Review Zeller et al. "Analysis of elastic incoherent neutron scattering data" by W. Doster

This article most likely presents the work of a pHD project (Zeller, an English version is available, http://www.theses.fr/s146914) and seems to be needed to validate the diploma at the Grenoble University. It is quite astonishing , that the supervisors, which act as coauthors, instrument responsibles like J. Peters, V. Garcia-Sakai and M. Zamponi did such a lousy job, not to check even the basic equations (Eq. 4, 5).

Most published papers in bio-molecular neutron scattering deduce structural motions of proteins from the narrow elastic part of the spectrum at zero frequency instead of studying the full frequency window. Moreover, many of these papers, in addition, focus on the narrow range near zero momentum exchange, deriving mean square displacements assuming the Gaussian approximation. This implies that the experimental elastic scattering functions are approximated by straight lines. In the past this simplistic approach led numerous incorrect conclusions. The authors now present a more detailed analysis by fitting elastic data derived from several back-scattering spectrometers at different resolution and Q-ranges by including non-Gaussian fitting functions.

The authors of this paper belong to the "dynamical heterogeneity group"(DH), created in 1991 by J. Smith (Qu. Rev. Biophys.) as an alternative to the original 2-component molecular model of Doster, Cusack. Petry, Nature , 1989, 337, 754. The non-Gaussian elastic scattering function of proteins is interpreted by site heterogeneity of intrinsically Gaussian processes. The non-Gaussian behavior is thus attributed to a distribution of site mean square displacements. The previous analysis is essentially based on computer simulations and DH models that the authors have proposed. All models discussed here belong to this class except the "Do model". The Do model, instead of focusing on distributions, intends to identify specific molecular motions, like rotational transitions of side chains, which are intrinsically non-Gaussian. The original Do concept is bimodal and assumes a combination of Gaussian processes in sequence with rotational transitions of site chains. In this view, to identify a particular molecular process, one choses the optimal instrument with proper parameters: To identify methyl rotation is not possible with Osiris. Aiming at distributions a much broader range of spectrometers may be applied. This is what the authors have done. In the Abstract they conclude:

"The Gaussian approximation…gives qualitatively similar results to models that include heterogeneity..". This very important result should be emphasized already in the title, which is presently not informative. Only this result from their rather technical study, which is of interest to a small group of specialists, could justify a publication in the more general context of J. Chem. Phys.

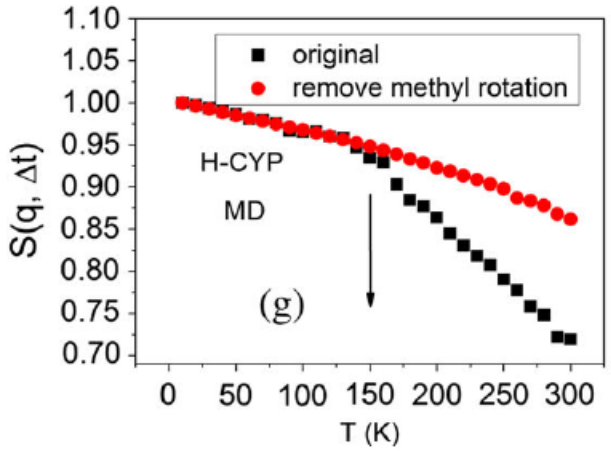
Major deficiencies:

1) I would not consider this as a valid scientific paper, since it does not properly discuss the literature, it does not even cite the relevant literature properly. In their letter to the Editor they motivate their work by recent papers of Frauenfelder, Vural, Doster and Peters. In the text however, their results are not discussed in this context: If ref. 6 is correct, an elastic peak does not exist for proteins, which is the basis of their analysis of lactalbumin presented here. Thus it is not possible to assess the relevance of their results. Their biased citations, excluding

alternative views, are annoying. This suggests that the authors support the Frauenfelder-Smith-Zaccai citation cartel, which exists in this field since 2001.

2) The sample is not properly characterized. It is not clear, which preparation of a-L was used. The protein was bought as a lyophilized powder at Sigma-Aldrich and was investigated without further purification. This is a serious restriction, since often such preparations contain buffers like acetate, which distort the neutron scattering spectrum. Ammonium acetate is a very common buffer, used with protein purification. In elastic neutron scattering experiments this leads to a low temperature onset of displacements due to fast rotating methyl groups around 100 K (Doster et al JCP 2013, ref. 11). Fig. 11 a) and b) suggest spurious buffer effects according to my experience.

