Hydration Water in Protein is crucial to the "Softening" of the Short Time Intraprotein Collective Vibrations of Specific Length Scale

Sow-Hsin Chen (sowhsin@mit.edu)
Department of Nuclear Science and Engineering, MIT

Collaborators:

Zhe Wang (MIT), Wei-Shan Chiang (MIT), Pei-Si Le (MIT), Kao-Hsiang Liu (MIT)
Ahmet Alatas (APS), Emiliano Fratini (U of Florence), Piero Baglioni (U of Florence)

This project is funded by *DE-FG02-90ER45429*, U.S. DOE.

An Invite Talk Given At BNL-NSLS-II Early Science Workshop on October 1, 2013.

Comparison of INS and IXS

1. The double differential cross-sections for INS and IXS are formally identical if one replace the coherent neutron scattering length of a nucleus b by $r_0 f(Q)$ of the atom, where $f(Q)$ is the electronic form factor, which has the maximum value Z.

$$\frac{d^2\sigma}{d\Omega d\omega} = \left(\frac{d\sigma}{d\Omega}\right)_0 S(Q,\omega)$$

$$\left(\frac{d\sigma}{d\Omega}\right)_0 = \frac{k_f}{k_i} b^2 \quad \text{for INS}$$

$$\left(\frac{d\sigma}{d\Omega}\right)_0 = \frac{k_f}{k_i} (\hat{\varepsilon}_i \cdot \hat{\varepsilon}_f)^2 r_0^2 f^2(Q) \quad \text{for IXS}$$

2. The scattering cross-section for x-rays is about 10 times smaller than that for thermal neutrons for hydrogen atom. A problem of neutron scattering from proteins is that the scattering cross-section is dominated by incoherent scattering from hydrogen atoms contained in it.

x-rays $(Zr_0)^2 = 7.95 \times 10^{-26} Z^2 \text{cm}^2$

neutrons $b^2 = 4.45 \times 10^{-25} \text{cm}^2$ for D^2

3. Neutrons are massive particles for which the energy is proportional to the wave number square while x-rays are photons without the rest mass for which the energy is proportional to the wave number.

x-rays: $\lambda[\text{Å}] = \dfrac{12400}{E(eV)}$; $k[\text{Å}^{-1}] = \dfrac{E[eV]}{1974}$

neutrons: $\lambda[\text{Å}] = \dfrac{0.286}{\sqrt{E[eV]}}$; $k[\text{Å}^{-1}] = 22.0\sqrt{E[eV]}$

Thus energy loss or gain of the order of $k_B T$ in a neutron scattering process would change the magnitude of the incident neutron wave-vector substantially. But for x-ray scattering, the same level of energy exchange will hardly change the magnitude of the wave-vector. Thus at a constant scattering angle, IXS will always keep a constant $\vec{Q}$ but not for INS.

4. The resolution and transmission functions change shape and normalization with energy transfer in INS while they stay practically constant for IXS. This simplifies the data analysis in the latter case.

5. IXS is capable of a much higher energy transfer at constant $\vec{Q}$. It however suffers from larger absorption for high Z materials. This is minimized by using higher energy x-rays.

Advantages of Inelastic X-Ray Scattering for Investigation of Collective Dynamics in Bio-Materials

1. One needs to use coherent scattering to obtain information on collective excitations such as phonons in biomaterials. Thomson scattering of x-rays from atomic electrons is a coherent scattering, irrespectively of atomic composition of a bio-molecule. There is no need to deuterate the bio-molecule as in a coherent inelastic neutron scattering experiment.

2. While the number of x-ray photons or neutrons at the sample are comparable (3×10^8 photons or neutrons per second), a synchrotron x-ray beam size is much smaller (200 μm × 100 μm) than that in an INS spectrometer. In practice, for a 40 wt% calf-thymus Na-DNA sample in the hexagonal liquid crystalline phase, a film of dimension 0.2 mm (thick) × 0.2 mm (wide) × 10 mm (long) on a quartz plate is sufficient, which is order of 0.4 mg of DNA molecules. Ordered 40 wt% calf-thymus Na-DNA samples of thickness 0.2 mm have been used successfully by us in APS. Protein samples we used were hydrated powder samples of 200 mg.

Decomposition of the Dynamic Structure Factor

*C. Y. Liao and S. H. Chen, "Theory of the generalized dynamic structure factor of polyatomic molecular fluids measured by inelastic x-ray scattering", Phys. Rev. E, **64**:021205 (2001).*

The Dynamic Structure Factor can be written as a weighted sum of the partial dynamic structure factor $S(k,\omega)$:

$$S(k,\omega) = \sum_{\alpha\beta} f_\alpha(k) f_\beta(k) \omega_\alpha \omega_\beta S_{\alpha\beta}(k,\omega)$$

where $f_\alpha(k)$ is the atomic form factor of the alpha-species of atoms $\omega_\alpha = \sqrt{N_\alpha / N}$ and $S(k,\omega)$ is given by the GTEE theory.

The structure factor measured by an X-rays diffraction experiment is thus

$$S(k) = \sum_{\alpha\beta} f_\alpha(k) f_\beta(k) \omega_\alpha \omega_\beta S_{\alpha\beta}(k)$$

where the partial structure factor $S_{\alpha\beta}(k)$ is given by

$$S_{\alpha\beta}(k) = \frac{1}{\sqrt{N_\alpha N_\beta}} \sum_{i\in\alpha, j\in\beta} \left\langle e^{i\vec{k}\cdot(\vec{r}_i - \vec{r}_j)} \right\rangle$$

The second moment sum rule can be shown to be

$$\left\langle \omega^2 \right\rangle = k^2 v_0^2(k) = k^2 \sum_\alpha f_\alpha^2(k) \omega_\alpha^2 \frac{k_B T}{m_\alpha}$$

Generalized Three Effective Eigenmode (GTEE) Theory

*C. Y. Liao and S. H. Chen, "Theory of the generalized dynamic structure factor of polyatomic molecular fluids measured by inelastic x-ray scattering", Phys. Rev. E, **64**:021205 (2001).*

The dynamic structure factor is given as,

$$S(k,\omega) = \frac{S(k)}{\pi}\mathrm{Re}\left\{\frac{\vec{I}}{i\omega\vec{I} + \vec{H}(k)}\right\}_{1,1} \qquad \vec{H}(k) = \begin{bmatrix} 0 & if_{un}(k) & 0 \\ if_{un}(k) & z_u(k) & if_{uT}(k) \\ 0 & if_{uT}(k) & z_T(k) \end{bmatrix}$$

with $f_{un}(k) = \sqrt{<\omega>^2} = kv_0(k)/\sqrt{S(k)}$ and $z_u(k)$, $f_{uT}(k)$, $z_T(k)$ all real numbers.

Hydrodynamic limit $(k \to 0)$

$$f_{un}(k) = kc_s / \sqrt{\gamma}$$

$$z_u(k) = \varphi k^2$$

$$z_T(k) = \gamma D_T k^2$$

$$f_{uT}(k) = kc_s\sqrt{(\gamma - 1)/\gamma}$$

Continued fraction expression

$$S(k,\omega) = \mathrm{Re}[S(k, z = i\omega)] = \left[z + \frac{f_{un}^2(k)}{z + z_u(k) + \dfrac{f_{uT}^2(k)}{z + z_T(k)}} \right]^{-1}$$

$$c_s = v_0\sqrt{\gamma / S(0)} \quad \gamma = c_p / c_v \quad \varphi = [(4/3)\eta + \zeta]/nm \quad D_T = \lambda / nmc_p$$

The three eigenvalues, to the order of $O(k^2)$, are:

$$z_h(k) = D_T k^2$$

$$z_\pm(k) = \pm ic_s k + \Gamma_s k^2$$

where $\Gamma_s = \varphi/2 + D_T(\gamma - 1)/2$ is the sound damping.

