Biological Physics

Hans Frauenfelder

Center for Nonlinear Studies, Los Alamos National Laboratory, Los Alamos, New Mexico 87545

Peter G. Wolynes

Department of Chemistry, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801

Robert H. Austin

Department of Physics, Princeton University, Princeton, New Jersey 08544

Physics and biology have interacted for at least two centuries. In the twentieth century, this interaction has become very strong and the overlap between the two fields has expanded enormously. In the present contribution, the authors sketch where physics has influenced biology and where investigations on biological systems have led to new physical insights. [S0034-6861(99)03902-1]

CONTENTS

I. Introduction S419
II. The Structures of Biological Systems S420
A. From Röntgen to synchrotrons and NMR S420
B. Biological systems S420
C. Myoglobin, the hydrogen atom of biology S420
III. The Complexity of Biological Systems S421
A. Distributions S421
B. The energy landscape of biomolecules S422
C. The Debye-Waller factor S422
IV. Dynamics S422
A. Fluctuations and relaxations S422
B. Proteins and glasses S423
C. Protein folding S423
V. Reaction Theory S424
A. Dynamic effects in chemical reactions S425
B. Tunnel effects S425
C. Gated reactions S425
VI. Bioenergetics and Physics S426
A. Charge transport S426
B. Light transduction in biology S426
VII. Forces S427
VIII. Single-Molecule Experiments S428
IX. The Future S429
Acknowledgments S429
References S429

I. INTRODUCTION

Physics and biology have interacted at least since Galvani and physicists have always been intrigued by biological problems. Erwin Schrödinger's book (1944) led many physicists to study biology. Despite its inspirational character and its stressing the importance of biomolecules, many of the detailed ideas in the book proved to be wrong and have had a limited impact on mainstream molecular biology. The connection between physics and biology has also been treated quite early in the *Reviews of Modern Physics*. In 1940, Loofbourow (1940) described the application of physical methods. Oncley *et al.* (1959) edited a study program on biophysical science; the report written by many outstanding scientists is still worth reading.

In the foreword to his article, Loofbourow wrote: "*I was tempted to use the title 'Biophysics' for this review as more succinctly delimiting the field discussed. But despite the obviously increasing interest in biophysical problems, there does not seem to be clear agreement, even among biophysicists, as to what the term biophysics means*." This confusion still exists. Here we use Stan Ulam's remark ("*Ask not what physics can do for biology, ask what biology can do for physics*") and define biological physics as the field where one extracts interesting physics from biological systems. Much like the terms physical chemistry and chemical physics, the terminological differences represent only psychological style and current attitude; the same person at different times could be thinking as a biophysicist or as a biological physicist.

The connection between biology and physics is a two-way street. However, the heavy traffic has gone one way. Many tools from physics have been adopted by researchers in the biological sciences. The return traffic, where biological ideas motivate physical considerations, has been less visible, but the study of biological systems has already led to some interesting results, particularly concerning the physics of complexity and of disordered systems. Here we focus on biological physics at the molecular level. However, biological physics is much broader. Organismal physiology has inspired much work, for example, the study of neural networks (J. Hertz *et al.*, 1991) and immunology (Perelson and Weisbuch, 1997). The mathematics of evolution and population biology has attracted much attention by theoretical physicists who have pioneered a mutually beneficial connection with computational and statistical physics.

Biophysics and biological physics cover an enormously broad field, and offer an exciting future. Unlike Janus, the Roman god who could see both to the past and the future, the present authors only know what has happened and even then have a limited view of that terrain. For the present review, in the spirit of the centennial celebration, we have looked mostly to the past and what has been successful. More than fifty years ago,

Loofbourow (1940) cited 1203 references in biological physics. Since then, the number of papers has increased nearly exponentially with a time constant of about 15 years. We had to make a biased selection in order to present a coherent story and so a great deal of important work is not mentioned. We cite reviews rather than original papers wherever possible but urge the reader to consult other books (Flyvbjerg *et al.*, 1997; Peliti, 1991) which present a broader picture than we can in these short pages.

II. THE STRUCTURES OF BIOLOGICAL SYSTEMS

In nearly every field of physics, experimental study of the structure of a system has been an essential first step leading to models and theories. Structural studies have also been crucial in biology.

A. From Röntgen to synchrotrons and NMR

The most important contribution of physics to molecular biology has been x-ray structure determination. Wilhelm Conrad Röntgen discovered x rays in 1895 and his discovery has affected all scientific fields (Haase *et al.*, 1997). Max von Laue introduced x-ray diffraction and W. L. Bragg determined the first crystal structures. Laue believed that the structure of biomolecules would never be solved. He was wrong. In 1953, James Watson and Francis Crick deduced the exquisite structure of the DNA double helix. In 1958 John Kendrew determined the structure of myoglobin. Shortly afterwards, Max Perutz solved the structure of the much larger hemoglobin. These structure determinations were heroic efforts and took years of work, but they showed how DNA and proteins are built and laid the foundation for an understanding of the connection between structure and function (Branden and Tooze, 1991).

Computers, synchrotron radiation, and improved detectors have changed the field radically. By the year 2000 about 25 000 structures will have been deposited in the Protein Data Bank. Moreover, cryogenic experiments permit the determination of nonequilibrium states produced for instance by photodissociation (Schlichting *et al.*, 1994). The x-ray diffraction technique has, however, two limitations: (1) X-ray diffraction requires good crystals, but not all proteins can be crystallized easily. This problem is particularly severe for the large and important class of membrane-bound proteins which require a heterogeneous environment. Even for those proteins which are soluble in water it is not always clear if the protein in a crystal has the same structure as in solution. (2) Water molecules, crucial for the function of biomolecules, are difficult to see with x rays; their positions must be inferred from the positions of the heavier atoms. The first limitation was overcome by another technique from physics, NMR (Wüthrich, 1986; Clore and Gronenborn, 1991). The main geometric information used in the NMR structure determination resides in short interproton-distance restraints derived from the observation of nuclear Overhauser effects. The second limitation is overcome by neutron diffraction (Schoenborn and Knott, 1996) which can locate hydrogen atoms directly because of their large scattering cross section and can distinguish hydrogen and deuterium, thus making labeling of exchangeable protons possible.

B. Biological systems

Living things can be viewed hierarchically: Genes (DNA)↔proteins↔organelles↔cells↔tissues ↔organs↔organisms. Explanations in terms of cause must ultimately deal with the last level of description (an extreme view is the dictum: ''Nothing in biology makes sense but in the light of the theory of evolution''). But for biological physics the first steps are intriguing enough to provide much inspiration and challenge. The genetic information is coded in the genes in the form of three-letter words on a linear unbranched DNA molecule. Organisms that have chromosomes have the DNA molecule wound around protein molecules (histones) for compact storage and access. Without this compactification, the enormous 3-m length of the DNA molecules in a human cell (with 1 billion basepairs) would not be able to fit as a random Gaussian coil within the 5-μm-diameter nucleus. The information for the construction of a particular protein is read and transcribed onto an RNA molecule. The RNA molecule is by itself also quite interesting because unlike DNA it is conformationally flexible due to its ability to basepair intramolecularly. Like proteins some RNA molecules can fold into three-dimensional catalytically active structures called ribozymes. Those RNA molecules destined to code for proteins are edited to decrease the error rate and this process also leads to interesting physics (Hopfield, 1978). The RNA molecule is then transported to a ribosome, where the protein assembly takes place. The protein is also built as a linear chain, but the building blocks of nucleic acids and proteins are different: Nucleic acids are built from four different nucleotides, proteins from twenty different amino acids. The RNA instructs the ribosome in which order the amino acids must be assembled to form the primary sequence of the protein. When the primary sequence emerges from the ribosome, it folds into the functionally active three-dimensional structure. Sometimes chaperone proteins are involved, but their role now seems to be one of correcting errors rather than being instructive.

