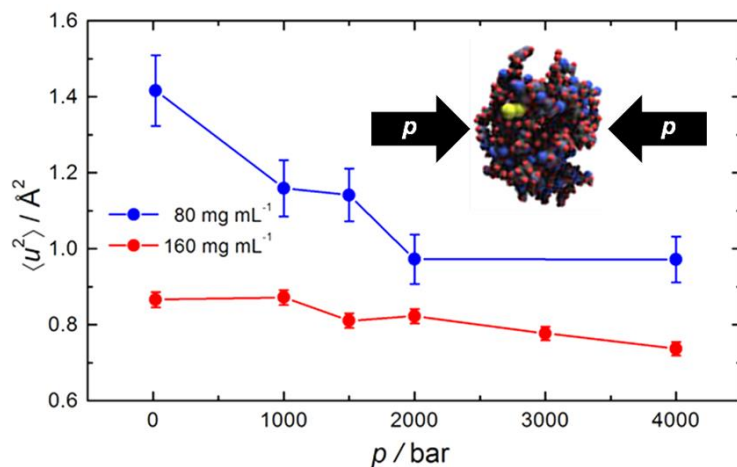**Comment by Wolfgang Doster at bioneutron.de on the publication:**

**Influence of Pressure and Crowding on Sub-nanosecond
Dynamics of Globular Proteins** M. Erlkamp, J. Marion, N. Martinez, C. Czeslik, J. Peters and R. Winter, J. Phys. Chem. B 119, 4842 (2015)

