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Introduction. Quantum catalysis in enzymes: beyond the transition state theory paradigm

Enzymes are catalysts that can achieve substantial rate enhancements over the uncatalysed reaction. Our understanding of the physical basis of enzyme catalysis is rooted in transition state theory (TST), but recent experimental and theoretical studies suggest that we need to progress beyond the TST paradigm. In particular, both experimental and theoretical studies highlight a potential role of quantum mechanical tunnelling in enzyme-catalysed hydrogen transfer—going beyond the Bell tunnel correction model of TST and based on a dissipative, full tunnelling depiction of the H-transfer reaction that has grown out of the Marcus formulism for electron transfer. This collection of reviews, dealing with recent insights from both the experimental and theoretical studies, is based on the invited lectures given at The Royal Society Discussion Meeting in November 2005. The contributions from leading practitioners in the field highlight the current controversies at the cutting edge of this important topic, reflected in the vigour of debate at the meeting. The foremost questions addressed were: How do enzymes work? What is the physical basis of the phenomenal rate enhancements achieved by enzymes? Do we have a theoretical framework that accounts for observed catalytic rates? What is the role of tunnelling phenomena and dynamics in enzyme catalysis?

This Discussion Meeting opened with a scene-setting contribution from Britton Chance, who identified the importance of tunnelling mechanisms in biology through his observations of quantum mechanical electron tunnelling in photosynthetic bacteria at cryogenic temperatures. In his classic 1966 paper with the late Don DeVault (DeVault & Chance 1996), he not only demonstrated the tunnelling signature of temperature-insensitive rate constants between 77 and 5 K for the millisecond oxidation of cytochrome *c* by the light-activated bacteriochlorophyll in the photosynthetic reaction centre, but also provided a theoretical basis for a long-distance electron tunnelling of over 20 Å. In his paper (Moser *et al.* 2006), Dutton reports on how Marcusian tunnelling parameters are selected and varied in biology in the natural engineering of transfer chains and catalytic sites of oxidoreductases. His Darwinian examination at the molecular scale yielded a remarkable simplifying picture—he showed that distance between cofactors varies with the height of the chemical barriers to be surmounted and the need to insulate against short circuits in energy conversion.

The measurement of competitive kinetic isotope effects and the temperature dependence of kinetic isotope effects has become the 'gold standard' for analysing experimentally the tunnelling regimes in enzyme systems. Kohen (Wang *et al.* 2006) describes the experimental methods, now widely adopted in the field, used to assess tunnelling behaviour in enzyme systems. He also discusses the recent data from a number of laboratories, including his own, that are consistent with environmentally coupled or vibrationally assisted tunnelling models. These models are also discussed by Alleman (Allemann *et al.* 2006) in his work on thermo- and mesophilic dihydrofolate reductase. He 'isolated' the chemical step using stopped-flow methods and presents kinetic data consistent with environmentally coupled models of H-tunnelling. Klinman further develops the theme of environmentally coupled hydrogen tunnelling by referring to her work with soybean lipoxygenase (Klinman 2006). She emphasizes recent studies with wild-type and mutant enzymes that exhibit very large primary deuterium kinetic isotope effects and the notion of 'distance sampling' required to bring donor and acceptor atoms sufficiently close to effect efficient wave function overlap. Experimental studies of tunnelling in B_{12}-dependent systems are presented by Banerjee (Banerjee *et al.* 2006). In this case, the transfer of a hydrogen atom is accompanied by a large kinetic isotope effect, well beyond the upper limit for semi-classical reactions in the absence of tunnelling.

Northrop (2006) reports on the pressure dependence of isotope effects in enzymes and he makes a case for hydrogen not tunnelling in yeast alcohol dehydrogenase. This is primarily based on the studies of ^{13}C isotope effects. He suggests protein domain motion as a possible cause for the observed effects. In his contribution, Nocera (Reece *et al.* 2006) presents the experimental studies on proton-coupled electron transfer—intrinsically a quantum mechanical effect since both the electron and proton tunnel. He discusses the means of disentangling the electron and proton tunnelling events, applying lessons learnt from model systems to biological systems.

Detailed insight, particularly at the atomic level, comes from theory and simulation; such studies are key to corroborating and extending findings emerging from experimental studies. Hammes-Schiffer (Hammes-Schiffer & Watney 2006) gives a clear exposition of how theory and simulation can provide valuable insight into the impact of enzyme motion on enzymic hydride transfer. Her studies of dihydrofolate reductase highlight a role for coupled motion, and that distal mutations can impact on the probability of sampling configurations conducive to hydride transfer. The role of protein motion in promoting tunnelling also featured in the presentation by Sutcliffe (Sutcliffe *et al.* 2006). He summarizes the experimental and computational studies of enzymic tunnelling observed in flavoprotein and quinoprotein enzymes. Computational studies of tunnelling in

One contribution of 16 to a Discussion Meeting Issue 'Quantum catalysis in enzymes—beyond the transition state theory paradigm'.

methylamine dehydrogenase are presented by Burton (Nunez *et al.* 2006). Different tunnelling paths are investigated, suggesting the existence of two major pathways, one dominated by tunnelling and the other dominated by a classical 'over-the-barrier' mechanism. Limbach presents results illustrating the application of the one-dimensional Bell–Limbach tunnelling model to several different hydrogen transfer reactions in small molecule systems (Limbach *et al.* 2006). His study suggests that the analysis of Arrhenius curves using the Bell–Limbach tunnelling model is a useful approach for the first screening of experimental data prior to more advanced physical descriptions and theoretical interpretations.

Reflecting the current controversy in the field, Warshel (Olsson *et al.* 2006) presents results that challenge not only a role for dynamical effects in enzymic reactions, but also the very existence of tunnelling in such reactions. He argues that TST remains secure in providing a satisfactory quantitative framework for studies of enzyme catalysis. In contrast, results illustrating that TST cannot fully describe the dynamics of enzyme-catalysed reactions are presented by Schwartz (Pineda & Schwartz 2006). His studies also address the question of how picosecond motions can be reconciled with the millisecond time-scale of enzymatic turnover. Onuchic's contribution (Onuchic *et al.* 2006) illustrates how recent theoretical advances have shed light on the impact of protein dynamics on electron tunnelling and biochemical reactivity. He illustrates how global transformations might involve large-scale motion and possibly partial unfolding during the reaction event.

This issue is closed by an inspiring 'summing up' from Marcus (2006). Having played a critical role in laying the foundations and subsequently developing our conceptual framework for understanding tunnelling mechanisms in biology, Marcus is ideally placed to present future perspectives also in the field. Our aim in organizing the meeting was to identify and address key issues in the field. This objective was met only owing to the excellent contributions by all authors (speakers) coupled with the lively debate at the Discussion Meeting. The organizers wish to thank all speakers and delegates for making this meeting a resounding success.

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