Dynamics of the Free-Energy Landscape of Proteins, explored with the Mössbauer Effect and Quasi-elastic Neutron Scattering

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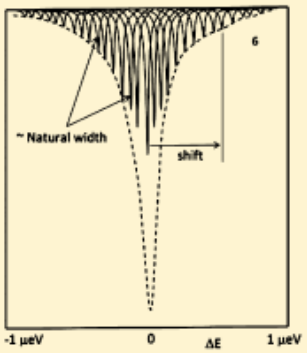
ABSTRACT: The Mössbauer effect and quasi-elastic neutron scattering (QENS) from hydrated proteins yield sharp elastic lines that are accompanied by broad wings. Conventionally, the elastic line and the broad wings are treated as separate phenomena. We show that there is no separation; the entire spectrum consists of Lorentzians with the natural line width. In protein crystals, the shifts of the individual lines from the elastic center above about 150 K are caused by beta fluctuations in the hydration shell. Vibrations cause shifts in the entire temperature range but are best seen below about 150 K. We construct a microscopic model for the dynamics that is based on a random walk of the proteins in their free-energy landscape. The model yields approximate values for the steps in the energy landscape. Remarkably, the quantum electrodynamic concept of gamma rays is needed to justify the model.



Comment by W. Doster, November 2017

1) The deceptive similarity of Mössbauer and neutron scattering data of heme proteins

The Mössbauer effect (ME) and neutron scattering (NS) are very different methods which therefore record different physical processes in proteins on different spatial and temporal scales. ME yields highly local information around embedded ^{57}Fe, only a single atom per protein, via a resonance absorption mechanism. NS by contrast is dominated by a global incoherent scattering process from protein hydrogens, which are distributed uniformly across the protein. The ME line width amounts to 4,7 neV, corresponding to an observation time of 141 ns. The resolution of neutron spectrometers are in the range of meV to μeV or typically 10 to 300 ps resolution time. The effective Q^2-value of ME is 50 A^{-2}, while a typical Q^2 value of NS is around 4 A^{-2} or less. The relevant spatial scale is thus less than 0.5 A for ME and 1 to 5 A for NS. Protein global diffusion can be recorded at temperatures as low as 250 K because of the neV resolution with ME (see fig. 2a), which requires higher temperatures and larger spatial scales for NS. Protein global diffusion demonstrates however that the homogeneous "spatial" scattering/resonance absorption theory of NS/ME (SSM) is correct in contrast to ELM.

When we first published the elastic NS data of hydrated myoglobin versus temperature in 1989, the results looked deceptively similar to ME: a linear temperature dependence of displacements (MSD) at low T (vibrations) and a cross-over to a super-harmonic increase above 200 K. With neutrons however, there was a dramatic improvement because of the observation of a temperature dependent non-Gaussian elastic scattering function. ME provides only a single high Q value, which does not allow to determine a truthful MSD. The MSD became feasible with NS as the low Q extrapolated slope of the scattering function. Second, with NS the first broad band temperature dependent inelastic protein spectrum was observed. All this is not feasible with ME.

Based on the elastic and inelastic data we realized already in 1989 that there were two onset temperatures, thus two molecular processes, interpreted at the time as torsional transitions and water induced small scale Gaussian translational motions. With ME only a single transition was observed. It is thus not clear whether the ME process is related in any way to the two NS processes.

The protein dynamical transition (PDT) was defined as the resolution controlled onset of the second process coupled to hydration water around 240 K on a 150 ps time scale. It took some time, documented in a series of publications by us from 2001 to 2013, to clarify the details. Not a single paper of this series is referenced here or in the later PNAS papers by HF.

