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Enzymology takes a quantum leap forward

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Michael J. Sutcliffe and Nigel S. Scrutton

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Enzymology takes a quantum leap forward **zymology takes a quantum leap forwar**
By Michael J. Sutcliffe¹ and Nigel S. Scrutton²

BY MICHAEL J. SUTCLIFFE¹ AND NIGEL S. SCRUTTON²
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Enzymes are biological molecules that accelerate chemical reactions. They are central Enzymes are biological molecules that accelerate chemical reactions. They are central
to the existence of life. Since the discovery of enzymes just over a century ago, we
have witnessed an explosion in our understanding of Enzymes are biological molecules that accelerate chemical reactions. They are central
to the existence of life. Since the discovery of enzymes just over a century ago, we
have witnessed an explosion in our understanding of have witnessed an explosion in our understanding of enzyme catalysis, leading to have witnessed an explosion in our understanding of enzyme catalysis, leading to
a more detailed appreciation of how they work. A key breakthrough came from
understanding how enzymes surmount the potential-energy barrier t a more detailed appreciation of how they work. A key breakthrough came from understanding how enzymes surmount the potential-energy barrier that separates reactants from products. The genetic engineering revolution has pro understanding how enzymes surmount the potential-energy barrier that separates reactants from products. The genetic engineering revolution has provided tools for $\overline{\circ}$ dissecting enzyme structure and enabling design of reactants from products. The genetic engineering revolution has provided tools for
dissecting enzyme structure and enabling design of novel function. Despite the huge
efforts to redesign enzyme molecules for specific appli dissecting enzyme structure and enabling design of novel function. Despite the huge
efforts to redesign enzyme molecules for specific applications, progress in this area
has been generally disappointing. This stems from ou efforts to redesign enzyme molecules for specific applications, progress in this area
has been generally disappointing. This stems from our limited understanding of the
subtleties by which enzymes enhance reaction rates. B has been generally disappointing. This stems from our limited understanding of the subtleties by which enzymes enhance reaction rates. Based on current dogma, the vast majority of studies have concentrated on understanding how enzymes facilitate passage of the reaction over a static potential-energy barrier. However, recent studies have revealed that passage through, rather than over, the barrier can occur. These studies reveal that quantum mechanical phenomena, d have revealed that passage through, rather than over, the barrier can occur. These have revealed that passage through, rather than over, the barrier can occur. These
studies reveal that quantum mechanical phenomena, driven by protein dynamics,
can play a pivotal role in enzyme action. The new millennium studies reveal that quantum mechanical phenomena, driven by protein dynamics,
can play a pivotal role in enzyme action. The new millennium will witness a flurry
of activity directed at understanding the role of quantum mec can play a pivotal role in enzyme action. The new millennium will witness a flurry
of activity directed at understanding the role of quantum mechanics and protein
motion in enzyme action. We discuss these new developments of activity directed at understanding the rol
motion in enzyme action. We discuss these
guide enzymology into the new millennium.

Neywords: hydrogen tunnelling; enzymes; quantum mechanics;

transition-state theory: isotone effect the contributions.
hydrogen tunnelling; enzymes; quantum :
transition-state theory; isotope effect

1. Introduction

1. Introduction
Enzymes facilitate life via a plethora of reactions (Fersht 1985). Not only do they
sustain life they are also involved in a myriad of processes that affect our everyday Enzymes facilitate life via a plethora of reactions (Fersht 1985). Not only do they
sustain life, they are also involved in a myriad of processes that affect our everyday
lives. These include applications in medicine, hous Enzymes facilitate life via a plethora of reactions (Fersht 1985). Not only do they sustain life, they are also involved in a myriad of processes that affect our everyday lives. These include applications in medicine, hous sustain life, they are also involved in a myriad of processes that affect our everyday
lives. These include applications in medicine, household detergents, fine chemical
synthesis, the food industry, bioelectronics and the lives. These include applications in medicine, household detergents, fine chemical synthesis, the food industry, bioelectronics and the degradation of chemical waste.
Over many years, much effort has been expended in the q synthesis, the food industry, bioelectronics and the degradation of chemical waste.
Over many years, much effort has been expended in the quest to create enzymes for
specific biotechnological roles. Prior to the early 1980 Over many years, much effort has been expended in the quest to create enzymes for specific biotechnological roles. Prior to the early 1980s, the only methods available for changing enzyme structure were those of chemical specific biotechnological roles. Prior to the early 1980s, the only methods available
for changing enzyme structure were those of chemical modification of functional
groups (Hirs 1967) or 'forced evolution' (Rigby *et al.* for changing enzyme structure were those of chemical modification of functional
groups (Hirs 1967) or 'forced evolution' (Rigby *et al.* 1974). These methods have
now been surpassed by knowledge-based (i.e. rational) site groups (Hirs 1967) or 'forced evolution' (Rigby *et al.* 1974). These methods have
now been surpassed by knowledge-based (i.e. rational) site-directed mutagenesis, and
the grafting of biological function into existing enz now been surpassed by knowledge-based (i.e. rational) site-directed mutagenesis, and
the grafting of biological function into existing enzyme molecules ('retrofitting'; see
Hecht (1996)). More recently, gene-shuffling tech the grafting of biological function into existing enzyme molecules ('retrofitting'; see
Hecht (1996)). More recently, gene-shuffling techniques (Stemmer 1994a) have been
used to generate novel enzymes. Rational redesign o

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reaction coordinate

Figure 1. Schematic representation of the energetics for an enzyme-catalysed reaction. For the reaction $(A-H + B \rightarrow A + H - B)$ to proceed reactants must pass over the potential-energy bar-Figure 1. Schematic representation of the energetics for an enzyme-catalysed reaction. For the reaction $(A-H + B \rightarrow A + H-B)$ to proceed, reactants must pass over the potential-energy bar-
rier to the product side via the so-call reaction $(A-H + B \rightarrow A + H-B)$ to proceed, reactants must pass over the potential-energy bar-
rier to the product side via the so-called transition state (denoted by \uparrow) at the top of the energy profile. The molecular vibrations of the reactive bond are superimposed on the energy profile rier to the product side via the so-called transition state (denoted by \ddagger) at the top of the energy
profile. The molecular vibrations of the reactive bond are superimposed on the energy profile
for the reaction. The g profile. The molecular vibrations of the reactive bond are superimposed on the energy profile
for the reaction. The ground-state vibration energy levels with different isotopic substitution
are shown. Each isotope gives r \downarrow \uparrow for the reaction. The grou
are shown. Each isotope gi
to surmount the barrier. producing better enzymes. However, with a few notable exceptions (see, for example,

producing better enzymes. However, with a few notable exceptions (see, for example, Clarke *et al.* 1989*a*, *b*; Nixon *et al.* 1998; Scrutton *et al.* 1990), rational approaches have been generally unsuccessful reiterati producing better enzymes. However, with a few notable exceptions (see, for example, Clarke *et al.* 1989*a*, *b*; Nixon *et al.* 1998; Scrutton *et al.* 1990), rational approaches have been generally unsuccessful, reitera **MATHEMATICAL,
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SCIENCES** Clarke *et al.* 1989*a*, *b*; Nixon *et al.* 1998; Scrutton *et al.* 1990), rational approaches have been generally unsuccessful, reiterating our poor level of understanding of how enzymes work. This has led to a more 'sh have been generally unsuccessful, reiterating our poor level of understanding of how
enzymes work. This has led to a more 'shotgun' approach to redesign, involving
random mutagenesis, producing modest success, but dependen enzymes work. This has led to a more 'shotgun' approach to redesign, involving
random mutagenesis, producing modest success, but dependent on being able to 'pull
out' an improved enzyme from a very large collection of ran random mutagenesis, producing modest success, but dependent on being able to 'pull
out' an improved enzyme from a very large collection of randomly modified enzymes
(Graham *et al.* 1994; Stemmer 1994*b*). However, develop out' an improved enzyme from a very large collection of randomly modified enzymes (Graham *et al.* 1994; Stemmer 1994*b*). However, development of a suitable test to identify an improved enzyme is intrinsically very diffi (Graham *et al.* 1994; Stemmer
identify an improved enzyme i
cannot, therefore, be ignored.
Enzymes are large biological identify an improved enzyme is intrinsically very difficult; the rational approach cannot, therefore, be ignored.
Enzymes are large biological molecules, usually proteins, that speed up chemi-

Enzymes are large biological molecules, usually proteins, that speed up chemical reactions. Molecules that speed up chemical reactions, but are unchanged afterwards, are known as catalysts. The substances that enzymes act cal reactions. Molecules that speed up chemical reactions, but are unchanged aftercal reactions. Molecules that speed up chemical reactions, but are unchanged after-
wards, are known as catalysts. The substances that enzymes act on are known as
substrates. Enzymes exhibit remarkable specificity for the wards, are known as catalysts. The substances that enzymes act on are known as
substrates. Enzymes exhibit remarkable specificity for their substrate molecules, and
can approach 'catalytic perfection' (Albery & Knowles 197 substrates. Enzymes exhibit remarkable specificity for their substrate molecules, and
can approach 'catalytic perfection' (Albery & Knowles 1976). A popular approach
to modelling catalysis has been to visualize an energy b can approach 'catalytic perfection' (Albery & Knowles 1976). A popular approach
to modelling catalysis has been to visualize an energy barrier that must be sur-
mounted to proceed from reactants to products (figure 1). Th to modelling catalysis has been to visualize an energy barrier that must be sur-
mounted to proceed from reactants to products (figure 1). The greater the height of
this energy barrier, the slower the rate of reaction. Enz mounted to proceed from reactants to products (figure 1). The greater the height of
this energy barrier, the slower the rate of reaction. Enzymes (like other catalysts)
reduce the amount of energy required to pass over thi this energy barrier, the slower the rate of reaction. Enzymes (like other catalysts) reduce the amount of energy required to pass over this barrier, thereby increasing reaction rate. The structure of the reactant at the to reduce the amount of energy required to pass over this barrier, thereby increasing
reaction rate. The structure of the reactant at the top of the barrier is energetically
unstable, and is known as the transition state. The reaction rate. The structure of the reactant at the top of the barrier is energetically unstable, and is known as the transition state. The energy required to pass over the barrier is surmounted by thermal excitation of th unstable, and is known as the transition state. The energy required to pass over the barrier is the activation energy; the barrier is surmounted by thermal excitation of the substrate. This classical over-the-barrier treat p *Phil. Trans. R. Soc. Lond.* A (2000) *Phil. Trans. R. Soc. Lond.* A (2000)

Figure 2. Tunnelling of wave function, Ψ , with kinetic energy E through a rectangular potential-energy barrier with height V . The narrower the barrier, the smaller the mass of the particle, \circ and the smaller the difference between V and E, the greater the tunnelling probability. If the tial-energy barrier with height V. The narrower the barrier, the smaller the mass of the particle,
and the smaller the difference between V and E, the greater the tunnelling probability. If the
amplitude of Ψ has not r and the smaller the difference between V and E, the greater the tunnelling probab amplitude of Ψ has not reached zero at the far side of the barrier, it will stop deresume the oscillation it had on entering the barrier theory (TST), has been used to picture enzyme-catalysed reactions over the last

theory (TST), has been used to picture enzyme-catalysed reactions over the last 50 years (Kraut 1988). However, recent developments indicate that this 'textbook' illustration is fundamentally flawed (at least in some circu theory (TST), has been used to picture enzyme-catalysed reactions 50 years (Kraut 1988). However, recent developments indicate that the illustration is fundamentally flawed (at least in some circumstances). TST considers o

illustration is fundamentally flawed (at least in some circumstances).
TST considers only the particle-like properties of matter. However, matter (espe-
cially those particles with smaller mass) can also be considered as h illustration is fundamentally flawed (at least in some circumstances).
TST considers only the particle-like properties of matter. However, matter (espe-
cially those particles with smaller mass) can also be considered as h IST considers only the particle-like properties of matter. However, matter (especially those particles with smaller mass) can also be considered as having wave-
like properties: this is known as the wave-particle duality o cially those particles with smaller mass) can also be considered as having wave-
like properties: this is known as the wave-particle duality of matter. For enzyme-
catalysed reactions, an alternative picture to TST has eme like properties: this is known as the wave-particle duality of matter. For enzyme-
catalysed reactions, an alternative picture to TST has emerged from considering the
wave-particle duality of matter. One important feature catalysed reactions, an alternative picture to TST has emerged from considering the
wave-particle duality of matter. One important feature of the wave-like properties of
matter is that it can pass through regions that woul wave–particle duality of matter. One important feature of the wave-like properties of matter is that it can pass through regions that would be inaccessible if it were treated as a particle, i.e. the wave-like properties me matter is that it can pass through regions that would be inaccessible if it were treated
as a particle, i.e. the wave-like properties mean that matter can pass through regions
where there is zero probability of finding it.

as a particle, i.e. the wave-like properties mean that matter can pass through regions
where there is zero probability of finding it. Thus, the pathway from reactants to
products in an enzyme-catalysed reaction may not nee where there is zero probability of finding it. Thus, the pathway from reactants to
products in an enzyme-catalysed reaction may not need to pass over the barrier, as
in TST with particle-like behaviour, but could pass thro products in an enzyme-catalysed reaction may not need to pass over the barrier, as
In TST with particle-like behaviour, but could pass through the barrier. This passing through the barrier (quantum tunnelling; figure 2) can be likened to passing from \blacktriangleright one valley to an adjacent valley via a tunnel, rather than having to climb over the mountain between. As the analogy suggests, this can significantly lower the energy required to proceed from reactants to products. Thus, quantum tunnelling may play
an important role in driving enzyme-catalysed reactions, especially for the transfer
of small nuclei, such as hydrogen. required to proceed from reactants
an important role in driving enzym
of small nuclei, such as hydrogen.
Quantum tunnelling is the estable m important role in driving enzyme-catalysed reactions, especially for the transfer small nuclei, such as hydrogen.
Quantum tunnelling is the established mechanism for enzyme-mediated transfer
the much smaller electron (D HS

