THE JOURNAL OF PHYSICAL CHEMISTRY B

Article

pubs.acs.org/JPCB

Temperature-Dependent Dynamics of Dry and Hydrated β-Casein Studied by Quasielastic Neutron Scattering

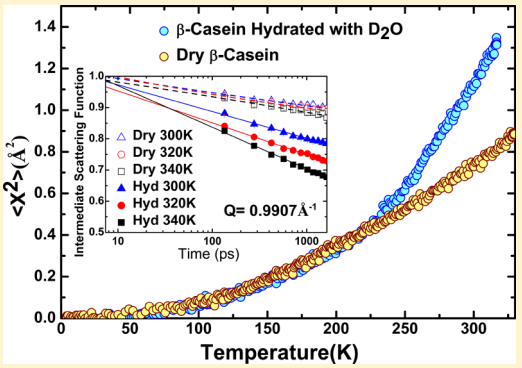
Gurpreet K Dhindsa,[†] Madhusudan Tyagi,[‡,§] and Xiang-qiang Chu*[,†]

[†]Department of Physics and Astronomy, Wayne State University, Detroit, Michigan 48201, United States

[‡]NIST Center for Neutron Research, Gaithersburg, Maryland 20899-8562, United States

[§]Department of Materials Science and Engineering, University of Maryland, College Park, Maryland 20742, United States

ABSTRACT: β-Casein is a component of casein micelle with amphillic nature and is recognized as a "natively disordered" protein that lacks secondary structures. In this study, the temperature and hydration effects on the dynamics of β-casein are explored by quasielastic neutron scattering (QENS). An upturn in the mean square displacement (MSD) of hydrated β-casein indicates an increase of protein flexibility at a temperature of ∼225 K. Another increase in MSD at ∼100 K, observed in both dry and hydrated β-casein, is ascribed to the methyl group rotations, which are not sensitive to hydration. QENS analysis in the energy domain reveals that the fraction of hydrogen atoms participating in motion in a sphere of diffusion is highly hydration dependent and increases with temperature. In the time domain analysis, a logarithmic-like decay is observed in the range of picosecond to nanosecond (β-relaxation time) in the dynamics of hydrated β-casein. This dynamical behavior has been observed in hydrated globular and oligomeric proteins. Our temperature-dependent QENS experiments provide evidence that lack of a secondary structure in β-casein results in higher flexibility in its dynamics and easier reversible thermal unfolding compared to other rigid biomolecules.



INTRODUCTION

Milk contains colloidal particles that are composed of complex associated proteins and calcium phosphates. These heterogeneous associated colloids are named "casein micelles".[1] The casein is a group of unique, specific proteins that constitutes approximately 80% of the total protein in cow's milk and other dairying species. It is composed of four main types of proteins: α_{S1}-casein (CN), α_{S2}-CN, β-CN, and κ-CN. All of them differ in structure, type, and degree of post-translational modification.[2] The casein proteins have different hydrophobic and hydrophilic regions along the protein chain and among them β-CN is most hydrophobic in nature. α_S-CN and β-CN are highly phosphorylated proteins, so they become high calcium ion binding proteins.[2] β-CN has been the subject of research for a long time because of its versatile properties, such as formation and stabilization of food emulsions,[3] binding sites of retinol and retinoic acid in milk,[4] and amphiphilic and self-assembly into stable micellar structure in aqueous solution.[2] The efficiency, capacity, and stability of β-CN makes it a good agent for entrapment as a drug carrier for oral delivery systems.[5−7]

Although caseins have important biological functions, they lack secondary structures and are recognized as "natively disordered" proteins.[8] There are various models proposed by different research groups to explain the internal structure of casein micelles and its components; however, no general agreement has been met.[9] Small angle neutron scattering (SANS), small-angle X-ray scattering (SAXS), and static light scattering (SLS) techniques were used to compare the different models to explain the internal structure of casein micelles.[10] Their SAXS study on casein micelles reports that "Yet their structure is still a puzzle and is continuously the subject of furious debates among the scientific community".[10,11] A hypothesis about the function of natively disordered proteins like casein[9,12] is that they might need a high structural flexibility to perform their function.[8,13] The flexibility of a protein, or protein "softness",[14] is generally known to be essential for its biological activities and is believed to be influenced by its hydration water and other solvents.[15−21] Some recent studies suggest that there are both solvent-dependent and solvent-independent fluctuations in the protein dynamics. Some aspects of protein dynamics are considered to be "slaved" to the solvent fluctuations (α-fluctuations), while some additional local protein motions (β-fluctuations) are independent of the solvent fluctuations.[22−26]

In this study, we use both elastic neutron scattering (ENS) and quasielastic neutron scattering (QENS) to investigate the temperature-dependent relaxational dynamics of both hydrated and dry β-CN. ENS provides information about the mean square displacement (MSD) of hydrogen atoms within protein molecules, which is traditionally used as a measure of the protein flexibility.[14] QENS has been proved to be a prevailing tool to study protein dynamics on the time scale of pico- to

Received: May 8, 2014
Revised: August 19, 2014

   dx.doi.org/10.1021/jp504548w | J. Phys. Chem. B XXXX, XXX, XXX−XXX

nanoseconds. The hydrogen atom has a large incoherent scattering cross-section compared to other atoms present in proteins. In the QENS study of protein dynamics, the incoherent scattering signal dominates only from nonexchangeable hydrogen atoms[27] that belong to the following groups: backbone hydrogen (H), methyl hydrogen (CH_3), methylene hydrogen (CH_2), and hydrogen atom from aromatic rings.[26] Therefore, QENS intensity selectively characterizes hydrogen atoms' motions in this protein. In this paper, QENS results are discussed in both the energy domain and time domain. In the energy domain, a model-independent analysis is employed to study the QENS spectra at different temperatures. In time domain, the Fourier transform of the QENS spectra and intermediate scattering functions (ISF) of hydrogen atoms in both dry and hydrated β-CN are analyzed by an asymptotic formula developed from mode coupling theory (MCT),[28−30] verifying that β-CN undergoes a logarithmic decay in its dynamics, which has been observed in other well-folded proteins.[30,31] The temperature dependence of the fitting parameters is consistent for both analyses. These results provide evidence that the natively disordered protein β-CN has a higher structural flexibility in its dynamics, which may result in an easier micellization[32] and thermal unfolding (or so-called reversible thermal denaturation) at lower temperatures compare to other well-folded proteins and rigid biomolecules.[31,33,34]

MATERIALS AND METHODS

Lyophilized β-casein (β-CN) was purchased from Sigma-Aldrich and used without further purification. For comparison purpose, both dry and hydrated protein samples were measured. The lyophilized protein powder was hydrated with D_2O vapor inside a glovebox for 24 h. The final hydration level of casein was 0.35 g of D_2O per gram of casein. This hydration level is chosen to make sure the protein has a monolayer of D_2O covering its surface to maintain its activity.[35] The hydration level was measured by directly measuring the weight of absorbed D_2O.

