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Reply to the Comment by S. Boresch and O. Steinhauser on the Letter by N. Nandi and B. Bagchi Entitled “Anomalous Dielectric Relaxation of Aqueous Protein Solution”

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In their Comment about the theoretical treatment of Nandi and Bagchi (referred to as NB)[1] on the dielectric relaxation of aqueous protein solution, Boresch and Steinhauser (referred to as BS)[2] claimed to find several shortcomings of the theoretical treatment of NB. To put the issue in perspective, we reconcile that the observed anomalies were explained by NB assuming that the total dipole moment correlation function is composed of the self- and cross-correlations between the three dynamical species present in the solution. Major objections about this work as raised by BS are 2-fold (i) presence of a factor of $(1/N)$ in the exact expression of dielectric function (eq 2.1 in ref 1) and (ii) interpretation of the correlation factor used in ref 1. We thank BS for their comments on our work, although we disagree with many of their comments. Our response is given below.

(1) Equation 2.1 is well-known in the literature (refs 9−11 in ref 1). The correct form is available in those references. Obviously, convergent results could not be obtained for the system under consideration with this $(1/N)$ factor. The misprint of the factor $(1/N)$ in ref 1 has already been corrected and published in a subsequent publication (see eq 2.3 in ref 3). We are sorry for this misprint. Unfortunately, BS missed this subsequent correction.

(2) The second objection is about the interpretation of the correlation factors used in the dipole moment correlation functions in ref 1. The equation used in ref 1 to relate the dielectric function to the dipole moment correlation function is exactly valid for a spherical system. The large sphere limit is taken by increasing the radius of the system while keeping the intrinsic properties of the system like density as unchanged. Thus, the correlation factor relevant to the exact expression (eq 2.3 in ref 3) is different from the Kirkwood g factor. While this large system limit is considered in all calculations done in ref 1, we failed to mention the factor due to the increase in radius of the sphere. This point is, however, explained in a subsequent publication by us.[3] To specify, the corrected correlation factor is mentioned as $g_{\_\{\mathrm{c}\}}$ in ref 3, which is different from the Kirkwood g factor.

Thus, the two major objections raised by BS have already been addressed in published form (ref 3).

We also address other related objections raised by BS as follows,

(3) The discrepancy between the interpretation of β-relaxation by NB and the interpretations by experimental studies published and simulations by BS and other workers is the source of objection of BS about its interpretation. It has already been explained in refs 1 and 3 that the results of these experimental studies were not quantitatively analyzed in terms of the total moment correlation function. Thus, the qualitative interpretations presented in the experimental studies cannot be a basis of objection to the quantitative approach presented in ref 1. Also, we note that the interpretation of the correlation function by NB and BS are rather different. The time correlation functions considered by Boresch et al.[4] are as follows (eqs 10 and 11 there),

$$\Phi_{\mathrm{p}}(t) = \Phi_{\mathrm{pp}}(t) + \Phi_{\mathrm{pw}}(t) = \langle \mathbf{M}_{\mathrm{p}}(0)\, \mathbf{M}_{\mathrm{p}}(t)\rangle + \langle \mathbf{M}_{\mathrm{p}}(0)\, \mathbf{M}_{\mathrm{w}}(t)\rangle \tag{1}$$

However, according to the scheme presented by NB (as done

$$\Phi_{\mathrm{w}}(t) = \Phi_{\mathrm{ww}}(t) + \Phi_{\mathrm{pw}}(t) = \langle \mathbf{M}_{\mathrm{w}}(0)\, \mathbf{M}_{\mathrm{w}}(t)\rangle + \langle \mathbf{M}_{\mathrm{p}}(0)\, \mathbf{M}_{\mathrm{w}}(t)\rangle \tag{2}$$

in ref 1), these correlation functions should read as follows

$$\Phi_{\mathrm{p}}(t) = \Phi_{\mathrm{pp}}(t) + \Phi_{\mathrm{pw}}(t) = \langle \mathbf{M}_{\mathrm{p}}(0)\, \mathbf{M}_{\mathrm{p}}(t)\rangle + \langle \mathbf{M}_{\mathrm{p}}(0)\, \mathbf{M}_{\mathrm{w}}(t)\rangle \tag{3}$$

