The simple explanation of the protein dynamical transition by Daniel et al. (2002)

M. R. Daniel, J. Finney and J. Smith, Faraday Discussion (2002) 122,163

Comment by Wolfgang Doster at bioneutron.de

I became aware of this paper accidentally in 2005, since the authors did not inform me about their ingenious solution. After their first hit, "disproving" the relevance of the PDT to enzyme activity, their second message is now, the PDT is not only irrelevant, it is also time-scale dependent!! Yes, that is exactly, what you should expect from a dynamical or glass transition, as defined by us in 1989 (Doster et al. Nature). Any MD simulation is time-scale dependent!

The idea of using two spectrometers for the same sample is not new (Doster et al.,1989, Doster, Settles 1998 in Hydration processes in biology)). Daniel et al. and Becker et al. apply elastic time-of flight (IN6) and back-scattering studies (IN16) to protein methanol-water solutions. Since they study solutions, the respective displacements display an odd temperature dependence never observed before. The results derived from the two spectrometers were identical!! except at intermediate temperatures, which is quite remarkable. The differences at intermediate temperatures are assigned to resolution effects, ignoring differences in Q and sample history.

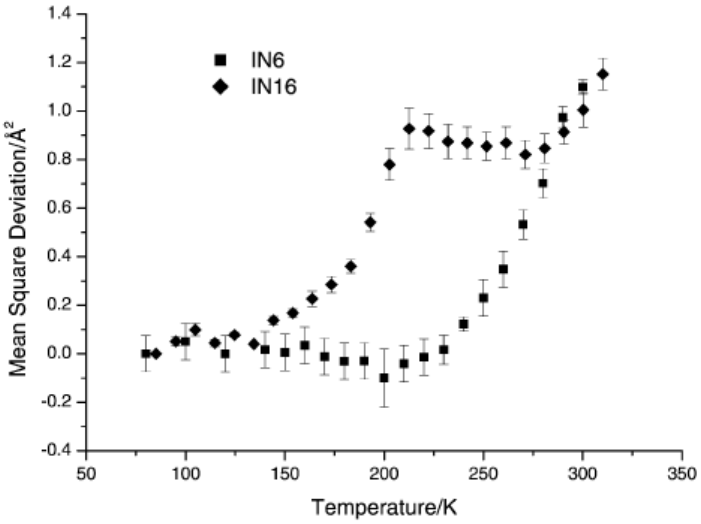
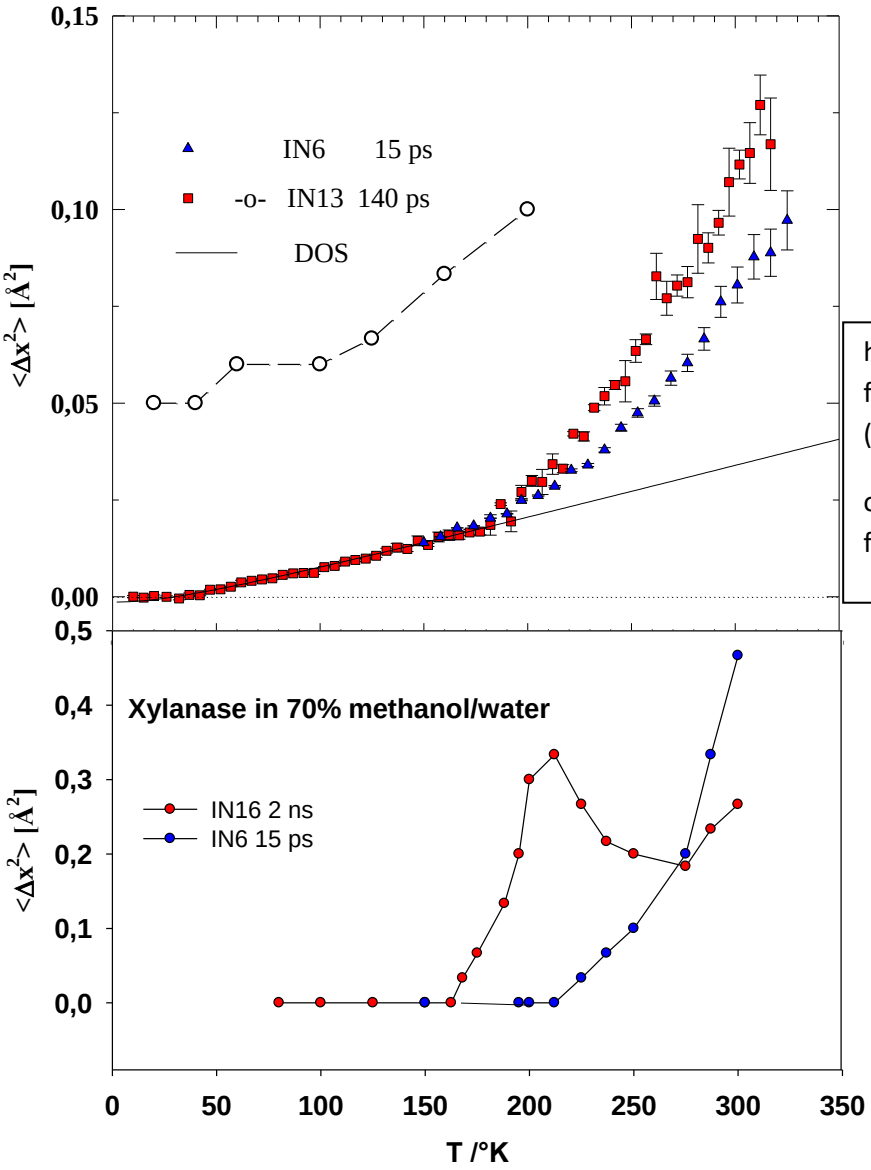


