# Quantum catalysis? A comment on tunnelling contributions for catalyzed and uncatalyzed reactions

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Appreciation for the contribution of nuclear quantum effects to chemical reactivity predates transition-state theory. Quantum corrections to rate constants for the reactions catalyzed by lactate dehydrogenase and formate dehydrogenase and the same reactions in water are estimated by Bell's one-dimensional approximate method and give tunnelling contributions to catalysis of 1.6 and 0.95, respectively. Published results for nuclear quantum effects, including both tunnelling and zero-point energies, estimated by the quantum classical path method for lactate dehydrogenase, carbonic anhydrase, glyoxylase I and lipoxygenase, together with the corresponding reactions in water, are reviewed: the respective contributions to catalysis are 0.66, 5, 1 and 1. In the absence of better evidence that an enzymic rate enhancement is due to a significantly larger quantum correction for the enzyme-catalyzed reaction than for an appropriate uncatalyzed reference reaction, it is suggested that the term "quantum catalysis" should be used with caution and restraint.

**Keywords:** tunnelling, nuclear quantum effects, catalysis, computational simulation

## QUANTUM CATALYSIS

"Quantum catalysis" was the title given to a summary[1] of a symposium on *Transition State Modelling in Catalysis* held in 1998, and the term was employed in the sense of the application of quantum mechanics to understanding complex catalytic reactions, just as others had previously coined similar phrases, for example, "quantum biochemistry" [2] or "quantum pharmacology";[3] the final sentence read, 'The age of quantum catalysis has begun, and it is entering a period where we can expect exponential growth for years to come.' In the sense intended, this prescient statement is indeed being fulfilled: a recent review of hybrid quantum/classical methods for biomolecular systems cited almost 200 applications published in this area in 2006-2007 alone,[4] and a recent issue of *Theochem* was devoted entirely to papers on theoretical modelling of heterogeneous catalysis.[5] Inasmuch as the term "quantum catalysis" simply telescopes the sense of "catalysis studied by means of quantum-mechanical methods", its usage is not being questioned here.

The term "quantum catalysis" has also recently been adopted by some in the field of quantum information theory to describe the triggering of a transformation by quantum entanglement.[6] I will not discuss this usage further.

The concern of this paper is to query another recent usage of the term "quantum catalysis", by some chemists and biochemists, to mean a rate enhancement due to the quantum character of nuclear motion in enzymic reactions.[7] In what sense may an experimentally observable rate acceleration be attributed to the particular choice of theoretical model used to describe the mechanism? To what extent does the evidence from computational simulation support the assertion that nuclear quantum effects (NQEs) contribute significantly to enhance the rates of enzyme-catalyzed

reactions as compared with the same reactions in solution? In order to develop the discussion, it is necessary first to consider the relationship between quantum theory and the transition-state theory (TST) of chemical reaction rates, and then to consider some implications of attempts to quantify contributions to catalysis. Next,

## SIMPLE CONSIDERATIONS

The implications of quantum theory for chemical reaction rates were recognised[8-12] before the formulation of TST[13-16] which, as pointed out clearly by Wigner,[17] was based strictly on classical mechanics.[18] In 1933, Bell discussed[19] the penetration by quantum-mechanical (QM) particles of a one-dimensional symmetrical Eckart potential energy barrier. He showed not only that there was a significant probability that light particles (e.g. protons) with energies lower than the height of the classical barrier would penetrate through from the reactant to the product side (tunnelling), but also that this probability was less than unity even for particles with energies above the barrier (non-classical reflection). This was in stark contrast with the classical-mechanical result that particles with energies above the barrier would always pass but those below would always fail to do so. By combining his expression for the barrier permeability with a Boltzmann distribution of energies, he considered the temperature dependence of his calculated rate coefficient and pointed out that the QM activation energy (from a plot of log $k$ against $1/T$) was smaller than the actual barrier height, and that it was not constant (i.e. the Arrhenius plot of calculated rate coefficients deviated significantly from linearity at low temperatures).[19] Bell's work was soon extended by Bawn and Ogden[20] to include the effects of zero-point energy and tunnelling upon reactions involving isotopes of hydrogen.

