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Supplementary Material for " Anomalous Dielectric Relaxation of Aqueous Protein Solutions " by Nilashis Nandi and Biman Bagchi

I. Details of the choice of parameters presented in Table I and used in the present calculation

(1) While the molecular volume of protein does not appear in theory, it is necessary to calculate the number of protein molecules (N_p) present in a given concentration of protein in solution from the molecular volume. The later is difficult to obtain in solution (Richards, F. M. *Ann. Rev. Biophys. Bioeng.*, **1977**, *6*, 151; Gerstein, M.; Chothia, C. *Proc. Nat. Acad. Sci. U.S.A.*, **1996**, *93*, 10167). In the present work the molecular volume is obtained by averaging the molecular volume obtained using the molecular weight data and the apparent densities of proteins in solution (Cohn, E. J.; Edsall, J. T. *Proteins, Amino acids and Peptides*, eds. by Cohn, E. J. ; Edsall, J. T. Reinhold Publishing Co., New York, **1950**, 370) and the molecular volume obtained from the molecular coordinates and electron density profiles (Rellick, L. M.; Becktel, W. J. *Biopolymers*, **1997**, *42*, 191). The molecular weights for myoglobin is taken as 17900 Dalton (Rellick, L. M.; Becktel, W. J. *Biopolymers*, **1997**, *42*, 191), the molecular weight of serum albumin is taken as 68360 Dalton (Minton, A. P. *Biophys. Chem.*, **1995**, *57*, 65) and the molecular weight for hemoglobin is taken as 68000 Dalton (Edsall , J. T.; Cohn, E. J *Proteins, Amino acids and Peptides*, eds. by Cohn, E. J. ; Edsall, J. T. Reinhold Publishing Co., New York, **1950**, 391).

(2) The data at 293.15^0 K are obtained from the experimentally known temperature variation of the time constants related to the δ relaxation (ref. 3 in the text).

(3) The high frequency dielectric constant is necessary for conversion of the gas phase dipole moment of a species to the solution values and vice versa. At present detailed quantitative analysis about the increment of the high frequency dielectric constant of solution from that of the bulk water value due to the presence of the protein molecules at the high frequency side of the dielectric spectrum is unavailable. However, the optical measurements in protein solutions indicate that the solution values are higher than the bulk (Doty, P.; Edsall, J. T. *Adv. Protein. Chem.*, eds. Anson, M. L.; Edsall, J. T.; Bailey, K. **1951**, *6*, 35). For

simplicity the high frequency permittivity is taken as equal to 2.5 in the present work for all three protein solutions studied here.

(4) The μ_p of MYG is obtained from ref. 3 and 14 in the text. Besides the permanent dipole correlation function, the transient or the fluctuating dipoles arising from the vibration of the charged residues in the vicinity of the hydration shell also contribute to the dipole moment correlation of protein (ref. 14 in the text). The time dependent decay of the effective dipole moment due to the fluctuation dipole can be described by a sum of decay processes and the corresponding decay time constants can be obtained either from simulation or from Raman lineshapes of the respective vibrations. However, in the present study we did not include the contribution of the fluctuation dipoles in the numerical calculation. The reasons for such exclusions are two fold. First, the magnitude of the fluctuation dipole μ_{eff} is of comparable magnitude with that of μ_p (Takashima, S. *Biophys. J.*, **1993**, *64*, 1550 and ref. 14 in the text) and is associated with N_p which is about 10^4 times smaller than the N_w. This makes the contribution of the fluctuation dipole term negligible compared to other terms. Secondly, the time constants associated with this term are in the femtosecond to few picoseconds only. Thus, their ultrafast dynamics have marginal role in the frequency range below the GHz region of the dielectric spectrum.

(5) The μ_p of BSA is taken from ref. 12 in the text.

(6) The μ_p of HEM is selected from (a) ref. 12 (b) Takashima, S. *Biophys. J.*, **1993**, *64*, 1550 and (c) Antosiewicz, J.; Porschke, D. *Biophys. J.*, **1995**, *68*, 655.

(7) The τ_p of MYG is taken from ref. 14 in the text.

(8) The τ_p of BSA is taken from (a) ref. 12 and (b) ref. 21 in the text

(9) The τ_p of HEM is taken from ref. 12.

