Mössbauer Effect in Proteins by Young, Frauenfelder and Fenimore PRL (2011)107, 158102

We have already discussed the simple view of the Mössbauer effect of Fenimore et al. PNAS (2004) 101, 14408 below. Since the authors never did such experiments, I find it remarkable that F. Parak, who provided the data is not a coauthor. It is also puzzling, why a closely related Mössbauer paper by Lichtenegger et al. Biophys.J. (1999) 76, 414 is not cited.

The presented material sometimes looks like a copy. Lichtenegger et al. had shown, that the PDT onset records the solvent relaxation time, which becomes comparable to the nuclear life time:

*"The line broadening **at the onset temperature** reflects diffusive motions that become resolved when the corresponding relaxation times have reached the level of 10 times the nuclear life time, a few microseconds…"*

This is one of the main conclusions presented here by Young et al as new. Another point is the attempt to disprove of a discontinuous spectral behavior associated with a dynamic transition. Besides the tiny spectra plotted on a linear scale, which will not prove anything, the PDT like a GT is structurally and spectrally continuous. Young et al thus try to disprove something, which was never proposed.

Specific Comments:

1) *"…the Lamb-Mössbauer relation is used to calculate the MSD of the sharp line. At about 180 K, this MSD increases sharply with temperature..as later(!!) also observed with neutron scattering and dubbed(!!) the protein dynamical transition (PDT)."* Several incompatible things are thrown together:
   - the MSD is the low Q- (or Gaussian) limit slope of the elastic scattering function. Since the effective Q^2 of Mössbauer is large (50 A^2), it is incorrect to derive an MSD outside the vibrational region.
   - the anharmonic onset, observed with Mössbauer and neutron scattering are first of all completely unrelated: One method probes the heme iron, while neutrons probe the protein protons. The resolution is quite different. An apparent anharmonic onset will always emerge for molecular processes probed with a fixed energy window, when its time scale enters the resolution window, for instance methyl group rotation or heme group jumps. The PDT was first defined and observed with neutron scattering as a two step transition of local and collective motions reflecting a protein-water glass transition (Doster et al. 1989). This was a very specific explanation requiring two onsets not just a single transition as observed by Parak. That a correlation exists was shown for the first time by Lichtenegger et al. 1999. In the context of the PDT Frauenfelder uses the terms, "babtized" or "dubbed" to suggest, that this is just a name. In fact the term "dynamical transition" is well established in physics, describing dynamical changes in the absence of structural changes: **Two such transition are known: the liquid-glass transition and the percolation transition.** By contrast the terms "conformational

substates" and "slaving", the Frauenfelder "β-relaxation" do not exist in other fields and are purely dubbed.

The conventional treatment has problems: The LMF relation is valid only for a harmonic potential, but proteins are not harmonic. The separation into a narrow line and a broad is misleading. In the conventional treatment, the broad line is homogeneous. The Heisenberg uncertainty relation implies that the nuclear life time is shortened by a factor of 100. No nuclear model exists, to our knowledge to understand such shortening. The PDT is caused by the incorrect separation into a sharp and a broad component and so is not a valid implication of the Mössbauer data. Our unified model solves these problems."

The LMF relation is valid for Gaussian processes, a property, which emerges because of particle conservation in the low Q-limit for all constrained motions. This has nothing to do with proteins being not harmonic. Frauenfelder has interpreted (with Petsko and Tsernoglu, Nature) nonlinear protein displacments (X-rays) by unharmonic potentials later shown to be incorrectly derived. A separation of a spectrum into components is always based on a model, which is never right or wrong. Thus the PDT cannot be the result of an incorrect separation. A model can only be more or less useful. Why this would shorten the nuclear life time by a factor of 100 remains a mystery without citation. The tiny spectra displayed in fig. 1 on a linear intensity scale can never be used to discriminate different types of models. The PDT by contrast was defined as a two-step feature of structural relaxation based on log-log-scale inelastic neutron scattering spectra and not just MSD onsets (Doster, Nature 1989, JNCS 2011) as incorrectly claimed by Frauenfelder.

