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Dynamics in Protein Powders on the Nanosecond–Picosecond Time Scale Are Dominated by Localized Motions

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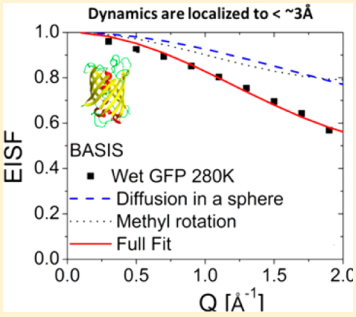
Supporting Information

ABSTRACT: We present analysis of nanosecond-picosecond dynamics of Green Fluorescence Protein (GFP) using neutron scattering data obtained on three spectrometers. GFP has a β-barrel structure that differs significantly from the structure of other globular proteins and is thought to result in a more rigid local environment. Despite this difference, our analysis reveals that the dynamics of GFP are similar to dynamics of other globular proteins such as lysozyme and myoglobin. We suggest that the same general concept of protein dynamics may be applicable to all these proteins. The dynamics of dry protein are dominated by methyl group rotations, while hydration facilitates localized diffusion-like motions in the protein. The latter has an extremely broad relaxation spectrum. The nanosecond–picosecond dynamics of both dry and hydrated GFP are localized to distances of ~1–3.5 Å, in contrast to the longer range diffusion of hydration water.



I. INTRODUCTION

The importance of protein dynamical processes in enzymatic activity is now widely recognized,[1] yet our understanding of internal protein dynamics remains limited. In contrast with many other soft materials (e.g., polymers, colloids), the dynamics of biological macromolecules lack a generally accepted framework describing multiple processes on different time and length scales. Numerous experimental tools have been used in the study of protein dynamics including NMR[2] and various optical and fluorescence based techniques,[3,4] as well as molecular dynamics (MD) simulations, which are becoming a powerful tool in analysis and visualization of biomolecular motions and function.[5]

Neutron scattering spectroscopy, however, holds a unique place in the study of protein dynamics due to the ability to directly probe atomic motions. Most importantly, it provides not only the characteristic time and energy of the motions, but also their geometry through the analysis of the wave-vector, Q, dependence.[6] Moreover, neutron scattering measurements yield the intermediate scattering function $S(Q,t)$ and dynamic structure factor $S(Q,E)$, which can be directly compared to MD-simulations.[7] This provides a thorough test of the results of simulations, and in return, simulations can help to interpret and visualize the results of neutron scattering measurements. Finally, the large difference in the incoherent neutron scattering cross sections of hydrogen and deuterium atoms provides unique possibilities to identify the part of the molecule contributing to particular motions.[8] Exciting new developments using spin polarization also open the possibility to study protein structure and dynamics by separating coherent and incoherent contributions to the scattered intensity.[9]

Several proteins have been already studied using neutron scattering.[10−25] Studies of protein solutions using the neutron spin echo (NSE) technique[21−24] have analyzed protein dynamics on the tens of nanoseconds time scale, where domain motions in alcohol dehydrogenase and phosphoglycerate kinase were identified.[22−24] These motions observed in solution have characteristic time scales of τ ~30–60 ns and amplitude of ~8–10 Å. Characteristic τ and amplitude were found to change upon binding of a substrate.[23] At shorter time and length scales, there are even more studies using time-of-flight and back-scattering spectrometers that analyze internal protein dynamics on time scales ranging from faster than ps to ~1 ns.[10−20,25,26] These studies show some similarities in the dynamics of various proteins and systematic differences with the dynamics of other biological macromolecules such as RNA and DNA.[10−30] From these studies stems the important role played by methyl groups in protein dynamics, especially in their dehydrated states.[11−13,15,16,28−31] In our recent studies[12,32,33] we combined neutron scattering, MD-simulations and dielectric spectroscopy data and identified several characteristic processes in dynamics of lysozyme on the picosecond–nanosecond time scale:

(i) low-frequency collective vibrations (the boson peak)
(ii) fast picosecond relaxations that are usually ascribed to a rattling of residues in a cage formed by neighbor residues and hydration water

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(iii) methyl group rotations that are essentially insensitive to hydration
(iv) localized diffusion that seems to be coupled to the dynamics of hydration water, has extremely broad spectrum, and is responsible for the dynamical transition
(v) jumps between different conformational states occurring on the several nanoseconds time scale

Analysis of neutron scattering data in myoglobin[14−16] has led to similar classification for processes (ii)−(iv) (although a different name has been used for localized diffusion) and emphasized the importance of process (iv) for biological function of proteins. Thus the major questions remaining are whether the same processes dominate the dynamics of other globular proteins on the ps−ns time scale, whether all these processes remain localized, and if the classification proposed for lysozyme is applicable to other proteins. In this article, we will test these questions by exploring the dynamics of green fluorescent protein (GFP), a 27 kDa globular protein with a barrel-like structure that differs significantly from other globular proteins (e.g., lysozyme, myoglobin, bacteriorhodopsin).

Neutron scattering data were obtained using several spectrometers to cover a broad frequency range from ∼1 ns to under 1 ps. Based on this analysis we demonstrate that the general concept described above for lysozyme is also applicable to dynamics of GFP. On the nanosecond−picosecond time scale, methyl group rotations dominate the dynamics of dehydrated GFP, while the hydration-induced motion can be described as a localized diffusion with a very broad spectrum. Based on our analysis we emphasize the localized nature of internal nanosecond−picosecond dynamics in globular proteins. This is in contrast to the dynamics of hydration water that exhibit diffusive behavior in this system.[33,34]

II. EXPERIMENTAL SECTION

Neutron scattering measurements were performed on dry GFP (lyophilized in D_2O) and GFP hydrated in D_2O (hydration level h ∼0.4). Details of the sample preparation can be found in ref 34. Three spectrometers were used: (1) the High Flux Back-Scattering spectrometer (HFBS) at the National Institute of Standards and Technology with a resolution of 0.9 μeV (∼1 ns) and Q-range of 0.25−1.75 Å^{-1};[35] (2) the Backscattering Spectrometer (BASIS) at the Oak Ridge National Laboratory (ORNL) Spallation Neutron Source (SNS) with a resolution of 3.5 μeV (∼300 ps) and Q-range of 0.2−2.0 Å^{-1};[36] and (3) the Cold Neutron Chopper Spectrometer (CNCS) at SNS with an elastic resolution of ∼60 μeV (∼20 ps) and an accessible Q-range of 0.5−4.0 Å^{-1}.[37] Combining the data from these 3 spectrometers provides information over more than four orders in frequency (and relaxation time) spanning from ∼1 μeV up to ∼10 meV (from ∼1 ns down to faster than ps). As we discuss in the text below, a broad frequency range is crucial for meaningful analysis of complex protein dynamics with strongly stretched relaxation spectra. In addition, we used elastic scans to estimate characteristic energy barriers controlling intramolecular motions.