Quantum tunnelling is the established mechanism for enzyme-mediated transfer of the much smaller electron (DeVault 1980; Marcus & Sutin 1985). Proteins are Quantum tunnelling is the established mechanism for enzyme-mediated transfer
of the much smaller electron (DeVault 1980; Marcus & Sutin 1985). Proteins are
electrical insulators; nevertheless, electrons can travel large d of the much smaller electron (DeVault 1980; Marcus & Sutin 1985). Proteins are
electrical insulators; nevertheless, electrons can travel large distances (up to $ca.3 \times 10^{-9}$ m) through them. This apparent paradox, of an e electrical insulators; nevertheless, electrons can travel large distances (up to $ca.3 \times 10^{-9}$ m) through them. This apparent paradox, of an electron passing through an electrical insulator, can be understood in terms of 10^{-9} m) through them. This apparent paradox, of an electron passing through an electrical insulator, can be understood in terms of the wave-like properties of the electron. Thus, the electron can pass via quantum tunne electrical insulator, can be understood in terms of the electron. Thus, the electron can pass via quantum tunn
which it would be excluded by its particle-like nature. *Phil. Trans. R. Soc. Lond.* A (2000)

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370 $M.$ J. Sutcliffe and N. S. Scrutton
In contrast to electron transfer via quantum tunnelling, the ground-breaking work In contrast to electron transfer via quantum tunnelling, the ground-breaking work
of Klinman and colleagues provides the only experimental indication of H-tunnelling
in enzyme molecules (currently five in total: see Bahns **MATHEMATICAL,
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SCIENCES** In contrast to electron transfer via quantum tunnelling, the ground-breaking work
of Klinman and colleagues provides the only experimental indication of H-tunnelling
in enzyme molecules (currently five in total; see Bahnso of Klinman and colleagues provides the only experimental indication of H-tunnelling
in enzyme molecules (currently five in total; see Bahnson & Klinman (1995)). This
arises conceptually because the mass of the H nucleus i in enzyme molecules (currently five in total; see Bahnson & Klinman (1995)). This arises conceptually because the mass of the H nucleus is approximately 1840 times greater than that of the electron. The probability of tun arises conceptually because the mass of the H nucleus is approximately 1840 times
greater than that of the electron. The probability of tunnelling decreases with increas-
ing mass, which significantly reduces the probabili greater than that of the electron. The probability of tunnelling decreases with increasing mass, which significantly reduces the probability of hydrogen versus electron tunnelling. Nevertheless, for those enzyme-catalysed ing mass, which significantly reduces the probability of hydrogen versus electron
tunnelling. Nevertheless, for those enzyme-catalysed reactions with a large activa-
tion energy, quantum tunnelling is an attractive means o tunnelling. Nevertheless, for those enzyme-catalysed reactions with a large activation energy, quantum tunnelling is an attractive means of transferring hydrogen from reactant to product. Until recently, quantum tunnelling tion energy, quantum tunnelling is an attractive means of transferring hydrogen from
reactant to product. Until recently, quantum tunnelling was thought to be significant
only at very low (cryogenic) temperatures. However, reactant to product. Until recently, quantum tunnelling was thought to be significant
only at very low (cryogenic) temperatures. However, deviations from classical TST
behaviour have been seen recently, implying that H-tun only at very low (cryogenic) temperatures. However, deviations from classical TST
behaviour have been seen recently, implying that H-tunnelling may be significant
at physiological temperatures. These results have, in the m \mathbf{H} behaviour have been seen recently, implying that H-tunnelling may be significant at physiological temperatures. These results have, in the main, been modelled as hybrid 'over' (TST) and 'through' (quantum-tunnelling) barri at physiological temperatures. These results have, in
hybrid 'over' (TST) and 'through' (quantum-tunnell
i.e. quantum correction models of TST (Bell 1980).
Our own studies have revealed, for the first time, the brid 'over' (TST) and 'through' (quantum-tunnelling) barrier transfer reactions,
c quantum correction models of TST (Bell 1980).
Our own studies have revealed, for the first time, that quantum tunnelling can be
e sole mean

Our own studies have revealed, for the first time, that quantum tunnelling can be the sole means by which an enzyme catalyses H-transfer during C-H bond breakage (Basran *et al.* 1999). The reaction pathway does not pass u Our own studies have revealed, for the first time, that quantum tunnelling can be the sole means by which an enzyme catalyses H -transfer during C $-H$ bond breakage the sole means by which an enzyme catalyses H-transfer during C-H bond breakage (Basran *et al.* 1999). The reaction pathway does not pass up the energy barrier prior to tunnelling, as with the quantum correction models o (Basran *et al.* 1999). The reaction pathway does not pass up the energy barrier prior
to tunnelling, as with the quantum correction models of TST, but tunnels through
the barrier from the starting (or ground) state. Para to tunnelling, as with the quantum correction models of TST, but tunnels through
the barrier from the starting (or ground) state. Paradoxically, reaction rates (as with
TST) are still highly dependent on temperature. This $\overline{0}$ the barrier from the starting (or ground) state. Paradoxically, reaction rates (as with TST) are still highly dependent on temperature. This observation is inconsistent with a pure 'ground-state' tunnelling reaction, since TST) are still highly dependent on temperature. This observation is inconsistent with a pure 'ground-state' tunnelling reaction, since the probability of tunnelling (and, thus, rate of reaction) is a function of barrier wi a pure 'ground-state' tunnelling reaction, since the probability of tunnelling (and, thus, rate of reaction) is a function of barrier width, but is independent of temperature. This apparent paradox is resolved by taking into account the temperature-
dependent natural breathing of enzyme molecules, which di ature. This apparent paradox is resolved by taking into account the temperature-
dependent natural breathing of enzyme molecules, which distorts the structure of
the protein to produce the geometry required for nuclear tun dependent natural breathing of enzyme molecules, which distorts the structure of
the protein to produce the geometry required for nuclear tunnelling (achieved by
reducing the width of the barrier between reactants and prod the protein to produce the geometry required for nuclear tunnelling (achieved by reducing the width of the barrier between reactants and products). In this dynamic view of enzyme catalysis, it is thus the width, and not th reducing the width of the barrier between reactants and products). In this dynamic
view of enzyme catalysis, it is thus the width, and not the height (as with TST), of
the energy barrier that controls the reaction rate.
Th Even of enzyme catalysis, it is thus the width, and not the height (as with TST), of
e energy barrier that controls the reaction rate.
The important criterion thus becomes the ability of the enzyme to distort and
ereby red **MATHEMATICAL,
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the energy barrier that controls the reaction rate.
The important criterion thus becomes the ability of the enzyme to distort and
thereby reduce barrier width, and not stabilization of the transition state with con-
comita The important criterion thus becomes the ability of the enzyme to distort and
thereby reduce barrier width, and not stabilization of the transition state with con-
comitant reduction in barrier height (activation energy). comitant reduction in barrier height (activation energy). We now describe theoretical
approaches to enzymatic catalysis that have led to the development of dynamic barcomitant reduction in barrier height (activation energy). We now describe theoretical
approaches to enzymatic catalysis that have led to the development of dynamic bar-
rier (width) tunnelling theories for H-transfer. Inde approaches to enzymatic catalysis that have led to the development of dynamic bar-
rier (width) tunnelling theories for H-transfer. Indeed, enzymatic H-tunnelling can
be treated conceptually in a similar way to the well-es rier (width) tunnelling theorie
be treated conceptually in a sin
electron transfer in proteins. electron transfer in proteins.
2. Enzyme catalysis in the classical world

2. Enzyme catalysis in the classical world
In the classical world (and biochemistry textbooks), TST has been used extensively
to model enzyme catalysis (Glasstone *et al.* 1941; Johnson *et al.* 1974; Kraut 1988) 2. Enzyme catalysis in the classical world
In the classical world (and biochemistry textbooks), TST has been used extensively
to model enzyme catalysis (Glasstone *et al.* 1941; Johnson *et al.* 1974; Kraut 1988).
The basi In the classical world (and biochemistry textbooks), TST has been used extensively
to model enzyme catalysis (Glasstone *et al.* 1941; Johnson *et al.* 1974; Kraut 1988).
The basic premise of TST is that the single-step r to model enzyme catalysis (Glasstone *et al.* 1941; Johnson *et al.* 1974; Kraut 1988). The basic premise of TST is that the single-step reaction converting substrate to product, $S \rightarrow P$, is instead treated as a two-step r product, $S \rightarrow P$, is instead treated as a two-step reaction over a static potential-

$$
S \rightleftharpoons X^{\ddagger} \xrightarrow{k} P,\tag{2.1}
$$

 $S \rightleftharpoons X^{\ddagger} \xrightarrow{k} P,$ (2.1)
where X^{\ddagger} is the transition state, which is treated as being in *quasi*-equilibrium with
the ground state of the substrate at the foot of the energy barrier. An equilibrium where X^{\ddagger} is the transition state, which is treated as being in *quasi*-equilibrium with the ground state of the substrate at the foot of the energy barrier. An equilibrium *Phil. Trans. R. Soc. Lond.* A (2000) *Phil. Trans. R. Soc. Lond.* A (2000)

THE ROYAL

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$$
k = \left(\frac{k_{\rm B}T}{h}\right) \exp\left(\frac{-\Delta G^{\ddagger}}{RT}\right). \tag{2.2}
$$

 $k = \left(\frac{B}{h}\right) \exp\left(\frac{B}{RT}\right)$. (2.2)
Where k_B is the Boltzmann constant, and h is Planck's constant. These simple
mathematical relationships probably account for the popularity of TST in modelling Where k_B is the Boltzmann constant, and h is Planck's constant. These simple mathematical relationships probably account for the popularity of TST in modelling enzyme catalysis. Where k_B is the I
mathematical relati
enzyme catalysis.
TST has been u athematical relationships probably account for the popularity of TST in modelling
zyme catalysis.
TST has been useful in providing a rationale for the so-called 'kinetic isotope
'ect' (KIE) (More O'Ferrall 1975: Van Hook 1

enzyme catalysis.

TST has been useful in providing a rationale for the so-called 'kinetic isotope

peffect' (KIE) (More O'Ferrall 1975; Van Hook 1971). The KIE is used by enzymolo-TST has been useful in providing a rationale for the so-called 'kinetic isotope
effect' (KIE) (More O'Ferrall 1975; Van Hook 1971). The KIE is used by enzymolo-
gists to probe various aspects of mechanism (Klinman 1978). I effect' (KIE) (More O'Ferrall 1975; Van Hook 1971). The KIE is used by enzymologists to probe various aspects of mechanism (Klinman 1978). Importantly, measured KIEs have also been used to monitor non-classical behaviour KIEs have also been used to monitor non-classical behaviour in enzyme-catalysed H-transfer reactions (Bahnson & Klinman 1995). The KIE arises because of the differential reactivity of, for example, a C-H (protium), a C-D (deuterium) and a C-T (tritium) bond. The electronic, rotational and translation differential reactivity of, for example, a $C-H$ (protium), a $C-D$ (deuterium) and a differential reactivity of, for example, a C–H (protium), a C–D (deuterium) and a C–T (tritium) bond. The electronic, rotational and translational properties of the H, D and T atoms are identical. However, by virtue of the C-T (tritium) bond. The electronic, rotational and translational properties of the H, D and T atoms are identical. However, by virtue of the larger reduced mass of C-T compared with that of C-D and C-H, the zero-point vib H, D and T atoms are identical. However, by virtue of the larger reduced mass of C-T compared with that of C-D and C-H, the zero-point vibrational energy of C-H is greater than that of C-D, which is greater than that of C C-T compared with that of C-D and C-H, the zero-point vibrational energy of C-H
is greater than that of C-D, which is greater than that of C-T (figure 1). In the
transition state, one vibrational degree of freedom is lost is greater than that of C-D, which is greater than that of C-T (figure 1). In the transition state, one vibrational degree of freedom is lost, which leads to differences between isotopes in ΔG^{\ddagger} . This leads, in turn between isotopes in ΔG^{\ddagger} . This leads, in turn, to an isotope-dependent difference in rate: the lower the mass of the isotope, the lower ΔG^{\ddagger} , and, thus, the faster the rate. between isotopes in ΔG^{\ddagger} . This leads, in turn, to an isotope-dependent difference in
rate: the lower the mass of the isotope, the lower ΔG^{\ddagger} , and, thus, the faster the rate.
The KIEs, therefore, have different rate: the lower the mass of the isotope, the lower ΔG^{\ddagger} , and, thus, t
The KIEs, therefore, have different values depending on the isotope:
 $k_H/k_D \approx 7$ and $k_H/k_T \approx 15$ at 25 °C (Schneider & Stern 1972).
For a single b is KIEs, therefore, have different values depending on the isotopes being compared:
 $f/k_D \approx 7$ and $k_H/k_T \approx 15$ at 25 °C (Schneider & Stern 1972).

