Dynamics and Rigidity in an Intrinsically Disordered Protein, β-Casein

ARTICLE *in* THE JOURNAL OF PHYSICAL CHEMISTRY B · JUNE 2014

Impact Factor: 3.3 · DOI: 10.1021/jp503788r · Source: PubMed

5 AUTHORS, INCLUDING:

Stefania Perticaroli
Oak Ridge National Laboratory
29 PUBLICATIONS **280** CITATIONS
SEE PROFILE

Jonathan D Nickels
Oak Ridge National Laboratory
44 PUBLICATIONS **548** CITATIONS
SEE PROFILE

THE JOURNAL OF PHYSICAL CHEMISTRY B

Article

pubs.acs.org/JPCB

Dynamics and Rigidity in an Intrinsically Disordered Protein, β-Casein

Stefania Perticaroli,*[,†,‡,§,#] Jonathan D. Nickels,*[,†,§,#] Georg Ehlers,[∥] Eugene Mamontov,[⊥] and Alexei P. Sokolov[†,‡,§]

[†]Joint Institute for Neutron Sciences, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831, United States

[‡]Chemical and Materials Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831, United States

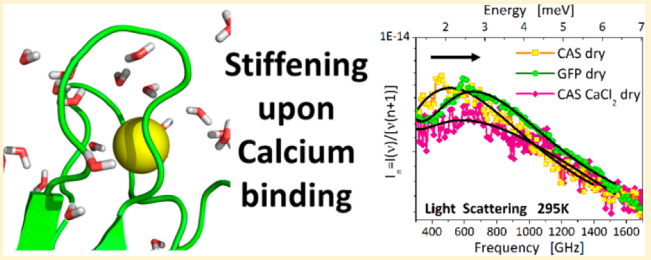
[§]Department of Chemistry, University of Tennessee, Knoxville, Tennessee 37996-1600, United States

[∥]Quantum Condensed Matter Division, Oak Ridge National Laboratory, P.O. Box 2008, Oak Ridge, Tennessee 37831, United States

[⊥]Chemical and Engineering Materials Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831, United States

Supporting Information

ABSTRACT: The emergence of intrinsically disordered proteins (IDPs) as a recognized structural class has forced the community to confront a new paradigm of structure, dynamics, and mechanical properties for proteins. We present novel data on the similarities and differences in the dynamics and nanomechanical properties of IDPs and other biomacromolecules on the picosecond time scale. An IDP, β-casein (CAS), has been studied in a calcium bound and unbound state using neutron and light scattering techniques. We show that CAS partially folds and stiffens upon calcium binding, but in the unfolded state, it is softer than folded proteins such as green fluorescence protein (GFP). We also see that some localized diffusive motions in CAS have a larger amplitude than in GFP at this time scale but are still smaller than those observed in tRNA. In spite of these differences, CAS dynamics are consistent with the classes of motions seen in folded protein on this time scale.



INTRODUCTION

Understanding the relationships between protein structure, dynamics, and function is one of the fundamental questions in molecular biophysics. Protein function is classically thought to be determined by its three-dimensional structure[1,2] and thus by the specific amino acid sequence. However, there are proteins, called intrinsically disordered proteins (IDPs), which are characterized by unstructured regions of significant size (>50 residues) and are nevertheless biologically active. These IDPs carry out functional roles in crucial areas, such as transcriptional regulation, translation, cellular signal transduction, and storage of small molecules.[3,4] Some of the sequence peculiarities of these proteins are now widely recognized. One of them is the presence of numerous uncompensated charged groups (often negative), which contributes to the lack of a compact and closely packed structure.[2] Many IDPs are also known to become more ordered or fold into stable secondary or tertiary structures upon binding to their targets, undergoing coupled folding and binding processes.[4] β-Casein (CAS), for instance, is a flexible negatively charged unfolded protein in its functional state, which binds and transports Ca^{2+} ions in the form of protein-bound nanoclusters.[5−7] The presence of such extended disordered regions often comes with a biological cost, as IDPs are increasingly being associated with human diseases. Indeed, disordered regions are sometimes the site of chromosomal translocations, such as in human leukemias,[8] and can also be related to protein folding diseases (Parkinson's, Huntington's, and polyglutamine diseases).[9,10] Finally, human proteins containing large sequences of extremely low complexity, which are commonly constituted by Ser, Gln, or acidic residues, have been connected to neurological diseases and cancer.[11]

Specific software and data sets have currently been developed to identify unfolded regions of proteins from the primary amino-acid sequence,[12] and advances in functional genomics are contributing to an increased understanding of the functions of disordered regions.[4] However, the dynamics and the polypeptide mobility of such IDPs are still unclear and poorly explored.[13,14] Moving beyond a static picture to a dynamic understanding of proteins is of crucial importance. This characterization of protein dynamics represents a stimulating challenge, since these processes are influenced by a variety of factors, such as protein chemical composition, folding conformations, packing conditions, electrostatic environment, and hydration water interactions.

The way that these factors combine to influence internal dynamics is still not completely understood. These factors are also important parameters in the structure and mechanical properties of these proteins. It is known that globular proteins with different secondary structures and rigidity properties, such as lysozyme, myoglobin, and GFP, show similar kinds of

Received: April 17, 2014
Revised: June 10, 2014

   dx.doi.org/10.1021/jp503788r | J. Phys. Chem. B XXXX, XXX, XXX−XXX

dynamics in the picosecond−nanosecond time scales.[15] However, the role played by disordered regions and unfolded portions on rigidity and dynamics of proteins still needs to be unraveled.

Single molecule experiments, conducted on aqueous solutions of short unstructured proteins and model peptides,[16,17] are among the few studies that examine flexibility and dynamics of IDPs. In these articles, global reconfiguration and folding of the peptide chains, which occur from tens to hundreds of nanoseconds, were characterized through the identification of intrachain contacts and diffusion constants. Wright and co-workers have also applied NMR spectroscopy and spectral density mapping to study partly folded apomyoglobin in aqueous solution.[18,19] These authors recognized different structural regions in the protein: a compact core made of α-helices, whose packing leads to a decrease in backbone flexibility, and largely disordered portions showing a high degree of mobility on a fast sub-nanosecond time scale. However, these methods do not provide a physical description of the motions involved[20] and do not allow the separation of distinct dynamical processes. Moreover, dynamics that are sensitive to the rigidity of the system and relaxation processes for which the secondary structure of the protein plays a relevant role occur also at faster time scales than those probed by single molecule and NMR techniques, extending from nanosecond to fractions of picosecond.[13,21,22] Neutron scattering spectroscopy on the other hand is well suited to explore biomolecule dynamics at these time scales. Gaspar et al. applied neutron spectroscopy to investigate relaxation processes of unfolded caseins and globular proteins,[13] and found that in aqueous solutions the unstructured proteins have the most flexible structures, followed by proteins made of α-helices and then by proteins composed mainly of β-sheets, in agreement with results obtained on protein powders.[23,24] However, their analysis was limited to the time window faster than 20 ps and was strongly affected by long-range (global) diffusion of the entire protein molecules in solution.

A controversial point which is still under debate concerns the relative flexibility of folded and unfolded proteins in the form of hydrated powders. This topic has been examined previously based on a comparison of tau protein and maltose binding protein.[14] In this study, they showed an increase in the sub-nanosecond mean-square displacement (MSD) in the IDP tau compared to the well folded maltose binding protein at room temperature. On the other hand, hydrated powders of folded wild-type staphylococcal nuclease and its partially unfolded mutant present similar mobility.[25] Close values of MSD have also been observed for native and alkali-denatured lysozyme[26] in similar time scales. It is now well recognized that an approach based only on the analysis of MSD presents some serious limitations and that the analysis of energy-resolved spectra, with their Q and temperature dependence, is considerably more informative. In particular, an exclusive study of elastic neutron scattering data to compare the rigidity of different folded/unfolded structures could be misleading. MSD is an integrated quantity that includes all kinds of dynamics (vibrations, rotations, conformational changes, and diffusion), and its analysis does not provide a description of the motions involved. Moreover, according to MD simulations,[21] only a fraction of the dynamics occurring in the nano- to picosecond time scales are sensitive to rigidity. It should also be noted that in the nanosecond time scale used in the mentioned studies a significant contribution to the MSD of protein powders comes from methyl group rotations, and it masks changes in MSD that may result from unfolding.[26] We also remark that the degree of folding and exposure to water can vary significantly among different IDPs and proteins of varied unfolded content.[4] Consequently, the dynamical and mechanical properties of such proteins, as well as the coupling between protein and hydration water motions,[14] can change as a function of the compactness of the conformation.

