Dynamics of Biopolymers and Their Hydration
Water Studied by Neutron and X-ray Scattering

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Why important?

• At ambient condition, a protein cannot function without its hydration water.

• How does it work under extreme conditions, such as at very low temperatures and under high pressure?

• Softening of the intra-protein phonons at a specific Q range above \( T_D \) is intimately correlated with the increase of biological activities of the proteins.
LYZ is an enzyme consisting of 129 amino acid residues. It has an ellipsoidal shape with dimensions $a \times b \times b = 2.25 \times 1.5 \times 1.5 \text{ nm}^3$ (prolate). Molecular weight: 14.4 kDa

Hydrated lysozyme protein. Protein surface is represented by a wireframe. It is surrounded by water molecules represented by red (oxygen) and white (hydrogen) spheres.

We use a hydration level $h = 0.30-0.35$. Picture by P. Kumar (BU).
Two Major Observations

Dynamics of hydration water controls the low temperature dynamical property of a protein at moderate pressures.

Experimental evidence of a logarithmic-stretching of the intermediate-time protein relaxational dynamics.

\[ F_H(Q,t) \approx \left[ f(Q,T) - H_1(Q,T) \ln \left( \frac{t}{\tau_\beta(T)} \right) + H_2(Q,T) \ln^2 \left( \frac{t}{\tau_\beta(T)} \right) \right] \]
Subtraction Method

Protein\( + \text{H}_2\text{O} \)

Protein\( + \text{D}_2\text{O} \)

Hydration Layer

Backscattering (HFBS) spectrometer in NCNR

Backscattering spectrometer is a special version of the crystal analyzer spectrometer, in which the analyzer angle $\theta_A$ is selected to be close to $\pi/2$.

\[
\frac{\delta\lambda_A}{\lambda_A} = \sqrt{(\cot \theta_A \Delta \theta_A)^2 + \left(\frac{\Delta d_A}{d_A}\right)^2}
\]

When $\theta_A = \pi/2$, $\cot \theta_A = 0$, the only contribution to the wavelength uncertainty comes from $\Delta d_A$, which is small. The energy resolution is then extremely good.

Backscattering (HFBS) spectrometer with a resolution of 0.8 $\mu$eV and dynamic range of $\pm$ 17 $\mu$eV was used, covering time range of 50 ps to 16 ns, and Q range of 0.2 Å$^{-1}$ to 2.0 Å$^{-1}$. 
Biological Function of Protein Hydration Water

- At low T globular proteins exist in a glassy state having no conformational flexibility and show very low biological activities.

- For hydrated proteins above about 220 K, the flexibility is restored, able to sample more conformational sub-states, thus becoming biologically active.

- This “dynamical transition” is common to many biopolymers. Believe to be triggered by their strong coupling with mobility of their hydration water, which shows a similar “dynamical transition” at about the same temperature.

- We show experimentally that this sudden switch in dynamical behavior of hydration water on Lysozyme, B-DNA and RNA occurs precisely at 220 K and can be described as a Fragile-to-Strong dynamic crossover (FSC).

- At FSC, the structure of hydration water makes a transition from predominantly high density form (HDL), a more fluid state, to predominantly low density form (LDL), a less fluid state, derived from the existence of a second critical point at an elevated pressure.
Lysozyme Hydration Water

$p = 1500$ bar

$Q = 0.75 \text{Å}^{-1}$
Structure Factor Measured by QENS Experiment

Lysozyme Hydration Water
p = 1500 bar
Q=0.75Å⁻¹

$S(Q,\omega)$ (meV⁻¹)

E(μeV)
Analysis of QENS Spectra of Protein Hydration Water

(A1) \[ S(Q,\omega) \text{ (meV)} \]

\[ \begin{align*}
\text{Exp. data} & \quad \text{Fit} \\
\text{Quasi-elastic Comp.} & \quad \text{Elastic Comp.}
\end{align*} \]

\[ T=230K \]

\[ Q=0.87\text{Å}^{-1} \]