The QENS experiment was performed on a high-flux backscattering spectrometer (HFBS) at the National Institute of Standards and Technology (NIST) Center for Neutron Research (NCNR).[36] In elastic mode, elastic intensity was recorded from 330 K down to 4 K with a ramp rate of 1 K/min. In the quasielastic mode, the energy resolution was 0.8 μeV (full-width at half-maximum, for the Q-averaged resolution value) and the accessible dynamic range was $\pm$17 μeV. The hydrated sample was measured at seven temperatures from 220 to 340 K with 20 K intervals, and the dry casein sample was measured from 260 to 340 K, at 20 K intervals. The resolution function was measured at 4 K, where the whole signal is expected to be completely elastic in nature.

The measured QENS spectra, $S_m(Q,\omega)$, gives the self-dynamic structure factor, $S_H(Q,\omega)$, of hydrogen atoms of β-CN protein convoluted with instrumental resolution, $R(Q,\omega)$,

$$S_m(Q,\omega) = S_H(Q,\omega) \otimes R(Q,\omega) \quad (1)$$

The measured dynamic structure factor is modeled as

$$S_m(Q,\omega) = [A_0\delta(Q,\omega) + (1 - A_0)S(Q,\omega) + B(Q,\omega)] \otimes R(Q,\omega) \quad (2)$$

where $\delta(\omega)$ is a delta function centered at zero energy transfer, A_0 is the fractional intensity of elastic scattering, $R(Q,\omega)$ is the resolution function, $B(Q,\omega)$ is the linear background, and $S(Q,\omega)$ is the model scattering function. We found a single Lorentzian was appropriate to fit the data of the entire temperature range and for all Q values, which is given by

$$S(Q,\omega) = \frac{1}{\pi}\frac{\Gamma(Q)}{\omega^2 + \Gamma^2(Q)}$$

The data fitting was done by the peak analysis software PAN in the package DAVE developed by NCNR.[37]

To study the dynamics of β-CN in the time domain, we make a Fourier transform of eq 1 and convert it to

$$F_m(Q,t) = F_H(Q,t)\, R(Q,t) \quad (3)$$

where $F_H(Q,t)$ is the intermediate scattering function (ISF) of the hydrogen atoms in β-CN and can be calculated from

$$F_H(Q,t) = F_m(Q,t)/R(Q,t) \quad (4)$$

where $F_m(Q,t)$ is the Fourier transform of measured QENS data, $F_m(Q,t) = \mathrm{FT}[S_m(Q,\omega)]$, and $R(Q,t)$ is the Fourier transform of resolution function. As both samples measured at 4 K were used as respective resolution functions in the Fourier transform, all normalizations are done correctly. The Fourier transform is obtained by using the FFT toolkit in the DAVE software package developed by NCNR.[37]

The $F_H(Q,t)$ can be fitted by an asymptotic expression derived from mode coupling theory (MCT):[29,30,38]

$$F_H(Q,t) \sim [f(Q,T) - H_1(Q,T)\ln(t/\tau_\beta(T)) + H_2(Q,T)\ln^2(t/\tau_\beta(T))]\exp(t/\tau_\alpha(Q,T)) \quad (5)$$

where $\tau_\beta(T)$ and $\tau_\alpha(Q,T)$ are the characteristic β- and α-relaxation times, $f(Q,T)$ is a temperature-dependent prefactor, which is proportional to the Debye−Waller factor for small Q values; i.e., $f(Q,T) = \exp[-A(T)Q^2]$. The Q-dependent parameters, $H_1(Q,T)$ and $H_2(Q,T)$, can be written as $H_1(Q,T) = h_1(Q)\,B_1(T)$ and $H_2(Q,T) = h_1(Q)\,B_2(Q,T)$, representing the first- and second-order logarithmic decay parameters.[29,39] In our experiment, the time range (up to 1 ns) is much shorter than the α-relaxation time range (microseconds to milliseconds); hence, the value of the last exponential factor can be approximated to unity. Equation 5 can be simplified as

$$F_H(Q,t) \sim [f(Q,T) - H_1(Q,T)\ln(t/\tau_\beta(T)) + H_2(Q,T)\ln^2(t/\tau_\beta(T))] \quad (6)$$

RESULTS AND DISCUSSION

1. MSD and Protein Flexibility. The "softness" or the flexibility of the protein can be quantified by the mean square displacement (MSD) $\langle x^2(T)\rangle$ of hydrogen atoms in protein molecules.[14,40,41] The MSD is calculated from the measured incoherent elastic neutron scattering intensity using the Debye−Waller factor derived from a Gaussian approximation

$$I_{el}(Q,T,\omega{=}0)/I_{el}(Q,T{=}4\mathrm{K},\omega{=}0) = \exp[-\frac{1}{3}Q^2\langle x^2\rangle] \quad (7)$$

where $I_{el}(Q,T,\omega{=}0)$ is the temperature-dependent elastic scattering intensity.

The measured MSDs $\langle x^2(T)\rangle$ for both dry and hydrated β-CN are plotted in Figure 1 as functions of temperature. Neither of the curves show a sharp drop at the freezing point (273 K),

dx.doi.org/10.1021/jp504548w | J. Phys. Chem. B XXXX, XXX, XXX−XXX

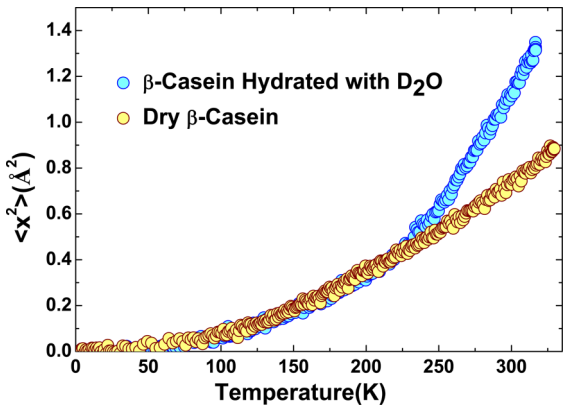


Figure 1. MSD, $\langle x^2(T)\rangle$, of dry β-CN and D_2O-hydrated β-CN plotted as functions of temperature. The dynamic transition is observed at $T \sim 225$ K in hydrated β-CN. Error bars are within the size of the symbols.

indicating no ice formation in our measured temperature range for either dry or hydrated samples. Two onsets inflected in the MSD slopes are observed in the hydrated sample. The first onset occurs at $T \sim 100$ K and is common for both samples. This anharmonic behavior has been observed in a number of proteins[16,42] and ascribed to the onset of methyl group rotations. Interestingly, both samples show the exact same MSDs until about 220 K, indicating that methyl group dynamics of β-CN is independent of hydration, which has been found to be the case for other proteins as well.[43−46] The second increase in the slope at $T \sim 225$ K is only observed in hydrated protein. Although a very recent study[47] demonstrated that the MSD of dry bovine serum albumin (BSA) powder exhibits a change in the slope in the vicinity of 240−260 K, our results do not show the same transition in dry β-CN, as the T-dependence of the MSD slope remains unchanged in the above T-range. A possible reason is due to their much different energy window (ca. millielectronvolts for a time-of-flight spectrometer) compared to our backscattering spectrometer with a microelectronvolt energy window. In the meV energy range, the instrument is more sensitive to localized fast motions rather than conformational fluctuations. Furthermore, BSA is a well-folded protein and is much more rigid than the natively disordered β-CN. Thus, the hydration effect can be more prominent for β-CN molecules.