In order to overcome such problems and compare rigidity and dynamics of unstructured and well-folded proteins, we studied CAS and green fluorescent protein (GFP) in the form of powders, so that global motions (diffusion and rotation of the entire molecule) are completely suppressed on the accessible time scale. It is known that proteins' rigidity properties are correlated to their biological function. In particular, the stiff 11-stranded β-barrel, that constitutes GFP, shields and protects the internal chromophore and enables the proton transfer that gives rise to fluorescence.[27] On the other hand, β-casein is an unfolded protein whose ability to bind multiple targets requires high flexibility.[28] Moreover, we note that these two proteins are very similar in size (molecular weights of GFP and CAS are 26.8 and 25 kDa, respectively) and the difference in dynamics should reflect their different structure.

Our approach consists of extending the detailed knowledge of the dynamics of globular folded proteins[15,23,29,30] to the study of unfolded secondary structures. We use neutron and light scattering techniques to compare the picosecond dynamics of dry and hydrated powders of folded GFP to those of natively unfolded CAS. We also analyzed β-casein samples containing $CaCl_2$ in order to monitor the effects of ion-binding and partial folding on the rigidity and dynamics of a natively unfolded protein. Our analysis explores in detail the low-frequency collective vibrations, known as the boson peak (BP). This feature occurs on the sub-picosecond time scale (∼2−4 meV; ∼0.5−1 THz; ∼0.16−0.33 ps) and appears in both neutron and light scattering spectra of all proteins, both at cryogenic and room temperature, as well as in dry and hydrated state.[31] The BP involves the entire protein, including backbone and amino-acid side chains and hydrophilic and hydrophobic components,[30,32,33] and represents a convenient tool to provide information about the local rigidity of proteins at a few nanometer length scale.[23]

The goal of the present work is to provide new insights into some open questions which are largely relevant to both biophysical and biomedical communities: (i) What is the relative rigidity of folded globular proteins and IDPs? (ii) What are the characteristic dynamics of unfolded proteins? (iii) How does folding influence the rigidity and dynamics of IDPs? (iv) What are the controlling factors and interactions involved?

MATERIALS AND METHODS

β-Casein from bovine milk and $CaCl_2$ were purchased from Sigma-Aldrich. Green fluorescent protein was provided by the Biodeuteration Lab of Oak Ridge National Laboratory.[34] Proteins were equilibrated in D_2O in order to replace all exchangeable hydrogen atoms by deuterium. β-Casein/$CaCl_2$ samples were obtained by adding 60 mg of $CaCl_2$ to D_2O solutions containing 100 mg of β-casein. This salt concentration (∼6.75 mM), chosen in the range explored by previous studies,[35] guarantees an excess of Ca^{2+} relative to the amount that can be captured by casein. It is known that in these solutions, both with or without calcium, CAS exists in the form

dx.doi.org/10.1021/jp503788r | J. Phys. Chem. B XXXX, XXX, XXX−XXX

of micelles.[35] All the samples were then lyophilized before use; upon freeze-drying of the solution, the Ca^{2+} in excess reforms the salt. Part of these powders was directly used as dry samples. The other portion was hydrated to a ratio, h, of 0.4 (h = mass of D_2O/mass of protein) by placing the powder in a closed hydration chamber. The secondary structure of all the proteins was checked through infrared spectroscopy in the frequency range of amides I and II (1500−1750 cm^{-1}). These measurements were carried out at room temperature using a JASCO FT/IR-6100 spectrometer equipped with an ATR-PRO 450-S device. The sample environment of IR measurements was open to the atmosphere. Each spectrum was measured at room temperature and with a resolution of 1 cm^{-1} in the 400−4000 cm^{-1} wavenumber range. The measurements were repeated three times in order to test the reproducibility of the results. After subtraction of a linear baseline, the spectra of casein samples were normalized to the integrated intensity in the 900−1770 cm^{-1} frequency range. Additional information can be found in the Supporting Information.

Neutron scattering experiments were performed at the Oak Ridge National Laboratory (ORNL) Spallation Neutron Source (SNS), at the Backscattering Spectrometer (BASIS),[36] and at the time-of-flight Cold Neutron Chopper Spectrometer (CNCS).[37] Elastic resolutions employed were 3.5 μeV (~300 ps) on BASIS and ~50 μeV (~20 ps) on CNCS; the accessible Q ranges were 0.2−2 and 0.2−4 $Å^{-1}$, respectively. This combination of spectrometers provides information over more than three orders in energy (and relaxation time) spanning from ~3.5 μeV up to ~20 meV (from hundreds of picoseconds to sub-picosecond). Neutron data on GFP were collected at 170 and 280 K, and CAS data, at 170 and 295 K.

Depolarized light scattering spectra were measured using a Sandercock type (3 + 3)-pass tandem Fabry−Pérot interferometer (laser light λ = 532 nm and three different mirror separations: 2, 1, and 0.4 mm) and a Jobin-Yvon T64000 triple monochromator (laser light λ = 647.1 nm), with 10−20 mW incident power on the sample. The frequency window covered in this case was ~24 GHz to 15 THz (~0.1−60 meV), and the temperatures investigated were 295 and 170 K. Polarized Brillouin light scattering spectra were also measured in order to calculate the Young's elastic modulus of dry CAS and GFP at 295 K. The procedure and the experimental conditions applied were the same as those described in ref 23; further details are reported in the Supporting Information.

Data Analysis. The formalism chosen to display neutron and light scattering spectra in the full frequency range is the imaginary part of the dynamic susceptibility, $\chi''(\nu)$. The neutron scattering dynamic susceptibility is related to the experimentally measured dynamic structure factor, $S(Q, \nu)$, in the energy gain side through the Bose occupation number $n_B(\nu) = [\exp(h\nu/kT) - 1]^{-1}$:[38]

$$\chi''_{NS}(\nu) \propto \frac{S(Q, \nu)}{n_B(\nu)} \tag{1}$$

In the case of light scattering experiments, the energy loss side of the spectra is measured; thus, the susceptibility is calculated according to the equation

$$\chi''_{LS}(\nu) \propto \frac{I(\nu)}{n_B(\nu) + 1} \tag{2}$$

where $I(\nu)$ is the measured intensity.

This representation is particularly convenient to visualize relaxation dynamics, since trivial temperature effects are taken into account and well-separated relaxation processes appear as separate peaks.

On the other hand, to present neutron and light scattering data in the boson peak region, we chose the spectral density formalism, I_n, since it enhances the quasielastic portion of the spectra and conveniently isolates the BP feature.

The spectral density of neutron spectra was calculated as

$$I_n^{NS}(Q, \nu) = \frac{S(Q, \nu)}{\nu \cdot n_B(\nu)} \tag{3}$$

whereas the light scattering spectral density was obtained using the equation[39]

$$I_n^{LS}(\nu) = \frac{I(\nu)}{\nu \cdot [n_B(\nu) + 1]} \tag{4}$$

To estimate the frequency of the boson peak, ν_{BP}, we fit the spectral densities $I_n(\nu)$, obtained from both neutron and depolarized light scattering experiments, according to the expression[23]

$$I_n(\nu) = \frac{A}{\nu^2 + \nu_0^2} + B \exp\left[-\frac{\left(\ln \frac{\nu}{\nu_{BP}}\right)^2}{2W^2}\right] + y_0 \tag{5}$$

where the first term represents the quasielastic scattering contribution approximated by a Lorentzian function with the width ν_0 and amplitude A and the second term describes the boson peak in terms of a log-normal function[40] with the frequency of the maximum ν_{BP}, width W, and amplitude B. The parameter y_0 = 0 in the case of neutron scattering data, and is left as a free fit parameter in light scattering spectra to account for a flat fluorescence background.[23]

Finally, to better compare GFP and CAS picosecond dynamics and to get some insights about the geometry of the motions and the fraction of atoms involved, we analyzed the elastic incoherent structure factor (EISF(Q)). EISF(Q) is defined as the fraction of neutrons scattered elastically by the protons diffusing in a confined geometry, relative to the total measured intensity:

$$\mathrm{EISF}(Q) = S_{EL}(Q, E \approx 0)/[S_{EL}(Q, E \approx 0) + S_{QE}(Q, E)] \tag{6}$$

where $S_{EL}(Q, E \approx 0)$ and $S_{QE}(Q, E)$ are the elastic and quasielastic intensities, respectively.