For finite k, $z_u(k)$, $f_{uT}(k)$, $z_T(k)$ become arbitrary functions of k. However, in most cases, the eigenvalues of the matrix $\vec{\mathcal{H}}$ consist of one real number Γ_h and a couple of conjugate complex numbers $\Gamma_s \pm i\Omega_s$. One can therefore write the NDSF in general in the hydrodynamic-like form:

$$\frac{S(k,\omega)}{S(k)} = \frac{1}{\pi}\left\{A_0 \frac{\Gamma_h}{\omega^2+\Gamma_h^2} + A_S \frac{\Gamma_S + b(\omega+\Omega_S)}{(\omega+\Omega_S)^2+\Gamma_S^2} + A_S \frac{\Gamma_S - b(\omega-\Omega_S)}{(\omega-\Omega_S)^2+\Gamma_S^2}\right\}$$

Damped Harmonic Oscillator limit ($f_{uT}(k)=0$)

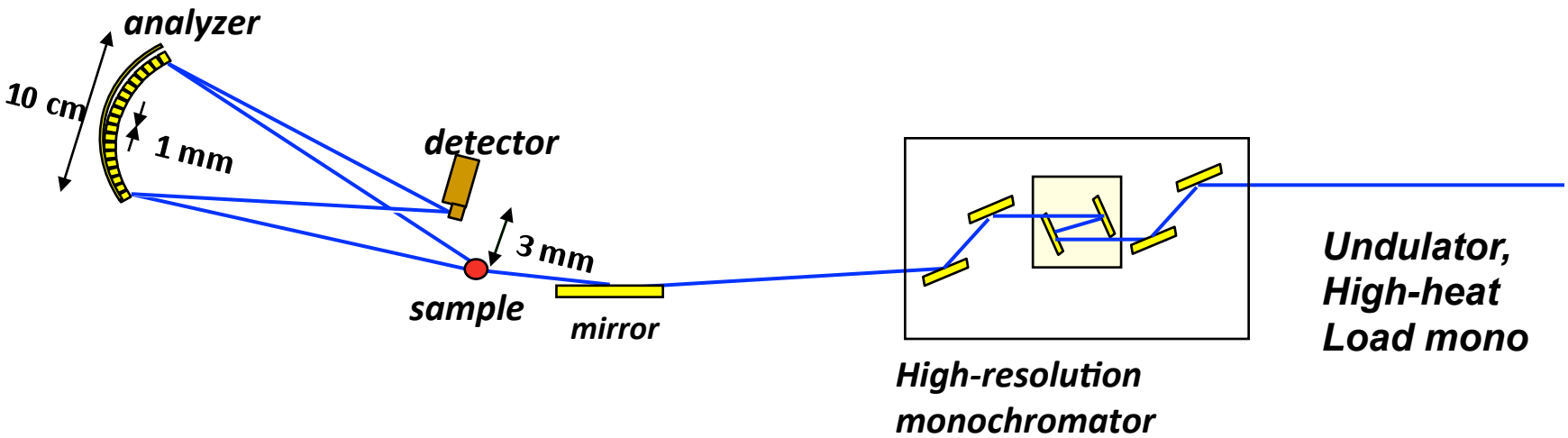
The amplitude of the central peak of $S(k,\omega)$ is zero, the side peaks can be written as :

$$S(k,\omega)/S(k) = \frac{1}{\pi}\frac{f_{un}^2(k)z_u(k)}{(\omega^2 - f_{un}^2(k))^2 + (\omega z_u(k))^2}$$

DHO Model (add a delta function central peak)

$$S(Q,E) = A\frac{E}{k_B T}[n(E)+1]\Big[f_g(Q)\delta(E) + [1-f_g(Q)] \times \frac{1}{\pi}\frac{\Gamma(Q)\Omega(Q)^2}{[E^2-\Omega(Q)^2]^2+[\Gamma(Q)E]^2}\Big],$$

HERIX-3 & HERIX-30 at APS



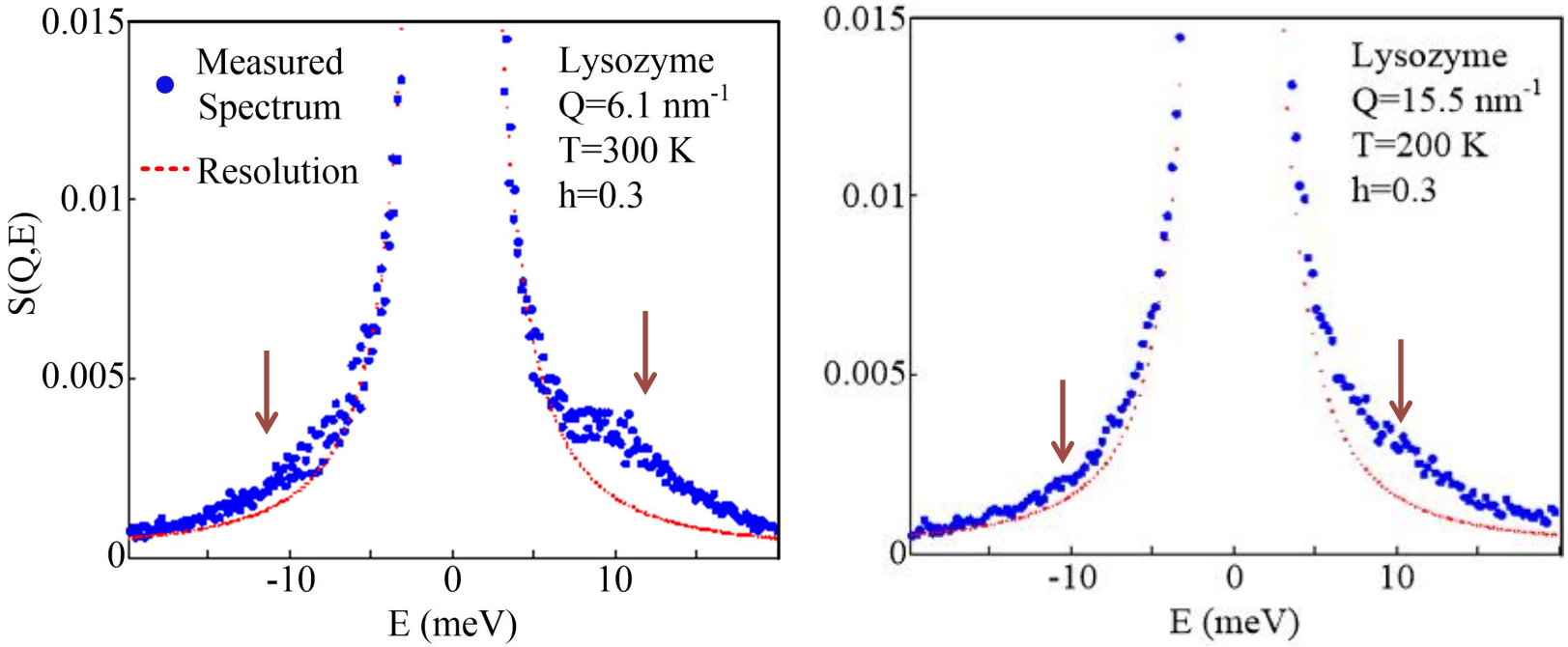
The current status of the HERIX programs at APS

	HERIX-3	HERIX-30
Sector	3	30
Working energy (keV)	21.6	23.7
Energy resolution (meV)	2-2.4	1.5-1.7
Number of analyzers	4	9
Maximum momentum transfer (nm^{-1})	35	72
Beam size at sample (VxH) (μm^2)	17x17	20x35

HERIX-30 is a high energy resolution (1.5 meV) 4 circle diffractometer for inelastic x-ray measurements used for phonon measurements in solids and liquids.

