Comment on "Evidence of Coexistence of Change of Caged Dynamics at T_g and the Dynamic Transition at T_d in Solvated Proteins" by Capaccioli et al. (J.Phys.Chem.B. 2012,116,1745)

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Elastic neutron scattering data of proteins in cryo-solvents are interpreted in terms of two transition temperatures, the glass temperature T_g and the dynamic transition temperature T_d, supposedly related to two processes called primitive- and β-relaxation.[1] Conventionally both temperatures were associated with the viscosity-coupled α-relaxation of the solvent observed at different time scales[2,3]. Here we focus on fig. 1 of ref.(1), which compares temperature dependent mean square displacements (MSD) of partially deuterated glycerol with those derived from a 50:50 mixture of lysozyme and per-deuterated glycerol. The MSD values of solvent and protein solution superimpose perfectly across the entire temperature range, which is remarkable for the following reasons:

(1) In previous neutron scattering work on hydrated proteins we have compared both static and time resolved MSDs of hydration water with those of water-coupled protein

motions,[2,3,5,6]. As expected, the MSD values of the solvent protons grow significantly faster than those of the protein residues both versus time and temperature. Moreover, their statistical properties are different: water is highly mobile and can perform long range diffusion, while protein residues are confined by covalent bonds. The apparent displacements of water thus increase with decreasing wave vector Q in contrast to those of localized protein side chains. The same differences are expected to occur for the MSD values of liquid glycerol and protein residues solvated by glycerol. The data in fig. 1 suggest instead identical displacements.

(2) Identical MSDs in fig. 1 could indicate, that the same molecular motions were recorded in both cases irrespective of the sample content, most likely the mobile glycerol molecules. Since pure glycerol is a bad solvent to proteins[4], in particular at high concentration, partial de-mixing and the formation of protein clusters may occur, explaining the above result. In our studies we chose myoglobin instead of lysozyme because of its very high solubility in hydrogenous solutions as demonstrated by small angle scattering experiments[5].

(3) Even if hydrogenated lysozyme is fully dissolved at a 1:1 ratio in per-deuterated glycerol, one cannot ignore the relatively large coherent cross-section of the solvent, partially masking the incoherent part of the protein. Our neutron polarization analysis studies of proteins in per-deuterated solvents indicates that the Q-dependent signal of glycerol will contribute at least 25 % to the total cross-section[8]. This number is comparable with the partial cross-section of methyl groups in proteins, giving rise to strong signals in powder studies[3]. We can test this idea with similar data taken at different protein concentrations, at a ratio of 50:50 and 80:20 lysozyme. According to Tsai et al[9],

the apparent MSD values decrease with increasing fraction of per-deuterated glycerol: The MSDs at 50:50 are lower than those at 80:20 including also the low temperature vibrational range. Tsai et al conclude, that "glycerol appears to facilitate anharmonic motions above T_d, but limits the amplitude of harmonic motions below T_d." The simplest and most straightforward explanation was not considered, the unavoidable change of the total cross-section with concentration, possibly leading to a switch between glycerol- and protein- dominated MSD recording. The larger protein MSD points to a contribution of methyl groups[3], which is absent in glycerol. More strikingly, the anharmonic onset temperature T_d shifts from 240 K at 50:50, typical for pure glycerol at this resolution, up to 300 K at 80:20. A $T_d \approx 300$ K was predicted by us for 90% glycerol at this resolution[6], as shown in fig. 1*.

(4) A shift in T_d of lysozyme with glycerol concentration is expected to occur also for following reason: Neutron scattering experiments, in contrast to dielectric relaxation[15], provide Q-dependent dynamic information . The authors of ref. (1) do not discuss, how the MSD values in fig. 1 were actually generated, their article does not include a single equation: From the literature it is clear that the MSDs of lysozyme in glycerol were derived from the low Q-slope of the elastic scattering function, $S(Q, \omega = 0) = S_{el}(Q) \propto \exp(-Q^2\langle u^2\rangle/3)$, with $Q^2 < 1$ Å^{-2}. By contrast, the MSD of partially deuterated glycerol by Wuttke et al.[10], superimposed in fig. 1, were measured using IN13 at much higher $Q^2 \approx 9$ -25 Å^{-2}. This leads to diverging results if the elastic scattering functions is not Gaussian within the entire Q-range. Moreover, glycerol is a liquid, which does not exhibit genuine elastic scattering. The Gaussian approximation is thus neither valid at high nor at low Q-values: $S(Q,\omega = 0) = 1/\pi\,(DQ^2)^{-1}$, where D denotes the self-diffusion coefficient. The

two data sets are thus Q-incompatible. Most important, Fujara et al[11] have derived the corresponding low-Q MSD values of the same partially deuterated glycerol sample: their results differ from those shown in fig. 1. Instead, from the non-Gaussian Q-dependence, fast sub-T_g processes are derived similar to what we have suggested for hydrated myoglobin[2]. Data above 260 K were omitted in ref. 11, "since the elastic analysis is no longer applicable as the α-process (!) enters into the dynamic regime of the spectrometer".