For a single barrier, the classical theory places an upper limit on the ob

 $k_{\rm H}/k_{\rm D} \approx 7$ and $k_{\rm H}/k_{\rm T} \approx 15$ at 25 °C (Schneider & Stern 1972).
For a single barrier, the classical theory places an upper limit on the observed KIE.
However, with enzyme-catalysed reactions, the value of th For a single barrier, the classical theory places an upper limit on the observed KIE.
However, with enzyme-catalysed reactions, the value of the KIE is often less than the
upper limit. This can arise because of the complex However, with enzyme-catalysed reactions, the value of the KIE is often less than the upper limit. This can arise because of the complexity of enzyme-catalysed reactions.
For example, enzymes often catalyse multi-step reac *AATHEMATICAL,
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CIENCES* upper limit. This can arise because of the complexity of enzyme-catalysed reactions.
For example, enzymes often catalyse multi-step reactions, involving transfer over
multiple barriers. In the simplest case, the highest ba For example, enzymes often catalyse multi-step reactions, involving transfer over multiple barriers. In the simplest case, the highest barrier will determine the overall reaction rate. However, in the case where two (or mo multiple barriers. In the simplest case, the highest barrier will determine the overall reaction rate. However, in the case where two (or more) barriers are of similar height, each will contribute to determining the overal reaction rate. However, in the case where two (or more) barriers are of similar height,
each will contribute to determining the overall rate; if transfer over the second barrier
does not involve breakage of a C-H bond, it each will contribute to determining the overall rate; if transfer over the second barrier
does not involve breakage of a C–H bond, it will not be an isotope-sensitive step,
thus leading to a reduction in the observed KIE. does not involve breakage of a C–H bond, it will not be an isotope-sensitive step,
thus leading to a reduction in the observed KIE. An alternative rationale for reduced
KIEs has also been discussed in relation to the struc thus leading to a reduction in the observed KIE. An alternative rationale for reduced KIEs has also been discussed in relation to the structure of the transition state. For isoenergetic reactions (i.e. the substrate and p KIEs has also been discussed in relation to the structure of the transition state. For isoenergetic reactions (i.e. the substrate and product have the same energy; the total
free energy change, $\Delta G = 0$), the transition state is predicted to be symmetrical and
vibrations in the reactive C-H bond are lost at free energy change, $\Delta G = 0$), the transition state is predicted to be symmetrical and
vibrations in the reactive C-H bond are lost at the top of the barrier. In this scenario,
the maximum KIE is realized. However, when t vibrations in the reactive C–H bond are lost at the top of the barrier. In this scenario, the maximum KIE is realized. However, when the transition state resembles much more closely the enzyme-substrate complex $(\Delta G < 0)$ The maximum KIE is realized. However, when the transition state resembles much \bigcup more closely the enzyme-substrate complex $(\Delta G < 0)$ or the enzyme-product com-
 \bigcirc plex $(\Delta G > 0)$, the presence of vibrational frequen more closely the enzyme-substrate complex $(\Delta G < 0)$ or the enzyme-product com-
plex $(\Delta G > 0)$, the presence of vibrational frequencies in the transition state cancel
with ground-state vibrational frequencies, and the KIE plex $(\Delta G > 0)$, the presence of vibrational frequencies in the transition state cancel
with ground-state vibrational frequencies, and the KIE is reduced. This dependence
of transition-state structure on the KIE has become with ground-state vib
of transition-state str
(Westheimer 1961). **PHILOSOPHICAL**
TRANSACTIONS

(Westheimer 1961).
† The Gibbs free energy is equivalent to the activation energy, ΔE_a , discussed elsewhere (providing [†] The Gibbs free energy is equivalent to the activation energy, ΔE_a , discussed elsewhere (providing that $\Delta E_a \gg RT$, which is the case in the systems discussed herein; $\Delta H = \Delta E_a - RT$). Note that by convention enzymologi [†] The Gibbs free energy is equivalent to the activation energy, ΔE_a , discussed elsewhere (providing that $\Delta E_a \gg RT$, which is the case in the systems discussed herein; $\Delta H = \Delta E_a - RT$). Note that by convention, enzymolog that $\Delta E_a \gg RT$, which is the case in the systems discussed herein; $\Delta H = \Delta E_a - RT$). No
convention, enzymologists consider reactions on the macroscopic scale and use the macrosc
free energy; this is equivalent to considering free energy; this is equivalent to considering the potential energy on the microscopic scale.
Phil. Trans. R. Soc. Lond. A (2000)

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M. J. Sutcliffe and N. S. Scrutton
3. A role for protein dynamics in classical transfers

The TST is probably an oversimplification when applied to enzyme catalysis; it was The TST is probably an oversimplification when applied to enzyme catalysis; it was
originally developed to account for gas-phase reactions. Solvent dynamics and the
natural 'breathing' of the enzyme molecule need to be inc The TST is probably an oversimplification when applied to enzyme catalysis; it was originally developed to account for gas-phase reactions. Solvent dynamics and the natural 'breathing' of the enzyme molecule need to be inc originally developed to account for gas-phase reactions. Solvent dynamics and the natural 'breathing' of the enzyme molecule need to be included for a more complete picture of enzymatic reactions. Kramers put forward a the natural 'breathing' of the enzyme molecule need to be included for a more complete
picture of enzymatic reactions. Kramers put forward a theory that explicitly recog-
nizes the role of solvent dynamics in catalysis (Gavis picture of enzymatic reactions. Kramers put forward a theory that explicitly recognizes the role of solvent dynamics in catalysis (Gavish 1986; Kramers 1940). For the reaction $S \rightarrow P$, Kramers (1940) suggested that this pr nizes the role of solvent dynamics in catalysis (Gavish 1986; Kramers 1940). For the reaction $S \rightarrow P$, Kramers (1940) suggested that this proceeds by a process of diffusion over a potential-energy barrier. The driving forc reaction $S \rightarrow P$, Kramers (1940) suggested that this proceeds by a process of diffusion over a potential-energy barrier. The driving force for the reaction is derived from random thermally induced structural fluctuations i sion over a potential-energy barrier. The driving force for the reaction is derived from
random thermally induced structural fluctuations in the protein, and these 'energize'
the motion of the substrate. This kinetic motio The motion of the substrate. This kinetic motion in the substrate is subsequently dis-
sipated because of friction with the surroundings, and enables the substrate to reach the motion of the substrate. This kinetic motion in the substrate is subsequently dis-
sipated because of friction with the surroundings, and enables the substrate to reach
a degree of strain that is consistent with it pro sipated because of friction with the surroundings, and enables the substrate to reach
a degree of strain that is consistent with it progressing from S to P (along the reac-
tion pathway): the so-called 'transient-strain' a degree of strain that is consistent with it progressing from S to P (along the tion pathway): the so-called 'transient-strain' model of enzyme catalysis. The constant, k , is related to the height of the potential-ener tion pathway): the so-called 'transient-strain' model of enzyme catalysis. The rate constant, k, is related to the height of the potential-energy barrier, ΔU , by

$$
k = (1/\tau)e^{(-\Delta U/RT)},\tag{3.1}
$$

where τ is the time constant of structural fluctuations (and is proportional to the local where τ is the time constant of structural fluctuations (and is proportional to the local viscosity). Equation (3.1) takes the same form as the phenomenological Arrhenius equation (equation (3.2)) which has been used t where τ is the time constant of structural fluctuations (and is proportional to the local viscosity). Equation (3.2)), which has been used to describe classical transfers over a static potential-energy barrier viscosity). Equation (3.1) takes
equation (equation (3.2)), which
static potential-energy barrier,

$$
k = Ae^{(-\Delta E_a/RT)}, \tag{3.2}
$$

where A is the so-called pre-exponential factor, and ΔE_a is the activation energy. where A is the so-called pre-exponential factor, and ΔE_a is the activation energy.
However, the dynamic nature of molecules is incorporated into ΔU , but not into ΔE . By acknowledging the dynamic nature of protein where A is the so-called pre-exponential factor, and ΔE_a is the activation energy.
However, the dynamic nature of molecules is incorporated into ΔU , but not into ΔE_a . By acknowledging the dynamic nature of protei However, the dynamic nature of molecules is incorporated into ΔU , but not into ΔE_a . By acknowledging the dynamic nature of protein molecules, Kramers' (1940) theory (but not TST) for classical transfers provides us ΔE_a . By acknowledging the dynamic nature of protein molecules, Kratheory (but not TST) for classical transfers provides us with a platform to develop new theories of quantum tunnelling in enzyme molecules. to develop new theories of quantum tunnelling in enzyme molecules.
4. Wave–particle duality and the concept of tunnelling

Tunnelling is a phenomenon that arises as a result of the wave properties of matter. The (de Broglie) wavelength, λ , of a particle can be calculated from its mass, m, and its kinetic energy, E , using the de Broglie equation:

$$
\lambda = h/(2mE)^{1/2}.\tag{4.1}
$$

 $\lambda = h/(2mE)^{1/2}$. (4.1)
Thus, the lighter the particle, the longer its de Broglie wavelength, and, as the
particle mass is reduced there is higher uncertainty in its position. Quantum tun-Thus, the lighter the particle, the longer its de Broglie wavelength, and, as the particle mass is reduced, there is higher uncertainty in its position. Quantum tun-
nelling is the penetration of a particle into a region Thus, the lighter the particle, the longer its de Broglie wavelength, and, as the particle mass is reduced, there is higher uncertainty in its position. Quantum tunnelling is the penetration of a particle into a region tha particle mass is reduced, there is higher uncertainty in its position. Quantum tunnelling is the penetration of a particle into a region that is excluded in classical mechanics (due to it having insufficient energy to over nelling is the penetration of a particle into a region that is excluded in classical
mechanics (due to it having insufficient energy to overcome the potential-energy
barrier). An important feature of quantum mechanics is t mechanics (due to it having insufficient energy to overcome the potential-energy
barrier). An important feature of quantum mechanics is that details of a parti-
cle's location and motion are defined by a wave function. The barrier). An important feature of quantum mechanics is that details of a particle's location and motion are defined by a wave function. The wave function is
a quantity which, when squared, gives the probability of finding cle's location and motion are defined by a wave function. The wave function is
a quantity which, when squared, gives the probability of finding a particle in a
given region of space. Thus, a non-zero wave function for a gi a quantity which, when squared, gives the probability of finding a particle in a
given region of space. Thus, a non-zero wave function for a given region means
that there is a finite probability of the particle being found given region of space. Thus, a non-zero wave function for a given region means
that there is a finite probability of the particle being found there. A non-zero
wave function on one side of the barrier will decay inside th that there is a finite probability of the particle being found there. A non-zero wave function on one side of the barrier will decay inside the barrier where its kinetic energy, E , is less than the potential-energy of t

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TRANSACTIONS

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nuclear configuration
Figure 3. Reaction coordinate diagram for a simple chemical reaction. The reactant A is con-
verted to product B. The B curve represents the potential surface of the reactant, and the P Figure 3. Reaction coordinate diagram for a simple chemical reaction. The reactant A is converted to product B. The R curve represents the potential surface of the reactant, and the P curve the potential-energy surface of Figure 3. Reaction coordinate diagram for a simple chemical reaction. The reactant A is converted to product B. The R curve represents the potential surface of the reactant, and the P curve the potential-energy surface of verted to product B. The R curve represents the potential surface of the reactant, and the P
curve the potential-energy surface of the product. Thermal activation leads to an over-the-barrier
process at transition state X. process at transition state X. The vibrational states have been shown for the reactant A. As
temperature increases, the higher energy vibrational states are occupied leading to increased
penetration of the P curve below th temperature increases, the higher energy vibrational states are occupied leading to increased

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SCIENCES** if $E > V$, it can pass over the barrier). On emerging at the other side of the barrier, the wave function amplitude is non-zero, and there is a finite probability that the particle is found on the other side of the barrier if $E > V$, it can pass over the barrier). On emerging at the other side of the bar-
rier, the wave function amplitude is non-zero, and there is a finite probability that
the particle is found on the other side of the barri if $E > V$, it can pass over the barrier). On emerging at the other side of the barrier, the way
the particle
(figure 2).
Ouantum the particle is found on the other side of the barrier, i.e. the particle has tunnelled (figure 2).
Quantum tunnelling in chemical reactions can be visualized in terms of a reaction