- Energy transfer −200 to 200 meV
- Maximum momentum transfer of 75 nm^{-1}
- Momentum resolution of 0.65 nm^{-1} (can be improved by slits)

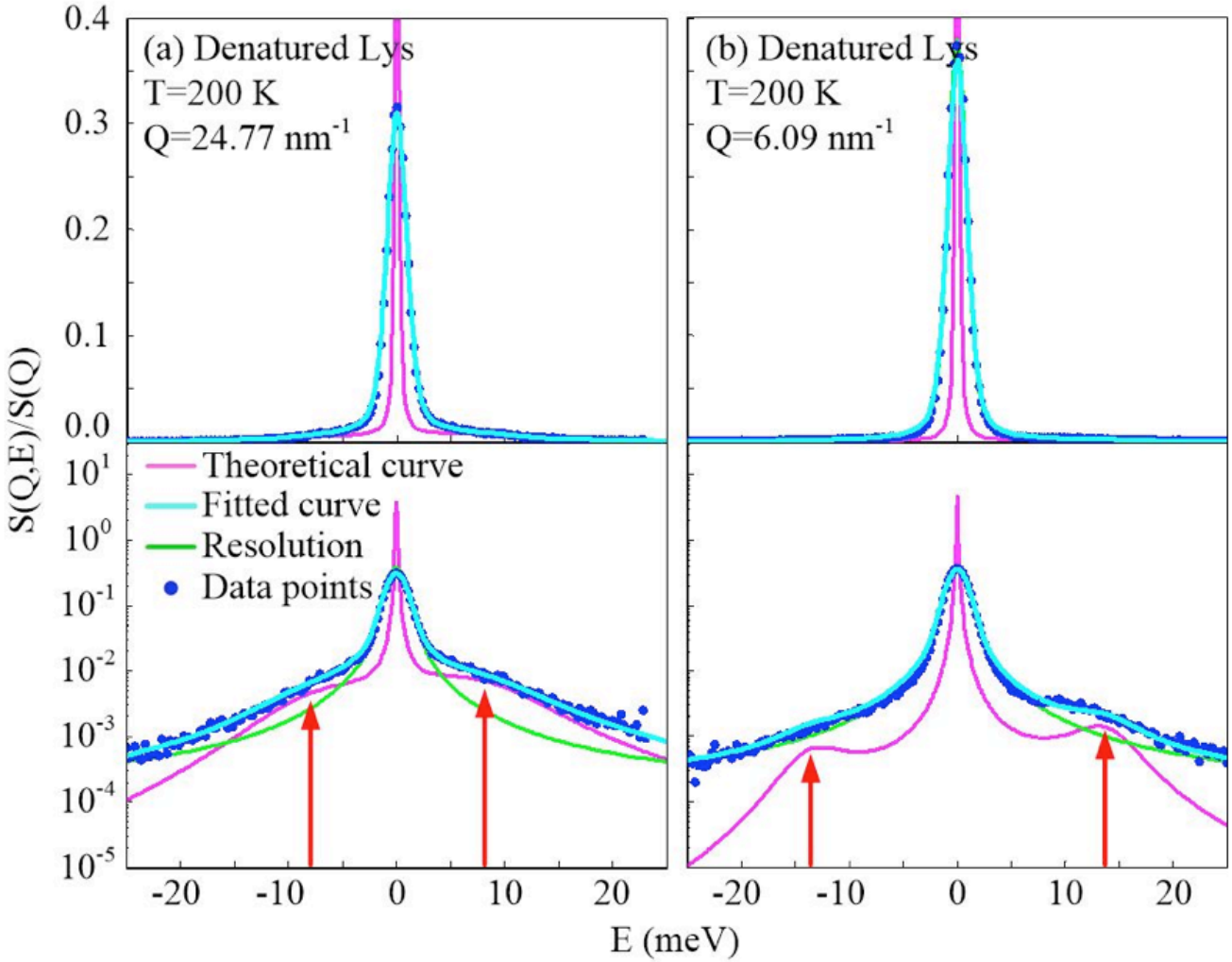
Evidence of Phonon-Like Excitations in Protein from IXS Spectra



- Inelastic X-ray scattering technique can be used to measure the collective dynamics in a protein molecule on the sub-picosecond time scale.
- On the sub-picosecond time scale, a protein sample exhibits phonon-like excitations based on intra-protein collective vibrations of constituent molecular groups.
- The position of the Brillouin peak, Ω_s, measured at a specific Q value, is the energy of the phonon-like excitation. In order to extract Ω_s one needs a proper liquid theory.

Z. Wang et al, *Hydration water in protein: key to the "softening" of short-time intra-protein collective vibrations of specific length scale,* to be published.

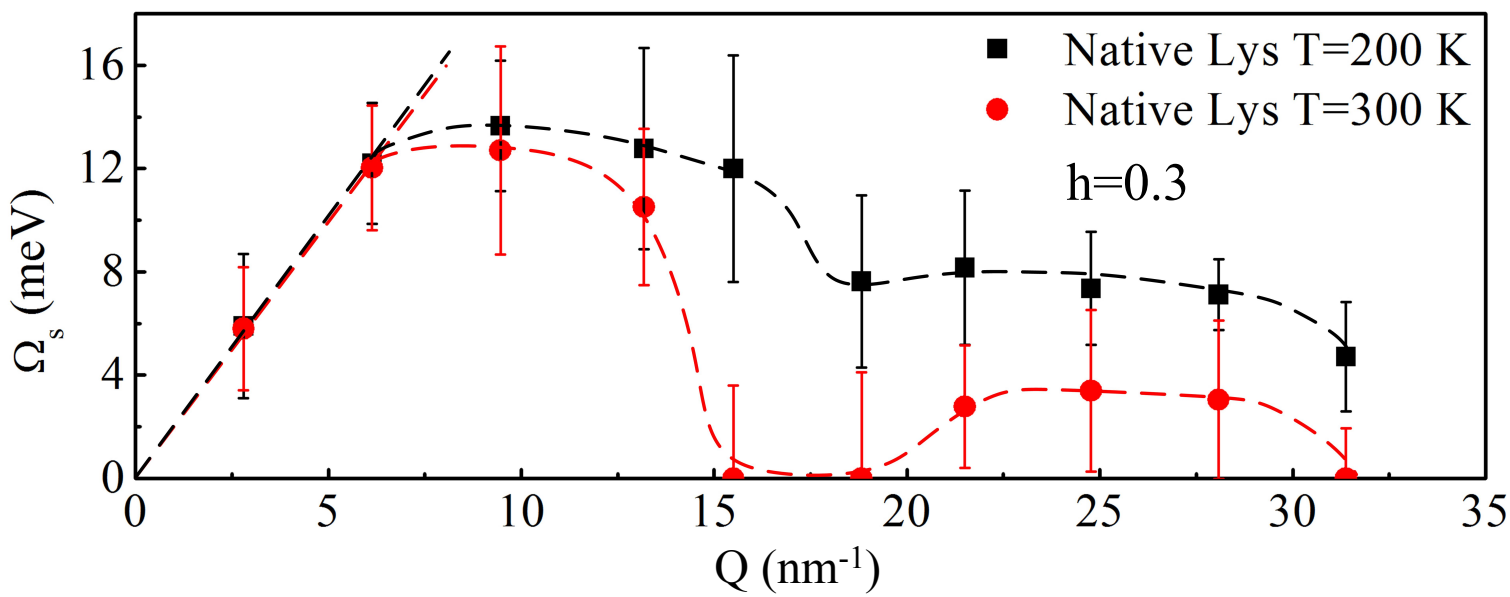
Rayleigh-Brillouin Spectra measured by IXS and Analysis by GTEE Theory