Specifically, the EISF(Q) values used in this work were extracted from the elastic scattering intensity of the spectra collected on the BASIS spectrometer.

The incoherent neutron scattering of protein samples can be interpreted as the time and space Fourier transform of the self-correlation function of hydrogen atoms in the biomolecule. $S(Q, E)$, for a given instrumental resolution $R(Q, E)$, can be written as a sum of processes contributing to the signal:[22]

$$S(Q, E, T) = \mathrm{DWF}(Q, T)[\sum_{i=1}^{n} P_i\{\mathrm{EISF}_i(Q)\delta(E) + [(1 - \mathrm{EISF}_i(Q))S_i(Q, E, T)]\} \otimes R(Q, E)] \tag{7}$$

dx.doi.org/10.1021/jp503788r | J. Phys. Chem. B XXXX, XXX, XXX−XXX

In this expression, P_i represents the fraction of hydrogen atoms participating in mode i in the energy (time) range accessible for the given spectrometer, $S_i(Q, E, T)$ is the spectrum of mode i at temperature T, DWF(T) is the Debye–Waller factor, and the delta function, $\delta(E)$, corresponds to the elastic part of the scattering function.

The fit of the Q dependence of the EISF(Q) can be used to obtain details about the geometry of the motions. In the case of methyl group rotations, the EISF(Q) can be modeled by the three-site jump model:

$$\mathrm{EISF_M}(Q) = 1 - P_\mathrm{M} + \frac{P_\mathrm{M}}{3}[1 + 2j_0(Qr\sqrt{3})] \quad (8)$$

where j_0 is the zero order Bessel function and r is the radius of the methyl group rotation.[22]

In the case of localized diffusion, the EISF(Q) can be written as

$$\mathrm{EISF_{LD}}(Q) = 1 - P_\mathrm{LD} + P_\mathrm{LD}\left[\frac{3j_1(QR)}{QR}\right]^2 \quad (9)$$

where j_1 is the first order Bessel function and R is the radius of a sphere in which the diffusion is localized. Among the numerous analytical models used to reproduce localized motions, there are two-site jumps in an asymmetric double-well potential, three-site jumps, and a localized diffusion model (diffusion within a sphere).[22,41−44] Our Q range and data accuracy are not sufficient to discriminate between these different models. Thus, we adopted the localized diffusion model on the basis of MD simulation findings[42] and previous analysis conducted on GFP,[15] as well as on other proteins.[43−45] The fit of the spectra and the derivation of the EISF(Q) have been performed using the DAVE software package as described in ref 15. A delta function to account for the elastic line and a single Lorentzian to approximate the quasielastic neutron scattering were used as components of the fit. The EISF(Q) was then calculated as the ratio between the obtained elastic intensity to the total scattering intensity resulting by fitting of the spectra at each Q. The use of a single Lorentzian to reproduce the quasielastic scattering in proteins is obviously not accurate, since their relaxation processes are strongly stretched.[46] Therefore, we do not use this analysis to estimate the corresponding relaxation time of the process. However, this approximation provides valuable qualitative information for the analysis of geometry of the atomic motion through the evaluation of the EISF-(Q).[15,22,43,45]

RESULTS AND DISCUSSION

Rigidity and Dynamics of Unfolded β-Casein. The spectral density, I_n, obtained from neutron scattering experiments on dry (empty symbols) and hydrated (closed symbols) protein powders shows a clear boson peak (Figure 1). Because these spectra do not exhibit any significant Q dependence, they were summed over all the measured Q (0.5−5 Å^{-1}) and normalized by the total cross section. The solid lines represent the fit to eq 5; the ν_BP values extracted are listed in Table 1. Depolarized light scattering data in the same spectral region show consistent trends (Table 1), as previously shown for a number of proteins.[23]

At both temperatures and hydration levels, the BP in CAS spectra is located at significantly lower frequency compared to GFP, indicating that the intrinsically unstructured protein is a considerably softer system. Also, at all temperatures and hydration conditions, the quasielastic intensity of CAS is higher than that in GFP, suggesting that the IDP shows higher flexibility and enhanced picosecond dynamics relative to the rigid β-barrel.

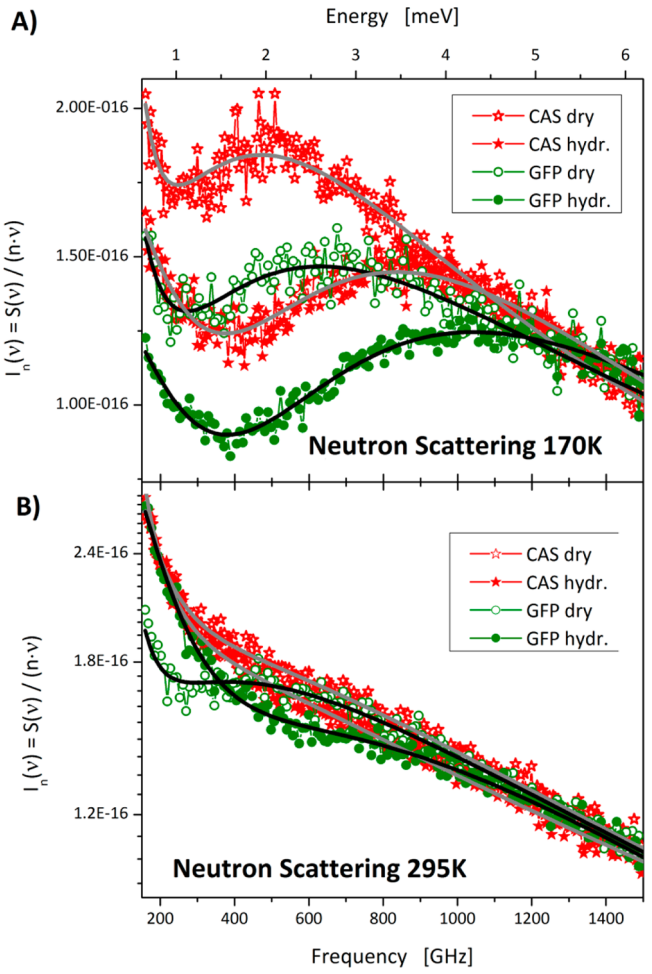


Figure 1. Neutron scattering spectra in the boson peak region presented as the spectral density for dry (empty symbols) and hydrated (closed symbols) proteins at 170 K (A) and 295 K (B). Solid lines represent the fit obtained applying eq 5 (gray for fit on CAS and black for the ones on GFP). The spectra, which were summed over all the measured Q (0.5−5 Å^{-1}), have been normalized at the high frequency tail.

Such evidence is also supported by our estimates of Young's modulus obtained in the GHz frequency range from Brillouin light scattering measurements as described in ref 23 and in the Supporting Information. Indeed, at 298 K, the elastic modulus of dry CAS powder is ∼7.0 GPa, which is smaller than the value obtained for GFP (∼9.7 GPa) and for other α-helical proteins previously studied.[23] Therefore, we can conclude that the native unfolded CAS system is more flexible than GFP at both length scales, the scale of a few nanometers (BP results) and at hundreds of nanometers (Brillouin data).