(figure 2).
Quantum tunnelling in chemical reactions can be visualized in terms of a reaction
coordinate diagram (figure 3). As we have seen, classical transitions are achieved
by thermal activation: nuclear (i.e. atomic Quantum tunnelling in chemical reactions can be visualized in terms of a reaction coordinate diagram (figure 3). As we have seen, classical transitions are achieved by thermal activation: nuclear (i.e. atomic position) di coordinate diagram (figure 3). As we have seen, classical transitions are achieved
by thermal activation: nuclear (i.e. atomic position) displacement along the R curve
distorts the geometry so that the intersection of the by thermal activation: nuclear (i.e. atomic position) displacement along the R curve distorts the geometry so that the intersection of the R and P curves is reached (the \geq so-called transition state). Quantum mechanic distorts the geometry so that the intersection of the R and P curves is reached (the \blacktriangleright so-called transition state). Quantum mechanics is based on the premise that energy is quantized (i.e. can have only specific, discrete values). Thus, in the reaction coordinate diagram, the quantized vibrational energy states of the reactant and product can be depicted (figure 3). At ambient temperatures dinate diagram, the quantized vibrational energy states of t
can be depicted (figure 3). At ambient temperatures, it is
ground-state vibrational energy levels that are populated.
Factors that enhance tunnelling are a small Geven be depicted (figure 3). At ambient temperatures, it is almost exclusively the \bigcirc ground-state vibrational energy levels that are populated.
 \bigcirc Factors that enhance tunnelling are a small particle mass (increa

ground-state vibrational energy levels that are populated.
Factors that enhance tunnelling are a small particle mass (increased de Broglie wavelength) and a narrow potential-energy barrier. To illustrate the effect of part Factors that enhance tunnelling are a small particle mass (increased de Broglie wavelength) and a narrow potential-energy barrier. To illustrate the effect of particle mass, consider the somewhat idealized rectangular pot mass, consider the somewhat idealized rectangular potential-energy barrier (height V , width ℓ), where the tunnelling probability, P , is related to the mass of the tun- $\frac{1}{0}$ nelling particle by the following relationship:

$$
P \propto \exp[(-2\ell\sqrt{2mV})/\hbar],\tag{4.2}
$$

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TRANSACTIONS

**MATHEMATICAL,
PHYSICAL
& ENGINEERING
SCIENCES**

374 *M. J. Sutcliffe and N. S. Scrutton*
where \hbar is $h/2\pi$. In biology, electron transfer is known to occur over large distances **IATHEMATICAL,
HYSICAL
: ENGINEERING
CIENCES** where \hbar is $h/2\pi$. In biology, electron transfer is known to occur over large distances (up to ca. 25 Å). Using the above expression, and given that the mass of protium is 1840 times that of the electron, the same pr (up to $ca. 25$ Å). Using the above expression, and given that the mass of protium is (up to ca. 25 Å). Using the above expression, and given that the mass of protium is 1840 times that of the electron, the same probability for protium tunnelling gives a transfer distance of 0.58 Å. This distance is simila 1840 times that of the electron, the same probability for protium tunnelling gives
a transfer distance of 0.58 Å. This distance is similar to the length of a reaction
coordinate, and is thus suggestive of high tunnelling a transfer distance of 0.58 Å. This distance is similar to the length of a reaction coordinate, and is thus suggestive of high tunnelling probability. The larger masses of deuterium and tritium lead to corresponding trans coordinate, and is thus suggestive of high tunnelling probability. The larger masses of deuterium and tritium lead to corresponding transfer distances of 0.41 Å and 0.34 Å , respectively, thus making KIE studies

respectively, thus making KIE studies attractive for the detection of H-tunnelling in
enzymes.
The effect of barrier width is readily illustrated by using a more realistic potentialenergy barrier shape (e.g. a truncated parabola). The temperature dependence of a The effect of barrier width is readily illustrated by using a more realistic pot
energy barrier shape (e.g. a truncated parabola). The temperature dependen
unimolecular (i.e. single-molecule) rate constant k may be repres delay a parassary. The same of k may
 $k(T) = AQ(T)e^{-V/RT}$,

$$
k(T) = AQ(T)e^{-V/RT},
$$
\n(4.3)

where V is the height of the barrier, and A is the temperature-independent frewhere V is the height of the barrier, and A is the temperature-independent frequency of collisions with the barrier. $Q(T)$, the tunnelling correction factor, is defined as the ratio of the quantum mechanical to the classi where V is the height of the barrier, and A is the temperature-independent frequency of collisions with the barrier. $Q(T)$, the tunnelling correction factor, is defined as the ratio of the quantum mechanical to the classi quency of collisions with the barrier. $Q(T)$, the tunnelling correction factor, is defined
as the ratio of the quantum mechanical to the classical barrier transmission rates,
which approaches unity at high temperatures (i as the ratio of the quantum mechanical to the classical based which approaches unity at high temperatures (i.e. the approaches V). For a truncated parabolic energy barrier

cated parabolic energy barrier
\n
$$
Q(T) = \frac{h\nu}{2k_{\rm B}T\sin(h\nu/2k_{\rm B}T)},\tag{4.4}
$$

 $Q(T) = \frac{W}{2k_BT\sin(h\nu/2k_BT)}$, (4.4)
where ν is the reaction coordinate frequency (e.g. the frequency of a vibrating C–H
bond) Treatment of the vibrating bond as a simple harmonic oscillator allows the $\sum_{B} B \sin(n\nu/2\kappa B T)$
where ν is the reaction coordinate frequency (e.g. the frequency of a vibrating C-H
bond). Treatment of the vibrating bond as a simple harmonic oscillator allows the
reaction coordinate frequency bond). Treatment of the vibrating bond as a simple harmonic oscillator allows the relation coordinate frequency to be formulated in terms of Hooke's law:

$$
\nu = \frac{1}{2\pi} \left(\frac{k_{\text{bond}}}{m}\right)^{1/2}.\tag{4.5}
$$

 $\nu = \frac{1}{2\pi} \left(\frac{300 \text{ rad}}{m} \right)$. (4.5)
is, therefore, related to the mass of the particle m and the force constant k_{bond} of
e vibrating bond. Large force constants produce high and narrow barrier shapes ν is, therefore, related to the mass of the particle m and the force constant k_{bond} of
the vibrating bond. Large force constants produce high and narrow barrier shapes
and therefore ν is large. Consequently in t ν is, therefore, related to the mass of the particle m and the force constant k_{bond} of
the vibrating bond. Large force constants produce high and narrow barrier shapes
and, therefore, ν is large. Consequently, i the vibrating bond. Large force constants produce high and narrow barrier shapes
and, therefore, ν is large. Consequently, in this regime, $Q(T)$ is large and transfer
is by quantum tunnelling. For low and wide barrier and, therefore, ν is large. Consequently, in this regime, $Q(T)$ is large and transfer is by quantum tunnelling. For low and wide barrier shapes (i.e. small values of t force constant), $Q(T)$ is small, and transfer is d is by quantum tunnelling. For low and wide barrier shapes (i.e. small values of the force constant), $Q(T)$ is small, and transfer is dominated by the classical route.
Different strategies are required for optimizing enzym

proceed by quantum tunnelling rather than classical transfer. For classical transfers, Different strategies are required for optimizing enzyme structure for reactions to
proceed by quantum tunnelling rather than classical transfer. For classical transfers,
the enzyme has evolved to reduce the height of the p proceed by quantum tunnelling rather than classical transfer. For classical transfers,
the enzyme has evolved to reduce the height of the potential-energy barrier and to
stabilize the transition state (rather than ground s the enzyme has evolved to reduce the height of the potential-energy barrier and to
stabilize the transition state (rather than ground state). In the quantum regime,
it is reduction of barrier width and not height that opti stabilize the transition state (rather than ground state). In the quantum regime,
it is reduction of barrier width and not height that optimizes rate. Quantum tun-
nelling from the ground state requires little or no struct it is reduction of barrier width and not height that optimizes rate. Quantum tun-
nelling from the ground state requires little or no structural reorganization of the
substrate, and the need to stabilize a transition state nelling from the ground state requires little or no structural reorganization of the substrate, and the need to stabilize a transition state is thus eliminated. Exclusion of water from the active sites of enzymes prevents substrate, and the need to stabilize a transition state is thus eliminated. Exclusion
of water from the active sites of enzymes prevents coupling of solvent motion to
the transferred par-
ticle. In the following sections, of water from the active sites of enzymes prevents coupling of solvent motion to
the transfer reaction, and this leads to a reduction of mass for the transferred par-
ticle. In the following sections, we review the evidenc the transfer reaction, and this leads to a reduction of mass for the transferred particle. In the following sections, we review the evidence for quantum tunnelling in biological catalysis and discuss the strategies employe ticle. In the following sections, we review the evidence for quantum tunnelling in
biological catalysis and discuss the strategies employed by enzymes to optimize the
transfer process. Surprisingly, and unlike for biologic biological catalysis and discuss the strategies employed by enzymes to optimize the
transfer process. Surprisingly, and unlike for biological electron transfers, reports of
H-tunnelling in enzymatic reactions have been res transfer process. Surprisingly, and unlike for biological electron transfers, reports of H-tunnelling in enzymatic reactions have been restricted to only a small number of enzyme molecules. The realization that H-tunnelli H-tunnelling in enzymatic reactions have been restricted to only a small number of enzyme molecules. The realization that H-tunnelling occurs in enzymes has been relatively recent (Basran *et al.* 1999; Cha *et al.* 1989; *et al.* 1999; Cha *et al.* 1989; Grant & Klinman 1989; Jonsson

Phil. Trans. R. Soc. Lond. A (2000)

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enzymes.

- $Enzymology takes a quantum leap forward$ 375
(i) the misconception that the much larger mass of the H nucleus is inconsistent
with tunnelling: and the misconception that with tunnelling; and % with tunnelling; and
(ii) the erroneous assumption that measured KIEs less than 7 are always indicative
- of classical H-transfer (Bruno & Bialek 1992).

of classical H-transfer (Bruno & Bialek 1992).
Our recent work has demonstrated that H-tunnelling in proteins is inextricably cou-
pled to protein dynamics (Basran *et al.* 1999). This provides a link to the established or classical H-transfer (Bruno & Blatek 1992).
Our recent work has demonstrated that H-tunnelling in proteins is inextricably cou-
pled to protein dynamics (Basran *et al.* 1999). This provides a link to the established
th pled to protein dynamics (Basran *et al.* 1999). This provides a link to the established theories for electron tunnelling in proteins. To provide a framework for the discussion of H-tunnelling in enzymes, protein-mediated theories for electron tunnelling in proteins. To provide a framework for the discussion

5. Electron tunnelling in proteins

5. Electron tunnelling in proteins
The transfer of electrons in proteins by a quantum mechanical tunnelling mechanism
is now firmly established (DeVault & Chance 1966: Marcus & Sutin 1985). Electron The transfer of electrons in proteins by a quantum mechanical tunnelling mechanism
is now firmly established (DeVault & Chance 1966; Marcus & Sutin 1985). Electron
transfer within proteins occurs between two 'centres' (kno The transfer of electrons in proteins by a quantum mechanical tunnelling mechanism
is now firmly established (DeVault & Chance 1966; Marcus & Sutin 1985). Electron
transfer within proteins occurs between two 'centres' (kn is now firmly established (DeVault & Chance 1966; Marcus & Sutin 1985). Electron transfer within proteins occurs between two 'centres' (known as redox centres, since one reduces the other, and in so doing is itself oxidiz transfer within proteins occurs between two 'centres' (known as redox centres, since
one reduces the other, and in so doing is itself oxidized): the 'electron donor' (which
is thereby oxidized) supplies an electron to the one reduces the other, and in so doing is itself oxidized): the 'electron donor' (which is thereby oxidized) supplies an electron to the 'electron acceptor' (which is thereby reduced). This can be modelled using quantum mechanics. If the wave function of the product state denoted $\Psi(P)$ then electron transfe duced). This can be modelled using quantum mechanics. If the wave function of the eactant state is denoted $\Psi(R)$ and the wave function of the product state denoted (P) , then electron transfer between two redox centres A reactant state is denoted $\Psi(R)$ and the wave function of the product state denoted $\Psi(P)$, then electron transfer between two redox centres A and B is viewed as a transition in state from $\Psi(R) = (a^*, b)$ to $\Psi(P) = (a, b^*)$. $\Psi(P)$, then electron transfer between two redox centres A and B is viewed as a transition in state from $\Psi(R) = (a^*, b)$ to $\Psi(P) = (a, b^*)$. Here a and b are functions that describe the nuclear and electronic motions of their transition in state from $\Psi(R) = (a^*, b)$ to $\Psi(P) = (a, b^*)$. Here a and b are functions that describe the nuclear and electronic motions of their respective redox centres.
The electron, denoted by an asterisk, is transferred that describe the nuclear and electronic motions of their respective redox centres.
The electron, denoted by an asterisk, is transferred from a to b during the course
of the reaction. This results in a change in charg The electron, denoted by an asterisk, is transferred from a to b during the course
of the reaction. This results in a change in charge distribution (since the location of
the electron changes). In turn, this alters th of the reaction. This results in a change in charge distribution (since the location of
the electron changes). In turn, this alters the position of polar groups around the
redox centres. Thus, electron transfer alters the the electron changes). In turn, this alters
redox centres. Thus, electron transfer alters
as well as electronic states of the protein.
It is well established that electron transi It is well as electronic states of the protein.
It is well established that electron transfer in proteins is driven by distortion in

