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Exploring protein energy landscapes

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1

GENERAL INTRODUCTION

1.1 Protein structure and dynamics

Proteins are indispensable elements in the machinery of life. [*Str88*] They contribute to the functioning of living systems in a multitude of ways. For instance, proteins supply the structural basis to fibrous tissue and muscle, are involved in the transport of small molecules, electrons, and energy, and catalyze chemical reactions. The human body alone, contains an estimate of 100,000 different proteins, each of which has a unique function. A particular protein is characterized by the sequence of the amino acids in the polypeptide chain, which is referred to as its primary structure. It is this sequence that determines the tertiary structure of a protein, which is the intricate three-dimensional arrangement of its atoms in the native state. Although each protein has a unique, well-defined tertiary structure, often regularly repeating conformations such as α-helices, β-sheets, and β-turns are observed. These structural elements comprise the so-called secondary structure of a protein.

The first protein to have its three-dimensional structure revealed was myoglobin, a small globular protein which has the function of transporting dioxygen in muscle tissue. In 1960, Kendrew et al. [*Ken60*] presented a structural model of myoglobin with a resolution of 2 Å, based on x-ray diffraction patterns. The three-dimensional structure of myoglobin is displayed in Fig. 1.1. Myoglobin is a very compact protein, consisting mainly of α-helix. Its dimensions are roughly $45 \times 35 \times 25$ Å, and it has a molecular weight of approximately 17 kD. Myoglobin contains 153 amino acids. Furthermore, it contains a heme group, which is the binding site for oxygen. The exploratory work of Kendrew et al. marked a milestone in the field of structural biology, and since then the structures of an ever growing number of proteins have been resolved.

The widespread interest in protein structure is based on the common view that structure and biochemical function are intimately related and many examples are available that support the general existence of such a relationship. When the first tertiary structures were being

Figure 1.1 *Structural model of myoglobin. The central heme is recognizable by its light shading.*

resolved, proteins were generally conceived of as rigid bodies. Earlier on, Schrödinger [*Sch44*] referred to them as aperiodic crystals, aperiodic in the sense that they do not exhibit a regularly repeating structural pattern like ordinary crystals, but crystalline in the sense that the position of each atom is fixed at a well defined point in space. However, experimental and computational evidence is rapidly accumulating that shows that this view needs to be adjusted. Proteins exhibit a multitude of motions ranging from local atomic fluctuations to complex global rearrangements. [*Bro88*] As a consequence, proteins exhibit structural heterogeneity. Therefore, the atomic coordinates obtained from x-ray diffraction and nuclear magnetic resonance studies should be considered as averages, either ensemble averages, or time averages of single proteins. This combination of crystalline properties and properties that are associated with disordered condensed phases, such as glasses and liquids, qualifies proteins as a unique state of matter.

A protein consists of both rigid and flexible groups of atoms. A substantial contribution to the conformational flexibility of a protein arises from fluctuations of the polypeptide

backbone. The polypeptide chain is made up of fairly rigid groups, the CONH units, linked by carbon-carbon single bonds that allow rotations. Substantial displacements arise from torsional motions around these single bonds. Although low amplitude fluctuations may occur independently on an atomic level, larger scale rearrangements are expected to occur in a concerted way. This is because proteins are densely packed, which implies spatial restrictions in the sense that for one atom or groups of atoms to move, neighboring groups have to give way. Since the dense packing is stabilized by energetically favorable interactions, such as hydrogen bonds and electrostatic interactions, the conformational flexibility is also limited by energetic factors, as the motions of groups are likely to modify these interactions.

Some of the conformational fluctuations of proteins are unmistakably functionally important. For instance, the functioning of a protein can involve a structural change induced by external stimuli or by thermal fluctuations. In fact, myoglobin is a good example of the need for conformational flexibility. The protein can exist in two states, an oxygen-poor state (Mb), and an oxygen-rich state (MbO_2). The functioning of the protein requires transitions between these states. Dioxygen binds to the heme pocket of the protein, but within the native state the heme pocket is not exposed to the surroundings. Therefore, in order to function, structural fluctuations need to occur that allow small ligands to reversibly bind to the heme pocket. [*Aus75*] Consequently, if we wish to understand the design and functioning of proteins it is not sufficient to just consider their tertiary conformations, but it is of equal importance to study the fluctuations around their average structures. Conformational changes of proteins can also exert a negative influence on the functioning of biological systems. For instance, the prions that cause bovine sprongiform encephalopathy (BSE) and human Creutzfeldt-Jakob disease are isoforms of normal cellular proteins that have adopted a structural change. [*Pru97*]

In general, we can state that the functioning of a protein requires a delicate balance between order and disorder. Order can be found in the need for a well-defined structure, while disorder manifests itself in structural indeterminism and dynamics. These properties are reflected in the shape and structure of the potential energy surface of a protein, often referred to as its *energy landscape*. In terms of the 'aperiodic crystal', i.e. assuming complete structural order, the tertiary structure of a protein should correspond to a minimum within a very steep well on its energy surface. However, the aforementioned disordered properties of proteins require their energy surfaces to be much shallower. Furthermore, they exhibit a rugged structure similar to the energy surfaces of disordered materials like glasses and polymers. [*Fra91*] More detailed information on the structure of protein energy landscapes will be provided in the next chapter.

