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Protein Dynamics, Tunneling, and All That*

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Abstract

We review some physical issues in the theory of reaction rates in proteins, starting with the classic observations of DeVault and Chance on "electron tunneling" in bacterial photosynthesis and their interpretation by Hopfield. These ideas lead to a family of generalized spin-boson models for reaction rates, and these models are used to explore the significance of quantum effects and the possibility of "dynamical specificity" – extreme sensitivity of the rate to changes in protein dynamics. We assess the success of these models in rationalizing kinetic and spectroscopic data both on photosynthetic electron transfer and on ligand binding to heme proteins, and conclude with some open questions.

1. Introduction

One of the basic tasks facing each cell of an organism is the control of chemical reaction rates. This task is accomplished by coupling each reaction to a specific protein catalyst, and the results are impressive: in the photosynthetic apparatus of bacteria [1], for example, a group of three proteins is responsible for "arranging" a sequence of electron transfer reactions whose rates span twelve orders of magnitude from $k \sim 10^{12}\,s^{-1}$ to $k \sim 1\,s^{-1}$. The performance of proteins as catalysts has traditionally been interpreted semi-empirically, in terms of a variety of chemical factors that can be mimicked in the reactions of smaller organic model compounds [2, 3]. But proteins are very large molecules, and there are surely aspects of protein dynamics which cannot be modelled by smaller structures; do these special dynamics contribute to the efficiency and specificity of catalysis? This question, which is of obvious biological interest, cuts across a variety of physical issues: How do we describe manifest themselves in spectroscopic experiments? What are the criteria for quantum vs. classical behaviour in large molecules? Is there in fact any physical basis for "dynamical specificity" in the determination and control of reaction rates?

In the last decade, two very different approaches to these problems have developed. One approach, which we review here, may be traced to Hopfield's 1974 [4] analysis of the DeVault–Chance experiments [5, 6] on "electron tunneling" in photosynthesis. An alternative viewpoint has grown largely out of experiments by the Illinois group [7–11] on the binding of small ligands to myoglobin and related proteins. These two approaches adopt qualitatively different descriptions of protein dynamics with correspondingly different conclusions about which physical issues are relevant to understanding biomolecular function. Each set of issues is closely related to theoretical problems of current interest in condensed matter physics. The goal of this short review is to indicate – at least from one point of view – the relations between these theoretical issues and emerging experiments. A more biological perspective on these ideas is presented elsewhere [12].

2. The DeVault–Chance reaction

Much of the interest in physical theories of protein reaction rates can be traced to the experiment of DeVault and Chance [5], who studied light-induced electron transfer in the photosynthetic bacterium *Chromatium vinosum*. Photon absorption by a primary electron donor P_{870} (P) triggers a sequence of reactions resulting in the radical cation P^{+}_{870}. A secondary donor, cytochrome c (C), then fills the hole on P_{870}; the key observation of DeVault and Chance concerns the temperature dependence of the reaction $CP^{+} \rightarrow C^{+}P$, shown in Fig. 1. At $T > 100\,K$ we see the conventional Arrhenius

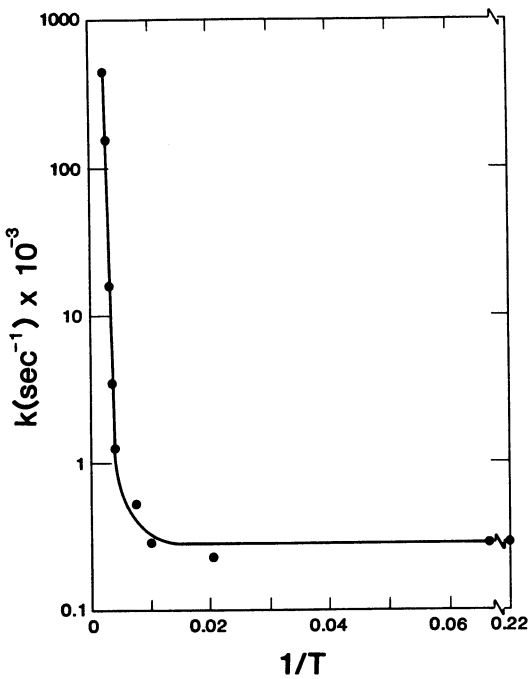

Fig. 1. Temperature dependence of the reaction rate $P^{+}C \rightarrow C^{+}P$ in *Chromatium vinosum*. The data may be summarized by the low temperature rate, $k(T = 0) = 2.8 \times 10^{2}\,s^{-1}$, the extrapolated infinite temperature rate, $k(T \rightarrow \infty) = 7.8 \times 10^{8}\,s^{-1}$, and the activation energy $E_a = 0.18\,eV$ such that $k(T > 150\,K) = k(T \rightarrow \infty)e^{-E_a/k_BT}$. After refs. [5] and [6].

* Based in part on discussions at the NORDITA workshop on the Physics of Biomolecules, Copenhagen, June 1985.

behaviour, with $k(T) \sim k(T \to \infty)e^{-E_a/k_BT}$, but for $T <$ 100 K the rate is temperature independent, $k(T) \sim k(T = 0)$.

Temperature-independent reaction rates are suggestive of tunneling processes, as had in fact been discussed in connection with photosynthesis for some time. The DeVault–Chance reaction was cited as evidence of *electron* tunneling in biomolecules, and analogies to electron tunneling across an insulating barrier between two metals or superconductors were pursued. These ideas were wrong because biomolecules, unlike metals, are dominated by discrete, localized electronic states. In the absence of an electronic continuum, electron transfer must be coupled to other degrees of freedom to guarantee irreversibility and dissipate the ~ 0.45 eV energy difference between initial and final states. Grigorov and Chernavskii [13] and Hopfield [4] independently suggested that the relevant degrees of freedom are molecular vibrations, and Hopfield showed how a theory of *vibrationally assisted* electron transfer could quantitatively account for the DeVault–Chance data.

Hopfield's semi-classical argument is schematized in Fig. 2. We have two electronic states, identified with reactants (CP^+) and products (C^+P). In addition there is some vibrational coordinate of the molecule(s), and the energy of each electronic state depends on this coordinate. Finally there is a small matrix element which connects the two states and an energy gap which separates them. Classically the molecule is described by a point moving on the potential surface; conservation of energy then requires that the transition between the two surfaces occur in the neighborhood of their crossing – the "transition state". The reaction rate is proportional to the probability of finding the molecule on the reactants' surface at the crossing. At high temperature this probability is $\sim e^{-E_a/k_BT}$ and we find an Arrhenius temperature dependence. At absolute zero thermal fluctuations can never carry the vibrational coordinates up to the transition state, but quantum zero-point motion can. Quantum fluctuations dominate thermal fluctuations at temperatures below $T_0 \sim \hbar\omega/2k_B$, where ω is the vibrational frequency, so we except a temperature-independent reaction rate at $T \ll T_0$ and Arrhenius behaviour at $T \gg T_0$.