The second change in MSD slope is highly hydration-dependent[16] and is generally recognized as the dynamic transition. This transition involves backbone fluctuations[26,48] and activation of hydrophilic side chains, which are strongly coupled to the relaxation rates of the hydrogen bonds that they form with hydration water.[49,50] In some recent studies,[51−53] it is suggested that the protein dynamic transition, shown as the anharmonic onset in the MSDs, depends on the energy resolution of the spectrometer. Here, our results clearly demonstrate that the local and global dynamics of proteins are affected by the hydration. While β-CN has both hydrophobic and hydrophilic regions,[2] the sharp increase in MSD of hydrated β-CN at 225 K represents the hydrophilic region. No such transition or anharmonic behavior is observed in the MSD of dry β-CN at 225 K, indicating that hydrophilic residues in β-CN protein is highly hydration-dependent.

2. Analysis of QENS Data in Energy Domain. The normalized QENS spectra for both dry and hydrated β-CN at

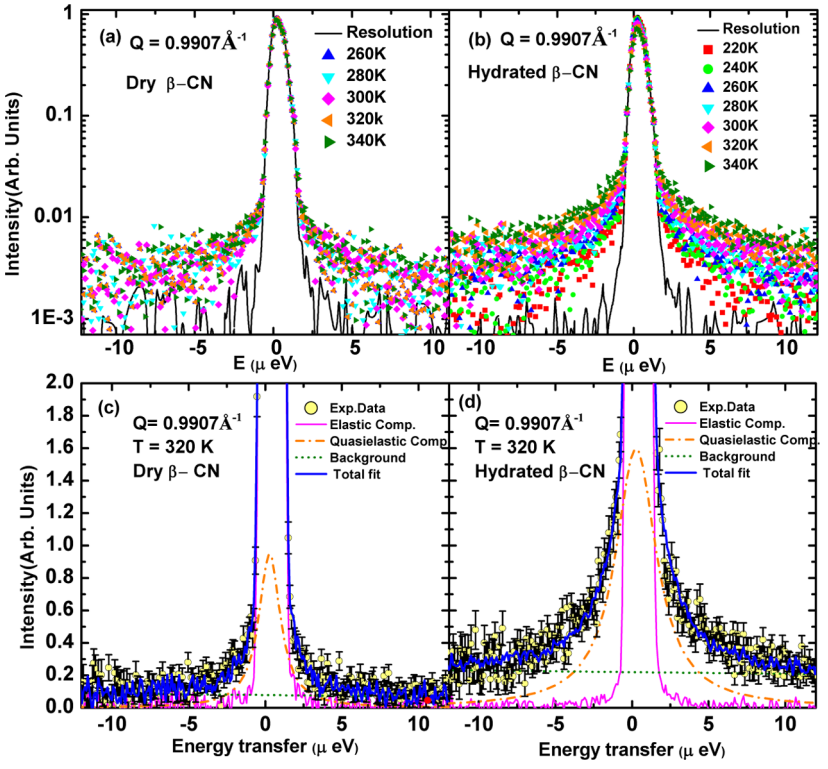


Figure 2. (a and b) Normalized QENS spectra of dry and hydrated β-CN at different temperatures from 220 to 340 and 260 to 340 K, respectively, at $Q = 0.9907$ Å^{-1}. (c and d) Analysis of the QENS spectra of dry and hydrated β-CN. The circles are the experimental data, and the blue lines represent the fitted curves. The orange and magenta lines represent the quasielastic scattering component and elastic scattering component, respectively. Error bars throughout the text represent one standard deviation.

dx.doi.org/10.1021/jp504548w | J. Phys. Chem. B XXXX, XXX, XXX−XXX

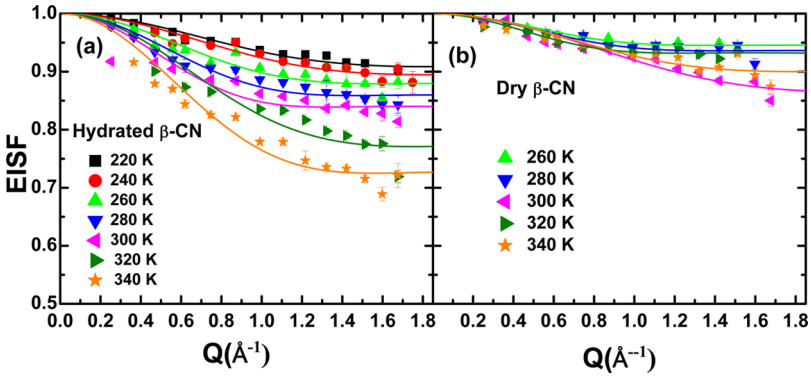


Figure 3. EISF of (a) hydrated and (b) dry β-casein at different temperatures fitted according to eq 8. The solid lines are the fitted curves. The error bars of the data points are within the symbols if not shown.

different temperatures are plotted in Figure 2a,b on a log scale. The broadening of central peaks from the resolution indicates the quasielastic scattering of hydrogen atoms in the protein molecules. The central peaks become broader as the temperature increases, implying faster dynamics of hydrogen atoms in the β-CN at higher temperatures. Comparing panels a and b of Figure 2, the central peaks for hydrated sample have more broadenings than that of the dry sample, which implies more dynamics in the hydrated sample. Figure 2c,d illustrates the analysis of QENS data by DAVE.[37] A single Lorentzian fitting agrees with the measured QENS data satisfactorily. The fitting parameters for dry and hydrated samples at the same temperatures (shown in Figures 3 and 4 and Table 1)

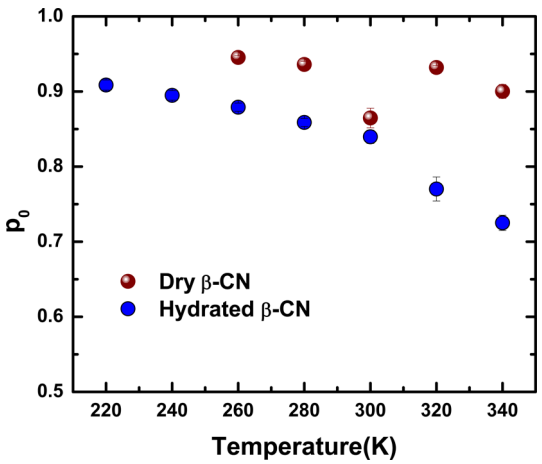


Figure 4. Fitting parameter, p_0, representing the fraction of immobile protons, plotted as a function of temperature for both dry and hydrated β-casein.

