This manuscript has been authored by UT-Battelle, LLC under Contract No. DE-AC05-00OR22725 with the U.S. Department of Energy. The United States Government retains and the publisher, by accepting the article for publication, acknowledges that the United States Government retains a non-exclusive, paid-up, irrevocable, world-wide license to publish or reproduce the published form of this manuscript, or allow others to do so, for United States Government purposes. The Department of Energy will provide public access to these results of federally sponsored research in accordance with the DOE Public Access Plan (<http://energy.gov/downloads/doe-public-access-plan>).

Microscopic relaxations in a protein sustained down to 160 K in a non-glass forming organic solvent

E. Mamontov^{a,*} and H. O'Neill^b

^a*Chemical and Engineering Materials Division, Neutron Sciences Directorate, Oak Ridge National Laboratory, Oak Ridge, TN 37831*

²*Biology and Soft Matter Division, Neutron Sciences Directorate, Oak Ridge National Laboratory, Oak Ridge, TN 37831*

*Phone: 1-865-771-1387. FAX: 1-865-574-6080. E-mail: mamontove@ornl.gov

Structured Abstract

Background

We have studied microscopic dynamics of a protein in carbon disulfide, a non-glass forming solvent, down to its freezing temperature of ca. 160 K.

Methods

We have utilized quasielastic neutron scattering.

Results

A comparison of lysozyme hydrated with water and dissolved in carbon disulfide reveals a stark difference in the temperature dependence of the protein's microscopic relaxation dynamics induced by the solvent. In the case of hydration water, the common protein glass-forming solvent, the protein relaxation slows down in response to a large increase in the water viscosity on cooling down, exhibiting a well-known protein dynamical transition. The dynamical transition disappears in non-glass forming carbon disulfide, whose viscosity remains a weak function of temperature all the way down to freezing at just below 160 K. The microscopic relaxation dynamics of lysozyme dissolved in carbon disulfide is sustained down to the freezing temperature of its solvent at a rate similar to that measured at ambient temperature.

Conclusions

Our results demonstrate that protein dynamical transition is not merely solvent-assisted, but rather solvent-induced, or, more precisely, is a reflection of the temperature dependence of the solvent's glass-forming dynamics.

General Significance

We hypothesize that, if the long debated idea regarding the direct link between the microscopic relaxations and the biological activity in proteins is correct, then not only the microscopic relaxations, but also the activity, could be sustained in proteins all the way down to the freezing temperature of a non-glass forming solvent with a weak temperature dependence of its viscosity.

Keywords: Protein; microscopic dynamics; non-aqueous solvent.

TOC Graphical Abstract

1. Introduction

From protein folding to self-organization of lipid bilayers in aqueous environments, the role played by the solvent in shaping the structure of biomolecules is broadly recognized. On the other hand, the solvent's influence on the microscopic dynamics of biomolecules is not understood nearly as well, beyond the general notion of dynamic coupling between the aqueous solvent and the hydrated/solvated biomolecules. There is a general consensus regarding the minimal hydration level needed for the appreciable protein enzymatic activity and the appearance of solvent-induced relaxations, $h = 0.2$ (h is measured in g of water per g of protein) [1]. As the hydration level is increased further, the amplitudes of the solvent and solute molecular motions increase, and there is a concomitant increase in the activity. However, the consensus ends as soon as the protein's activity and microscopic dynamics need to be analyzed as a function of temperature at a given hydration level, as is commonly done to separate the vibration and relaxation degrees of freedom in biomolecules. It is well known empirically that the emergence of the microscopic relaxations on warming up (as opposed to vibrational degrees of freedom, which are always active) requires the presence of a solvent, and the temperature of the relaxation onset depends on the solvent's properties. Are biomolecules enslaved [2-4] to the hydration water (or, broadly speaking, the solvent), or does the hydration water (or a solvent) act as a plasticizer [5] to the motions of biomolecules?

In order to address such questions, it is instructive to consider a reverse scenario, when a system of solvated biomolecules is gradually cooled down from ambient to very low temperatures. Initially exhibiting both vibration and relaxation degrees of freedom, the biomolecules lose the latter on cooling down in a response to the slowing down of the solvent's motions. Interestingly, practically every solvent used to date in the studies of microscopic dynamics of biomolecules, including the most common aqueous solvents, has been a glass-former with non-Arrhenius temperature dependence of its viscosity. With such solvents, the rapid loss of the relaxation degrees of freedom on cooling down, which is just the reverse of the protein "dynamical transition" [6] to the relaxation-active state on warming up, is a result of the rapid (characterized by super-Arrhenius temperature dependence) loss of either the plasticizing capabilities, or the fluctuation rate [7] of the solvent thermal bath.