- IXS spectra for the denatured lysozyme sample at $T = 200$ K and $h = 0.3$ for two Q values, (a) at 24.77 nm^{-1} and (b) at 6.09 nm^{-1}. The upper panel shows data in linear scale, while the lower panel is in logarithmic scale. Thus one can check the fitting quality for both the Rayleigh peak and Brillouin peak. The blue points are the measured spectral data points. The pink line is the extracted NDSF (multiplied by the detailed balance factor). The cyan line is the fitted spectral intensity. For the lower panels, the green line shows the energy resolution function of the spectrometer. The red arrows indicate the position of the Brillouin peaks.

Z. Wang et al., to be published (figure 1) * C. Liao and S.-H. Chen, Phys. Rev. E, 2001, **64**, 021205. (GTEE theory)

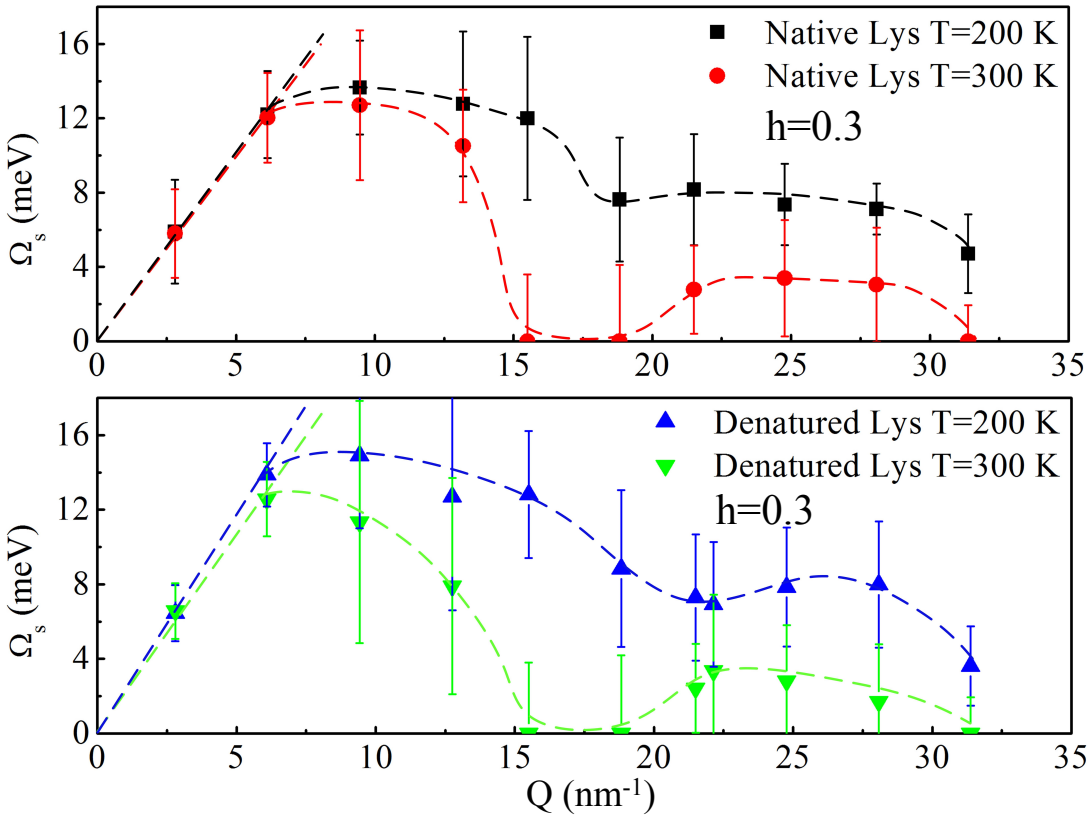
Phonon Energy Softening in Hydrated Sample

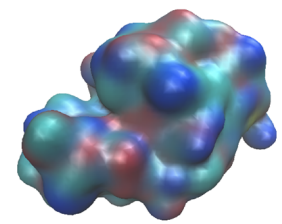


- The above figure shows the dispersion relations of the phonon-like excitations at
- T = 200 K and 300 K for hydrated native lysozyme sample (h=0.3).
- Within the Q range of about 12~31 nm^{-1}, the phonon energy exhibits a substantial softening for the higher temperature (300 K).
- This Q range corresponds to the length scale of 2 – 5.2 Å, which is comparable to the length scale of the spatial order of protein secondary structures (4 ~ 5 Å).

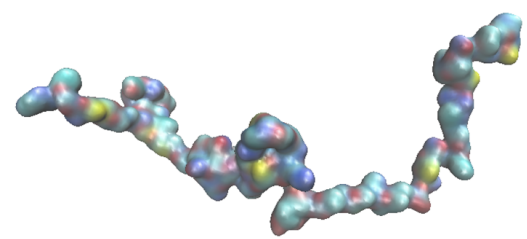
Z. Wang et al., to be published.

Irrelevance of the Protein Tertiary and Secondary Structures to the Phonon Energy Softening