For the two protein-water processes we have introduced in 1989 (Doster et al. Nature) the notion α- and β-relaxation in analogy to processes observed in viscous liquids. This concept was based on extended thermal and IR work on hydrated proteins published in 1986 (Doster et al. Biophys.J.). β-relaxation was assigned to fast local hydrogen bond fluctuations (water-amide groups) at the protein-water interface. α-relaxation by contrast involves a restructuring of hydration water positions due to lateral or perpendicular diffusion.

Frauenfelder picks up our terminology, but confuses the picture by introducing new definitions:

"*We have shown earlier, that dielectric fluctuations in the hydration shell of Mb predict the onset of the PDT…"*

such conclusions were already presented in 1999 by Lichtenegger et al. for Mössbauer spectra of myoglobin, not only for water but also for glycerol-water and sucrose-water as solvents. Lichtenegger showed for the first time that the PDT onset varies with the solvent viscosity, associated with the α-relaxation. A secondary process (β) was never detected by Mössbauer spectroscopy of proteins.

By replacing the term α-relaxation with β-relaxation, Frauenfelder confuses the picture without giving a sound physical explanation. The main difference: β-relaxation is not supposed to depend on the bulk solvent viscosity. Lichtenegger just had shown the opposite.

The surface viscosity can however differ from the bulk due to surface effects. Long range diffusion along the surface definitely implies a viscosity. The properties of hydration water approach those of the bulk continuously with distance from the protein surface. A clear interface was never observed in contrast to what is suggested here.

"*Two types of fluctuations in the protein environment called α and β are involved… The α-fluctuations are structural. Their rate coefficient is inversely proportional to the viscosity of the medium. They can be neglected in protein crystals (*no proof is given*), but they are important in viscous liquids… The β_F fluctuations originate in the hydration shell and influence internal protein motions. They depend on hydration and vanish if the protein is dehydrated".*

Hydration shell fluctuations are thus "dubbed" β without any deep reasoning. Numerous experiments (NMR, Neutron scattering, see comments on hydration water below) and simulations with protein-adsorbed water have demonstrated the existence of translation diffusion and thus α-relaxation also in protein crystals. In Doster et al. JCP (2013) we have shown that the LMF can be reproduced by the viscosity dependent structural relaxation and is incompatible with secondary processes.

"The protein dynamic transition is not required since the full Mössbauer spectrum is explained without invoking a dynamic transition. In reality, the apparent abrupt increase in the MSD occurs when k_β becomes larger than $k_{M\ddot{o}}$."

Both structure and spectra of a super-cooled liquid vary continuously across the glass transition even if observed above T_g on a short time scale below 100 s. This is common knowledge, there is no need to publish this in PRL.

Lichtenegger et al. write in 1999:

"*Similar to our results with (solvent exposed) ^{57}FePFC in sucrose/water, the extra loss of area in the Mössbauer probes in glycerol/water is accompanied by a broadening of the central Lorentzian line and additional broad wings in the spectrum that have to be accounted for by a second Lorentzian component. Alternatively a single non-Lorentzian component, a Cole-Davidson function, could fit the data just as well. Furthermore, the resulting average relaxation times were compatible to those derived for the viscoelastic α-relaxation of the glycerol by other methods (Nienhaus et al., 1991). This result allows one to assign the line broadening to the structural relaxation of the solvent. For myoglobin in 75% glycerol/water the onset of anharmonic mean square displacements occurs at ;215 K (Fig. 8a and Franke, 1992). The broadening of the central line becomes prominent at slightly higher temperatures between 220 K and 230 K. The viscoelastic relaxation time of 75% glycerol/water in this temperature interval decreases from 2 ms to 200 ns (Kleinert et al., 1998), which is in the range of the 57Fe-nuclear lifetime. This result, as in the case of theiron salt discussed above, is consistent with the notion that the broadening of the resonance line reflects the viscoelastic relaxation of the glycerol/water mixture. The coupling of the heme displacements to the solvent may involve the propionic acid side chains of the heme, which are exposed to the solvent. With myoglobin in the more viscous solvent, 80% sucrose/water, we observe the same spectral features except that the onset temperature is up-shifted to 240 K…"*

