Experimental mapping of short-wavelength phonons in proteins

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Graphical abstract

Public summary

- Quantum phenomena in biology have long fascinated people around the world
- This work presents a direct experimental observation of phonons, the quantum vibrations in a protein
- The collective excitations or phonons in proteins were detected by utilizing inelastic neutron scattering technique at Oak Ridge National Laboratory
- Our results illustrate the flexibility-activity relationship in proteins by mapping the temperature and hydration dependence of these collective excitations
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Phonons are quasi-particles, observed as lattice vibrations in periodic materials, that often dampen in the presence of structural perturbations. Nevertheless, phonon-like collective excitations exist in highly complex systems, such as proteins, although the origin of such collective motions has remained elusive. Here we present a picture of temperature and hydration dependence of collective excitations in green fluorescent protein (GFP) obtained by inelastic neutron scattering. Our results provide evidence that such excitations can be used as a measure of flexibility/softness and are possibly associated with the protein’s activity. Moreover, we show that the hydration water in GFP interferes with the phonon propagation pathway, enhancing the structural rigidity and stability of GFP.

INTRODUCTION

The motion of life, specifically protein dynamics, plays a vital role in biological activities. The timescale of internal motions in protein ranges from femtoseconds to milliseconds, analogous to supercooled liquids and glasses. Such internal motions are essential for enzyme catalysis, conformational transitions, binding affinities, transfer of ions, transport of metabolic energy (ATP hydrolysis), and the folding-unfolding mechanism. The conventional ideas of biological processes come from the observation of slow binding and conformational changes in proteins occurring on a timescale of a few microseconds to milliseconds. However, recent studies have stressed that harmonic vibrations (on the order of femtoseconds to picoseconds) are precursors of the biological activities in proteins and enzymes.

Despite a long history of study of protein dynamics, it remains elusive how the softness of a protein is associated with its activity. Moreover, many studies have reported that protein becomes flexible or soft upon hydration required for activity. Hydration water is believed to affect fast and slow motions in proteins by forming hydrogen bond networks. However, the collective excitations (fast motion) in proteins are highly damped and short-lived (on the order of tens of femtoseconds to picoseconds) because of glass-like behavior, making them experimentally challenging to measure.

Phonon-like collective excitations in proteins have been investigated using inelastic X-ray scattering (IXS), and however, IXS has a limited energy resolution, especially for studying low-frequency collective excitations. Compared with IXS, coherent inelastic neutron scattering (INS) technique is exceptionally suitable for investigating low-frequency collective excitations and has been applied successfully to study collective density fluctuations in amorphous materials, glasses, or liquids. Moreover, INS has a significant advantage when studying biological samples because it has much better resolution at the biologically relevant energy and length scales but causes almost no radiation damage compared with IXS. However, a large amount of fully deuterated protein samples is required for high-resolution INS experiments because of the huge incoherent scattering cross-section of hydrogen atoms to neutrons, hindering mapping and analysis of such collective motions in past decades.

In this work, a powerful state-of-the-art INS spectrometer allowed us to map the temperature and hydration dependence of collective excitations in proteins with high precision and relate them to a protein’s biological activity. Here we report INS study of a model protein, perdeuterated green fluorescent protein (dGFP), dry and hydrated, to investigate low-frequency intra-protein collective motions on the order of femtoseconds to picoseconds. GFP has a remarkable regular structure that is stable and resistant to unfolding so that even the water molecules on the outside of the barrel form “stripes.” Therefore, GFP is a suitable model system for INS measurements. In our experiment, we observed propagating acoustic modes from both samples. Further analysis of such excitations helped us to understand the relations between protein flexibility/softness and its activity from a physical perspective.

RESULTS

The INS experiments were performed on a fine-resolution Fermi chopper spectrometer, SEQOIA, at the Spallation Neutron Source (SNS), Oak Ridge National Laboratory (ORNL). Details regarding the measurements, sample preparation, and rationale behind using the perdeuterated samples for INS measurements are described in the supplemental information. The hydrated sample of dGFP had a hydration level of h = 0.37 (i.e., 0.37 g of D2O per 1 g of dry dGFP). Such a hydration level forms at least a monolayer of hydration water around the protein and is linked to its bioactivity. The UV-visible (UV-vis) emission spectra of protonated GFP (hGFP) and dGFP samples are substantially the same (Figure S1), confirming that the deuteration has no adverse effect on the protein’s activity in the in vitro experiment. Here we considered dry (yellow) and hydrated (bright green) samples of dGFP inactive and active states, respectively, in terms of their optical properties (Figure 1). The measured INS spectra or dynamic structure factors S(Q,E) are shown in Figure S2 for dry and D2O-hydrated dGFP samples at different temperatures as a function of neutron momentum and energy transfers (Q and E).

