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Water Rotational Relaxation and Diffusion in Hydrated Lysozyme

Massimo Marchi,*,‡ Fabio Sterpone,‡ and Matteo Ceccarelli†,§

Contribution from the Commissariat à l'Energie Atomique, DSV-DBJC-SBFM, Centre d'Études, Saclay, 91191 Gif-sur-Yvette Cedex, France, and CECAM, Centre Européen de Calcul Atomique et Moleculaire, Ecole Normale Superieure de Lyon, 46 Allée d'Italie, 69364 Lyon, France

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Abstract: This paper is concerned with the dynamics of water around a small globular protein. Dipolar second-rank relaxation time and diffusion properties of surface water were computed by extensive molecular dynamics simulations of lysozyme in water which lasted a total of 28 ns. Our results indicate that the rotational relaxation of water in the vicinity of lysozyme is 3−7 times slower than that in the bulk depending on how the hydration shell is defined in the calculation. We have also verified that the dynamics of water translational diffusion in the vicinity of lysozyme have retardations similar to rotational relaxation. This is a common assumption in nuclear magnetic relaxation dispersion (NMRD) studies to derive residence times. In contrast to bulk water dynamics, surface water is in a dispersive diffusion regime or subdiffusion. Very good agreement of dipolar second-rank relaxation time with NMRD estimates is obtained by using appropriate dimensions of the hydration shell. Although our computed second-rank dipolar retardations are independent of the water model, SPC/E describes more realistically the time scale of the water dynamics around lysozyme than does TIP3P.

I. Introduction

In the past few years, sophisticated experimental techniques have been developed to study the structural and dynamic properties of hydration waters in biomolecular systems, such as proteins and DNA. The expected, but yet elusive, goal of these studies is the identification of the role of water in biological phenomena such as enzyme activity, protein folding, and denaturation.

In this paper, we focus on the dynamics of hydration waters. Two experimental techniques have been used in the past to extract the time scales of the water molecules neighboring a protein: inelastic neutron scattering[1−4] and nuclear magnetic relaxation dispersion (NMRD).[5−12]

Inelastic neutron scattering experiments derive the diffusion constant of the waters in the sample from measurements of their quasi-elastic neutron scattering cross section. Such techniques are typically applied to samples in powder form, where a controlled number of water molecules surrounds the protein. Thus, the "effective" diffusion constant of water in deuterated C-phycocyanin has been estimated to be similar to that of bulk water for fully hydrated samples[1] and smaller for lower hydrations[3] − up to 4 times for a hydration of 0.2 g of H_2O per gram of protein. Subtraction techniques were first used by Settles and Doster[2] on myoglobin and more recent applied to deuterated C-phycocyanin.[4] Results obtained from the latter investigation indicate that water diffusion is slowed 15 times with respect to bulk waters,[4] in contrast with earlier experiments on the fully hydrated sample. Even larger slowing downs were inferred in ref 2 for hydrated myoglobin.

In the past few years, NMRD techniques have instead provided a more consistent picture of the diffusion of surface water around proteins. The behavior of the longitudinal relaxation rate measured by ^2H and ^{17}O NMRD depends in part on the time relaxation, τ_S, of the dipolar second-rank correlation functions (see, for instance, refs 7, 13). Simple physical arguments show that the residence time of the surface waters, or τ_w, is directly proportional to τ_S. For many hydrated proteins, the second-rank retardation, τ_S/τ_{bulk}, estimated from NMRD is within 4.9−5.4, meaning that the translational diffusion constant of the surface waters is about 5 times smaller than that in the bulk. This result is in sharp contrast with the neutron scattering experiments at the highest hydration which seem to predict

* To whom correspondence should be addressed. E-mail: marchi@villon.saclay.cea.fr.
† Present address: CSCS/ETHZ, Via Cantonale, CH-6928 Manno, Switzerland.
‡ Commissariat à l'Energie Atomique.
§ Centre Européen de Calcul Atomique et Moleculaire.

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retardation much smaller or much larger than 5, depending on the technique used to analyze the experimental data.