The relaxation rate of the protein solvents can be varied in several ways. For example, irrespective of the specifics of the hydrated surface, the temperature dependence of the relaxation

rate in the hydration water varies with the hydration level [8,9]. Besides, the relaxations of the aqueous solvent can be affected by co-solvents [10-31], which often provide an added benefit of cryo-protection, suppressing freezing and thus enabling wide temperature range studies of bulk-like protein solutions that are possible only for hydrated powders if pure water solvent is used. Nevertheless, even in the extreme case of a water-free solvent (e.g., pure glycerol), qualitatively, the scenario remains the same: the non-Arrhenius relaxation dynamics of the solvated protein can be understood as a result of coupling to the non-Arrhenius relaxations of the solvent.

In a departure from the previous studies of microscopic dynamics of solvated proteins, in this work we have used carbon disulfide (CS_2), a non-glass forming solvent that does not exhibit a strong temperature dependence of the relaxation rate on cooling down from ambient temperature until a rapid crystallization at the freezing temperature of *ca.* 160-162 K. This ambient-to-freezing temperature range, free of both solvent crystallization and a strong solvent viscosity increase, is sufficiently wide to test the dynamics of the solvated protein over the temperature range of the commonly observed protein dynamical transition. Carbon disulfide provides a rather uncommon solvent thermal bath with Arrhenius-type temperature dependence of the relaxation rate and no hydrogen bonding to the solvated protein molecules.

2. Materials and methods

2.1 Sample preparation

We used two samples in the liquid form: CS_2 and lysozyme dissolved in CS_2 at 10 mg/mL, and three samples in the powder form: dry lysozyme and lysozyme hydrated with D_2O and H_2O vapors to $h = 0.22$ g of solvent per gram of protein. As commonly done in neutron scattering experiments, H_2O - and D_2O -hydrated lysozyme was used to probe the dynamics of the water solvent and the protein itself, respectively.

Lysozyme (Sigma-Aldrich) was initially treated by dissolving it in with H_2O or D_2O followed by lyophilization. In the case of D_2O exchange, the protein sample was sequentially dissolved in D_2O and lyophilized three times to ensure complete exchange of labile H atoms in the protein with D atoms. The D_2O -dissolved lysozyme was used to prepare the dry and D_2O -hydrated powder samples, and the H_2O -dissolved lysozyme was used to prepare the H_2O -hydrated powder sample.

To prepare the lysozyme-CS₂ sample, the lyophilized protein (prepared from H₂O) was dissolved in CS₂ to a final concentration of 10 mg/mL. While initial breakdown of small chunks of the protein powder into barely visible particles was accomplished in about an hour, it required several hours of stirring (overnight) for complete dissolution. The final sample was essentially colorless, uniform, and somewhat transparent, but less transparent than pure carbon disulfide (that is, exhibited some turbidity, as one can see in Figure 1). It was not possible to fully dissolve lysozyme at higher concentration (e.g., 20 mg/mL).

All three powder samples used lysozyme powders that had been dissolved in carbon disulfide for a full week prior to the solvent evaporation and subsequent lyophilization of the protein (followed by the final hydration for the $h=0.22$ samples). Therefore, the purpose of measuring the lysozyme powder samples was two-fold. Besides providing a canonical example of hydrated protein dynamical transition for comparison with the protein dissolved in CS₂, we would like to verify that prolonged dissolution in CS₂ does not alter the protein in such a way as to render meaningless the comparison of the CS₂-exposed lysozyme with the lysozyme data widely available in the literature. Such a concern may be only partially warranted, since even the purposely denatured lysozyme still exhibits the same dynamical transition as native lysozyme [32]. Furthermore, it is known from the literature that the effects of CS₂ exposure on proteins are mostly limited to covalent cross-linking [33-37]. In fact, carbon disulfide is known to react spontaneously with amine groups to form dithiocarbamates on gold surface [38-41]. Nevertheless, for the sake of better comparison, we would like to make sure that lysozyme in all samples, including hydrated powders, had a prior exposure to CS₂.

2.2 Neutron scattering experiment

Quasielastic neutron scattering (QENS) measurements were performed on a backscattering spectrometer BASIS [42] operated with an energy resolution of 3.4 μ eV (full-width at half-maximum for the Q -averaged resolution value) and a useful dynamic range of accessible energy transfer of ± 100 μ eV. We used 2.5 mm thick annular aluminum sample holders with the outer diameter of 29 mm to load a CS₂ sample and a 10 mg/mL lysozyme in CS₂ sample. With a beam height of 30 mm, the protein sample had about 70 mg of lysozyme in the solvent exposed to the neutron beam. All the scattering data, elastic and dynamic, were collected on cooling down with a ramp rate of 1 K/min. Besides the elastic intensity scans that monitored the temperature

dependence of the scattering intensity integrated over $\pm 3.4 \mu\text{eV}$ energy transfer range, the dynamic data over $\pm 100 \mu\text{eV}$ energy transfer range were obtained by summing the data over (10 min)/(10 K) slices, thereby yielding the data points with 10 K increments. The data collected at the baseline temperature of 10 K was used for the resolution function. Besides, the diffraction pattern was collected at ambient and baseline temperature using diffraction detectors at the BASIS.