Besides the Born-Oppenheimer separation of nuclear and electronic motions and the Boltzmann distribution of energies for reactant molecules, the key assumptions of TST are (a) that molecules pass through the transition state only once on their way to becoming products and (b) that motion along the reaction coordinate in the transition state (the saddle point on the potential energy surface, TS, in conventional TST) is separable from other motions.

At the TS, each normal mode with positive curvature in the potential energy surface has a real vibrational frequency $\nu$ and a classical partition function $Q_{cl}$. Following Wigner,[12] the quantum correction factor $\Gamma$ is the quantum partition function $Q_{qu}$ divided by $Q_{cl}$, given by eq. (1); in this expression the origin of the quantized energy levels is the potential energy of the saddle point, where $k_B$ is Boltzmann's constant and $h$ is Planck's constant, $T$ is the absolute temperature and $u = h\nu/k_BT$.

$$\Gamma = Q_{qu} / Q_{cl} \quad = \quad (e^{-u/2}/1 - e^{-u}) / u^{-1} \quad = \quad (u/2)/\sinh(u/2) \qquad (1)$$

The factor $e^{-u/2}$ appearing in the numerator is due to the zero-point energy of the vibrational mode that arises in consequence of the Heisenberg Uncertainty Principle: even in the lowest quantum state of a vibrational mode the momentum and the position of a molecule cannot both be known precisely. The molecular structure is delocalized in a manner described by the wavefunction for the vibrational mode.

The single normal mode with negative curvature in the potential energy surface at the TS has an imaginary vibrational frequency $\nu^{\ddagger}$, and the Uncertainty Principle implies delocalization of the structure along this mode: there is a finite probability that the molecule tunnels through the barrier with energy less than that of the saddle point. Bell pointed out that it is "illogical to consider the tunnel effect as some special or additional quantum effect, or to ignore it, if we accept the existence of zero-point energy."[21,22] Within the assumption of separability, the quantum correction for the

reaction-coordinate mode is a factor $\Gamma^{\ddagger}$ which, for an inverted parabolic barrier, is given by eq. 2.[23]

$$\Gamma^{\ddagger} = (|u^{\ddagger}|/2)/\sin(|u^{\ddagger}|/2) \quad (2)$$

## CATALYSIS

To say that an enzyme catalyzes a chemical reaction is to imply that the rate is enhanced in the presence of the enzyme as compared to what it is in its absence; it does not mean simply that the enzyme is involved in the reaction mechanism. Quantifying the extent of catalysis requires that an appropriate uncatalyzed reaction is available for comparison. In Schowen's words, 'A catalyst must have a reaction to catalyze; catalysis implies an "uncatalyzed" or standard reaction.'[24] The choice of the standard (or reference) reaction is arbitrary, provided that it has the same stoichiometry as the enzyme-catalyzed reaction. It has been pointed out that understanding enzyme catalytic power is a process of successive redefinition of the reference reaction: 'many discussions of the catalytic factors which contribute to enzymic activity constitute…simply a step-by-step redefinition of the standard reaction until the binding energy of the standard transition state becomes zero'.[24] When the reference reaction contains within it all the same features as the enzymic reaction, the catalysis is accounted for by those features, whatever they may be.

From a theoretical point of view, a logical and meaningful choice is the reaction in water of the same reacting moieties with the same spatial disposition as occur in the enzymic mechanism. This excludes factors associated with bringing these moieties together, but allows a clear focus upon the important issue of how the protein environment affects the reactivity differently from the aqueous environment. A simple justification for comparing the rate of an enzyme-catalyzed reaction with the rate of a corresponding uncatalyzed reaction in water is that the natural environment of the enzyme is also aqueous: most biology occurs in water.