III. DATA ANALYSIS

The neutron scattering intensity from all the spectrometers was converted to the dynamic structure factor, $S(Q,E)$, using software provided by the user facilities. In simple terms the incoherent scattering of protein samples can be interpreted as the time and space Fourier transform of the self-correlation function of hydrogen atoms in the protein. In the general case, $S(Q,E)$ for a given instrumental resolution $R(Q,E)$ can be presented as a sum of several processes:[11]

$$S(Q,E) = \mathrm{DWF}(T)\left[\sum_{i=1}^{n} P_i\{EISF_i(Q)\delta(E) + [(1 - EISF_i(Q))S_i(Q,E,T)]\} \otimes R(Q,E)\right] \quad (1)$$

where P_i defines the fraction of H-atoms participating in the particular mode i, $S_i(Q,E,T)$ represents the spectrum of the mode i at temperature T, and the delta function, $\delta(E)$, represents the elastic part of the scattering function. $\mathrm{DWF}(T)$ is the Debye−Waller Factor which approximates harmonic vibrational motions and can be defined as[13]

$$\mathrm{DWF}(T) = \exp\left[-\frac{1}{3}Q^2\frac{\mathrm{d}\langle r^2\rangle}{\mathrm{d}T}T\right] \quad (2)$$

The $\mathrm{EISF}_i(Q)$ (Elastic Incoherent Structure Factor) provides the geometry of the given motion i. In particular case of methyl group rotations the three-site jump model applies

$$\mathrm{EISF}_{\mathrm{methyl}}(Q) = 1 - P_{\mathrm{methyl}} + \frac{P_{\mathrm{methyl}}}{3}[1 + 2j_0(Qr\sqrt{3})] \quad (3)$$

where j_0 is the zero order Bessel function and r ∼1.03 Å is the radius of the methyl group rotation. In the case of localized diffusion

$$\mathrm{EISF}_{\mathrm{LD}}(Q) = 1 - P_{\mathrm{LD}} + P_{\mathrm{LD}}\left[\frac{3j_1(QR)}{QR}\right]^2 \quad (4)$$

where j_1 is the first order Bessel function, and R is the radius of a sphere in which the diffusion is localized. Thus a fit of the Q-dependence of the EISF provides estimates of the geometry of the motion. There are different approximations to describe the EISF of this motion, including two-site jumps in an asymmetric double-well potential.[14,38] However, the limited Q-range of our data is not sufficient to differentiate these models (one can see discussion in ref 38 for more details). However, earlier MD-simulations[33] show that the observed motion is consistent with the localized diffusion model. In any case, we use this approximation to estimate a characteristic localization length of the hydration induced process.

In our analysis we fit the spectra using the DAVE software package to obtain the EISFs.[39] Specifically, the spectrum is fit by a delta function to represent the elastic line and a single Lorentzian to approximate the quasielastic neutron scattering (QENS); we do not use a flat background in this work as proposed elsewhere.[40] EISF is then defined as the ratio of the obtained elastic intensity to the total scattering intensity as obtained by fitting of the spectra at each Q value measured. We emphasize that the approximation of the QENS spectrum by a single Lorentzian is not accurate, because the spectra of proteins is usually strongly stretched.[12,32] As such, the obtained width of the Lorentzian does not provide any reliable estimate of the characteristic relaxation time of the process. It might depend strongly on resolution and available energy range of the spectrometer and should be interpreted with caution. This is the reason we do not analyze the obtained single Lorentzian fit line widths in this paper, although they are presented in Figures S1, S2, and S3 in the Supporting Information (SI). On the

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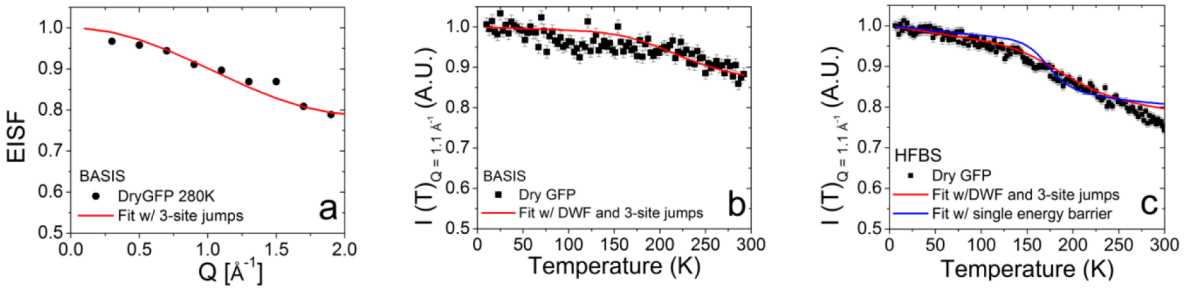

Figure 1. (a) EISF of dry GFP at T = 280 K measured on the BASIS spectrometer (symbols). The solid line presents a three-site jump model (eq 3) with all parameters fixed to methyl group rotation. (b) and (c) Temperature dependence of elastic intensity in dry GFP (symbols) at Q = 1.1 Å^{-1} measured on BASIS (b) and HFBS (c) spectrometers. Solid lines show the fit by the methyl group rotation with a Gaussian distribution of energy barriers, while the blue line in (c) represents the best fit with a single energy barrier 15.5 kJ/mol. All the fit lines include DWF.

contrary, the EISF is useful in that it provides the geometry of the motion that dominates the accessible energy range of the spectra, and the estimated P_i corresponds to the fraction of hydrogen atoms moving in the energy (time) range accessible for the given spectrometer. If the motion is slower or faster than the fitted spectral range, it will not be included in the P_i estimated using this approach. This means that the real P_i can be higher or equal to the estimated P_i from the fit.