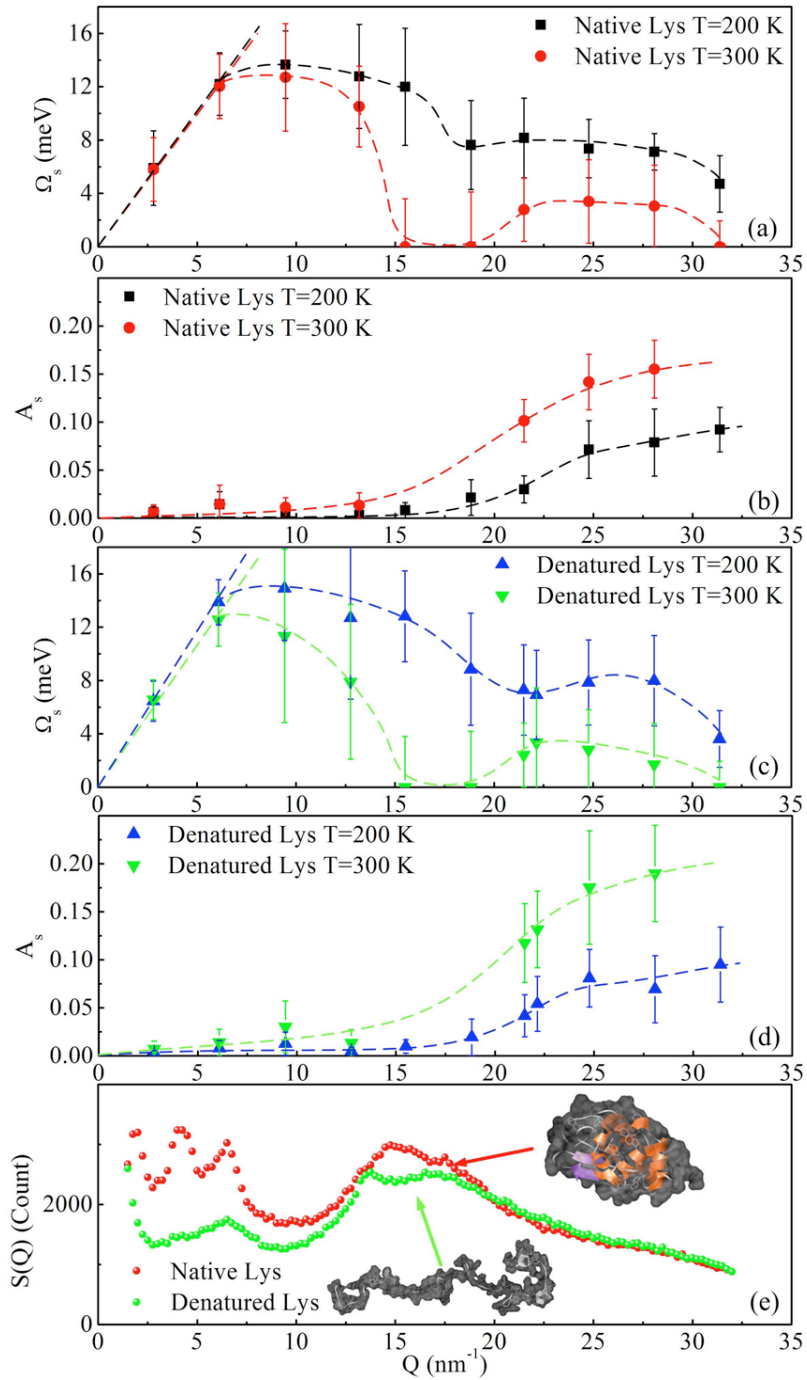
Native



Denatured

- The phonon energy softening appears in both the hydrated native lysozyme and hydrated denatured lysozyme.
- The phonon energy softening does not depend significantly on the protein tertiary structure and secondary structure.

Z. Wang et al. *Hydration water in protein: key to the "softening" of short time intraprotein collective vibrations of specific length scale* (to be published).



(a) Dispersion relations of the phonon-like excitations.

(b) Fractional area of the Brillouin peaks A_s at two temperatures for hydrated lysozyme at $h = 0.3$. Black squares and red circles indicate $T = 200$ K and 300 K. respectively.

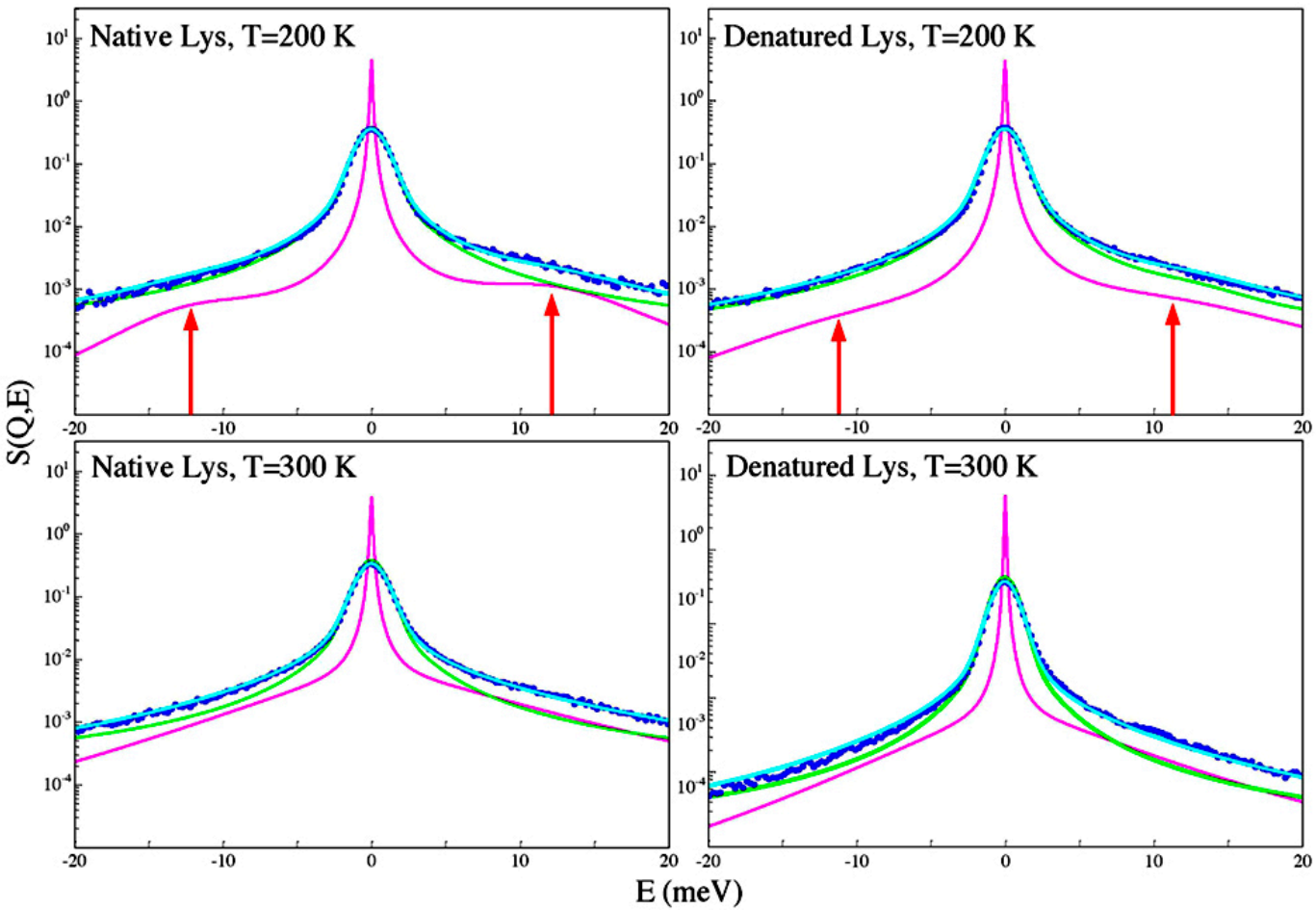
(c) Dispersion relations of the phonon-like excitations.

(d) Fractional area of the Brillouin peaks A_s at two temperatures for hydrated denatured lysozyme at $h = 0.3$. Green up triangles and blue down triangles indicate $T = 200$ K and 300 K, respectively.

(e) $S(Q)$ curves and 3D graphical representations for the above two samples. The dashed lines are drawn to guide the eyes.

Z. Wang et al., to be published.

Rayleigh-Brillouin Spectra measured by IXS and Analysis by GTEE Theory



- Model used for analysis: the Generalized Three Effective Eigenmode Theory (GTEE).*
- Notice for the cases of S(Q,E)/S(Q) at T=300 K, Q = 15.5 nm^{-1} phonon energies are zero.