The difference between the two sets of equations is in the cross-

$$\Phi_{\mathrm{w}}(t) = \Phi_{\mathrm{ww}}(t) + \Phi_{\mathrm{wp}}(t) = \langle \mathbf{M}_{\mathrm{w}}(0)\, \mathbf{M}_{\mathrm{w}}(t)\rangle + \langle \mathbf{M}_{\mathrm{w}}(0)\, \mathbf{M}_{\mathrm{p}}(t)\rangle \tag{4}$$

correlation terms (compare the cross terms in eqs 2 and 4 (however, eqs 3 and 4 seem to be more a natural way of expressing the cross-correlation terms of two species rather than eqs 1 and 2). Also, the weight of a relaxation component in the scheme of Boresch et al. depends on the distance dependent g factor. Thus, the approaches presented in refs 1 and 4 are significantly different and the results are, not surprisingly, different. The difference arising from different ways of evaluating the cross-correlation terms has been evaluated and will be published elsewhere.

(4) Their objection about the use of the slow relaxation time in the cross-correlation term was not based on any quantitative argument. A large number of experimental evidences suggest that the dynamics of water near a biomolecule is slowed compared to the bulk and is often in the nanosecond range.[1,3] Boresch et al.[4] considered the modification of the static properties of the molecules (water and protein) due to their mutual presence (by considering the distance dependent g factor). It seems that they neglected the dynamical features of water in the hydration shell arising out of the dynamics of hydrogen bond formation and breaking in their simulation. It is not understandable why they favor modification in the structure of water due to the mutual correlation of protein and water (in the form of a distance dependent g factor) but oppose the modification (slowing down of the relaxation time) of the dynamics of water, which is quite well-known in the literature. Recent simulations by Balasubramanian and Bagchi of the orientational relaxation of water on a micellar surface[5] found explicit slowing down by more than 2 orders of magnitude in the orientation of water due to hydrogen bonding. This seems

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to confirm the basic assumption of Nandi and Bagchi about the slowing down of the dynamics of water near the protein surface.

(5) NB never claimed that the linear concentration dependence could not be obtained by other means. However, the fact that the weight of each component is proportional to its concentration (or number density), as pointed out by BS themselves, raises the question, why was the weight of protein contribution dominant in the simulation (ref 4) despite its low concentration? We want to address this issue in the future.

We note that no published theoretical or simulation studies available during the publication of ref 1 were aimed at understanding the experimentally observed anomalies in terms of the contribution of these three species to the total dipole moment correlation function. Recognition of the heterogeneous and multicomponent nature of the aqueous protein solution and the consideration of the contribution of three dynamic components present in the system in explaining the dielectric properties were the major ideas of this work. The literature on dielectric relaxation of solvated proteins (including that by the group of BS) published before ref 1 never aimed to understand the anomalies in a coherent way and also neglected the importance of cross-correlations. This is also mentioned in ref 4. We also note that simulation studies published after ref 1 include systematic contribution of the cross correlation terms[4] and the results thus obtained are much better than those obtained previously in terms of the explanation of the anomalies mentioned before. Thus, NB rightfully claimed this theory as the “first, unified” theory. However, the fact that the scheme of representation of the dipole correlation function of an aqueous protein system was presented in ref 1, for the first time, was ignored in the works by Steinhauser and co-workers (see related comment and the introduction section of ref 4 and related references therein).

To summarize, the points of objections raised by BS have already been addressed and published elsewhere and we clarified them further here. We also addressed other issues raised by them. It is already mentioned in the beginning that the key point of the theoretical approach of ref 1 is the consideration of the contribution of different dynamical species present in the protein solution in terms of the explicit consideration of the self- and cross-correlation terms. While the interpretation of these correlation terms (in terms of different correlation factors, etc.) could be different for different approaches, the general scheme is no doubt successful when considering its clarity and success in explaining the anomalies. This is further supported by the betterment of the simulation results based on similar theoretical approaches taken by subsequent simulations (published by the lab of BS) after ref 1 by NB compared to the previous results. Recent simulation results about the orientational dynamics of water near the micellar surface also provided unequivocal support to the basic assumptions of the theory.[5] The verdict given by BS does not change these facts.

References and Notes

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(4) Boresch, S.; Hochtl, P.; Steinhauser, O. *J. Phys. Chem. B* **2000**, *104,* 8743.

(5) Balasubramanian, S.; Bagchi, B. *J. Am. Chem. Soc.* Submitted for publication.