When the reaction occurs as a result of zero-point motion we actually are observing vibrational tunneling, since the transition state is in a forbidden region for a classical system with the zero-point energy $\hbar\omega/2$. Thus the onset of temperature independence at $T < T_0$ reflects a crossover to tunneling as originally suggested, but the tunneling is in the vibrational sector – the coordinates of atomic nuclei rather than electrons. Do the electrons tunnel at all? The electronic dynamics are summarized by the energy gap ε and the matrix element V. If the donor and acceptor sites are far apart then V is probably controlled by electron motion in a classically forbidden region, and the dynamics of this electron tunneling contribute an overall factor $\sim V^2$ to the rate but are irrelevant for temperature dependence. Unless one can experimentally test calculations of the matrix element V itself it is unlikely that "electron tunneling" could be proven.

We can give a very different description of the model in Fig. 2. This is a theory of two electronic states interacting with a single phonon mode with phonon energy $\hbar\omega$. To dissipate the electronic energy gap ε we must emit $N \sim \varepsilon/\hbar\omega$ phonons, and since we are adopting a semi-classical picture we don't worry that this is not exactly an integer; at finite

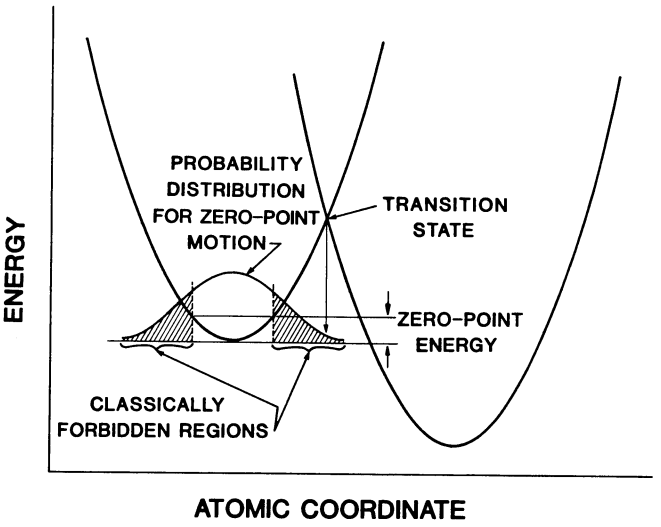

Fig. 2. A semi-classical picture of electron transfer as described in the text. The transition occurs at the crossing of the two potential surfaces (the "transition state"), and we explicitly note that this point can be reached by quantum zero-point motion, but this requires motion in a classically forbidden region tunneling.

temperature we can absorb m and emit $N + m$ phonons. Each absorption or emission process contributes a dimensionless electron-phonon coupling S defined below, Bose–Einstein factors for stimulated and spontaneous emission, and combinatorial factors since we don't care about the order of events. This leads us to guess

$$k \sim (---)e^{-S(2\bar{n}+1)} \sum_{m=0}^{\infty} \frac{S^m S^{N+m}}{m!(N+m)!} (\bar{n})^m (\bar{n}+1)^{N+m}, \qquad (1)$$

where $\bar{n} = (e^{-E_a/k_BT} - 1)^{-1}$ is the thermal mean number of phonons $e^{-S(2\bar{n}+1)}$ normalizes the probabilities for phonon emission and absorption, and (——) is everything we left out. Remarkably, in terms of temperature dependence eq. (1) misses very little: it indicates a T-independent rate for $\bar{n} \ll 1$ and asymptotes to Arrhenius behaviour with $E_a = (\varepsilon - S\hbar\omega)^2/4S\hbar\omega$ at $\bar{n} \gg 1$. Thus we can understand the Arrhenius law as a result of stimulated phonon emission, with no reference to the transition state!

If the activation energy calculated by counting multiphonon absorption and emission processes is to agree with the semi-classical picture then the electron–phonon coupling must be

$$S^{1/2} = \frac{1}{2}\left(\frac{\text{displacement between reactants and products}}{\text{R.M.S. zero} - \text{point displacement fluctuations}}\right). \qquad (2)$$

To get a feeling for orders of magnitude, consider the lowest stretching mode of an alpha-helical protein segment. Based on computer simulations [14] and Brillouin scattering experiments, [15] this mode may be understood by taking the helix to be a uniform elastic rod of Young's modulus $Y = 2 \times 10^{10}\,N/m^2$, density $\varrho = 1.3 \times 10^3\,kg/m^3$, and area $A = 5 \times 10^{-19}\,m^2$. The vibrational frequency is $\omega = (Y/\varrho)^{1/2}L^{-1}$, with L the length of the helix; in conventional units we have $\hbar\omega \sim 20\,cm^{-1}$ $(10\,Å/L)$. The zero-point motion (fluctuation in helix length) for this mode is $\delta Q^2_{z.p.} = \hbar/2(\varrho AL)\omega \sim (0.046\,Å)^2$ independent of L. Strong electron-phonon coupling ($S \sim 1$) can thus be achieved with extremely small structural

changes ($\Delta Q \sim 0.1$ Å) between reactants and products; displacements of this order are observed crystallographically upon oxidation of cytochrome *c* [16], one of the participants in the DeVault–Chance reaction.

Qualitatively these arguments allow us to understand crossover from temperature independence to Arrhenius behaviour in terms of strong coupling between the electronic states of proteins and some important phonon mode. Can we make a quantitative theory and address some of the issues summarized in the Introduction?

3. Generalized spin-boson models

We start simply by taking Fig. 2 literally. A single normal coordinate Q with frequency ω is coupled to a two-state system or effective spin $\frac{1}{2}$ such that the equilibrium position of Q depends on the electronic state; spin up will be identified with the reactants, spin down with the products. Adding the energy gap and the matrix element, we have the Hamiltonian

$$H = \frac{\varepsilon}{2}\sigma_z + V\sigma_x + \tfrac{1}{2}[\dot{Q}^2 + \omega^2(Q - \tfrac{1}{2}g\sigma_z)^2]. \tag{3}$$

This model still does not describe irreversible approach of σ_z to its equilibrium value; rather eq. (3) predicts that energy flows coherently back and forth between electron and phonon degrees of freedom. A related pathology is that at least for small V the spectral functions of Q – which are measured in infrared or Raman spectroscopy – are infinitely sharp. At the very least we must add to our model some degrees of freedom which are responsible for vibrational relaxation of Q, and it will be these "heat bath coordinates" which ultimately absorb the excess electronic energy.