Our analysis of the QENS spectra obtained on HFBS and BASIS spectrometers reveals no significant Q dependence in its width (see also Figures S1−S3 in SI), while significant increase in QENS intensity with Q. This is consistent with earlier analysis of lysozyme spectra[12] and reflects the localized nature of the motions on the accessible time scale. This observation also allows us to sum up the spectra measured at different Q's to improve the statistics of QENS spectra for their spectral shape analysis. Thus the analysis that follows is based on spectra summed over all Q, and only the Q-dependence of the QENS intensity (via the EISF) is used to estimate geometry and type of motions.

We have also evaluated the temperature dependence of the elastic intensity measured using the HFBS and BASIS spectrometers. Equation 1 can be rewritten for the elastic scattering as follows:[41]

$$S(Q, E, T)_{el} = \mathrm{DWF}(T)\left(\sum_{i=1}^{n} P_i\left\{\mathrm{EISF}_i(Q)\delta(E) + \frac{2}{\pi}\left[(1 - \mathrm{EISF}_i(Q))\arctan\left(\frac{\Gamma_{res}}{\Gamma_i(E, T)}\right)\right]\right\}\right) \quad (5)$$

where Γ_{res} is the width of the resolution function and Γ_i is the width of the QENS spectra of the i mode assuming its Lorentzian shape. This leads to the arctan term which is an estimate of the integral of the Lorentzian distribution for the respective process, i, falling outside the elastic line width. For methyl group rotations, the characteristic relaxation time usually follows Arrhenius behavior, such that $\tau = \tau_0 \exp(E_a/kT)$.[42,43] It is known that methyl group rotations in proteins have a broad distribution of relaxation times and energy barriers, caused by variations in chemical structure (different amino-acids) and in positions of the methyl groups in the protein.[12,44] This heterogeneity can be approximated by a Gaussian distribution of energy barriers $g_i \propto \exp[-(E_0 - E_i)^2/2\Delta E^2]$. Here E_0 is the averaged energy barrier and ΔE is the width of the distribution. Using this approximation we can express the decrease of elastic intensity caused by methyl group rotation:[42,43]

$$S(Q, E, T)_{el} = \mathrm{DWF}(T)\mathrm{EISF}_{methyl} + \frac{2}{\pi}(1 - \mathrm{EISF}_{methyl})\sum_i g_i \arctan\left(\frac{\Gamma_{res}}{\Gamma_i(T)}\right) \quad (6)$$

IV. RESULTS AND DISCUSSION

The spectra of dry GFP exhibits strong QENS intensity similar to those of other studied dry proteins.[12,13,15,33] In agreement with earlier analysis of lysozyme,[12,33] the width of the QENS spectra of dry GFP is essentially Q-independent, suggesting that scattering from local motions is the primary contribution to the spectra. However, the QENS intensity does change with Q and allows us to calculate the EISF(Q), as shown in Figure 1a for the spectra measured on BASIS at T = 280 K. The experimental data can be reproduced well by a model of methyl group rotation using eq 3 with no adjustable parameters. We fixed P_{methyl} = 0.26 to the fraction of all nonexchangeable hydrogen atoms on methyl groups and r = 1.03 Å as the radius of the methyl group. This result confirms that the QENS spectra of dry GFP in the time range ∼10−350 ps are dominated by methyl group rotations. This is consistent with earlier studies of dehydrated lysozyme,[45] myoglobin,[15] and several other proteins.[13]

Next we analyze the temperature dependence of the elastic intensity in dry GFP using eq 6 with fixed P_{methyl} = 0.26 and r = 1.03 Å to determine the average value of the energy barrier, E_0, and the width, ΔE, of the Gaussian distribution of the energy barriers. DWF(T) was estimated from the slope of the mean-squared displacements at T < 100 K where methyl group contributions remain minor, $d\langle r^2\rangle/dT \sim 4 \times 10^{-4}$ Å^2/K. τ_0 was also fixed to the known value for the methyl group libration, $\tau_0 = 2.8 \times 10^{-14}$ s.[42] In this way the model has only two free fit parameters: the average value E_0 and the width ΔE of the Gaussian distribution of the energy barriers. Figure 1c illustrates that the temperature dependence of elastic intensity cannot be described by a single energy barrier. The elastic intensity measured on the BASIS and HFBS spectrometers can be well reproduced using the fit (Figure 1b and c), providing estimates of the energy barrier distribution with $E_0 = 15.5 \pm 2$ kJ/mol and $\Delta E = 3.5 \pm 1$ kJ/mol. The HFBS data, however, reveal extra decay in elastic intensity at T > 250 K that cannot be accounted

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for by methyl groups alone (Figure 1c). These results suggest that other relaxation processes are contributing to the QENS spectra of dry GFP on the time scale ~1 ns at higher T. This conclusion is consistent with another recent neutron study[13] and with recent simulations[46] that have also identified additional relaxations in dry GFP.

Another approach to verify the obtained distribution of energy barriers is a comparison of the expected QENS spectra from methyl groups to the measured dynamic structure factor

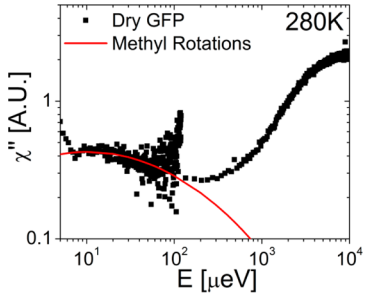

Figure 2. Susceptibility spectra of dry GFP (combined BASIS and CNCS data) at T = 280 K (symbols) and the relaxation spectra of the methyl group process (line) calculated with the parameters estimated from the temperature dependence of elastic intensity. This comparison confirms that methyl groups dominate the dynamics in dry GFP on the nanosecond−picosecond time scale.

of the dry GFP (Figure 2). For this comparison we have used susceptibility presentation instead of $S(Q,E)$

$$\chi''(Q, E) \propto \frac{S(Q, E)}{n_B(T, E)} \tag{7}$$

where n_B is the temperature Bose factor. This presentation has the advantage of showing well separated relaxation processes as peaks, with the maximum providing an estimate of the characteristic relaxation time, and the shape of the peaks providing information on the stretching of the processes. The QENS spectrum of dry GFP shows a maximum at E ~10−15 μeV (τ ~60 ps). This agrees reasonably well with the expected spectrum of methyl groups at T = 280 K (Figure.2). At frequencies higher than ~100 μeV contributions of fast picosecond relaxation and of the boson peak vibrations enter the susceptibility spectra, becoming the predominant feature.