C. Myoglobin, the hydrogen atom of biology

As an example of a typical protein, we discuss myoglobin (Mb). Myoglobin stores oxygen (O_2), facilitates oxygen diffusion, and mediates oxidative phosphorylation in muscles (Wittenberg and Wittenberg, 1990). In phosphorylation, the free-energy donor molecule ATP is formed as a result of the transfer of electrons from NADH to oxygen. This process clearly involves physics. Myoglobin also binds carbon monoxide (CO). The reversible binding processes

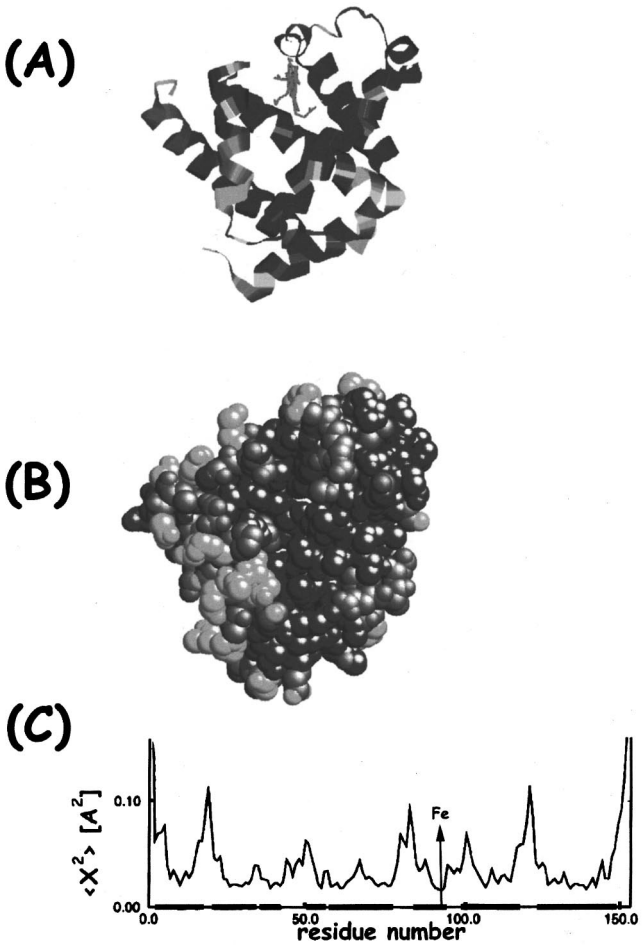

FIG. 1. Myoglobin (a) Skeleton, showing the protein backbone. The Debye-Waller factors averaged over the amino acid group are gray-scale coded, a lighter gray equals more movement. (b) Computer-produced space-filling view of myoglobin, again gray-scale coded in terms of Debye-Waller factors. (c) Debye-Waller factors. The mean-square deviations, plotted as a function of the amino acid number for deoxyMb. The data for parts (a) and (b) are taken from the Brookhaven Protein Data bank entry IMBC (Kuriyan, Wilz, Karplus, and Petsko). The data for part (c) are from F. Parak, personal communication.

$$\mathrm{Mb}+\mathrm{O_2}\leftrightarrow\mathrm{MbO_2},$$

$$\mathrm{Mb}+\mathrm{CO}\leftrightarrow\mathrm{MbCO} \tag{1}$$

can be used to study reaction theory, protein dynamics, and protein function. CO and O_2, and other molecules that are bound are called ligands. Myoglobin consists of 153 amino acids, also called residues. Its secondary structure consists of eight alpha helices. These fold into a boxlike tertiary structure, shown in Fig. 1(a), with approximate dimensions $2\times3\times4$ nm^3 that encloses a heme group (protoporphyrin *IX*) with an iron atom at its center. The ligand, O_2 or CO, binds at the iron. At first glance the structure is complicated, but it does contain some elements of a rough symmetry—a polyhedral arrangement of cylindrical helices.

III. THE COMPLEXITY OF BIOLOGICAL SYSTEMS

Biological systems, in particular biomolecules, are ideal systems to study complex phenomena. Biological systems are reproducible and thus offer many experimental advantages over other seemingly complex systems such as disordered magnetic alloys.

A. Distributions

A "simple" system such as an atom or a small molecule has a unique ground state and its properties have, within the limits of the uncertainty relation, sharp values. At finite temperatures small molecular systems usually sample configurations in the vicinity of this ground state. The transformations of small molecules then satisfy very simple phenomenological laws familiar in elementary chemistry. The time dependence of simple unimolecular reactions is usually given by a single exponential and the temperature dependence follows an Arrhenius law,

$$k(H,T)=A\exp(-H/RT), \tag{2}$$

where H is a barrier height, R the gas constant, and A the preexponential factor. In complex systems, such as, for instance, glasses, the behavior is different (Richert and Blumen, 1993). The time dependence of relaxation phenomena is usually nonexponential in time and can often be described by a power law or a stretched exponential:

$$N(t)=N(0)\exp\{-[k(T)t]^{\beta}\}, \tag{3}$$

where β is less than 1. The rate coefficient, $k(T)$, often does not follow the Arrhenius relation Eq. (2), but can be approximated by, for instance, the Ferry relation (Fig. 2):

$$k(H^*,T)\approx A\exp[-(H^*/RT)^2]. \tag{4}$$

For many years, reactions observed in proteins were assumed always to be simple and were described by Eqs. (1) and (2). As in the chemistry of small molecules, deviations from these laws were ascribed to mechanisms involving the concatenation of a few elementary steps. The impression that proteins were simple was fortified by the structures inferred from x-ray diffraction. These showed each atom in a unique position, but actually this is the result of the model usually used in data reduction. A study of the reaction Eq. (1) at low temperatures changed the picture (Austin *et al.*, 1975). The binding of CO or O_2 was not exponential in time; between 40 K and about 200 K it could be described by a distribution of barrier heights H:

$$N(t)=N(0)\int g(H)\exp(-H/RT)dH. \tag{5}$$

Here $g(H)dH$ gives the probability of finding a barrier between H and $H+dH$.

The appearance of a distribution rather than a single value for H in MbCO is not an exception. Both at low temperatures and at room temperature at short times, protein properties must be described by distributions.

There are two different explanations: Either all myoglobin molecules are identical, but processes are intrinsically nonexponential in time (homogeneous case), or different myoglobin molecules are different (heterogeneous case). The experiment gives a clear answer: Each myoglobin molecule rebinds exponentially, but different molecules are different. This conclusion is supported by many experiments (Nienhaus and Young, 1996). Particularly convincing are spectral hole-burning experiments. If protein molecules with identical primary sequence indeed differ in tertiary structure, spectral lines should be inhomogeneously broadened and it should be possible to use a sharp laser line to burn a hole into the band. This phenomenon is indeed observed; it proves the inhomogeneity and permits the study of many protein characteristics (Friedrich, 1995).