Table 1. Fitting Parameter a at Different Temperatures for Both Dry and Hydrated β-Casein

temp (K)	a_{dry} (Å)[a]	$a_{hydrated}$ (Å)
220	N/A	2.39 ± 0.25
240	N/A	2.41 ± 0.21
260	3.30 ± 0.25	2.83 ± 0.20
280	3.21 ± 0.35	3.08 ± 0.19
300	2.05 ± 0.21	3.37 ± 0.38
320	3.67 ± 0.23	2.56 ± 0.29
340	2.44 ± 0.33	2.89 ± 0.17

[a]N/A = not applicable.

demonstrate that hydrated β-CN has a much larger quasielastic scattering component than the dry one, while their elastic scattering components are comparable. This fact is consistent with the observation of the raw data shown in Figure 2a,b.

The elastic incoherent structure factor (EISF), defined as the ratio of the elastic intensity to the total intensity, i.e., A_0 in eq 2, is plotted as a function of Q in Figure 3. EISF provides the information on the fraction of atoms that are mobile and immobile in the range of the spectrometer. It contains the information on the geometry of motion of atoms exhibiting translational or rotational motion and represents the probability that a particle can be found in the same volume of space at some subsequent time.[54,55] As the QENS data is fitted by using a single Lorentzian, we expect the EISF curves to be fitted well with the expression of a single diffusive motion of atoms. As this diffusion is expected to be isotropic, we started with the expression for diffusive motion within a sphere, which is given by $(3j_1(Qa)/Qa)^2$, where j_1 denotes the spherical Bessel function of the first kind of order 1. In the expression above, a is the radius of the diffusive sphere, in which atoms diffuse freely. The complete model can be written as[56]

$$\mathrm{EISF} = p_0 + p_1(3j_1(Qa)/Qa)^2 \quad (8)$$

where p_0 denotes the immobile fraction of protons and p_1 denotes the mobile fraction of atoms and $p_1 + p_0 = 1$. We use the above model to fit the EISF of both dry and hydrated samples, to better compare the two cases with the same set of parameters. Although in the recent literature[16,57,58] it is suggested to fit the EISF of the dry sample with a model for methyl rotations, the difference between the two analyses are indistinguishable in our measured Q and energy range for the low hydration and dry samples.[16] Moreover, our current data show that a much smaller mobile fraction of hydrogen atoms participates when only methyl groups contribute to the dynamics, which justifies the approach that we have used in this study. The fitted curves of EISF are shown as solid lines in Figure 3.

The mobile population of atoms undergoes the diffusive motions in spheres as the EISF is reasonably fit with the corresponding model (eq 8), as can be seen in Figure 3. The deviations on the low Q side could be due to some small multiple scattering contribution to the observed data. The fitting parameter p_0 is plotted in Figure 4 as a function of temperature for both dry and hydrated samples. Apparently, the population of the immobile hydrogen atoms, p_0, is much lower for hydrated sample than the dry one at all temperatures,

dx.doi.org/10.1021/jp504548w | J. Phys. Chem. B XXXX, XXX, XXX−XXX

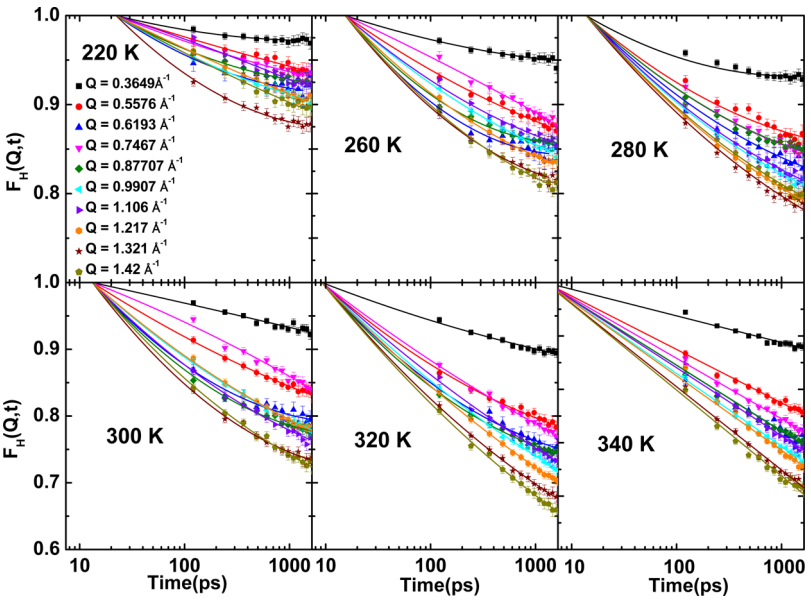


Figure 5. Analysis of the ISF of hydrated β-CN in the β-relaxation region at 10 different Q-values. The upper three panels show the results at lower temperatures T = 220, 260, and 280 K; the lower three panels show higher temperatures T = 300, 320, and 340 K. The solid lines represent the fitted curves.

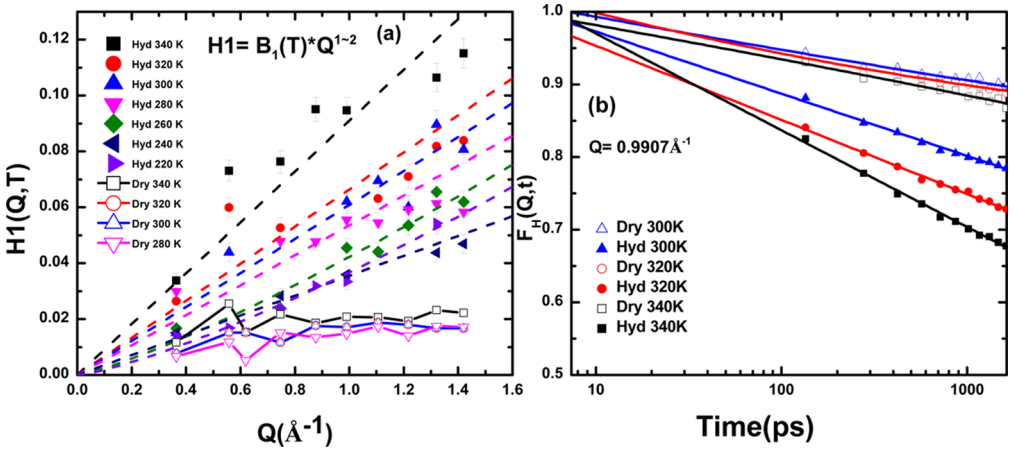


Figure 6. (a) Analysis of the fitting parameter $H_1(Q,T)$ of both samples as a function of Q at different temperatures. For the hydrated sample (solid symbols), $H_1(Q,T)$ is fitted by a power law, $H_1(Q,T) = B_1(T)Q^{\beta}$, at temperatures T = 220, 240, 260, 280, 300, 320, 340 K. For the dry β-CN (open symbols), $H_1(Q,T)$ does not show much temperature or Q dependence. (b) Comparison of the ISF(Q,t) of hydrated and dry β-CN at three different temperatures, T = 300, 320, 340 K. The open symbols represent data belongs to dry β-CN, while solid symbols belong to hydrated β-CN. The error bars of the data points are within the symbols if not shown.