We can now compare the distribution of energy barriers for methyl rotation obtained for GFP to literature data for three other dry globular proteins, lysozyme, myoglobin, and apoferritin, as well as a polypeptide (Table 1). There is no large difference in the averaged activation energy and the width of the distribution among these systems. According to results from MD simulations on lysozyme[12] and myoglobin,[31] the broad distribution of energy barriers controlling methyl rotation is caused by both the varied chemical structure of the different methyl containing residues and the unique position of each group in the protein. However, the results on polyalanine suggest that broad distribution of methyl's τ can exist even in chemically identical residues. This observation suggests that the position of the methyl group, its fluctuating environment, might play a more important role in the distribution of energy barriers than specific chemical structure of the residue.

We turn now to the dynamics of hydrated GFP, and compare the QENS spectra in the dry and hydrated states. Figure 3a

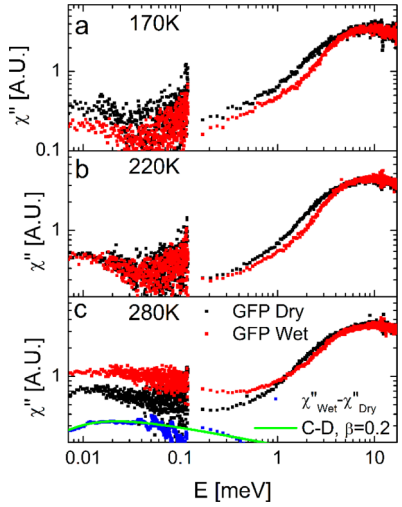

Figure 3. Susceptibility spectra (combined BASIS and CNCS data) for dry and hydrated GFP at 3 temperatures: (a) 170 K, (b) 220 K, and (c) 280 K. The difference between spectra of hydrated and dry GFP at T = 280 K presents the spectrum of hydration-induced process (c). The solid line in (c) represents the Cole−Davidson distribution function with the stretching exponent β = 0.2. Accuracy of the data is not sufficient for a meaningful fit.

shows that the susceptibility is higher in dry GFP than in hydrated GFP at low T = 170 K. We ascribe this difference to the suppression of the protein fast picosecond relaxation by frozen water that has been previously discussed for myoglobin,[47] lysozyme,[48] and GFP.[34] Increasing temperature to T = 220 K leads to very similar QENS spectra at energies ~1−200 μeV for both dry and hydrated GFP, while faster dynamics remain more depressed in the hydrated biomolecule (Figure 3b). Finally, at the highest studied temperature, T = 280 K, the QENS spectrum of hydrated GFP is significantly more intense than the spectrum of dry GFP (Figure 3c). This extra intensity is related to the hydration-induced relaxation process that enters the resolution window of our spectrometers.

The spectrum of the hydration-induced process as revealed by a subtraction of these two spectra in Figure 3c appears to be

Table 1. Comparison of Methyl Rotation Parameters[a]

	Lys[12]	Mb[44]	Mb[63]	Apo[62]	GFP	polylanine[63]
P_m	0.26	0.22	0.25	0.24	0.26	0.75
$E_{0,avg}$ (kJ/mol)	16.6	15.6	15.1	17	15.5 ± 2	15.9
ΔE (kJ/mol)	5.8	3.4	3.4	4	3.5 ± 1	5.0

[a]Methyl rotation parameters estimated for different globular proteins using neutron scattering measurements, P_m is a fraction of all non-exchangeable hydrogen atoms on methyl groups, E_0 is the energy maximum, and ΔE is the width of the distribution of the energy barriers controlling methyl dynamics. (Lys: Lysozyme, Mb: Myoglobin, Apo: Apoferritin, GFP: Green Fluorescent Protein).

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extremely broad. It can be described reasonably well by Cole–Davidson or Cole–Cole distribution functions with a stretching exponent β ~0.2–0.4 (Figure.3c), in agreement with earlier analysis of other proteins.[14,32] Only the combination of several neutron scattering spectrometers can fully cover such a broad relaxation spectrum. Traditional use of a single spectrometer and the fit of QENS spectra by a few Lorentzians leads to estimates of the QENS width and relaxation times that are highly dependent on the spectrometer resolution and accessible energy range. To the best of our knowledge, a similar combination of neutron scattering spectrometers has been applied only to the analysis of lysozyme dynamics which also revealed an extremely broad spectrum of the hydration-induced process.[32] The broad spectrum of the hydration-induced process was also suggested from analysis of neutron scattering spectra of myoglobin.[49] Based on current results for GFP and earlier results for lysozyme[32] and myoglobin,[49] we suggest that the broad spectral shape (strong stretching) is a characteristic feature of the hydration-induced relaxation in globular proteins. MD simulations of lysozyme[33,50] demonstrate that all structural units (backbone, side groups, loops, etc.) are involved in this relaxation process. As a result the distribution of energy barriers and relaxation times for this process are understandably very broad and suggests a reason for the strongly stretched relaxation spectrum that is observed. This picture is consistent with detailed NMR studies of lysozyme that revealed a distribution of relaxation time from 11 ps up to 1.3 ns depending on residue analyzed.[51]

It is important to note that hydration water relaxes on a similar time scale and also has rather broad relaxation spectrum.[28,52] The neutron scattering spectra of lysozyme hydration water were fit by stretched exponential Kohlarusch–Williams–Watts (KWW) function with the stretching exponent β ~0.5,[52] while neutron scattering spectra of GFP hydration water were fit by Cole–Cole function with the stretching parameter α ~0.6.[28] This stretching is ascribed to strong heterogeneity in interaction of water molecules to protein surface residues with different polarities. Because protein dynamics is strongly coupled to translational motions of hydration water,[53,54] broad relaxation spectrum of the latter provides an additional mechanism of broadening for hydration-induced protein relaxation. Yet, the stretching of the protein relaxation is obviously higher than that of its hydration water.

This hydration induced process leads to a strong decrease in the elastic intensity for hydrated GFP at T > 220 K (Figure 4). The analysis shows that the decay of elastic intensity in hydrated GFP is significantly stronger than that expected from methyl group rotation at T above ~200–220 K (Figure 4). Comparing the different onset temperatures of this process for two different spectrometers in Figure 4, we clearly see the effect of the different instrumental resolutions. The deviation from the line describing methyl groups' contribution appears in the HFBS data (resolution ~1 ns) at lower T than in the BASIS data (resolution ~300 ps). This indicates that the process is not the result of a particular phase transition, but is caused by the entry of the relaxation process into the respective instrumental windows as it becomes faster with increasing temperature. This point has been discussed in many earlier publications[42,55–57] and is out of the scope of the current paper.