It has been suggested recently that enzymes have evolved to catalyze hydrogen-transfer reactions by means of quantum tunnelling; in other words, tunnelling is primarily responsible for the catalytic power.[25-28] If true, this implies that the quantum correction $\Gamma^{\ddagger}$ is much larger for the enzyme-catalyzed reaction than for an (arbitrarily chosen) uncatalyzed standard reaction. If the standard reaction is chosen to be that in water (as suggested above), this hypothesis can be tested very simply, within the assumption of separability of motion, by evaluating the one-dimensional factor $\Gamma^{\ddagger}$ for the reaction in each medium according to eq. 2, in which for a given temperature the only variable is the magnitude of the imaginary reaction-coordinate frequency, $|\nu^{\ddagger}|$. The contribution of QM tunnelling to catalysis is the quotient $(\Gamma^{\ddagger})_{enz} / (\Gamma^{\ddagger})_{aq}$. Alternatively, more reliable estimations of the values of $\Gamma^{\ddagger}$ for catalyzed and uncatalyzed reactions may be made using more sophisticated theoretical methods.

The published transactions of a recent Discussion Meeting[7] on the subject of "Quantum Catalysis in Enzymes" contain many interesting papers discussing evidence (*e.g.* kinetic isotope effects and deviations in Arrhenius plots) for significant contributions from tunnelling to rate constants in many enzyme-catalyzed reactions. It is noteworthy, however, that only one of these papers provides a comparison of (computer simulated) catalyzed and uncatalyzed reactions; it concludes that QM contributions are similar for reactions in enzymes and in solution and thus do not contribute to catalysis.[29] None of the other papers provides any positive evidence for tunnelling being primarily responsible for enzyme catalytic power, in the sense

defined here, by demonstrating a greater value for $\Gamma^{\ddagger}$ in an enzyme-catalyzed reaction as compared with *any* standard reaction. Undeniably there are significant contributions from tunnelling to rate constants and kinetic isotope effects in many enzyme-catalyzed reactions, with substantial values for $(\Gamma^{\ddagger})_{enz}$, but currently there does seem to be a paucity of evidence that NQEs contribute significantly to rate enhancements for these reactions as compared with any uncatalyzed reaction.

It has been acknowledged that it is extremely difficult experimentally to study both catalyzed and uncatalyzed reactions with the same mechanism.[25,30] Notably, Finke and co-workers designed experimental systems specifically to test the hypothesis presented above; for a reaction with and without enzyme, they reported similar results for each of three criteria for tunnelling (magnitudes of a kinetic isotope effect, difference in activation energies and ratio of Arrhenius pre-exponential factors for isotopologous reactions) and concluded that QM tunnelling was not enhanced by the enzyme.[31,32] However, in the context of a critique of reference reactions for the assessment of tunneling in enzymic reactions, Schowen has pointed out that it is not logically necessary that enzymic enhancement of tunneling is always accompanied by observable changes in these three values, thereby maintaining an open verdict on the matter.[33]

It is perfectly feasible to study both catalyzed and uncatalyzed reactions with the same mechanism by means of computational simulation.[34] It has been argued that a distinct merit of this approach is that it allows for a clear distinction between those contributions to catalysis that arise from differences in chemical mechanism between the enzymic reaction and its reference reaction in solution and those that arise specifically from differences in the protein and solvent environments.[35] We will now briefly review the results of comparisons of tunnelling contributions for catalyzed and uncatalyzed reactions by means of computational simulation. The first two examples below apply the simple treatment of eq. 2 to reactions for which the imaginary (transition) frequency of the TS has been computed both in an enzyme active site and in water by this author and his collaborators. The remaining examples are taken from the published work of other authors.

## EXAMPLE 1: LACTATE DEHYDROGENASE (LDH)

Some years ago we performed hybrid QM/classical calculations to locate and characterise a family of six TSs for the reduction of pyruvate to lactate within the active site of LDH.[36] The mean value and standard deviation of $|\nu^{\ddagger}|_{enz}$ for these first-order saddle points was 834 ± 37 $cm^{-1}$ at the AM1/CHARMM22 level. We also characterized a single TS for reduction of pyruvate to lactate in water within an encounter complex of the substrate with a nicotinamide hydride donor and an imidazolium proton donor; the extent of the QM region was the same as in the enzymic reaction. The (previously unpublished) value of $|\nu^{\ddagger}|_{aq}$ for the TS in aqueous solution was 573 $cm^{-1}$ at the AM1/TIP3P level. Inserting these frequencies into eq. 2 yields $(\Gamma^{\ddagger})_{enz}$ = 2.25 and $(\Gamma^{\ddagger})_{aq}$ = 1.40 at 300 K. The contribution of QM tunnelling to catalysis is $(\Gamma^{\ddagger})_{enz}$ / $(\Gamma^{\ddagger})_{aq}$ = 1.6.