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CIENCES* It is well established that electron transfer in proteins is driven by distortion in
the nuclear (protein) geometry of the reactant state. This is facilitated by the nat-
ural, thermally activated, breathing of the protein the nuclear (protein) geometry of the reactant state. This is facilitated by the natural, thermally activated, breathing of the protein molecule. Thermal activation of the reactant state leads to overlap with the potential the nuclear (protein) geometry of the reactant state. This is facilitated by the natthe reactant state leads to overlap with the potential-energy curve for the product state; the point of overlap is the nuclear geometry that is compatible with elecstate; the point of overlap is the nuclear geometry that is compatible with electron tunnelling. At this intersection point, there is an energy barrier through which the electron must tunnel to arrive on the product side. tron tunnelling. At this intersection point, there is an energy barrier through which tron tunnelling. At this intersection point, there is an energy barrier through which
the electron must tunnel to arrive on the product side. The theory for protein-
mediated electron transfer reactions illustrates an impo the electron must tunnel to arrive on the product side. The theory for protein-
mediated electron transfer reactions illustrates an important role for protein dynamics
in driving the tunnelling process. The importance of d mediated electron transfer reactions illustrates an important role for protein dynamics in driving the tunnelling process. The importance of dynamic fluctuations in the protein can be appreciated by considering those react ics in driving the tunnelling process. The importance of dynamic fluctuations in
the protein can be appreciated by considering those reactions that have a non-
zero thermodynamic driving force for the electron transfer rea the protein can be appreciated by considering those reactions that have a non-
zero thermodynamic driving force for the electron transfer reaction. Since tunnelling
is significant only between states of nearly equal energy zero thermodynamic driving force for the electron transfer reaction. Since tunnelling
is significant only between states of nearly equal energy, tunnelling is unlikely in
such instances. However, dynamic fluctuations in th U such instances. However, dynamic fluctuations in the protein overcome this prob-
Olem. These equalize the energy between the reactant and product at the intersuch instances. However, dynamic fluctuations in the protein overcome this problem. These equalize the energy between the reactant and product at the intersection point of the R and P curves (i.e. their configurations are lem. These equalize the energy between the reactant and product at the intersection point of the R and P curves (i.e. their configurations are identical), thus enabling transfer by quantum tunnelling. The term 'vibrationa section point of the R and P curves (i.e. their configurations are identical), thus
enabling transfer by quantum tunnelling. The term 'vibrationally assisted tunnelling'
is, therefore, appropriate for protein electron tran enabling transfer by quantum tunnelling. The term 'vibrationally assisted tunnelling'
is, therefore, appropriate for protein electron transfer reactions. As described below,
our recent work has also demonstrated a similar is, therefore, appropriate for protein electron transfer reactions. As described below,
our recent work has also demonstrated a similar role for dynamic fluctuations of
the protein during enzyme-catalysed H-tunnelling. Ele our recent work has also demonstrated a similar role for dynamic fluctuations of
the protein during enzyme-catalysed H-tunnelling. Electron transfer theory there-
fore provides a useful framework for understanding enzymati the protein during enzyme-catalysed H-tunnelling. Electron transfer theory therefore provides a useful framework for understanding enzymatic H-tunnelling. Despite this, until very recently, tunnelling derivatives of TST (B *Phil. Trans. R. Soc. Lond.* A (2000) **Phil.** Trans. *R. Soc. Lond.* A (2000)

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Figure 4. The static barrier (TST-derived) model of H-tunnelling and definition of tunnelling
regimes (a) H-tunnelling regimes (b) A static barrier indicating transfer to the product side in Figure 4. The static barrier (TST-derived) model of H-tunnelling and definition of tunnelling regimes. (a) H-tunnelling regimes. (b) A static barrier indicating transfer to the product side in each of the regimes shown in regimes. (a) H-tunnelling regimes. (b) A static barrier indicating transfer to the product side in each of the regimes shown in (a). In regimes II and III, thermal activation may be required to populate higher vibrational each of the regimes shown in (a) . In regimes II and III, thermal activation may be required to

populate higher vibrational energy states of the reactive C–H bond.
take into account the fluctuating nature of the enzyme—have been used to account
fully for enzymatic H-tunnelling. As a backdrop to the very recent dynami take into account the fluctuating nature of the enzyme—have been used to account fully for enzymatic H-tunnelling. As a backdrop to the very recent dynamic treat-
ments of H-tunnelling in enzymes, we describe below static take into account the fluctuating nature of the enzyme—have been used to account
fully for enzymatic H-tunnelling. As a backdrop to the very recent dynamic treat-
ments of H-tunnelling in enzymes, we describe below static ments of H-tunnelling in enzymes, we describe below static barrier approaches, i.e. tunnelling correction theories of TST, that have been applied to some enzyme systems.

6. TST and corrections for H-tunnelling

In TST, a static barrier is used to represent the reaction. The temperature dependence of such a reaction can be understood in terms of the Arrhenius equation $(3, 2)$) Deviations from classical behaviour are usefully pro In TST, a static barrier is used to represent the reaction. The temperature depen-In TST, a static barrier is used to represent the reaction. The temperature dependence of such a reaction can be understood in terms of the Arrhenius equation (equation (3.2)). Deviations from classical behaviour are usef dence of such a reaction can be understood in terms of the Arrhenius equation (equation (3.2)). Deviations from classical behaviour are usefully probed via the kinetic isotope effect ($\S 2$). For non-enzymatic reactions, **MATHEMATICAL,
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SCIENCES** (equation (3.2)). Deviations from classical behaviour are usefully probed via the kinetic isotope effect $(\S 2)$. For non-enzymatic reactions, several factors—in addition to inflated KIEs (i.e. KIEs above 7)—have been u kinetic isotope effect (§2). For non-enzymatic reactions, several factors—in addition
to inflated KIEs (i.e. KIEs above 7)—have been used to indicate quantum tun-
nelling of hydrogen. A particularly striking indication of to inflated KIEs (i.e. KIEs above 7)—have been used to indicate quantum tunnelling of hydrogen. A particularly striking indication of quantum tunnelling is the observation of curvature in the Arrhenius plot (i.e. $\ln(\text{rate})$ observation of curvature in the Arrhenius plot (i.e. $\ln(\text{rate})$ versus $1/T$) over an extensive temperature range. Interestingly, this has been observed in non-enzymatic radical reactions (Bromberg *et al.* 1972; Brunton *e* observation of curvature in the Arrhenius plot (i.e. $\ln(\text{rate})$ versus $1/T$) over an extensive temperature range. Interestingly, this has been observed in non-enzymatic extensive temperature range. Interestingly, this has been observed in non-enzymatic
radical reactions (Bromberg *et al.* 1972; Brunton *et al.* 1976; Wang & Williams
1972). However, curvature in Arrhenius plots is not a u radical reactions (Bromberg *et al.* 1972; Brunton *et al.* 1976; Wang & Williams 1972). However, curvature in Arrhenius plots is not a useful indicator of quantum tunnelling because the limited experimental temperature r 1972). However, curvature in Arrhenius plots is not a useful indicator of quantum
tunnelling because the limited experimental temperature range available in studies
using enzymes makes it impossible to detect any curvature tunnelling because the limited experimental temperature range available in studies
using enzymes makes it impossible to detect any curvature. An alternative approach
is to estimate, from the Arrhenius plot, the activation using enzymes makes it impossible to detect any curvature. An alternative approach
is to estimate, from the Arrhenius plot, the activation energy for the reaction and
the pre-exponential factors. Large differences in the is to estimate, from the Arrhenius plot, the activation energy for the reaction and
the pre-exponential factors. Large differences in the activation energies for protium
and deuterium transfer $(\Delta \Delta E_a > 5.4 \text{ kJ mol}^{-1})$ (Bel the pre-exponential factors. Large differences in the activation energies for protium
and deuterium transfer $(\Delta \Delta E_a > 5.4 \text{ kJ mol}^{-1})$ (Bell 1980), and values deviating
from unity for the ratio of Arrhenius pre-exponential and deuterium transfer $(\Delta \Delta E_a > 5.4 \text{ kJ mol}^{-1})$ (Bell 1980), and values deviating
from unity for the ratio of Arrhenius pre-exponential factors $(A^H:A^D \neq 1)$ (More
O'Ferrall 1975; Schneider & Stern 1972), can indicate non from unity for the ratio of Arrhenius pre-exponential factors $(A^H:A^D \neq 1)$ (More O'Ferrall 1975; Schneider & Stern 1972), can indicate non-classical behaviour. In conjunction with inflated KIEs, these parameters have be O'Ferrall 1975; Schneider & Stern 1972), conjunction with inflated KIEs, these para-
quantum tunnelling in enzyme molecules.
Small deviations from classical behaviors conjunction with inflated KIEs, these parameters have been used to demonstrate quantum tunnelling in enzyme molecules.
Small deviations from classical behaviour have been reported for the enzymes

yeast alcohol dehydrogenase (YADH) (Cha *et al*. 1989), bovine serum amine oxidase (BSAO) (Grant & Klinman 1989), monoamine oxidase (MAO) (Jonsson *et al*. 1994) and glucose oxidase (GO) (Kohen *et al*. 1997). More recently, the enzyme

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lipoxygenase has been shown to catalyse H-transfer by a more extreme quantumtunnelling process (Jonsson *et al*. 1996). In this case, the apparent activation energy lipoxygenase has been shown to catalyse H-transfer by a more extreme quantum-
tunnelling process (Jonsson *et al.* 1996). In this case, the apparent activation energy
was found to be much smaller than for reactions cataly tunnelling process (Jonsson *et al.* 1996). In this case, the apparent activation energy was found to be much smaller than for reactions catalysed by YADH, BSAO, MAO or GO, suggesting a correlation between apparent activa or GO, suggesting a correlation between apparent activation energy and the extent of tunnelling. Use of a static (TST-like) barrier in the treatment of H-tunnelling in or GO, suggesting a correlation between apparent activation energy and the extent
of tunnelling. Use of a static (TST-like) barrier in the treatment of H-tunnelling in
enzymes has allowed the construction of (hypothetical of tunnelling. Use of a static (TST-like) barrier in the treatment of H-tunnelling in
enzymes has allowed the construction of (hypothetical) relationships between the
reaction rate and temperature (Jonsson *et al.* 1996). reaction rate and temperature (Jonsson *et al.* 1996). These relationships are readily visualized in the context of a temperature-dependence plot \int (a plot of ln k/T versus $1/T$ and are observed in studies that employ isotope (i.e. H, D and T) substitution within the reactive bond. The plot can be divided into four regimes (figure 4): $1/T$) and are observed in studies that employ isotope (i.e. H, D and T) substitution within the reactive bond. The plot can be divided into four regimes (figure 4): regime I describes classical (TST) behaviour, conforms t tion within the reactive bond. The plot can be divided into four regimes (figure 4):
regime I describes classical (TST) behaviour, conforms to the unimolecular rate law
and is characterized by large values of ΔH^{\ddagger} a and is characterized by large values of ΔH^{\ddagger} and a $A^{\prime H}:A^{\prime D}$ ratio of approximately 1 (the prime is used to distinguish this ratio from the A^H : A^D ratio calculated from the classical Arrhenius plot). Regimes II-IV reveal the effects of quantum tunnelling on the temperature dependence of the reaction rate; the extent of quantum tunnelling increases from regime II to regime IV. In regime II, protium tunnels more extensively than deuterium, thus giving rise to inflated value nelling increases from regime II to regime IV. In regime II, protium tunnels more extensively than deuterium, thus giving rise to inflated values for the KIE, and an $A'^{H}:A'^{D}$ ratio less than 1. Regime III is character nelling increases from regime II to regime IV. In regime II, protium tunnels more
extensively than deuterium, thus giving rise to inflated values for the KIE, and an
 $A'^{\rm H}:A'^{\rm D}$ ratio less than 1. Regime III is charact extensively than deuterium, thus giving rise to inflated values for the KIE, and an $A'^{H}:A'^{D}$ ratio less than 1. Regime III is characterized by extensive tunnelling of both protium and deuterium, and the $A'^{H}:A'^{D}$ r $A'^{\rm H}:A'^{\rm D}$ ratio less than 1. Regime III is characterized by extensive tunnelling of both protium and deuterium, and the $A'^{\rm H}:A'^{\rm D}$ ratios are difficult to predict. Finally, regime IV is the predicted regime for t both protium and deuterium, and the $A'^{H}:A'^{D}$ ratios are difficult to predict. Finally,
regime IV is the predicted regime for transfer solely by ground-state tunnelling. In
this case, the $A'^{H}:A'^{D}$ ratio equals the regime IV is the predicted regime for transfer solely by ground-state tunnelling. In this case, the $A'^{H}:A'^{D}$ ratio equals the KIE and the reaction rate is not dependent on temperature (the reaction passes through, and on temperature (the reaction passes through, and not over, the barrier, thus there is temperature (the reaction passes through, and not over, the barrier, thus there is
temperature-dependent term).
Relationships between reaction rate and temperature can, therefore, be used to
tect non-classical behaviour i

no temperature-dependent term).
Relationships between reaction rate and temperature can, therefore, be used to
detect non-classical behaviour in enzymes. Non-classical values of the $A^{\prime H}:A^{\prime D}$ ratio
 $(A^{\prime H}:A^{\prime D} \neq 1$ Relationships between reaction rate and temperature can, therefore, be used to
detect non-classical behaviour in enzymes. Non-classical values of the $A'^{\text{H}}:A'^{\text{D}}$ ratio
 $(A'^{\text{H}}:A'^{\text{D}} \neq 1)$ and $\Delta \Delta H^{\ddagger}$ (great detect non-classical behaviour in enzymes. Non-classical values of the $A'^{H}:A'^{D}$ ratio $(A'^{H}:A'^{D} \neq 1)$ and $\Delta \Delta H^{\ddagger}$ (greater than 5.4 kJ mol⁻¹, i.e. greater than the difference in zero-point vibrational energi $(A'^{H}:A'^{D} \neq 1)$ and $\Delta \Delta H^{\ddagger}$ (greater than 5.4 kJ mol⁻¹, i.e. greater than the difference in zero-point vibrational energies of the C-H and C-D bonds) have been the criteria used to demonstrate H-tunnelling in th ference in zero-point vibrational energies of the C-H and C-D bonds) have been
the criteria used to demonstrate H-tunnelling in the enzymes mentioned above. A
major prediction from this static-barrier (TST-like) plot is t the criteria used to demonstrate H-tunnelling in the enzymes mentioned above. A major prediction from this static-barrier (TST-like) plot is that tunnelling becomes more prominent as the apparent ΔH^{\ddagger} decreases. Thi major prediction from this static-barrier (TST-like) plot is that tunnelling becomes
more prominent as the apparent ΔH^{\ddagger} decreases. This holds for the enzymes listed
above, but the correlation breaks down for enzyme more prominent as the apparent ΔH^{\ddagger} decreases. This holds for the enzymes listed
above, but the correlation breaks down for enzymes catalysing the breakage of C–H
bonds (Basran *et al.* 1999): a direct result of the above, but the correlation breaks down for enzymes catalysing the breakage of C-H
bonds (Basran *et al.* 1999): a direct result of the type of potential-energy barrier
that is used. Temperature-independent tunnelling is a bonds (Basran *et al.* 1999): a direct result of the type of potential-energy barrier
that is used. Temperature-independent tunnelling is a direct result of invoking a
static (Eyring-like) potential-energy barrier. Howeve that is used. Temperature-independent tunnelling is a direct result of invoking a static (Eyring-like) potential-energy barrier. However, an alternative approach comes from invoking a fluctuating (Kramers-like) potential-e from invoking a fluctuating (Kramers-like) potential-energy barrier (Chandrasekhar from invoking a fluctuating (Kramers-like) potential-energy barrier (Chandrasekhar
1943; Kramers 1940). This is conceptually more realistic as it takes into account
the dynamic motion of the protein. These dynamic effects 1943; Kramers 1940). This is conceptually more realistic as it takes into account
the dynamic motion of the protein. These dynamic effects will give rise to more
complex temperature dependencies for rates of H-transfer th complex temperature dependencies for rates of H-transfer than those illustrated in \mathbf{H} figure 4. The role of protein dynamics in driving enzymatic H-tunnelling is discussed THE
SOCI below.