3) The main assumption of Gaussian heterogeneity models is that side-chain rotations, which produce intrinsically non-Gaussian scattering functions, are not relevant (ref. 15, 22). This assumption disagrees with simulations and experimental data. Removing the methyls either by selective deuteration or in the computer simulation proves the drastic influence of side chain rotation. The figure below is taken from a recent simulation of lysozyme with and without methyl by Liu et al. (PRL 2017, July):



Peters, Kneller write (JCP 2013): *Applied to the neutron scattering data from human acetylcholinesterase, it reveals a strong increase of the motional heterogeneity at the two transition temperatures T = 150 K and T = 220 K, respectively, which can be located with less ambiguity than with the Gaussian model. We find that the first transition is essentially characterized by a change in the form of the elastic scattering profile and the second by a homogeneous increase of all motional amplitudes. These results are in agreement with previous combined experimental and simulation studies of protein dynamics, which attribute the first transition to an onset of methyl rotations and the second to more unspecific diffusion processes involving large amplitude motions"* This conclusion is very close to ref. 2 and Doster/Settles BBA 2005. There a bimodal distribution is analysed (ref. 23). The heterogeneity distributions after 30 years of simulations now seen to converge to a bimodal shape (Vural et al., Biophys. J.

2018), suggesting that heterogeneity is not the dominant feature of protein motions, which is stressed by the present study.

4) The role of the instrumental resolution

The authors have applied several back-scattering spectrometers with different Q-ranges and instrumental resolution. However the role of the instrumental resolution is not taken into account by their model equations. This question was first dealt with by "elastic resolution spectroscopy" in Physica B 3011,65 (2001) and by Doster et al. JCP 2013. The analysis also ignores the existence of quasi-elastic scattering at zero frequency (Doster et al. JCP 139,45105, 2013) and protein diffusion at 0,8 g/g .

5) Low Q: At low Q the relevance of coherent scattering increases, (Gaspar BBA 2010). Spin echo experiments record only coherent scattering. This implies that their suggested approach will address different scattering processes at low Q than at high Q. Multiple scattering plays a bigger role than generally assumed (ref. 23 and cited literature).
6) Gaussian approximation

One of the disturbing features of this group is their lack of a sound understanding of their basic equation, the Lamb-Mössbauer factor. With neutron scattering one can determine elastic displacements at finite resolution on an absolute scale. There is no space for conventions unless one measure displacements in inches or light-years. In the past, Zaccai and Smith have produced a lot of confusion in the literature with their arbitrary and still varying definitions. There is a subtlety, since the prefactor of the Gaussian displacement exponent <dr^2> is 1/3 for elastic scattering but 1/6 for time resolved scattering. The reason is that with elastic scattering one measures only the half width of the displacement distribution, while in the time domain with the intermediate scattering function it is the full width of the spectrum. Time resolved displacements are thus, at the same time, twice the ones determined by Gaussian elastic scattering:

<r^2(t)> = 2 <r^2(w = 0, dw = 1/t)>

Anyway, the reference (12) given in the manuscript by Rahman concerns fluctuations in liquids. It is well known, that liquids do not exhibit an elastic peak, their LMF is zero, thus this paper cannot be referenced to justify equ. (9).

7) Equs. 4 and 5 are wrong. To submit a paper to JCP with incorrect basic equations shows the lack of quality even with instrument responsibles as coauthors.

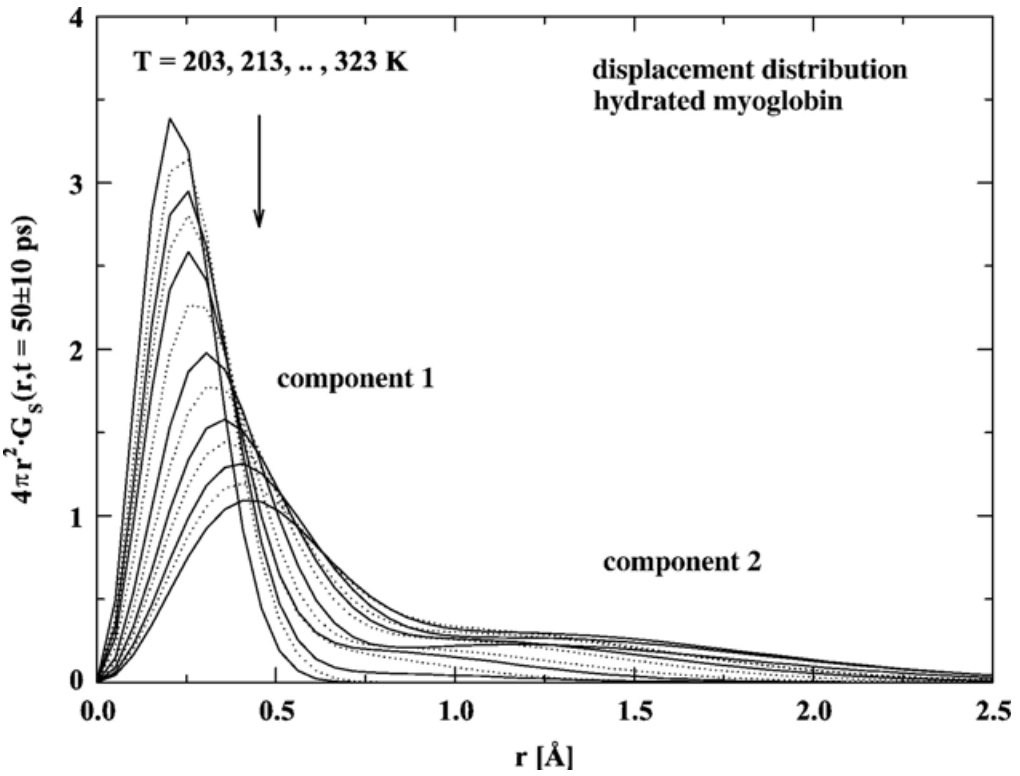
Conclusion:

This manuscript investigates the experimental basis of the DH concept. According to their conclusion, a Gaussian treatment, ignoring heterogeneity is reasonable in most cases. Done much earlier (30 years) this study may have avoided some of the current errors in this field. However, the elastic scattering functions of proteins are not Gaussian due to rotational motions. This conclusion is outside the range investigated by the authors.

Review of the revised version (short version):

1) Reviewer #1 briefly characterizes the three models. According to his view model 3 is not a model of dynamic heterogeneity, it comprises just two states and all sites perform the same motions.
   Page 13: In this model each hydrogen atom can be found in one of two different harmonic wells, separated by a distance d and by a free energy difference ΔG. The model will be referred as the Do model…
   This is a common misquotation still presented in the revised version:
   a) All sites in the Doster, Cusack Petry model of 1989 are assumed to be dynamically identical. There is no "site" heterogeneity in this model. But each site performs two kinds of motions, which is a specific dynamic heterogeneity. The reduced dynamic heterogeneity model, which is discussed here, reflects site heterogeneity only. This is inconsistent with the standard definition of dynamic heterogeneity in condensed matter physics, which is based on non-exponential relaxation. This goes beyond site disorder. The authors assume incorrectly that the Do model reflects anharmonicity only, excluding heterogeneity.
   b) The two kinds of motion model of ref. 2 cannot be reduced to a single motion between two-states: The main topic of ref. 2 "the dynamical transition" refers to the Gaussian diffusive component and not the two state transition. Nakagawa et al. (ref. 22) confused "bimodal" with the "double well", which has become an often cited misquotation. Equ. 16 and 17 are thus not accurately characterized. Moreover, their Do model is obsolete. It was redefined by three equivalent states of mainly methyl groups (Doster/Settles BBA 2005). These points were already made in the first review, implying that the authors are not willing to correct their position by including methyl groups explicitly.
2) The authors even introduce a fourth model, which is supposed to be the most relevant model with IN13: a "bimodal fitting model" in p. 27, citing the equations of Nakagawa of ref. 18. However a bimodal analysis is not performed there, the authors compare instead a Gaussian distribution (fig. 3) with an exponential distribution (fig. 4) of SN. For the bimodal case no distribution is shown, only displacements are given in fig. 5. The displayed linear temperature dependence of one type across the entire temperature range is physically impossible except for vibrations. Their bimodal approach does not yield reasonable relaxation processes. Even for the bimodal X-ray B-factors of fig. 6, only Gaussian and exponential distributions are shown. This paper is definitely not supporting the bimodal approach. As a second case the authors discuss a paper by Combet / Zanotti (ref. 42), where just transitions of displacements involving hydration water are shown. But again the topic of this paper is not a bimodal distribution, nothing is said about it in the conclusion, no distribution is displayed. The main point is the coupling of the protein to the hydration water, based on dynamic transition temperatures. That the first bimodal distribution analysis with IN13 data is not cited here is a revealing provocation.

Fig. 8. Displacement distribution (myoglobin, D_2O-hydrated (0.35 g/g)) at a fixed time (50 ps) versus temperature derived from data in Fig. 6, instrument: IN13, ILL. Doster Settles BBA 2005 1749, 173



Obviously the authors refuse to cite this paper like other collegues of the heterogeneity group, which is a defining property in addition to misquotation of the Do model. If such a basic paper of the field, which presents many other first time results, methyl rotation, dynamics of hydration water, time resolved displacements, is not cited, there has to be very strong reasons. Recently even Vural et al. BJ. 2018 (ref. 22) did cite this paper at least in the preprint, that I have seen.

The paper questions the relevance of the heterogeneity approach:
The average rotational barrier of methyl groups in myoglobin is in the range of 10 kJ/mol [6,17,48]. The solid lines in Figs. 6 and 7 represent fits assuming tortional transitions of methyl groups to be the dominant molecular process. The reasonable agreement with experimental data produced by the model suggests that rotational transitions, essentially of methyl groups, are likely to be the origin of the non-Gaussian displacement distribution. Dynamical heterogeneity seems to be of minor importance. Torsional transitions occur in the hydrated, the dry as well as in the vitrified state. The rates may differ however.

There is an obvious tendency, that the heterogeneity group is trying to present the model as the genuine result of their own research (Vural et al. BJ. 2018, ref. 22) that they have battled against for 30 years. In this context the remarks in this manuscript fit in.