3. Results

The diffraction patterns presented in Fig. 1 demonstrate that, even at the relatively low concentration of 10 mg/mL, there is sufficient excess intensity evident at Q below $1 \text{ \AA}^{-1}$ that originates from the predominantly incoherent scattering from the dissolved lysozyme. This is possible largely because the neutron scattering cross-section of CS_2 is completely coherent, so the scattering intensity from the solvent concentrates near the maxima of its structural factor, and there is no Q -independent incoherent scattering signal from the solvent. Distinguishing the incoherent signal from a protein at 10 mg/mL would be impossible with a hydrogenated solvent and difficult even with a deuterated solvent, because of the sizable incoherent scattering cross-section of deuterium that yields a substantial Q -independent signal overlapping with the signal

Figure 1. Bottom panel: the diffraction patterns collected at 10 K and 300 K. Compared to the CS_2 buffer alone, the excess intensity due to the predominantly incoherently scattering dissolved lysozyme is evident below $ca. 1 \text{ \AA}^{-1}$. Top panel: images of 10 mg/mL lysozyme- CS_2 solution in a glass vial.

from the dissolved protein. Thus, besides being a non-glass former, CS_2 has an important added benefit for our experiment of having zero incoherent neutron scattering cross-section.

The data in Fig. 1 also define the Q -range suitable for the QENS data analysis, which, for the dissolved protein sample, involves subtraction of the background spectra measured from the CS_2 buffer. Thus, for data analysis, the elastic intensity scans and dynamic QENS data were binned into three Q values: 0.5, 0.7, and 0.9 $\text{\AA}^{-1}$.

The temperature dependence of the energy-resolved elastic intensity measured on cooling down is presented in Fig. 2. The right panel demonstrates that the data from the CS_2 -exposed

Figure 2. The energy-resolved elastic scattering intensity as a function of temperature measured on cooling down. Left panel: protein-free carbon disulfide buffer and the lysozyme dissolved in CS_2 (with the buffer signal subtracted). Right panel: reference lysozyme powder samples.

lysozyme is qualitatively no different from the data on a native lysozyme and numerous other proteins known from the literature. As we have mentioned in the sample preparation section,

despite known reaction of carbon disulfide with amine groups to form dithiocarbamates, this was fully expected, yet warranted verification. The dry lysozyme shows a gradual increase in the elastic scattering as the temperature is decreased, which can be mostly accounted for by the thermal Debye-Waller factor, since relatively few non-vibrational degrees of freedom (primarily methyl group rotations) are excited in a dry protein. The D₂O-hydrated lysozyme shows a faster increase in the elastic scattering on cooling due to gradual “freezing out” of the relaxation degrees of freedom down to *ca.* 220 K. Below this temperature, once the relaxation degrees of freedom become frozen on the time scale of the measurement, as defined by the energy resolution at the elastic line, further increase in the elastic scattering again mostly reflects vibrational Debye-Waller temperature dependence. The relaxations in the protein are driven by the hydration water, as evident from the data for H₂O-hydrated lysozyme, where the signal is dominated by the hydration water rather than hydrated protein. The relaxations in the hydration water become frozen below *ca.* 220 K. Such behavior of hydration water is fairly generic, although the hydration level greatly influences the dynamics of the hydration water (and, as a consequence, the dynamics of the host, if the latter is “soft” enough to sustain relaxation motions) [9]. The dependence of the hydration water dynamics on the host has been demonstrated [43], but the meaningful comparison between different hosts is hindered by difficulties in matching the hydration level, which itself strongly influences the relaxations in the hydration water [9]. Elastic intensity scans of hydrated proteins show little difference when the data were obtained on cooling down, as in the current work, or warming up, as often reported in the studies of protein dynamical transition above *ca.* 220 K. The hydration water is a glass-forming solvent [44].

The left panel in Fig. 2 shows the elastic intensity scans for the standalone solvent and the dissolved lysozyme. To facilitate the comparison, an identical temperature range (defined by freezing of CS₂) was chosen for both the samples of interest (left panel) and the control samples (right panel). It is evident that between the room temperature and the solvent freezing (a step-like increase in the intensity just below 160 K), there is no dynamical transition for the solvent or solute. The replacement of a glass-forming solvent (such as hydration water, an aqueous cryo-protective solvent, pure glycerol, etc.) with a non-glass forming carbon disulfide thus eliminates the protein dynamical transition.