^y Previous studies of H-tunnelling in enzymes have been analysed in terms of the phenomenological [†] Previous studies of H-tunnelling in enzymes have been analysed in terms of the phenomenological Arrhenius plot (e.g. Jonsson *et al.* (1996), i.e. a plot of $\ln(k)$ versus $1/T$). Although the Arrhenius plot approaches † Previous studies of H-tunnelling in enzymes have been analysed in terms of the phenomenological
Arrhenius plot (e.g. Jonsson *et al.* (1996), i.e. a plot of $\ln(k)$ versus $1/T$). Although the Arrhenius plot
appears linea Arrhenius plot (e.g. Jonsson *et al.* (1996), i.e. a plot of $\ln(k)$ versus $1/T$). Although the Arrhenius plot appears linear in the accessible temperature range, it is in fact curved and asymptotically approaches infinity appears linear in the accessible temperature range, it is in fact curved and asymptotically approaches
infinity at high temperatures. Apparent linearity in the accessible temperature range does not compro-
mise data analys infinity at high temperatures. Apparent linearity in the accessible temperature range does not co
mise data analysis in this regime, but temperature-dependence studies should strictly be analy
terms of the equation describ terms of the equation describing a unimolecular reaction, which can be conveniently written as:

$$
\ln(k/T) = \ln(k_{\rm B}/h) + \Delta S^{\ddagger}/R - \Delta H^{\ddagger}/RT.
$$

The activation parameter ΔH^{\ddagger} is calculated from the slope of the plot.

Phil. Trans. R. Soc. Lond. A (2000)

nuclear configuration

nuclear configuration
Figure 5. The dynamic barrier model of H-tunnelling. Reactant (R) and product (P) energy
curves for distortion of the protein scaffold. The intersection point (X) is the optimum geometry Figure 5. The dynamic barrier model of H-tunnelling. Reactant (R) and product (P) energy curves for distortion of the protein scaffold. The intersection point (X) is the optimum geometry required for H-transfer. At the Figure 5. The dynamic barrier model of H-tunnelling. Reactant (R) and product (P) energy
curves for distortion of the protein scaffold. The intersection point (X) is the optimum geometry
required for H-transfer. At the curves for distortion of the protein scaffold. The intersection point (X) is the optimum geometry
required for H-transfer. At the intersection point, transfer can be by the classical (I), ground-state
tunnelling (IV) or in tunnelling (IV) or intermediate regimes (II) and (III). In regimes II and III, additional thermal activation (other than that required to distort the protein scaffold to the optimum geometry tunnelling (IV) or intermediate regimes (II) and (III). In regimes II and III, additional thermal
activation (other than that required to distort the protein scaffold to the optimum geometry
for transfer, i.e. the R-P int activation (other than that required to distort the protein scaffold to the optimum geometry
for transfer, i.e. the R-P intersection) may reflect (i) partition into higher vibrational levels of
the reactive C-H bond; and/o the reactive C–H bond; and/or (ii) transfer via a combination of classical (over-the-barrier) and quantum mechanical routes.

7. H-tunnelling driven by protein dynamics

7. H-tunnelling driven by protein dynamics
Vibrational enhancement (i.e. thermal activation) in non-enzymatic H-tunnelling
reactions has also been considered but these thermal fluctuations are usually re-Vibrational enhancement (i.e. thermal activation) in non-enzymatic H-tunnelling
reactions has also been considered, but these thermal fluctuations are usually re-
stricted to the reacting species (Borgis & Hynes 1991–1996 Vibrational enhancement (i.e. thermal activation) in non-enzymatic H-tunnelling
reactions has also been considered, but these thermal fluctuations are usually re-
stricted to the reacting species (Borgis & Hynes 1991, 1996 reactions has also been considered, but these thermal fluctuations are usually restricted to the reacting species (Borgis & Hynes 1991, 1996; Suarez & Silbey 1991).
In recent years, attempts have been made to model, theor stricted to the reacting species (Borgis & Hynes 1991, 1996; Suarez & Silbey 1991).
In recent years, attempts have been made to model, theoretically, enzymatic H-
tunnelling by incorporating thermal vibrations. However, a In recent years, attempts have been made to model, theoretically, enzymatic H-
tunnelling by incorporating thermal vibrations. However, and importantly, none of
these approaches have been verified experimentally. Recently, tunnelling by incorporating thermal vibrations. However, and importantly, none of these approaches have been verified experimentally. Recently, the kinetic data for BSAO have been re-evaluated for thermally activated subs these approaches have been verified experimentally. Recently, the kinetic data for **BSAO** have been re-evaluated for thermally activated substrate vibrations, but with
the protein molecule treated as rigid (Antoniou & Schwartz 1997). Computational
molecular-dynamics simulation studies have also suggeste the protein molecule treated as rigid (Antoniou & Schwartz 1997). Computational
molecular-dynamics simulation studies have also suggested a dynamic role for the
protein molecule in enzymatic H-tunnelling (Bala *et al.* 199 molecular-dynamics simulation studies have also suggested a dynamic role for the protein molecule in enzymatic H-tunnelling (Bala *et al.* 1996; Hwang *et al.* 1991; Hwang & Warshel 1996). Indeed, some theoretical treatme protein molecule in enzymatic H-tunnelling (Bala *et al.* 1996; Hwang *et al.* 1991; Hwang & Warshel 1996). Indeed, some theoretical treatments have recognized the role of thermal motion in the protein in H-tunnelling (Dog Hwang & Warshel 1996). Indeed, some theoretical treatments have recognized the role of thermal motion in the protein in H-tunnelling (Dogonadze *et al.* 1977; Sumi & Ulstrop 1988), but have failed to predict the experimen role of thermal motion in the protein in H-tunnelling $\&$ Ulstrop 1988), but have failed to predict the experimental verification of these theories is lacking.
The only (to the best of our knowledge) theoretical to Ulstrop 1988), but have failed to predict the experimentally observed KIE; again,
perimental verification of these theories is lacking.
The only (to the best of our knowledge) theoretical treatment of H-transfer by tun-
ll

experimental verification of these theories is lacking.
The only (to the best of our knowledge) theoretical treatment of H-transfer by tun-
nelling to explicitly recognize the role of protein dynamics, and relate this in The only (to the best of our knowledge) theoretical treatment of H-transfer by tunnelling to explicitly recognize the role of protein dynamics, and relate this in turn to the observed KIE, was described by Bruno $\&$ Bial nelling to explicitly recognize the role of protein dynamics, and relate this in turn to
the observed KIE, was described by Bruno & Bialek (1992). This approach has been
termed vibrationally enhanced ground-state tunnellin

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key feature of this theory—and one that sets it apart from many other theoretical key feature of this theory—and one that sets it apart from many other theoretical
approaches—is that tunnelling occurs from the ground-state vibrational energy levels
of the substrate, i.e. there is no thermal activation o key feature of this theory—and one that sets it apart from many other theoretical
approaches—is that tunnelling occurs from the ground-state vibrational energy levels
of the substrate, i.e. there is no thermal activation o approaches—is that tunnelling occurs from the ground-state vibrational energy levels
of the substrate, i.e. there is no thermal activation of the substrate. The temperature
dependence of the reaction is, therefore, attribu of the substrate, i.e. there is no thermal activation of the substrate. The temperature dependence of the reaction is, therefore, attributed to the natural thermally induced breathing of the enzyme molecule, thus shortenin

dependence of the reaction is, therefore, attributed to the natural thermally induced
breathing of the enzyme molecule, thus shortening the distance the hydrogen must
tunnel. Thus, the natural breathing of the enzyme molec breathing of the enzyme molecule, thus shortening the distance the hydrogen must
tunnel. Thus, the natural breathing of the enzyme molecule can be visualized in the
context of the familiar R and P potential-energy curve d tunnel. Thus, the natural breathing of the enzyme molecule can be visualized in the context of the familiar R and P potential-energy curve depiction encountered in discussions of electron transfer in proteins ($\S 5$). H-t context of the familiar R and P potential-energy curve depiction encountered in discussions of electron transfer in proteins $(\S 5)$. H-tunnelling does not occur until the geometry of the protein is distorted so that the geometry of the protein is distorted so that the R and P curves intersect (figure 5).
At the intersection point (X) of the two curves, the nucleus tunnels; the average geometry of the protein is distorted so that the R and P curves intersect (figure 5).
At the intersection point (X) of the two curves, the nucleus tunnels; the average
tunnelling probability is decreased when heavier iso At the intersection point (X) of the two curves, the nucleus tunnels; the average
tunnelling probability is decreased when heavier isotopes (e.g. deuterium) are trans-
ferred, thus giving rise to a KIE greater than 1. At tunnelling probability is decreased when heavier isotopes (e.g. deuterium) are transferred, thus giving rise to a KIE greater than 1. At the intersection point, tunnelling is from the vibrational ground states, since vibr ferred, thus giving rise to a KIE greater than 1. At the intersection point, tunnelling is from the vibrational ground states, since vibrational quanta are comparable to barrier height (Bruno & Bialek 1992), and, therefor is from the vibrational ground states, since v
barrier height (Bruno & Bialek 1992), and, tl
lead to a classical 'over-the-barrier' transfer.
Clearly protein dynamics is hypothesized rrier height (Bruno & Bialek 1992), and, therefore, vibrational excitation would
ad to a classical 'over-the-barrier' transfer.
Clearly, protein dynamics is hypothesized to play a major role in driving H-
nnelling in enzym

lead to a classical 'over-the-barrier' transfer.
Clearly, protein dynamics is hypothesized to play a major role in driving H-
tunnelling in enzymes. However, like all hypotheses, this requires experimental ver-
ification. Clearly, protein dynamics is hypothesized to play a major role in driving H-
tunnelling in enzymes. However, like all hypotheses, this requires experimental ver-
ification. The activation energy of the reaction is associat tunnelling in enzymes. However, like all hypotheses, this requires experimental ver-
ification. The activation energy of the reaction is associated with distortion of the
protein molecule. Following the tunnelling event, ification. The activation energy of the reaction is associated with distortion of the protein molecule. Following the tunnelling event, rapid movement away from the intersection point along the P curve prevents coherent o ō protein molecule. Following the tunnelling event, rapid movement away from the
intersection point along the P curve prevents coherent oscillations of the H nucleus
between the R and P curves. As such, the reaction is model intersection point along the P curve prevents coherent oscillations of the H nucleus
between the R and P curves. As such, the reaction is modelled in much the same way
as electron transfer in proteins (i.e. Fermi's golden between the R and P curves. As such, the reaction is modelled in much the same way as electron transfer in proteins (i.e. Fermi's golden rule applies and the non-adiabatic
regime operates). A key prediction of this theory is that H-tunnelling can occur even
when the value of the KIE is less than 7, thus regime operates). A key prediction of this theory is that H-tunnelling can occur even
when the value of the KIE is less than 7, thus suggesting that (contrary to current
dogma) KIEs may be poor indicators of quantum tunnel when the value of the KIE is less than 7, thus suggesting that (contrary to current dogma) KIEs may be poor indicators of quantum tunnelling in enzymes. This is an important point, since static barrier models of H-tunnelli dogma) KIEs may be poor indicators of quantum tunnelling in enzymes. This is an
important point, since static barrier models of H-tunnelling suggest that H-tunnelling
does not occur when the KIE is less than 7. This indica important point, since static barrier models of H-tunnelling suggest that H-tunnelling
does not occur when the KIE is less than 7. This indicates that detailed temperature-
dependence studies are required to demonstrate, u does not occur when the KIE is less than 7.
dependence studies are required to demon
feature of an enzyme-catalysed reaction.
The fluctuating enzyme model of H-tur pendence studies are required to demonstrate, unequivocally, that tunnelling is a
ature of an enzyme-catalysed reaction.
The fluctuating enzyme model of H-tunnelling can be divided into two reaction
monents: (i) a thermall

feature of an enzyme-catalysed reaction.
The fluctuating enzyme model of H-tunnelling can be divided into two reaction
components: (i) a thermally activated nuclear reorganization step, and (ii) the H-
tunnelling event at The fluctuating enzyme model of H-tunnelling can be divided into two reaction
components: (i) a thermally activated nuclear reorganization step, and (ii) the H-
tunnelling event at the intersection point of the potential-e components: (i) a thermally activated nuclear reorganization step, and (ii) the H-
tunnelling event at the intersection point of the potential-energy curves. This leads to
three possible rate-limiting regimes in which eit