B. The energy landscape of biomolecules

Why do distributions occur? A possible answer was implicitly contained in a visionary talk by Cyrus Levinthal (1969) who asked if the final conformation after folding necessarily has to be the one of lowest free energy. He concluded that it did not have to be the case, but that it must be a metastable state in a sufficiently deep well to survive possible perturbations. If the lowest state is not reached, the observation of a distribution of activation barriers, Eq. (5), can be explained by saying that the protein can assume a very large number of related, but different conformational substates that are only potentially related to the ground-state structure. It must be described by an energy landscape (Frauenfelder, Sligar, and Wolynes, 1991; Frauenfelder *et al.*, 1997). To completely describe an energy landscape, the energy of the protein should be given as function of the $3N$-6 (>1000) coordinates of all atoms. It is not enough to exhibit the energy function. The organization of the hyperspace that results from the energy function must be understood. Each substate is a valley in this hyperspace. The activation barriers in the different substates are different and the observed $g(H)$ is explained. A one-dimensional schematic of an energy landscape is given in Fig. 2. The energy barriers between different valleys (different conformational substates) range from about 0.2 kJ/mol to about 70 kJ/mol. The kinetic observations suggest that the energy landscape might have a hierarchical structure, arranged in a number of tiers, with different tiers having widely separated average barrier heights. A strictly hierarchical energy landscape arises in other complex systems such as spin glasses. Understanding the nature of such a hierarchy remains a hot topic.

C. The Debye-Waller factor

Can the existence of an energy landscape and conformational substates be reconciled with the apparently unique structure that emerges from the x-ray diffraction? Yes! Debye and Waller proved that for a harmonic oscillator with mean-square deviation, $\langle x^2 \rangle$, the intensity

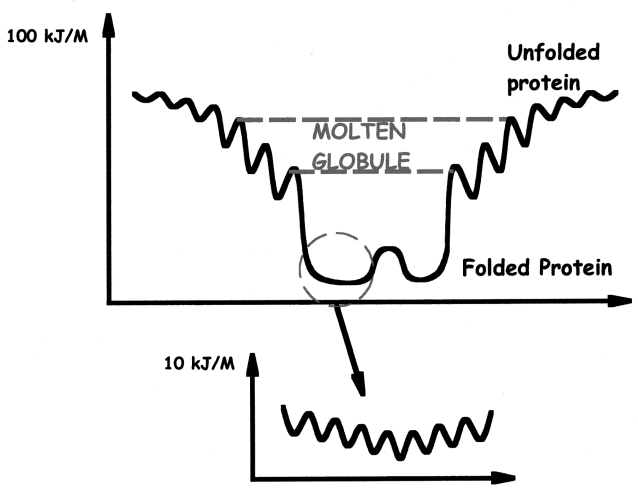

FIG. 2. The energy landscape. The main figure shows the funnel in the energy landscape that leads towards the folded protein. The vertical axis is the difference in enthalpy between the folded and the unfolded state; the difference between the unfolded and folded states is of the order of 100 kJ/mol. The horizontal axis is simply a crude one-dimensional representation of a many-dimensional space of coordinates for the amino acids. The bistable minima in the figure are meant to represent the possibility of the protein existing in more than one metastable conformation. The lower figure shows the energy landscape magnified by roughly 10 for the folded protein; it gives H^*, Eq. (4), as a function of one conformation coordinate. Note that H^* characterizes the roughness of the energy landscape and not the height of an Arrhenius barrier.

of an x-ray-diffraction spot, with wavelength λ at the scattering angle θ decreases by a factor (Willis and Pryor, 1975):

$$f_{DW} = \exp(-16\pi^2 \langle x^2 \rangle \sin^2 \theta / \lambda^2). \quad (6)$$

If proteins have substates, it should show up in the Debye-Waller factor for the individual atoms. This effect is indeed observed. In Fig. 1(c) the $\langle x^2 \rangle$ for deoxy Mb are plotted versus the amino acid number. The figure shows that the $\langle x^2 \rangle$ in different parts of the proteins differ. The same plot for a crystalline solid would show a uniform and smaller $\langle x^2 \rangle$ that would vanish at $T=0$. At present, $\langle x^2 \rangle$ are routinely determined in x-ray structures of proteins (Rejto and Freer, 1996).

IV. DYNAMICS

Motions are essential in biology, from the transport of oxygen by hemoglobin to muscle action. Study of the motions of biomolecules is a central part of biological physics.

A. Fluctuations and relaxations

Fluctuations at equilibrium and relaxations from nonequilibrium states are essential for the function of proteins. The processes can be described in terms of the

energy landscape (Fig. 2). Fluctuations correspond to equilibrium transitions among the conformational substates in the folded protein; relaxation processes correspond to transitions towards equilibrium from an out-of-equilibrium state. The folding of the nascent polypeptide chain is the prototype of a relaxation process. Near equilibrium, fluctuations and relaxations are directly connected, but this is not always the case if the system is strongly perturbed.

One goal of the studies of relaxation and fluctuation phenomena is to connect them to structural entities within the protein. Another is to determine their functional importance. Not all motions of a biomolecule are directly relevant to its biological role. Piecing together the information from experiments, theory, and computation (McCammon and Harvey, 1987; Brooks *et al.*, 1988; Nienhaus and Young, 1996) yields striking results. Differential scanning calorimetry and flash-photolysis studies indicate, for instance, that the distribution of relaxation times in myoglobin is extremely broad. Additional information comes from inelastic neutron scattering, spectral hole burning, optical spectroscopy, NMR, the Mössbauer effect, and other techniques. A synthesis that connects structure, energy landscape, dynamics, and function has not yet been achieved.

B. Proteins and glasses

Proteins and glasses share one fundamental property, the existence a large number of nearly isoenergetic minima and some of the terminology from glasses is now also used for proteins (Frauenfelder *et al.*, 1991). Significant protein motions involve transitions between substates. The harmonic vibrations occur within the substates and are possibly too fast to be directly involved in most physiology, although the role of harmonic and anharmonic effects in biological processes remains an active area of research (Christiansen and Scott, 1990). As the temperature is lowered, the transitions become slower. An arbitrary glass temperature T_g can be defined as the temperature where the transition rate is 10^{-5} s^{-1}. In the simplest view, the protein moves above T_g, and is metastable below T_g. The existence of a hierarchy of conformational substates complicates the situation, because motions in different tiers freeze out at different temperatures. Some motions occur even at 100 mK.

The glass transition in proteins is more involved than in an ordinary structural glass. If a protein is embedded in a glass-forming solvent, T_g for large-scale protein motions is very similar to T_g of the surrounding glass. The motions are slaved to the solvent. Protein and surrounding must consequently be treated together and the environment can control protein motions. This fact may be used by biological systems for control.

C. Protein folding

The complexity of biomolecules ultimately derives from the information contained in the sequence of nucleotide bases in DNA. To a strict reductionist, the task of biological physics is to decode this message or at least to describe how the phenomena of biology at different levels emerge from this kernel of information. Remarkably, the first few steps of information flow from the DNA to biological behavior, while physically complex, can be algorithmically understood in a simple way. With high but not perfect reliability, because of RNA editing, the sequence of a protein can be inferred from the DNA, which encodes it. This simplicity seems to derive ultimately from both having a complex biological apparatus to transfer the information from DNA into protein, including error-correction machinery. But the simplicity also comes from the simple structure of DNA, which can accommodate extraordinarily different sequences in the same structural format—just as many different words can all be written in a book using the same typeface. The next stage of information flow—how the protein molecule obtains a three-dimensional shape which allows it to function in the ways previously described—has been particularly inspiring to the current generation of biological physicists. There are several reasons for this. First, this self-organization can proceed without additional biological machinery and thus can be studied in detail in the test tube. The spontaneous act of folding is quite remarkable in that the complex motion of the protein transfers the information contained in a one-dimensional sequence of data into a three-dimensional object: sculpture by Brownian motion. Folding resembles a phase transition like crystallization, but is much more complex, since there are so many different shapes a protein can have. Since folding must usually occur before any further functioning but is also directly related to the genetics, the understanding of folding intellectually intersects the study of molecular evolution and the origin of life. Finally, there is an important practical motivation for the study of folding. Sequence data are cheap while structural data are still expensive. Even the frightening rate of experimental determination of protein structures cannot keep up with the more horrifying rate of acquisition of the DNA sequence data, which ultimately encodes it. Understanding protein folding can improve the capability of predicting protein structure from sequence. This engineering goal of making structure predictions pinpoints a place where theory can be of direct economic value.