The dynamic behavior of hydration water at the surface of proteins has also been investigated by theoretical means. The main aim of a large number of molecular dynamics (MD) simulations of hydrated proteins has been to compute water residence time from survival probabilities[14] of the water−protein bond. Some of these works[15−20] have correlated the residence time of single residues to their chemical identity, secondary and tertiary structures, and accessible surface. Other studies[20−25] have focused more on the general features of water dynamics near the protein surface. In a previous study,[25] the survival probability of water attachment obtained from two independent 9 ns simulations of hydrated lysozyme distinguished three peculiar temporal scales of the hydration dynamics. Two among these, with subnanosecond mean τ_w, are characteristic of surface hydration water; the slower time scale ($\tau_w \approx 2-3$ ns) is associated with buried waters in hydrophilic pores or in superficial clefts. Puzzlingly, our computed residence times for the bulk-exchanging surface water, 14.2 and 12.7 ps for the two simulations, are shorter than that estimated from NMRD, or 26 ps. Indeed, as remarked previously,[7] MD studies[21,22] have in the past predict surface water diffusion in time scales at least 2−3 times smaller than NMRD.

In this paper, we report results on the second-rank spin−spin relaxation and diffusion of surface waters around a globular protein. Extensive molecular dynamics simulations of hydrated hen egg white lysozyme were carried out for a total of 28 ns. Two independent simulations of 9 ns each discussed in ref 25 were run with TIP3P[26] water, while the SPC/E[27] model was used for an additional simulation of 10 ns long. For both models of water, we have computed the retardation effect due to the protein on the solvent dynamics. Anticipating our results, we find that the rotational relaxation of the water in the vicinity of lysozyme is 3−7 times slower than that in bulk depending on the definition of hydration shell. Similar retardations are also found for the diffusion of water around the protein. This finding verifies a common assumption used by NMRD to derive residence times. With the choice of solvation shell consistent with hydration numbers obtained from protein accessible surface, we find very good agreement with dipolar second-rank relaxation time estimated from NMRD. Although our results on rotational retardations are independent of the water model, SPC/E describes realistically the dynamics of water hydration.

II. Methods

A. Simulation. In a previous investigation,[25] we reported on the dynamic properties of hydration water based on two MD simulations of hen egg white lysozyme in TIP3P water. The two runs, named lysoA and lysoB, are 9 ns NPT simulations, at $T = 300$ and $P = 0.1$ MPa, of a lysozyme molecule in 3954, lysoA, and 3966, lysoB, waters. The two trajectories were commenced from initial conditions differing in the choice of crystallization waters included in the calculation. For lysozyme we used a multicomponent potential function developed and parametrized by MacKerell et al.[28] Our simulation technique includes r-RESPA (reversible reference system propagation algorithm)[29] coupled with smooth particle mesh Ewald (SPME)[30] to handle electrostatic interactions, and constraints on covalent bonds entailing hydrogens.[31] For the present study, an additional 10 ns simulation, lysoC, was carried out with SPC/E water starting from the same initial coordinates lysoB, by adjusting the bond lengths and angle of the TIP3P water molecules to those of the SPC/E model. For each run, coordinates were stored for analysis every 240 fs.

B. Analysis of MD Trajectories. As a first probe of the time scales of hydration, the survival probabilities for the water−protein bonds are computed as the function:[14]

$$N_w(t) = \frac{1}{N_t}\sum_{n=1}^{N_t}\sum_j P_j(t_n,t) \tag{1}$$

where N_t is the number of the simulation time frames, and the function $P_j(t_n,t)$ takes the values of 1 if the j-th water molecule is within a certain cutoff of the solute between time t_n and $t_n + t$, and zero otherwise. The cutoff radii are determined for each couple of water and protein atoms − w and p, respectively − from the function:

$$R_{cut} = f(r_w + r_p) \tag{2}$$

where the coefficient f is a number near 1, and r_w and r_p are the atomic van der Waals radii as obtained from the simulation force field. As in ref 25, time scales were extracted from a reduced survival probability

$$\Delta N_w(t) = N_w(t) - N_w(t_{sim}) \tag{3}$$

ΔN_w was then fitted by one stretched exponential function combined with two simple exponentials

$$\Delta N_w(t) \simeq n_{st}\exp\left(-\left(\frac{t}{\tau_{st}}\right)^{\gamma}\right) + \sum_{i=2}^{3} n_i \exp\left(-\frac{t}{\tau_i}\right) \tag{4}$$