The simplest description of vibrational relaxation is that Q is subject to linear frequency-dependent damping $\gamma(\Omega)$. By this we mean that the classical Langevin equation of motion for Q is

$$\frac{\mathrm{d}^2Q(t)}{\mathrm{d}t^2} + \int \frac{\mathrm{d}\Omega}{2\pi} \mathrm{i}\Omega\gamma(\Omega) \int \mathrm{d}t' \, \mathrm{e}^{-\mathrm{i}\Omega(t-t')}Q(t') + \omega^2 Q(t) = F(t) + \delta F(t), \tag{4}$$

where $F(t)$ is an externally applied force and $\delta F(t)$ is chosen from a stationary Gaussian ensemble of functions with spectral density

$$S_F(\Omega) \equiv \int \mathrm{d}\tau \, \mathrm{e}^{+\mathrm{i}\Omega\tau}\langle \delta F(t+\tau)\,\delta F(t)\rangle = 2k_B T \,\mathrm{Re}\,\gamma(\Omega). \tag{5}$$

Following Senitzky [17], we can construct a microscopic model of dissipation by coupling Q to some generalized coordinate of the heat bath, so that

$$H = \frac{\varepsilon}{2}\sigma_z + V\sigma_x + \tfrac{1}{2}[\dot{Q}^2 + \omega^2(Q - \tfrac{1}{2}g\sigma_z)^2] + QX + H_{\mathrm{bath}}(X). \tag{6}$$

The dynamics of this model reproduces these of eq. (4) if $X(t)$ responds linearly to its conjugate force and if the response function (determined by the details of H_{bath}) is chosen as $\tilde{\chi}(\Omega) = \mathrm{i}\Omega\gamma(\Omega)$. Since we are only concerned with linear response of the bath we are free to use any description of the bath coordinates which generates the correct dissipative coefficient; as emphasized by Caldeira and Leggett [18] this implies that we can model the bath as a collection of harmonic oscillators:*

$$H \to \frac{\varepsilon}{2}\sigma_z + V\sigma_x + \tfrac{1}{2}[\dot{Q}^2 + \omega^2(Q - \tfrac{1}{2}g\sigma_z)^2] + \frac{1}{2}\sum_\mu [\dot{X}_\mu^2 + \omega_\mu^2(X_\mu - C_\mu Q)^2], \tag{7}$$

where the dissipative coefficient is identified as

$$\gamma(\Omega) = (-\mathrm{i}\Omega)\lim_{\delta\to 0^+}\sum_\mu \frac{(C_\mu\omega_\mu)^2}{\omega_\mu^2 - (\Omega + \mathrm{i}\delta)^2}. \tag{8}$$

The Hamiltonian of eq. (7) is a special case of the spin-boson model which has recently been extensively studied in connection with macroscopic quantum phenomena.† In contrast to the macroscopic case, we are primarily concerned with systems in which $\varepsilon \gg k_B T, V, \hbar\omega$, and where the spectral density of Bose variables retains a resolvable feature at ω rather than being smooth from $\Omega \to 0$ to some cutoff $\Omega_C \gg \omega$. Furthermore, eq. (7) is but the simplest example of a whole family of generalized spin-boson models for reaction rates in proteins: not only can several normal modes of the molecule be displaced in the transition from reactants to products, these modes can change frequency and rediagonalize, and even their couplings to the heat bath may depend on the electronic state. These additional effects result in spin-boson couplings quadratic in the Bose operators (i.e., $\sim\sigma_z Q_\alpha Q_\beta$) rather than linear coupling ($\sim\sigma_z Q$) as in the standard model; while such terms arise naturally in a molecular context (vibrational spectra of reactants and products are usually different!), they do not seem to have any simple macroscopic analog. Some progress in defining and solving these more general models has been made, but here we focus primarily on the simplest case, eq. (7).

We should say at the outset that the dynamics generated by eq. (7) are not completely understood. Everything discussed here is true for "small V" (perturbatively),‡ and there are several arguments available for how small is small – some of these arguments are themselves approximate, however, and the situation is not completely clear.

After all this prologue, we obtain the order V^2 rate constant k for $|\sigma_z = \uparrow\rangle \to |\sigma_z = \downarrow\rangle$ from eq. (7) by a simple argument [25]. First transform to "physical phonons" – elementary excitations whose energy (at $V = 0$) is indepen-

* Strictly speaking, Caldeira and Leggett argued for a much more general representation of the bath, still in terms of oscillators but with non-linear couplings to Q. Our case is their "strictly linear dissipation", which is much simpler. An alternative argument for this description of dissipation in biomolecules is given in ref. [19].

† Some recent papers are refs. [20–23]. For a systematic review see Leggett et al. [24].

‡ This is often called the "non-adiabatic" limit, since for small V the Born–Oppenheimer (adiabatic) approximation to eq. (3) breaks down. We eschew this terminology because the criteria for Born–Oppenheimer breakdown are in general different from the criteria for perturbative calculation of the rate constant [25]. Furthermore, there are situations [26] in which the rate is non-perturbative in V but the dynamics of eq. (7) do *not* correspond to damped motion on the ground adiabatic potential surface of eq. (3). Finally, when an effective two-level system is obtained by truncating the full molecular electronic Hamiltonian, the validity of the adiabatic approximation for the model Hamiltonian may have nothing to do with the validity of the Born–Oppenhemier approximation for the real molecule.

dent of σ_z:

$$Q \to Q + \tfrac{1}{2} g\sigma_z \qquad x_\mu \to x_\mu + \tfrac{1}{2} C_\mu g\sigma_z.$$

This transformation is carried out with

$$U = \exp\left\{+ \mathrm{i}\frac{g\sigma_z}{2\hbar}(\dot{Q} + \Sigma_\mu C_\mu \dot{x}_\mu)\right\}$$

and gives

$$\begin{aligned} U^+HU &= \frac{\varepsilon}{2}\sigma_z + \tfrac{1}{2}[\dot{Q}^2 + \omega^2 Q^2] \\ &\quad + \frac{1}{2}\sum_\mu [\dot{x}_\mu^2 + \omega_\mu^2 (x_\mu - C_\mu Q)^2] \\ &\quad + V(\sigma_+ F + \sigma_- F^+), \end{aligned} \tag{9}$$

where

$$F = \exp\left\{+ \mathrm{i}\frac{g}{\hbar}(\dot{Q} + \Sigma_\mu C_\mu \dot{x}_\mu)\right\}.$$

This Hamiltonian is equivalent to a spin one-half in a static magnetic field ($\sim \varepsilon$) along $\hat{z}$ and a fluctuating magnetic field ($\sim VF$) in the $\hat{x} - \hat{y}$ plane. If we are interested in dynamics on time scales much longer than the correlation time for the fluctuating field — if the reaction rate is much slower than the vibrational relaxation rate — then the conventional theory of relaxation in magnetic resonance applies, and for $\varepsilon \gg V\langle F\rangle$ we have

$$k = \frac{2V^2}{\hbar^2}\int \mathrm{d}\tau\, \mathrm{e}^{+\mathrm{i}\varepsilon\tau/\hbar}\langle F^+(\tau)F(0)\rangle. \tag{10}$$

The correlation function $\langle F^+(\tau)F(0)\rangle$ can be related to the response functions of Q by standard methods, and we find

$$\begin{aligned} k = \frac{2V^2}{\hbar^2}\int \mathrm{d}\tau \exp\Bigg\{ &+ \mathrm{i}\frac{\varepsilon\tau}{\hbar} - \mathrm{i}\frac{g^2}{\hbar}\int\frac{\mathrm{d}\Omega}{2\pi}\sin(\Omega\tau)\frac{\gamma'(\Omega)}{\Omega} \\ &\times\frac{\omega^4}{|-\Omega^2 - \mathrm{i}\Omega\gamma(\Omega) + \omega^2|^2} - \mathrm{i}\frac{g^2}{\hbar}\int\frac{\mathrm{d}\Omega}{2\pi}[1 - \cos(\Omega\tau)] \\ &\times\frac{\gamma'(\Omega)}{\Omega}\,\frac{\omega^4\coth(\hbar\Omega/2k_BT)}{|-\Omega^2 - \mathrm{i}\Omega\gamma(\Omega) + \omega^2|^2}\Bigg\}, \end{aligned} \tag{11}$$

with $\gamma'(\Omega) = \mathrm{Re}\,\gamma(\Omega)$. Note that even for Ohmic dissipation [$\gamma(\Omega) = \gamma$, a constant] there are no divergences at either high or low frequency, although we shall see that other things go wrong in this case. The dimensionless coupling $S = g^2\omega/\hbar$.