(10) The τ_{h1} of MYG is taken from ref. 3 in the text. For the assignment of weight of the time constant see item (13) below.

(11) The τ_{h1} of BSA is taken from ref. 21 in the text. For the assignment of weight of the time constant see item (13) below.

(12) The τ_{h1} of HEM is taken from ref. 22 in the text. For the assignment of weight of the

time constant see item (13) below.

(13) The τ_{h2} of the proteins (as well as τ_{h1}) are outcome of dynamic exchange of water in the hydration shell, slow motion of water on the protein surface, smaller diffusion constant of water near the protein and slow diffusion of water from bulk to hydration shell and vice versa and is observed in the δ dispersion. The dynamic origin of the slowness is explained in ref. 15 of the text. In the present study we represent the bimodal decay by two time constant where the slower time constant have larger weight compared to the faster one due to the reasons explained above. Recent measurements on the rotational time constant of water associated with protein also indicates that these time constants are rather slow (ref. 19). See text for detail.

(14) The μ_w of water is taken from *Water : A Comprehensive Treatise*, ed. Franks, F.;**1982**, *1*, Plenum Press, N.Y. The data at 293.15^0 K is obtained from the same reference from the known temperature dependence.

(15) The μ_h is assumed as equal to that of bulk water.

(16) g_p of all proteins are taken as unity. See ref. 2 in the text also.

(17) τ_w is taken from Barthel, J. and Buchner, R. *Pure. Appl. Chem.*, **1991**, *63*, 1473.

II. Detailed analysis of the sensitivity of the theoretical result to the choice of different parameters and the analysis of the relative contributions of the relaxation terms in Eq. 2.3 and their roll in concentration cross over

We have varied different parameters appeared in the theory for a reasonable range around the values used in the present work (shown in Table I). The resultant variation in the dielectric constant (indicated by $\Delta\epsilon_0$) in the three different frequency regions are calculated. The $\Delta\epsilon_0$ indicates the average of the variation of the static dielectric constant in the high and in the low frequency end of the frequency region under consideration. The results are shown in Table II. While the molecular weight of protein (MW_p) does not directly appear as a parameter of the theory, it is necessary to calculate the number of protein molecules (see footnote 1 of Table I.).

In order to quantitatively elaborate the observed cross over we analyzed the static contributions to the dielectric function described by Eq. 2.3. When the concentration of the protein is increased the total static correlation also increases. The static value of the right hand side of the Eq. 2.3 (denoted by R) is plotted in Fig. 7(a). R increases with concentration. However, self and cross correlation of all the components composing R do not increase monotonically. In Fig. 7(b) we have plotted the individual terms constituting R (denoted by $R(ij)$; $R = \sum R(ij)$) and in Fig. 7(c) we have plotted only the the relative percentage of contribution of the number density terms contributing to R ($N(ij) = N_{ij}^2 \times 100/N^2$, where i and j refer to protein, hydration shell water or bulk water). It is shown that while the amount of water in the hydration shell increases with the increasing protein concentration, the amount of the bulk water molecule decreases in the system. Thus, with an increase in concentration the bulk water content decreases making the dip in the $\epsilon(\omega)$ in the high frequency region. We present the magnitudes of the different components contributing to the dielectric function given by Eq. 2.3 in the Table III.

Table II The average variation of the static dielectric constant of different frequency regions in the dielectric function with the variation of paramaters in the theory. The protein solution is whale myoglobin (MYG) at 298.15^0 K.

Parameter	Variation range	$\Delta\epsilon_0$ (< 10MHz)	$\Delta\epsilon_0$ (10 MHz - 1000MHz)	$\Delta\epsilon_0$ (> 1000 MHz)
τ_p	±10 ns	∓0.01	∓0.01	0.00
τ_{h1}	±10 ps	0.00	∓0.15	∓0.15
τ_{h2}	±10 ns	∓1.53	∓0.74	0.00
τ_w	±1 ps	0.00	±0.04	±0.09
μ_p	±10 D	∓0.02	∓0.02	∓0.015
μ_h	±1 D	±1.12	±0.64	±1.33
μ_w	±1 D	±22.75	±22.94	±23.18
g_p	±0.2	0.00	0.00	0.00
g_h	±0.2	∓0.06	∓0.07	∓0.01
g_w	±0.2	±1.47	±1.45	±1.49
MW_p	±1000 Dal	±0.54	±0.53	±0.54