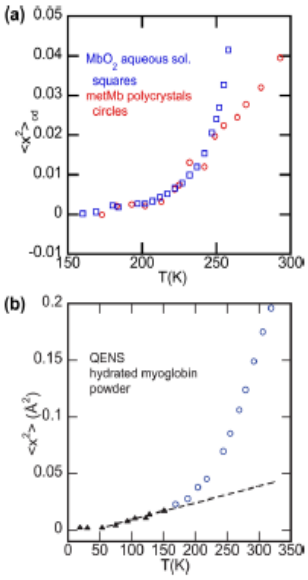


Figure 2. (a) Mean-square displacement for MbO_2 in aqueous solution (squares) from Mössbauer,[4] metMb polycrystals (circles).[5] (b) Mean-square displacement for Mb powder (circles), $h = 0.4$, from QENS.[6]

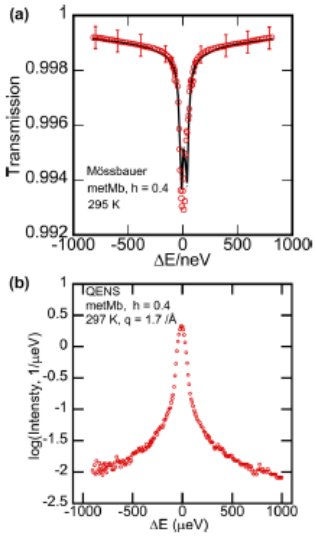


Figure 3. (a) A Mössbauer transmission spectrum. Metmyoglobin crystal, 295 K, $h = 0.4$. The solid line is a one-parameter fit, assuming that the broadening is caused by external fluctuations. Data from Parak and Achterhold.[43] (b) A QENS spectrum of perdeuterated metmyoglobin polycrystals, 297 K, $q = 1.7$ A^{-1}.[49]

Fig. 2 compares ME and NS MSD values of myoglobin in different environments. The MSD extra increase in fig. 2a (ME) above 250 K with myoglobin in solution, reflects the translational diffusion of myoglobin around 250 K mentioned above (Keller/Debrunner PRL 45 (1980)68, Lichtenegger et al. BJ 1999, vol.76). Apart from this difference, fig. 2a) and b) look virtually identical. HF implies, that *Doster et al. did discover nothing new with MS, ME is the primary technique.* Both data sets look apparently similar, because hydrated proteins or crystals were studied versus the temperature in the same low temperature range.

Apart from this similarity fig. 2 is quite misleading, because a single onset temperature of NS and ME around 200 K is suggested. Both methods are supposed to reflect the onset of the same process. This conclusion is wrong, it is incompatible even with the absolute MSD values: NS at high T yields a MSD around 0.2 A^2, with ME, it is only 0,035 A^2. To derive an MSD with ME, one must assume a Gaussian distribution of protein displacements from a single Q-value, which yields at best a lower limit. The nature of the myoglobin ME displacements versus environment was first studied by

Lichtenegger et al. Biophys. J. 1999, vol.76, 414: "Heme-solvent coupling of myoglobin sucrose". It is shown that the ME onset temperatures vary with the viscosity and the viscoelastic relaxation time near the protein surface. For the first time, the onset temperature is related to the ratio of the solvent relaxation time to the Mössbauer life time. For 75% glycerol water, one has:

$$\tau_{Möss} \sim \tau_{sol}(T_{on}) = \tau_{\alpha}(T_{on})$$

at the onset temperature. With sucrose one observes T_{on}~ 240 K, which suggests a coupling to the surface viscosity. In contrast HF writes:

"By contrast β-relaxation in supercooled liquids and glasses are essentially independent of the viscosity..(HF et al. PNAS 2009,106, 5129.)

With NS but not ME, a transition is even observed in the dry state (around 180 K) due to side chain rotation (Doster et al. BBA 2005).

The same differences show up in fig. 3: 3a) records motions of the heme iron, by contrast, fig. 3 b shows from NS hydration water dynamics of per-deuterated myoglobin.