Analysis of neutron and dielectric spectroscopy data for lysozyme[32] suggests that the hydration-induced process has a non-Arrhenius temperature dependence, and is strongly coupled to dynamics of hydration water. This fact together with the very broad spectrum of the process (Figure 3c) complicates analysis of the temperature dependence of elastic intensity and characteristic relaxation time of the process. Nevertheless, we can analyze the EISF of this process in both BASIS and HFBS energy windows (Figure 5). The process appears to be localized and can be described by a model for

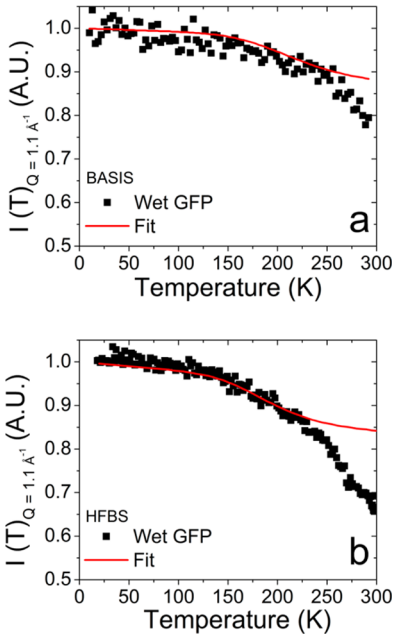

Figure 4. Temperature dependence of elastic intensity in hydrated GFP at Q = 1.1 A^{-1} measured on BASIS spectrometer (a) and on HFBS spectrometer (b). Symbols are experimental data and the lines represent the contribution of methyl groups.

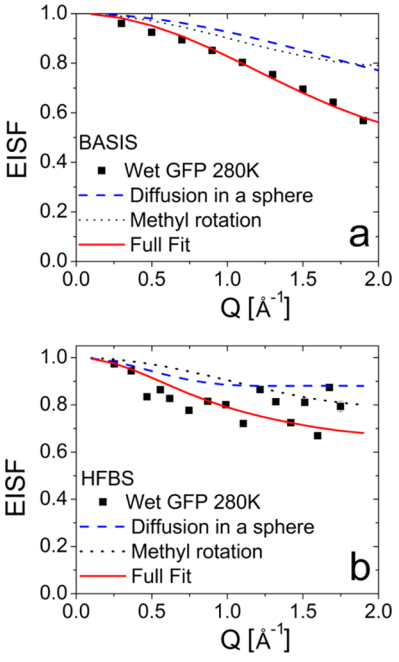

Figure 5. EISF for hydrated GFP measured on BASIS spectrometer (a) and HFBS spectrometer (b). Symbols are experimental data, dotted lines show methyl group contribution, dashed lines show the fit with diffusion on a sphere model, and the solid lines show the sum of those two contributions.

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either a 2-site jump, 3-site jump, or by localized diffusion. The accessible Q-range and accuracy of the data do not allow us to differentiate these models. Following the suggestion from simulation, that H-atoms undergo localized diffusion motion in proteins,[33,46] we fit the EISFs to the localized diffusion model (eq 4), in addition to the contribution of methyl rotations that we estimated for the dry protein. The fit provides an estimate of the radius of localized diffusion at $R \sim 1$ Å and $P_i = 0.4$ in the case of BASIS spectra (Figure 5a). The distance R agrees well with the recent simulations, while P_i suggests that only half of the non-methyl hydrogen atoms contribute to this process in the energy window of BASIS (faster than ∼300 ps). The estimated localization length $R \sim 1$ Å might depend slightly on the specific model used (diffusion in a sphere, or multiple-site jumps), but it provides a characteristic scale of the motion.

Similar analysis of HFBS (∼1 ns) data provides the EISF level higher than EISF estimated from the BASIS data. This discrepancy appears due to the limitations of the Lorentzian approximation described above, stemming from the limited energy window available in the fitting of the spectra used to estimate the EISFs. Modeling of the EISF obtained on the HFBS spectrometer using the localized diffusion or the two-site jump models results in $P_i = 0.1$ and $R \sim 3.5$ Å. This analysis shows much larger amplitude of the motion. It is possible that another slower process contributes to the spectra in the slower relaxation window accessible at HFBS. According to simulations,[33,46] this slower process might be jumps between various conformational states and can have larger length of atomic motions. Unfortunately, we cannot obtain more accurate information about the hydration-induced dynamics of GFP from these neutron scattering spectra.

The presented analysis clearly demonstrates that, at these nanosecond−picosecond time scales, the intramolecular dynamics of GFP in both the dry and hydrated states are localized. Geometry of hydrogen atoms in all the motions described above is restricted to distances ∼1−3 Å. Our spectrometers probe distances larger than $r \sim 2\pi/Q_{max} \sim 3$ Å. The estimated localization length is also consistent with earlier analysis of lysozyme[12,32,45] and also with simulations of both lysozyme and GFP.[33,46] Localization length of the hydration induced process in myoglobin was also found to be ∼1.3 Å.[16]

Such strong localization of protein intramolecular motions is in contrast to the diffusive-like motions of hydration water.[33,34] Currently, most of the authors agree that picosecond−nanosecond dynamics of protein and its hydration water are strongly coupled.[15,16,32,58] This coupling has been demonstrated from analysis of differently deuterated protein samples[15,16] and from comparison of neutron and dielectric spectroscopy data,[32] time-resolved optical spectroscopy,[58,59] and MD-simulations.[53,54] Both protein fluctuations and hydration water have comparable relaxation times of ∼15−50 ps. It is obvious that translational motion of hydration water should be active to enable ∼1−3 Å scale motions for protein surface residues. This leads to significant coupling of hydration-induced processes to translational motion of hydration water. Despite this coupling there are distinct differences in the protein and hydration water dynamics. Hydration water exhibits strongly Q-dependent dynamics that reflect diffusive motions of the water molecules.[33,34] This means that the characteristic time scale of hydration water motions depends on the probe distance (measured Q). In contrast, the intramolecular dynamics of hydrated protein at ambient temperature are localized with characteristic relaxation time being essentially Q-independent in the studied Q-range. From this point of view, the time scale of protein dynamics should be compared to the time scale of hydration water dynamics on the length scale comparable to the same distance ∼1−3 Å.