The static structure factors S(Q) of dry and hydrated samples are compared at a series of temperatures, T = 150-270 K (Figures 1A and 1B). S(Q) was calculated using the relation S(Q) = |S(Q,E)|dE, where the integration of energy depends on each Q value (Figure S2). While integrating the energy transfer (ÆE) at each specific Q value, the same energy ranges were considered for dry and hydrated samples. For the dry sample, a small peak at Q < 0.6 Å−1 represents the distance between the secondary structures (i.e., central α-helix and β-barrel strand, approximately equal to 10.5 Å). The prominent peak observed at Qdry = 1.4 Å−1 corresponds to the spacing between the β-barrel strands being nearly equal to 4.5 Å. The additional broader peak at Q ~ 2.9 Å−1 represents the local structure, a fluorophore, and amino acid residues. On the other hand, for the hydrated sample, the peak at Q ~ 0.6 Å−1 shifts to a slightly lower Q, possibly because of a slight expansion of the β-barrel to accommodate the water molecules. The prominent peak at Qhyd ~ 1.4 Å−1 becomes broader and then shifts toward a slightly higher Q value, Qhyd ~ 1.6 Å−1. The additional broader peak at Q ~ 2.9 Å−1 flattens out because of the change in the local structure (Figures 1A, 1B, and S3). There is also a sign of ice crystals in the hydrated sample (see supplemental information for more details).

Here we hypothesized that the softness or stiffness (opposite of softness) of a protein upon hydration depends predominantly on the tertiary structure in 3 dimensions. The temperature dependence of S(Q) can be observed above Q ~ 1.6 Å−1 for dry and hydrated dGFP, where S(Q) values decrease with an increase in temperature. The overlap of S(Q) below Q ~ 1.4 Å−1 confirms the scattering signal dominated by protein in both samples, nearly independent of temperature (Figures 1A, 1B, and S3). However, above Q ~ 1.4 Å−1, the higher intensity in the hydrated sample indicates that the protein hydration shell makes it more rigid than the dry sample.

The measured INS signal arises from the detection of collective excitations in dGFP (Figure 2). The dynamic coherent structure factor S(Q,E) was fitted using a damped harmonic oscillator (DHO) model with the following expression:

\[
S(Q, E) = \left[ I_0(Q) \delta(E) + g(E) I_1(Q) \right] \left\{ \frac{1}{(E - \Omega(Q))^2 + (I(Q))^2} \right\}^{-1} \left\{ \frac{1}{(E + \Omega(Q))^2 + (I(Q))^2} \right\} + (A + E + B) \Theta(R, Q, E) \quad (\text{Equation 1})
\]

Understanding the relations between protein flexibility/softness and its activity from a physical perspective.
where \( R(Q, E) \) is the instrumental resolution function, and \((A \cdot E + B)\) is the background term. The additional “slop” term \((A \cdot E + B)\) in the background of Equation 1 was included to improve the overall fit quality even though the positions of the fitted inelastic features were not affected by the fit with or without this term. Such a sloped background term likely originates from the additional experimental background (e.g., from epithermal neutrons) that is roughly constant as a function of time of flight and yields a sloped background upon data conversion to energy transfer. \( I(Q) \) and \( \delta(E) \) are the elastic intensity and the delta function, respectively, in the elastic component, and \( I_1(Q), \Omega(Q) \) and \( R(Q) \) are the inelastic intensity, excitation energy, and damping factor, respectively, of the DHO function describing the collective phonon excitations. Also, the Bose thermal factor \( g(E) \) or the temperature-dependent correction in the DHO function \( g(E) \) is given by

\[
g(E) = \frac{1}{1 - \exp(-E/k_BT)}
\]