(A2) \[ S(Q,\omega) \text{ (meV)} \]

\[ \begin{align*}
\text{Exp. data} & \quad \text{Fit} \\
\text{Resolution}
\end{align*} \]

\[ T=230K \]

\[ Q=0.87\text{Å}^{-1} \]

(B1) \[ S(Q,\omega) \text{ (meV)} \]

\[ \begin{align*}
\text{Exp. data} & \quad \text{Fit}
\end{align*} \]

\[ T=210K \]

\[ Q=0.87\text{Å}^{-1} \]

(B2) \[ S(Q,\omega) \text{ (meV)} \]

\[ \begin{align*}
\text{Exp. data} & \quad \text{Resolution}
\end{align*} \]

\[ T=210K \]

\[ Q=0.87\text{Å}^{-1} \]
Analysis of QENS Spectra of Protein Hydration Water

An intuitive method to extract the alpha-relaxation time from the Intermediate Scattering Function

Proteins Remain Soft at Lower Temperature under Pressure


MSDs of H-atom in lysozyme and its hydration water show the same crossover temperature $T_D = T_L$ at six pressures.
Pressure Dependence of the Dynamic Crossover (FSC) in Lysozyme Hydration Water

Proteins Remain Soft at Lower Temperature under Pressure

Pressure Dependence of the Average Translational Relaxation Time $\langle \tau_T \rangle$ at $T_L$ and $E_A$ decrease linearly with pressure

$T_L$ and $E_A$ decrease linearly with pressure

$E_A/k_B T_L(P)$ remains constant as function of pressure

Widom Line in 1-D and 2-D Confined Water and Its Liquid-Liquid Critical Point $C_{L-L}$

Fractional Stokes-Einstein Relation in Protein Hydration Water

Predicted the power-law exponent $\xi = 2/2.3$ ($d = 2$) for FA models of strong glassy liquids, very close to the experimental value 0.82 shown in figure B.

Dynamical Crossover and Breakdown of the Stokes–Einstein Relation in Confined Water and in Methanol-Diluted Bulk Water

Appearance of a Well-defined Boson Peak at and below 220K

Protein lysozyme
\[ E_B = 3 \text{ meV} \]

\( T_C = T_L = 220K \)
\( T_C : \) protein “glass transition” temp.
\( T_L : \) dynamic crossover temp.

Protein hydration water
\[ E_B = 5 \text{ meV} \]

Y. Zhang, S.-H. Chen, et al. (to be published)
Evidence of the synchronization of the crossover-temperature between biopolymers and their hydration water

Each panel shows the temperature dependence of the MSD of hydrogen atoms in both the biopolymer and its hydration water, respectively. It shows evidence that the crossover temperatures of the two systems, the biopolymer and its hydration water, are closely synchronized. (A): MSD of hydrated lysozyme; (B): MSD of the hydrated B-DNA; (C): MSD of the hydrated RNA. The arrow signs indicate the approximate positions of the crossover temperature in both the biopolymer \( (T_D) \) and its hydration water \( (T_L) \). Note that the scale on the left hand side is for MSD of the hydration water and that on the right hand side is for the biopolymer.
The extracted $\langle \tau_T \rangle$ from fitting of QENS spectra by RCM plotted in the log scale vs. $1/T$. Panels (A), (B), and (C) show clearly well defined cusp-like dynamic crossover behavior in each case. The dashed line represents fitted curves using the VFT law, while the solid line is the fitting according to the Arrhenius law. FSC temperatures are respectively, (A) 220 K, hydration water in lysozyme; (B) 222 K, hydration water in DNA; and (C) 220 K, hydration water in RNA. All three temperatures are essentially the same within the experimental error of 5 K suggesting universality of $T_L$ in hydration water of all biopolymers.
Coincidence of the Dynamic Crossover and MSD Crossover Temperature


Hydrated RNA

$h = 0.5\, \text{g H}_2\text{O}/1\, \text{g RNA}$

$T_L = 220\, \text{K}$

- **Exp. result**
- **Arrhenius law**
- **VFT law**
- $\langle x_{\text{H}_2\text{O}}^2 \rangle$
“MD simulation based on a realistic hydrated lysozyme powder model reproduces the dynamic crossover phenomenon of the hydration water.”
Hydration Level Dependence of the Crossover Temperature for Hydrated Lysozyme
MD Simulation by Chansoo Kim et al, to be published.
Comparison between single-particle dynamics of protein and its hydration water

