Feb. 8. 2018

Review of the revised version by W. Doster (open review submitted to Biophys. J.)

Determination.. by Vural et al.

1) Bimodal Distribution

The authors came from very far, in 2008, it was a Weibull distribution

Smith, Displacement distribution of SN, Biophysical Journal 94, 4812

(2008)

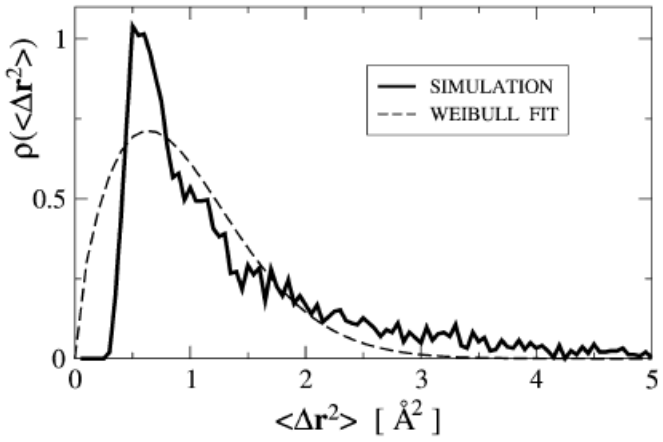

FIGURE 3 Distribution of atomic mean-square displacements from a molecular dynamics simulation of crystalline Staphylococcal nuclease calculated from a 1-ns trajectory and with $\Delta t = 40$ ps corresponding to the IN13 energy/time resolution. Simulation details are described elsewhere (40). The simulation data is fitted using a Weibull distribution, Eq. 4, with the parameters $\alpha = 1.68$ and $\beta = 1.09$.

Now it is changed to bimodal. Their revised version thus **confirms** our original analysis of 2005. In table I even the interpretation of peak 2 is now attributed to methyl groups as in Doster/Settles, BBA 2005. Smith et al derive these conclusions after ignoring our work for 13 years. The converging results question the usefulness of the dynamic heterogeneity concept. Bimodal means two processes not complex energy landscapes.

Response to Peer Reviewer #2

> Contrast this with the analysis of Ref. 29 (revised text). Lacking the MD simulation step, the authors were obliged to invoke a 'minimal model' to describe the elastic scattering, consisting of two components, each of which was assumed to be Gaussian. Thus in Fig. 8 of their paper the authors themselves make the Gaussian approximation for individual atoms and derive a distribution of displacements, similar to the approach in our manuscript. However, they then twist around and *assume* that the motion of one of the components is torsional, citing an MD simulation by Loncharich and Brooks, in which torsional motions were seen but neutron properties were not calculated. Now, there is no doubt that torsional motions are activated with temperature, and indeed methyl rotations play a role, as detailed by us and others. Moreover, the corresponding scattering can be non-Gaussian for individual atoms. However, to leap from this to the assumption that the origin of the non-Gaussian scattering is torsional is not justified

The authors downgrade and misquote our work. They start even a priority conflict. We were not obliged to use a minimal model. We had further information beyond the scattering function. We did not make the Gaussian approximation for individual atoms. Our intention was not to copy the DH model, we applied the Gaussians as formal expansion to Fourier transform the scattering function. We could also have used exponentials. Our goal was to derive the density correlation function G(r, t) from scattering data at fixed time, which is the fundamental displacement distribution not some ρ(s). It turned out that two Gaussians were already enough with temperature dependent parameters for our purpose. Thus we could picture the evolution of the G(r, T). Since the two components are well separated and smooth, the two-Gauss approximation reproduces some essential properties, but it is not the correct G(r, t, T) of course.

With a bimodal distribution the DH approach reaches two limitations:

(1) There are just two processes in the data, for this you don't need distributions. Frauenfelder is out. This was already obvious in 1989. Of course dynamical heterogeneity exists since proteins

are not homo-polymers. But this does not show up in the elastic scattering functions. Not primarily. All methyl groups have identical elastic structure factors. It will show up instead in producing a distribution of relaxation times.

(2) In proteins, rotational transitions are relevant, which excludes Gaussian local distributions no matter what has been simulated. Gaussians may be sufficient if one is only interested in MSDs and not in higher moments. That is our experience with the two Gauss model.

5 closing remarks

To close, we return to an issue of long standing and continuing interest: is the EISF, Eq. (21), a Gaussian in Q (i..e. $I(Q, t = \infty) = \mathrm{e}^{-Q^2\langle z^2\rangle}$ where $\langle z^2\rangle$ is a constant) or not and if not why not? In their pioneering paper, Doster et al. (6) observed that the EISF of myoglobin at 300 K was not Gaussian in Q. They reproduced and explained the non-Gaussian EISF using a two-state model of H motion. To further explain it Doster and Settles (29) identified both torsional and linear H motions in myoglobin, introduced two MSDs, $\langle z_1^2\rangle$ and $\langle z_2^2\rangle$, to describe them which led to an EISF that had two Gaussian components (see also the $\rho(s)$ in the first paragraph of section 4.1). This two MSD EISF reproduced the EISF of myoglobin well.