\((\text{Equation 2})\), where \( k_BT \) is the Boltzmann constant and \( T \) is the absolute temperature. The DHO model has been implemented successfully to study Brillouin-like inelastic side peaks in liquids and amorphous materials, including biomolecules. The DHO model has been implemented successfully to study Brillouin-like inelastic side peaks in liquids and amorphous materials, including biomolecules.24,26,38

**Figures 2A and 2B illustrate the fitting of measured INS spectra \( S(Q, E) \) using Equation 1 at \( T = 150 \) K and \( Q = 0.64 \) Å\(^{-1}\). The Brillouin-like side peaks of DHO are observed at the energy positions \( Q \) that indicate the excitation energy of the collective motions. The dispersion curves of collective excitations can be determined from these DHO side peaks at all measured temperatures for both samples (Figures 2C, 2D, 3, and 4), where the black dashed lines represent the acoustic branches of phonon energy dispersion fitted with Equation 3 in the region \( Q \leq 1.1 \) Å\(^{-1}\). The dispersion curves overlaid on top of 2-dimensional (2D) raw INS spectra for dGFP samples at \( T = 150 \) K and \( Q = 0.64 \) Å\(^{-1}\) confirm that the calculated excitation energies are larger than the instrumental resolution (Figures 2C and 2D). Furthermore, the bending of the energy dispersion curve occurs at the boundary of the acoustic branch (i.e., \( Q_m \approx 1.1 \) Å\(^{-1}\)), suggesting the existence of a pseudo-Brillouin zone with a finite group velocity up to the value of \( Q_m \), after which a plateau develops or \( \Omega(Q) \) starts to deviate from the usual Q dependence.

The value of \( Q_m \approx 1.1 \) Å\(^{-1}\) corresponds to the topological disorder length scale in the sample or half of the distance to the nearest pseudo-reciprocal lattice point. The plateau, or the lack of significant dispersion at higher Q-values above \( Q_m \), indicates the end of such wave propagation, exemplifying the localized collective modes.

Furthermore, SEQUOIA has an energy resolution of less than 2 meV and a dynamic range of ~30 meV (Figure S2), capable of resolving acoustic phonons in proteins. As a result, we observed low-frequency coherent motions in dry and hydrated samples of dGFP, as confirmed by the energy dispersion curves (Figures 3 and 4). Such motions arise from periodic vibrations of predominant secondary structures, such as \( \beta \)-barrels, in dGFP. Here the phonon-like propagation takes place on a length scale larger than 5 Å, where \( Q_m = 1.1 \) Å\(^{-1}\) can be considered a boundary above which the excitations are localized. This size resembles the protein secondary and tertiary structures participating in biological activity. On the other hand, the localized excitations at \( Q > Q_m \) are mainly attributed to vibrations of fluorophore plus amino acid residues for the dry sample and fluorophore plus amino acid residues plus D\(_2\)O for the hydrated sample, which are on a length scale of 2.0–5.0 Å.

The energy dispersion curves \( \Omega \) versus \( Q \) show the propagation of acoustic modes in the longer wavelength-limit \( Q \leq Q_m \) at all measured temperatures of \( T = 150–270 \) K for dGFP samples (Figures 3A, 3B, and 4). We designate these modes as acoustic because extrapolation of the excitation energy in the dispersion curve is approaching the value zero in the limit of low Q. Because of amorphous protein samples that lack a preferred orientation, it is not easy to explicitly differentiate between longitudinal and transverse acoustic modes. However, the calculated values of low-frequency phonon-like excitations can be attributed to the transverse acoustic mode, which is in good agreement with recently published molecular dynamics simulations and experiments. Furthermore, for \( Q \geq Q_m \), an apparent softening of the localized intra-protein vibrations with an increase in temperature is observed, as indicated by the decrease in the excitation energy in both samples, whereas the acoustic mode is nearly temperature independent. It suggests that dry and hydrated proteins become softer as the temperature increases.