Comparison of Ballistic $\alpha$-Decay and Log $\beta$-Decay for two different temperatures:
- $T = 250$ K
- $T = 310$ K

Plots show:
- O-O Self Correlation Function of hydration water
- Center-of-mass of protein amino acids

Graphs illustrate the decays at different times ($t$ in ps) with distinct features such as Plateau and $\alpha$-Decay.
Examples of logarithmic decay in the intermediate time scale

Slope of sandpiles

Spin-glass Magnetization
Nordblad et al, 1986, Phs Rev B, 33, 645

Polymer blends

Simple Glass Formers

Micellar Copolymer Dynamics
Chen et al, 2003 Science, 300, 619

Short-Range Attractive Colloids
Logarithmic beta decay according to Mode Coupling Theory

Gotze, Sperl et al. (2002): log decay is predicted by MCT for systems close to a higher-order glass transition singularity.

In MCT formalism, the transition is denoted as $A_{n+1}$.

$A_2 = \text{sharp (fold) transition ergodic-nonergodic state at } x_c$

$A_3 - A_4 = \phi_q(t)$ can be approximated by a logarithmic expansion when $\epsilon$ is small,

$$\phi_q(t) \sim \left[ f_q - H'_q \ln(t / \tau_\beta) + H''_q \ln^2(t / \tau_\beta) \right]$$

$f_q, H'_q, \text{ and } H''_q$ are functions of $q$ and of $\epsilon$

$\tau_\beta$ is only function of $\epsilon$

Competition between 2 different arrest mechanisms, e.g. excluded volume effect and short-range attraction.

Three different protein systems at the physiological temperature

Powder (h ~ 0.30)  Crystal (h ~ 0.38)  Solution (h ~ 8.0)

Two different proteins at the physiological temperature

Lysozyme

RNAse

Backbone vs Side-chains motions of lysozyme

Backbone at 310 K

Side-chains at 310 K
Intermediate Scattering Functions in the time range 1 ps - 250 ns

Self-intermediate scattering functions for the amino acidic residues of lysozyme at $T = 310$ K. 12 different wave vectors are displayed, from 0.8 Å$^{-1}$ to 9.6 Å$^{-1}$ with a 0.8 Å$^{-1}$ interval (from top to bottom).

A) COM

B) Hydrogens.

The red continuous lines are the best fits with the equation

$$\phi^S_q(t) \sim [f_q - H'_q \ln(t/\tau^\beta) + H''_q \ln^2(t/\tau^\beta)\exp(-t/\tau^\alpha)]$$

Evidence of Logarithmic Decay in the $\beta$-relaxation region of time between 2 ps and 2 ns

Q-vector and temperature dependence of the self-intermediate scattering functions for the COM of the amino acid residues. A) $T = 310$ K; B) $T = 280$ K; C) $T = 320$ K.

10 different wave vectors are displayed, from $1.6 \, \text{Å}^{-1}$ to $8.8 \, \text{Å}^{-1}$ with a $0.8 \, \text{Å}^{-1}$ interval (from top to bottom). The red continuous lines are the best fits with Eq. 2 (panel A) and Eq. 1 (B and C).

$$\Phi_q^S(t) \sim [f_q - H'_q \ln(t/\tau^\beta) + H''_q \ln^2(t/\tau^\beta)] \exp(-t/\tau_q^\alpha)$$

Analysis of the beta relaxation based on MCT

$q$-values: from 1.6 to 8.0 Å$^{-1}$ with 0.8 Å$^{-1}$ interval

\[
\phi_q(t) \sim \left[ f_q - H_q \ln(t/\tau_\beta) + H^*_{q} \ln^2(t/\tau_\beta) \right]
\]

$T = 280$ K

$T = 340$ K

Q-dependence of MCT parameters

\[ \phi_q^S(t) \sim [f_q - H'_q \ln(t/\tau^\beta) + H''_q \ln^2(t/\tau^\beta)] \exp\left(-t/\tau_q^\alpha\right) \]

*Upper panel:* \( f_q \), Debye-Waller factor.