Fig. 3b should thus be a very good case to prove the existence of a local β_h process of hydration water. Instead Achterhold et al. Phys. Rev. E (2011) analyze their data using the diffusion model of Swingi and Sjölander. They demonstrate that hydration water can perform long range diffusion interrupted by short rattling attachments. Moreover here is ample evidence from neutron scattering, NMR and simulations that hydration water at high temperatures migrates by translational diffusion along the protein surface (Settles, Doster, 1996, Faraday Discussion 103, Doster, Settles BBA 2005, Doster et al. PRL 2010, Schiro et al. March 2015 Nature Comm. "Translational diffusion of hydration water correlates with protein function.." and simulations by the Smith group. β-relaxation or localized motion takes over on approaching the glass transition below 200 K (Doster, J. Noncryst. Solids 2011, 357,622, Rosenstihl et al. J. Noncryst. Solids 407,449, 2015: "dynamics of interfacial water".

The figure below (Doster et al. JCP 2013) compares the experimental ME data of Fe^{57} in pure glycerol and of myoglobin in 75% glycerol/water. For pure glycerol and 75 % glycerol water dielectric relaxation data as well as frequency dependent specific heat data of the α-process are given in the literature. Without adjustments using the literature data, we can reproduce the LMF of Fe/glycerol and of myoglobin/glycerol using the correct resolution function of ME. The shape of the instrumental resolution function is more relevant to the LMF than the "distribution of solvent relaxation times". By contrast, the β-process of glycerol given in the literature cannot account for the data as shown by the red dashed curve.

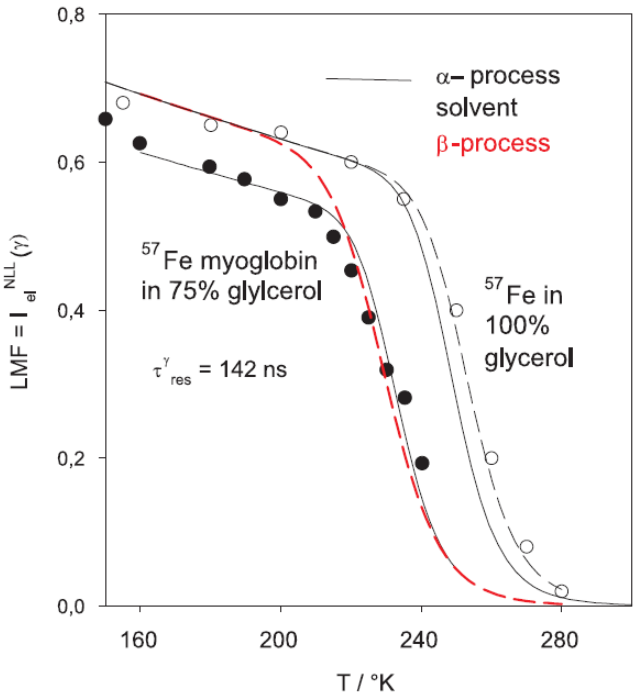


FIG. 3. Lamb-Mössbauer factor of ^{57}Fe in 100% glycerol[42] and myoglobin in 75% glycerol water[45] and simulations according to the Lorentz-Lorentz model of Eq. (20b): $\tau_{res} = 142$ ns, EISF = 0, τ_c(T) for α-relaxation[19,46] and β-relaxation (red).[19] The rates of the perfect fit, dashed line, differ by less than a factor of two from the bulk α-relaxation rates.[46]

2) Spectral heterogeneity and the protein dynamical transition (PDT)

"The PDT has generated a flood of papers, but no explanation is universally accepted. We show that the PDT is not a real transition in the protein, but that the change in the slope is caused by the onset of β_h-fluctuations, which simulate the transition…"

HF switched to our model (Doster et al. 1989) with "α" replaced by the term "β" relaxation.

