

Papers for Wed, Nov 15: (1) Eur. J. Biochem. (2002) 269, 3113-3121. Michael J. Knapp & Judith P. Klinman, Environmentally coupled hydrogen tunneling – Linking catalysis to dynamics. **(2) Phil. Trans. R. Soc B (2006) 361, 1323-1331.** Judith P. Klinman, Linking protein structure and dynamics to catalysis: the role of hydrogen tunneling.

The goal of this discussion is to learn about the experimental observations that provide evidence for quantum mechanical tunneling in the mechanism of hydrogen transfer reactions and how the theory used to explain the phenomenon has and is continuing to evolve in light of the observation of unexpected temperature dependent behaviors. Current models point to a process where the hydrogen transfer always occurs by tunneling but relies on protein dynamical modes to maximize the probability of tunneling.

The two papers are both reviews of the key observations from studies of many enzymes. We'll focus on the first as it provides a nice description the historical progression of experimental observations and the associated progression in theories/models developed to explain the tunneling contribution to the rate of hydrogen transfers. The second, more recent review augments the earlier one with new insights from both old and new data. As you read the papers consider the following points for discussion. – Where you're not familiar with the concepts, please raise questions.

1) Marcus theory argues that the electron transfers between two centers occur by quantum mechanical tunneling because it's wave function occupies a large volume due to its small mass. The rate of electron transfers however, which can still have significant energies of activation, are not then limited by the transfer itself but rather by energy-requiring structural reorganization processes.

2) The deBroglie wavelength is another way to envision the magnitude of the uncertainty in the position of the particle, i.e., it's wave function, and is inversely dependent on the mass of the particle.

3) Key aspects of transition state or absolute rate theory:

- a) the reactants must reach an energetically activated vibrational level in order to pass through the TS and over the barrier
- b) occasionally, if wave function overlap is optimal, hydrogens may tunnel through the barrier, but this is not the dominant mode for hydrogen transfer – just a correction
- c) processes that require enthalpy for activation to the excited vibrational state will be temperature dependent
- d) isotope effects on the rate are proposed to be due to the difference in zero point energies and hence a difference in the energies of activation.

4) Recall the Arrhenius equation is: $k = Ae^{-E_{act}/RT}$, where E_{act} is isotope dependent as noted above but A is proposed to be a constant that is isotope independent. Note that this predicts that there should not be an isotope effect on A , i.e. $A_H/A_D = 1$. (Recall what an Arrhenius plot is and hence where one obtains the parameters from the plot.) In this context, the semiclassical model with its tunneling correction cannot explain a small but finite E_{act} and an $A_H/A_D \gg 1$.

5) Transfer of a particle between donor and acceptor by tunneling is a probabilistic event that only depends on the overlap of the wave functions for the particle in the two states (bound to donor and to acceptor). In a *static* structural model, i.e., where donor and acceptor are held rigid, the extent of H, D or T tunneling will depend on the relative distances between donor and acceptor and is expected to be temperature independent.

6) Since proteins are not “static” but dynamic, the probability of tunneling will vary with the dynamic motions of the protein. Since the motions are energy dependent, the probability for tunneling also becomes energy dependent, which can lead to an energy of activation for the rates of hydrogen transfer, but a lack of difference between the energies of activation for the isotopes and hence temperature independence of the k_H/k_D , but a large isotope effect on the prefactor A_H/A_D .

7) So the question is whether there is a model that can explain the observations well in the different types of systems that have been studied. The model in the Knapp and Klinman review is similar in form to Marcus theory and works well to explain the soybean lipoxygenase data described in the review. However, it is less clear how to explain the data for the behavior of the thermophilic enzymes – as mentioned both in the review and for the hyperthermophile mentioned in paper (2) in terms of where the gating becomes most significant. This point is one of the main issues open for discussion – how to connect changes in protein flexibility that occur in thermophilic enzymes as a function of temperature with the changes in the apparent degree of tunneling which is observed to be greater at their optimal temperature for growth and activity.