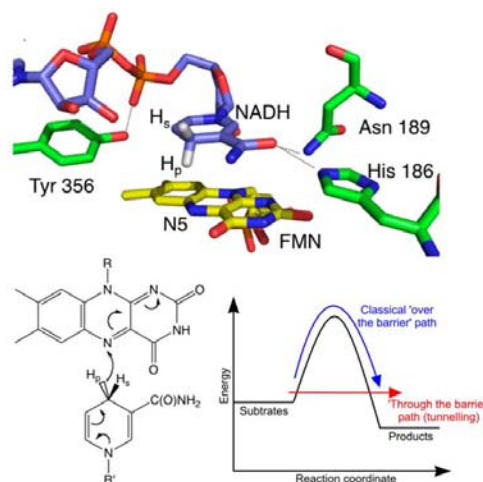


Nigel Scrutton – Enzymes in the Quantum World

While enzymes are efficient catalysts the precise origin of their catalytic power still remains unresolved after more than a century of research. The dominant paradigm of enzyme catalysis remains transition state theory (TST), where the rate of the reaction is determined by the height of the reaction barrier. A theme of research activity in my group is to uncover the physical principles that contribute to the catalytic power of enzymes based on detailed kinetic, spectroscopic and structural characterisation of enzyme systems. Current foci are on dynamics and coupling to reaction chemistry, especially in relation to reactions that proceed through quantum effects such as quantum mechanical tunneling (electron and hydrogen transfer), quantum spin transitions, photon capture and related phenomena. Our approach is to integrate fast reaction biophysical methods (laser photolysis, stopped-flow etc), with theory and computation, coupled to the use of model enzyme systems and structure determination.

It is often possible to probe experimentally the height (energy) of the reaction barrier by measuring the temperature dependence of the reaction rate and analyzing these data in terms of Arrhenius or Eyring theory. However, it is likely that more than half of all known enzyme-catalyzed reactions involve one or more hydrogen (H) transfers and it is now becoming apparent that in many cases, these H-transfers occur in part, or in full, by quantum mechanical tunneling. Unlike classical over-the-barrier (TST) reactions, the rate of a tunneling reaction – which proceeds through the reaction barrier rather than over it – is governed by the overall shape, and particularly the width, of the reaction barrier. The role that protein motions play to actively promote such hydrogen tunneling is vigorously debated at present. Based mainly on indirect evidence from the measurement of kinetic isotope effects (KIEs) as a function of temperature, we have used new theoretical models to relate protein motions to hydrogen transfer. The common feature of all these models is that motions of the enzyme are invoked not just to explain the relatively slow physical steps of the reaction such as substrate binding and product release but that they all rely on fast enzyme motions that couple to the reaction coordinate and promote the actual chemical step. Computational studies have supported a need for these fast promoting motions and we are now ‘chasing’ these motions experimentally using ultrafast (femtosecond) laser methods through our BBSRC LoLa grant award.



Studies of the temperature dependence of kinetic isotope effects are now used as standard in the field to demonstrate tunneling behaviour. However, it is not possible to experimentally deduce any real information about the shape of the reaction barrier from the temperature dependence of the reaction rate. We use alternative experimental probes of barrier shape, and particularly barrier width, to study such reactions. Hydrostatic pressure is a tractable experimental condition with which to probe enzymatic reactions in solution. We recently pioneered variable pressure stopped-flow approaches to study the pre-steady state (single-turnover) kinetics of hydride transfer in enzymes. We analyzed the combined temperature and pressure-dependence of the rate and KIE for this reaction in terms of a Marcus-like full H-tunneling model. The key assumption in this modeling was that hydrostatic pressure ‘squeezes’ the enzyme and consequently compresses the reaction barrier. This ‘squeezing’ has been contentious in the field, but we recently provided direct spectroscopic evidence for barrier compression that supports a squeezing motion. We have also developed full tunneling theory to include pressure effects on the tunneling rate constant, providing a mathematical framework for analysis and modeling of experimental data. More recently we have been able to quantitatively model the effect of changing barrier width on reactions and to determine the effects on quantum tunneling *versus* ‘over the barrier’ contributions to the reaction.

We are now testing rigorously the nonadiabatic full tunneling model for enzymatic H-transfer

using a combination of P and T variance and a number of enzyme systems which are both thermally and light activated. This will involve the use of fast reaction methods (stopped-flow, laser flash and equilibrium perturbation, in combination with isotope effects, numerical modelling and computational analysis at variable pressure). Our aim is to test robustly the nonadiabatic 'deep tunneling' model for enzymatic H transfer, and to provide a rigorous mathematical understanding of catalysis that will address the shortcomings of transition state theory in relation to biological H-transfer reactions. We will then extend to studies with heavier nuclei (carbon, nitrogen etc).