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*Figure 1. Static structure factors of dry and D\(_2\)O hydrated dGFP samples, measured by INS at temperatures of 150–270 K (A and B). Static structure factors of (A) dry and (B) D\(_2\)O-hydrated dGFP samples, measured by INS as functions of wave-vector transfer \( Q \) at temperatures of 150–270 K. The error bars are within the size of the symbols.*
The apparent increase in the half-width at half-maximum (HWHM) of the inelastic side peaks or the phonon damping parameter ($\Gamma$) with temperature (Figures 3C and 3D) suggests an increase in damping of intra-protein collective vibrations because of emerging relaxation and diffusion processes or anharmonic motion. It corresponds to the lifetime ($\tau = 2/\kappa \Gamma$) of the collective excitations and is fitted with the relation $\Gamma(Q) = AQ^2$ at low $Q$ values (dashed line). $Q_m$ refers to the values of $Q$, above which $\Omega(Q)$ and $\Gamma(Q)$ start to deviate from their usual $Q$ dependence.
lines in Figures 3C and 3D) as in ordinary glasses but becomes flat or deviates from the usual $Q^2$-dependence for $Q > Q_m$ because of the localized excitations. Furthermore, it is interesting to note that the protein energy dispersion curve also follows the relation $\Omega(Q_m) = \Gamma(Q_m)$, similar to glasses and glass-forming liquids. In addition, the ratio $Q_m/Q_p$ measures the fragility of the sample and is found to be $\sim 0.8$ and $\sim 0.7$ for dry and hydrated dGFP, respectively, much larger than the values observed in glasses and glass-forming liquids such as o-terphenyl, glycerol, and SiO$_2$. This signifies that GFP has considerable dynamic heterogeneity (ascribe to its secondary and tertiary structures) compared with glasses, so that a slight temperature change can reorganize structures in many orientations or a large number of conformational substrates in a rugged free energy landscape (Figure 5A). The complete set of comparison of energy dispersion curves between the dry and hydrated protein samples at temperatures of $T = 150$–$260$ K (Figure 4) suggests that the presence of water molecules in GFP increases the energy of the collective excitations and increases its rigidity.

The energy dispersion curves in the acoustic branch can be fitted with a simplified relation given by the Born-von Karman lattice dynamics theory in one dimension, expressed as

$$E(\text{meV}) = 4.192 \times 10^{-3} \frac{v(m/s)}{Q_m(\text{Å}^{-1})} \sin \left( \frac{\pi Q_m(\text{Å}^{-1})}{2 Q_m(\text{Å}^{-1})} \right)$$

(Equation 3)

where $v$ is the velocity of the collective wave propagation. The fittings are shown in Figures 2C, 2D, and 4 as dashed lines. Although the model only considers the nearest-neighbor interactions mainly applicable to the single crystals, it successfully fits the data from the amorphous form of the protein samples. The calculated velocities of the acoustic mode from dry and hydrated dGFPs are compared in Figure 5B, indicating that the propagation is slightly faster in the hydrated sample because of reduced softness. The sound velocities are barely temperature
dependent, with average values of $1.194 \pm 13$ m/s and $1.269 \pm 9$ m/s for dry and hydrated dGFP samples, respectively, very close to the fast transverse sound speed of water, which is about $1,500$ m/s. Because these sound velocities are due to the low-frequency (transverse) acoustic mode, these values are much smaller than the velocities in proteins observed using IXS (12,30,31) due to the high-frequency (longitudinal) acoustic mode, which are close to the fast sound velocity of heavy water, about $3,300$ m/s. The calculated velocities in this study are in good agreement with the average sound velocity of about $1,200$ m/s in lysozyme (in solution), obtained using ultrasound velocimetry, and of $1,605$ m/s in amorphous myoglobin, obtained using the Mössbauer effect. Also, the sound velocity of a transverse acoustic mode in a protein active-site mimic (chloro(octaethyloporphyrinato)iron(III)) has been found to be nearly $1,011$ m/s, using IXS and vibrational spectroscopy techniques. The slightly higher velocity in hydrated dGFP than in the dry one is due to a slight increase in stiffness caused by the presence of water molecules inside the β-barrels and water’s strong coupling to the protein surface. Our result is qualitatively consistent with a previously reported result from an incoherent elastic neutron scattering experiment, where dry dGFP was softer than its hydrated counterpart in the temperature range of $100$ K. However, the two experiments are technically different, providing dissimilar dynamical information. Incoherent elastic neutron scattering measures the self-correlated dynamics of hydrogen atoms. In contrast, coherent INS measures the collective excitations contributed mainly by nitrogen, deuterium, carbon, and oxygen atoms in the sample.