This paragraph was added. It again misquotes our work, in that we would explain the non-Gaussian structure factor by a two-state model.

In 1989 we explained both elastic and inelastic spectra by two processes, thus a bimodal distribution of processes:

(1) There were two transition temperatures at 150 and 240 K

(2) The elastic scattering function was not Gaussian but could be fitted by a combination of two processes. It was not just a two state model as stated above

(3) There were two processes observed in the inelastic spectrum Elastic scattering model:

a) Rotational transition of side chains, we used a two state model for simplicity, but in the paper it is stated that a three state model also fits the data. The displacements were on the order of 1,5 A, thus only rotational transitions could be considered.

b) Small scale Gaussian displacements, which were sensitive to the water content.

Both components were combined in sequence to account for the total scattering function. At this stage we assumed identical sites with two different processes for simplicity . In 2005 we proposed a parallel model of two kinds of sites. We could identify one of the two processes as methyl group rotations. This assignment was done first in 2001 by ERS with a bimodal time domain intermediate scattering function at room temperature Doster et al. Physica B 301 (2001) 65–68.

Moreover we could predict the MSD of one component versus temperature from the dynamic parameters of the methyl group.

Nakagawa et al. 2004 do not show or analyse a bimodal distribution. They had the funny explanation that our two state model may be the origin of the bimodal non-Gaussian scattering. The same group published an extensive DH paper in Phys.Rev.E (2007) without citing our work in BBA 2005. This shows the attitude. We were cited again in 2010 BBA negatively!

2) Incorrect basic equation

Their basic equations 19-22 are still incorrect. In the bioneutron community it is generally ignored that elastic scattering at $\omega = 0$ is not fully elastic, there is a quasi-elastic component, which can be even dominant. This is not a matter of resolution primarily. Their new treatment thus misses the point. I proposed to read our recent work Doster et al. JCP 2013, fig. 6, in this context, which explains how to derive correctly dynamic information from elastic scattering experiments. This is a very basic paper, but it is totally ignored by the inner circle of bioneutron scattering people. My insight with their equations is limited, I would check a missing pre-Gauss factor (always the problem with Gaussians) in the integral of equ. 19. After inserting this factor there will be a quasi-elastic component at $\omega = 0$ even after normalization.

3) Ethical problems: 20 years of exclusion and misquotation

The authors strongly suggest to the Editor to exclude me as a referee. They don't give a reason. I will give it. Personally I should be very interested in getting this paper published, it confirms my work unintentionally. However this exclusion has a much wider background and it now goes on for 20 years until today. J. Zaccai excluded me totally as a referee, I never reviewed a single Zaccai paper, although he vastly used and misused our ideas. Most of what is known today in bioneutron scattering was first published by the Munich group. Normally in a scientifically correct procedure each bioneutron paper literature list would start with a series of Doster et al. papers. Instead this list was replaced by Frauenfelder papers. In

the latest Review by the Smith group (Vural et l BBA 2017) number 1 citation is again the Frauenfelder/Wolynes 1991 Science paper on energy landscapes, which is totally obsolete and has nothing to do with neutron scattering. Hydration water does not exist in this world yet. Later it will "slave" the protein although the landscape is still dominating protein dynamics, that's the logic. The severe attack of Frauenfelder on neutron scattering theory in PNAS is obviously supported by Smith. By contrast the basic paper of the field of 1989 is cited in his Review as number 67!! There we show that, instead of energy landscapes, a two component molecular dynamic models is sufficient, now confirmed in this paper. It is interesting what is not cited: The new development of "elastic resolution spectroscopy" with several papers since 2001 up to 2013 (JCP) is completely ignored. No, not quite, the primitive version of it by Magazu is cited. My experience in 20 years was that first my work is ignored, then when my results are reproduced by others, they are republished under a different name often with much less quality. ERS becomes RENS and in a different context α-relaxation turns into β-relaxation. Similar things will happen with the "bidmodal distribution". It is interesting to look at the literature list of Nakagawa et al. 2004, again the same downgrading of Doster et al.

Since about 2002 there is a general agreement by the "inner circle" not to cite my work except the "pioneer" paper of 1989. This is why J. Smith has a problem citing our BBA 2005 paper. There is a lot of evidence for this plot in the literature and directly by the testimony of collaborators. Frauenfelder played a big role, but also Zaccai and Smith. Martin Weik presented a list of relevant bioneutron papers at a Rome conference in 2009, my papers were classified as irrelevant except of course the "pioneer" paper. The editor of PNAS at that time, Gene Stanley

warned me, that Frauenfelder was very determined to move me out of his way. This would be the main goal for the rest of his life. Scientifically it was a big mistake by Smith and Zaccai to open the field to Frauenfelder. But for their career it was a push. There are too many Frauenfelder slaves and corruption in this field . As a result of excluding critical workers, like me, there is a dramatic decline in scientific quality, which is documented at my Web site bioneutron.de.

Stopp discriminating my work. 20 years are enough!