Several approaches to evaluating the integral in eq. (11) may be found in the literature Straightforward power-series expansion in $g^2 \sim S$ gives us a rigorous version of eq. (1), with separate terms for each of the possible multiple phonon absorption and emission events. This expansion allows us to look very carefully at a highly non-classical effect, namely the resonant dependence of the reaction rate on the ratio $\varepsilon/\hbar\omega$. When this quantity is close to an integer the rate is enhanced; when it is "far" from integral the rate is suppressed. A very different approximation allows us to see the smooth part of $k(\varepsilon)$, although it discards the resonances entirely, and this is a short-time expansion of the exponent in eq. (11). If this expansion is carried to $O(\tau^2)$ we find that $k(\varepsilon)$ is Gaussian; this is called the "energy-gap law" and is the basis for analysis of a wide variety of data on electronic transitions in molecules and solids [27]. Each of these approaches given sensible results only if $\gamma(\Omega) \to 0$ as $\Omega \to \infty$, that is if the dynamics of the heat bath have some finite correlation time.

If a large number of modes are independently coupled to the reaction then the single correlation function $\langle F^+(\tau)F(0)\rangle$ is replaced by a product $\Pi_\mu\langle F_\mu^+(\tau)F_\mu(0)\rangle$ of correlation functions, one from each mode. Under these conditions we can use the central limit theorem to justify the short-time expansion and hence the energy-gap law provided that certain convergence criteria are met [25]. These conditions are equivalent to demanding that $\gamma(\Omega)$ vanish sufficiently rapidly as $\Omega \to \infty$, as above.

The energy-gap law gives the same form for the reaction rate as Hopfield's semi-classical analysis schematized in Fig. 2. We can make a more systematic approximation by noting that eq. (11) is of the form

$$k = \frac{2V^2}{\hbar^2}\int \mathrm{d}\tau\, \mathrm{e}^{\mathrm{i}S(\tau)/\hbar}, \tag{12}$$

so that as $\hbar \to 0$ the integral is dominated by τ's near saddle points τ_S, $S'(\tau_S) = 0$. This calculation again hinges on the behaviour of $\gamma(\Omega \to \infty)$: in the absence of a high-frequency cutoff $S(\tau)$ cannot be analytically continued into the complex τ-plane, which means that we cannot deform the contour of integration to pass through τ_S. If we introduce a cutoff the approximation can be made to work and the leading terms are essentially cutoff-independent. With this caveat, the saddle-point method gives a remarkably good picture of $k(\varepsilon)$, including both the smooth energy-gap law results and the quantum resonances; details of the saddle point calculations and a comparison with direct numerical integration will be given elsewhere.

If the phonon mode is underdamped the integral in eq. (11) has multiple saddle points spaced approximately by $\Delta\tau_s \sim 2\pi/\omega$. Focusing on $\tau_s^{(0)}$ — the saddle point closest to the origin — we find the energy-gap law with quantum corrections,

$$\begin{aligned} k^{(0)}(\varepsilon) &\sim A\,\mathrm{e}^{-G} \\ G &\sim \frac{(\varepsilon - S\hbar\omega)^2}{2S(\hbar\omega)^2(2\bar{n}+1)} - \frac{(\varepsilon - S\hbar\omega)^3}{6S(\hbar\omega)^3(2\bar{n}+1)^5} \\ &\quad \times[3 + (2\bar{n}+1)^2] + \cdots \\ A &\sim (2V^2/\hbar)[2S(\hbar\omega)^2(2\bar{n}+1) + \cdots]^{-1/2}, \end{aligned} \tag{13}$$

where these and all other results are for $\gamma(\omega)/\omega \ll 1$.

To understand this as a semi-classical expansion note that ε, $\lambda = S\hbar\omega$, and k_BT are energies which are finite as $\hbar \to 0$, while of course $\hbar\omega$ is not. We have

$$\lim_{\hbar\omega\to 0} G \sim \frac{(\varepsilon - \lambda)^2}{4\lambda k_BT} - \frac{(\varepsilon - \lambda)^3}{48\lambda^2 k_BT}\left(\frac{\hbar\omega}{k_BT}\right)^2 + \cdots,$$

so that these first quantum corrections to the energy-gap law are significant for

$$\hbar\omega/k_BT \sim 2\left(\frac{3\lambda}{\varepsilon - \lambda}\right)^{1/2}.$$

What does this mean? Suppose that we can go into the molecule and change only quantum energies $\sim \hbar$ while keeping classical energies $\sim \lambda$, ε, k_BT fixed. Then the rate constant changes by

$$|\Delta(\ln k)| \sim \frac{\Delta(\hbar\omega)}{(\hbar\omega)}\cdot\frac{|\varepsilon - \lambda|^3}{24\lambda^2 k_BT}\left(\frac{\hbar\omega}{k_BT}\right)^2.$$

When

$$\hbar\omega/k_BT \sim 2\left(\frac{3\lambda}{\varepsilon - \lambda}\right)^{1/2}, \quad |\Delta(\ln k)| \sim \frac{\Delta(\hbar\omega)}{(\hbar\omega)}\cdot\frac{(\varepsilon - \lambda)^2}{2\lambda k_BT},$$

so a given fractional change in the quantum energy has the same significance as the same fractional change in temperature. Obviously these effects are of greatest interest when $\varepsilon > \lambda$; otherwise the criterion for quantum effects to be significant in $k^{(0)}(\varepsilon)$ is just $\hbar\omega/k_B T \sim 1$.

Additional saddle points give smooth functions $k^{(n)}(\varepsilon)$ multiplied by $\sim\cos(2\pi n\varepsilon/\hbar\omega)$. The inclusion of saddle points further and further away from $\tau = 0$ thus gives us a picture of $k(\varepsilon)$ at increasingly higher resolution (as we expect from time-energy uncertainty) and at improved resolution "ripples" are evident at energy gaps which meet the quantum resonance condition. At $T = 0$ terms $\sim\cos(2\pi n\varepsilon/\hbar\omega)$ are attenuated by $\sim\exp(-2\pi n\gamma\varepsilon/\hbar\omega^2)$, so the significance of the quantum effects depends both on a conventional "classicalness" parameter $\varepsilon/\hbar\omega$ and on a purely dynamical parameter γ/ω: for appropriate dynamics quantum effects can be significant even if the energy quanta are very small.