Elimination of the dynamical transition in the proteins has been observed, e.g., for lysozyme in trehalose [31]. The high glass transition temperature of trehalose of 390 K suppresses the relaxations in the protein. In this work, on the other hand, the protein in CS_2 does not lose its relaxation degrees of freedom; instead, the relaxation dynamics shows very little temperature dependence, thereby unraveling the dynamical transition. To demonstrate this, we have analyzed the temperature dependence of the QENS data, taking rather uncommon approach in the studies of protein dynamical transition, which tend to rely exclusively on the elastic intensity scans. Fig. 3 shows an example of the QENS data and the best fits obtained using a

Figure 3. The QENS data (symbols) and the overall fits (pink solid lines) as described in the text. Blue solid lines: elastic component of the fit. Red solid lines: inelastic component of the fit. The data collected at $T=260$ K, $Q=0.7$ $\text{\AA}^{-1}$, shown over the limited energy transfer range of ± 40 μeV to emphasize the QENS signal, are used as a representative example. For the lysozyme dissolved in CS_2 , the buffer signal has been subtracted.

resolution-convolved superposition of a delta function centered at zero energy transfer and a model scattering function plus a linear background:

$$I(E) = [x\delta(E) + (1-x)S(E)] \otimes R(E) + (C_1E + C_2) \quad (1)$$

Regarding the choice of the model scattering function, $S(E)$, from which the width of the QENS signal is derived, a simple Lorentzian

$$S(E) = \frac{1}{\pi} \frac{\Gamma}{E^2 + \Gamma^2} \quad (2)$$

surprisingly proved adequate for all samples but one, the hydration H₂O in lysozyme. For that sample, a Lorentzian fit was clearly unsatisfactory, and we used instead a Cole-Cole distribution function:

$$S(E) = \frac{(E/E_0)^{-\alpha} \cos \frac{\pi\alpha}{2}}{1 + 2(E/E_0)^{1-\alpha} \sin \frac{\pi\alpha}{2} + (E/E_0)^{2(1-\alpha)}} \quad (3)$$

Here the parameter E_0 is analogous to the half-width at half maximum (HWHM) of a Lorentzian function, which is the limiting case of Eq. (3) when the “stretching” parameter $\alpha = 0$. At each measurement temperature, the QENS broadening parameter $\Gamma(Q)$ (or $E_0(Q)$ for the hydration H₂O in lysozyme) was determined at $Q = 0.5, 0.7$, and $0.9 \text{ \AA}^{-1}$. Then its Q dependence was fit as shown in Fig. 4 insets to obtain the temperature dependence of the QENS signal broadening as shown in the main Fig. 4 panels. We determined and plotted in Fig. 4 either the slope, in the units of $\mu\text{eV}/\text{\AA}^{-2}$, for the translational diffusion Q -dependence of a solvent ($\Gamma(Q)$ or $E_0(Q) \sim Q^2$), or the Q -independent average, in the units of μeV , for the localized motions in lysozyme.

4. Discussion

We can now make a comparison between the relaxation dynamics of lysozyme in contact with water and carbon disulfide solvents. At ambient temperature, the $\sim 10 \mu\text{eV}$ signal from the hydrated lysozyme indicates dynamics on ~ 60 ps time scale. As the temperature is decreased, the QENS broadening narrows, until it plateaus at $\sim 6 \mu\text{eV}$ (~ 100 ps). This temperature dependence is clearly related to the behavior of the water solvent, which, as a glass-former, shows a pronounced slowing down on cooling down, until its dynamics becomes unresolvable at the resolution of the current experiment below 220 K.

On the contrary, the dynamics of a non-glass forming CS₂ solvent slows down much more gradually, only by about a factor of 2.5 between 300 and 200 K. Remarkably, in this temperature range, the signal from the dissolved lysozyme hardly shows any temperature dependence at all, remaining at $\sim 10 \mu\text{eV}$ (~ 60 ps). In agreement with the elastic intensity scans, there is no protein dynamical transition in lysozyme dissolved in carbon disulfide, except for the

trivial effect of the solvent freezing below *ca.* 160 K. This is a consequence of our careful choice of a non-glass forming solvent with a weak temperature dependence of the relaxation dynamics. Obviously, the phenomenon of the protein dynamical transition is not merely solvent-assisted, but rather solvent-induced, or, more precisely, is a reflection of the temperature dependence of the solvent's glass-forming dynamics.