## EXAMPLE 2: FORMATE DEHYDROGENASE (FDH)

Recently hybrid QM/classical calculations were performed to locate and characterise a family of ten TSs for the reduction of carbon dioxide to formate within the active site of FDH.[37] The mean value and standard deviation of $|\nu^{\ddagger}|_{enz}$ for these first-order saddle points was 783 ± 12 $cm^{-1}$ at the AM1/OPLS-AA level.[38] A single TS for

reduction of carbon dioxide to formate in water within an encounter complex of the substrate with a nicotinamide hydride donor and an imidazolium proton donor was also characterized; the extent of the QM region was the same as in the enzymic reaction. The value of $|\nu^{\ddagger}|_{aq}$ for the TS in aqueous solution was 808 $cm^{-1}$ at the AM1/TIP3P level.[38] Inserting these frequencies into eq. 2 yields $(\Gamma^{\ddagger})_{enz}$ = 1.97 and $(\Gamma^{\ddagger})_{aq}$ = 2.08 at 300 K. The contribution of QM tunnelling to catalysis is $(\Gamma^{\ddagger})_{enz}$ / $(\Gamma^{\ddagger})_{aq}$ = 0.95.

## MULTI-DIMENSIONAL TUNNELLING

The simple expression given in eq. 2 for the quantum correction in the reaction-coordinate assumes that this mode is separable from the other (real) vibrations, which is valid only in the immediate vicinity of the saddle point where the potential energy is approximately quadratic. It has long been recognised that a simple one-dimensional treatment is invalid whenever the de Broglie wavelength is large compared to the quadratic region.[39] The quantity *u* in eqs. 1 and 2 is equivalent to the ratio of the de Broglie wavelength to the Boltzmann-average vibrational amplitude at a given temperature:[40] in the two examples given above, values of $|u^{\ddagger}|$ between 2.7 and 4.1 were found at 300 K, corresponding to "small to moderate tunnelling" for which eq. 2 should estimate $\Gamma^{\ddagger}$ with "fair accuracy".[21] Nonetheless, a multi-dimensional treatment of NQEs would be preferable.

Truhlar and co-workers have developed a plethora of approximate methods for multidimensional tunnelling[41] based upon a classical reaction coordinate, separate from all the other (quantal) degrees of freedom, with quantum and nonseparability effects included in a transmission coefficient. Ensemble-averaged variational transition-state theory with multidimensional tunnelling (EA-VTST/MT) has been applied with success to a number of enzymic reactions:[42-46] this method considers a range of different possible tunnelling paths, some close to the one-dimensional classical reaction path and others distant from it, at a range of different energies, to find the best compromise between path length and effective potential along the path in order to maximise the tunnelling. A recent book chapter reviewing the application of this method is entitled "*Quantum Catalysis in Enzymes*",[46] but it does not discuss catalysis in the sense defined above. At the time of writing, no example has yet been published in which this method has been applied to an enzyme-catalyzed reaction and to the same reaction in water; the first publication of this nature is awaited with much interest.

A different approach to NQEs on chemical reaction rates employs the centroid path integral method, in which a small number of atoms (often a transferring hydrogen together with the donor and acceptor) are quantised and treated as a closed ring of quasiparticles each experiencing a fraction of the external potential acting on the real particle. A practical approximation is Warshel's Quantised Classical Path (QCP) method the QM partition function for the TS is obtained by running classical trajectories for the quasiparticles to find the probability distribution for their centre of mass.[47] The difference in activation free energies computed classically and by the QCP method provides an estimate for $\Gamma^{\ddagger}$ that includes both zero-point energy and tunnelling contributions inseparably. In view of Bell's remark, quoted above, it is perfectly logical to consider both effects together. Similar methods have been recently reviewed by Gao and co-workers.[48]