tunnelling event at the intersection point of the potential-energy curves. This leads to
three possible rate-limiting regimes in which either (i) nuclear reorganization is rate-
limiting, (ii) quantum tunnelling is rate-li three possible rate-limiting regimes in which either (i) nuclear reorganization is rate-
limiting, (ii) quantum tunnelling is rate-limiting, or (iii) both factors contribute to the
observed rate. The value of the KIE is af limiting, (ii) quantum tunnelling is rate-limiting, or (iii) both factors contribute to the observed rate. The value of the KIE is affected directly by these steps. When nuclear reorganization is rate limiting, the KIE is Sobserved rate. The value of the KIE is affected directly by these steps. When nuclear reorganization is rate limiting, the KIE is unity (since this is independent of isotope) and reaction rates are dependent on solvent v protein structure can reorganize). In the quantum-tunnelling limiting regime, the and reaction rates are dependent on solvent viscosity (i.e. the ease with which the
protein structure can reorganize). In the quantum-tunnelling limiting regime, the
KIE is not dependent on solvent viscosity and is not uni protein structure can reorganize). In the quantum-tunnelling limiting regime, the KIE is not dependent on solvent viscosity and is not unity (since tunnelling rate is a function of isotope). However, when both nuclear reor KIE is not dependent on solvent viscosity and is not unity (since tunnelling rate
is a function of isotope). However, when both nuclear reorganization and quantum
tunnelling contribute to the observed rate, the KIE is visc is a function of isotope). However, when both nuclear reorganization and quantum
tunnelling contribute to the observed rate, the KIE is viscosity dependent, as viscosity
increases the nuclear reorganization step becomes ra tunnelling contribute to the observed rate, the KIE is viscosity dependent, as viscosity
increases the nuclear reorganization step becomes rate limiting, and, thus, the KIE
tends to unity. In experimental studies, measurem increases the nuclear reorganization step becomes rate limiting, and, thus, the KIE tends to unity. In experimental studies, measurements of (i) increased viscosity or (ii) decreased temperature effects on the KIE may be u tends to unity. In experimental studies, measurements of (i) increased viscosity or (ii) decreased temperature effects on the KIE may be used to discriminate between these possible regimes, since both would be expected to $\frac{1}{0}$ distortion of the protein. ssible regimes, since both would be expected to selectively perturb geometrical
stortion of the protein.
VEGST assumes that H-transfer occurs entirely by quantum mechanical tun-
lling. The model is therefore, appropriate f

distortion of the protein.
VEGST assumes that H-transfer occurs entirely by quantum mechanical tunnelling. The model is, therefore, appropriate for those enzymes catalysing groundnelling. The model is, therefore, appropriate for those enzymes catalysing ground-
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380 $M. J. \textit{Sutcliffe}$ and N. S. Scrutton
state tunnelling (see below). The model is likely to be incomplete for those enzymes state tunnelling (see below). The model is likely to be incomplete for those enzymes
in which tunnelling occurs just below the saddle point of the energy surface (i.e.
the reactant passes up the energy barrier before tunne state tunnelling (see below). The model is likely to be incomplete for those enzymes
in which tunnelling occurs just below the saddle point of the energy surface (i.e.
the reactant passes up the energy barrier before tunne in which tunnelling occurs just below the saddle point of the energy surface (i.e.
the reactant passes up the energy barrier before tunnelling); in these situations, H-
transfer is likely to occur by a combination of clas the reactant passes up the energy barrier before tunnelling); in these situations, H-
transfer is likely to occur by a combination of classical and quantum mechanical
behaviour (Garrett & Truhlar 1980; Truhlar & Gordon 19 H-transfer is by a combination of classical and quantum mechanical effects, the actibehaviour (Garrett & Truhlar 1980; Truhlar & Gordon 1990). In the case where H-transfer is by a combination of classical and quantum mechanical effects, the activation energy will reflect partitioning of energy into a wid H-transfer is by a combination of classical and quantum mechanical effects, the activation energy will reflect partitioning of energy into a wide range of modes within the protein, e.g. changes in protein geometry, bond an protein, e.g. changes in protein geometry, bond angles of reacting substrate, etc., as
well as thermal excitation of the reactive C–H bond. However, experimental verifica-
tion of VEGST would demonstrate the importance of protein, e.g. changes in protein geometry, bond angles of reacting substrate, etc., as
well as thermal excitation of the reactive C–H bond. However, experimental verifica-
tion of VEGST would demonstrate the importance of well as thermal excitation of the reactive C–H bond. However, experimental verifica-
tion of VEGST would demonstrate the importance of protein dynamics in enzymatic
H-tunnelling. By analogy, therefore, protein dynamics wou tion of VEGST would demonstrate the importance of protein dynamics in enzymatic H-tunnelling. By analogy, therefore, protein dynamics would also be expected to play a major role in those enzymes where H-tunnelling is not f H-tunnelling. By analogy, therefore, protein dynamics would also be expected to play
a major role in those enzymes where H-tunnelling is not from the ground state, but
from an excited state of the substrate molecule. Exper a major role in those enzymes where H-tunnelling is not from the ground state, but
from an excited state of the substrate molecule. Experimental verification of a role
for protein dynamics is, thus, a key milestone in deve for protein dynamics is, thus, a key milestone in developing theories for enzymatic

8. Experimental demonstration of VEGST

8. Experimental demonstration of VEGST
Kinetic data for BSAO were originally analysed in terms of the tunnelling correction
derivatives of TST (Grant & Klinman 1989), but the data are also consistent with— Kinetic data for BSAO were originally analysed in terms of the tunnelling correction
derivatives of TST (Grant & Klinman 1989), but the data are also consistent with—
although not verification of—VEGST theory (Bruno & Bia Kinetic data for BSAO were originally analysed in terms of the tunnelling correction
derivatives of TST (Grant & Klinman 1989), but the data are also consistent with—
although not verification of—VEGST theory (Bruno & Bial derivatives of TST (Grant & Klinman 1989), but the data are also consistent with—although not verification of —VEGST theory (Bruno & Bialek 1992). Alternatively, the BSAO data can also be interpreted in terms of an H-tunnelling reaction driven
by substrate oscillations (Antoniou $\&$ Schwartz 1997). Thus, ambiguity remains con-
cerning the correct theoretical treatment of the BSAO the BSAO data can also be interpreted in terms of an H-tunnelling reaction driven
by substrate oscillations (Antoniou & Schwartz 1997). Thus, ambiguity remains con-
cerning the correct theoretical treatment of the BSAO kin by substrate oscillations (Antoniou & Schwartz 1997). Thus, ambiguity remains concerning the correct theoretical treatment of the BSAO kinetic data. This ambiguity arises because the complex temperature dependence of the r arises because the complex temperature dependence of the reaction can be modelled in a variety of ways. Our recent studies on enzymatic C-H bond cleavage have, arises because the complex temperature dependence of the reaction can be modelled in a variety of ways. Our recent studies on enzymatic C–H bond cleavage have, however, provided verification of VEGST theory, and also, for elled in a variety of ways. Our recent studies on enzymatic C–H bond cleavage have,
however, provided verification of VEGST theory, and also, for the first time, proved
the existence of a ground-state H- and D-tunnelling r however, provided veri
the existence of a grou
(Basran *et al.* 1999).
Our KIE and temper the existence of a ground-state H- and D-tunnelling regime in an enzyme molecule (Basran *et al.* 1999).
Our KIE and temperature-dependent studies of the reaction catalysed by the bac-

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SCIENCES** (Basran *et al.* 1999).

Our KIE and temperature-dependent studies of the reaction catalysed by the bac-

terial enzyme methylamine dehydrogenase (MADH) have revealed that the rate of

reduction of the enzyme redox centre Our KIE and temperature-dependent studies of the reaction catalysed by the bac-
terial enzyme methylamine dehydrogenase (MADH) have revealed that the rate of
reduction of the enzyme redox centre (tryptophan tryptophylquin terial enzyme methylamine dehydrogenase (MADH) have revealed that the rate of
reduction of the enzyme redox centre (tryptophan tryptophylquinone (TTQ)) by sub-
strate has a large, temperature-independent KIE (Basran *et al* reduction of the enzyme redox centre (tryptophan tryptophylquinone (TTQ)) by substrate has a large, temperature-independent KIE (Basran *et al.* 1999). Reduction of this redox centre is a convenient way of following C–H b strate has a large, temperature-independent KIE (Basran *et al.* 1999). Reduction of
this redox centre is a convenient way of following C–H bond breakage in this enzyme,
since breakage of the bond and reduction of the cof this redox centre is a convenient way of following C-H bond breakage in this enzyme,
since breakage of the bond and reduction of the cofactor occur simultaneously. A plot
of $\ln k/T$ versus $1/T$ reveals that ground-state qu since breakage of the bond and reduction of the cofactor occur simultaneously. A plot
of $\ln k/T$ versus $1/T$ reveals that ground-state quantum tunnelling is responsible for
the transfer of the hydrogen nucleus. This is ind of $\ln k/T$ versus $1/T$ reveals that ground-state quantum tunnelling is responsible for
the transfer of the hydrogen nucleus. This is indicated by the linear and parallel
nature of the plots for C-H and C-D bond breakage, w the transfer of the hydrogen nucleus. This is indicated by the linear and parallel
nature of the plots for C–H and C–D bond breakage, which should be compared
with regime IV of the corresponding hypothetical plot for a sta nature of the plots for C–H and C–D bond breakage, which should be compared
with regime IV of the corresponding hypothetical plot for a static potential-energy
barrier (figure 6). However, contrary to the static potentialwith regime IV of the corresponding hypothetical plot for a static potential-energy
barrier (figure 6). However, contrary to the static potential-energy barrier model
for H-tunnelling, reaction rates are strongly dependent barrier (figure 6). However, contrary to the static potential-energy barrier model
for H-tunnelling, reaction rates are strongly dependent on temperature (apparent
activation $\Delta H^{\ddagger} \sim 45 \text{ kJ mol}^{-1}$) and, importantly, for H-tunnelling, reaction rates are strongly dependent on temperature (apparent activation $\Delta H^{\ddagger} \sim 45 \text{ kJ mol}^{-1}$) and, importantly, ΔH^{\ddagger} was found to be independent of isotope. These observations indicate that $\overline{\mathbf{S}}$ dent of isotope. These observations indicate that thermal distortion of the protein scaffold—but not vibrational excitation of the substrate—are required to drive Hdent of isotope. These observations indicate that thermal distortion of the protein scaffold—but not vibrational excitation of the substrate—are required to drive H-
transfer. Thus, a fluctuating energy surface is a featur scaffold—but not vibrational excitation of the substrate—are required to drive H-
transfer. Thus, a fluctuating energy surface is a feature of the tunnelling process.
The VEGST equivalent of regime IV of the static barrier transfer. Thus, a fluctuating energy surface is a feature of the tunnelling process.
The VEGST equivalent of regime IV of the static barrier plot (figure 6) recognizes
that thermal motions of the protein molecule are requi The VEGST equivalent of regime IV of the static barrier plot (figure 6) recognizes that thermal motions of the protein molecule are required to distort the protein scaffold into conformations compatible with H-tunnelling. ă

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Figure 6. Expected temperature dependence (in the accessible temperature range) in regime IV Figure 6. Expected temperature dependence (in the accessible temperature range) in regime IV
in the context of figure 5. Ground-state tunnelling occurs in regime IV. The experimental data
for MADH are apparently linear in Figure 6. Expected temperature dependence (in the accessible temperature range) in regime IV
in the context of figure 5. Ground-state tunnelling occurs in regime IV. The experimental data
for MADH are apparently linear in for MADH are apparently linear in regime IV, but, as noted in the text, this linearity will
probably not extend to cryogenic temperatures.