Figure 4. The QENS broadening as a function of temperature. For the hydrated and solvated proteins, the broadening is Q -independent; thus, the data points (in the units of μeV) are obtained simply by averaging over the three Q points, as illustrated by the horizontal lines drawn through the filled symbols in the insets. For the solvents, either CS_2 or water, the broadening shows a DQ^2 dependence characteristic of the translational diffusion; thus, the data points (in the units of $\mu\text{eV}/\text{\AA}^{-2}$) are the slopes, as illustrated by the lines drawn through the open symbols in the insets.

Our experiment demonstrates that, as long as a relatively low viscosity of a non-glass forming solvent is retained even at low temperatures, where traditional solvents would have become glass-like, microscopic relaxation dynamics of the solvated protein can be sustained at a level comparable to the dynamics at ambient temperature. In fact, the role of the rigid cage formed by a glass-like solvent at sufficiently low (for the solvent) temperature in suppressing the

protein dynamics below the level of a dry protein powder has been discussed in the literature for both aqueous [45] and non-aqueous [31] solvents.

The role of a non-glass forming non-polar solvent such as carbon disulfide in sustaining the microscopic relaxations in a protein down to low temperatures is not only to provide a thermal bath, but also to eliminate a glass-forming aqueous solvent from a contact with the protein. The microscopic protein dynamics on pico-to-nanosecond time scale is governed by the hydration shell rather than bulk solvent [3,4]. Even in a solvent with a potential for preferential protein hydration, such as an aqueous salt solution, the protein is coupled to the dynamics of its immediate hydration shell, whose chemical composition and dynamics temperature dependence may differ greatly from those of the bulk solvent [46]. Thus, a solvent designed to sustain the low-temperature protein dynamics should be chemically homogeneous, with no potential for preferential hydration, while the protein should be lyophilized prior to exposure to the solvent.

In this study, we chose a model protein lysozyme so that a comparison could be made between the standard solvent (water) and the solvent of interest (carbon disulfide). While illustrative, the limitation of this approach is that the assessment of the enzymatic activity of lysozyme relies on the presence of water [1,47]. As a consequence, we could not test an intriguing idea that the sustained microscopic protein dynamics could be associated with the protein biological activity sustained down to very low temperatures. There have been reports in favor of [48-51] and against [10,13,14] the hypothesis of a direct link between the onset of the microscopic dynamics and the biological activity in proteins. However, such measurements in commonly encountered aqueous environments are complicated by the limitations of transport of products and reactants at high solvent viscosities associated with low temperatures. A non-glass forming solvent such as CS₂ exhibits low viscosity all the way down to freezing temperature and can be used to test whether a solvated protein could sustain bioactivity at a level comparable to the ambient temperature bioactivity if the protein can function in a completely water-free environment. Several studies of proteins in organic solvents have demonstrated that proteins often remain folded and may show substantial biological activity in aqueous-free media [52-54]. An important point, specifically emphasized in reference [52] is that, although it is commonly believed that proteins will unfold and/or aggregate in non-polar organic solvents, this notion usually comes from studies of proteins in aqueous-organic mixtures, whereas proteins in pure organic solvents tend to be much more stable. We emphasize again that we used carbon disulfide

to dissolve lyophilized lysozyme, not lysozyme exposed to ambient humidity. At the same time, we cannot rule out that a combination of carbon disulfide and lysozyme happens to be especially favorable from the standpoint of protein stability in a non-polar solvent.

5. Conclusion

The solvent that we have chosen for the present study of microscopic protein dynamics, carbon disulfide, has several interesting and useful properties. It is a non-polar solvent that could dissolve water-free, lyophilized lysozyme, at least at a 10 mg/mL concentration. Unlike any protein solvent utilized in the previously reported studies of temperature dependence of protein microscopic relaxations, carbon disulfide is a non-glass former, with a rather weak temperature dependence of viscosity all the way down to freezing below 160 K. It does not form hydrogen bonds with the protein, while still providing a thermal bath to the protein. Importantly for neutron scattering experiments, the neutron scattering cross-section of CS₂ is completely coherent, so the scattering intensity from the solvent concentrates near the maxima of its structural factor, and there is no Q -independent incoherent scattering signal from the solvent. This is very useful for studying bulk-like protein solutions, since distinguishing the incoherent signal from a protein at 10 mg/mL concentration would be difficult even with a deuterated solvent, because of the sizable incoherent scattering cross-section of deuterium. In a stark contrast with a hydrated lysozyme, the lysozyme dissolved in CS₂ shows no protein dynamical transition, while sustaining its microscopic relaxations at a rate similar to that measured at ambient temperature. This demonstrates that protein dynamical transition is not merely solvent-assisted, but rather solvent-induced, or, more precisely, is a mere reflection of the temperature dependence of the solvent's glass-forming dynamics. In fact, it has been suggested [55] that the protein dynamical transition as measured in hydrated proteins is a manifestation of a generic relationship between the solvent (not necessarily aqueous) and the soft matter host (not necessarily biologically relevant). Finally, we hypothesize that, if the long debated idea regarding the direct link between the microscopic relaxations and the biological activity in proteins is correct, then not only the microscopic relaxations, but also the activity, could be sustained in proteins all the way down to the freezing temperature of a non-glass forming solvent with a weak temperature dependence of its viscosity.