The residence time for the stretched exponential part can be computed as average relaxation time, or

$$\langle\tau_{st}\rangle = \frac{\tau_{st}}{\gamma}\Gamma\left(\frac{1}{\gamma}\right) \tag{5}$$

with Γ as the gamma factorial function. For all trajectories, we verified that the function in eq 4 gives a better χ^2 than the fit performed with a 4 exponential function involving an extra parameter.

To investigate the rotational relaxation dynamics of water, we have computed the functions:

$$P_1(t) = \langle\cos\theta(t)\rangle \tag{6}$$

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$$P_2(t) = \left\langle \frac{3}{2}\cos^2\theta(t) - \frac{1}{2} \right\rangle \quad (7)$$

where $\theta(t)$ is the angle between vectors in the molecular fixed frame at times τ and $\tau + t$. The average is intended over a given fraction of the total water molecules. This can be limited to surface waters or to all the waters of the simulation. The dipole moment of each water was used as vector in the calculation of the two correlation functions. Calculations for the O−H vectors of water were also carried out.

Voronoi polyhedrons[32] were used to compute the accessible surface area for lysozyme along the trajectories. The calculation was carried out using the recursive algorithm described in ref 33. In our investigation, the Voronoi protein surface for lysozyme was computed from the MD generated trajectories. The accessible surface area for lysozyme was computed by adding up the contributions of all the facets of the Voronoi polyhedrons shared by protein atoms and water. No explicit hydrogens were included in these calculations. The accessible surface areas were then averaged over the length of each run.

Finally, diffusion of water near the protein surface was monitored by computing the mean square displacement of water oxygens, or $\langle|\mathbf{r}(t)|^2\rangle$.

All simulations and analyses described in this paper were performed with the parallel version of the program ORAC.[34,35]

III. Results

A. Survival Probabilities. Previous studies have dealt with the dynamics of hydration by computing the relaxation of the survival probability of the water−protein bond, thus extracting relaxation or "residence" times, τ_w. In very much the same spirit, as reported in ref 25, we have computed residence times for hydrated lysozyme. In Table 1 we present new results for the τ_w of SPC/E and TIP3P water as the cutoff parameter f varies. Noticeably, the time scales of lysoC are always longer than those obtained from the lysoB results. This is less marked on the longer time scales which, as seen in ref 25, concern only more strongly bound waters. For both water models, we observe that the shortest τ_w, $\langle\tau_s\rangle$, becomes shorter as f decreases. The longer τ_w's are relatively less affected by changes in f, and the general trend is to increase with smaller f's.

This behavior can be interpreted in light of the results of our previous paper. In ref 25 we found that the shortest living water−protein bonds are likely to be broken by exchanges to the bulk solvent. Indeed, in this case, water attachment implies on average only 1−2 residues. On the contrary, slower waters attached to any given site exchange to different protein sites or residues − up to 35 for the slowest waters. These results were confirmed here also for SPC/E waters of trajectory lysoC. Consequently, when f decreases, fastest waters exchanging with the bulk have a smaller space to diffuse and shorter τ_w. This is not the case for slower waters which diffuse all along the hydration shell and, at the same time, are sufficiently close to the protein not to be affected by changes in the hydration cutoff.

Table 1 indicates that for $f = 1-1.1$, the protein number of hydration including all waters (i.e., $n_{st} + n_2 + n_3$) is close to the value used in NMRD estimates of τ_w. Thus, for those values of f, MD simulations carried out with TIP3P and SPC/E water seem yet again to greatly underestimate $\tau_w$with respect to the NMRD results $\tau_w = 26$ ps. Indeed, with $f = 1$, our smallest $\langle\tau_{st}\rangle$'s are 7.9 and 12.9 ps for TIP3P and SPC/E, respectively.