In fig. 7 Lichtenegger et al. compare the Fe diplacements of myoglobin in two solvents with the functional property of ligand escape in a flash photolysis experiment. log Nout(T) varies continuously with temperature, but shows the same viscosity shift:

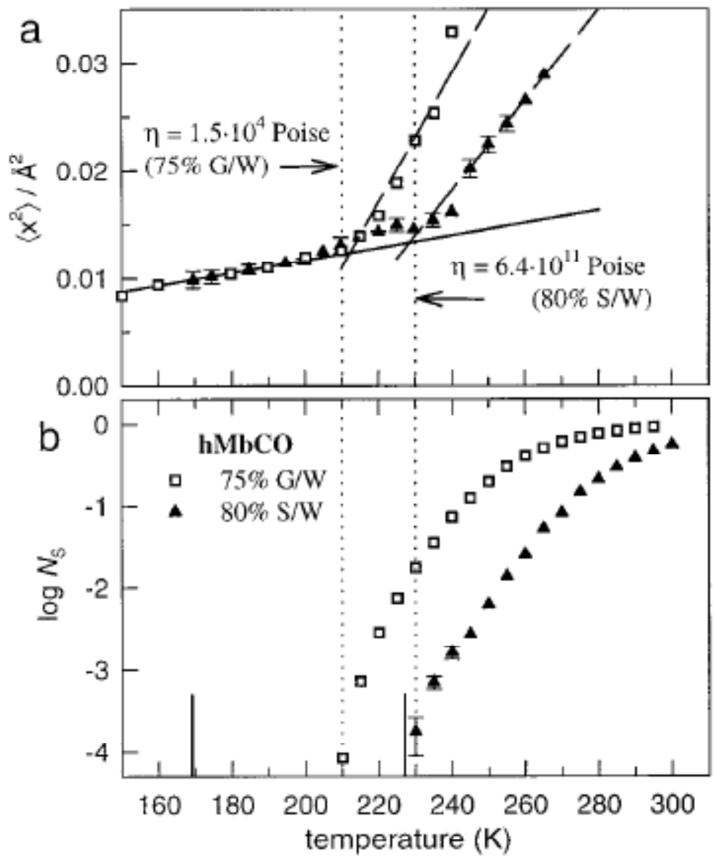

FIGURE 7 (*a*) Mean square displacements of the heme iron of myoglobin in 75% glycerol-water (□; Franke, 1992) and 80% sucrose/water (▲). The bulk solvent viscosity at respective onset temperature is also given. The lines are drawn to guide the eye. (*b*) Escape fraction N_s of MbCO in 80% sucrose/water (▲, 80% S/W), 75% glycerol-water (□, 75% G/W) versus temperature. The bars indicate the calorimetric glass temperatures T_g of 80% sucrose/water and 75% glycerol-water at ~230 K and ~170 K, respectively.

The central ideas of Young et al. were thus published already by Lichtenegger et al. 12 years earlier. The same is true for Fenimore et al. PNAS 2004. The latter publication implies a dramatic reversal of Frauenfelder's view of the PDT: before it was explained as a de-trapping transition out of the wells of an energy landscape (see also W. Doster, Comment to the PDT puzzle of Magazu, JPCB (2012)116 ,6066. The energy landscape of HF is no longer controlling protein dynamics. Now it is the solvent.

That Lichtenegger et al is not cited by Young et al. constitutes a clear case of scientific misconduct. The author try to suggest that their ideas are new and original.