The displacements of atoms between different conformational states of globular proteins appear to be very small, just a few angstroms. This differentiates the intramolecular dynamics of proteins from the usual structural relaxation in other soft materials. This localization might be related to rather restricted backbone motions as long as proteins stay folded. In other words, on the nanosecond−picosecond time scale globular proteins cannot have intramolecular atomic displacements on scale of, e.g., ∼10 Å when the protein itself has a size of ∼20−50 Å. This conclusion implies that protein structure on ps−ns time scale remains rather well-defined with only small fluctuations around the equilibrium positions. However, on time scales of several ns and longer, given sufficient hydration, domain motions lead to larger atomic displacements that have been observed in the NSE experiments and estimated to have the amplitude of ∼5−10 Å.[22−24]

V. CONCLUSIONS

The presented analysis of the dynamics of GFP demonstrates that the recently proposed classification of protein motions[12,32−34] can be applied to proteins other than lysozyme. Although GFP has a very specific barrel-like structure the qualitative picture of the protein dynamics remains reasonable for this protein as well. In the dry state, the dynamics of GFP on the nanosecond−picosecond time scale are dominated by methyl group rotations, with some minor contributions from other processes approaching the nanosecond time range. Comparison of methyl group dynamics in various globular proteins suggests that the distribution of energy barriers for methyl rotations does not change dramatically with the identity of the protein or its specific structure.

Hydration of the protein leads to suppression of its fast dynamics at lower temperature, but induces additional local processes on the nanosecond−picosecond time scale at temperatures above ∼220 K. This hydration-induced process is a kind of localized diffusion or jumps between conformational states, both of which would be localized to distances ∼1−3.5 Å. The hydration-induced process(es) has(have) extremely broad QENS spectra, suggesting a broad distribution of relaxation times. We emphasize that this feature is typical for similarly hydrated globular proteins such as lysozyme. Based on this analysis we suggest that intramolecular dynamics of globular proteins are indeed strongly localized and that the various conformational states sampled by the protein on nanosecond−picosecond time scales differ in atomic positions by only a few Å. In that respect the structure of the globular proteins remains rather well-defined as long as they stay folded. This, however, is not the case for all biological macromolecules. For example, it has been shown that ns−ps dynamics of t-RNA exhibit significantly larger displacements, up to ∼7 Å.[60,61]

■ ASSOCIATED CONTENT

Supporting Information

Lorentzian line widths resulting from the fitting procedure used to calculate the EISF in Figures 1a and 5a,b. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Henzler-Wildman, K. A.; Lei, M.; Thai, V.; Kerns, S. J.; Karplus, M.; Kern, D. A Hierarchy of Timescales in Protein Dynamics is Linked to Enzyme Catalysis. *Nature* **2007**, *450*, 913−916.

(2) Kay, L. E. Protein Dynamics from NMR. *Nat. Struct. Biol.* **1998**, *5*, 513−517.

(3) Lippincott-Schwartz, J.; Snapp, E.; Kenworthy, A. Studying Protein Dynamics in Living Cells. *Nat. Rev. Mol. Cell Biol.* **2001**, *2*, 444−456.

(4) Weiss, S. Measuring Conformational Dynamics of Biomolecules by Single Molecule Fluorescence Spectroscopy. *Nat. Struct. Biol.* **2000**, *7*, 724−729.

(5) Karplus, M.; McCammon, J. A. Molecular Dyanmics Simulations of Biomolecules. *Nat. Struct. Biol.* **2002**, *9*, 646−652.

(6) Bee, M. *Quasielastic Neutron Scattering: Principles and Applications in Solid State Chemistry, Biology, and Materials Science*; Adam Hilger: Bristol, U.K., 1988.

(7) Smith, J.; Kuczera, K.; Karplus, M. Dynamics of Myoglobin: Comparison of Simulation Results with Neutron Scattering Spectra. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 1601−1605.

(8) Sears, V. F. Neutron Scattering Lengths and Cross Sections. *Neutron News* **1992**, *3*, 26−37.

(9) Gaspar, A. M.; Buschc, S.; Appavou, M. S.; Haeussler, W.; Georgii, R.; Su, Y.; Doster, W. Using Polarization Analysis to Separate the Coherent and Incoherent Scattering From Protein Samples. *Biochim. Biophys. Acta* **2010**, *1804* (1), 76−82.

(10) Gabel, F.; Bicout, D.; Lehnert, U.; Tehei, M.; Weik, M.; Zaccai, G. Protein Dynamics Studied by Neutron Scattering. *Q. Rev. Biophys.* **2002**, *35*, 327−367.

(11) Fitter, J.; Lechner, R. E.; Buldt, G.; Dencher, N. A. Internal Molecular Motions of Bacteriorhodopsin: Hydration-Induced Flexibility Studied by Quasielastic Incoherent Neutron Scattering Using Oriented Purple Membranes. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 7600−7605.

(12) Roh, J. H.; Curtis, J. E.; Azzam, S.; Novikov, V. N.; Peral, I.; Chowdhuri, Z.; Gregory, R. B.; Sokolov, A. P. Influence of Hydration on the Dynamics of Lysozyme. *Biophys. J.* **2006**, *91*, 2573−2588.

(13) Telling, M. T. F.; Clifton, L.; Combet, J.; Frick, B.; Howells, S.; García Sakai, V. Lyophilised Protein Dynamics: More than Just Methyls? *Soft Matter* **2012**, *8*, 9529−9532.

(14) Doster, W.; Cusack, S.; Petry, W. Dynamical Transition of Myoglobin Revealed by Inelastic Neutron Scatting. *Nature* **1989**, *337*, 754−756.

(15) Doster, W.; Settles, M. Protein-Water Displacement Distributions. *Biochim. Biophys. Acta* **2005**, *1749*, 173−186.

(16) Doster, W. The Protein-Solvent Glass Transition. *Biochim. Biophys. Acta* **2010**, *1804*, 3−14.

(17) Zaccai, G. How Soft is a Protein? A Protein Dynamics Force Constant Measured by Neutron Scattering. *Science* **2000**, *288*, 1604−1607.

(18) Stadler, A. M.; Garvey, C. J.; Bocahut, A.; Sacquin-Mora, S.; Digel, I.; Schneider, G. J.; Zaccai, G. Thermal Fluctuations of Haemoglobin from Different Species: Adaptation to Temperature via Conformational Dynamics. *J. R. Soc. Interface* **2012**, *9*, 2845−2855.