The precise criterion for attenuation of the quantum ripples at finite temperature is complicated to exhibit in closed form. If we expand in $\varepsilon - \lambda$, as in eq. (13), the lowest order result is an attenuation of terms $\sim\cos(2\pi n\varepsilon/\hbar\omega)$ by $\sim\exp(-2\pi nS\gamma k_B T/\hbar\omega^2)$, so the reaction is fully classical only if $2\pi S(\gamma k_B T/\hbar\omega^2) \gg 1$. If we imagine that the structural change between reactants and products consists of a ~ 0.3 Å lengthening of a ~ 10 Å segment of alpha helix, then from above we have $\hbar\omega \sim 20\,\mathrm{cm}^{-1}$ and $S \sim 10$, so that quantum effects are significant at 300 K unless $\gamma \gg 6 \times 10^9\,\mathrm{s}^{-1}$. In fact, $\gamma < 10^9\,\mathrm{s}^{-1}$ has been directly observed [15] at 300 K for $\hbar\omega \sim 0.3\,\mathrm{cm}^{-1}$ acoustic modes of alpha helical polymers.* Thus even though $k_B T \sim 10\hbar\omega$ and the structural changes are $\sim$ ten times the quantum zero-point motion, the molecule need not behave classically! This conclusion is closely related to the fact that measurements on a mechanical system at frequency ω can be limited by quantum noise even when $k_B T \gg \hbar\omega$ provided that the damping γ is sufficiently small [29]. We emphasize that these comments refer to what is *possible*; whether these extreme quantum effects are significant for the functional behaviour of particular proteins remains to be seen.

To summarize, the basic semi-classical result

$$k_{\mathrm{s.c.}} \sim \frac{2V^2}{\hbar[2S(\hbar\omega)^2(2\bar{n}+1)]^{1/2}} \times \exp[-(\varepsilon - S\hbar\omega)^2/2S(\hbar\omega)^2(2\bar{n}+1)] \qquad (14)$$

provides the skeleton on which we hang all the quantum corrections. These consist both of deviations from the energy-gap law in the "wings" of the Gaussian and of ripples which come from resonance effects. The quantum effects allow the rate constant to depend on the vibrational frequency even when all classical energies are held fixed, which means that quantum mechanics provides a pathway for control of the reaction rate not present in the classical limit.

Even in the classical limit the reaction rate can be extremely sensitive to dynamical parameters of the molecule. Thus when ω changes (e.g., from molecule to molecule in an inhomogeneously broadened ensemble – see below) $\lambda = S\hbar\omega$ will change unless the frequency shift was produced purely by an effective mass added to that mode alone. If the system operates in the tail of the energy-gap law – so the activation energy is much larger than $k_B T$ – small changes in λ have enormous effects on the rate constant.

How does this story change when we consider molecules with many vibrational modes? As noted above, there are some conditions under which the accuracy of the semi-classical approximation imroves as we add more modes. This is only true, however, in the limit of asymptotically large numbers of modes; with a small number of modes the addition of one more introduces new quantum effects associated with resonances at multiples of the new phonon energy. Since data is scarce, our strategy in rationalizing experiments on proteins is to start with a single-mode model and build up. *Caveat emptor.*

4. The data revisited

In the fully semi-classical limit of eq. (14), the rate constant is determined by four parameters: the electronic energy gap ε and matrix element V, the vibrational frequency ω and the coupling S. As noted in Fig. 1, the DeVault–Chance data can be summarized by three parameters, and we will take $\varepsilon = 0.45$ eV from electrochemical experiments [1]. All the parameters of the model can thus be determined (Table I), although two very different coupling constants are in fact consistent with the data.

Are the parameters of Table I reasonable? Certainly $\hbar\omega \sim 200\,\mathrm{cm}^{-1}$ is a region where modes involved in active site dynamics have been identified in Raman spectra of cytochrome *c* and related proteins [30]. From the discussion above $S \sim 6$ corresponds to structural changes ~ 0.25 Å between reactant and products, which is what one sees crystallographically [16]; $S \sim 60$ (the other possibility) would seem unlikely in light of these data. It should be emphasized that the crystallographic results came as something of a surprise, since most analyses of cytochrome electron transfer based on chemical analogies had suggested much larger "conformational changes", and unrefined crystal structures tended to support this conclusion.

Table I. *Parameters for a quasi-harmonic theory of the DeVault– Chance reaction*

Parameter	Undercoupled case	Overcoupled case
ε	0.45 eV	0.45 eV
$\hbar\omega$	$185\,\mathrm{cm}^{-1}$	$193\,\mathrm{cm}^{-1}$
S	6.1	60.8
V	8×10^{-5} eV	1.4×10^{-4} eV

* Clearly the frequency dependence of vibrational relaxation rates is of critical importance in applying the Brillouin scattering results of ref. [15] to more typical globular proteins. In particular, Kosic et al. [28] have found very strong frequency and temperature dependences of phonon lifetimes in amino acid crystals, and they use this result to argue that long phonon lifetimes are impossible in proteins at interesting temperatures. The Brillouin scattering results on both dry and hydrated helices suggest that the dominant relaxation pathways are internal to the polymer, presumably the breakup of one phonon into two. For quasi-one-dimensional structures like the alpha helix the frequency and temperature dependence of this relaxation rate is much less severe than that described by Kosic et al. Normal mode calculations on the small protein BPTI [43] show the density of vibrational states has a very weak frequency dependence at low frequency, which again suggests that two-phonon breakup will be rather weakly frequency and temperature dependent. From these and other arguments [19] we see no reason to doubt that the long phonon lifetimes *observed* in model systems may be relevant to globular proteins.

Hopfield [31] pointed out that these theories of electron transfer have an unambiguous spectroscopic signature – in addition to the normal reaction, which is a *radiationless* transition, there should be a radiative transition in which photon absorption is associated with prompt electron transfer. This absorption takes place between the two electronic states which represent reactants and products, so that the position and shape of this "charge-transfer band" depend on the same electron–phonon couplings that control the reaction rate. This band has been found [32, 33] for the DeVault–ted Chance reaction, and its properties are those expected from theory with the "undercoupled" ($S \sim 6$) parameters of Table I.

The search for the charge-transfer band is the first instance in which the dynamical theory of reaction rates in proteins has been put to an unambiguous and non-trivial test. It is somewhat remarkable that – for the DeVault–Chance reaction – the very simplest example of the general theory allows us to rationalize the functional (i.e., kinetic) behaviour of a protein in terms of a dynamical model which is in turn consistent with direct and indirect spectroscopic tests, and even the once puzzling structural data fit neatly into the picture. There are several indications that the simplicity will not extend to all reactions, even in bacterial photosynthesis, as will become evident below.