We will show in the following that this disagreement is not due to inaccuracies in the interaction potentials as previously suggested,[7] but lies instead in comparing the wrong quantities.

Table 1. Lysozyme Residence Times Computed by Fitting the Reduced Survival Probability $\Delta N_w(t)$ with One Stretched Exponential and Two Exponentials

SPC/E Simulation lysoC

f	$\langle\tau_{st}\rangle$	n_{st}	τ_2	n_2	τ_3	n_3
1.0	12.9	436.2	374.0	16.8	2321.7	9.6
1.1	14.6	516.2	301.1	26.0	2110.0	12.1
1.2	16.8	573.8	235.6	39.2	1927.7	15.4
1.3	22.1	636.2	270.7	42.2	1949.2	15.5

TIP3P Simulation lysoB

f	$\langle\tau_{st}\rangle$	n_{st}	τ_2	n_2	τ_3	n_3
1.0	7.9	441.9	124.6	20.5	1157.6	15.0
1.1	8.2	510.8	112.9	35.8	1383.9	16.5
1.2	9.9	576.9	112.0	45.8	1369.0	17.5
1.3	12.7	644.7	125.5	48.2	1371.1	17.6

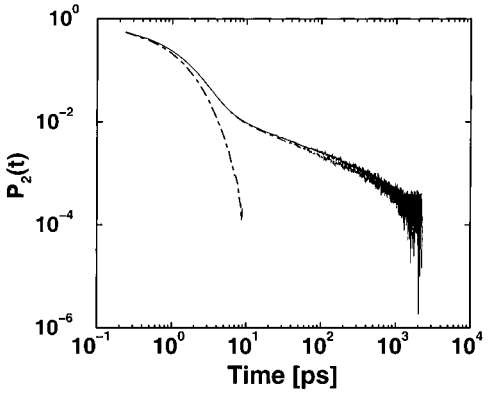

Figure 1. Second-rank dipole−dipole correlation functions for simulation lysoA (−), lysoB (· · · · ·), and bulk TIP3P water (· − · − ·) in a log−log scale. The correlation functions for lysoA and lysoB are plotted up until oscillations to negative values.

B. Rotational Relaxation. We first point out that NMRD can only measure the second-rank retardation of surface water with respect to the bulk, not residence times directly. Assuming that the mechanism of rotational and translational diffusions is rate-limited by hydrogen-bond disruption, rotational and translation diffusion constants are similar. Thus, since the ratio of the first-rank and second-rank correlation times in an isotropic rotational diffusion model is 3, we get finally:[7,13]

$$\tau_w = 3(\rho_S + 1)\tau_{Sbulk} \quad (8)$$

where $\rho_S + 1 = \tau_S/\tau_{Sbulk}$ is the second-rank rotational retardation, while τ_S and τ_{Sbulk} are the second-rank relaxation of surface and bulk water, respectively. We point out that the bulk water residence time $\tau_w^{bulk} \simeq 3\tau_{Sbulk}$ corresponds to the time required by a water molecule to cover 3 Å, that is, the diameter of a water molecule.

In Figure 1 we present the comparison between the second-rank dipolar correlation function $P_2(t)$ for simulation lysoA and lysoB with the result for bulk TIP3P water. The functions of lysoA and lysoB were computed here by adding the contribu-

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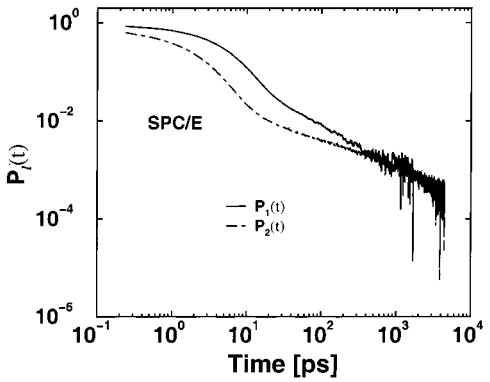

Figure 2. Comparison between the first (−) and second (· − · − ·) rank dipole−dipole correlation functions for simulation lysoC involving SPC/E water. As for Figure 1, the correlation functions are plotted up until oscillations to negative values.