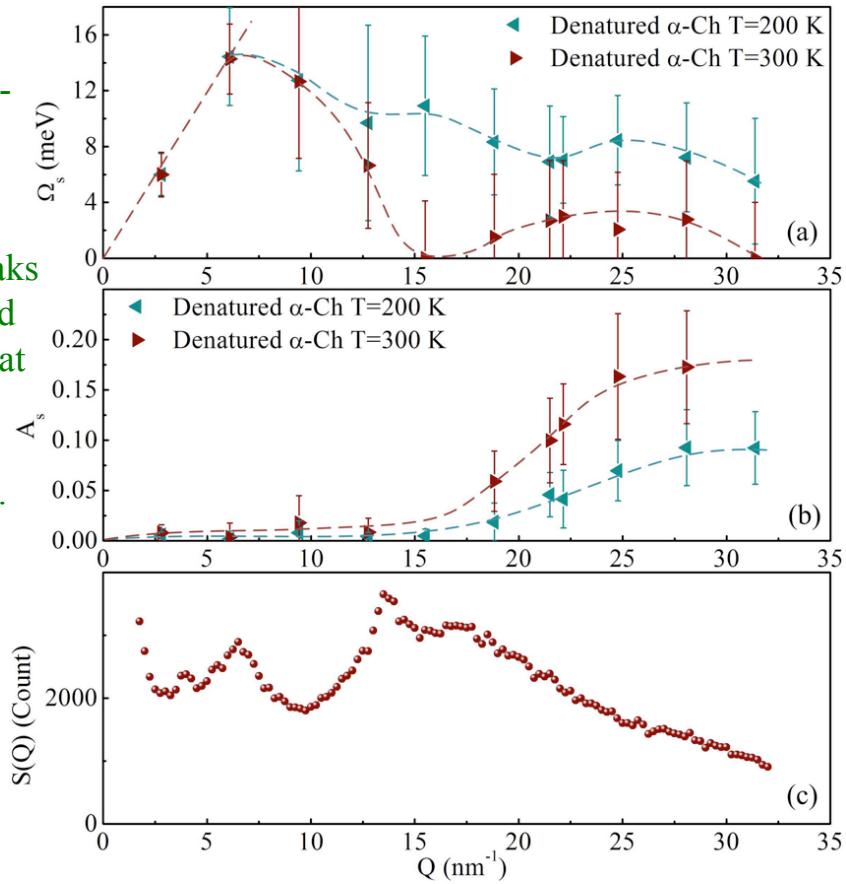
* C. Liao and S. H. Chen., Phys. Rev. E, 2001, **64**, 021205. (GTEE)

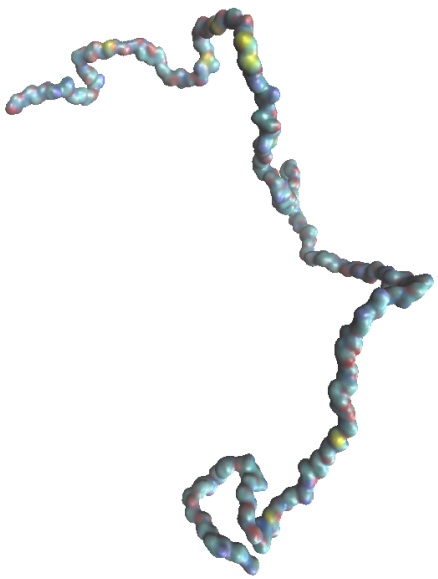
Irrelevance of the Protein Tertiary and Secondary Structures to the Phonon Energy Softening

(a) Dispersion relations of the phonon-like excitations.

(b) Fractional area of the Brillouin peaks A_s at two temperatures for hydrated denatured α-Chymotrypsinogen A at h = 0.3. Dark cyan left triangles and brown right triangles indicate T = 200 K and 300 K, respectively.

(c) S(Q) of the hydrated denatured α-Chymotrypsinogen A at h = 0.3.



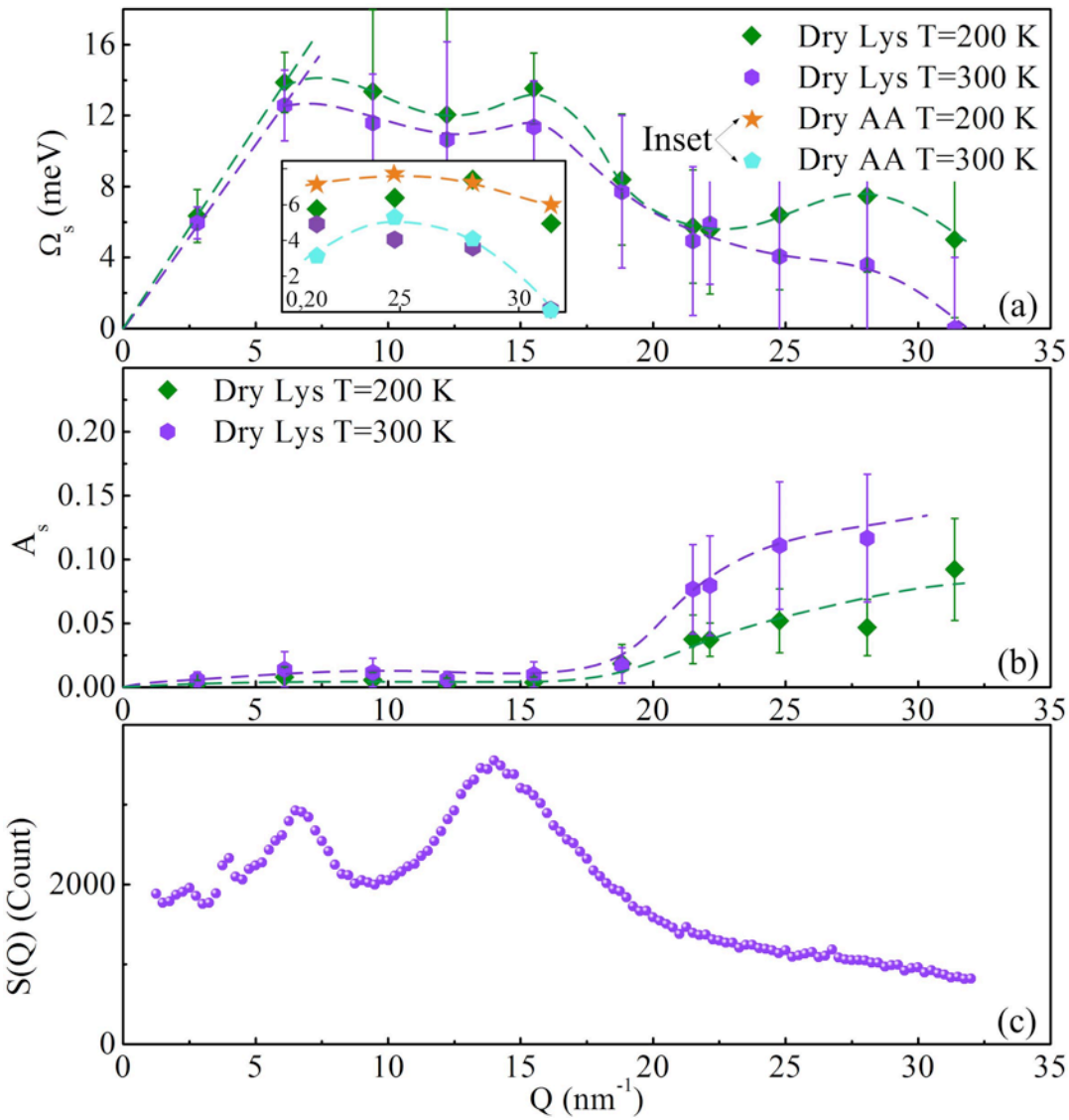


Denatured Chymo

- For the hydrated denatured α-Chymotrypsinogen A, the structure is more unfolded than that of the denatured lysozyme.
- Similar softening still appears. This confirms the conclusion that the "phonon energy softening" is only weakly dependent on the protein tertiary and secondary structures.