The basic pattern of temperature independence at low temperature and activated kinetics at high temperature can also be seen in the binding of small ligands [oxygen (O_2), carbon monoxide (CO), . . .] to heme proteins [hemoglobin (Hb), myoglobulin (Mb), . . .], but these reactions exhibit many new features. The binding of CO to Mb [7], for example, can apparently be resolved into several steps, of which the first involves the ligand breaking through the solvation shell of the protein and the last can be isolated by looking only at temperatures $T \leqslant 160\,\mathrm{K}$, where the CO is trapped in the neighborhood of the active site (heme pocket). Experimentally these reactions can be initiated by flash photolysis from the liganded (Mb · CO) state, and the progress of the rebinding reaction (return of the ligand to the active site iron) can be followed spectroscopically over a wide range of time scales. The most striking observation is that the time course of the rebinding reaction is not exponential. Although no single rate constant describes the rebinding, we can arbitrarily define a "rate" as the inverse of time required for the reaction to reach 25% completion [8]. A plot of the logarithm of this rate vs. $1/T$ gives results qualitatively similar to those for the DeVault–Chance reaction, but the temperature dependence continues to lower temperature, $T_0 \sim 20\,\mathrm{K}$.

Non-exponential kinetics can be explained by (1) each molecule is non-exponential and all molecules are identical, or (2) each molecule is exponential but different molecules have different rate constants. "Each molecule is exponential" means that it has a constant probability per unit time of reacting and hence no memory of the time at which photolysis occurred; if we give a periodic series of flashes there will be no memory of when the first flash occurred and therefore the kinetics will be periodic. Experimentally one sees the predicted periodicity [7]. No finite experiment can prove that each molecule is *exactly* exponential, but the available data place stringent bounds on the extent of non-exponential behaviour in single molecules [34].

To summarize, the data indicate that heme proteins in frozen solution exhibit a temperature dependent *distribution* of reaction rates $P(k|T)$, so that experiments on an ensemble of molecules yield the non-exponential reaction time course

$$N(t;\,T) \;=\; \int_0^\infty \mathrm{d}k\; P(k|T)\,\mathrm{e}^{-kt}. \tag{15}$$

The question raised by this result is clear: What is the physical origin of the rate constant distibution? This is a biologically interesting question because freezing the solution has apparently trapped myoglobin molecules into states or environments which endow the protein with different functional behaviour, i.e., different reaction rates. If we have a workable theory of how protein function is controlled we must be able to explain how the presumably subtle differences among molecules of the experimental ensemble can generate reaction rates which differ by many orders of magnitude.

We have seen that reaction rates can depend sensitively on protein vibrational frequencies, both in the classical limit and as a result of quantum effects. Molecules in solution often have a *distribution* of vibrational frequences, and in some cases the width of this distribution can be understood [35] in terms of interactions with the "solvent shell" around the solute molecule – each solvent molecule in the shell contributes to a sovlent shift of the vibrational frequency, and the number of molecules in the shell fluctuates as they exchange with the bulk. For proteins the size of myoglobin there are ~ 100 "bound waters" in the solvation shell [36, 37], and at room temperature [38] they exchange among the available sites at $\sim 10^9\,\mathrm{s}^{-1}$. This time scale is long compared to the vibrational relaxation times of most Raman or infrared-active modes, so these spectroscopies see an inhomogeneously broadened ensemble, but most chemical reactions are sufficiently slow that one observes a rate constant averaged over the ensemble. The situation changes dramatically if the solvent is frozen or otherwise solidified (e.g., by embedding in the plastic PVA), at which point the time for exchange among solvent binding sites can become macroscopically long. Under these conditions the reaction proceeds at a rate $k(\omega)$ for molecules with vibrational frequency ω, but the distribution of ω is essentially static. We will observe the non-exponential reaction time course

$$N(t) \;=\; \int \mathrm{d}\omega\; P(\omega)\,\mathrm{e}^{-k(\omega)t}, \tag{16}$$

where $P(\omega)$ is the (normalized) inhomogeneous lineshape. If a large number of solvent molecules contribute to the broadening then $P(\omega)$ is Gaussian with mean $\bar{\omega}$ and standard deviation $\Delta\omega$.

We have tried [19] to interpret the Mb–CO data in terms of eq. (16) – essentially we are asserting that "dynamical specificity", an extreme dependence of the reaction rate on vibrational frequency, in fact occurs in the system and that freezing the solution allows us to see more of this specificity by stopping the protein from averaging over its frequency distribution. Again we try a single mode model, and by analogy with the DeVault–Chance reaction we see that temperature dependence down to $T \sim 20\,\mathrm{K}$ requires $\hbar\omega \sim 20\,\mathrm{cm}^{-1}$. A candidate for such a low-frequency mode is the breathing of the F alpha helix – this segment contains the only covalent link to the active site iron, and it is known from crystallography that ligand binding induces structural changes throughout the F helix [36]. From the experiments and simulations discussed above we can make rough esti-

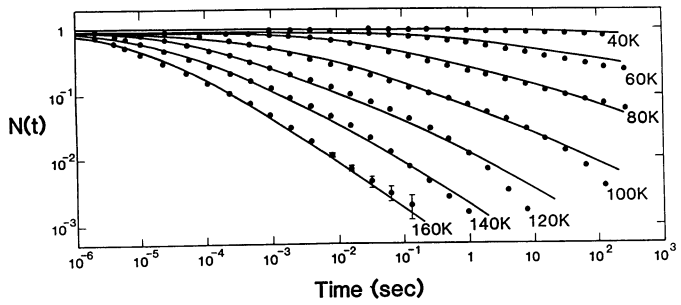

Fig. 3. A single mode simulation of the last step in CO binding to Mb, as described in the text. Data points from ref. [7] and theoretical lines from ref. [19].

mates of the properties of this mode; we take $\hbar\omega = 20\,\mathrm{cm}^{-1}$, $\hbar\gamma = 0.01\,\mathrm{cm}^{-1}$, and note that very large coupling constants ($S \sim 50$) are consistent with the crystallographic data – even larger coupling constants may be relevant since we are approximating all the structural changes by motion along a single mode. To complete our model we need the energy gap, which we take as $\varepsilon = 0.9\,\mathrm{eV}$ from ref. [7], the matrix element V – which sets the absolute scale of the reaction time course but is not involved with either temperature dependence or non-exponentiality – and the inhomogeneous linewidth $\Delta\omega$. In practice the only two parameters which must be fit to the temperature-dependent time course are S and $\Delta\omega$.

The results of this single-mode simulation are shown in Fig. 3, with $S = 170$, $V = 1.1\,\mathrm{cm}^{-1}$, and $\hbar\Delta\omega = 1.6\,\mathrm{cm}^{-1}$. A simple dynamical model again provides a successful rationalizing of the kinetic data; the essential feature of this rationalization is strong coupling to a low frequency ($\sim 20\,\mathrm{cm}^{-1}$) vibrational mode. Recent experiments [39] on Mb · CO have shown that the means-square displacement of the iron atom, as inferred from the recoil-free fraction in the Mössbauer spectra, exhibits a temperature dependence from 4 K to 100 K consistent with strong coupling to a single mode at $25\,\mathrm{cm}^{-1}$. A $25\,\mathrm{cm}^{-1}$ mode is also visible in the temperature dependence of the electric field gradient at the iron nucleus (quadrupole splitting), so this mode can apparently modulate the crystal field and hence couple to spin changes as required for ligand binding.