ABSTRACT: *"The influence of hydrostatic pressure on the internal sub-nanosecond dynamics of highly concentrated lysozyme in aqueous solution was studied by elastic incoherent neutron scattering (EINS) up to 4 kbar. We found, with increasing pressure a reduction in the dynamics of H atoms of folded lysozyme, suggesting a loss in mobility.."*
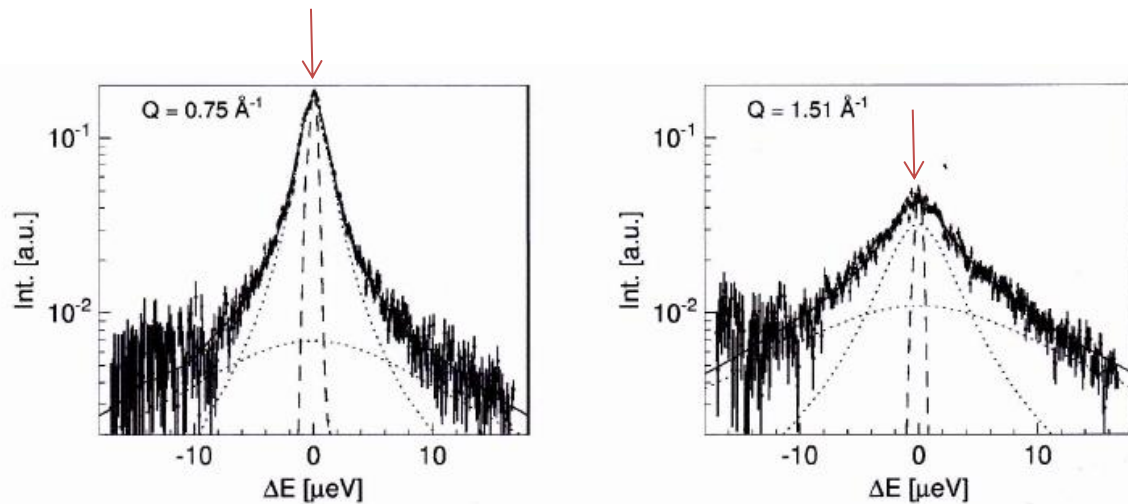
The cover figure above shows their main result, the "hydrogen" mean squares displacement $<u^2>$ of two lysozyme D_2O-solutions versus pressure at different protein concentrations. The higher concentration sample (red curve) has almost no pressure dependence (note the small error bars, even the dip at 1,5 kbar could be real), while the blue curve at half the concentration exhibits much larger error bars and larger displacements, with a depression at low pressure. Why should the internal motional amplitudes of the compact protein lysozyme vary so much with concentration? And why is the pressure effect so strongly depending on concentration? The authors offer a catch word explanation "crowding", implying aggregation at the higher concentration. The enhanced aggregation is not really supported by their small angle data with slightly different samples. Moreover the displacements of compact proteins are not very sensitive to protein environment unless the hydration shell is removed. This suggests that the recorded displacements are not really internal.
First of all, the recorded displacements are too large (even after division by 3) compared to what is shown for lysozyme-D2O (hydrated or crystal) on the top of the Critical Review page.
The samples are in solution, so one can expect that global protein diffusion contributes significantly to the apparent hydrogen displacement. Did the authors take that into account? The answer is no. The authors were obviously not aware of the relevant literature (Longeville et al., Myoglobin in crowded solutions, Structure and Diffusion, Chem.Phys. 292 (2003) 412 and Doster et al. Elastic Scattering Analysis, J.Chem.Phys. 139, (2013) 45105, Monkenbusch et al. J. Chem. Phys. 143(2015) 75101).
To derive dynamics from elastic scattering, which reflects the rigid aspect of the molecule, as proposed here, you have to understand the complete spectrum. The figure below shows the back-scattering spectrum of a concentrated protein solution at two Q-values on a log scale, comparable to the data presented above (Busch et al. MRS Bull. 2006):
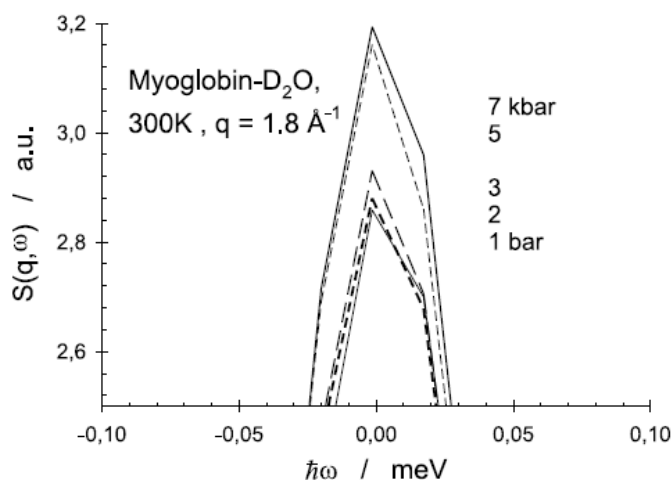
Also shown is a three-component fit of the spectrum comprising an elastic (dashed), and two quasi-elastic lines (dotted). The narrow quasi-elastic line, which broadens with Q was assigned to protein diffusion, the second line reflects internal motion convoluted with diffusion broadening. The intensity of the elastic line decreases with Q due to both, internal displacements and translational displacements.

Erlkamp et al. only record the intensity at zero energy exchange (red arrow), thus they cannot discuss the real sub-nanosecond structural dynamics including global diffusion. The figure also shows, that by focusing on the elastic intensity, one ignores the quasi-elastic spectral contribution at zero frequency (Doster et al. 2013). At the large $Q^2<u^2>$ values the elastic intensity has dropped down to 3% and becomes comparable to or less than the inelastic spectral intensity at zero frequency. Then assumptions brake down.

The effect of protein diffusion provides a plausible alternative explanation, both to the enhanced magnitude and the concentration dependence of the apparent mean square displacement.