**DISCUSSION**

The flexibility and stability of biomolecules are seemingly conflicting. However, both are essential for biochemical and biophysical processes such as molecular reactions, protein-ligand interactions, conformational fluctuations, and free energy of protein folding. Our work reveals the relation of fast coherent motion to a protein’s intrinsic flexibility regarding temperature and hydration. The experimentally obtained energy dispersion curves show propagation of the acoustic mode in dGFP, which is nearly independent of temperature in dry and hydrated samples (Figure 5B), whereas the phonon energy of intra-protein localized collective modes decreases with temperature (Figures 3A and 3B). This indicates that dry and hydrated proteins become softer, or more flexible, as temperature increases. The larger energy values of transverse acoustic propagation and localized modes in dGFP upon hydration suggest that the protein becomes significantly more rigid or more stable, because of accommodation of water molecules in the β-barrels. This fact is consistent with the theory that proteins must keep their native structures thermally stable, which is necessary for their biological activity. In this way, the phonon behaviors measured in our current study bridge these two conflicting properties of proteins (i.e., stability and flexibility) and further relate them to protein bioactivities, specifically upon hydration and in their native states.

Here we observe experimentally the collective or phonon-like excitations in proteins that are due to harmonic vibrations of amino acid residues and secondary/tertiary structural segments. Our results provide experimental evidence that the free energy landscape of a protein is rugged even in the lowest free energy basin because of harmonic motions (shown schematically in Figure 5A), where the protein structure hops around neighboring conformational substates separated by small energy barriers that are lower than $k_BT$. Furthermore, the collective modes can measure the softness or flexibility of the protein, which are related to the protein’s activity. The measured excitation energy of the order of a few meV in protein is a dynamic entity that can be used as a precursor of a protein’s activity. Moreover, we observed an apparent temperature and hydration dependence of collective excitations in GFP that are connected to its optical properties (i.e., dry and hydrated samples emitted yellow and bright green fluorescent light, respectively). Interestingly, the phonon energy of acoustic and localized collective modes increases significantly upon hydration, indicating that GFP has a more rigid/stable but flexible structure in its native state. We attribute this to the mechanical packing and folding necessary for the activity of GFP upon hydration.

**MATERIALS AND METHODS**

The details of protein sample preparation and neutron scattering experiments are described in the supplemental information.

**REFERENCES**

1. Frauenfelder, H., Sligar, S.G., and Wolynes, P.G. (1991). The energy landscapes and motions of proteins. Science 254, 1598–1603.
2. Henzler-Wildman, K., and Kern, D. (2007). Dynamic personalities of proteins. Nature 450, 964–972.
3. Hay, S., and Scrutton, N.S. (2012). Good vibrations in enzyme-catalysed reactions. Nat. Chem. 4, 161–168.
4. Schwartz, S.D., and Schramm, V.L. (2009). Enzymatic transition states and dynamic motion in barrier crossing. Nat. Chem. Biol. 5, 551–558.
5. Klinman, J.P., and Kohen, R. (2013). Hydrogen tunneling links protein dynamics to enzyme catalysis. Annu. Rev. Biochem. 82, 471–496.
6. Berko, S.J., Hammes, G.G., and Hammes-Schiffer, S. (2008). Free-energy landscape of enzyme catalysis. Biochemistry 47, 3317–3321.
7. Cheatham, T., and Kohen, R. (2013). Relationship of femtosecond-picosecond dynamics to enzyme-catalyzed H-transfer. Top. Curr. Chem. 337, 1–39.
8. Zacca, G. (2000). How soft is a protein? A protein dynamics force constant measured by neutron scattering. Science 288, 1604–1607.
9. Conti-Nibali, V., and Havenith, M. (2014). New insights into the role of water in biological function: studying solvated biomolecules using terahertz absorption spectroscopy in conjunction with molecular dynamics simulations. J. Am. Chem. Soc. 136, 12800–12807.
10. Gabel, F., Bicout, D., Lehniert, U., et al. (2002). Protein dynamics studied by neutron scattering. Q. Rev. Biophys. 35, 327–367.
11. Sokolov, A.P., Rohl, J.H., Mamontov, E., and Garcia-Sakai, V. (2008). Role of hydration water in dynamics of biological macromolecules. Chem. Phys. 345, 212–218.
12. Mamontov, E., and Chu, X-Q. (2012). Water–protein dynamic coupling and new opportunities for probing it at low to physiological temperatures in aqueous solutions. PCCP 14, 11573–11588.
13. Chu, X-Q., Lagi, M., Mamontov, E., et al. (2010). Experimental evidence of logarithmic relaxation in single-particle dynamics of hydrated protein molecules. Soft Matter 6, 2623–2627.
14. Chu, X-Q., Mamontov, E., O’Neill, H., and Zhang, Q. (2012). Apparent decoupling of the dynamics of a protein from the dynamics of its aqueous solvent. J. Phys. Chem. Lett. 3, 380–385.
15. Mamontov, E., O’Neill, H., Zhang, Q., and Chatoth, S.M. (2013). Temperature dependence of the internal dynamics of a protein in an aqueous solvent: decoupling from the solvent viscosity. Chem. Phys. 424, 12–19.
16. Hong, L., Smolin, N., Lindner, B., et al. (2011). Three classes of motion in the dynamic neutron-scattering susceptibility of a globular protein. Phys. Rev. Lett. 107, 148102.