The low frequency mode which is essential to our analysis of the kinetic data may also be relevant to anomalies in the Mössbauer spectra of myoglobin at higher temperature. In addition to the usual Mössbauer process – which is a "vacuum-to-vacuum" transition – there are quasi-elastic processes which involve the coherent absorption and emission of equal numbers of phonons so that there is no net recoil but only a broadening of the line by $\sim\gamma$, the vibrational relaxation rate. Again using the "typical" alpha helix parameters discussed above, we found [19] that this mechanism could generate the observed [40, 41] broad-line components of the Mössbauer spectrum in the $\gtrsim 200\,\mathrm{K}$ range. The more recent low temperature experiments directly measure an essential parameter of this description, namely the magnitude of coupling between the low frequency mode and iron atom displacement, and this measurement is in good agreement with our theoretical estimate. Taken together, the "high"- and low-temperature Mössbauer data – which might have been expected to probe dynamical processes on very different time/energy scales – can be understood surprisingly well in terms of a very simple model of active site dynamics, one in which the low energy excitation spectrum is dominated by single narrow phonon mode at $\hbar\omega \sim 25\,\mathrm{cm}^{-1}$. This picture could be tested much more quantitatively if the broad-line components were detected in Mb · CO or Mb · O_2, where the careful low-temperature experiments have been done and where there are no complications due to spin degrees of freedom.

Further analysis of the low temperature Mössbauer data [39] allows us to draw some remarkable if tentative conclusions. Specifically, the $25\,\mathrm{cm}^{-1}$ mode in Mb · CO saturates $\sim 29\%$ of the relevant sum rule for iron atom motion in one dimension. A second mode at $220\,\mathrm{cm}^{-1}$ (also identified in Raman spectra [42] as the stretching of the active site iron against the His of the F Helix) was detected by its contibution to the temperature dependence of the 2nd-order Doppler shift, and this saturates $\sim 65\%$ of the relevant sum rule. Most importantly, no other significant discrete modes were found, and with some care stringent bounds could probably be placed on the contribution of a continuum background. This is astounding because the density of states of the molecule as whole almost assuredly does not have such sharp features – see for example the normal mode calculations on the small protein BPTI [43] and the neutron scattering spectra of hexokinase [44]. Other modes likely to couple to the iron atom are motion of the C and O relative to Fe ($577\,\mathrm{cm}^{-1}$ and $512\,\mathrm{cm}^{-1}$) [45] and motion of Fe relative to the heme nitrogens (controversial, but probably ~ 300–$500\,\mathrm{cm}^{-1}$), but all of these are above the energy range which can be probed by temperature-dependent Mössbauer spectroscopy; again the key result is the apparent existence of sharp features in the low energy region. It would seem that the protein must be "engineered" so that active site dynamics are dominated such narrow spectal features – or equivalently by a small number of modes – rather than by the featureless continuum. This situation, for which the Mössbauer data provide the first evidence, is exactly what we require for maximum "dynamical specificity" in the sense outlined above.

5. Outlook

The analyses of the DeVault–Chance reaction and Mb · CO binding reviewed here are encouraging. They indicate that generalized spin-boson Hamiltonians have a chance to describe important dynamical factors in the control of reaction rates in proteins and to provide a framework within which increasingly sophisticated spectroscopic data can be assimilated and related to the functional behaviour of these molecules. It is not at all clear, however, that the basic assumptions behind these models have been put to a serious test. These key assumptions are (1) that a small number of electronic states are sufficient for the description of interesting chemical reactions and (2) that all non-electronic degrees of freedom may be described by a set of weakly interacting, damped phonon modes; in the analysis above we have used rather more stringent versions of these assumptions, but this is not essential. We consider these ideas in turn:

(1) Truncation of the full molecular electronic Hilbert space to a small, discrete subspace is a tricky business. Sethna [46] was probably the first to emphasize the dangers of the truncation approximation for tunneling of impurities in solids. Macroscopic quantum mechanics problems have provided the motivation for careful justification of this approximation

in selected cases [24, 47, 48], although as emphasized by Chakravarty and Kivelson [47] these arguments demonstrate only that the truncated model generates the correct thermodynamics and not necessarily the correct real-time evolution; even disregarding this caveat it does not seem that the arguments in the literature are sufficient to justify or invalidate truncation in cases of relevance to biomolecules. Part of the problem is that the electronic configurations of interest are far from single-particle states – the different spin states of the iron atom in the reactants and products of the Mb · CO reaction, for example, are clearly strongly correlated multi-electron states.

The theoretical question of whether truncation of the electronic Hamiltonian provides a viable approximation scheme is related to a practical question about the interpretation of temperature dependent reaction rates. Specifically, we have considered here truncated models in which the phonons couple only to σ_z, and in these models the reaction rate factors (at least perturbatively) into a constant "electronic factor" $\sim V^2$ and a temperature dependent multi-phonon density of states. If truncation fails – or if rigorous truncation schemes lead to a coordinate-dependent matrix element $V(Q)$ – the factorization fails, and this means that most of our intuition about transition states and activation energies goes out the window. Again, there are fairly solid results in the cases of interest for macroscopic quantum mechanics [24], where such "non-Condon effects" have been shown to be neglibible. These arguments do not seem to tell us very much about the problems in biomolecules, and in the case of electron transfer some simple models suggest that non-Condon effects could be quite substantial [41].

It should be evident that the whole question of truncation in the electronic dynamics is open. No available results should be construed as invalidating this approximation, so it seems that truncation provides a good starting point for data analysis, but we are far from understanding its limitations. Would the breakdown of this approximation have clear experimental signatures?

(2) The description of protein dynamics within a single electronic state by a small set of weakly interacting, damped phonon modes is the subject of considerable controversy. Specifically, the Illinois group [7–11] has suggested that the myoglobin data should be interpreted in terms of a qualitatively different physical picture – the "conformational substates" model, in which the dynamics are dominated by diffusive hopping among different locally stable structures, rather than coherent vibrations around a single equilibrium structure as in the models considered here. Space does not permit a detailed comparison of these two points of view; we have tried to do this in ref. [12]. As emphasized by Stein [50] and by Ansari et al. [11], if the substates model is qualitatively correct then the physical issues in protein dynamics are closely related to physical issues in the dynamics of glasses and spin glasses – disordered systems. If the "quasi-harmonic" models discussed here are qualitatively correct, then as we have indicated the relevant issues are related to macroscopic quantum mechanics and the dynamics of crystalline solids – ordered systems. Clearly there are some philosophical questions – are biological systems ordered or disordered? – but perhaps these are best left to the reader.