These results seem consistent and may be related to the different rigidities required by the two proteins to carry out their biological function. They are also in line with the findings obtained by Gaspar et al. on concentrated solutions of casein proteins, which display a higher degree of intramolecular mobility relative to folded proteins on the picosecond time scale.[13] The higher flexibility of the IDP might be ascribed to an increase in conformational freedom of the backbone, which is

dx.doi.org/10.1021/jp503788r | J. Phys. Chem. B XXXX, XXX, XXX−XXX

Table 1. Boson Peak Frequencies Obtained from the Fit of the Neutron Scattering Data (170 and 295 K) and of the Depolarized Light Scattering Spectra (295 K) with eq 5[a]

	neutron scattering				light scattering	
	170 K		295 K		295 K	
ν_{BP} (meV)	dry	hydr.	dry	hydr.	dry	hydr.
CAS	2.21 (±0.06)	4.02 (±0.05)	1.58 (±0.01)	2.65 (±0.09)	2.23 (±0.04)	2.92 (±0.09)
GFP	2.84 (±0.08)	4.73 (±0.04)	2.17 (±0.09)	3.67 (±0.09)	2.89 (±0.01)	3.13 (±0.01)
CAS−$CaCl_2$					2.95 (±0.05)	3.28 (±0.02)

[a]The uncertainties in parentheses were provided by the fit.

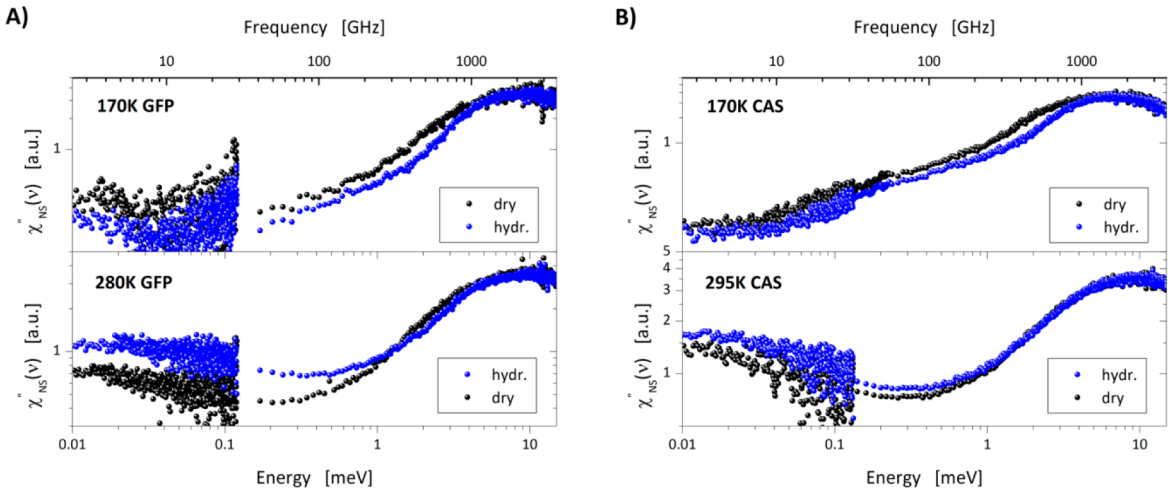


Figure 2. Susceptibility spectra (combined BASIS and CNCS neutron data) for GFP (panel A) and CAS (panel B) samples. Dry samples are displayed in black and hydrated ones in blue, at both low (top) and high (bottom) temperature. The spectra correspond to $Q = 1.1$ Å^{-1}. GFP data were reported in ref 15.

related to the presence of a lower number of intramolecular hydrogen bonds interactions per residue along the chain. Another explanation might reside in the differences in the chemical composition of IDP and folded proteins,[14] since different types of amino acids display different nano- to picosecond dynamics.[47] However, in the literature, there is contrasting evidence regarding this point. In some cases, the backbone fluctuations seem to vary only marginally as a function of sequence,[48] whereas local interactions, which lead to motional restriction, were pointed out for some other proteins.[46,49]

In order to better understand the origin of these differences in rigidity between folded GFP and unfolded CAS, we now turn to the study of the relaxation dynamics of these systems, analyzing the neutron scattering susceptibility spectra of GFP (Figure 2A) and CAS (Figure 2B), obtained in the broader energy range at both low and high temperature.

Recently, it has been shown that in the nanosecond−picosecond time scales four processes mainly contribute to the neutron scattering spectra of GFP, as well as of other globular proteins:[15] methyl group rotations, essentially unaffected by hydration, which approach the nanosecond time range; localized diffusion-like motions in the protein which have an extremely broad relaxation spectrum and are enhanced by the presence of water; fast picosecond dynamics; and BP vibrations. BP and localized diffusion processes are correlated to the rigidity of the system.[21,23] The nanosecond−picosecond dynamics of GFP occurring in both dry and hydrated states are localized to short distances of ∼1−3.5 Å, in contrast to the longer range diffusion of hydration water.[29] The fast picosecond relaxation is usually ascribed to the local rattling motions of residues in a cage formed by neighbor units (other residues and hydration water molecules).[41−43,50] It is located between ∼100 GHz and 1 THz, corresponding to relaxation times of ∼1 ps or faster (0.1−0.3 ps).[42,43] We notice that the tail of the jumps between different conformational states occurring on the several nanosecond time scale can also contribute to the dynamics of globular protein in the studied energy range.[21,41,43,50]

The spectra of GFP and CAS in the studied energy range are similar and exhibit analogous evolution in response to variations in temperature and hydration level (Figure 2). Therefore, we can conclude that CAS powders seem to exhibit the same types of dynamics detected for GFP and other globular proteins. Unfortunately, we cannot directly overlap the full spectra of the two proteins at high temperature, since data for GFP were taken at 280 K and the CAS ones at 295 K. Eventual differences in the spectra could reflect changes in the dynamics not only due to the different nature of the two proteins but also to the 15 K difference in the measurement temperature.

However, we can notice that the methyl groups' rotations of the two proteins on the low-frequency side of our spectra occur at comparable energy scales and appear as very broad distributions in both cases. This is in agreement with the well-recognized notion that methyl group rotations in proteins have broad distributions of energy barriers and relaxation times (∼10−350 ps),[15,44,51] due to differences in the chemical composition of the amino acids and in the position of the methyl groups along the polypeptide chain.[43,52] This large

dx.doi.org/10.1021/jp503788r | J. Phys. Chem. B XXXX, XXX, XXX−XXX

contribution is compatible with a high fraction of all nonexchangeable hydrogen atoms located on methyl groups, P_M, which in CAS corresponds to a value of ~0.29. The methyl content P_M in GFP is a bit smaller (P_M = 0.26).[15] We remark that a higher content in methyl groups can plasticize dynamics more strongly and increase the flexibility of CAS relative to GFP. Indeed, methyl groups are known to act as internal plasticizing agents in proteins; their rotations remain active even at low temperatures and dry conditions and facilitate the dynamics of proteins due to their low activation energies.[53]

Fast picosecond dynamics are suppressed by the presence of hydration water in both proteins at T = 170 K. Frozen water stiffens the systems relative to their dry states. This restriction of the amino-acid residue fluctuations is particularly evident in GFP, probably due to its barrel-like structure.[29] When hydration water becomes mobile at higher temperatures, it facilitates protein dynamics and hydration-induced relaxation process(es) enters the resolution window of the spectrometers. Those hydration-induced motions show a very broad distribution of relaxation times, consistent with the data reported for other globular proteins.[1,15,41,50]

In order to get a deeper understanding regarding the geometry of motions and the fraction of hydrogen atoms involved in these picosecond dynamics, we analyzed the EISF(Q) of both dry and hydrated CAS and compared to the results obtained for GFP samples in the same BASIS window (~300 ps) (Figure 3). The spectra of both proteins in dry and hydrated states exhibit strong QENS characterized by an essentially Q-independent width (Figure S2 in the Supporting Information). This has been observed also for other proteins[15,42,43,51,54] and suggests that the dominant contribution to the scattering comes from local motions. However, the QENS intensity does change with Q and can be used to calculate the EISF(Q). As reported in ref 15, the experimental Q dependence of EISF(Q) of dry GFP (Figure 3A) can be adequately reproduced by a model of methyl group rotation (eq 8), fixing P_M to the fraction of nonexchangeable hydrogen atoms on methyl groups (P_M = 0.26) and r = 1.03 Å[22] as the radius of the methyl group rotation. Interestingly, the contribution of methyl groups' rotations (with the same r value and P_M = 0.29) alone is not sufficient to appropriately model the EISF(Q) of dry CAS. An additional contribution accounting for localized dynamics is required even in the dry state; about 2% of hydrogen atoms of dehydrated CAS move with an amplitude of 4 Å on the time scale faster than 300 ps. In fact, our data show that the EISF(Q) of GFP and CAS show significant differences also in the hydrated case (Figure 3B). The EISF(Q) profiles of both hydrated proteins can be described as the sum of methyl group rotations and localized diffusion motions (inset Figure 3), for which the model of diffusion on a sphere has been applied (eq 9). However, in the case of hydrated GFP at 280 K, the fit provides an estimate of the radius of localized diffusion at R ~ 1 Å and P_{LD} = 0.4, whereas the values obtained for hydrated CAS at 295 K are R ~ 4 Å and P_{LD} = 0.16. Nevertheless, before drawing conclusions, we need to point out that data of hydrated GFP collected on the HFBS spectrometer (~1 ns) at 280 K and treated with the same analysis give R ~ 3.5 Å and P_{LD} = 0.1.[15] This suggests that part of the differences observed between the two hydrated proteins in the BASIS window might be related to the 15 K temperature difference in the experiments. However, we see that on picosecond time scale hydration-induced motions in hydrated CAS have larger amplitude (R ~ 4 Å) compared to

