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# Neutron scattering and protein dynamics

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Neutrons play an important role in the study of proteins. The best known example is the determination of protein structures using neutron diffraction. Less well known, but possibly even more important in the future, is the determination of protein fluctuations using neutron scattering. Here, the background is sketched and some recent measurements are described that show how a relevant and revealing range of relaxation rates can be explored.

## 1. Dynamics

Working proteins are dynamic systems; they fluctuate in order to work. We have recently introduced a unified model of protein dynamics that explains some functional motions quantitatively (Frauenfelder *et al.*, 2009). The model is based on three experimentally founded concepts: the existence of conformational substates (CS), a hierarchically organized energy landscape (EL) and two types of fluctuations, called $\alpha$ and $\beta_{\rm h}$. Even in their ground state, proteins can assume a very large number of different conformations or CS that can be described by an energy landscape (Austin *et al.*, 1975; Frauenfelder *et al.*, 1979). The barriers between the CS that determine the fluctuation rates of the conformational transitions are organized into a hierarchy (Ansari *et al.*, 1985). Initially, it was assumed that the barriers that control the dominant fluctuations are properties of the protein itself. However, experiments with myoglobin (Mb) proved that the barriers are produced by the environment. The $\alpha$ fluctuations originate in the bulk solvent. Their rate coefficient, $k_\alpha(T)$, is related to the solvent viscosity $\eta(T)$ by the Maxwell relation $k_\alpha(T) = G/\eta(T)$, where $G$ is the infinite-frequency shear modulus. The $\alpha$ fluctuations control, for instance, the shape of the protein and the opening and closing of a gate that permits the entry and exit of small molecules and folding. If the proteins are crystallized, the viscosity is extremely large and the $\alpha$ fluctuations are essentially absent. The $\beta_{\rm h}$ fluctuations originate in the hydration shell and influence internal protein motions. The coefficient $k_\beta(T)$ depends on hydration and essentially vanishes if the protein is dehydrated.

The relation between the external fluctuations and the protein motions require further study. Some connections have already been made using myoglobin (Mb; Frauenfelder *et al.*, 2009). The $\beta_{\rm h}$ fluctuations in the hydration shell were measured using dielectric relaxation spectroscopy. The coupled protein motion was observed by using the Mössbauer effect in $^{57}$Fe (Frauenfelder *et al.*, 2009). Motions such as the opening of a channel in Mb for the entry and exit of small molecules follow the $\alpha$ fluctuations in the solvent over a wide temperature range (Fenimore *et al.*, 2002). The advantage of using the Mössbauer effect to monitor internal protein fluc-

tuations is that one observes a well defined position, usually the heme iron in heme proteins. There are two disadvantages: only proteins that contain $^{57}$Fe can be investigated and the range over which motions can be seen is limited. Neutron scattering is a technique that can overcome both of these disadvantages. It permits the measurement of motions inside and outside the protein over a very broad time range.

## 2. Neutron scattering

The unique feature of neutron scattering in probing matter is that it can explore the motion of atoms and molecules in the nanoscopic space and time domain centered around 1 nm and 1 ns, respectively. This time and length scale is intermediate between the microscopic atomic scale and the macroscopic scale, which is exactly the arena of action in molecular biology. Neutron spectroscopy can explore atomic and molecular motion between $10^{-14}$ and $10^{-6}$ s by the combination of different experimental techniques, from time-of-flight (TOF) to neutron spin echo (NSE) spectroscopy. Biological matter always contains a large number of H atoms, which scatter neutron radiation very strongly compared with the other atoms present. Most of the scattering of hydrogen is incoherent, which reveals the behavior of single atoms. Except when working with sizable single crystals, in proteins neutron scattering directly follows the behavior of individual H atoms in space and time, naturally as an average over all H atoms present. Mössbauer spectroscopy provides similar (but a lot more restricted in space and time and less direct) information on Fe atoms. Mössbauer experiments revealed a long time ago that proteins showed the onset of some motion of an unidentified nature with increasing temperature at about 200 K (Keller & Debrunner, 1980; Parak *et al.*, 1981). This motion was detected by measuring the temperature dependence of the mean-square displacement of the Fe atom and was subsequently confirmed by neutron scattering on the H atoms in the protein and dubbed 'dynamical transition' (Doster *et al.*, 1989). The 'transition' temperature coincides with the onset of functionality in hydrated myoglobin in $O_2$ transport, but the 'transition' is absent in dry myoglobin, which is a further indication of its relation to the function. The nature of the 'transition' was unclear for many years and it was simply fitted by the universal first tool of physicists, a double-well potential. A combination of dielectric spectroscopy and the Mössbauer effect recently showed that there is no 'dynamical transition', but that dielectric fluctuations in the hydration shell of myoglobin are responsible for the rapid increase in the mean-square displacement above about 200 K (Frauenfelder *et al.*, 2009). The fluctuations are similar to the $\beta$ fluctuations in supercooled liquids and are denoted as $\beta_h$, where the subscript indicates that they originate in the hydration shell. The apparent onset at about 200 K arises from the fact that the fluctuations become fast enough at this temperature to be observed in the time window of the Mössbauer effect.