This is a dramatic correction to the old de-trapping substate model of 1996. "*At any given instant, an individual protein molecule is in a specific conformational substate. It usually does not stay there but hops to other substates and explores the energy landscape. This exploration depends critically on temperature. At low temperatures, each protein remains frozen in a particular CS. At room temperature, the protein moves through the CS..*" In between there is a dynamical de-trapping transition from frozen to mobile states. The ME PDT was explained by HF, Keller, Debrunner and Parak (ref.4, 5) as a detrapping transition involving low energy conformational states. By contrast, in "the Mössbauer effect of proteins" (PRL 2011, 107 158102) HF writes " *the PDT is caused by an incorrect separation into a sharp and a broad component and so is not a valid implication of Mössbauer data. The entire spectrum is inhomogeneous, composed of sharp lines. In the conventional treatment, the broad component is homogeneous,… which implies that the nuclear life time is shortened*

by a factor of 100. No nuclear model exists to our knowledge to understand such a shortening.."

This strikingly incorrect interpretation of Mössbauer spectroscopy ignores that the broad component is not broadended by the nuclear life time, but by diffusive displacement of the heme iron, a stochastic process, which has a much shorter life time than $\tau_{mö}$.
Thus the strong Frauenfelder statement, that the Mössbauer broad line is inhomogeneous, collapses.
The ME absorption mechanism is different from the heterogeneity model. Briefly: The emitted γ- quantum has a finite coherence length due to the nuclear life time, which affects the absorption. Spatial diffusion is a stochastic process which further degrades the coherence length. The ME absorption thus records the decay of a phase correlation function similar to incoherent scattering experiments.

The basic equation of the ME spectrum (excluding quadrupole, hyperfine interactions and chemical shifts) looks similar to the basic NS equation (see comment to HF 2014). The ME resonance absorption cross-section $\sigma(k,\omega)$ is given by (Chong et al. Eur. Biophys. J. 2001, 30,319) the Fourier transform of a phase factor correlation function:

$$\sigma(k,\omega) \sim FT\{ \Sigma_j <e^{ikr_j(t)} e^{-ikr_j(0)}> R(t/\tau_{Mö}) \} \qquad \text{equ. (1)}$$

where k is the wave vector of the gamma radiation and $h\omega = E - E_a$ with E_a being the resonance energy of the nuclear transition. $\tau_{Mö} \cong 141$ ns is the nuclear life time. $r_j(t)$ denotes the position of the Fe atom (j). The ME resolution function $R(t/\tau_{Mö})$ is an exponential with a resulting Lorentzian minimal half with of $1/\tau_{Mö}$: If the phase correlation function in equ.(1) follows an exponential decay due to spatial motion of the iron with time τ_c, the combined homogeneous half width of the Lorentzian $\sigma(k,\omega)$ will be $\Gamma_{mö} = 1/\tau_{ME} + 1/\tau_c$. If there is a finite $EISF_{ME}(k)$ as in solids, there will be an elastic line in addition as with NS. So the decomposition of the "spatial model" into elastic and quasi-elastic components is unavoidable if these methods record the decay of the phase correlation function**. The ELM is totally inconsistent with the established theory of the Mössbauer effect (Parak, Rep-Prog.Phys. 66 (2003)103.**

Summary:

1) The question of inhomogeneous spectra remains obscure. However a convincing physical interpretation exists for the quasi-elastic broadening: It reflects the decay of a spatial phase correlation function, exemplified by the global diffusion of the protein.
2) ME and NS data should be treated with accounting for the major differences. The effective line widths differ by a factor of 1000.
3) the water model of β-relaxation is not consistent with NS and NMR data and simulations. The correlated density fluctuations of protein and solvent determine the experimental quantities. Dielectric relaxation is just one of several methods to measure water dynamics, which has no build-in molecular spatial scale like NS. NMR, quasi-elastic neutron scattering and simulations prove the existence of translational diffusion and viscosity of hydration water. There is no "bound water" (β-relaxation) according to NL Wüthrich.