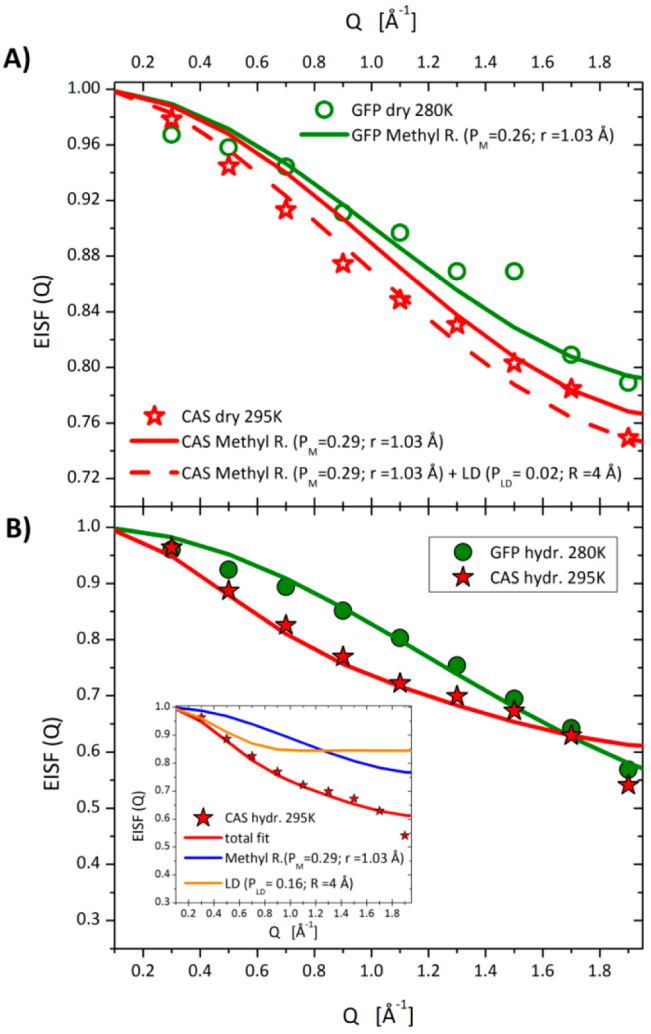


Figure 3. EISF(Q) for GFP and CAS in dry (A) and hydrated states (B), measured on a BASIS spectrometer (~300 ps). Symbols are experimental data, lines represent the total fits (red for CAS samples and green for GFP). GFP data were reported in ref 15. (A) The continuous red line represents the fit of CAS data with methyl groups' rotations only. The dashed line represents the fit including an additional term for localized motions. (B) Inset: EISF(Q) of hydrated CAS (stars). The blue line shows methyl group contribution, the orange line shows the fit with diffusion on a sphere model, and the red line (total fit) represents the sum of these two components.

the case of hydrated GFP. We can also argue that the hydration-induced motions of CAS experience higher amplitudes relative to globular proteins and there is not such a drastic increase in the number of flexible regions participating in the motions on time scale faster than 300 ps. It is possible that the larger amplitude motions observed in CAS are significantly slower than the smaller amplitude motions observed in GFP. As a result, the smaller fraction of these motions (P_{LD} = 0.16) appears in the accessible time window. It is also worth mentioning that the amplitude of the diffusion motions of fully hydrated tRNA at 300 K and a time scale of ~1 ns, calculated using the diffusion on a sphere model, has been proved to be almost twice as large as in hydrated globular proteins.[54] These differences were ascribed to a more open structure of hydrated tRNA and to the presence of a larger fraction of hydrophilic sites that can interact with water relative to globular proteins. In light of these data, we can picture a quite complex scenario for

dx.doi.org/10.1021/jp503788r | J. Phys. Chem. B XXXX, XXX, XXX−XXX

CAS: this protein is expected to be largely unfolded in its native state, but it has a high content of hydrophobic regions. Indeed, the fraction of hydrophobic residues in CAS, calculated as the ratio of the number of hydrophobic residues to the number of total residues, is 0.40, which is significantly higher than that of many globular proteins (for instance, GFP's fraction of hydrophobic residues is 0.28), other IDPs and also tRNA. Our results indicate that localized diffusion motions in CAS behave intermediately between well-folded globular proteins and biomolecules (e.g., tRNA) with open structure fully interacting with water.

Rigidity and Dynamics of Partially Folded β-Casein (Calcium Binding). Genome structure analysis assigns β-casein to the group of natively unfolded calcium-binding phosphoproteins.[5] Caseins are thought to have an open and flexible conformation, with only few tracts of folded secondary structures.[7,28] These IDPs have large binding surface areas which offer many possible points of contact for substrates.[55] This allows the interaction with multiple target molecules; indeed, caseins can bind to a range of integrin receptors on cell surfaces.[28]

In aqueous solution, β-casein undergoes self-association, which is promoted by surface hydrophobicity, and forms micelles whose limiting size depends on the ionic strength.[35,56] CAS and the other calcium-sensitive caseins (α_{s1}- and α_{s2}-casein but not k-) sequester Ca^{2+} ions in the form of protein-bound nanoclusters.[57] The partial folding of the primary structure with the clustering of phosphorylated residues, which creates the phosphate centers, is fundamental in calcium sequestration.[5] The five phosphoserine residues of bovine β-casein are all contained in the N terminal portion of the molecule (residues 1−40), which carries essentially all of the protein's net charge as well as most of the protein's predicted α-helical residues. The other regions contain a number of proline- and glutamine-rich sequences both of which fail to fold into stable conformations.[55] Indeed, proline residues are frequently found in β-turns and, lacking an amide proton to participate in hydrogen bonds, destabilize other regular secondary structures, whereas glutamine residues prevent the specific formation of β-sheets by competitive side chain−backbone hydrogen bonding interactions which stabilize an extended (β-strand) conformation. The C terminal of CAS (residues 136−209) consists of many apolar residues (resulting in its high hydrophobicity[6]) and only two short stretches of potential β-structure, as predicted by secondary structural algorithms.[58]

The secondary structure composition of CAS has also been determined by several experimental studies.[6,7,59] Many of these investigations were carried out using FT-IR and Raman techniques, which have the advantage of sampling the structure of the entire protein backbone. The only results available for CAS in the form of lyophilized powder were obtained by Byler et al. using Raman spectroscopy.[7] These authors showed that β-casein is largely disordered even in the absence of water, and that dry milk casein micelles binding calcium present a larger content of folded secondary structures. However, we point out that such results do not refer to β-casein exclusively, since the lyophilized micelles containing calcium ions were formed by a mixture of different types of caseins.