## EXAMPLE 3: LDH AGAIN

The reaction catalyzed by LDH was simulated by Hwang *et al*.[49] using a combination of the QCP and Empirical Valence Bond (EVB) methods. Quantum corrections $(\Gamma^{\ddagger})_{enz}$ = 82 and $(\Gamma^{\ddagger})_{aq}$ = 124 at 300 K were obtained. These values are substantially larger than those estimated above by means of the simple eq. 2, but the contribution of nuclear QM effects to catalysis is $(\Gamma^{\ddagger})_{enz} / (\Gamma^{\ddagger})_{aq}$ = 0.66. Note that (a) this ratio includes both tunnelling and zero-point energy, (b) the QCP method would be expected to be more reliable for estimation of tunnelling at energies significantly lower than the effective barrier height, (c) the TS studied by these workers was dominated by hydride transfer whereas that studied by Williams and co-workers was for an alternative LDH mechanism with dominant proton transfer.[36,50,51] Note also that it is quite feasible for quantum corrections to make either positive, insignificant or negative contributions to catalysis.[52]

## EXAMPLE 4: CARBONIC ANHYDRASE

Hwang and Warshel[53] applied the same EVB/QCP methodology to the proton-transfer reaction catalyzed by carbonic anhydrase and estimated that NQEs contributed to lower the activation free energy for the enzymic reaction by about 1 kcal $mol^{-1}$ more than for the same reaction in water at 300 K. This corresponds to $(\Gamma^{\ddagger})_{enz} / (\Gamma^{\ddagger})_{aq} \approx 5$. Note that there was about an order of magnitude error in the computed value of $k_{cat}$ for the enzyme-catalyzed reaction.

## EXAMPLE 5: GLYOXYLASE I

A similar approach was adopted by Åqvist and co-workers[54] in their study of the hydride-transfer reaction catalyzed by glyoxylase I: they found that the reduction in the activation free energy due to nuclear quantum effects was "almost identical" at $\approx 2.5 \pm 0.2$ kcal $mol^{-1}$ in both the enzyme and aqueous solution at 300 K. Thus quantum corrections enhance the rate by a factor of about 66 but do not contribute to catalysis.

## EXAMPLE 6: LIPOXYGENASE

The EVB/QCP approach was used by Olsson *et al*.[55] in their study of hydrogen-atom transfer catalyzed by lipoxygenase. Once again, the contribution of NQEs to $k_{cat}$ was found to be "very similar" for the enzymic reaction and its counterpart in water, reducing the free energy of activation at 300 K by about 6 kcal $mol^{-1}$ in each case (corresponding to a factor of about $2 \times 10^4$) but with no additional rate enhancement caused by the enzyme over that found in aqueous solution.

## OVER THE BARRIER OR THROUGH THE BARRIER?

Several discussions of the role of in QM tunnelling in enzymic reactions have been couched in terms of a dichotomy between mechanisms that take the transferring hydrogen *either* over the top of the classical potential energy barrier *or else* through it (for example, refs. 27,56-58). This language seems to imply that an enzyme has the capability to "choose" between a classical mechanism and a quantum mechanism, as if it could switch the quantum nature of any atom "on" or "off". Tunnelling is not an alternative pathway that may be followed, as an option along with (say) a one-step or a two-step mechanism. For a reaction involving rate-determining hydrogen transfer, it

may be that a much greater proportion of the reactive flux occurs at energies below the classical barrier for one mechanism as opposed to another, but one cannot say for either mechanism that a particular percentage of the reaction proceeds by tunnelling while the remainder proceeds over the barrier, as if some reactive events were quantum and others classical. In computational simulations, of course, one may choose to use a theoretical model that either does or does not include NQEs. Within a treatment that does include them, all mechanisms are QM and, for a given mechanism, all individual reactive events are QM, even those involving energies above the classical barrier height (in consequence of non-classical reflection).