Acknowledgments

The authors are grateful to Q. Zhang for her generous help with sample preparation and handling. The neutron scattering experiments at Oak Ridge National Laboratory's (ORNL) Spallation Neutron Source were supported by the Scientific User Facilities Division, Office of Basic Energy Sciences, U.S. Department of Energy (DOE). The authors acknowledge ORNL's Center for Structural Molecular Biology (Project ERKP291) supported by the Office of Biological and Environmental Research, U.S. DOE. ORNL is managed by UTBattelle, LLC, for the U.S. DOE under Contract No. DE-AC05-00OR22725.

References

- [1] J. A. Rupley, G. Careri, Protein hydration and function, *Adv. Protein Chem.* 41 (1991) 37-172.
- [2] P. W. Fenimore, H. Frauenfelder, B. H. McMahon, F. G. Parak, Slaving: Solvent fluctuations dominate protein dynamics and functions, *Proc. Nat. Acad. Sci.* 99 (2002) 16047-16051.
- [3] P. W. Fenimore, H. Frauenfelder, B. H. McMahon, R. D. Young, Bulk-solvent and hydration-shell fluctuations, similar to alpha- and beta-fluctuations in glasses, control protein motions and functions, *Proc. Nat. Acad. Sci.* 101 (2004) 14408-14413.
- [4] H. Frauenfelder, G. Chen, J. Berendsen, P. W. Fenimore, H. Jansson, B. H. McMahon, I. R. Stroe, J. Swenson, R. D. Young, A unified model of protein dynamics, *Proc. Nat. Acad. Sci.* 106 (2009) 5129-5134.
- [5] W. Doster, M. Settles, Protein-water displacement distributions, *Biochim. Biophys. Acta* 1749 (2005) 173-186.
- [6] W. Doster, S. Cusack, W. Petry, Dynamical transition of myoglobin revealed by inelastic neutron-scattering, *Nature* 337 (1989) 754-756.
- [7] R. D. Young, P. W. Fenimore, Coupling of protein and environment fluctuations, *Biochim. Biophys. Acta* 1814 (2011) 916-921.
- [8] D. Russo, J. R. D. Copley, J. Ollivier, J. Teixeira, On the behaviour of water hydrogen bonds at biomolecular sites: Dependences on temperature and on network dimensionality, *J. Mol. Struct.* 972 (2010) 81-86.

- [9] E. Mamontov, H. O'Neill, Q. Zhang, W. Wang, D. J. Wesolowski, Common features in the microscopic dynamics of hydration water on organic and inorganic surfaces, *J. Phys.: Condens. Matt.* 24 (2012) 064104 (7 pages).
- [10] R. M. Daniel, J. C. Smith, M. Ferrand, S. Hery, R. Dunn, J. L. Finney, Enzyme activity below the dynamical transition at 220 K, *Biophys. J.* 75 (1998) 2504-2507.
- [11] R. M. Daniel, J. L. Finney, V. Reat, R. Dunn, M. Ferrand, J. C. Smith, Enzyme dynamics and activity: Time-scale dependence of dynamical transitions in glutamate dehydrogenase solution, *Biophys. J.* 77 (1999) 2184-2190.
- [12] L. Cordone, M. Ferrand, E. Vitrano, G. Zaccai, Harmonic behavior of trehalose-coated carbon-monooxy-myoglobin at high temperature, *Biophys. J.* 76 (1999) 1043-1047.
- [13] J. M. Braggar, R. V. Dunn, R. M. Daniel, Enzyme activity down to -100 degrees C, *Biochim. Biophys. Acta* 1480 (2000) 278-282.
- [14] R. V. Dunn, V. Reat, J. Finney, M. Ferrand, J. C. Smith, R. M. Daniel, Enzyme activity and dynamics: xylanase activity in the absence of fast anharmonic dynamics, *Biochem. J.* 346 (2000) 355-358.
- [15] V. Reat, J. L. Finney, A. Steer, M. A. Roberts, J. Smith, R. Dunn, M. Peterson, R. Daniel, Cryosolvents useful for protein and enzyme studies below -100 degrees C, *J. Biochem. Biophys. Meth.* 42 (2000) 97-103.
- [16] V. Reat, R. Dunn, M. Ferrand, J. L. Finney, P. M. Daniel, J. C. Smith, Solvent dependence of dynamic transition in protein solutions, *Proc. Nat. Acad. Sci.* 97 (2000) 9961-9966.
- [17] A. M. Tsai, D. A. Neumann, L. N. Bell, Molecular dynamics of solid-state lysozyme as affected by glycerol and water: A neutron scattering study, *Biophys. J.* 79 (2000) 2728-2732.
- [18] A. M. Tsai, T. J. Udovic, D. A. Neumann, The inverse relationship between protein dynamics and thermal stability, *Biophys. J.* 81 (2001) 2339-2343.
- [19] A. Paciaroni, S. Cinelli, G. Onori, Effect of the environment on the protein dynamical transition: A neutron scattering study, *Biophys. J.* 83 (2002) 1157-1164.
- [20] A. Paciaroni, A. Orecchini, S. Cinelli, G. Onori, R. E. Lechner, J. Pieper, Protein dynamics on the picosecond timescale as affected by the environment: a quasielastic neutron scattering study, *Chem. Phys.* 292 (2003) 397-404.
- [21] F. Gabel, M. Weik, B. P. Doctor, A. Saxena, D. Fournier, L. Brochier, F. Renault, P. Masson, I. Silman, G. Zaccai, The influence of solvent composition on global dynamics of

