

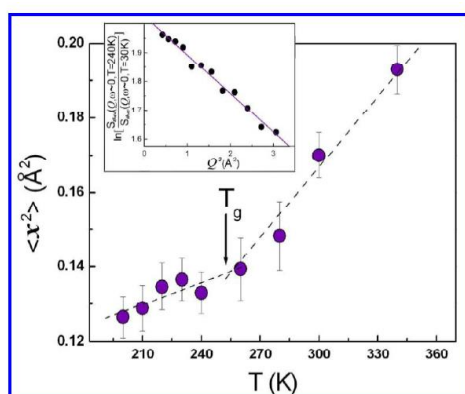
**Does a dry protein undergo a glass transition? J. Phys. Chem. B. (2014) 118, 2791**

by A. Frontzek, S. Strokov, J. Embs and S. Lushnikov

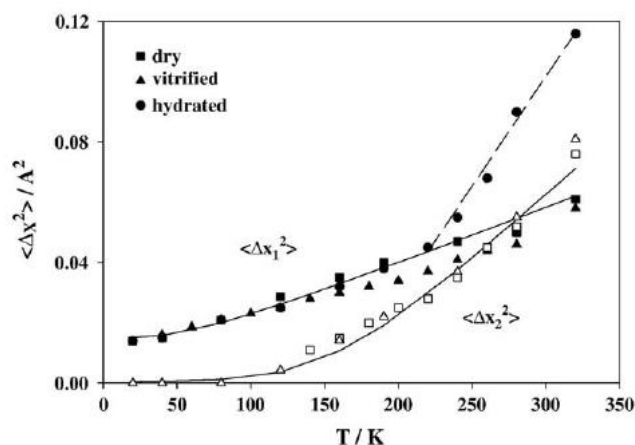
Comment by Wolfgang Doster at bioneutron.de

Glass transition in a dry protein? Depends how you define it! Most workers in the field of bio-preservation, including those of bio-neutron scattering would exclude it: Dehydration generally immobilizes proteins in a glassy state, which is the essence of the preservation mechanism. This does not mean that dry or solvent-vitrified proteins are completely immobilized as implied by Zaccai (Science 2000, see review below). Internal motions, such as transitions of side chains and internal ligand migration prevail. (Doster, Settles, BBA 2005).

I was therefore not too much surprised about quasi-elastic broadening observed with a dry protein. What surprised me, however, was the sharp transition in the T-dependent displacements near 250 K (authors, actually it is 273 K!!) with de-hydrated albumin.



Numerous similar experiments with dry proteins of variable structure did not show any enhancement. The figure below shows a comparison of dry/hydrated myoglobin (Doster, Settles, BBA 2005, 1749, 173) and a two component displacement analysis: Component 2 was assigned to hydration-independent methyl group rotation, component 1 varies with hydration (IN13, ILL). The transition of the hydrated system occurs around 210 K with no transition observed with the dry or sucrose-vitrified protein:



Is albumin really special from other proteins or does their result point to a different origin?

Before I suggest an alternative explanation, I review some statements of the authors which also surprised me:

### 1) Neutron scattering :

a) **Sample preparation:** The sample was bought from SIGMA and used without further purification. Such preparations often contain residual salts such as acetate, leading to spurious quasi-elastic broadening by acetyl methyl groups. The sample preparation is not fully described or it has not been done properly: To put a dry protein into a can is not a trivial task. If this is not done carefully under a dry atmosphere (helium) in a glove box, the sample will definitely pick up water. In my view this is what happened here.

b) **Interpretation of uncorrelated dynamics:**

The authors are interested in “uncorrelated dynamics” which they incorrectly identify with incoherent scattering. They mix up “uncorrelated dynamics” with single particle displacements, which will be highly correlated with motions of neighboring particles in a dense protein system.

c) **Cross sections:** “*Since the scattering cross-section of hydrogen (80.3 barn) is much bigger than the coherent (1.8 barn) in contrast to deuterium....., there is no need for future (?) separation of both contributions preliminary to the data analysis.*”