indicating that when hydrated, more hydrogen atoms are observable within our measurement dynamic window. Moreover, the decrease in p_0 value with an increase in temperature indicates that more hydrogen atoms are participating in motion as the temperature goes up. The parameter a, representing the radius of the diffusive sphere, is shown in Table 1 for both samples. It is clear that for hydrated sample with increasing temperature, both the fraction of mobile atoms (p_1) and the radius of sphere (a) increase significantly, indicating that more hydrogen atoms undergo diffusive motions with larger amplitudes. Such temperature dependence of parameters is not clearly evident for the dry sample. The stronger temperature dependence of the mobile fraction and radius of sphere in hydrated sample, together with the fact that the mobile fraction is much smaller in dry sample, clearly show that motions of hydrogen atoms in methyl groups and side chains together are highly hydration-dependent. Additionally, in Table 1, the sphere radius a has a value of ~3 Å for both dry and hydrated samples, which is much larger than the radius of a methyl group (~1 Å). Thus, the measured dynamics in β-casein demonstrates a primarily slow relaxation process. Considering that the bovine β-casein lacks tertiary structure and its primary structure suggests amphiphilic nature,[31] our results reveal that the increase in hydration of β-casein leads to an increase in the flexible regions in the protein, which is crucial for β-caseins to form micellelike structures.

Interestingly, when temperature goes even higher, say to 320 and 340 K, a decrease in volume of diffusion is observed (decrease in the radius a). It might be due to the partially thermal unfolding (or so-called thermal denaturation) of the proteins at these higher temperatures.[59,60] The partially thermal unfolding is suggested to correlate with the relaxation in the hydrogen bond network formed between the protein and its hydration water.[34,49,59,61] Upon thermal unfolding, the hydro-

dx.doi.org/10.1021/jp504548w | J. Phys. Chem. B XXXX, XXX, XXX−XXX

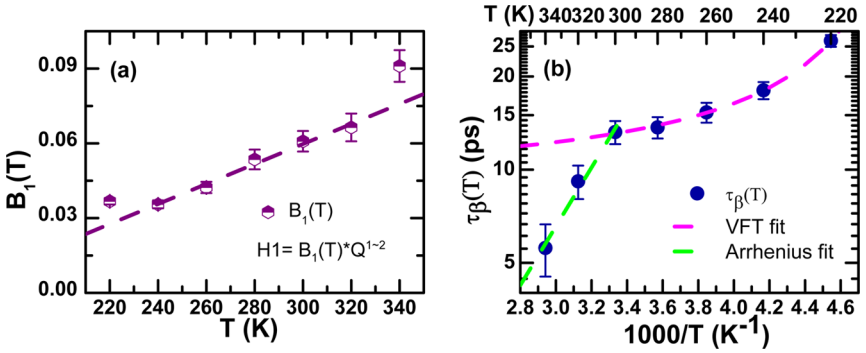


Figure 7. (a) Linear fitting of the parameter $B_1(T)$ as a function of temperature for hydrated β-CN. (b) Arrhenius plot of the relaxation time $\tau_\beta(T)$ as a function of inverse of temperature on a log scale.

gen bond network collapses and the flexibility of the protein is reduced. The number of hydrogen atoms with diffusive motion (p_1) increases markedly, given that more hydrogen atoms are able to diffuse than in the native state, while the radius of the sphere in which the hydrogen atoms move is reduced, because of less flexibility in the protein molecule. These results are consistent with the previous observation of thermal denaturation (heat-induced unfolding) of protein in solution[59] and suggest the thermal unfolding of hydrated β-casein at 320 and 340 K.

3. Time Domain Analysis. The ISF $F_H(Q,t)$, referred to as the density−density correlation function, is a primary tool to unravel the relaxational dynamics in the protein molecules. In this paper, the ISFs of both samples are analyzed by an asymptotic expression derived from the MCT (eq 6). The MCT is a theory originally developed to describe glass-forming liquids.[37,38] Here it is employed to describe the dynamics of proteins because proteins and glass-forming liquids share common dynamical behaviors in many aspects.[28,42,62−66] The MCT has predicted a logarithmic decay in the β-relaxation range of protein dynamics and has been proven to be efficient in fitting protein dynamics in time domain both by molecular dynamics (MD) simulations[29] and experiments.[30,33] In Figure 5, the ISF of the hydrated β-CN is analyzed according to eq 6 at 6 temperatures and 10 Q-values. Four fitting parameters, $A(T)$, $\tau_\beta(T)$, $H_1(Q,T)$, and $H_2(Q,T)$, are obtained by fitting curves at all 10 Q-values together, where $A(T)$ and $\tau_\beta(T)$ are Q-independent parameters and $H_1(Q,T)$ and $H_2(Q,T)$ Q-dependent parameters. The fitting results show that the value of $A(T)$ is very close to 0. This is because the prefactor $f(Q,T)$ goes to 1 at all Q-values at a specific short time $\tau_\beta(T) \sim 10$ ps, which is much shorter than our measured time range (above 100 ps).

Figure 6a compares the Q-dependent parameter $H_1(Q,T)$ of both dry and hydrated β-CN. $H_1(Q,T)$ represents quantitatively the slope of the decay, which can be observed qualitatively in Figures 5 and 6b. For the dry sample, $H_1(Q,T)$ hardly shows any Q or T dependence, due to the lack of hydrogen motions detected in our measurement time window. For the hydrated sample, $H_1(Q,T)$ increases with Q and follows a power law in Q at small Q-values; i.e., $H_1(Q,T) = B_1(T)Q^\beta$, where β is a value between 1 and 2 and $B_1(T)$ is a temperature-dependent parameter, which is plotted in Figure 7a. Figure 6b shows the comparison of relaxational dynamics of hydrated and dry β-CN at $Q = 0.99$ Å^{-1} at three different temperatures in the time domain. It is clearly shown that the slope of the decay $H_1(Q,T)$ of the dry sample is much smaller than that of the hydrated sample, which means that the relaxational dynamics of dry β-CN is much slower than that of the hydrated one and is much less Q- or temperature-dependent. This provides another evidence that the dynamics of β-CN is highly hydration-dependent and solvent plays an important role in the activation of proteins.[24]

