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Review

Spectroscopy of proteins at low temperature. Part I: Experiments with molecular ensembles

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Abstract

We discuss aspects of the physics of proteins at low temperature as they are reflected in highly resolved optical spectra of molecular probes. Typical probe molecules are heme-like dyes, aromatic amino acids, but also extended molecular aggregates in light harvesting complexes. We put emphasis on the interactions of the probe with its protein environment, on the range of these interactions, on their specific behavior in external fields, as well as on the characteristic parameters of the protein which can be determined with optical techniques at low temperatures but are not easily accessible otherwise. However, the focus of the review is on spectral diffusion physics of proteins, i.e. on their motion in conformational phase space, and on how this motion is reflected in the optical spectra. These structure changing-processes reflect the non-ergodic nature of low temperature proteins. They are most clearly detected at low temperature where the resolution of the experiment is close to the ultimate limit as given by the natural linewidth and where the dynamics become slow enough to be conveniently measured. In part I we discuss aspects of ensemble experiments, in part II we focus on experiments with single protein complexes. We offer lines of reasoning which may serve as guidelines for an understanding of the phenomena.

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Contents

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1. Introduction

Proteins work at ambient temperature, for instance, as transporters, charge carriers, enzymes, signal transducers, etc. In almost all cases, the function of a protein stops at sufficiently low temperature, even if the protein is still intact. Then, a quite obvious question is: can we learn anything useful on biological molecules, for instance on their structure, their functioning, their general dynamics, from low temperature experiments?

There are many important properties of proteins with physiological relevance, which are revealed in low temperature experiments, only. A representative example in this context is their heterogeneity which has a drastic influence on protein dynamics and function: although most proteins show well resolved x-ray diffraction patterns, their structure is as a rule not unique. They can exist in a variety of structures, a fact which is most clearly revealed in low temperature spectroscopic experiments. At low temperatures conformational dynamics become so slow that most of these structures may eventually freeze out. This freezing process results in a static distribution of the associated barriers which separate the various structures. Structural heterogeneity manifests itself in a dramatic increase in dynamic heterogeneity, i.e. in broad rate distributions which cover many orders of magnitude in time, as was shown, for the first time, in the low temperature CO rebinding experiments in myoglobin [1,2]. Structural heterogeneity is also at the heart of the folding theories [3]. And, as it seems, an understanding of the functional motions of a protein in a solvent at physiological temperatures requires understanding of protein motions at non-physiological, i.e. at low temperatures, as was recently pointed out by Lubchenko et al. [4].

The conclusions from the early optical experiments were supported by low temperature elastic x-ray scattering investigations of proteins: proteins are characterized by a huge static mean square displacement of their building blocks, another very direct indication that protein ensembles exist in a broad variety of structures [5].

The fact that proteins do exist in various structures despite the fact that well resolved x-ray diffraction patterns are obtained, points to a peculiar feature of the protein state of matter: there must be motions which change the structure for a short while, obviously strongly anharmonic motions with large amplitude displacements. On the other hand, a well resolved average static structure points to a harmonic solid. Indeed, a protein is something between a liquid and a solid. This specific feature is also seen in low temperature experiments: at around 200 K, proteins perform a so-called dynamic transition, where they switch from a harmonic behavior to strongly anharmonic motions with very large amplitudes. For the first time, this transition was observed in Mößbauer experiments [6,7] and has been found in x-ray [8] and neutron scattering experiments [9] later as well. In addition, the transition is clearly revealed