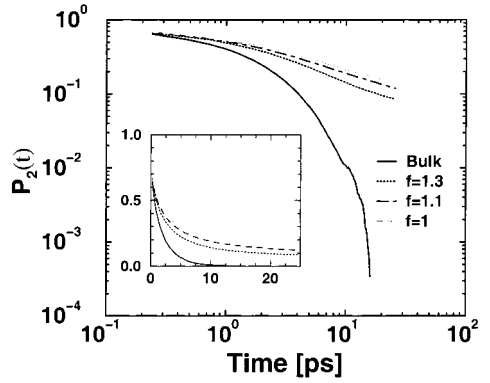

Figure 3. Log−log plot of the dipolar second-rank rotational correlation function, $P_2(t)$, versus time. Functions computed from the hydration waters of shell $f = 1.0$, 1.1, 1.3 are shown and compared with the result for bulk water. All of the data are obtained from simulation lysoC involving SPC/E water. Only the fast decaying part of the function is plotted here. In the inset, we show $P_2(t)$ in a X−Y scale.

tions from all water molecules in the system. They both show a power law decay at times longer than 10 ps, also seen for $P_2(t)$ of simulation lysoC involving SPC/E water (data not shown). This behavior is likely to be due to waters more strongly bound to the protein and hindered in their rotational relaxation. At shorter times, the relaxation of lysoA and lysoB is stretched exponential in nature, and the two curves in Figure 1 overlap. The average relaxation time, or $\langle\tau_{st}\rangle$, computed after fitting the initial decay of the three functions, shows a bulk water retardation of 1.08 for both simulations. Figure 2 shows that both first- and second-rank dipolar correlation functions have a similar decay structure. After first eliminating the power law decay region, we have fitted the rest with a stretched exponential function and obtained that the ratio between the first- and second-rank averaged relaxation times is $\langle\tau_s^1\rangle/\langle\tau_s^2\rangle = 2.7$. This indicates that in the rotational fast diffusion regime our system closely behaves like a isotropic rotational diffusion model for which $\langle\tau_s^1\rangle/\langle\tau_s^2\rangle = 3$.

More interesting are our results on the $P_2(t)$ for the hydration waters. In Figure 3 we show the fast decaying region of the second-rank correlation function for simulation lysoC at different values of f and compare with results for SPC/E bulk water. This picture underlines that the rotational relaxation of surface waters depends crucially on the choice of the hydration shell around the protein. Tables 2 and 3 present the average $\langle\tau_{st}\rangle$ and retardation as a function of f determined by a stretched exponential

Table 2. Surface Water Second-Rank Rotational Properties of Simulation lysoB[a]

TIP3P water	bulk	$f = 1.3$	$f = 1.1$	$f = 1.0$	exp[b]
τ_S [ps]	0.76	2.19	3.82	4.90	
τ_S/τ_{Sbulk}		2.9	5.1	6.5	5.1 (4.4[c])
N_S		700	546	468	440[d] (528[e])
$N_S\rho_S$ [10^3]		1.3	2.2	2.6	1.8 ± 0.2

[a] τ_S and τ_{Sbulk} are the averaged second-rank relaxation time of surface and bulk water, respectively. N_S is the number of hydration water in the vicinity of the protein and $\rho_S = \tau_S/\tau_{Sbulk} - 1$. [b] Experimental results are from ref 6. [c] Retardation computed from experimental $N_S\rho_S$ and N_S derived from simulation Voronoi accessible surface. [d] Estimated in ref 6. [e] Estimated from Voronoi accessible surface of simulation. See text for further explanations.