The temperature dependent $B_1(T)$ is fitted linearly in Figure 7a. The characteristic relaxation time $\tau_\beta(T)$ is plotted versus $1000/T$ (the so-called Arrhenius plot) in Figure 7b. Below 300 K, $\tau_\beta(T)$ can be fitted with the Vogel−Fulcher−Tammann (VFT) law, $\tau_\beta(T) = \tau_1(T)\exp[DT_0/(T - T_0)]$, where D is a dimensionless parameter providing measurement of fragility and T_0 is the ideal glass transition temperature. According to the glass transition theory, the VFT behavior typically represents the α-process. Such apparent non-Arrhenius behavior of relaxation time is a common feature in the dynamics of biomolecules and has been observed in many previous works.[31,33,53,67] Above $T \sim 300$ K, the temperature dependence of $\tau_\beta(T)$ does not follow the VFT law anymore. It switches to an Arrhenius-like behavior. Since it is hard to determine whether this behavior is an Arrhenius behavior due to limited data points (three points here), we use a linear temperature dependence to fit the data points as a guide to the eyes (the green dashed line in Figure 7b). Notice that this switch in $\tau_\beta(T)$ at 300 K is consistent with our analysis in energy domain shown in Figure 4 and Table 1. This high-temperature transition in hydrated β-casein is possibly related to the reversible thermal denaturation or thermal unfolding of the protein, which has been observed in hydrated lysozyme and its hydration water in previous experiments[31,34] and has also been observed in hydrated tRNA.[33] It is highly hydration-dependent and is suggested to correlate with the relaxation in the hydrogen bond network formed between protein and hydration water.[34,49,61] The lower transition temperature in β-casein (300 K) comparing to other biomolecules (320 K for tRNA[33] and 345 K for lysozyme[34]) indicates that the lack of secondary structure will result in thermal unfolding or thermal denaturation of β-casein at relatively lower temperatures compare to other well-folded proteins and rigid biomolecules. This observation confirms that natively disordered β-casein has relatively higher structural flexibility compare to well-folded proteins.

CONCLUSION

In summary, the relaxational dynamics of a natively disordered protein β-casein has been studied by quasielastic neutron scattering (QENS) both in energy and time domain to study

dx.doi.org/10.1021/jp504548w | J. Phys. Chem. B XXXX, XXX, XXX−XXX

the effect of hydration and temperature. The temperature-dependent mean square displacement (MSD) of hydrogen atoms within β-casein molecules demonstrates two onsets (~100 and ~225 K) inflected in its slope in the hydrated sample, while only one onset (~100 K) is observed in the dry sample. These results indicate that the activation of natively disordered β-casein relies on both methyl group rotations, which is independent of hydration, and the backbone fluctuations and activation of hydrophilic side chains, which are highly hydration-dependent. This is consistent with the previous literature that β-casein has higher flexibility in its dynamics compared to other well-folded proteins.[8]

From the analysis of the EISF in the energy domain, it is shown that for hydrated β-casein, as temperature increases, both the of mobile fraction of hydrogen atoms and the radius of the free diffusive sphere increase significantly. However, such temperature dependence of parameters is not evident for the dry sample. This indicates that such motion in hydrogen atoms is highly hydration-dependent. At even higher temperatures of 320 and 340 K, a partial unfolding of hydrated β-CN causes a decrease in volume of diffusion.

In time domain, we found that the relaxational dynamics of dry β-CN is much slower than that of the hydrated one and is much less Q- or temperature-dependent. This provides another evidence that solvent plays an important role in the activation of proteins.[24] The intermediate scattering functions (ISF) of the hydrated β-CN demonstrate a logarithmic-like decay, which is predicted by mode coupling theory (MCT) and has been observed in well-folded proteins[30,31] and other biopolymers, such as tRNA.[33] The high-temperature transition in the characteristic β-relaxation time $\tau_\beta(T)$ reveals that the lack of secondary structure in β-CN will result in easier unfolding at lower temperatures compare to other more rigid biomolecules.

AUTHOR INFORMATION

Corresponding Author

*E-mail: chux@wayne.edu.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was funded and supported by Wayne State University. We acknowledge the support of the National Institute of Standards and Technology, U.S. Department of Commerce, in providing some of the neutron research facilities used in this work. This work utilized facilities supported in part by the National Science Foundation under Agreement No. DMR-0944772. Identification of commercial products does not imply endorsement by the National Institute of Standards and Technology nor does it imply that these are the best for the purpose.

REFERENCES

(1) Fox, P. Milk Proteins: General and Historical Aspects. *Advanced Dairy Chemistry—Volume 1 Proteins*; Springer: New York, 2003; pp 1–48.

(2) Swaisgood, H.; Fox, P. Chemistry of the Caseins. *Advanced Dairy Chemistry—Volume 1: Proteins*, 2nd ed.; Springer: New York, 1992; pp 63–110

(3) Dickinson, E.; Semenova, M. G.; Belyakova, L. E.; Antipova, A. S.; Tsapkina, E. N.; Ritzoulis, C. Analysis of Light Scattering Data on the Calcium Ion Sensitivity of Caseinate Solution Thermodynamics: Relationship to Emulsion Flocculation. *J. Colloid Interface Sci.* **2001**, *239* (1), 87–97.

(4) Bourassa, P.; N'soukpoe-Kossi, C.; Tajmir-Riahi, H. Binding of Vitamin A with Milk α- and β-Caseins. *Food Chem.* **2012**, *138*, 444–453.

(5) Shapira, A.; Assaraf, Y. G.; Livney, Y. D. Beta-Casein Nanovehicles for Oral Delivery of Chemotherapeutic Drugs. *Nanomed.: Nanotechnol., Biol. Med.* **2010**, *6* (1), 119–126.

(6) Shapira, A.; Assaraf, Y. G.; Epstein, D.; Livney, Y. D. Beta-Casein Nanoparticles as an Oral Delivery System for Chemotherapeutic Drugs: Impact of Drug Structure and Properties on Co-Assembly. *Pharm. Res.* **2010**, *27* (10), 2175–2186.

(7) Bachar, M.; Mandelbaum, A.; Portnaya, I.; Perlstein, H.; Even-Chen, S.; Barenholz, Y.; Danino, D. Development and Characterization of a Novel Drug Nanocarrier for Oral Delivery, Based on Self-Assembled β-Casein Micelles. *J. Controlled Release* **2012**, *160* (2), 164–171.

(8) 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.

(9) Dalgleish, D. G. On the Structural Models of Bovine Casein Micelles—Review and Possible Improvements. *Soft Matter* **2011**, *7* (6), 2265–2272.

(10) Cunsolo, A.; Kodituwakku, C. N.; Bencivenga, F.; Frontzek, M.; Leu, B. M.; Said, A. H. Transverse Dynamics of Water across the Melting Point: A Parallel Neutron and X-ray Inelastic Scattering Study. *Phys. Rev. B* **2012**, *85* (17).