Our FT-IR spectra obtained for the dry samples of β-casein with and without $CaCl_2$ are shown together with the spectrum of the neat salt in Figure 4. Additional details regarding the spectrum of $CaCl_2$ and assignments of frequencies in the region of amides I (1570−1750 cm^{-1}) and II (1450−1570 cm^{-1}) to

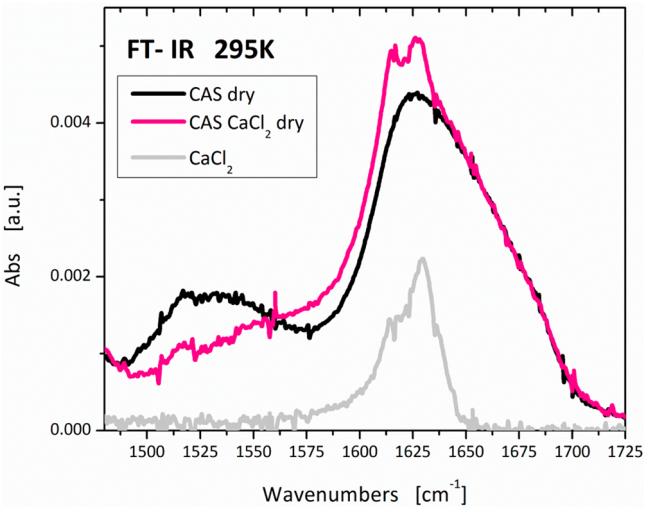


Figure 4. FT-IR spectra of dehydrated CAS (black line) and CAS−$CaCl_2$ (pink line) samples in the amide I and amide II region, collected at 295 K. The spectra of the two proteins were normalized to the integrated area between 900 and 1770 cm^{-1}. Absorbance of pure $CaCl_2$ (gray line) is also shown for qualitative comparison (spectrum not normalized).

different secondary structures for caseins and globular proteins[6,59] (Table S1, Supporting Information) are summarized in the Supporting Information. The amide I band cannot be easily used to identify differences in the folding of the two casein samples. The spectral changes in this frequency range might also depend on aggregation phenomena[60] and on the presence of $CaCl_2$[61,62] in excess, containing the calcium that was not captured by casein and reformed the salt upon freeze-drying during the sample preparation.

On the other hand, the amide II region is not influenced by these additional contributions and clearly indicates that a decrease in the extended regions (~1520−1535 cm^{-1}) and an increase in the α-helical contribution (1540 cm^{-1}) occur in the structure of CAS upon Ca^{2+} binding.[59] Our findings support Byler's trends obtained for the mixed casein micelles.[7] This evidence suggests that CAS undergoes a partial folding process and assumes a less extended, more compact conformation.

In order to verify how this affects the rigidity and dynamical properties of the systems, we bring our attention to the collective BP vibrations. The BP spectra obtained from light scattering experiments on GFP, CAS, and CAS−$CaCl_2$ samples at 295 K are displayed in Figure 5, together with the corresponding best fits. The resulting boson peak frequencies obtained using eq 5 are listed in Table 1. Partially folded β-casein samples show very similar BP frequencies (Table 1) and amplitudes slightly smaller than those of GFP.

This direct comparison suggests that CAS samples, both in dry and hydrated conditions, stiffen considerably in the presence of Ca^{2+}, reaching rigidity levels comparable, and even slightly higher, than those of the folded β-barrel. These results are also consistent with the picture emerging from the investigation of the picosecond dynamics. In the dry state and especially in the presence of hydration water, the amplitudes of picosecond dynamics of CAS−$CaCl_2$ are strongly reduced compared to unfolded CAS (Figure 5A). The analysis of the Q dependence of the EISF(Q) on the BASIS spectrometer shows that the hydration-induced dynamics are still localized to 4 Å in hydrated samples containing $CaCl_2$, but that the fraction of

dx.doi.org/10.1021/jp503788r | J. Phys. Chem. B XXXX, XXX, XXX−XXX

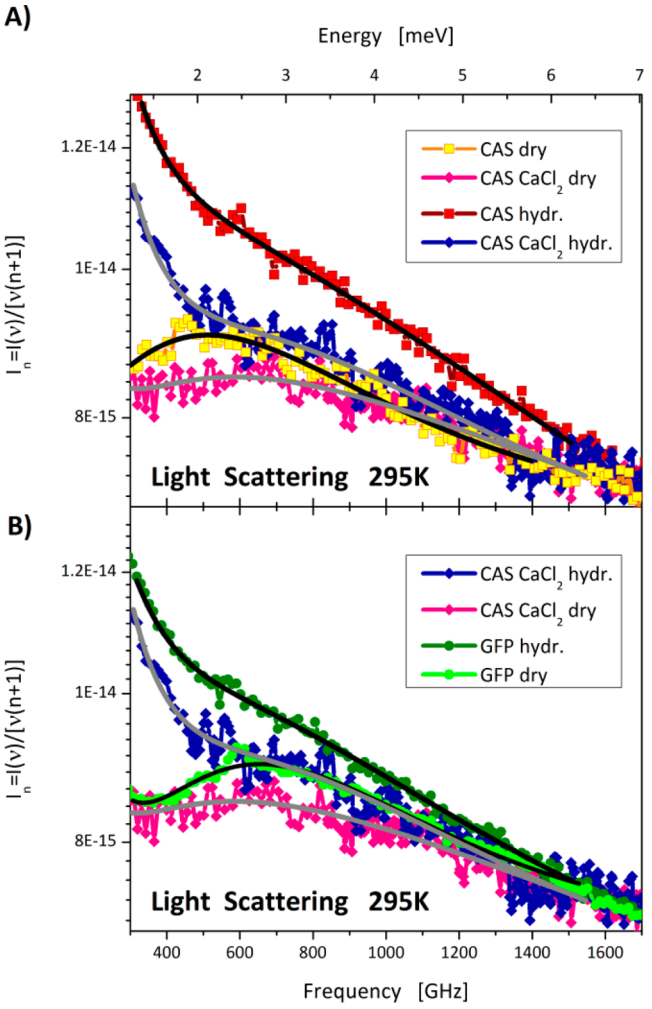


Figure 5. Depolarized light scattering spectra of the boson peak region for dry and hydrated systems, presented as the spectral density (295 K). (A) Comparison between CAS (squares) and CAS−$CaCl_2$ (diamonds) samples. (B) Comparison between CAS−$CaCl_2$ (diamonds) and GFP (circles). The data have been normalized at the high frequency tail. The continuous lines represent the fit obtained using eq 5.

hydrogen atoms contributing to the motions decreases to ∼0.14 (Figure S3 in the Supporting Information). We suggest that such large amplitude motions occur mostly in the unfolded regions of β-casein and the P_{LD} reduction in the samples containing calcium is related to a decrease in the amount of this disordered fraction of the molecule.

Electrostatic forces and hydration interactions are strongly coupled in this system. We conclude that this suppression of the local motions of CAS in the presence of calcium ions can be ascribed to a decrease in the number of fluctuations of the hydrogen-bonding network between the compact conformation of CAS micelles and hydrating water.

It is interesting to notice that an opposite trend had been observed in the case of folded and unfolded tRNA hydrated powders.[63] The local dynamics of hydrated tRNA strongly increase in the presence of Mg^{2+} ions, which screen the negative charge of the polynucleotide and stabilize its folded structure. Once again, one reason behind the different trends between hydrated proteins and tRNA was identified in the high fraction of hydrophilic sites in tRNA,[54] which can interact with the solvent and reach large flexibility. Answering this question is anyway difficult at a molecular level. Indeed, the extent of direct ion−molecule contacts, the influence of water on the location of the ion, and the degree to which the hydration of the ion is perturbed create a variety of different environments. Devising a comprehensive framework of ion−biomolecule−water interactions, that accounts for the overall ion dependence of the biomolecule folding, remains a major challenge and still requires further exhaustive studies.

CONCLUSIONS

We have related structural and folding properties of proteins to their rigidity and dynamics in the picosecond time scale. Neutron and light scattering experiments were used to compare the natively unfolded β-casein with and without calcium present, to GFP, a well-folded β-barrel of similar size. Unfolded β-casein is softer than the folded GFP, both under dry and hydrated conditions, as well as at low (170 K) and high temperature (295 K). This is consistent with the trend in rigidity properties observed previously and with a simple picture of their respective functions: GFP maintains a certain rigidity to facilitate the proton transfer associated with fluorescence, while CAS requires a flexibility to reversibly bind calcium and interact with multiple targets. We also demonstrated in the dry state that these differences in rigidity between the native states hold not only at probe lengths of a few nanometers but also at probe lengths over hundreds of nanometers.