Table 3. Surface Water Second-Rank Rotational Properties of Simulation lysoC[a]

SPC/E water	bulk	$f = 1.3$	$f = 1.1$	$f = 1.0$	exp[b]
τ_S [ps]	1.85	5.68	9.77	13.00	
τ_S/τ_{Sbulk}		3.1	5.3	7.0	5.1 (4.5[c])
N_S		681	530	453	440[d] (517[e])
$N_S\rho_S$ [10^3]		1.4	2.3	2.7	1.8 ± 0.2

[a] See caption to Table 2 for explanations. [b] Experimental results are from ref 6. [c] Retardation computed from experimental $N_S\rho_S$ and N_S derived from simulation Voronoi accessible surface. [d] Estimated in ref 6. [e] Estimated from Voronoi accessible surface of simulation. See text for further explanations.

fit to the fast relaxation of $P_2(t)$ for simulations lysoB and lysoC, respectively.

In the same table we also report the hydration number, N_S, and compare with the value used in NMRD experiments. On the basis of crystallographic results, the latter was estimated to 440 molecules. This number was obtained by dividing the accessible surface of the protein X-ray structure by 15 Å. Instead, 528 and 517 water molecules are obtained if one uses the Voronoi accessible surface obtained from runs lysoB and lysoC, respectively. These values are in good agreement with N_S computed at hydration $f = 1.1$.

The two models of water, TIP3P and SPC/E, compute very similar τ_S/τ_{Sbulk} for any given dimension of the hydration shell. Retardation is closer to the estimated experimental value when $f = 1.1$. In particular, for the two simulations, τ_S/τ_{Sbulk} is 5.1, lysoB, and 5.3, lysoC, while its experimental estimate is around 4.4−5.1 − the smallest values being obtained by using the averaged accessible surfaces from our simulations. Finally, we have computed $N_S\rho_S$ for the three surface shell and obtain values between 1.3−2.6 and 1.4−2.7 for lysoB and lysoC, respectively. Again, the values for $f = 1.1$ are in both cases in very good agreement with the experimental value of 1.8. It is important to point out that our error on retardation and $N_S\rho_S$ is on the order of 10%.

C. Diffusion. Finally, we turn our attention to the diffusion of surface water. It is well known that TIP3P and SPC/E models give different diffusion constants for water. We have repeated here the calculation at the same condition of temperature and pressure used for the lysozyme simulations and found $D = 5.2\ 10^{-5}$ cm^2/s and $D = 2.6\ 10^{-5}$ cm^2/s for TIP3P and SPC/E, respectively. To extract the residence times, we follow NMRD work[7] and define it as the time required by a water molecule to cover 3 Å, that is, the diameter of a water molecule. In Table 4 we report τ_w for TIP3P and SPC/E water.

Next, we investigate the diffusion dynamics of the surface water in simulations lysoB and lysoC up to a 40 ps time scale.

Table 4. Diffusion of Surface Water for Simulations Carried out with TIP3P, lysoB, and SPC/E, lysoC, Water Models[a]

	TIP3P		SPC/E	
f	α	τ_w [ps]	α	τ_w [ps]
1.0	0.60	18.8 (7.2)	0.59	32.3 (6.2)
1.1	0.62	14.2 (5.5)	0.61	26.8 (5.2)
1.3	0.64	8.3 (3.2)	0.64	17.0 (3.3)
bulk	0.98	2.6 (1)	0.94	5.2 (1)

[a] α is the exponent in eq 9, and τ_w is the residence time defined as the time needed by the system to diffuse 3 Å. In brackets are the residence times relative to bulk water.

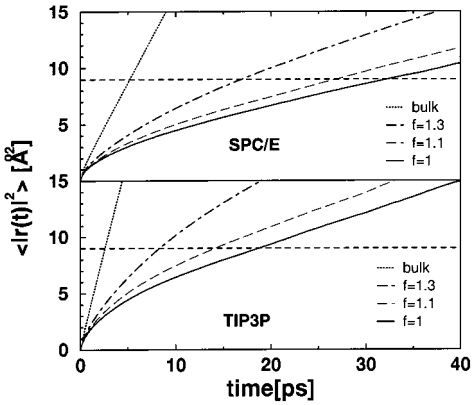

Figure 4. Oxygen water mean square displacement as a function of time as obtained from simulation lysoB and lysoC. Results for $f = 1.0$, 1.1, 1.3 and bulk water are compared.