Appreciating that protein folding is sculpture by Brownian motion has led to a view which unites the study of the folding process with the investigation of protein motions that occur in the folded protein. Understanding folding, however, requires a broader picture of the energy landscape that includes not only the states that are excited during function but also those far from equilibrium in which the protein is found in, early after the molecule is synthesized. Directly upon synthesis, the protein molecule is nearly a random coil much like many artificial polymers in solution. The molecule condenses into a more compact, but still highly fluctuating, structure and finally chooses to organize itself into the much smaller set of structures that are involved in function

and whose average is obtained by x-ray diffraction and NMR. The protein starts out soft and squishy like molecular spaghetti and becomes harder and more organized: an aperiodic crystal in Schrödinger's language. Many of these folding transformations can be described using the language of phase transitions in small systems. The mesoscopic size of proteins, along with their reproducibility, makes the study of the folding process an excellent test bed for statistical physics of small systems, much as the study of nuclei was for quantum mechanics in the 1940s. It is the variety of possible structures, along with the relative specificity of the structures actually formed, that brings a truly novel element into the physics of folding. The complexity of the protein sequence might suggest that the sequence could be treated as specific but random. Thus, phase transitions in a folding protein would resemble those seen in disordered magnetic alloys or spin glasses, which are known to have complex energy landscapes. The analysis of spin glass-like models of folding has, therefore, been very useful. However, an important consequence of the analysis of the folding of random heteropolymers is the realization that achieving organized structures probably requires a preselection of protein sequences so that the energy landscape of a protein is, in some respects, simplified from the worst case of a highly disordered system. The spin-glass landscape has many alternate basins, statistically similar but in detail different. Likewise, for a random heteropolymer, each alternate basin could act as a trap for the configurational motions of the molecule impeding the folding process. Some specially chosen sequences have landscapes that eliminate these traps leaving primarily only one dominant basin in which the minima required for function can coexist. Thus the complexity of the landscape is partially removed by selective evolution. This aspect of landscapes needed for efficient folding is known as the ''principle of minimal frustration'' (Bryngelson and Wolynes, 1987). Using the theory of spin glasses and polymer theory, the principal of minimal frustration can be translated into a quantitative statement about the statistical characteristics of the energy landscape, namely the depth of the main basin must exceed the amount by which the energy varies from configuration to configuration, the ''ruggedness'' of the landscape. These statistical characteristics are related to underlying thermodynamic transition temperatures, one being a glass transition driven by the ruggedness, the other equilibrium folding temperature related to the mean basin depth. The simplified energy landscape, which now allows rapid folding, can be described as a funnel (see Fig. 2). A single basin with many minima dominates although within it there are side basins, which can act transiently as traps. In a funnel landscape, sculpture by Brownian motion becomes easy and a folded state is nearly inevitable at a low enough temperature.

The energy-landscape description inherent in this funnel picture has influenced the experimental work on folding. Levinthal's argument that pointed out the difficulty of finding a folded state had inspired an experimental program of searching for specific paths to the folded state and emphasized finding slow folders with intermediates. In contrast, the energy-landscape picture suggested that many of these studies actually were exploring traps that weren't helping the folding process. Landscape ideas shifted the emphasis to the faster folding proteins whose kinetics do not show intermediates. Studying these molecules using protein engineering (Fersht, 1995) and fast spectroscopic methods (Eaton *et al.*, 1998) has yielded much information about the structure of the landscape. The new emphasis in experiment is on characterizing the ensembles of different structures, not trying to find individual specific ones that occur in the folding process.

A fruitful question has been to find out how evolution was able to select sequences that would obey the minimal frustration principle and lead to funnel-like landscapes. This question reminds us of the ''Hoyle paradox'' named after the astronomer who whimsically argued that the difficulty of biological design buttresses the case for an extraterrestrial origin of life in a steady-state universe (Hoyle, 1957). Statistical physics shows that this ''design problem'' is intrinsically easier than the search problem faced by a random sequence with its complex energy landscape. This has been made clear by the development of many algorithms that ''design proteins'' on lattices that fold readily in a computer simulation. The emerging ability of chemists to design laboratory proteins from scratch reinforces this lesson.

One way in which a funneled landscape can be achieved is for the folded structure to be particularly symmetric. This property has led to a search for ''magic numbers'' in the database of known protein structures, much like the earlier search for magic numbers in nuclear abundance. The occurrence of certain super families of protein structures seem to be explicable on the grounds that some structures are particularly appropriate for funneled landscapes. The quantitative nature of the energy-landscape approach to protein folding allows statistical physics to be used as a new tool in creating protein-structure prediction algorithms. The statistical examination of the simplified energy landscapes of proteins used for computational structure prediction allows one to assess which models are better and which are worse as prediction schemes. Using optimization strategies to find energy functions that lead to minimally frustrated landscapes for known sequences with known structures also provides a route to approximate energy functions for use in structure prediction (Onuchic *et al.*, 1997). Physically based algorithms that come from this approach are now competitive with others that use the evolutionary trees of proteins to predict their structure by analogy to related proteins of known structure.

V. REACTION THEORY

The complex motions of proteins and their self-organization into three-dimensional structures have been inspirational for physicists. But even for investigat-

ing simple chemical transformations, biomolecules, in particular proteins, have proved to be excellent laboratories.

A. Dynamic effects in chemical reactions

Biological reactions occur in condensed phases. For many years, textbooks used the transition-state theory to describe such reactions. This theory, however, does not take the effect of the surrounding into account and it cannot, therefore, adequately describe biological reactions. Fortunately, a better theory exists, created by Kramers in 1940 (Frauenfelder and Wolynes, 1985; Hänggi *et al.*, 1990). The interaction of the reacting system with the environment is characterized by a friction coefficient which, by Stokes law, is approximately proportional to the viscosity η. At small viscosities, the system must make a Brownian walk in energy to move over the barrier and the rate coefficient k is proportional to η. At high viscosities, the system moves like through molasses and must diffuse over the barrier; k then is inversely proportional to η. While many studies of this rate theory concentrated on small molecule reactions in liquids, the biomolecular problems were an important proving ground for the theory.

Experimentally, the viscosity effect has, for instance, been studied in the case of the binding of CO to myoglobin [Eq. (1)]. Binding involves a series of steps. The CO enters the protein from the solvent and moves into a cavity near the heme iron (Fig. 1). Once there, it can establish a covalent bond with the iron atom at the center of the heme group. The viscosity dependence of the different steps has been examined. The results support the Kramers equation, but with modifications. Both the viscosity of the solvent and of the protein must be considered. As the action moves deeper into the protein, the effect of the environment is attenuated, and even in the high-friction range, the rate coefficient is not proportional to $1/\eta$, but to $\eta^{-\kappa}$, with $\kappa<1$.