There are still many similarities in the dynamics of the disordered protein CAS and folded proteins such as GFP. However, in this study, interesting differences have emerged. Despite the substantial differences in electrostatic environment and conformational and rigidity properties, CAS and GFP show the same types of dynamics in the picosecond time scale, which also vary in a similar way in response to temperature and hydration changes. Methyl group rotations, localized diffusion motions, rattling of atoms within the cage formed by neighbor units, and boson peak collective vibrations are common to both systems. However, a subtle additional localized diffusion contribution is present in the CAS samples: ∼2% of its hydrogen atoms moving with an amplitude of 4 Å (in the measured time window) even in the case of the dry CAS powder. These localized diffusion motions in CAS undergo large plasticization in the presence of water, and are localized to larger distances ($R \sim 4$ Å) than was observed in GFP or other globular proteins but smaller than those observed in tRNA. These large amplitude motions are probably related to the unfolded regions of the molecule. This result can be ascribed to an intermediate behavior of CAS being characterized by an open conformation and high hydrophobicity which limits its interaction with water.

Our analysis also reveals that partial folding and compact packing significantly modify the rigidity and dynamical properties of β-casein. FT-IR results show that, in the presence of Ca^{2+}, the α-helical content of CAS increases and the protein assumes a less extended, more compact conformation. As a consequence, the boson peak of CAS shifts to higher frequencies and decreases in amplitude, indicating a rigidity comparable, and even higher, than that of GFP. This result is corroborated by the analysis of the picosecond localized dynamics. When the polypeptide chain assumes a more folded and tightly packed conformation, the picosecond localized dynamics of β-casein are depressed and show amplitudes similar (or even slightly smaller) to those of GFP. This

dx.doi.org/10.1021/jp503788r | J. Phys. Chem. B XXXX, XXX, XXX−XXX

variation is related to an ∼10% decrease in the number of flexible regions participating in the diffusion-like motions and not to a reduction of the characteristic length scale. Our results revealed an opposite trend compared to the case of tRNA hydrated powders,[63] which in the presence of Mg^{2+} ions fold and show increased mobility at picosecond−nanosecond time scales.

This comparison of the dynamics of β-casein and other biomacromolecules demonstrates important similarities, and differences, that make disordered proteins so intriguing. We have shown that the picosecond scale motions of CAS are strongly influenced by electrostatic forces and folding conditions, in addition to interactions with water molecules. These relationships have a deep influence on protein function and might suggest how disordered regions can be used for applications in design and development of novel protein-based materials.

■ ASSOCIATED CONTENT

Supporting Information

Brillouin light scattering spectra and Young's moduli of GFP and CAS dry powders, curve fitting of QENS spectra of casein samples, characteristic frequencies and assignments of different secondary structures from vibrational spectroscopies from the literature. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: spertica@utk.edu.

*E-mail: nickelsjd@ornl.gov.

Author Contributions

#These authors contributed equally. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We acknowledge Department of Energy support through the EPSCoR program (grant DE-FG02-08ER46528) and by the Scientific User Facilities Division, Office of Basic Energy Sciences, U.S. DOE. The research at the Oak Ridge National Laboratory facilities is sponsored by UT-Battelle, LLC, for the U.S. Department of Energy under Contract No. DEAC05-00OR22725. We also thank the SNS-User Chemistry Lab., Hugh O'Neill and Qiu Zhang for providing GFP and Paola Sassi for helpful discussions.

■ REFERENCES

(1) Doster, W.; Cusack, S.; Petry, W. Dynamic instability of liquidlike motions in a globular protein observed by inelastic neutron scattering. *Phys. Rev. Lett.* **1990**, *65*, 1080.

(2) Uversky, V. N. What does it mean to be natively unfolded? *Eur. J. Biochem.* **2002**, *269*, 2−12.

(3) Wright, P. E.; Dyson, H. J. Intrinsically unstructured proteins: re-assessing the protein structure-function paradigm. *J. Mol. Biol.* **1999**, *293*, 321−331.

(4) Dyson, H. J.; Wright, P. E. Intrinsically unstructured proteins and their functions. *Nat. Rev. Mol. Cell Biol.* **2005**, *6*, 197−208.

(5) Smyth, E.; Clegg, R. A.; Holt, C. A biological perspective on the structure and function of caseins and casein micelles. *Int. J. Dairy Technol.* **2004**, *57*, 121−126.

(6) Farrell, H., Jr; Wickham, E.; Unruh, J.; Qi, P.; Hoagland, P. Secondary structural studies of bovine caseins: temperature dependence of β-casein structure as analyzed by circular dichroism and FTIR spectroscopy and correlation with micellization. *Food Hydrocolloids* **2001**, *15*, 341−354.

(7) Michael Byler, D.; Farrell, H. M., Jr; Susi, H. Raman spectroscopic study of casein structure. *J. Dairy Sci.* **1988**, *71*, 2622−2629.

(8) Yang, X. J. The diverse superfamily of lysine acetyltransferases and their roles in leukemia and other diseases. *Nucleic Acids Res.* **2004**, *32*, 959−976.

(9) McCampbell, A.; Fischbeck, K. H. Polyglutamine and CBP: fatal attraction? *Nat. Med.* **2001**, *7*, 528−530.

(10) Nucifora, F. C., Jr; Sasaki, M.; Peters, M. F.; Huang, H.; Cooper, J. K.; Yamada, M.; Takahashi, H.; Tsuji, S.; Troncoso, J.; Dawson, V. L. Interference by huntingtin and atrophin-1 with cbp-mediated transcription leading to cellular toxicity. *Science* **2001**, *291*, 2423−2428.

(11) Karlin, S.; Brocchieri, L.; Bergman, A.; Mrázek, J.; Gentles, A. J. Amino acid runs in eukaryotic proteomes and disease associations. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 333−338.

(12) Dunker, A. K.; Lawson, J. D.; Brown, C. J.; Williams, R. M.; Romero, P.; Oh, J. S.; Oldfield, C. J.; Campen, A. M.; Ratliff, C. M.; Hipps, K. W.; Ausio, J.; Nissen, M. S.; Reeves, R.; Kang, C.; Kissinger, C. R.; Bailey, R. W.; Griswold, M. D.; Chiu, W.; Garner, E. C.; Obradovic, Z. Intrinsically disordered protein. *J. Mol. Graphics Modell.* **2001**, *19*, 26−59.

(13) 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*, 573−582.

(14) Gallat, F.-X.; Laganowsky, A.; Wood, K.; Gabel, F.; van Eijck, L.; Wuttke, J.; Moulin, M.; Härtlein, M.; Eisenberg, D.; Colletier, J.-P. Dynamical coupling of intrinsically disordered proteins and their hydration water: comparison with folded soluble and membrane proteins. *Biophys. J.* **2012**, *103*, 129−136.

(15) 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*, 11548−11555.

(16) Nettels, D.; Gopich, I. V.; Hoffmann, A.; Schuler, B. Ultrafast dynamics of protein collapse from single-molecule photon statistics. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 2655−2660.

(17) Bieri, O.; Wirz, J.; Hellrung, B.; Schutkowski, M.; Drewello, M.; Kiefhaber, T. The speed limit for protein folding measured by triplet−triplet energy transfer. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 9597−9601.

(18) Eliezer, D.; Chung, J.; Dyson, H. J.; Wright, P. E. Native and non-native secondary structure and dynamics in the pH 4 intermediate of apomyoglobin. *Biochemistry* **2000**, *39*, 2894−2901.

(19) Dyson, H. J.; Wright, P. E. Equilibrium NMR studies of unfolded and partially folded proteins. *Nat. Struct. Mol. Biol.* **1998**, *5*, 499−503.

(20) Jane Dyson, H.; Ewright, P. Insights into the structure and dynamics of unfolded proteins from nuclear magnetic resonance. *Adv. Protein Chem.* **2002**, *62*, 311−340.

(21) Hong, L.; Glass, D. C.; Nickels, J. D.; Perticaroli, S.; Yi, Z.; Tyagi, M.; 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.

(22) Fitter, J.; Lechner, R.; Buldt, G.; Dencher, N. 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.

(23) Perticaroli, S.; Nickels, J. D.; Ehlers, G.; O'Neill, H.; Zhang, Q.; Sokolov, A. P. Secondary structure and rigidity in model proteins. *Soft Matter* **2013**, *9*, 9548−9556.