In Figure 4 the mean square displacement for different hydration shells is plotted as a function of time. For the sake of comparison, diffusion data from bulk water simulations are also shown. The difference between diffusion of bulk water and of the water in the protein vicinity is striking; for all hydration shells investigated, surface water is in a dispersive diffusion regime and obeys a power law

$$\langle |\mathbf{r}(t)|^2 \rangle \simeq t^{\alpha} \tag{9}$$

with $\alpha < 1$. For all *f*'s and models of water considered here, we find a value of α close to 0.6 − see Table 4. α has a very small dependence on the shell size and does not depend on the water model. The latter finding provides a strong indication that the protein surface roughness and not temporal disorder − which depends on the energetics of water binding − is likely to be responsible for the diffusion dispersive regime.[22]

In the two panels of Figure 4, the intercept between the line $\langle |\mathbf{r}(t)|^2 \rangle = 9$ Å and the mean square displacement for hydration water marks the residence times. The results on τ_w summarized in Table 4 are consistent with the second-rank rotational relaxation discussed in the previous section. The residence times relative to bulk water − shown in brackets in Table 4 − follow closely the rotational retardation computed for different hydration shells. We find that for hydration shell $f = 1.1$ the SPC/E model gives a value of $\tau_w = 26.8$ ps in excellent agreement with the NMRD experimental estimate of 26 ps.

IV. Discussion and Conclusion

This investigation has provided a consistent picture of water dynamics around a protein in solution. Both water models used here, TIP3P and SPC/E, in the vicinity of the protein give a very similar dynamic retardation relative to the bulk. The absolute value of the relaxation time is instead different and mirrors differences in the self-diffusion coefficients of TIP3P and SPC/E. We have clearly identified the dependence of the dynamics on the hydration shell size considered in the calculation. For values of the parameter *f* close to 1.1 we find a second-rank retardation and $N_S\rho_S$ of both models of water in excellent agreement with NMRD estimates and measurements. We have also verified that the dynamics of rotational and translational diffusions are strictly related, as rotational and translation retardation relative to bulk water are in good agreement with each other for any given value of the parameter *f*. This finding gives a computational basis for extracting residence time from NMRD experiments.

Our results also indicate that, at least for proteins in solution, diffusion in the first solvation shell is retarded with respect to bulk of 5−7 times considering an *f* of 1.1−1.0. If this result agrees very well with NMRD measurements, it contrasts with neutron scattering estimates of hydration dynamics which have given a diffusion coefficient relative to bulk water of 1.1 or 15 depending on the data analysis technique employed. It is beyond the scope of our investigation to explain this discrepancy; here we limit ourselves to observe that neutron scattering experiments are carried out on rehydrated lyophilized protein powder, while NMRD is performed on solvated proteins. In the latter, surface waters exchange primarily with the bulk, while in the former, no bulk water exists, and exchanges are possible only among the surface waters themselves. Thus, our findings that waters diffusing on the protein surface have residence times 10−20 times longer than bulk exchanging waters might explain the greater retardation found in protein powders. Nevertheless, given that previous MD studies[24] on protein powder overestimate 3−4 times the hydration water diffusion coefficient with respect to neutron results of Settles and Doster,[2] further investigations are needed.

To conclude, we remark that our extensive simulation runs on hydrated lysozyme have provided evidence that the present models of water and water−protein interactions are adequate to reproduce the experimental water dynamics near a protein relative to bulk. On the other hand, SPC/E which better reproduces self-diffusion of water provides a more realistic picture of water hydration than does the TIP3P model. Our calculation has also shown that the assumption, used by NMRD to estimate water residence times, that rotational and translational diffusions have identical time scales is fundamentally correct. All these results give a stronger credibility to molecular simulation in the investigation of protein hydration.

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