Z. Wang et al., *Hydration water in protein: key to the "softening" of short time intra-protein collective vibrations of specific length scale* (to be published).

Temperature dependence of phonon dispersion relation and population of dry lysozyme



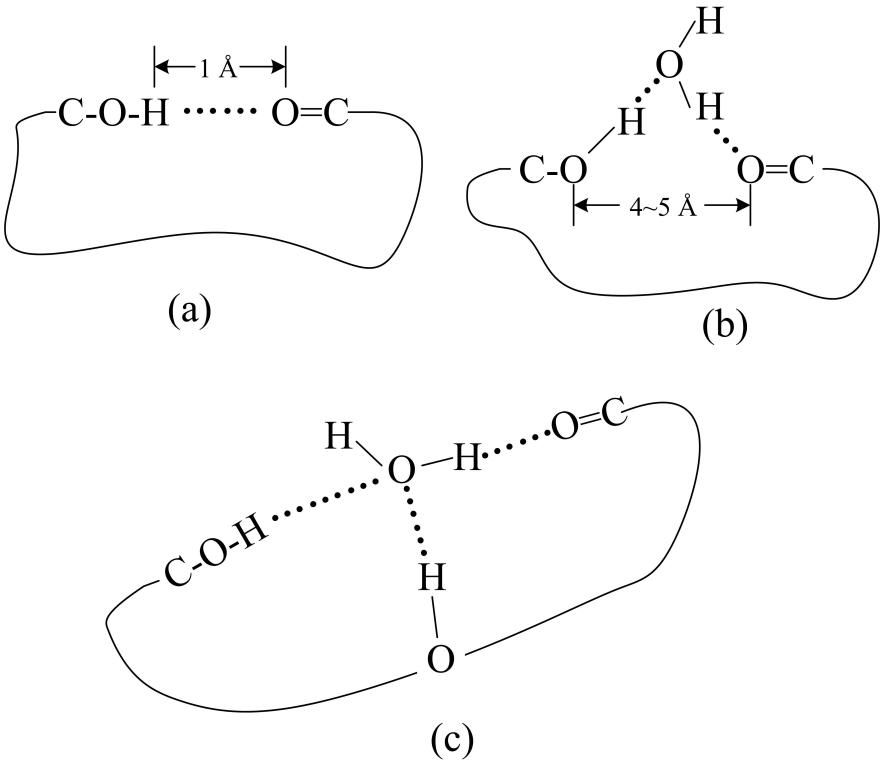
(a) Dispersion relations of the phonon-like excitations. Inset in (a) Dispersion relations of the phonon-like excitation for the dry lysozyme sample and the dry mixture of amino acid molecules in the Q range of 20 – 35 nm^{-1} at two temperatures. Orange stars and cyan pentagons indicate T = 200 K and 300 K for the dry mixture of amino acids, respectively. For clarity, we did not add error bars here. The dashed lines are drawn to guide the eyes.

(b) Fractional area of the Brillouin peaks A_s at two temperatures for the dry lysozyme sample. Green diamonds and violet hexagons indicate T = 200 K and 300 K, respectively.

(c) $S(Q)$ of the dry lysozyme sample.

Z. Wang et al., to be published.

The Way that Hydration Water Induces the "Phonon Energy Softening" at T = 300 K



(a) Without water, hydrophilic interactions in protein saturate internally. The total surface area of the protein is thus smaller.

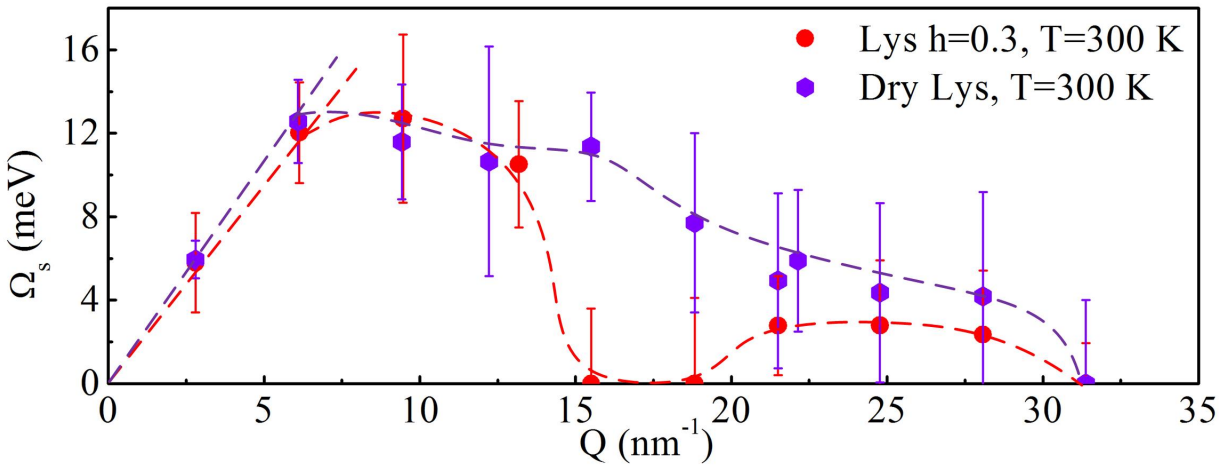
(b), (c) The presence of water allows the structure to expand by formation of hydrogen bonds.

- Water molecule can expand the protein structure at the length scale of 4 – 5 Å by introducing two or three hydrogen bonds.
- With hydration water, the accessible conformational space for collective motions in such length scales (4 – 5 Å) increases.
- Water expands the protein surface area, introduces fluctuations and reduces the energy barriers. In other words, water makes the protein "softer" in these time and length scales.

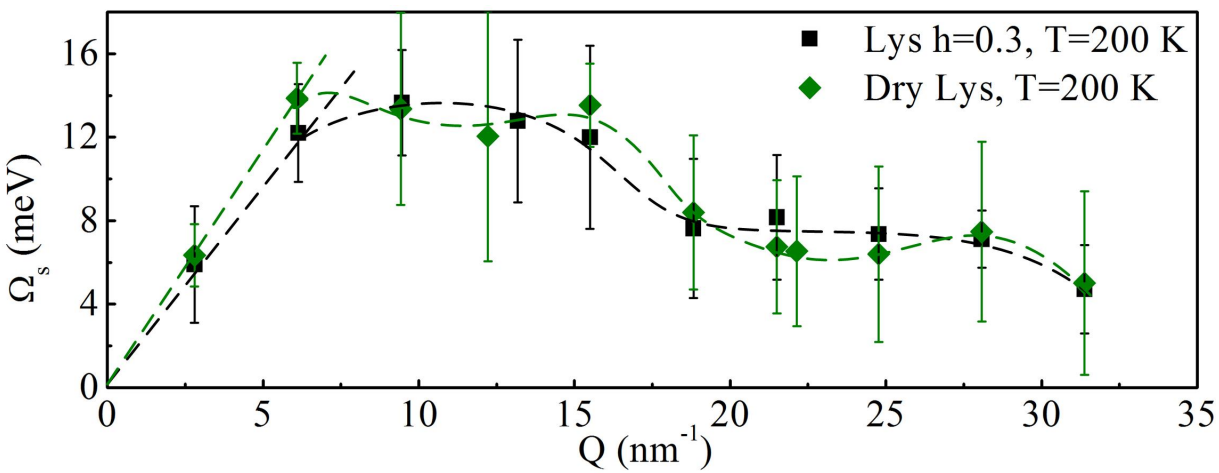
H. Wang et al., J. Phys. Chem. B, 1997, **101**, 1077.