This seems to be the accepted view of those workers, which are cited by the authors. Their sloppy statement ignores the cross-section of all other atoms of the protein and the Q-dependence of coherent scattering. Obviously the authors were not aware of the polarized neutron scattering study by Gaspar et al. (BBA 1084 (2010), 76) separating coherent from incoherent scattering of dry and hydrated proteins and the recent discussion by Doster et al. (JCP, 2013).

d) **Equations, dynamic models:** a model of the *theoretical dynamical, incoherent quasi-elastic scattering function* is presented, which is actually a model of the complete structure factor including the elastic component: The elastic structure factor is introduced. But then in the derivation of the mean square displacements the combined elastic intensity is used, which contains a quasi-elastic component. The non-Gaussian nature of the elastic scattering function of proteins due to side-chain rotation is ignored completely (Doster et al. Nature 1989). The fit shown in fig. 2 for a single temperature emphasizes the rather uninteresting vibrational tail and the Boson peak.

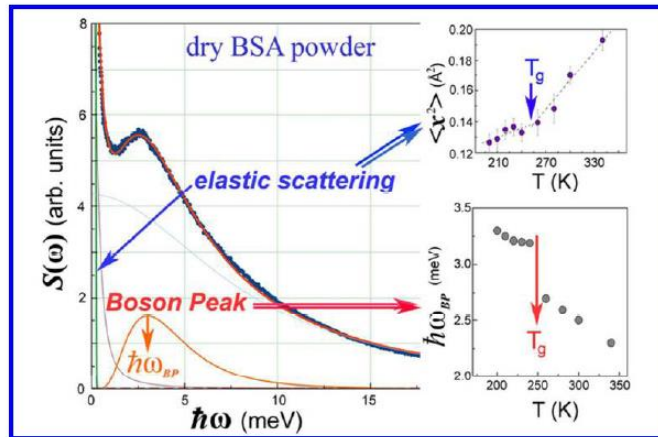


fig. 2, showing the four (!) component fit at 240 K and the temperature dependent Boson peak frequency with step at  $T_g$ .

- e) No temperature dependent fits of the data in fig. 3 are shown, which would be essential to justify a T-dependent transition around 250 K. In fact it is obvious from the spectra in fig. 3, that the main change occurs at much higher temperature: There is a dramatic enhancement of the quasi-elastic spectrum above 280 K:

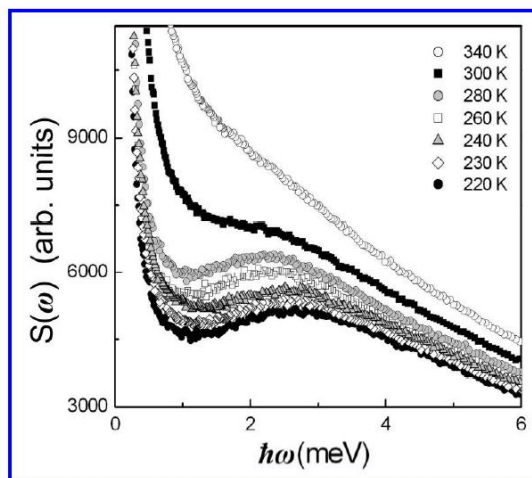


Figure 3. The dynamic structure factors of dry BSA measured at different temperatures.

- f) **Boson peak:** The properties of a Boson peak in the context of proteins was first discussed for hydrated myoglobin by Cusack and Doster in Biophys. J. (1990): Their main results were:
- 1) The Boson peak does not shift with temperature!
  - 2) It is independent of protein structure...
  - 3) The peak frequency shifts with hydration (Diehl et al. Biophys. J. 73 (1997) 2726). With dehydration, the Boson peak shifts to lower frequency, independent of the temperature.
  - 4) The biological relevance of the Boson peak and theoretical fitting models of its shape were discussed by Leyser et al. PRL 82 (1999) 1987. All these highly relevant papers are ignored, which is one of the main reasons for the final

incorrect conclusion: The apparent temperature dependence of the Boson peak frequency is taken as evidence of a glass transition.