(19) Paciaroni, A.; Orecchini, A.; Cinelli, S.; Onori, G.; Lechner, R. E.; Pieper, J. Protein Dynamics on the Picosecond Timescale as Affected by the Environment: A Quasielastic Neutron Scattering Study. *Chem. Phys.* **2003**, *292*, 397−404.

(20) Paciaroni, A.; Stroppolo, M. E.; Arcangeli, C.; Bizzarri, A. R.; Desideri, A.; Cannistraro, A. S. Incoherent Neutron Scattering of Copper Azurin: A Comparison with Molecular Dynamics Simulation Results. *Eur. Biophys. J.* **1999**, *28*, 447−456.

(21) Bu, Z.; Biehl, R.; Monkenbusch, M.; Richter, D.; Callaway, D. J. Coupled Protein Domain Motion in Taq Polymerase Revealed by Neutron Spin-Echo Spectroscopy. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 17646−17651.

(22) Biehl, R.; Hoffmann, B.; Monkenbusch, M.; Falus, P.; Préost, S.; Merkel, R.; Richter, R. Direct Observation of Correlated Interdomain Motion in Alcohol Dehydrogenase. *Phys. Rev. Lett.* **2008**, *101*, 138102−4.

(23) Inoue, R.; Biehl, R.; Rosenkranz, T.; Fitter, J.; Monkenbusch, M.; Radulescu, A.; Richter, D. Large Domain Fluctuations on a 50-ns Timescale Enable Catalytic Activity in Phosphoglycerate Kinase. *Biophys. J.* **2010**, *99*, 2309−2317.

(24) Brás, A. R.; Pasquino, R.; Koukoulas, T.; Tsolou, G.; Holderer, O.; Radulescu, A.; Richter, D. Structure and Dynamics of Polymer Rings by Neutron Scattering: Breakdown of the Rouse Model. *Soft Matter* **2011**, *7*, 11169−11176.

(25) Perticaroli, S.; Nickels, J. D.; O'Neill, H.; Zhang, Q.; Ehlers, G.; Sokolov, A. P. Secondary Structure and Rigidity in Model Proteins. *Soft Matter* **2013**, DOI: 10.1039/C3SM50807B.

(26) Gaspar, A. M.; Appavou, M. S.; Busch, S.; Unruh, T.; Doster, W. Dynamics of Well-Folded and Natively Disordered Proteins in Solution: A Time-of-Flight Neutron Scattering Study. *Eur. Biophys. J.* **2008**, *37* (5), 573−582.

(27) Khodadadi, S.; Roh, J. H.; Kisliuk, A.; Mamontov, E.; Tyagi, M.; Woodson, S. A.; Briber, R. M.; Sokolov, A. P. Dynamics of Biological Macromolecules: Not a Simple Slaving by Hydration Water. *Biophys. J.* **2010**, *98*, 1321−1326.

(28) Nickels, J. D.; Curtis, J. E.; O'Neill, H.; Sokolov, A. P. Role of Methyl Groups in Dynamics and Evolution of Miomolecules. *J. Biol. Phys.* **2012**, *38*, 497−505.

(29) Doster, W.; Diehl, M.; Petry, W.; Ferrand, M. Elastic Resolution Spectroscopy: A Method to Study Molecular Motions in Small Biological Samples. *Phys. B: Condens. Matter* **2001**, *301* (1), 65−68.

(30) Doster, W.; Diehl, M.; Gebhardt, R.; Lechner, R. E.; Pieper, J. TOF-Elastic Resolution Spectroscopy: Time Domain Analysis of Weakly Scattering (Biological) Samples. *Chem. Phys.* **2003**, *292* (2), 487−494.

(31) Krishnan, M.; Kurkal-Siebert, V.; Smith, J. C. Methyl Group Dynamics and the Onset of Anharmonicity in Myoglobin. *J. Phys. Chem. B* **2008**, *112*, 5522−5533.

(32) Khodadadi, S.; Pawlus, S.; Sokolov, A. P. Influence of Hydration on Protein Dynamics: Combining Dielectric and Neutron Scattering Spectroscopy Data. *J. Phys. Chem. B* **2008**, *112*, 14273−14280.

(33) Hong, L.; Smolin, N.; Lindner, B.; Sokolov, A. P.; Smith, J. C. Three Classes of Motion in the Dynamic Neutron-Scattering Susceptibility of a Globular Protein. *Phys. Rev. Lett.* **2011**, *107*, 148102−5.

(34) Nickels, J. D.; O'Neill, H.; Hong, L.; Tyagi, M.; Ehlers, G.; Weiss, K. L.; Zhang, Q.; Yi, Z.; Mamontov, E.; Smith, J. C.; Sokolov, A. P. Dynamics of Protein and its Hydration Water: Neutron Scattering Studies on Fully Deuterated GFP. *Biophys. J.* **2012**, *103*, 1566−1575.

(35) Meyer, A.; Dimeo, R. M.; Gehring, P. M.; Neumann, D. A. The High Flux Backscattering Spectrometer at the NIST Center for Neutron Research. *Rev. Sci. Instrum.* **2003**, *74*, 2759−2777.

(36) Mamontov, E.; Herwig, K. W. A Time-of-Flight Backscattering Spectrometer at the Spallation Neutron Source, BASIS. *Rev. Sci. Instrum.* **2011**, *82*, 085109−085118.

(37) Ehlers, G.; Podlesnyak, A. A.; Niedziela, J. L.; Iverson, E. B.; Sokol, P. E. The New Cold Neutron Chopper Spectrometer at the Spallation Neutron Source: Design and Performance. *Rev. Sci. Instrum.* **2011**, *82*, 085108−085113.