Fig. 2 Mean square displacement of GDH in 70% methanol in water, as a function of temperature *T*, measured on both IN6 and IN16.

figure based on Daniel et al.l Biophys.J. (1999) 77 2184

The IN16 long-time displacements increase up to 200 K, going through a maximum at 210 K, then there is a slightly decreasing plateau, finally the data merge with the IN6 short time data above 250 K. The IN6 short time data decrease above 100 K with increasing temperature, then at 230 K, there is a drastic enhancement until the MSDs merge with the long time MSDs at 300 K. This behavior is hardly compatible with incoherent scattering from a liquid solution. The elastic scattering method should be applied with great care to such samples, since the solvent, which does not exhibit genuine elastic scattering, plays a dominant role. Moreover, global rotational and translational diffusion of protein molecules become significant in the spectrum. Neither global protein nor solvent diffusion are taken into account by Becker and Daniel et al 2002., although this was discussed by Perez et al. in 1999. Uncorrected experiments performed with liquid samples at different resolution will mostly reflect global diffusion, depending on Q, with ill-defined elastic intensities. Moreover the authors used a 70% methanol-water solvent, which, in addition to the severe damping effect of methanol on the biological activity, partially crystallizes at low temperatures, generating Bragg peaks.

Thus not only freezing and melting, accompanied by uncontrollable freeze concentration occurs, but also a significant coherent background emerges. It is thus not surprising that the resulting ‘incoherent’ displacements show an anomalous behavior with a pronounced maximum at a certain temperature depending on the frequency window as shown in figure 2. The cusp at 220 K is totally inconsistent with incoherent scattering. Second moments of thermal displacement distributions and stochastic displacements never decrease with increasing temperature. With this anomaly, the proposed ‘simple’ explanation collapses, it mostly reflects the complex properties of the solvent and less the protein or the frequency window of their spectrometer. This example illustrates, why experimental data obtained with protein solutions first have to be corrected for solvent scattering and global diffusion, before the elastic analysis of protein-internal motions described above, can be applied.



hydrated lysozyme (0.35 g/g) taken from Hydration Processes in Biology (1998) Ed. Bellissent-Funel

open circles: same sample taken from Magazu et al JPC B 2011??

this paper and Dunn et al. Biochem. 2000

The unpublished figure above compares our hydrated lysozyme data taken at two different resolutions (Doster, Settles 1998), with the Xylanase methanol-water solution of Daniel Smith and Finney. The lysozyme displacements clearly show enhanced displacements at the higher resolution even at high temperatures. Note that the Magazu data taken with the same sample and resolution disagree with our measurements even after dividing them by a factor of 2. Our data were presented at a Les Houches seminar in 1998, which was published in a book Hydration processes in biology, edited by M.C. Bellissent. J. Finney, who had attended the same seminar, published the

GDH experiment in methanol-water one year, later: Daniel et al. Biophys.J. (1999) 77 2184. The time-scale effect was presented there as a genuine new idea. Our lysozyme experiment was not cited. That's it for John Finney.

With IN16, the peak is almost certainly reflecting the ice formation and melting, probably cubic ice, which melts at 210 K. I have done similar experiments with myoglobin in 70 % methanol- water. The protein denatured and aggregated. The authors concede in their paper the existence of Bragg peaks, which is claimed to be irrelevant to their analysis. This is obviously untrue. J. Smith even tried to reproduce the peak at 210 K with simulations. Their "simple explanation" is just too simple to be true.

Finally, their "dynamical transition" has little to do with what we had introduced in 1989 (Doster et al. Nature). The original definition involved two steps (see 1989 comment), a resolution-independent pre-transition at 180 K , induced by an increasing amplitude of fast H-bond fluctuations and a slower resolution dependent onset at 240 K, due to coupling of protein fluctuations to glass-forming structural relaxation of hydration water. Two spectrometers, back-scattering and IN6, were used already in our original study in 1989. The methanol-water system does not show a glass-forming property, which is essential for observing a PDT. Their anharmonic onset is not a PDT.