 ps. The resonant frequency is $\omega_4$ = 175 cm$^{-1}$. Roy and Bagchi [130] gave a resonant frequency of $\omega_4$ = 200 cm$^{-1}$ and a damping constant such that $\omega_4 \tau_4 = 2$. They also calculated the frequency-dependent friction for outer sphere electron transfer reactions in water out to $\omega \tau = 5$, where $\tau = 0.1$ ps.

Figure 5 in [129] shows, for $\omega \sim 100$ cm$^{-1}$, that $\text{Im} \epsilon(\omega) \sim 2$ (see also figure 18 in [131]) which is an order of magnitude larger than the contribution from the slowest relaxation ($i = 1$).

A parameterization of the higher frequency part of the dielectric function has been given in [132]. There are features at frequencies of about 180, 600, 1800 and 3200 cm$^{-1}$. The first can be assigned to hindered translation of the hydrogen-bonded network (the O···O stretch of the O–H···O of the water–hydrogen bonds). Hindered rotation (libration) is the origin of the second feature. Figure 3 in [128] shows a plot of the frequency dependence of the right-hand side of (44). Again, there is a substantial contribution from the librational band in the range 600–800 cm$^{-1}$.

We can model a peak at a frequency $\omega_r$ in the spectral density by (19) with the corresponding friction kernel, (20). If $z, \omega_r \gg \Gamma$ then

$$\hat{\gamma}(z) \sim \frac{\gamma_r \Gamma}{z}$$  \hspace{0.5cm} (46)

and for the typical values of $\gamma_r \sim \Gamma \sim 100$ cm$^{-1}$, $\omega_b \sim v = 2\pi k_B T \sim 1000$ cm$^{-1}$, this gives $\hat{\gamma}(v) \ll v$, which again justifies using the weak-friction limit in reaction rate theory.

6. Quantum transition state theory describes the experimental data

Quantum transition state theory predicts that for $T > T_0$, the H/D KIE is given by

$$\frac{k_H}{k_D} = \sqrt{2} \frac{\sinh(\hbar \omega_0/2k_B T) \sin(\hbar \omega_b/2\sqrt{2}k_B T)}{\sinh(\hbar \omega_0/2\sqrt{2}k_B T) \sin(\hbar \omega_b/2k_B T)}.$$  \hspace{0.5cm} (47)
Figure 3. Comparison of the measured temperature dependence of the KIE [48] for the enzyme methylmalonyl-CoA mutase and several synthetic molecules with similar H-atom abstraction reactions (NpCb, AdoCbl and 8-MeOAdoCbl) with quantum transition state theory at temperatures above which tunneling is possible. These systems all have KIEs and Arrhenius parameters that differ by factors of 5–10 from the semi-classical values traditionally claimed to hold in the absence of tunneling (see table 1). The use of the synthetic molecules allows coverage of a much wider temperature range (from 10 to 120°C) than possible with enzymes. The solid line is a fit of equation (47) to the experimental data with two free parameters, the barrier frequency, $\omega_b$, and the oscillation frequency in the reactant well, $\omega_0$. The value obtained for $\omega_0$ is comparable with typical C–H stretch frequencies. The value obtained for $\omega_b$ is comparable with estimates from quantum chemistry calculations (see table 3) of similar reactions. The value of the crossover temperature $T_0 \simeq 250\,\text{K} = -20\,\text{°C}$ implied by the fitted value of $\omega_b$ and equation (8) is consistent with the domain of validity of (47) (i.e. $T > T_0$). This figure shows that it is not necessary to invoke quantum tunneling to obtain a quantitative description of the experimental data for this enzyme and its synthetic analogues.

Note that this expression depends only on two parameters, $\omega_0$ and $\omega_b$. Figures 3 and 4 show the fits of equation (47) to experimental data for different enzymes that show kinetic anomalies typical of systems that have been argued to exhibit tunneling. The first thing to notice is that the quantum transition state theory result can reproduce these experimental results. Secondly, the values of $\omega_0$ and $\omega_b$ obtained from the fits are comparable to typical C–H stretch frequencies and to barrier frequencies that have been obtained from quantum chemistry calculations (table 3). The values of $\omega_b$ obtained for both fits imply a crossover temperature $T_0$ below room temperature, indicating that our description is self-consistent in that we are in the temperature regime above the crossover.
Figure 4. Comparison of the temperature dependence of the KIE (for tritium substitution) measured for flavoenzyme monoamine oxidase [30] with quantum transition state theory at temperatures above which tunneling is possible. The solid line is a fit of equation (36) to the experimental data with two free parameters, the barrier frequency, \( \omega_b \), and the oscillation frequency in the reactant well, \( \omega_0 \). The value obtained for \( \omega_b \) is comparable with estimates from quantum chemistry calculations (see table 3) for an amineoxidase enzyme which give a barrier frequency of around 1000 cm\(^{-1}\) [104]. The value of 2100 cm\(^{-1}\) for the reactant well oscillation frequency is about two-thirds of typical carbon hydrogen stretch frequencies, but is comparable to values of the stretch frequency calculated near transition states [62, 66]. The value of the crossover temperature \( T_0 \approx 240 \text{K} \) implied by the fitted value of \( \omega_b \) and equation (8) is consistent with the domain of validity of (47). This figure shows that it is not necessary to invoke quantum tunneling to obtain a quantitative description of the experimental data for this enzyme.

7. Conclusions

The path integral approach we have reviewed has several benefits for elucidating questions concerning the role of quantum effects in hydrogen transfer reactions in enzymes. It allows a full quantum mechanical treatment of the role of both temperature and the environment of the active site. Quantum tunneling is described by the instantons (bounce solutions), which are periodic solutions to the semi-classical equations of motion in imaginary time. An important result is that these solutions only exist below some temperature, \( T_0 \), which is determined by the curvature of the top of the barrier. Above this temperature the only role of quantum effects concerns quantum fluctuations about the transition state. We have shown that for two specific classes of enzymes a quantitative description of the temperature dependence of KIEs is possible in terms of such a quantum transition state theory. This suggests that contrary to what is often claimed, quantum tunneling does not necessarily play a significant role in hydrogen transfer reactions catalyzed by enzymes.
Acknowledgments

We thank P Curmi, P Davies, H Grabert, N Hush, M Karplus, J Klinman, H A McKenzie, P Meredith, S Olsen, B J Powell, J Reimers, H F Schaefer, M F Smith, and W Yang for helpful discussions. This work was supported by the Australian Research Council.

References

[1] Ball P 2004 Nature **431** 396
[23] Marcus R 2006 *Phil. Trans. R. Soc. B* **361** 1445
[34] Basran J, Sutcliffe M J and Scrutton N S 1999 *Biochemistry* **38** 3218

[79] Bell R 1959 Trans. Faraday Soc. 55 1