(38) Doster, W. The Dynamical Transition of Proteins, Doncepts and Misconceptions. *Eur. Biophys. J.* **2008**, *37* (5), 591−602.
(39) Azuah, R. T.; Kneller, L. R.; Qiu, Y.; Tregenna-Piggott, P. L. W.; Brown, C. M.; Copley, J. R. D.; Dimeo, R. M. DAVE: A Comprehensive Software Suite for the Reduction, Visualization, and Analysis of Low Energy Neutron Spectroscopic Data. *J. Res. Natl. Inst. Stand. Technol.* **2009**, *114*, 341−358.
(40) Nickels, J. D. Instrumental Resolution Effects in Neutron Scattering Studies of Protein Dynamics. *Chem. Phys.* **2013**, DOI: 10.1016/j.chemphys.2012.11.021.
(41) Grapengeter, H. H.; Alefeld, B.; Kosfeld, R. An Investigation of Micro-Brownian Motions in Polydimethylsiloxane by Complementary Incoherent-Neutron-Scattering and Nuclear-Magnetic-Resonance Experiments Below Room Temperature. *Colloid Polym. Sci.* **1987**, *265*, 226−233.
(42) Frick, B.; Fetters, L. J. Methyl-Group Dynamics in Glassy Polyisoprene - A Neutron Backscattering Investigation. *Macromolecules* **1994**, *27*, 974−980.
(43) Arrighi, V.; Higgins, J. S.; Burgess, A. N.; Howells, W. S. Rotation of Methyl Side Groups in Polymers: A Fourier Transform Approach to Quasielastic Neutron Scattering. 2. Polymer Blends. *Macromolecules* **1995**, *28*, 4622−4630.
(44) Wand, A. J. Dynamic Activation of Protein Function: A View Emerging from NMR Spectroscopy. *Nat. Struct. Biol.* **2001**, *8*, 926−931.
(45) Roh, J. H.; Novikov, V. N.; Gregory, R. B.; Curtis, J. E.; Chowdhuri, Z.; Sokolov, A. P. Onsets of Anharmonicity in Protein Dynamics. *Phys. Rev. Lett.* **2005**, *95*, 038101.
(46) Hong, L.; Glass, D. C.; Nickels, J. D.; Perticaroli, S.; Yi, Z.; Madhusudan, T.; O'Neill, H.; Zhang, Q.; Sokolov, A. P.; Smith, J. C. Elastic and Conformational Softness of a Globular Protein. *Phys. Rev. Lett.* **2013**, *110*, 028104.
(47) Diehl, M.; Doster, W.; Petry, W.; Schober, H. Water-Coupled Low-Frequency Modes of Myoglobin and Lysozyme Observed by Inelastic Neutron Scattering. *Biophys. J.* **1997**, *73* (5), 2726−2732.
(48) Caliskan, G.; Kisliuk, A.; Tsai, A. M.; Soles, C. L.; Sokolov, A. P. Protein Dynamics in Viscous Solvents. *J. Chem. Phys.* **2003**, *118*, 4230.
(49) Doster, W.; Cusack, S.; Petry, W. Dynamic Instability of Liquid like Motions in a Globular Protein Observed by Inelastic Neutron Scattering. *Phys. Rev. Lett.* **1990**, *65*, 1080−1083.
(50) Khodadadi, S.; Curtis, J. E.; Sokolov, A. P. Water Versus Protein Contributions. *J. Phys. Chem. B* **2011**, *115*, 6222−6226.
(51) Buck, M.; Boyd, J.; Redfield, C.; MacKenzie, D. A.; Jeenes, D. J.; Archer, D. B.; Dobson, C. M. Structural Determinants of Protein Dynamics: Analysis of 15N NMR Relaxation Measurements for Main-Chain Nuclei of Hen Egg White Lysozyme. *Biochemistry* **1995**, *34*, 4041−4055.
(52) Chen, S. H.; Liu, L.; Fratini, E.; Baglioni, P.; Faraone, A.; Mamontov, E. Observation of Fragile-to-Strong Dynamic Crossover in Protein Hydration Water. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103* (24), 9012−9016.
(53) Tournier, A. L.; Xu, J.; Smith, J. C. Translational Hydration Water Dynamics Drives the Protein Glass Transition. *Biophys. J.* **2003**, *85*, 1871−1875.
(54) Tarek, M.; Tobias, D. J. Role of Protein-Water Hydrogen Bond Dynamics in the Protein Dynamical Transition. *Phys. Rev. Lett.* **2002**, *88*, 138101−4.
(55) Daniel, R. M.; Smith, J. C.; Ferrand, M.; Héry, S.; Dunn, R.; Finney, J. L. Enzyme Activity Below the Dynamical Transition at 220 K. *Biophys. J.* **1998**, *75* (5), 2504−2507.
(56) Magazú, S.; Migliardo, F.; Benedetto, A. Mean Square Displacements from Elastic Incoherent Neutron Scattering Evaluated by Spectrometers Working with Different Energy Resolution on Dry and Hydrated (H_2O and D_2O) Lysozyme. *J. Phys. Chem. B* **2010**, *114* (28), 9268-−9274.
(57) Doster, W.; Busch, S.; Gaspar, A. M.; Appavou, M. S.; Wuttke, J.; Scheer, H. Dynamical Transition of Protein-Hydration Water. *Phys. Rev. Lett.* **2010**, *104* (9), 098101−5.
(58) Li, T.; Hassanali, A. A.; Kao, Y. T.; Zhong, D.; Singer, S. J. Hydration Dynamics and Time Scales of Coupled Water-Protein Fluctuations. *J. Am. Chem. Soc.* **2007**, *129*, 3376−3382.
(59) Zhang, L.; Yang, Y.; Kao, Y. T.; Wang, L.; Zhong, D. Protein Hydration Dynamics and Molecular Mechanism of Coupled Water−Protein Fluctuations. *J. Am. Chem. Soc.* **2009**, *131* (30), 10677−10691.
(60) Roh, J. H.; Briber, R. M.; Damjanovic, A.; Thirumalai, D.; Woodson, S. A.; Sokolov, A. P. Dynamics of tRNA at Different Levels of Hydration. *Biophys. J.* **2009**, *96*, 2755−2762.
(61) Roh, J. H.; Tyagi, M.; Briber, R. M.; Woodson, S. A.; Sokolov, A. P. The Dynamics of Unfolded versus Folded tRNA: The Role of Electrostatic Interactions. *J. Am. Chem. Soc.* **2011**, *133*, 16406−16409.
(62) Telling, M. T.; Neylon, C.; Clifton, L.; Howells, S.; van Eijck, L.; García Sakai, V. Thermal Motion in the Multi-Subunit Protein, Apoferritin, as Probed by High Energy Resolution Neutron Spectroscopy. *Soft Matter* **2011**, *7*, 6934−6941.
(63) Schiró, G.; Caronna, C.; Natali, F.; Cupane, A. Direct Evidence of the Amino Acid Side Chain and Backbone Contributions to Protein Anharmonicity. *J. Am. Chem. Soc.* **2010**, *132*, 1371−1376.