*Middle panel:* first coefficient, \( H'_q \).

*Bottom panel:* \( H''_q \), the dashed line at 0 intersects the curves at different \( q \) values depending on the temperature.

\( \tau^\beta \) is chosen at each \( T \) so that \( f_q \) is independent of \( T \).

Q-dependence of the alpha-relaxation time

\[ \phi(t) \sim \left[ f_q - H'_q \ln(t / \tau^\beta) + H''_q \ln^2(t / \tau^\beta) \right] \exp\left(-t / \tau^\alpha_q\right) \]

- a stretched exponential form does not improve the fitting
- the correlators decay to 0, further proof that the protein dynamics are liquid-like
- we can extract a diffusion constant

\[ T = 310 \text{ K} \]
\[ q\text{-values: 2.4, 3.2, 4.0, 4.8, 5.6, 6.4 Å}^{-1} \]

Non-Arrhenius temperature dependence of the alpha-relaxation time and liquid-like diffusive behavior of protein interior

Values of the diffusion constant similar to the ones of a simple glass former close to $T_C$

The VFT temperature $T_0 \sim 270$ K can also be measured by dielectric spectroscopy…

$1/\tau_\alpha = Dq^2$

$T_0 = 272$ K
$T_C = 285$ K

New Wide Dynamic Range BASIS at SNS

BASIS is a backscattering spectrometer well suited for probing diffusive and relaxational motions of glassy materials, but can also be effectively used for studying some types of collective excitations in condensed matter, such as boson peak. In the quasi-elastic regime of operation, BASIS can be used to probe dynamic processes on the pico- to nano-second time scale.
QENS Spectral Analysis of BASIS Data on Hydrated Lysozyme Powder
Experimental Evidence of the Logarithmic Decay of $\beta$-relaxation Region

Log-Decay Fitting Results

\[ F(Q,T) = f(Q) - H_1(Q,T)\ln(t/\tau_\beta) + H_2(Q,T)\ln^2(t/\tau_\beta) \]

\[ H_1(Q,T) = B_1(T)Q^\beta \quad B_1(T) = 0.086(T/T_c - 1)^{1/2} \]

\( T_C \) is the critical MCT temperature and is chosen to make \( B_1(T) \) linearly dependent on \( |(T-T_C)/T_C|^{1/2} \).

Our results show \( T_C \) is about \( 210 \pm 10 \) K, very close to the well-known dynamic transition temperature in protein \( T_D \sim 220 \) K.

HERIX will become a user instrument in January 2007. It works with up to nine analyzers in parallel with an energy resolution around 1.6 meV. The flux at the sample position will be $10^9$ photons/second in a spot of 30 x 10 microns.
Model fitting of the measured IXS spectra of BSA. The blue open circles, magenta solid line, green dashed line and red solid line represent respectively the measured data, DHO model fitted curve, resolution function and Brillouin component of the DHO model. The arrow signs (red) show the Stokes and the anti-Stokes components of the phonon-like mode excitation energy at this Q.

The upper panel shows the fitted results at Q=4.5, 5.6, 6.7 nm\(^{-1}\) (from left to right) at 170 K. No dispersion was observed in this Q range.

The lower panel shows the fitted results at Q=24.6, 27.9, 31.2 nm\(^{-1}\) (from left to right) at T=170, 220, 250 K (from up to down). At each temperature, clear Q-dependence of the phonon excitation energy is shown in the figure. At a given Q value, the phonon energy decreases substantially when temperature exceeds \(T_D\).

Biological significance of phonons in globular proteins

Many hydrated globular proteins exhibit a universal dynamic transition at $T_D = 220K$, below which the biological activity of a protein sharply diminishes.

We studied the phonon-like excitations of two structurally different proteins, lysozyme and BSA, using inelastic x-ray scattering above and below $T_D$ at APS.

We found the excitation energies of the high $Q$ phonons show a marked softening above $T_D$. It suggests that the softening of the phonons at a specific $Q$ range is intimately correlated with the increase of biological activities of the proteins.