Raman and infrared spectroscopies (and to some extent the temperature dependence of the recoil-free fraction in Mössbauer spectroscopy) probe primarily the single-phonon density of states. The interesting phenomena – chemical reactions, optical absorption – are multi-phonon processes. The essence of the models discussed here is that single phonon spectra can be used to understand multi-phonon events, and very different multi-phonon events can be related to one another. Do we have any experimental evidence which bears directly on these points?

In the case of the DeVault–Chance reaction we have two multi-phonon processes which can be compared, the (dark) electron transfer and the charge-transfer absorption band. The consistency of these two phenomena with the same quasi-harmonic model Hamiltonian is evidence that phonon-phonon interactions are qualitatively insignificant on the energy scales of relevance to the biological function of these molecules.

In the case of myoglobin, Mössbauer, Raman, and infrared spectroscopies define a discrete set of modes (at 25, 220, 512, 577, 1944 cm^{-1}) which are certainly involved in active site dynamics, and most importantly the Mössbauer results provide evidence against significant coupling to modes with $25 < \hbar\omega < 220\,cm^{-1}$. For $T < 160\,K$, where the final step of ligand binding is well isolated, this means that if a quasi-harmonic model is correct all of the temperature dependence in the ligand binding rate must be carried by the $25\,cm^{-1}$ mode. While the single mode simulations [19] of Fig. 3 are consistent with this idea, they do not provide a stringent test. The key is to do a realistic multi-mode simulation, and work toward this goal is in progress.

For systems with well resolved optical absorption spectra we can test the quasi-harmonic approximation by trying to reconstruct the absorption spectrum from phonon energies and electron-phonon couplings measured in Raman scattering; ideally this should be done as a function of temperature, to determine whether temperature-dependent spectrum shifts can be understood in terms of increasing phonon populations rather than more non-linear effects (e.g., a glass transition) [11, 50]. We note that for the visual pigment rhodopsin and for the primary donor of photosynthesis, understanding the optical absorption spectra is of more than academic interest. The possibility of "Raman reconstruction" of absorption spectra has received considerable attention in the literature [51], but there remain signficant theoretical problems in extracting the relevant information from the Raman specta. This is an area in which interaction between theory and experiment is already quite good; understanding will hopefully soon progress to the point where the very detailed experiments currently feasible can be used to test models of protein dynamics rather than our calculational abilities.

Whatever model may provide an appropriate description of protein dynamics, there are a number of remarkable phenomena which provide clear theoretical challenges. Since much discussion has been focussed on a relatively narrow set of experiments, it seems appropriate to conclude with some indication of the wonders which the biological world presents:

(1) Anomalous temperature dependence. The relatively simple pattern of temperature dependence observed by DeVault and Chance is apparently understandable in terms of very simple models. At least two other electron transfer steps in the photosynthetic reaction center* have temperature-

* Several reports have appeared very recently [52–55].

independent rates at below some T_0 but *slow down* at $T > T_0$; one of these reactions shows clear non-exponential decays, with the degree of non-exponentiality dependent upon the functional state of the reaction center. Although a variety of scenarios for this temperature dependence have been proposed, we do not feel that any of these can be considered convincing and certainly none has been put to a decisive test. The existence of this anomalous behaviour puts us on our guard about the indiscriminate use of the Arrhenius law in biomolecules. In the case of myoglobin, for example, the temperature dependent distribution of reaction rates at $T > 40\,\mathrm{K}$ has been modeled [7–11] as a temperature independent distribution of activation energies through $k \sim A\,\mathrm{e}^{-E_a/k_B T}$. This procedure can be made to work, but there is no direct evidence that each molecule in the ensemble obeys this relation; this is important because considerable significance has been attached [56] to the prefactor A which can be extraced from such an analysis. Although the story told in this review began nearly twenty years ago with the study of temperature dependent reaction rates, it is evident that we still do not fully understand how to go beyond the Arrhenius law in the analysis of experiments.

(2) Spin-phonon dynamics. Many biomolecules have metal atoms in non-trivial magnetic configurations which are important for biological function. In myoglobin Mössbauer results [39] show that the crystal field at the active site iron in Mb · CO is strongly modulated by a phonon mode at $\hbar\omega = 25\,\mathrm{cm}^{-1}$, which is comparable to the width of the spin-two quintent in free Mb [57] or in the photolyzed state [58] $(\mathrm{Mb}\cdots\mathrm{CO})^*_{S=2}$. This means that we can have resonantly enhanced spin–phonon interactions, and hence that the usual perturbative adiabatic elimination arguments [59] for the spin-Hamiltonian formalism may fail; this is another instance in which biomolecules present a very well understood problem from condensed matter in a new and ill-understood parameter regime. It is tempting to suggest that spin-phonon interaction may help us understand the remarkably broad zero-field transitions in free Mb [60], or help resolve the significant problems which arise in the analysis of high magnetic field Mössbauer spectra in Mb and Hb [61]. Since ligand binding to the heme proteins is accompanied by spin state changes at the iron atom, it would be difficult to conclude that we understand the reaction rate until we have a convincing analysis of these experiments which directly probe the spin degrees of freedom.

Photosynthesis provides another example in which spin dynamics are of functional significance. Magnetic field effects in the primary electron donor/ acceptor pair have been extensively studied [62, 63], and there have been attempts to relate (following Anderson's [64] theory of superexchange) the parameters which describe these effects to the more fundamental electronic matrix elements which control the electron transfer rate. While this works for one later step in the photosynthetic pathway [65] it fails for the primary step [66]. One way out of this dilemma is to postulate an intermediate acceptor, but the evidence for such an intermediate in the sence required to explain the magnetic effects is not what it used to be [67]. Another possibiltiy is that electron–phonon coupling (which was neglegible in the solid-state problem originally considered by Anderson) can significantly modify the relation between magnetic interactions and electronic matrix elements [68]. Although experiments on magnetic field effects in photosynthesis have progressed to considerable sophistication, these basic physical issues in the interpretation of such experiments remain to be understood.*

(3) Ultrafast reactions. The initial events of photosynthesis [1] and vision [69] occur on a picosecond time scale; the very low quantum yields for fluorescence from rhodopsin [70] and the broad holes which can be burned [71] in the spectrum of the primary donor of bacterial photosynthesis are suggestive of even faster events. This time scale is comparable to the vibrational relaxation times measured for chlorophyll in solid matrices (for example), so that during these events the molecules involved are not necessarily at the temperature of their environment. Depending in detail on some of the parameters for these reactions it is even possible that they occur before the relaxation mechanisms have a chance to destroy quantum mechanical coherence, which would have significant experimental (and conceptual) consequences [68, 72]. Again this is an example where a familiar process (photon-induced electronic transitions) have been driven, presumably by evolutionary pressure, into a regime where our intuition does not help us; serious theoretical issues must be resolved before we even know the correct language to describe these events. The impressive pace of spectroscopic and structural experiments on these systems – and many other biophysical systems not discussed – provides, as we hope has become clear, excellent opportunities for interaction between theory and experiment.

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