B. Tunnel effects

The Arrhenius and the Kramers equations contain the factor $\exp(-H/RT)$ and therefore predict that the reaction rate should vanish in the limit $T\rightarrow 0$. It has, however, been known since Hund's work in 1927 that quantum tunneling takes over at low temperatures (Goldanskii *et al.*, 1989). The theory has been worked out in detail (Hänggi *et al.*, 1990), but for some insight, a simple expression suffices. The rate coefficient k_t for tunneling of a particle with mass M through a barrier of height H and width d can be approximated by

$$k_t \approx A \exp[-\pi^2 d(2ME)^{1/2}/h], \tag{7}$$

where h is Planck's constant. This relation shows that tunneling is essentially temperature independent and decreases exponentially with increasing distance and mass. Indeed, electrons tunnel easily, and electron tunneling is crucial in photosynthesis. Protons are also known to tunnel, but for heavier particles, the barrier H and the distance d must be very small for tunneling to be measurable. Here proteins again provide an excellent laboratory. The last step in the binding of CO to heme proteins is the bond formation Fe-CO. The low-temperature data (Austin *et al.*, 1975) show that the barrier H for this step can be as small as a few kJ/mol. Distances d for bond formation are of the order of 1 nm. Equation (7) then implies that tunneling of CO should be observable below, say, 20 K. The rate coefficient for the CO binding indeed follows an Arrhenius law down to about 20 K, but then becomes essentially temperature independent, implying tunneling. Since Eq. (7) indicates that k_t depends on mass, it is also possible to study the isotope effect in tunneling.

C. Gated reactions

The treatment so far assumed static barriers. In a fluctuating protein, however, the barriers themselves fluctuate. An example is the entrance of ligands into myoglobin. It is known that even isonitriles, molecules much larger than CO, can enter myoglobin and bind. The x-ray structure of myoglobin shows, however, no channel where molecules could enter and leave. Thus fluctuations must open channels. This opening must involve large-scale motions of the protein that are coupled to the solvent and hence depend on solvent viscosity.

The theory of fluctuating barriers involves two cases. The fluctuations can either be energetic or geometric. The rate coefficient for passage through a gate depends on the rate coefficient k_f of the fluctuations that open the gate and the rate coefficient k_p for passage of the ligand through the open gate. The calculations yield a fractional-power dependence on viscosity. A resonance occurs when $k_p \sim k_f$ (Gammaitoni *et al.*, 1998). This stochastic resonance between fluctuation (noise) and transition leads to an enhancement of the effective passage rate. The enhancement has been observed in many systems, but it is not yet clear if proteins take advantage of it.

In chemical reactions, both nuclei and electrons move. The discussion so far has only considered nuclear motions, assuming that the electrons adjust to the nuclear position. There are, however, situations where this assumption fails. Consider the binding of CO to the heme iron. Before binding the Fe-CO system is in a quintuplet (q) state, in the bound state it is in a singlet (s) state. If the matrix element connecting s and q, $\Delta = V_{sq}$, vanishes, the free CO cannot bind; it will remain on the diabatic curve q. If Δ is very large, the system will change from q to s in the transition region, the reaction will be adiabatic, and the CO can bind. The condition for adiabaticity and the probability of changing from q to s have been calculated by Zener (Zener, 1932), and by Stueckelberg (Frauenfelder and Wolynes, 1985). In the intermediate case, the transition $q \rightarrow s$ depends on Δ. Here is another case where biomolecules may use quantum mechanics to regulate a reaction (Redi *et al.*, 1981).

VI. BIOENERGETICS AND PHYSICS

Bioenergetics, accounting for how energy flows in biological systems, has been a major inspiration for physicists. We often forget that Mayer, a physician, who was contemplating why sailors' blood in the tropics was of a different color than in temperate climates, took the first steps toward the law of conservation of energy in thermodynamics. The myoglobin example derives ultimately also from understanding respiration, the first step of energy transformation for animals. Later steps in energy transduction involve setting up transmembrane potential gradients ultimately caused by the transfer of electrons and protons between different biomolecules. For plants, the transformation of light into chemical and electrical energy has led also to much good physics. Both charge transport and light energy transduction very early forced biological physicists to face the quantum. In both areas, the progress of biology would have been impossible without the contributions of physical scientists.

A. Charge transport

Charge transport in molecular systems is generally different from the free flow studied in simple metals. An isolated charge in a biomolecule strongly perturbs its environment and actually acts much like a polaron. Distortions of the molecular framework must accompany the motion of the charge, dramatically affecting the rate of charge-transfer processes. The study of biological electron transfer, however, brought new surprises. The environmental distortions accompanying charge transport in proteins at low temperature can occur by quantum-mechanical tunneling and involve nonadiabatic effects.

Biological electron transfer was one of the first tunneling processes observed in complex molecular systems. Electron transfer in biology occurs over large physical distances. In ordinary electrochemical reactions, the molecules transferring charge come nearly into contact, but the big proteins separate the small prosthetic groups in which the labile electrons reside often by tens of angstroms. While physicists were familiar with such large-distance electron transfer processes as occurring by electron tunneling in metal-oxide metal junctions, this idea was controversial among biologists and chemists for quite some time. Hopfield pointed out the analogy and suggested that charge transfer could be mediated and controlled by tunneling through the intervening protein medium. At first glance, an exponential decay of the tunneling probability with characteristic length of approximately 1 Å fits many experimental data. Crucial experiments that used protein engineering to place the electron donor and acceptor sites in well-defined locations confirmed the outline of this electron tunneling picture but showed there was a still deeper aspect that involved the structure of the pro-tein. Theorists finally showed that charge is transported in biomolecules through quantum-mechanical tunneling of holes, mostly along the covalent backbone of the molecules (Onuchic *et al.*, 1992). Tunneling rates for electrons can be predicted by finding the tubes along which these holes are transported. It seems likely that evolution has made use of the details of the tunneling process in modulating charge transport in many systems.

B. Light transduction in biology

Both the processes of vision in animals and photosynthesis in plants have brought forth new biomolecular physics. Our vision is sensitive at the single-photon level. The process starts with a photoinduced isomerization, which is one of the fastest processes in biology occurring in <1 ps. This speed makes it comparable to many of the processes of vibrational-energy flow in small molecules. While much is known about the process, it remains a controversial area which attracts laser physicists with powerful new ultrafast techniques and theoreticians developing new computational methods for quantum dynamics.

The study of photosynthesis has a longer history and has been even more fruitful. One of the first steps in photosynthesis is the capture of light energy by chlorophyll molecules in a so-called antenna system. In the 1940s, J. R. Oppenheimer reasoned that the transfer of energy within the photosynthesis apparatus could occur by a process analogous to internal conversion in nuclear physics. This process was independently described later by Förster in greater detail and is now known as Förster transfer—a general mechanism for energy flow between electronically excited molecules. Recent experiments suggest that this transfer is not quite so simple as Förster imagined. The transfer occurs so rapidly that quantum-mechanical coherence is not completely lost. Recent structural characterization of the light-harvesting apparatus has allowed Schulten and his co-workers to give a more complex quantum-mechanical description of this process (Hu and Schulten, 1997).

In photosynthesis, light energy is ultimately transduced into chemical and electronic energy through the apparatus of the photosynthetic reaction center. Here the excitation of a chlorophyll molecule by the photon's energy initiates a series of charge-transfer processes. Again, the first steps are so fast that the quantum dynamics of the nuclear motion needs to be accounted for as well as the electron tunneling *per se*. Theorists have brought to bear much of the heavy machinery of quantum dynamics to address this problem, ranging from large-scale molecular dynamics coupled with polaron theory to real time-dependent quantum Monte Carlo methods. In many of these processes, the precise tuning of energy levels of the molecules, probably largely through electrostatics seems to play a crucial role. This fine-tuning represents a puzzle that needs to be reconciled with the disorder intrinsic in the energy landscape of proteins, suggesting that there are still mysteries to be resolved.