It is striking that their literature on neutron pressure experiments is very incomplete:

The first elastic- inelastic neutron scattering spectrum versus pressure of a protein including pressure denaturation was published by Doster and Gebhardt (Chem. Phys. 282 (2003) 393). The figure shows the peak elastic intensity versus pressure and the denaturation transition at 4 kbar: The pressure effect is small except in the transition region. The elastic intensity is enhanced in the aggregated, immobilized denatured state. This effect is probably related to the results of Erlkamp et al., showing a step down in the displacements at low concentration to those of higher concentration.
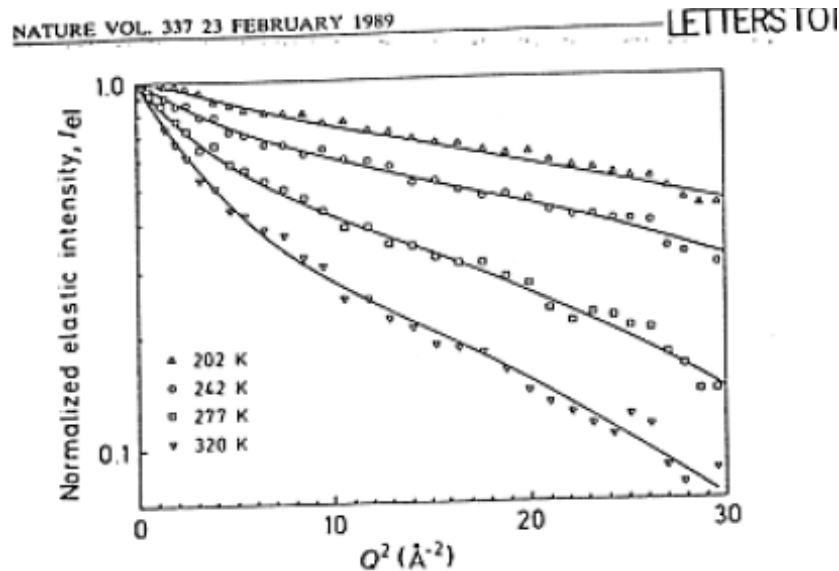

More recently (2011) Appavou et al. published an inelastic neutron scattering study of hemoglobin solutions under pressure (Eur. Biophys. J. 40, 705). The neutron structure factor of myoglobin versus

pressure is shown in the Protein Folding Handbook Vol 1 p. 99: Pressure-Temperature Phase Diagrams of Proteins by Doster and Friedrich.

 For their analysis Erlkamp et al. rely on the rather questionable paper by Tehei, Daniel, Zaccai (ref. 58) . Equation (1) applies to a single Gaussian process ~exp $(-Q^2<u^2>/3)$ but disagrees with the referred equ.(1) of Tehei et al. Moreover the range of validity of the Gaussian approximation in neutron spectroscopy is not equivalent to the Guinier approximation of elastic low angle scattering. The magnitude of $Q^2<u^2>$ is rather irrelevant. Proteins are complex systems with multi-component dynamics, there will be a combination of processes with different types of displacements leading to non-Gaussian scattering functions at all Q-values (Doster, Settles, protein-water displacement distributions, BBA 1749 (2005) 173).
Already the first elastic - inelastic neutron scattering study of a protein (Doster et al. Nature 1989) revealed a non-Gaussian IN13 elastic scattering function which is reproduced in the figure below. The non-Gaussian feature was explained by a two-component inelastic spectrum of internal motions including side chain rotation.

This scattering function generally applies to globular proteins.
The Gaussian scattering function recorded by Erlkamp et al. thus indicates that the non-Gaussian internal displacements are overwhelmed by Gaussian diffusion. This is well known since Perez et al. Biophys. J. (1999) 77, 454. To clarify this point requires a spectral analysis similar to the one displayed above. The Gaussian elastic model promoted in ref. 58 has been criticized frequently. It condenses the full spectral information to a low frequency and low Q limit. The boiled down data set opens a large range to possible interpretations and thus lacks any scientific value. Similar arguments apply to a related paper by Marion et al. PCCP 2015,17,3157. Finally, "dynamics of well folded and natively disordered proteins in solution" studied with neutron TOF was published by Gaspar et al. EBJ (2008) 37,573. It shows that global diffusion matters even at the TOF resolution in contrast to the assumptions by Tehei et al.