- human butyrylcholinesterase powders: A neutron-scattering study, *Biophys. J.* 86 (2004) 3152-3165.
- [22] M. T. Cicerone, C. L. Soles, Fast dynamics and stabilization of proteins: Binary glasses of trehalose and glycerol, *Biophys. J.* 86 (2004) 3836-3845.
- [23] A. L. Tournier, V. Reat, R. Dunn, R. Daniel, J. C. Smith, J. Finney, Temperature and timescale dependence of protein dynamics in methanol: water mixtures, *Phys. Chem. Chem. Phys.* 7 (2005) 1388-1393.
- [24] E. Cornicchi, M. Marconi, G. Omori, A. Paciaroni, Controlling the protein dynamical transition with sugar-based bioprotectant matrices: A neutron scattering study, *Biophys. J.* 91 (2006) 289-297.
- [25] D. Russo, The impact of kosmotropes and chaotropes on bulk and hydration shell water dynamics in a model peptide solution, *Chem. Phys.* 345 (2008) 200-211.
- [26] H. Jansson, F. Kargl, F. Fernandez-Alonso, J. Swenson, Dynamics of a protein and its surrounding environment: A quasielastic neutron scattering study of myoglobin in water and glycerol mixtures, *J. Chem. Phys.* 130 (2009) 205101 (13 pages).
- [27] S. Magazu, F. Migliardo, F. Affouard, M. Descamps, M. T. F. Telling, Study of the relaxational and vibrational dynamics of bioprotectant glass-forming mixtures by neutron scattering and molecular dynamics simulation, *J. Chem. Phys.* 132 (2010) 184512 (9 pages).
- [28] B. Varga, F. Migliardo, E. Takacs, B. Vertessy, S. Magazu, M. T. F. Telling, Study of solvent-protein coupling effects by neutron scattering, *J. Biol. Phys.* 36 (2010) 207-220.
- [29] X. Chu, E. Mamontov, H. O'Neill, Q. Zhang, Apparent decoupling of the dynamics of a protein from the dynamics of its aqueous solvent, *J. Phys. Chem. Lett.* 3 (2012) 380-385.
- [30] E. Mamontov, X. Chu, Water-protein dynamic coupling and new opportunities for probing it at low to physiological temperatures in aqueous solutions, *Phys. Chem. Chem. Phys.* 14 (2012) 11573-11588.
- [31] V. Garcia Sakai, S. Khodadadi, M. T. Cicerone, J. E. Curtis, A. P. Sokolov, J. H. Roh, Solvent effects on protein fast dynamics: implications for biopreservation, *Soft Matter* 9 (2013) 5336-5340.
- [32] E. Mamontov, H. O'Neill, Q. Zhang, Mean-squared atomic displacements in hydrated lysozyme, native and denatured, *J. Biol. Phys.* 36 (2010) 291-297.

