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Comment

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Reply to "Comment on 'Puzzle of the Protein Dynamical Transition'"

Salvatore Magazù,* Federica Migliardo, and Antonio Benedetto

Department of Physics, University of Messina, Viale Ferdinando Stagno D'Alcontres n° 31, P.O. Box 55, 98166 S. Agata, Messina, Italy

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Doster's Comment[1] is addressed to the protein dynamical transition (PDT) that represents a hot topic within the biophysical community; PDT is referred to as an abrupt increase of the *measured* atomic mean square displacement (MSD) as a function of temperature that is present in hydrated proteins with respect to dry proteins; it is usually registered at a temperature value T_d in the range T = 200−240 K. Interest in PDT has been stimulated also by the fact that the measurable functional activity of proteins is supposed to appear at about the same temperature range, although so far only a few examples have been reported. The basic understanding of the mechanism underlying PDT is controversial, and various interpretative models have been proposed; e.g., see refs 2−9.

The main aim of our paper[10] is to formulate an explicative hypothesis for PDT and to clarify the connection between the PDT and the so-called dynamical fragile-to-strong crossover (FSC) registered in the characteristic relaxation time as a function of temperature in hydrated proteins. No reference is made to the "de-trapping model" and to the "glass transition scenario", and no mention about the connection between "T_{on}", "T_g", and T_d is suggested in our paper;[10] therefore, our position is neither a "killer argument" against the "de-trapping model", neither "confirms the original glass transition scenario": T_{on} refers to a system property, whereas T_d refers to an operatively defined experimental observable, as mentioned at the beginning of the Reply.

In our study, we show that a kink in the *measured* MSD temperature behavior appears at a temperature value for which the system relaxation time intersects the instrumental resolution time of the employed spectrometer. Starting from this result, it is shown, from the theoretical, simulative, and experimental point of view, that PDT does not require *necessarily* any discontinuous change in the temperature behavior of the system relaxation time, and hence, it is not *necessarily* connected to a real transition in the system dynamical properties. In particular, in addition to the support of theoretical and simulation findings, in order to get an experimental validation, a set of different neutron scattering experiments on dry and hydrated lysozyme with different neutron spectrometers, working at different instrumental energy resolutions, were taken into account, i.e., with an elastic energy resolution (fwhm) of 1 μeV, corresponding to a characteristic time of about 2192 ps, by IN10 at Institute Laue Langevin (ILL); fwhm = 8 μeV, corresponding to a characteristic time of about 274 ps, by IN13 at ILL; fwhm = 200 μeV, corresponding to a characteristic time of 11 ps, by IN4 at ILL; and fwhm = 0.85 μeV, corresponding to a characteristic time of 2740 ps by HFBS at NIST.

The presence of the above-mentioned kink in the temperature behavior of the *measured* MSD leads to two considerations: (a) the PDT may be coincident with this kink; (b) the temperature behavior of the system relaxation time can be extracted from different *measured* MSD temperature behaviors, obtained on the same system with different elastic incoherent neutron scattering (EINS) measurements by varying the instrumental energy resolution used, i.e., (τ_{RES}, T_{kink}), as discussed in our paper.[10]

As far as the point (a) is concerned, due to the fact that the PDT kinks in the MSDs of hydrated lysozyme *measured* with different energy resolutions occur at the same temperature value for which the system intermediate scattering function intersects each resolution function of the spectrometers used, i.e., at T = 200, 220, 240, and 300 K, it can be inferred that the PDT is (i) a finite instrumental resolution effect that occurs when the system relaxation time intersects the instrumental resolution time and that (ii) it does not imply any transition in the dynamical properties of the system and hence (iii) it is not due to FSC in the temperature behavior of the system relaxation time.[4]

These conclusions on the nature of the PDT are also confirmed by taking into account both (1) the effects on the dynamical behavior of hydrated lysozyme due to the presence of disaccharides and (2) experimental data on hydrated protein systems as a function of the hydration level h. In particular, concerning point (1), the addition of disaccharides is shown to shift the system relaxation time toward a higher value:[11] in such a case, in agreement with our hypothesis, the PDT occurs at a higher temperature, i.e., T = 255 K instead of T = 220 K (see Figure 5c in ref 10).[12] As far as point (2) is concerned, since the increment of hydration level in hydrated proteins leads to a decrement of the system relaxation time,[13] this latter enters the resolution energy windows at a lower temperature: in agreement with our hypothesis, for a given instrumental energy resolution, higher hydration levels show lower *dynamical transition* temperature values.[14]

It should also be mentioned that other recent results are in agreement with our PDT interpretation, and we use this Reply to cite a few of them. For instance, A. P. Sokolov et al. in ref 15 study the dynamics of tRNA at different levels of hydration; they clearly show that the *dynamical transition* temperature in the *measured* MSD shifts to a lower temperature value when the hydration level increases (Figure 1 in ref 15). Furthermore, H.

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Frauenfelder et al. in refs 6 and 9 propose a new unified model of protein dynamics; more specifically, the authors show experimentally that "the rapid increase of the MSD at ≈200 K is not a '*dynamic transition*'",[6] and "introduce a new interpretation of the Mossbauer effect in proteins and demonstrate that no *dynamical transition* is required [...] the apparent abrupt increase in the MSD occurs when k_β (i.e., the rate $1/\tau_\beta$ of the β fluctuations originated in the hydration shell) becomes larger than k_{Mo} (i.e., the characteristic rate $1/\tau_{Mo} = 1/140$ ns of the Mossbauer effect in ^{57}Fe)".[9] These recent results are in clear agreement with the explicative hypothesis on the nature of the PDT proposed in our paper.[10]

Finally, concerning point (b), it is shown that the temperature behavior of the system relaxation time can be extracted from different *measured* MSD temperature behaviors, obtained by EINS measurements at different instrumental energy resolutions, starting from the conclusion that the obtained PDT temperatures (T_d) are the temperature values at which the system relaxation time intersects the resolution time, i.e., $\tau = \tau_{RES}$. This idea is also at the base of the resolution elastic neutron scattering (RENS),[16] where more extensive theoretical and simulation findings are presented. From the experimental point of view, in Figure 1 of ref 10, these points (τ_{RES}, T_d) obtained by an instrumental resolution of 0.85 μeV (2740 ps, 200 K), of 1 μeV (2192 ps, 220 K), of 8 μeV (274 ps, 240 K), and of 200 μeV (11 ps, 300 K) on hydrated lysozyme have been reported: at T = 220 K, a change from Arrhenius to non-Arrhenius behavior has been registered. As a result, we can affirm that FSC is due neither to the finite energy resolution effects nor to numerical errors in data analysis protocol, differently from what W. Doster et al. have suggested.[8]

AUTHOR INFORMATION

Notes

The authors declare no competing financial interest.

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