plot, therefore, has a non-zero value for the slope, the value of which is the energy plot, therefore, has a non-zero value for the slope, the value of which is the energy
required to distort the protein into the geometry compatible with H-tunnelling. With
MADH, it has thus been possible to quantify the ene plot, therefore, has a non-zero value for the slope, the value of which is the energy
required to distort the protein into the geometry compatible with H-tunnelling. With
MADH, it has thus been possible to quantify the ene required to distort the protein into the geometry compatible with H-tu
MADH, it has thus been possible to quantify the energy term associat
tural distortion of the protein during an enzyme-catalysed reaction.
The temperatu ADH, it has thus been possible to quantify the energy term associated with struc-
ral distortion of the protein during an enzyme-catalysed reaction.
The temperature dependence in regime IV (ground-state tunnelling) for VEG

tural distortion of the protein during an enzyme-catalysed reaction.
The temperature dependence in regime IV (ground-state tunnelling) for VEGST
contrasts markedly with that for the static barrier model. Although there is The temperature dependence in regime IV (ground-state tunnelling)
contrasts markedly with that for the static barrier model. Although there
energy term in this regime for the VEGST model $(\Delta H^{\ddagger} \sim 45 \text{ kJ mol}^{-1})$, t
linea g) for VEGST
ere is a sizeable
), the apparent
pably does not contrasts markedly with that for the static barrier model. Although there is a sizeable
energy term in this regime for the VEGST model $(\Delta H^{\ddagger} \sim 45 \text{ kJ mol}^{-1})$, the apparent
linearity seen in the accessible temperature r energy term in this regime for the VEGST model $(\Delta H^{\ddagger} \sim 45 \text{ kJ mol}^{-1})$, the apparent
linearity seen in the accessible temperature range for MADH probably does not
extend to lower temperatures. At low temperatures, nucle extend to lower temperatures. At low temperatures, nuclear vibrations will be frozen, extend to lower temperatures. At low temperatures, nuclear vibrations will be frozen,
thus preventing distortion of the nuclear scaffold into geometries compatible with
hydrogen tunnelling. Thus, over a large temperature r thus preventing distortion of the nuclear scaffold
hydrogen tunnelling. Thus, over a large tempera
dependencies of the reaction rate are predicted.
Ground-state tunnelling driven by protein dyna hydrogen tunnelling. Thus, over a large temperature range, complex temperature
dependencies of the reaction rate are predicted.
Ground-state tunnelling driven by protein dynamics (VEGST) is the only theoret-

dependencies of the reaction rate are predicted.
Ground-state tunnelling driven by protein dynamics (VEGST) is the only theoret-
ical treatment consistent with our work on MADH. As indicated above, a prediction
of VEGST is Ground-state tunnelling driven by protein dynamics (VEGST) is the only theoret-
ical treatment consistent with our work on MADH. As indicated above, a prediction
of VEGST is that ground-state tunnelling may occur even when ical treatment consistent with our work on MADH. As indicated above, a prediction
of VEGST is that ground-state tunnelling may occur even when the KIE is less than
7; a regime interpreted previously as indicating classical of VEGST is that ground-state tunnelling may occur even when the KIE is less than
7; a regime interpreted previously as indicating classical behaviour. The KIE with
MADH is large (approximately 18), and thus the presence o 7; a regime interpreted previously as indicating classical behaviour. The KIE with MADH is large (approximately 18), and thus the presence of tunnelling is predicted by current dogma. However, our recent analysis of H-tunn MADH is large (approximately 18), and thus the presence of tunnelling is predicted by current dogma. However, our recent analysis of H-tunnelling in trimethylamine λ dehydrogenase (TMADH) has indicated that, under cert to current dogma), ground-state tunnelling occurs even when the KIE is less than 7 dehydrogenase (TMADH) has indicated that, under certain conditions (and contrary
to current dogma), ground-state tunnelling occurs even when the KIE is less than 7
(Basran, Sutcliffe & Scrutton, unpublished results). This to current dogma), ground-state tunnelling occurs even when the (Basran, Sutcliffe & Scrutton, unpublished results). This observat
to the validity of VEGST in describing enzymatic H-tunnelling. to the validity of VEGST in describing enzymatic H-tunnelling.
9. Significance of H-tunnelling in enzymes

9. Significance of H-tunnelling in enzymes
Both MADH and TMADH catalyse the breakage of stable C–H bonds. These are dif-
ficult reactions if viewed in terms of the classical TST approach to catalysis, but the cult reactions if viewed in terms of the classical TST approach to catalysis, but the ficult reactions if viewed in terms of the classical TST approach to catalysis, but the *Phil. Trans. R. Soc. Lond.* A (2000) *Phil. Trans. R. Soc. Lond.* A (2000)

382 *M. J. Sutcliffe and N. S. Scrutton*
structural plasticity of MADH and TMADH (in common with other enzymes) prostructural plasticity of MADH and TMADH (in common with other enzymes) provides a means of circumventing this problem by facilitating ground-state tunnelling.
Vibration-driven ground-state tunnelling may, therefore, be a c structural plasticity of MADH and TMADH (in common with other enzymes) provides a means of circumventing this problem by facilitating ground-state tunnelling.
Vibration-driven ground-state tunnelling may, therefore, be a c vides a means of circumventing this problem by facilitating ground-state tunnelling.
Vibration-driven ground-state tunnelling may, therefore, be a common mechanism
for the breakage of C–H bonds by enzymes, and this may ext Vibration-driven ground-state tunnelling may, therefore, be a common mechanism
for the breakage of C–H bonds by enzymes, and this may extend to other types of
H-transfer reactions.
The dynamic barrier approach to catalysis for the breakage of C-H bonds by enzymes, and this may extend to other types of

H-transfer reactions.
The dynamic barrier approach to catalysis has major implications for how H-
transfer reactions—and, indeed, other reactions—are modelled theoretically. Given
the dynamic nature of protein molecules, i The dynamic barrier approach to catalysis has major implications for how H-
transfer reactions—and, indeed, other reactions—are modelled theoretically. Given
the dynamic nature of protein molecules, it is perhaps surprisin transfer reactions—and, indeed, other reactions—are modelled theoretically. Given
the dynamic nature of protein molecules, it is perhaps surprising that the indiscrim-
inate use of TST has persisted for so long. For classi the dynamic nature of protein molecules, it is perhaps surprising that the indiscriminate use of TST has persisted for so long. For classical transfers, Kramers's theory seems appropriate, and this is an excellent platform seems appropriate, and this is an excellent platform from which to develop theories
of quantum tunnelling in enzymes. For those reactions that proceed by quantum
tunnelling, it is the energy barrier width that is important seems appropriate, and this is an excellent platform from which to develop theories
of quantum tunnelling in enzymes. For those reactions that proceed by quantum
tunnelling, it is the energy barrier width that is important of quantum tunnelling in enzymes. For those reactions that proceed by quantum
tunnelling, it is the energy barrier width that is important in determining reaction
rate. Tunnelling probability depends on the mass of the tra tunnelling, it is the energy barrier width that is important in determining reaction
rate. Tunnelling probability depends on the mass of the transferred particle, the net
driving force and the height and width of the reac rate. Tunnelling probability depends on the mass of the transferred particle, the net
driving force and the height and width of the reaction barrier. Proteins can facilitate
this by (i) reduction of mass (e.g. exclusion o driving force and the height and width of the reaction barrier. Proteins can facilitate
this by (i) reduction of mass (e.g. exclusion of water), (ii) an equalization of energy
states for reactants and products (Albery & Kn this by (i) reduction of mass (e.g. exclusion of water), (ii) an equalization of energy states for reactants and products (Albery & Knowles 1976; Nambiar *et al*. 1983), and, most importantly, (iii) a reduction in barrier states for reactants and products (Albery & Knowles 1976; Nambiar *et al.* 1983), and, literature. The exploitation of protein dynamics to equalize energy states and shorten tunnelling distance is, however, less well appreciated, but, nevertheless, pivotal.

10. Enzymology into the new millennium

An in-depth understanding of biological catalysis is central to the successful exploita-An in-depth understanding of biological catalysis is central to the successful exploita-
tion of enzymes by mankind. At the end of the last century, the 'lock-and-key' mech-
anism propounded by Emil Fischer—in which the en An in-depth understanding of biological catalysis is central to the successful exploita-
tion of enzymes by mankind. At the end of the last century, the 'lock-and-key' mech-
anism propounded by Emil Fischer—in which the en tion of enzymes by mankind. At the end of the last century, the 'lock-and-key' mechanism propounded by Emil Fischer—in which the enzyme accommodates a specific substrate like a lock does a key—opened the door to our unders anism propounded by Emil Fischer—in which the enzyme accommodates a specific
substrate like a lock does a key—opened the door to our understanding of enzyme
catalysis. This has evolved to take account of protein motion in substrate like a lock does a key—opened the door to our understanding of enzyme catalysis. This has evolved to take account of protein motion in the 'induced-fit' model of catalysis (Bennet & Syeitz 1978; Koshland 1973), i catalysis. This has evolved to take account of protein motion in the 'induced-fit'
model of catalysis (Bennet & Syeitz 1978; Koshland 1973), in which the enzyme
has one conformation in the absence, and another conformation model of catalysis (Bennet & Syeitz 1978; Koshland 1973), in which the enzyme
has one conformation in the absence, and another conformation in the presence,
of substrate. The induced-fit model of catalysis recognizes pref has one conformation in the absence, and another conformation in the presence,
of substrate. The induced-fit model of catalysis recognizes preferred complemen-
tarity to the transition state and has provided a conceptual f of substrate. The induced-fit model of catalysis recognizes preferred complementarity to the transition state and has provided a conceptual framework for TST (Kraut 1988). Now, as we move into the new millennium, our under tarity to the transition state and has provided a conceptual framework for TST (Kraut 1988). Now, as we move into the new millennium, our understanding has progressed yet further by highlighting the role of (i) protein dyn (Kraut 1988). Now, as we move into the new millennium, our understanding has
progressed yet further by highlighting the role of (i) protein dynamics and (ii) quan-
tum tunnelling in enzyme catalysis. Thus, the rules underp progressed yet further by highlighting the role of (i) protein dynamics and (ii) quan-
tum tunnelling in enzyme catalysis. Thus, the rules underpinning our design and
understanding of enzymes have changed significantly. Im tum tunnelling in enzyme catalysis. Thus, the rules underpinning our design and
understanding of enzymes have changed significantly. Important areas in which these
rules apply include enzyme redesign, the production of cat understanding of enzymes have changed significantly. Important areas in which these
rules apply include enzyme redesign, the production of catalytic antibodies, design
of enzyme inhibitors (drugs and pesticides), enzymati rules apply include enzyme redesign, the production of catalytic antibodies, design
of enzyme inhibitors (drugs and pesticides), enzymatic fine chemical synthesis, and
use of enzymes in bulk processing (e.g. paper manufact gents). use of enzymes in bulk processing (e.g. paper manufacture, food industry and deter-
gents).
Enzyme redesign strategies currently attempt to reduce the activation energy (i.e.

gents).
Enzyme redesign strategies currently attempt to reduce the activation energy (i.e.
the barrier height) by seeking maximum complementarity with the transition state
and destabilization of the ground state. This is t Enzyme redesign strategies currently attempt to reduce the activation energy (i.e.
the barrier height) by seeking maximum complementarity with the transition state
and destabilization of the ground state. This is the appro the barrier height) by seeking maximum complementarity with the transition state
and destabilization of the ground state. This is the approach adopted in producing
catalytic antibodies. Here, an animal's immune system is e and destabilization of the ground state. This is the approach adopted in producing catalytic antibodies. Here, an animal's immune system is exposed to a transition state analogue, thus inducing antibodies with surface comp catalytic antibodies. Here, an animal's immune system is exposed to a transition state analogue, thus inducing antibodies with surface complementarity to the transition state. Although, in principle, this is an elegant app state analogue, thus inducing antibodies with surface complementarity to the transition state. Although, in principle, this is an elegant approach to producing novel catalysts, in practice it is usual for catalytic antibod sition state. Although, in principle, this is an elegant approach to producing novel catalysts, in practice it is usual for catalytic antibodies to have poor catalytic rates. These studies imply that knowledge of the trans These studies imply that knowledge of the transition state alone is not sufficient to *Phil. Trans. R. Soc. Lond.* A (2000)

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develop a good catalyst. Insight into additional factors required for efficient catalysis develop a good catalyst. Insight into additional factors required for efficient catalysis
has come from recent work. An important determinant of catalytic efficiency is the
role of protein dynamics. The structural plastici develop a good catalyst. Insight into additional factors required for efficient catalysis
has come from recent work. An important determinant of catalytic efficiency is the
role of protein dynamics. The structural plastici has come from recent work. An important determinant of catalytic efficiency is the role of protein dynamics. The structural plasticity of protein molecules is important in driving both classical and quantum mechanical tran role of protein dynamics. The structural plasticity of protein molecules is important
in driving both classical and quantum mechanical transfers. As we have seen, in
quantum mechanical transfers, distortion of the enzyme m quantum mechanical transfers, distortion of the enzyme molecule transiently compresses barrier width and equalizes reactant and product energy states. In contrast to quantum mechanical transfers, distortion of the enzyme molecule transiently com-
presses barrier width and equalizes reactant and product energy states. In contrast to
classical models of catalysis, for vibrationally drive presses barrier width and equalizes reactant and product energy states. In contrast to classical models of catalysis, for vibrationally driven ground-state tunnelling, max-
imum complementarity with the ground state should classical models of catalysis, for vibrationally driven ground-state tunnelling, max-
imum complementarity with the ground state should be sought. Additionally, the
exclusion of water will reduce the mass of the transferre imum complementarity with the ground state should be sought. Additionally, the exclusion of water will reduce the mass of the transferred particle (thus increasing tunnelling probability). The challenge will therefore be t exclusion of water will reduce the mass of the transferred particle (thus increasing
tunnelling probability). The challenge will therefore be to incorporate these new
aspects into programmes of rational enzyme redesign and tunnelling probability). The challenge will therefore be to incorporate these new
aspects into programmes of rational enzyme redesign and to provide a unified theory
for enzyme-catalysed reactions. Over the last century, o aspects into programmes of rational enzyme redesign and to provide a unified theory
for enzyme-catalysed reactions. Over the last century, our understanding of catalysis
has been based primarily on static pictures of enzym for enzyme-catalysed reactions. Over the last century, our understanding of catalysis
has been based primarily on static pictures of enzymes and enzyme-ligand com-
plexes. As we look to the new millennium, our quest for a has been based primarily on static pictures of enzymes and enzyme-ligand com-
plexes. As we look to the new millennium, our quest for a better understanding will
be driven by an appreciation of a role for protein dynamics plexes. As we look to the new millennium, our quest for a better understanding will
be driven by an appreciation of a role for protein dynamics—both experimental and
computational—in driving enzyme-catalysed reactions.

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THE ROYAL

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His research involves the development and use of com and joined Leicester University as a Lecturer in 1998, where he is currently Reader.
His research involves the development and use of computational methods to address
one of the major challenges in the biomolecular science His research involves the development and use of computational methods to address
one of the major challenges in the biomolecular sciences, understanding the relation-
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