The notion of "over the barrier or through the barrier" may arise from thinking about the contribution of NQEs as a source of rate enhancement, in which the QM description of events corresponds to a faster catalyzed reaction and the classical description corresponds to a slower uncatalyzed reaction. This is not a fruitful way to consider the origins of enzyme catalytic power. Instead an enzyme-catalyzed reaction should be compared with a standard uncatalyzed reaction; the selection of the latter is arbitrary, although it may be argued that the same reaction in aqueous solution is an appropriate choice where possible. If computational simulations performed with and without inclusion of QM effects on the motions of the reacting nuclei indicate a larger role for quantum corrections in the enzyme than in water, then one may talk meaningfully about the contribution of tunnelling to catalysis.

## CONCLUSIONS

Table 1 summarises the examples considered here of computational simulations for enzyme-catalyzed reactions and the same uncatalyzed reactions in water. These computational methods use microscopic theories based upon atomistic descriptions in which the comparison between the catalyzed and uncatalyzed reactions does not involve any adjustable parameters; they are not phenomenological models that merely provide a satisfactory fit to observed data. The ratios $(\Gamma^{\ddagger})_{enz} / (\Gamma^{\ddagger})_{aq}$ are all very small values, regardless of whether they are obtained by means of the simple one-dimensional correction given by eq. 2 or by the sophisticated QCP method. For some reactions NQEs are slightly more significant in the enzymic environment and for some they are slightly more significant in the aqueous environment, but clearly their contribution to enzyme catalysis is not the dominant factor for any of these reactions.

It is not in dispute that NQEs – primarily tunnelling – may contribute significantly to reducing the effective free energy of activation for a hydrogen-transfer reaction catalyzed by an enzyme. The question at issue is whether these effects contribute significantly more for an enzyme-catalyzed reaction than for the same uncatalyzed reaction in water. The results collected in Table 1 do not constitute compelling evidence in support of a significantly greater extent of tunnelling in the enzyme-catalyzed reactions. It is entirely possible that a particular enzyme may indeed contain structural features that serve to enhance tunnelling relative to a reaction in the absence of the enzyme, but the (admittedly small) range of reactions considered here does not provide evidence for this happening.

It is not universally accepted that the extent of enzyme catalysis should be determined by comparison with the same reaction in water. The key point of the present paper does not depend upon the particular choice of this as the standard uncatalyzed reaction, even though the examples considered here do so. It would be

sufficient to demonstrate that an enzymic rate enhancement was due to $(\Gamma^{\ddagger})_{enz}$ / $(\Gamma^{\ddagger})_{standard}$ for any appropriate choice of standard reaction.

Until suitable experiments can be performed, accompanied by computational simulations with reliable treatments of NQEs, for a wider selection of reactions catalyzed by enzymes and appropriate uncatalyzed reactions, it would perhaps be advisable to exercise great caution in the use of the term "quantum catalysis". By all means let us employ QM methods to study catalysts, catalytic behaviour and enzyme mechanisms in computational simulations, and let us employ appropriate experimental methods as diagnostics for tunnelling. But in discussions of the origins of enzyme catalytic power, let us refrain from adopting any concept as a contributing factor unless it can be quantified; this voluntary restraint should include the possible contribution to catalysis that arises in computational simulations due to the quantum description of nuclear motions.

**Table 1**. Quantum corrections and contributions to catalysis for catalyzed and uncatalyzed reactions at 300 K.

| reaction | method | $(\Gamma^{\ddagger})_{enz}$ | $(\Gamma^{\ddagger})_{aq}$ | $(\Gamma^{\ddagger})_{enz}$ / $(\Gamma^{\ddagger})_{aq}$ |
|---|---|---|---|---|
| LDH | eq. 2 | 2.25 | 1.40 | 1.6 |
| FDH | eq. 2 | 1.97 | 2.08 | 0.95 |
| LDH | QCP | 82 | 124 | 0.66 |
| carbonic anhydrase | QCP | - | - | ~ 5 |
| glyoxylase I | QCP | 66 | 66 | ~ 1 |
| lipoxygenase | QCP | $2 \times 10^4$ | $2 \times 10^4$ | ~ 1 |

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