Table III The magnitudes of the different components contributing to the static dielectric constant of the myoglobin solution at solution at 298.15^0 K. The concentrations (c) are expressed in mg/ml. The protein, water in the hydration shell and bulk water are indicated by 'p','h' and 'w', respectively as in the text. Note that each of the three cross correlation terms in the table is a sum of two individual cross correlation terms each with different dynamics while their static values are same. For details see Eq. 2.3 and relevant discussions in the text.

c	[p-p]	[2× (p-h)]	[2× (p-w)]	[h-h]	[2× (h-w)]	[w-w]
170	0.000049	0.002785	0.011098	0.039693	0.316291	0.630084
161	0.000043	0.002463	0.010597	0.035100	0.302036	0.649761
150	0.000037	0.002101	0.009968	0.029947	0.284109	0.673837
99	0.000015	0.000847	0.006832	0.012067	0.194717	0.785523
82	0.000010	0.000566	0.005718	0.008071	0.162973	0.822661
77	0.000009	0.000496	0.005385	0.007065	0.153479	0.833567

Figure captions for the supplementary material

Fig.5 The the real part of the complex permittivity of aqueous bovine serum albumin solution (concentration is 63.5 mg/ml) calculated from the present theory (indicated by solid line) and that from the experiment (indicated by line with +) (ref. 21 in the text) at 298.15^0 K.

Fig.6 The concentration dependence of the real part of the complex permittivity of aqueous equine hemoglobin solution calculated from the present theory and that from the experiment (ref. 22 in the text) at 298.15^0 K. The solid line corresponds to the theoretical plot with concentration 75 mg/ml, the line with empty square corresponds to the experimental plot with concentration 75 mg/ml, the line with solid triangle corresponds to the theoretical plot with concentration 98 mg/ml, the line with ($*$) corresponds to the experimental plot with concentration 98 mg/ml, the line with (+) corresponds to the theoretical plot with concentration 147 mg/ml, the line with (x) corresponds to the experimental plot with concentration 147 mg/ml.

Fig. 7 (a) Plot of the static counterpart of the right hand side of Eq. 2.3 (denoted by R) with variation in concentration. (b) The plot of R and its individual components (c) The plot of number density related terms, $N(ij)$ ($= N_{ij}^2 \times 100/N^2$, where i and j refer to protein, hydration shell water or bulk water) for the individual terms in R with variation in concentration. The details are discussed above in the supplementary material.

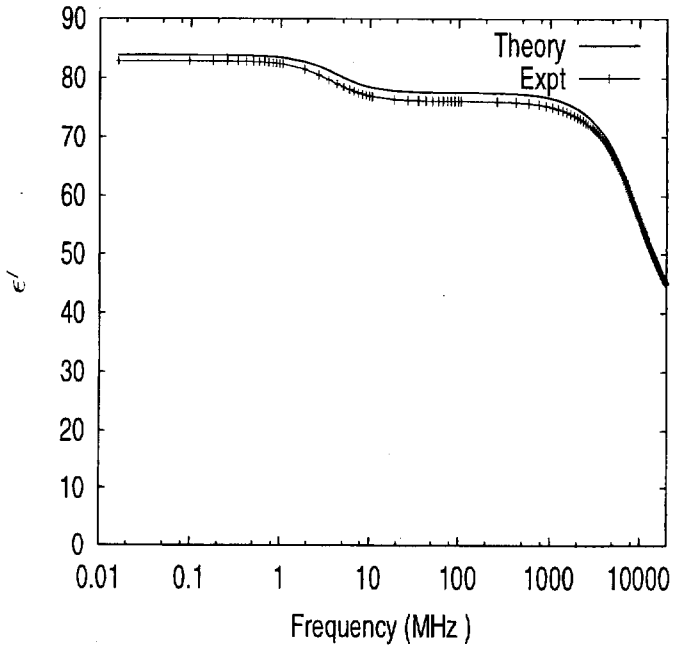


FIG. 5.