(24) Perticaroli, S.; Nickels, J. D.; Ehlers, G.; Sokolov, A. P. Rigidity, secondary structure, and the Universality of the Boson Peak in Proteins. *Biophys. J.* **2014**, *106*, 2667−2674.
(25) Nakagawa, H.; Kamikubo, H.; Kataoka, M. Effect of conformational states on protein dynamical transition. *Biochim. Biophys. Acta, Proteins Proteomics* **2010**, *1804*, 27−33.
(26) Mamontov, E.; O'Neill, H.; Zhang, Q. Mean-squared atomic displacements in hydrated lysozyme, native and denatured. *J. Biol. Phys.* **2010**, *36*, 291−297.
(27) Tsien, R. Y. The green fluorescent protein. *Annu. Rev. Biochem.* **1998**, *67*, 509−544.
(28) Fisher, L. W.; Torchia, D. A.; Fohr, B.; Young, M. F.; Fedarko, N. S. Flexible Structures of SIBLING Proteins, Bone Sialoprotein, and Osteopontin. *Biochem. Biophys. Res. Commun.* **2001**, *280*, 460−465.
(29) 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*, 1566−1575.
(30) Nickels, J. D.; Perticaroli, S.; O'Neill, H.; Zhang, Q.; Ehlers, G.; Sokolov, A. P. Coherent Neutron Scattering and Collective Dynamics in the Protein, GFP. *Biophys. J.* **2013**, *105*, 2182−2187.
(31) 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*, 2726−2732.
(32) Kurkal-Siebert, V.; Smith, J. C. Low-temperature protein dynamics: a simulation analysis of interprotein vibrations and the boson peak at 150 K. *J. Am. Chem. Soc.* **2006**, *128*, 2356−2364.
(33) Tarek, M.; Tobias, D. J. Effects of solvent damping on side chain and backbone contributions to the protein boson peak. *J. Chem. Phys.* **2001**, *115*, 1607−1612.
(34) Luo, G.; Zhang, Q.; Castillo, A. R. D.; Urban, V.; O'Neill, H. Characterization of sol− gel-encapsulated proteins using small-angle neutron scattering. *ACS Appl. Mater. Interfaces* **2009**, *1*, 2262−2268.
(35) Müller-Buschbaum, P.; Gebhardt, R.; Roth, S.; Metwalli, E.; Doster, W. Effect of calcium concentration on the structure of casein micelles in thin films. *Biophys. J.* **2007**, *93*, 960−968.
(36) Mamontov, E.; Herwig, K. W. A time-of-flight backscattering spectrometer at the Spallation Neutron Source, BASIS. *Rev. Sci. Instrum.* **2011**, *82*, 085109−085109−10.
(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*, 085108−085108−6.
(38) Bée, M. *Quasielastic neutron scattering: principles and applications in solid state chemistry, biology, and materials science*; Adam Hilger: Bristol, England; Philadelphia, PA, 1988; p xii, 437 p.
(39) Nielsen, O. F. Chapter 2. Low-frequency spetroscopic studies of interactions in liquids. *Annu. Rep. Prog. Chem., Sect. C: Phys. Chem.* **1993**, *90*, 3−44.
(40) Malinovsky, V.; Novikov, V.; Sokolov, A. Log-normal spectrum of low-energy vibrational excitations in glasses. *Phys. Lett. A* **1991**, *153*, 63−66.
(41) Doster, W.; Cusack, S.; Petry, W. Dynamical transition of myoglobin revealed by inelastic neutron scattering. *Nature* **1989**, *337*, 754−756.
(42) 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.
(43) Roh, J.; Curtis, J.; Azzam, S.; Novikov, V.; Peral, I.; Chowdhuri, Z.; Gregory, R.; Sokolov, A. Influence of hydration on the dynamics of lysozyme. *Biophys. J.* **2006**, *91*, 2573−2588.
(44) 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.
(45) Telling, M. T. F.; Clifton, L.; Combet, J.; Frick, B.; Howells, S.; Sakai, V. G. Lyophilised protein dynamics: more than just methyls? *Soft Matter* **2012**, *8*, 9529−9532.
(46) Alexandrescu, A. T.; Shortlet, D. Backbone dynamics of a highly disordered 131 residue fragment of staphylococcal nuclease. *J. Mol. Biol.* **1994**, *242*, 527−546.
(47) 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.
(48) Frank, M. K.; Clore, G. M.; Gronenborn, A. M. Structural and dynamic characterization of the urea denatured state of the immunoglobulin binding domain of streptococcal protein G by multidimensional heteronuclear NMR spectroscopy. *Protein Sci.* **1995**, *4*, 2605−2615.
(49) Farrow, N. A.; Zhang, O.; Forman-Kay, J. D.; Kay, L. E. Characterization of the backbone dynamics of folded and denatured states of an SH3 domain. *Biochemistry* **1997**, *36*, 2390−2402.
(50) Khodadadi, S.; Pawlus, S.; Sokolov, A. Influence of hydration on protein dynamics: combining dielectric and neutron scattering spectroscopy data. *J. Phys. Chem. B* **2008**, *112*, 14273−14280.
(51) Doster, W. Settles, M., Protein−water displacement distributions. *Biochim. Biophys. Acta, Proteins Proteomics* **2005**, *1749*, 173−186.
(52) Wand, A. J. Dynamic activation of protein function: a view emerging from NMR spectroscopy. *Nat. Struct. Mol. Biol.* **2001**, *8*, 926−931.
(53) Nickels, J. D.; Curtis, J. E.; O'Neill, H.; Sokolov, A. P. Role of methyl groups in dynamics and evolution of biomolecules. *J. Biol. Phys.* **2012**, *38*, 497−505.
(54) Roh, J.; Briber, R.; Damjanovic, A.; Thirumalai, D.; Woodson, S.; Sokolov, A. Dynamics of tRNA at different levels of hydration. *Biophys. J.* **2009**, *96*, 2755−2762.
(55) Holt, C.; Sawyer, L. Caseins as rheomorphic proteins: interpretation of primary and secondary structures of the αS1-, β- and κ-caseins. *J. Chem. Soc., Faraday Trans.* **1993**, *89*, 2683−2692.
(56) Andrews, A. L.; Atkinson, D.; Evans, M. T. A.; Finer, E. G.; Green, J. P.; Phillips, M. C.; Robertson, R. N. The conformation and aggregation of bovine β-casein A. I. Molecular aspects of thermal aggregation. *Biopolymers* **1979**, *18*, 1105−1121.
(57) Holt, C. An equilibrium thermodynamic model of the sequestration of calcium phosphate by casein micelles and its application to the calculation of the partition of salts in milk. *Eur. Biophys. J.* **2004**, *33*, 421−434.
(58) Kumosinski, T.; Brown, E.; Farrell, H., Jr. Three-Dimensional Molecular Modeling of Bovine Caseins: An Energy-Minimized< i> β</i>-Casein Structure. *J. Dairy Sci.* **1993**, *76*, 931−945.
(59) Kumosinski, T. F.; Farrell, H. M., Jr. Determination of the global secondary structure of proteins by Fourier transform infrared (FTIR) spectroscopy. *Trends Food Sci. Technol.* **1993**, *4*, 169−175.
(60) Sassi, P.; Giugliarelli, A.; Paolantoni, M.; Morresi, A.; Onori, G. Unfolding and aggregation of lysozyme: A thermodynamic and kinetic study by FTIR spectroscopy. *Biophys. Chem.* **2011**, *158*, 46−53.
(61) Lu, Y.; Deng, G.; Miao, F.; Li, Z. Sugar complexation with calcium ion. Crystal structure and FT-IR study of a hydrated calcium chloride complex of D-ribose. *J. Inorg. Biochem.* **2003**, *96*, 487−492.
(62) Horikoshi, K.; Hata, K.; Kawabata, N.; Ikawa, S.-I.; Konaka, S. Vibrational spectra and conformation of polyethylene glycol complexed with calcium and magnesium chlorides. *J. Mol. Struct.* **1990**, *239*, 33−42.
(63) Roh, J. H.; Tyagi, M.; Briber, R.; 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.