VII. FORCES

One of the grand themes of subatomic physics has been the exploration of the forces through which elementary particles interact. For biological physics, an understanding of the forces between biomolecules, organelles, and cells is of equal importance, but there is a difference. In subatomic physics, the forces and the underlying entities were unknown. In biological physics the force is well known, it is the electromagnetic interaction. In principle the Schrödinger equation with a suitable potential should describe all phenomena. The difficulty comes from the complexity of the systems that interact which leads to a description in terms of effective ''forces'' that are really not fundamental, but correspond to suitable approximations for the interactions between larger objects. The situation is like that of deducing the properties of nuclei directly from QCD.

The building blocks in the primary sequence of proteins and nucleic acids are held together by covalent bonds. These bonds are quite strong with binding potentials on the order of 1 to 2 eV. Rupture of these bonds, done by enzymes in biology, are ''violent'' events and the subject of a great deal of work. Such events which involve moving atoms apart to the point of dissociation are highly nonlinear and have attracted a great deal of interest within the theoretical physics community. Davidov (Davidov, 1987) proposed that not only was the catalytic event the result of nonlinear force-displacement relationships but that the transport of the energy used in catalysis was due to the movement of a solitonic elastic wave propagating down the backbone of the protein. A soliton, in an over-simplified view, is a nonlinear wave which moves in a highly dispersive medium where the phase velocity of the wave is a strong function of the frequency of the wave. When the amplitude of the wave in the medium is of the appropriate size the nonlinear modulation of the phase velocity exactly cancels the dispersion and the wave travels without spreading. Solitons exist and are very important but the relevance to biological systems is still very much in doubt (Christiansen and Scott, 1990) and await new experiments. The critical event in enzyme catalysis, the breakage of a bond, still remains the province of the chemist. Perhaps in the future aspects of nonlinear dynamics and energy flow will help us obtain insight into general aspects of this complex event.

At a lower-energy scale we consider the weaker forces that determine how biological polymers self-interact as they bend and twist and approach other molecules. Electrostatic, van der Waals, entropic, and undulation (elasticity) forces determine the three-dimensional structure of biopolymers and the interactions between biological entities up to the cell. Rather old discussions of the first two forces are still relevant (Gabler, 1978), but the entropic and undulation forces are not yet universally appreciated.

Entropic ''forces'' are due to phase-space considerations and seek disorder, fighting enthalpic forces which want to bring objects together (Leikin, Parsegian, and Rau, 1993). An interesting aspect of biological polymers (and solvents like water) is the relatively large magnitude of both the entropic (S) and the enthalpic (H) energies due to the large number of atoms which are linked together by the covalent backbone. Elementary thermodynamic arguments show that the fractional occupation of a state B which lies higher in free energy Δg above a ground state A is given by

$$\frac{N_B}{N_A+N_B}=[1-\tanh(\Delta G/kT)]/2, \tag{8}$$

where $\Delta G=\Delta H-T\Delta S$. Since ΔH and ΔS can be large for complex biomolecules, the temperature dependence of the populations of the two states can be steep. The midpoint temperature T_M is given by $\Delta H/\Delta S$ and the width of the transition ΔT is given by $k/\Delta S$. Thus the entropic contribution to the transitions is critical and for large molecules dominating.

The undulation forces are due to physical strains in the surface of biomembranes, and bring into play enthalpic considerations. The deformation of a cell, under complex cytoskeleton control, is directly concerned with the undulation force. The idea behind the undulation force is simple (Albersdorfer *et al.*, 1997). A biological object has a complex surface containing elements that interact with other elements through one or more of the first three forces. The movement of these elements towards or away from each other strains the connecting parts, adding an elastic energy to the interaction term. Consider a biological membrane of thickness d and Young's modulus E (the elastic modulus is a general concept which can be used to characterize any material). The bending modulus κ_M of such a membrane is

$$\kappa_M=\frac{Ed^3}{12(1-\nu^2)}, \tag{9}$$

where ν is Poisson's ratio. The bending energy H_{bend} stored in a membrane then is

$$H_{bend}=\frac{1}{2}\kappa_M\int_{surface}dA\left(\frac{\partial^2 u}{\partial x^2}+\frac{\partial^2 u}{\partial y^2}-C_0\right)^2, \tag{10}$$

where u is the magnitude of the membrane normal vector and C_0 is the spontaneous curvature of the membrane due to asymmetrical sides (Gruner, 1994). Variations on this theme, done in a far more sophisticated way than we have outlined here, can be applied at many different length scales to understand the deformation of biological polymers, membranes and organelles.

The undulation force is mechanical and thus can have a long range, just as when you pull on a rope the tension is transmitted over a long distance. If the object through which the force is transmitted is heterogeneous in composition due either to local Young's modulus or through local variations in the entropy density there is a complex dependence of the force with distance as the strained medium responds. The strain dependence of the force can be highly nonlinear. The strain dependence of the bending energy can be viewed as another form of ''energy landscape.'' However, now the energy landscape is that of the bond itself. Thus it is not only the affinity of

two ligands for each other, but also the landscape of the binding surface, that can make a great deal of biological difference. Implications are only recently being explored, and are amenable to the analytical tools of the physicist.

One example of this basic idea is the use of the DNA-sequence-dependent Young's modulus of DNA to predict the binding coefficient of a protein repressor (Hogan and Austin, 1987) which was known to strain noncontacted regions of the double helix. Through the use of elastic-energy considerations it is possible to predict quantitatively the dependence of the binding of a protein which induces helical strain on a basepair sequence.

VIII. SINGLE-MOLECULE EXPERIMENTS

Just as physicists have recently learned how to image single atoms and hold single electrons in confining traps, biological physicists are learning to study single biomolecules (Moerner, 1996; Nie and Zare, 1997). Unlike sodium atoms or electrons, biomolecules are individuals. New insight into biomolecular dynamics and function may result if the distributions discussed earlier are observed on single molecules. We give as an example of single-molecule experiments the stretching of DNA molecules. DNA is particularly easy to study at the single-molecule level because it is incredibly long and has a large value for its persistence length. The challenge of studying individual protein molecules is still very much in its infancy. Different approaches permit the study of the reaction kinetics and thermodynamics of individual proteins. The key is to use extreme dilution so that only a single biomolecule is in the reaction volume. Reactions or excitations are then induced in the same biomolecule many times and observed, for instance, through fluorescence. Such studies can provide additional information on the energy landscape and explore for instance the role of intermittency (Zeldovich *et al.*, 1990).

Nature not only knows chemistry and physics well, she uses them as an excellent engineer. She has built sophisticated linear and rotary motors even at the molecular level (Kreis and Vale, 1993). Linear motors, powered by the splitting of the fuel molecule ATP, actively transport molecules and organelles along the cytoskeleton from one part of the cell to another. Rotary motor proteins are powered by a flux of ions between the cytoplasm and the periplasmic lumen, transforming the ion flux into a rotary motion to drive bacterial flagella (Schuster and Khan, 1994). Motor proteins are exciting for biological physics both because they can be studied in single-molecule experiments and because they have given rise to sophisticated theoretical work (Jülicher *et al.*, 1997). Some of the ideas underlying the protein motors which involve the phenomena of rectified Brownian motion had already been discussed by Feynman (1963).

Single-DNA-molecule experiments are well advanced. A *single* DNA molecule is a polymer with a diameter of a few nm, but can have a length up to about 50 mm (Austin *et al.*, 1997). The mechanical property of individual DNA molecules can be studied for instance by attaching one end to a glass plate and the other end to a magnetic bead. A different technique uses microfabricated arrays to measure the static and dynamic properties of DNA (Bakaijn *et al.*, 1998). Such experiments permit a comparison of the properties of individual DNA molecules with theory.