- [33] V. Amarnath, D. C. Anthony, W. M. Valentine, D. G. Graham, The molecular mechanism of the carbon disulfide mediated cross-linking of proteins, *Chem. Res. Toxicol.* 4 (1991) 148-150.
- [34] W. M. Valentine, V. Amarnath, D. G. Graham, D. C. Anthony, Covalent cross-linking of proteins by carbon disulfide, *Chem. Res. Toxicol.* 5 (1992) 254-262.
- [35] W. M. Valentine, D. G. Graham, D. C. Anthony, Covalent cross-linking of erythrocyte spectrin by carbon disulfide in vivo, *Toxicol. Appl. Pharmacol.* 121 (1993) 71-77.
- [36] W. M. Valentine, V. Amarnath, K. Amarnath, F. Rimmele, D. G. Graham, Carbon disulfide mediated protein cross-linking by N,N-Diethyldithiocarbamate, *Chem. Res. Toxicol.* 8 (1995) 96-102.
- [37] W. M. Valentine, V. Amarnath, D. G. Graham, D. L. Morgan, R. C. Sills, CS₂-mediated cross-linking of erythrocyte spectrin and neurofilament protein: Dose response and temporal relationship to the formation of axonal swellings, *Toxicol. Appl. Pharmacol.* 142 (1997) 95-105.
- [38] Y. Zhao, W. Perez-Segarra, Q. Shi, A. Wei, Dithiocarbamate assembly on gold, *J. Am. Chem. Soc.* 127 (2005) 7328-7329.
- [39] H. Zhu, D. M. Coleman, C. J. Dehen, I. M. Geisler, D. Zemlyanov, J. Chmielewski, G. J. Simpson, A. Wei, Assembly of dithiocarbamate-anchored monolayers on gold surfaces in aqueous solutions, *Langmuir* 24 (2008) 8660-8666.
- [40] A. L. Eckermann, J. A. Shaw, T. J. Meade, Kinetic dispersion in redox-active dithiocarbamate monolayers, *Langmuir* 26 (2010) 2904-2913.
- [41] Y. Niu, A. I. Matos, L. M. Abrantes, A. S. Viana, R. G. Yin, Antibody oriented immobilization on gold using the reaction between carbon disulfide and amine groups and its application in immunosensing, *Langmuir* 28 (2012) 17718-17725.
- [42] E. Mamontov, K. W. Herwig, A time-of-flight backscattering spectrometer at the spallation neutron source, *BASIS, Rev. Sci. Inst.* 82 (2011) 085109 (10 pages).
- [43] S. Khodadadi, J. H. Roh, A. Kisliuk, E. Mamontov, M. Tyagi, S. A. Woodson, R. M. Briber, A. P. Sokolov, Dynamics of biological macromolecules: Not a simple slaving by hydration water, *Biophys. J.* 98 (2010) 1321-1326.
- [44] W. Doster, The protein-solvent glass transition, *Biochim. Biophys. Acta* 1804 (2010) 3-14.

- [45] J. D. Nickels, H. O'Neill, L. Hong, M. Tyagi, G. Ehlers, K. L. Weiss, Q. Zhang, Z. Yi, E. Mamontov, J. C. Smith, A. P. Sokolov, Dynamics of protein and its hydration water: Neutron scattering studies on fully deuterated GFP, *Biophys. J.* 103 (2012) 1566-1575.
- [46] E. Mamontov, H. O'Neill, Q. Zhang, S. M. Chathoth, Temperature dependence of the internal dynamics of a protein in an aqueous solvent: Decoupling from the solvent viscosity, *Chem. Phys.* 424 (2013) 12-19.
- [47] G. Careri, E. Gratton, P-H. Yang, J. A. Rupley, Correlation of IR spectroscopic, heat capacity, diamagnetic susceptibility and enzymatic measurements on lysozyme powder, *Nature* 284 (1980) 572-573.
- [48] F. Parak, E. W. Knapp, A consistent picture of protein dynamics, *Proc. Nat. Acad. Sci.* 81 (1984) 7088-7092.
- [49] B. F. Rasmussen, A. M. Stock, D. Ringe, G. A. Petsko, Crystalline Ribonuclease-A loses function below the dynamic transition at 220 K, *Nature* 357 (1992) 423-424.
- [50] M. Ferrand, A. J. Dianoux, W. Petry, G. Zaccai, Thermal motions and function of bacteriorhodopsin in purple membranes – effects of temperature and hydration studied by neutron-scattering, *Proc. Nat. Acad. Sci.* 90 (1993) 9668-9672.
- [51] A. Ostermann, R. Waschipky, F. G. Parak, G. U. Nienhaus, Ligand binding and conformational motions in myoglobin, *Nature* 404 (2000) 205-208.
- [52] A. M. Klibanov, Improving enzymes by using them in organic solvents, *Nature* 409 (2001) 241-246.
- [53] P. J. Halling, What can we learn by studying enzymes in non-aqueous media?, *Phil. Trans. R. Soc. Lond. B* 359 (2004) 1287-1297.
- [54] D. S. Clark, Characteristics of nearly dry enzymes in organic solvents: implications for biocatalysis in the absence of water, *Phil. Trans. R. Soc. Lond. B* 359 (2004) 1299-1307.
- [55] E. Mamontov, V. K. Sharma, J. M. Borreguero, M. Tyagi, Protein-style dynamical transition in a non-biological polymer and a non-aqueous solvent, *J. Phys. Chem. B* 120 (2016) 3232-3239.