(11) Bouchoux, A.; Gésan-Guiziou, G.; Pérez, J.; Cabane, B. How To Squeeze a Sponge: Casein Micelles under Osmotic Stress, a SAXS Study. *Biophys. J.* **2010**, *99* (11), 3754–3762.

(12) Mcmahon, D. J.; Oommen, B. Supramolecular Structure of the Casein Micelle. *J. Dairy Sci.* **2008**, *91* (5), 1709–1721.

(13) Uversky, V. N. What Does It Mean To Be Natively Unfolded? *Eur. J. Biochem.* **2002**, *269* (1), 2–12.

(14) Zaccai, G. Biochemistry—How Soft Is a Protein? A Protein Dynamics Force Constant Measured by Neutron Scattering. *Science* **2000**, *288* (5471), 1604–1607.

(15) Fenimore, P. W.; Frauenfelder, H.; Magazù, S.; Mcmahon, B. H.; Mezei, F.; Migliardo, F.; Young, R. D.; Stroe, I. Concepts and Problems in Protein Dynamics. *Chem. Phys.* **2013**, *424*, 2–6.

(16) 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* (7), 2573–2588.

(17) Caliskan, G.; Kisliuk, A.; Sokolov, A. P. Dynamic Transition in Lysozyme: Role of a Solvent. *J. Non-Cryst. Solids* **2002**, *307*, 868–873.

(18) Fenimore, P. W.; Frauenfelder, H.; Mcmahon, B. H.; Parak, F. G. Slaving: Solvent Fluctuations Dominate Protein Dynamics and Functions. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99* (25), 16047–16051.

(19) Chu, X. Q.; Faraone, A.; Kim, C.; Fratini, E.; Baglioni, P.; Leao, J. B.; Chen, S. H. Proteins Remain Soft at Lower Temperatures under Pressure. *J. Phys. Chem. B* **2009**, *113* (15), 5001–5006.

(20) Cardoso, M. B.; Smolensky, D.; Heller, W. T.; O'Neill, H. Insight into the Structure of Light-Harvesting Complex II and Its Stabilization in Detergent Solution. *J. Phys. Chem. B* **2009**, *113* (51), 16377–16383.

(21) Zanotti, J.-M.; Bellissent-Funel, M.-C.; Parello, J. Hydration-Coupled Dynamics in Proteins Studied by Neutron Scattering and NMR: The Case of the Typical EF-Hand Calcium-Binding Parvalbumin. *Biophys. J.* **1999**, *76* (5), 2390–2411.

(22) Frauenfelder, H.; Fenimore, P. W.; Chen, G.; Mcmahon, B. H. Protein Folding Is Slaved to Solvent Motions. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103*, 15469–15472.

(23) Fenimore, P. W.; Frauenfelder, H.; Mcmahon, B.; Young, R. Bulk-Solvent and Hydration-Shell Fluctuations, Similar to α-and β-Fluctuations in Glasses, Control Protein Motions and Functions. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101* (40), 14408–14413.

(24) Frauenfelder, H.; Chen, G.; Berendzen, J.; Fenimore, P. W.; Jansson, H.; Mcmahon, B. H.; Stroe, I. R.; Swenson, J.; Young, R. D. A