**g) Dynamical transition, glass transition**

*“First of all, the dynamic transition temperature, as identified as a change of slope in the T-dependent displacements has been shown to depend on the resolution of the spectrometer, that is not the case for a glass transition..”*

The authors thus neither understand the nature of the glass transition, which is a time scale dependent phenomenon, nor the nature of the Protein Dynamical Transition, which exists only in the context of a GT.

**2) Results and Alternative Explanation: The Freeze-Drying Transition.**

I am proposing a “possible” explanation of the transition at 250 K in contrast to the one presented in this paper, which in my view can be excluded. The figure below is taken from Leyser et al. (PRL 1999, 82, 2987) for dry and hydrated myoglobin (other proteins show identical results). The most important result is the down-shift of the Boson peak frequency from 3.2 meV (hydrated) to 2.2 meV (dry), which quite general depending on hydration or solvent but not on protein structure.

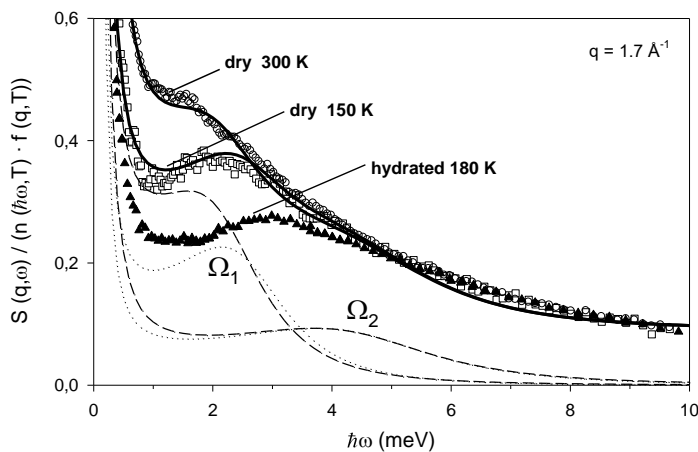


fig. 1 of Leyser et al. PRL 1999 of dry myoglobin with fits of the Boson peak.

Now we compare this result with fig. 4 of Frontzek et al., which shows a transition in the Boson peak frequency of “dry” albumin at 250 K from about 3.2 to 2.7 meV, decreasing with increasing temperature:

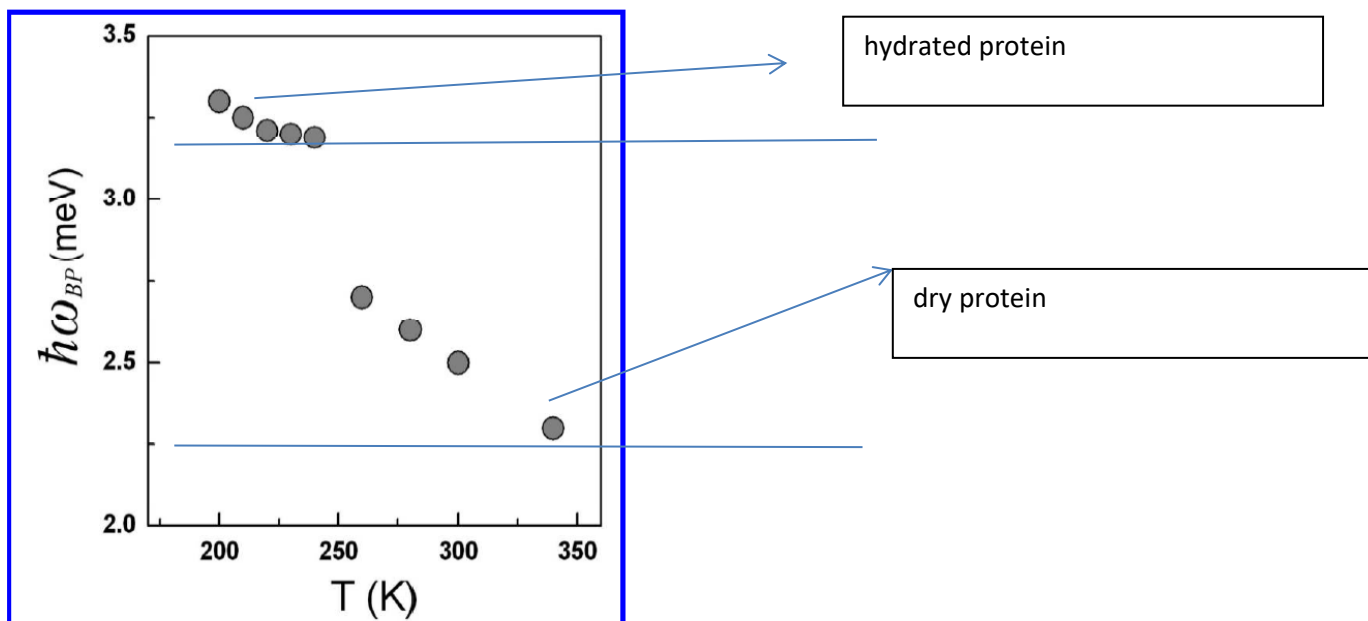
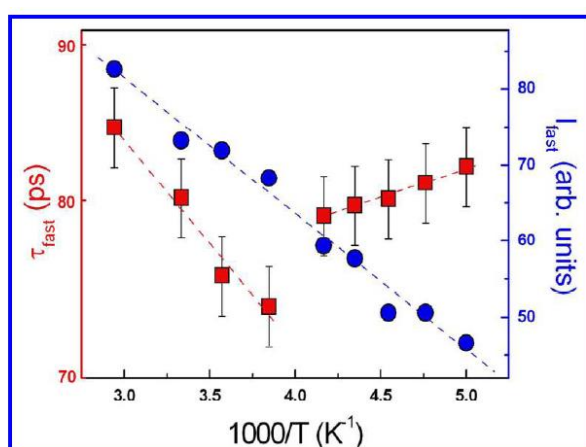


Figure 4. Temperature dependence of the frequency of boson peak maximum for dry BSA powder.  
70x70mm (600 x 600 DPI)

There is not much room for interpretation: The data suggest, that their albumin sample at low temperatures was hydrated given a Boson peak frequency around 3.2 meV. A frequency above 3 meV is incompatible with a dry protein. Then, above about 250 K, the water in the protein starts to evaporate and the protein now approaches a dry (glassy) state by freeze drying. The Boson peak frequency is thus approaching the 2.2 meV of a dry protein. The water vapour either condenses at the sample holder walls or contributes as a gas to the enhancement of quasi-elastic broadening above 280 K in fig. 3. This freeze-drying transition also explains the very strange behavior of the two correlation times with temperature in fig. 5, 6 (red squares, blue circles refer to amplitude).



Their fast correlation time is first decreasing with decreasing temperature and then increasing! Both correlation times show a discontinuity at 250 K. This is incompatible with a standard glass transition, where the structural relaxation times diverge. It is ironic, however, that the authors are probably right with their idea of a glass transition. However, their glass transition

from mobile to rigid occurs with increasing temperature, since the protein is dried by evaporation of protein-adsorbed water above 250 K.

The experiment probably should be repeated under more controlled conditions. The problem with neutron scattering is, that this requires more beam time, which you get only if you publish first. So the tendency is, to publish no matter what has been observed even if the results look strange. You sell it as a striking new result. Then with more beam time you can later correct with a second paper. So instead of doing serious science by performing a confirming experiment before publication, which results in a correct paper (or no paper), it is easier and better for the career to publish two papers.

This paper by authors, who are obviously new in the field, should not have been published . It is a good example of what I describe in the main text of this homepage: No special expertise is required to perform, analyze and publish a bio-neutron scattering experiment, especially if it concerns protein dynamics. That something is wrong with this paper is obvious, therefore the referees should have interfered. For this failure, I mainly blame the referees and not so much the authors. I have some doubts whether the referee system of JPCB in the bio-neutron field is working properly. I complained about it several times earlier as a referee of some other papers, which was ignored. When I tried to submit a Comment after my report had been ignored, it was rejected by the Editor arguing, that I had been a referee.