A long thin polymer molecule such as DNA is entropically extensible like a rubber band. The origin of this entropic elasticity is connected to the mechanical rigidity of the polymer. The mechanical rigidity κ_P of the polymer is a function of the modulus of elasticity E and the cross-sectional shape of the (long axis is the z axis) polymer in the xy plane:

$$\kappa_P = E \int x^2 dx\, dy. \tag{11}$$

While the rigidity tries to keep the DNA straight, the thermal forces buffeting the molecule act to bend it in random directions. The molecule is constantly in motion. The interplay between Brownian agitation and rigidity, then, determines the *persistence length* P of the DNA—the length scale on which the directionality of the polymer is maintained,

$$P = \frac{\kappa_P}{k_B T}. \tag{12}$$

Zooming in to scales shorter than P, the molecule appears straight. But looking from a distance, the molecule appears to be randomly coiled. For DNA in normal physiological conditions, $P \approx 50$ nm which is considerably longer than the molecular diameter of 12 nm but much smaller then the length of the total molecule. When the length L of the polymer is much greater than the persistence length the polymer acts as a linear hookean spring with effective spring coefficient $k_S \sim (3k_B T)/(2PL)$. However, as the strain increases at some force $F_{stretch}$ the polymer no longer responds in a linear manner to applied stress:

$$F_{stretch} \approx \frac{k_B T}{P}. \tag{13}$$

At room temperature $F_{stretch} \approx 0.1$ pN. This value is surprisingly small, weaker than the typical force generated by individual motor proteins and similar in magnitude to the typical drag forces acting on micron-sized objects as they are transported in the cell. When the applied force is stronger than $F_{stretch}$, the elasticity becomes nonlinear. It becomes harder and harder to stretch the DNA as it straightens out and the end-to-end separation approaches the contour length L. By pulling hard on the ends of a DNA molecule, it is possible to "wring out" all of the entropy and straighten the polymer. By pulling harder yet, might one stretch the molecular backbone, just as one can stretch a nylon thread? If so, the elasticity would correspond to the straining of chemical bonds along the DNA axis, and would therefore be of enthalpic, rather than entropic, origin.

There has been an explosion of work on single DNA molecules based upon the rather simple physical modeling that makes this molecule so accessible. The work basically has divided into two parts: studies on the fundamental statistical mechanics of long thin polymers (Perkins *et al.*, 1997; Bakajin *et al.*, 1998) and biological applications involving the influence of supercoiling and overstretching on the DNA and the onset of nonlinearities (Cluzel *et al.*, 1996; Smith *et al.*, 1996). The beauty of this work is the smooth junction between physics and biology, theory and experiment.

IX. THE FUTURE

It took a long time until the energy levels of atoms, small molecules, nuclei, and particles were well enough known so that fundamental theories could be constructed. Since organisms are made of biological molecules, it might have been thought that fundamental theories for biology could now be built up easily. The great complexity of these systems, however, has required a repetition of that earlier development now at a new level. Our present understanding of the energy landscapes and motions of biomolecules is probably no further along than the theory of the Bohr atom or the early shell model of the nucleus, despite the heavy mathematics already being used. It has been amazing how some simple ideas have emerged only recently and already proved unexpectedly useful. Today the physicist interested in biology is in a good position to provide such pictures for the future of biology both at the molecular and higher levels. We have emphasized the molecular aspects of biological physics in this brief review. The far greater problems of the brain loom ahead for those physicists that are brave (Hopfield, 1986).

Many scientists believe that each biological situation is unique, the results of unpredictable quirks of evolution. If so, the quest of biological physics to search for generalizations is quixotic. However, just the last few years of progress suggest that there is plenty of room to find new general concepts and principles through the study of biological systems. Therefore we have no doubt that the study of biological systems will continue to inspire the development of new physics. Ultimately, however, physics must transcend biology. The principles gleaned from biological physics should be extended to other systems of the same complexity as natural organisms. Barring the discovery of life on other planets, these more general objects of study will have to be constructed by us. Perhaps they already have been (Langton, 1988). One hundred years from now, the *Reviews of Modern Physics* will certainly contain discussions of what has been learned in biological physics. The only question is whether its authors will be carbon-based life forms like us.

ACKNOWLEDGMENTS

We thank our friends from both sides of the divide for many inspiring discussions and for many collaborative efforts. The work of H.F. was performed under the auspices of the U.S. Department of Energy. P.G.W. has been supported by the National Science Foundation and the National Institutes of Health. R.H.A. has been supported in part by the National Institutes of Health and the Office of Naval Research.

REFERENCES

Albersdorfer, A., T. Feder, and E. Sackmann, 1997, ''Adhesion-induced domain formation by interplay of long-range repulsion and short-range attraction force: a model membrane study,'' Biophys. J. **73**, 245–257.

Austin, R. H., K. W. Beeson, L. Eisenstein, H. Frauenfelder, and I. C. Gunsalus, 1975, ''Dynamics of ligand binding to myoglobin,'' Biochemistry **13**, 5355–5373.

Austin, R. H., J. P. Brody, E. C. Cox, T. Duke, and W. Volkmuth, 1997, ''Stretch genes,'' Phys. Today **50**(2), 32–38.

Bakajin, O. B., T. A. J. Duke, C. F. Chou, S. S. Chan, R. H. Austin, and E. C. Cox, 1998, ''Electrohydrodynamic stretching of DNA in confined environments,'' Phys. Rev. Lett. **80**, 2737–2740.

Branden, C., and J. Tooze, 1991, *Introduction to Protein Structure* (Garland, New York).

Brooks III, C. L., M. Karplus, and B. M. Pettitt, 1988, *Proteins—A Theoretical Perspective of Dynamics, Structure, and Thermodynamics* (Wiley, New York).

Bryngelson, J. D., and P. G. Wolynes, 1987, ''Spin glasses and the statistical mechanics of protein folding,'' Proc. Natl. Acad. Sci. USA **84**, 7524–7528.

Christiansen, P. L., and A. C. Scott, 1990, Eds., *Davydov's Soliton Revisited* (Plenum, New York).

Clore, G. M., and A. M. Gronenborn, 1991, ''Two-, three-, and four-dimensional NMR methods for obtaining larger and more precise three-dimensional structures of proteins in solution,'' Annu. Rev. Biophys. Biophys. Chem. **20**, 29–63.

Cluzel, P., A. Lebrun, C. Heller, R. Lavery, J.-L. Viovy, D. Chatenay, and F. Caron, 1996, ''DNA: an extensible molecule,'' Science **271**, 792–794.

Davidov, A. S., 1987, *Solitons in Molecular Systems* (D. Reidel, Boston).

Eaton, W. A., J. Hofrichter, V. Munoz, E. R. Henry, and P. A. Thompson, 1998, ''Kinetics and dynamics of loops, α-helices, β-hairpins, and fast-folding proteins,'' Accounts Chemical Research, 745–753.

Fersht, A. R., 1997, ''Nucleation Mechanisms in Protein-Folding,'' *Current Opinion in Structural Biology* **7**(1), 3–9.

Feynman, R. P., R. B. Leighton, and M. Sands, 1963, *The Feynman Lectures on Physics* (Addison-Wesley, Reading, MA), Vol. 1, Chap. 46.

Flyvbjerg, H., J. Hertz, M. H. Jensen, O. G. Mouritsen, and K. Sneppen, 1997, Eds., *Physics of Biological Systems* (Springer, Berlin).

Frauenfelder, H., and P. G. Wolynes, 1985, ''Rate theories and puzzles of hemeprotein kinetics,'' Science **229**, 337–345.