Unified Model of Protein Dynamics. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106* (13), 5129−5134.
(25) Nickels, J. D.; O'Neill, H.; Hong, L.; Tyagi, M.; Ehlers, G.; Weiss, K. L.; Zhang, Q.; Yi, Z.; Mamontov, E.; Smith, J. C. Dynamics of Protein and Its Hydration Water: Neutron Scattering Studies on Fully Deuterated GFP. *Biophys. J.* **2012**, *103* (7), 1566−1575.
(26) Schiró, G.; Caronna, C.; Natali, F.; Cupane, A. Molecular Origin and Hydration Dependence of Protein Anharmonicity: An Elastic Neutron Scattering Study. *Phys. Chem. Chem. Phys.* **2010**, *12* (35), 10215−10220.
(27) Gabel, F.; Bicout, D.; Lehnert, U.; Tehei, M.; Weik, M.; Zaccai, G. Protein Dynamics Studied by Neutron Scattering. *Q. Rev. Biophys.* **2002**, *35* (4), 327−367.
(28) 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* (8), 1080−1083.
(29) Lagi, M.; Baglioni, P.; Chen, S. H. Logarithmic Decay in Single-Particle Relaxation of Hydrated Lysozyme Powder. *Phys. Rev. Lett.* **2009**, *103* (10), 108102.
(30) Chu, X.-Q.; Lagi, M.; Mamontov, E.; Fratini, E.; Baglioni, P.; Chen, S.-H. Experimental Evidence of Logarithmic Relaxation in Single-Particle Dynamics of Hydrated Protein Molecules. *Soft Matter* **2010**, *6* (12), 2623−2627.
(31) Chu, X. Q.; Gajapathy, M.; Weiss, K. L.; Mamontov, E.; Ng, J. D.; Coates, L. Dynamic Behavior of Oligomeric Inorganic Pyrophosphatase Explored by Quasielastic Neutron Scattering. *J. Phys. Chem. B* **2012**, *116* (33), 9917−9921.
(32) Mikheeva, L. M.; Grinberg, N. V.; Grinberg, V. Y.; Khokhlov, A. R.; De Kruif, C. G. Thermodynamics of Micellization of Bovine *β*-Casein Studied by High-Sensitivity Differential Scanning Calorimetry. *Langmuir* **2003**, *19* (7), 2913−2921.
(33) Chu, X. Q.; Mamontov, E.; O'Neill, H.; Zhang, Q. Temperature Dependence of Logarithmic-Like Relaxational Dynamics of Hydrated tRNA. *J. Phys. Chem. Lett.* **2013**, *4* (6), 936−942.
(34) Zhang, Y.; Lagi, M.; Liu, D. Z.; Mallamace, F.; Fratini, E.; Baglioni, P.; Mamontov, E.; Hagen, M.; Chen, S. H. Observation of High-Temperature Dynamic Crossover in Protein Hydration Water and Its Relation to Reversible Denaturation of Lysozyme. *J. Chem. Phys.* **2009**, *130* (13), 135101.
(35) Rupley, J. A.; Careri, G. Protein Hydration and Function. *Adv. Protein Chem.* **1991**, *41*, 37.
(36) 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* (5), 2759−2777.
(37) Azuah, R. T.; Kneller, L. R.; Qiu, Y.; Tregenna-Piggott, P. L.; Brown, C. M.; Copley, J. R.; 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* (6), 341−358.
(38) Sciortino, F.; Tartaglia, P.; Zaccarelli, E. Evidence of a Higher-Order Singularity in Dense Short-Ranged Attractive Colloids. *Phys. Rev. Lett.* **2003**, *91* (26), 268301.
(39) Götze, W.; Sperl, M. Logarithmic Relaxation in Glass-Forming Systems. *Phys. Rev. E* **2002**, *66* (1), 011405.
(40) Reat, V.; Dunn, R.; Ferrand, M.; Finney, J. L.; Daniel, P. M.; Smith, J. C. Solvent Dependence of Dynamic Transitions in Protein Solutions. *Proc. Natl. Acad. Sci. U. S. A.* **2000**, *97* (18), 9961−9966.
(41) Paciaroni, A.; Cinelli, S.; Onori, G. Effect of the Environment on the Protein Dynamical Transition: A Neutron Scattering Study. *Biophys. J.* **2002**, *83* (2), 1157−1164.
(42) Doster, W.; Cusack, S.; Petry, W. Dynamical Transition of Myoglobin Revealed by Inelastic Neutron-Scattering. *Nature* **1989**, *337* (6209), 754−756.
(43) Roh, J.; Novikov, V.; Gregory, R.; Curtis, J.; Chowdhuri, Z.; Sokolov, A. Onsets of Anharmonicity in Protein Dynamics. *Phys. Rev. Lett.* **2005**, *95* (3), 038101.
(44) Wood, K.; Tobias, D. J.; Kessler, B.; Gabel, F.; Oesterhelt, D.; Mulder, F. A.; Zaccai, G.; Weik, M. The Low-Temperature Inflection Observed in Neutron Scattering Measurements of Proteins Is Due to Methyl Rotation: Direct Evidence Using Isotope Labeling and Molecular Dynamics Simulations. *J. Am. Chem. Soc.* **2010**, *132* (14), 4990−4991.
(45) Doster, W.; Settles, M. Protein−Water Displacement Distributions. *Biochim. Biophys. Acta, Proteins Proteomics* **2005**, *1749* (2), 173−186.
(46) Telling, M. T.; Howells, S.; Combet, J.; Clifton, L.; Sakai, V. G. Mean Squared Displacement Analysis of An-Harmonic Behaviour in Lyophilised Proteins. *Chem. Phys.* **2013**, *424*, 32−36.
(47) Frontzek, A. V.; Strokov, S. V.; Embs, J. P.; Lushnikov, S. G. Does a Dry Protein Undergo a Glass Transition? *J. Phys. Chem. B* **2014**, *118* (11), 2796−2802.
(48) 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* (4), 1371−1376.
(49) Miao, Y.; Yi, Z.; Glass, D. C.; Hong, L.; Tyagi, M.; Baudry, J.; Jain, N.; Smith, J. C. Temperature-Dependent Dynamical Transitions of Different Classes of Amino Acid Residue in a Globular Protein. *J. Am. Chem. Soc.* **2012**, *134* (48), 19576−19579.
(50) Tournier, A. L.; Xu, J.; Smith, J. C. Translational Hydration Water Dynamics Drives the Protein Glass Transition. *Biophys. J.* **2003**, *85* (3), 1871−1875.
(51) Magazu, S.; Migliardo, F.; Benedetoo, 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*, 9268−9274.
(52) Capaccioli, S.; Ngai, K. L.; Ancherbak, S.; Paciaroni, A. Evidence of Coexistence of Change of Caged Dynamics at T-G and the Dynamic Transition at T-D in Solvated Proteins. *J. Phys. Chem. B* **2012**, *116* (6), 1745−1757.
(53) Magazu, S.; Migliardo, F.; Benedetto, A. Puzzle of Protein Dynamical Transition. *J. Phys. Chem. B* **2011**, *115* (24), 7736−7743.
(54) Nickels, J. D.; García Sakai, V.; Sokolov, A. P. Dynamics in Protein Powders on the Nanosecond−Picosecond Time Scale Are Dominated by Localized Motions. *J. Phys. Chem. B* **2013**, *117* (39), 11548−11555.
(55) Fujiwara, S.; Plazanet, M.; Matsumoto, F.; Oda, T. Internal Motions of Actin Characterized by Quasielastic Neutron Scattering. *Eur. Biophys. J. Biophys. Lett.* **2011**, *40* (5), 661−671.
(56) Volino, F.; Dianoux, A. Neutron Incoherent Scattering Law for Diffusion in a Potential of Spherical Symmetry: General Formalism and Application to Diffusion inside a Sphere. *Mol. Phys.* **1980**, *41* (2), 271−279.
(57) Bee, M. *Quasielastic Neutron Scattering*; Adam Hilger: Philadelphia, PA, 1988.
(58) 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* (15), 7600−7605.
(59) Russo, D.; Perez, J.; Zanotti, J. M.; Desmadril, M.; Durand, D. Dynamic Transition Associated with the Thermal Denaturation of a Small Beta Protein. *Biophys. J.* **2002**, *83* (5), 2792−2800.
(60) Liu, D. Z.; Weeks, M. G.; Dunstan, D. E.; Martin, G. J. Temperature-Dependent Dynamics of Bovine Casein Micelles in the Range 10−40° C. *Food Chem.* **2013**, *141* (4), 4081−4086.
(61) Wood, K.; Frolich, A.; Paciaroni, A.; Moulin, M.; Hartlein, M.; Zaccai, G.; Tobias, D. J.; Weik, M. Coincidence of Dynamical Transitions in a Soluble Protein and Its Hydration Water: Direct Measurements by Neutron Scattering and MD Simulations. *J. Am. Chem. Soc.* **2008**, *130* (14), 4586−4587.
(62) Angell, C. A.; Ngai, K. L.; Mckenna, G. B.; Mcmillan, P. F.; Martin, S. W. Relaxation in Glassforming Liquids and Amorphous Solids. *J. Appl. Phys.* **2000**, *88* (6), 3113.
(63) Rasmussen, B. F.; Stock, A. M.; Ringe, D.; Petsko, G. A. Crystalline Ribonuclease-A Loses Function below the Dynamic Transition at 220 K. *Nature* **1992**, *357* (6377), 423−424.

(64) Leyser, H.; Doster, W.; Diehl, M. Far-Infrared Emission by Boson Peak Vibrations in a Globular Protein. *Phys. Rev. Lett.* **1999**, *82* (14), 2987−2990.
(65) Green, J. L.; Fan, J.; Angell, C. A. The Protein-Class Analogy—Some Insights from Homopeptide Comparisons. *J. Phys. Chem.* **1994**, *98* (51), 13780−13790.
(66) Etchegoin, P. Glassylike Low-Frequency Dynamics of Globular Proteins. *Phys. Rev. E* **1998**, *58* (1), 845−848.
(67) Khodadadi, S.; Pawlus, S.; Roh, J.; Garcia Sakai, V.; Mamontov, E.; Sokolov, A. The Origin of the Dynamic Transition in Proteins. *J. Chem. Phys.* **2008**, *128* (19), 195106.