The importance of the $\beta_h$ fluctuations for understanding protein dynamics calls for extending the measurements of neutron scattering to as broad as possible time and temperature ranges. Here, in an extensive neutron spectroscopy study we identified the time scale and spectral characteristics of this motion. Fig. 1 shows the observed neutron scattering spectra obtained by TOF spectroscopy as a function of temperature for myoglobin hydrated to 0.8 g water per 1 g of protein (Mezei *et al.*, 2009). The spectra show the frequency distribution of the motion; 1 meV corresponds to 240 GHz or, if we consider relaxation processes, to 0.6 ps. Quantitative analysis of the intensity of these spectra reveals that the atomic motion they reflect is the origin of the enhanced temperature-dependence of the atomic mean-square displacement discussed in the previous paragraph. The onset of this 'quasi-

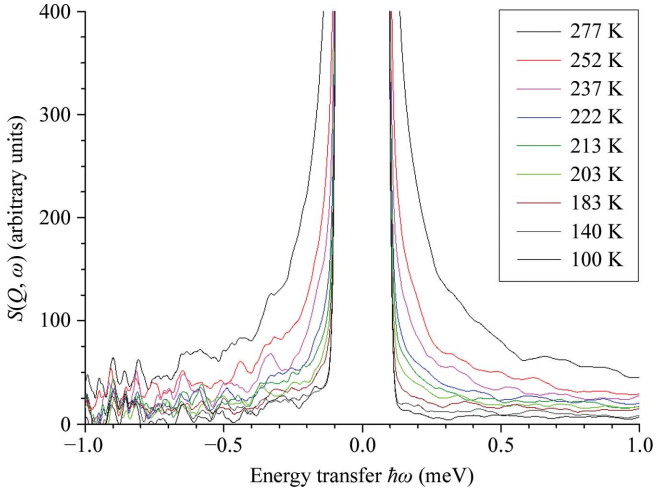


**Figure 1**
Temperature-dependence of the neutron scattering spectra at momentum transfer $Q \simeq 1$ Å$^{-1}$ in hydrated myoglobin (0.8 g $D_2O$ per 1 g protein). TOF neutron spectroscopy data obtained using the NEAT spectrometer at HZ Berlin.

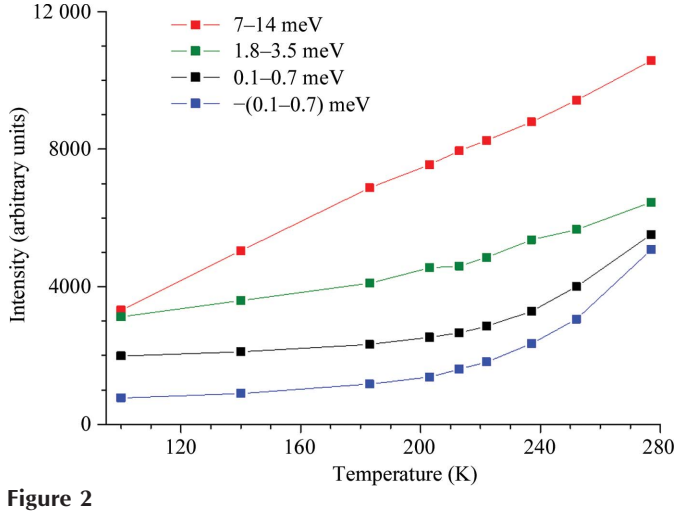


**Figure 2**
Temperature-dependence of the scattering intensities integrated over various energy (frequency) domains for the spectra of hydrated myoglobin partially shown in Fig. 1. The slight downturn above 200 K with increasing temperature in the curve for the 7–14 meV bracket is consistent with the drop in the Debye–Waller factor caused by the upturn of the scattering intensities at the lower energy brackets. (For greater clarity, the curves have been slightly shifted vertically to avoid overlap.)

elastic' process (*i.e.* centered around zero frequency) is shown in Fig. 2 for different energies (*i.e.* frequencies). Complementary NSE experiments showed that the observed onset of motion extends further towards longer times over the whole accessible time scale up to 10 ns (equivalent to 0.06 μeV or 14 MHz). Further important information from the much higher resolution NSE data is that the spectra shown in Fig. 1 cannot be explained by the broadening of the central line around zero frequency.

In this study, myoglobin samples have been explored with different kinds of hydration. Using heavy water ($D_2O$) for hydration has the same physico-chemical effect, but makes the hydration water practically invisible compared with the H atoms of the protein molecule itself. Comparison with $H_2O$-hydrated samples shows that the average amplitude of the H motion is considerably smaller (about 2/3) in the protein than in the hydration water shell, but the spectrum is similar and also covers the time domain of 1 ps to beyond 10 ns. More recent additional information (to be published) confirmed the same behavior for the lower hydration level of 0.4 g water per 1 g protein.

## 3. Conclusions

These observations provide unambiguous evidence that function in myoglobin is related to the motion of atoms in the broad nanoscopic time domain of 1 ps to 10 ns and longer. This time domain is slow compared with the temperature-induced vibrations of the atoms around their equilibrium positions, which concentrates on the 0.05–0.5 ps domain. On the other hand, it is many orders of magnitude faster than any flow-like motion governed by the viscosity, which is in the range corresponding to solid glasses in these samples.

The spectroscopic features correspond to those of the $\beta$ relaxation, which has been extensively explored in glasses and is fundamentally absent in crystalline solids, but is present in protein crystals. They arise from the atomic/molecular disorder, which inevitably creates regions with less rigid structure, where on a scale of a couple of atomic distances (nm) the atomic motion has to overcome lower energy barriers than in the bulk of the sample. The infinite variety of slightly different local structures in these regions (which is analogous to the variety of conformational substates in proteins) leads to the broad distribution in the 'landscape' of energy barriers and hence local relaxation times. Motion in these mobile regions also leads to molecular-level rearrangements, which can make a mobile region more rigid and, *vice versa*, a rigid region more mobile, with time (Russina *et al.*, 2000).

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