Frauenfelder, H., S. G. Sligar, and P. G. Wolynes, 1991, ''The Energy Landscapes and Motions of Proteins,'' Science **254**, 1598–1603.

Frauenfelder, H., A. R. Bishop, A. Garcia, A. Perelson, P. Schuster, D. Sherrington, and P. J. Swart, 1997, Eds., *Landscape Paradigms in Physics and Biology* (Elsevier, Amsterdam); reprinted from Physica D **107**, 117–435 (1997).

Friedrich, J., 1995, "Hole burning spectroscopy and physics of proteins," Methods Enzymol. **246**, 226–259.
Gabler, R., 1978, *Electrical Interactions in Molecular Biophysics* (Academic, New York).
Gammaitoni, L., P. Hänggi, P. Jung, and F. Marchesoni, 1998, "Stochastic resonance," Rev. Mod. Phys. **70**, 223–287.
Goldanskii, V. I., L. I. Trakhtenberg, and V. N. Fleurov, 1989, *Tunneling Phenomena in Chemical Physics* (Gordon and Breach, New York).
Gruner, S. M., 1994, "Coupling between bilayer curvature activity and membrane protein activity," in *ACS Advances in Chemistry Series No. 235, Biomembrane Electrochemistry*, edited by M. Blank and I. Vodanoy (ACS Books, Washington, DC).
Haase, A., G. Landwehr, and E. Umbach, 1997, Eds., *Röntgen Centennial—X-rays in Natural and Life Sciences* (World Scientific, Singapore).
Hänggi, P., P. Talkner, and M. Borkovec, 1990, "Reaction-rate theory: fifty years after Kramers," Rev. Mod. Phys. **62**, 251–341.
Hertz, J., A. Krogh, and R. G. Palmer, 1991, *Introduction to the Theory of Neural Computation* (Addison-Wesly, Reading, MA).
Hogan, M. E., and R. H. Austin, 1987, "The importance of DNA stiffness in protein-DNA binding specificity," Nature (London) **329**, 263–265.
Hopfield, J. J., 1978, "Origin of the genetic code: A testable hypothesis based on tRNA structure, sequence, and kinetic proofreading," Proc. Natl. Acad. Sci. USA **75**, 4334–4338.
Hopfield, J. J., 1986, in *Lessons of Quantum Theory. Niels Bohr Centenary Symposium* (North-Holland, Amsterdam), p. 295–314.
Hoyle, F., 1957, *The Black Cloud* (Harper, New York).
Hu, X., and K. Schulten, 1997, "How nature harvests sunlight," Phys. Today **50**(8), 28–34.
Jülicher, F., A. Ajdari, and J. Prost, 1997, "Modeling molecular motors," Rev. Mod. Phys. **69**, 1269–1281.
Kreis, T., and R. Vale, 1993, *Cytoskeletal and Motor Proteins* (Oxford University Press, New York).
Langton, C. G., 1988, *Artificial Life: Proceedings of an Interdisciplinary Workshop on the Synthesis and Simulation of Living Systems* (Addison-Wesley, Reading, MA).
Leikan, S., V. A. Parsegian, and D. C. Rau, 1993, "Hydration Forces," Annu. Rev. Phys. Chem. **44**, 369–395.
Levinthal, C., 1969, "How to fold graciously," in *Mössbauer Spectroscopy in Biological Systems*, Vol. 22-24, edited by P. Debrunner, J. C. M. Tsibris, and E. Münck (University of Illinois Engineering, Urbana).
Loofbourow, J. R., 1940, "Borderland problems in biology and physics," Rev. Mod. Phys. **12**, 267–358.
McCammon, J. A., and S. C. Harvey, 1987, *Dynamics of Proteins and Nucleic Acids* (Cambridge University Press, New York).
Moerner, W. E., 1996, Ed., "Single molecules and atoms," Acc. Chem. Res. **29**, 561–613.
Nie, S., and R. N. Zare, 1997, "Optical detection of single molecules," Annu. Rev. Biophys. Biomol. Struct. **26**, 567–596.
Nienhaus, G. U., and R. D. Young, 1996, "Protein Dynamics," Encyclopedia Appl. Phys. **15**, 163–184.
Oncley, J. L., F. O. Schmitt, R. C. Williams, M. D. Rosenberg, and R. H. Bolt, 1959, "Biophysical Science," Rev. Mod. Phys. **31**, 1–1072.
Onuchic, J. N., D. N. Beratan, J. R. Winkler, and H. B. Gray, 1992, "Pathway analysis of proton-electron transfer reactions," Annu. Rev. Biophys. Biomol. Struct. **21**, 349–377.
Onuchic, J. N., Z. Luthey-Schulten, and P. G. Wolynes, 1997, "Theory of protein folding: The energy landscape perspective," Annu. Rev. Phys. Chem. **48**, 545–600.
Parak, F., private communication.
Peliti, L., 1991, Ed., *Biologically Inspired Physics* (Plenum, New York).
Perelson, A. S., and G. Weisbuch, 1997, "Immunology for Physicists," Rev. Mod. Phys. **69**, 1219–1267.
Perkins, T. T., D. E. Smith, and S. Chu, 1994, "Direct observation of tube-like motion of a single polymer chain," Science **264**, 819–822.
Perkins, T. T., D. E. Smith, and S. Chu, 1997, "Single polymer dynamics in an elongational flow," Science **276**, 2016–2021.
Redi, M. J., J. Hopfield, and B. Gerstman, 1981, "Hemoglobin-carbon monoxide binding rate. Low temperature magneto-optical detection of spin-tunneling," Biophys. J. **35**, 471–484.
Rejto, P. A., and S. T. Freer, 1996, "Protein conformational substrates from x-ray crystallography," Prog. Biophys. Mol. Biol. **66**, 167–196.
Richert R., and A. Blumen, 1994, Eds., *Disorder Effects on Relaxation Processes* (Springer, Berlin).
Schlichting, I., J. Berendzen, G. N. Phillips, Jr., and R. M. Sweet, 1994, "Crystal structure of photolysed carbonmonoxy-myoglobin," Nature (London) **371**, 808–812.
Schoenborn, B. P., and R. B. Knott, 1996, Eds., *Neutrons in Biology* (Plenum, New York).
Schrödinger, E., 1944, *What is Life?* (Cambridge University Press, Cambridge, England).
Schuster, S. C., and S. Khan, 1994, "The bacterial flagellar motor," Annu. Rev. Biophys. Biomol. Struct. **23**, 509–539.
Smith, S. B., Y. Cui, and C. Bustamante, 1996, "Overstretching B-DNA: The elastic response of individual double-stranded and single-stranded DNA molecules," Science **271**, 795–799.
Wang, J., and P. Wolynes, 1993, "Passage through fluctuating geometrical bottlenecks. The general Gaussian fluctuating case," Chem. Phys. Lett. **212**, 427–433.
Willis, B. T. M., and A. W. Pryor, 1975, *Thermal Vibrations in Crystallography* (Cambridge University Press, London).
Wittenberg, J. B., and B. A. Wittenberg, 1990, "Mechanisms of cytoplasmic hemoglobin and myoglobin function," Annu. Rev. Biophys. Biophys. Chem. **19**, 217–241.
Wüthrich, K., 1986, *NMR of Proteins and Nucleic Acids* (Wiley, New York).
Zeldovich, Ya. B., A. A. Ruzmaikin, and D. D. Sokoloff, 1990, *The Almighty Chance* (World Scientific, Singapore).
Zener, C., 1932, "Non-adiabatic crossing of energy levels," Proc. R. Soc. London, Ser. A **137**, 696–702.