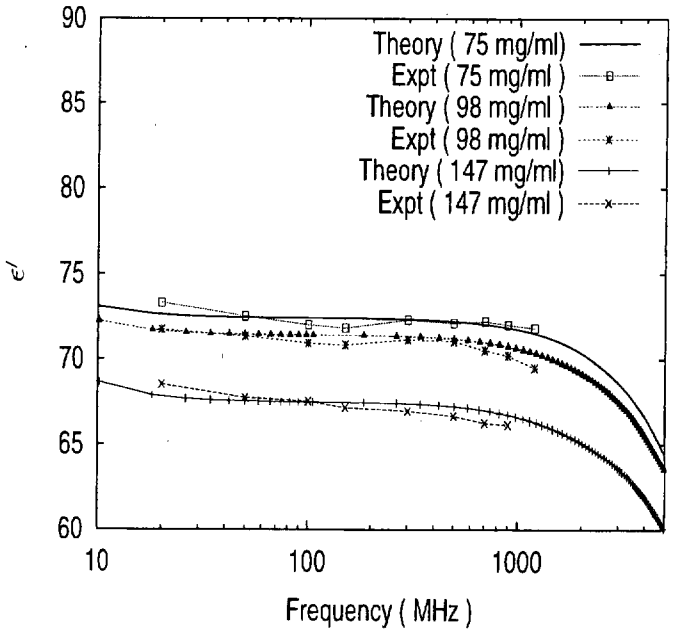
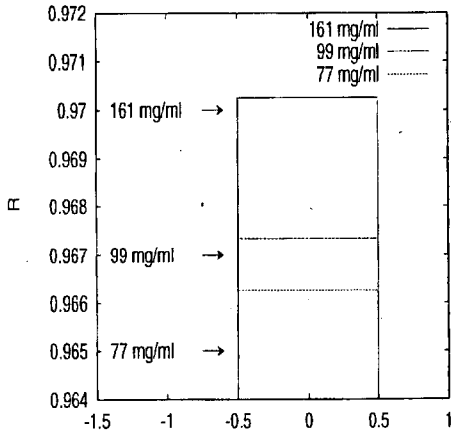
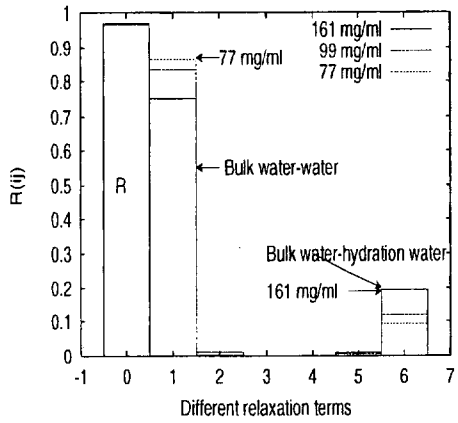


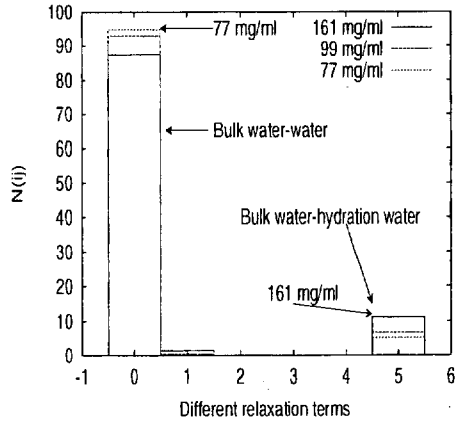
FIG. 6.


161 mg/ml
99 mg/ml
77 mg/ml
R
0.972
0.971
0.97
0.969
0.968
0.967
0.966
0.965
0.964
-1.5
-1
-0.5
0
0.5
1

7(a)


161 mg/ml
99 mg/ml
77 mg/ml
77 mg/ml
Bulk water-water
R
Bulk water-hydration water
161 mg/ml
R(ij)
Different relaxation terms

7(b)


77 mg/ml
161 mg/ml
99 mg/ml
77 mg/ml
Bulk water-water
Bulk water-hydration water
161 mg/ml
N(ij)
Different relaxation terms

7(c)