17. Qin, Y., Wang, L., and Zhong, D. (2016). Dynamics and mechanism of ultrafast water-protein interactions. Proc. Natl. Acad. Sci. U S A 113, 8424–8429.

18. Nickels, J.D., Perticaroli, S., O’Neill, H., et al. (2013). Coherent neutron scattering and collective dynamics in the protein, GFP. Biophys. J. 105, 2182–2187.

19. Liu, D., Chu, X.-Q., Lagi, M., et al. (2008). Studies of phononlike low-energy excitations of protein molecules by inelastic x-ray scattering. Phys. Rev. Lett. 101, 1–4.

20. Li, M., Chu, X.-Q., Fratini, E., et al. (2011). Phonon-like excitation in secondary and tertiary structure of hydrated protein powders. Soft Matter 7, 9648.

21. Wang, Z., Bertrand, C.E., Chiang, W.S., et al. (2013). Inelastic X-ray scattering studies of the short-time collective vibrational motions in hydrated lysozyme powders and their possible relation to enzymatic function. J. Phys. Chem. B 117, 1186–1195.

22. Shrestha, U.R., Bhowmik, D., Van Delinder, K.W., et al. (2017). Collective excitations in protein as a measure of balance between its softness and rigidity. J. Phys. Chem. B 121, 923–930.

23. Leu, B.M., Alatas, A., Sinn, H., et al. (2010). Protein elasticity probed with two synchrotron-based techniques. J. Chem. Phys. 132, 1–7.

24. Cunsolo, A., Kodituwakku, C.N., Bencivenga, F., et al. (2012). Transverse dynamics of water across the melting point: a parallel neutron and x-ray inelastic scattering study. Phys. Rev. B 85, 174305.

25. Teixeira, J., Bellissent-Funel, M.C., Chen, S.H., and Dornen, B. (1985). Observation of new short-wavelength collective excitations in heavy water by coherent inelastic neutron scattering. Phys. Rev. Lett. 54, 2681–2683.

26. Suck, J.-B. (2008). Inelastic neutron scattering applied to the investigation of collective excitations in topologically disordered matter. Condens. Matter Phys. 11, 7.

27. Li, X.Y., Zhang, H.P., Lan, S., et al. (2020). Observation of high-frequency transverse phonons in metallic glasses. Phys. Rev. Lett. 124, 225902.

28. Bellissent-Funel, M.C., Teixeira, J., Chen, S.H., et al. (1989). Low-frequency collective modes in dry and hydrated proteins. Biophys. J. 56, 713–716.

29. Phillips, G.N. (1997). Structure and dynamics of green fluorescent protein. Curr. Opin. Struct. Biol. 7, 821–827.

30. Leu, B.M., Sage, J.T., Silvernail, N.J., et al. (2011). Bulk modulus of a protein active-site mimic. J. Phys. Chem. B 115, 4469–4473.

31. Yoshida, K., Hosokawa, S., Baron, A.Q., and Yamaguchi, T. (2010). Observation of high-frequency transverse phonons in dry and hydrated proteins. Biophys. J. 103, 1566–1576.

32. Granroth, G.E., Kolesnikov, A.I., Sherline, T.E., et al. (2010). SEQUOIA: a newly operating high-resolution inelastic neutron scattering facility. Sci. Rep. 1, 012058.