1.2 Structural dynamics and spectral diffusion

Recently, optical line narrowing techniques such as hole burning and photon echo spectroscopy have proven to be instrumental in elucidating the low-temperature dynamic

properties of glasses. [*Nar90, Mei94a, Mei94b, Koe96*] The applicability of these techniques to the study of dynamics in low-temperature condensed phases relies on a phenomenon called *spectral diffusion*. The term spectral diffusion refers to the fluctuations of the optical resonance frequency of a dye molecule due to structural fluctuations of the matrix it is dissolved in. With the use of photon echo and hole burning spectroscopy such fluctuations can be studied over an extremely broad time window, ranging from picoseconds to several days. In this way, crucial information on the dynamic properties of the matrix can be obtained. Apart from their extremely broad dynamic range, optical line narrowing techniques are an extremely sensitive probe for structural dynamics, as their resolution is only limited by the homogeneous linewidth, which, at liquid helium temperatures, can be as small as a few thousandths of wavenumbers. The techniques are applicable to any matrix that can be doped with an optical probe. The fact that heme proteins, such as myoglobin and cytochrome *c*, contain an optical center, the heme group, as an intrinsic part of their structure, makes them particularly suitable for optical studies. Some preliminary experiments have clearly demonstrated the general applicability of optical line narrowing techniques to heme proteins, [*Box87, Kol87, Zol91a, Sai92*] and have opened the way for an extensive study of their low-temperature dynamics and energy landscapes.

1.3 Complex systems

Apart from their biological significance, the dynamic behavior of biological systems is receiving a rapidly increasing interest from workers in the physical sciences. This tendency should be viewed in the light of a shifting interest from 'simple' to 'complex' systems. As it turns out, it is not always easy to distinguish simplicity from complexity. However, an illustration of some borderline cases may show that the study of complex systems calls for a different scientific approach. Physical or chemical systems may be characterized as simple because of their low dimensionality, such as atoms and small molecules, an inherent symmetry, like that is present in perfect crystals, or the absence of interactions among particles in ideal gasses. A common motive in the treatment of simple systems is that an attempt is made to describe and understand the system in terms of the properties of their building blocks. For instance, the structure and dynamics of small molecules are treated in terms of the properties of and the interactions between the atoms they are made up of. For complex systems, such an approach is certainly not feasible, and may even prove to be obsolete. To give an extreme example, one cannot understand the behavior of a human being by solving the Schrödinger equation for his brain, nor is it helpful to predict the trend of the global economy by considering each human factor individually. This does not mean that we are unable to study these systems, it just means that a different approach is required. In the same way, physicists are able to study complex states of matter, including glasses, spin glasses, polymers, colloids, and biological systems. An important aspect of the study of complexity is that systems of a wildly different nature, may share common properties, such

as hierarchical organization, self-similarity, self-organization and hysteresis. Defining such properties, and finding their possible common origin is of major importance to understand complex behavior. This is also true on an interpretational level, as is illustrated by the general applicability of the landscape paradigms that are being used in this thesis. [*Lan96*] The same concepts can, for instance, be applied in tackling the traveling salesman problem, [*Buc96*] or in obtaining insight into the process of biological evolution.

One thing that the study of simple and complex systems have in common is the need for well defined model systems. Like atomic physics started with the simplest of atoms, the hydrogen atom, the natural course in the study of complex systems is to start out with characterizing the 'simplest' complex systems, and from that point on tackling more challenging systems. As it turns out, many biological systems, including proteins, are ideal model systems. One reason for this is that the structures of many biological systems are well known, which both facilitates both computational approaches, and the interpretation of experiments. This certainly holds for proteins, which have a number of additional advantages. They are often easily accessible, while their relatively modest size makes it possible to prepare and study large ensembles. Proteins should be of particular interest to those physicists involved with structurally disordered systems, like glasses and polymers. The dynamics of proteins are often described as being 'glass-like', which originates from similarities between their potential energy surfaces. One of the goals of this thesis is to find how far this analogy extends, and to explain their differences in terms of their energy landscapes.

1.4 Scope and organization of this thesis

The aim of this thesis is to study low-temperature protein dynamics by performing spectral diffusion experiments on heme proteins. Secondly, an attempt will be made to construct an image of the protein's energy landscape based on the results of these experiments. Furthermore, the protein-glass analogy will be critically evaluated.

The main part of this thesis is written in such a way to be accessible to an audience that is as wide as possible, and requires little background information, either for those interested in protein structure and dynamics, or those with a general interest in spectral diffusion phenomena in disordered materials.

Chapters 2 and 3 provide an introduction into the concepts and methods that will be applied in the remainder of this thesis. These include the protein energy landscape and existing models for its description, as well as experimental methods, and a quantitative model for the study of spectral diffusion. Experimental details are presented in chapter 4. In chapter 5 we will present the results of spectral diffusion experiments on Zn-substituted myoglobin and cytochrome c. Based on these results, and the recent results of other groups, a highly detailed description of the energy landscape of myoglobin will be presented. Finally, in chapter 6, we will reflect on ultrafast protein dynamics at low temperature, by studying

the pure dephasing in Zn-myoglobin.