(5) In the standard view of the protein dynamical transition, T_g and T_d have the same origin, specified by a step in the specific heat: the viscosity coupled α-process, observed on different time scales[2,3]. This view is disputed by assignments given in the insert of fig. 1, where T_d is associated instead with the JG secondary (β) process. We focus on the Mössbauer effect, sensitive to small scale(β) motions, where the difference in relaxation times is most pronounced. Instead of displacements, we consider a more basic quantity, the Lamb Mössbauer factor (LMF). Approximating the solvent dynamics by single exponential process leads to a Lorentz-Lorentz model[14]:

$$LMF = \exp(-A \cdot T) \quad [1 + \tau_{res} / \tau_c(T)]^{-1} \qquad (1)$$

'A' is the temperature coefficient of the vibrational DWF and $\tau_{res} \approx 141$ ns denotes the resolution determined by the ^{57}Fe life time. To simulate the LMF, we use as input the average correlation times $\tau_c(T)$ of α- or β-relaxation taken from either specific heat[12] or dielectric relaxation[1] experiments.

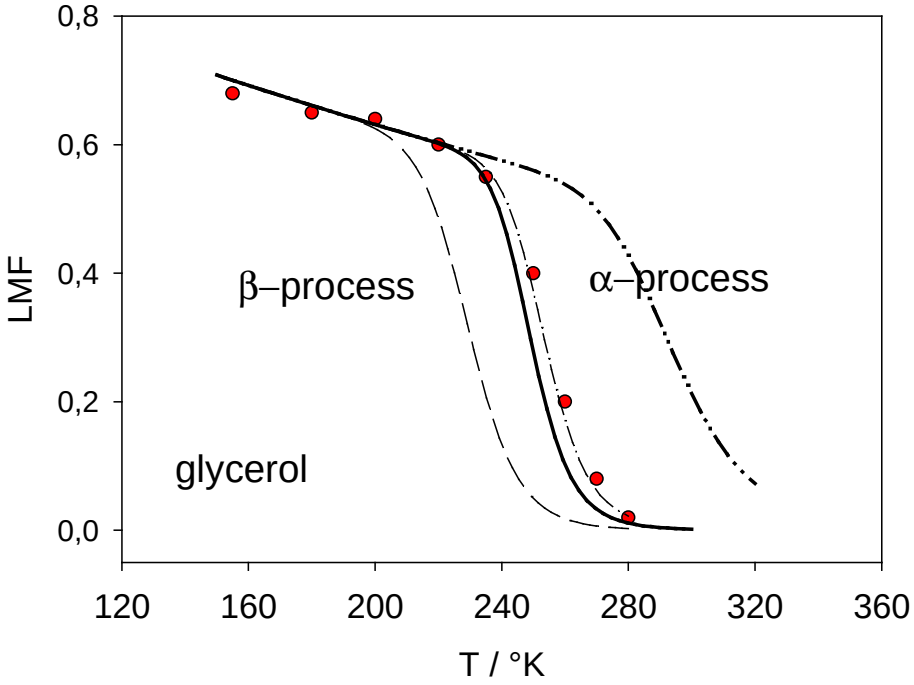

fig. 1: Lamb Mössbauer factor of 99% glycerol[12] and simulations according to equ.1 with τ_{res} = 141 ns, A = 0.0023/°K, $\tau_c = \tau_0 \exp[H/R(T-T_K)]$, (full line): H/R = 2310 K, τ_0 = 0,6 (1,4 dashed-dotted) 10^{-15}s, T_K =129 K [13], dashed: H/R = 6900 K, τ_0 = 1,3 10^{-20}s, T_K = 0 adapted by a fit to data in fig. 1 from ref. (1) . Dashed-d-dotted: τ_{res} = 1 ns.*

The comparison of simulation and experiment in fig. 1* suggests, that the iron couples directly to the viscosity dependent α-process. Although τ_0 derived from the data (dashed-dotted) is by a factor ≈ 2 larger than $\tau_{0\alpha}$ (full line), suggesting a slightly enhanced viscosity near the Mössbauer nucleus, the $T_d \approx 240$ K is correctly predicted. By contrast, the β-relaxation model predicts $T_d \approx 210$ K. The MSD onset temperature implies a time scale around 10^{-6} s [12] and not 10^{-7} s as assumed in the insert of fig. 1. Analogous conclusions were derived for ^{57}Fe in 80% sucrose and ^{57}Fe myoglobin in 75% glycerol and 80 % sucrose[14]. To summarize, the interpretation of fig. 1 in ref. 1 seems inconclusive. For glycerol, a single temperature is sufficient indicating a spectral crossing of the α-process.

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