33. Rupley, J.A., Gratton, E., and Careri, G. (1983). Water and globular proteins. Trends Biochem. Sci. 8, 18–22.

34. Yang, F., Moss, L.G., and Phillips, G.N. (1996). The molecular structure of green fluorescent protein. Nat. Biotechnol. 14, 1246–1251.

35. Mamontov, E., De Francesco, A., Formisano, F., et al. (2012). Water dynamics in a lithium chloride aqueous solution probed by Brillouin neutron and x-ray scattering. J. Phys. Condens Matter 24, 1–9.

36. Comicioli, E., Sebastiani, F., De Francesco, A., et al. (2011). Collective density fluctuations of DNA hydration water in the time-window below 1 ps. J. Chem. Phys. 133, 1–7.

37. Orecchini, A., Paciarotti, A., Franceschi, A., et al. (2008). Collective dynamics of protein hydration water by Brillouin neutron spectroscopy. J. Am. Chem. Soc. 131, 4664–4669.

38. Papino, E.A., Rodriguez-Tinoco, C., Kirsch, M., et al. (2013). Acoustic-like dynamics of amorphous drugs in the THz regime. Sci. Rep. 3, 1–5.

39. Wang, Z., Zhang, W.S., Le, P., et al. (2014). One role of hydration water in proteins: key to the “softening” of short time intraprotein collective vibrations of a specific length scale. Soft Matter 10, 4298–4303.

40. Paciarotti, A., Orecchini, A., Haertlein, M., et al. (2012). Vibrational collective dynamics of dry proteins in the terahertz region. J. Phys. Chem. B 116, 3861–3865.

41. Mamontov, E., Vakhрушев, S.B., Kumzerov, Y.A., et al. (2009). Acoustic phonons in chrysotile asbestos probed by high-resolution inelastic x-ray scattering. Solid State Commun. 149, 589–592.

42. Sette, F., Kirsch, M.H., Masciovecchio, C., et al. (1998). Dynamics of glasses and glass-forming liquids studied by inelastic X-ray scattering. Science 280, 1550–1555.

43. Burké, E. (2000). Phonon spectroscopy by inelastic x-ray scattering. Rep. Prog. Phys. 63, 171–232.

44. Conti Nibali, V., D’Angelo, G., Paciarotti, A., et al. (2014). On the coupling between the collective dynamics of proteins and their hydration water. J. Phys. Chem. Lett. 5, 1181–1186.

45. Pfeffer, H., and Heremans, K. (2002). Apparent sound velocity of lysozyme in aqueous solutions. Phys. Rev. Lett. 86, 225–230.

46. Leu, B.M., Sage, J.T., Silvernail, N.J., et al. (2011). Observation of phononlike low-energy excitations of protein molecules by inelastic x-ray scattering. Phys. Rev. Lett. 107, 1–4.

47. Shrestha, U.R., Bhowmik, D., Van Delinder, K.W., et al. (2017). Collective excitations in protein as a measure of balance between its softness and rigidity. J. Phys. Chem. B 121, 923–930.

48. Levy, Y., and Onuchic, J.N. (2006). Water mediation in protein folding and molecular recognition. Annu. Rev. Biophys. Biomol. Struct. 35, 389–415.

49. Frauenfelder, H., Parak, F., and Young, R.D. (1988). Conformational substates in proteins. Proc. Natl. Acad. Sci. U S A 104, 18049–18054.

50. Tarek, M., and Tobias, D.J. (2002). Role of protein-water hydrogen bond dynamics in the protein dynamical transition. Phys. Rev. Lett. 88, 138101.

51. Nickels, J.D., O’Neill, H., Hong, L., et al. (2012). Dynamics of proteins and its hydration water-neutron scattering studies on fully deuterated GFP. Biophys. J. 103, 1566–1576.

52. Isaksen, G.V., Aqvist, J., and Brandsdal, B.O. (2014). Protein surface softness is the origin of enzyme cold-adaptation of trypsin. PLoS Comput. Biol. 10, e1003813.

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AUTHOR CONTRIBUTIONS

X.C. designed the research. X.C., E.M., H.M.O., Q.Z., and A.I.K. performed the experiments. U.R.S. analyzed the data. X.C. and U.R.S. wrote the paper with input from all authors.

DECLARATION OF INTERESTS

The authors declare no competing interests.

SUPPLEMENTAL INFORMATION

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