Z. Wang et al., J. Phys. Chem. B, 2013, **117**, 1186. (figure taken from)

Hydration water makes the protein "soft" at T = 300 K, shown as follows:



But **NOT** at T=200 K:



- May be due to a qualitative difference in the hydration water mobility at T=200 K and 300 K (that is the change from predominantly LDL form to predominantly HDL form).

Z. Wang et al., to be published.

Summary

- In sub-picosecond time scale, the "softness" of a protein can be estimated from the energy of the intra-protein phonon-like excitation, or equivalently, the frequency of the collective vibration in the protein molecule.

- Proteins with significant enzymatic functions are "soft" in the sense that they exhibit low phonon energies. This is not the case for dry proteins or proteins at low temperatures (below ~220 K) that lack significant enzymatic functions.

- Hydration water is crucial to this "softness" in sub-picosecond time scale:

  (1) protein can be "softened" by increasing amount of hydration water at room temperature;

  (2) water helps protein to become "softer" as temperature increases.

- However, under low temperatures (below 220 K), water does not work as a "plasticizer" any more.

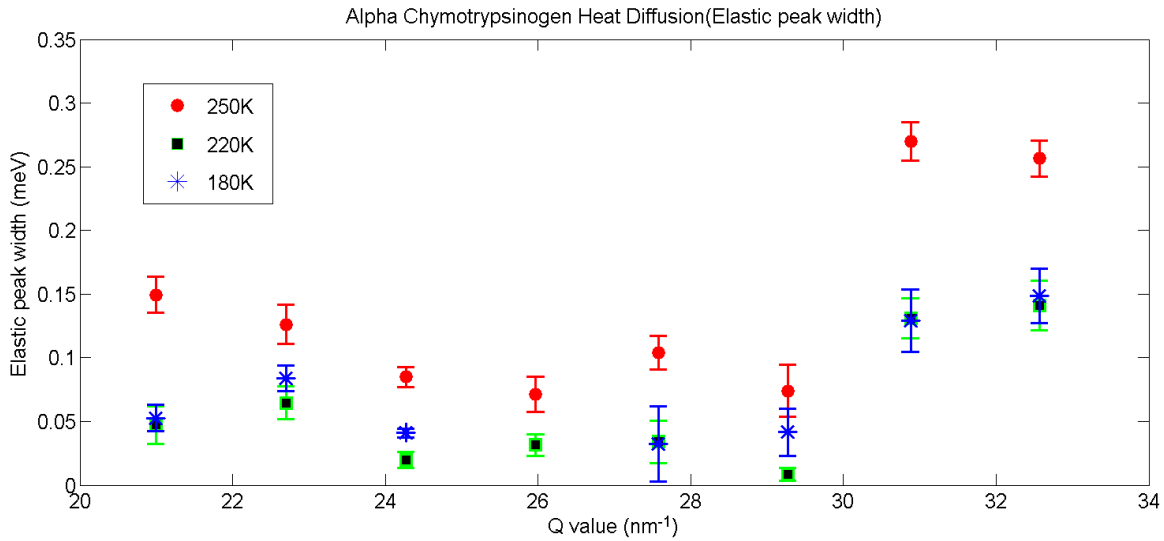
Central Peak Width Γ_h (thermal diffusion rate)

This is the first time that this relaxation time is experimentally detected.

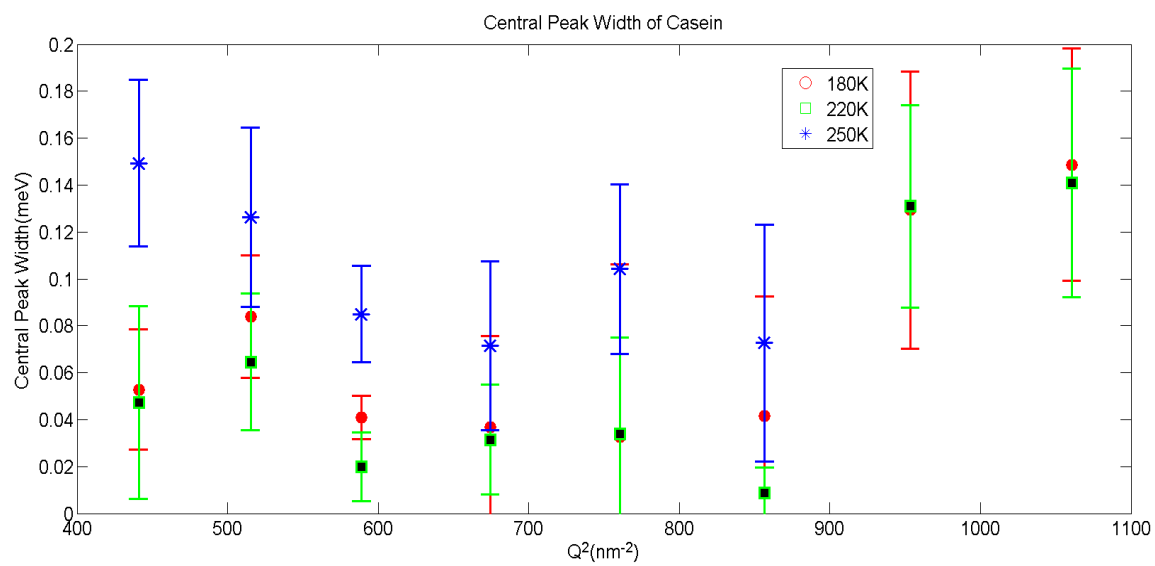
Central peak width shows a shallow minimum at Q_{min}= 26 1/nm as Q increases.

The magnitude of Γ_h is of the order of 0.1meV, which is measurable by the next generation IXS spectrometer being built at APS and NSLS-II right now.

Alpha-chymotrypsinogen



Casein



Initial Performance & Capabilities

Performance Comparison :

Courtesy of Dr. Yong Cai of NSLS-II, BNL

Facility (Beamline)	ΔE (meV)	ΔQ** (nm^{-1})	E (keV)	$I_{inc.}$ @ ΔE (photons/sec)	Beam Size (V×H μm^2)	Flux Density (photons/sec/μm^2)	Sharp Res. Tails
ESRF (ID28)	1.6	0.5	21.7	6×10^9	7×12	7.1×10^7	–
APS (30-ID-C)	1.5	0.6	23.8	2×10^9	15×35	3.8×10^6	–
SPring-8 (43LXU)	1.5	0.5	21.7	$\sim5\times10^{10}$	20×35	$\sim7.1\times10^7$	–
NSLS-II (IXS)	1.0	0.2	9.1	$\sim1.6\times10^{10}$ (4B HRM)*	5×7	**$\sim5\times10^8$**	yes

* Performance for a IVU22-3 m at 500 mA (flux 8×10^{14} phs/sec/0.1% bw) and 30% spectral efficiency for the 4B monochromator.
** Assuming 5 mrad acceptance angle.

Count Rate Estimates (single analyzer):

Sample	Thickness (μm)	ESRF ID28 (cnts/sec)	NSLS-II IXS (cnts/sec)
v-SiO_2	2100/190	~0.2	**~0.2**
H_2O (HP)	1000/1000	~1.0	**~8**
La_2CuO_4	75.3/7.13	~0.5	~0.04
Solid O_2	10/10	~1.5	**~2.2**

(ESRF data courtesy of M. Krisch)

Unique research strength for low-Z materials and high-pressure DAC systems (small beam size, highest flux density)

Thank you for your attention!

This project is funded by *DE-FG02-90ER45429*, U.S. DOE.