Experimental Evidence of Fragile-to-Strong Dynamic Crossover in DNA Hydration Water

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We used high-resolution quasielastic neutron scattering spectroscopy to study the single-particle dynamics of water molecules on the surface of hydrated DNA samples. Both H2O and D2O hydrated samples were measured. The contribution of scattering from DNA is subtracted out by taking the difference of the signals between the two samples. The measurement was made at a series of temperatures from 270 K down to 185 K. The Relaxing - Cage Model was used to analyze the quasielastic spectra. This allowed us to extract a Q-independent average translational relaxation time \( \langle \tau_T \rangle \) of water molecules as a function of temperature. We observe clear evidence of a fragile-to-strong dynamic crossover (FSC) at \( T_L = 222 \pm 2 \) K by plotting \( \log(\tau_T) \) vs. \( T \). The coincidence of the dynamic transition temperature \( T_L \) of DNA, signaling the onset of anharmonic molecular motion, and the FSC temperature \( T_L \) of the hydration water suggests that the change of mobility of the hydration water molecules across \( T_L \) drives the dynamic transition in DNA.

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It is known that hydrated bio-macromolecules show sharp slowing down of their functions (kinetics of biochemical reactions) within a temperature interval \( T \sim 250-200 \) K. It was also found, from neutron and X-ray scattering, or from Mössbauer spectroscopy, that the measured mean-squared atomic displacement \( \langle x^2 \rangle \) of the bio-molecules exhibits a sharp rise in the same temperature range \([1, 2, 3, 4, 5]\). This sharp increase in \( \langle x^2 \rangle \) was taken as a sign for a dynamic transition (or sometimes called glass-transition) in the bio-molecules occurring within this temperature range. In most of these papers, the authors suggest that the transition is due to a strong rise of anharmonicity of the molecular motions above this transition temperature \([1]\). Later on, it was demonstrated that the dynamic transition can be suppressed in dry bio-molecules \([2]\), or in bio-molecules dissolved in trehalose \([3]\). Moreover, it can be shifted to a higher temperature for proteins dissolved in glycerol \([4]\). Thus the dynamic transition can be controlled by changing the surrounding solvent of the bio-molecules.

On the other hand, it was found some time ago from Raman scattering that supercooled bulk water has a dynamic crossover transition at 220 K \([4]\), similar to that predicted by Mode-Coupling theory \([4]\). Approximate coincidence of these two characteristics temperatures, one for the slowing down of bio-chemical activities and the other for the dynamic crossover in water, suggests a relation between the dynamic transition of bio-molecules and that of their hydration water \([5]\).

Another striking experimental fact is that this dynamic transition temperature, as revealed by change of slope in \( \langle x^2 \rangle \) vs. temperature plot, occurs at a universal temperature range from 250 to 200 K in all bio-molecules examined so far. This list includes globular proteins, DNAs, and t-RNAs. This feature points to the plausibility that the dynamical transitions are not the intrinsic properties of the bio-molecules themselves but are imposed by the hydration water on their surfaces.

However, \( \langle x^2 \rangle \) (mostly coming from hydrogen atoms) is an integrated quantity of motion, arising from different types of molecular motions: both vibrations and librations of hydrogen atoms with respect to their binding center in the molecules, as well as large amplitude transitions between conformational substates of the macromolecule. Therefore, it is difficult to identify the microscopic processes underlying this transition and to pinpoint the actual dynamical transition temperature from the inspection of \( \langle x^2 \rangle \) only. On the other hand, dynamical quantities, such as the self-diffusion coefficient, the viscosity, and the structural relaxation time (or the so-called \( \alpha \)-relaxation time), could show a sharper transition as a function of temperature and pressure if there is a genuine dynamic transition in the hydration water.

In this paper, we demonstrate decisively using high-resolution quasielastic neutron scattering (QENS) spectroscopy that there is a sharp dynamic crossover, identified to be a fragile-to-strong dynamic crossover (FSC), temperature of the hydration water in DNA at \( T_L = 222 \pm 2 \) K. This change of mobility of the water molecules across \( T_L \) drives the dynamic transition in DNA which happens at the same temperature. We have recently found the same dynamic crossover temperature of \( T_L = \)
220 K for hydration water in protein lysozyme\textsuperscript{1}, which further supports our conjecture that it is a change of mobility of the hydration water which triggers the dynamic transition in bio-molecules.

Highly polymerized (calf thymus) DNA, sodium salt, was obtained from Sigma (D1501, batch number 091K7030) and used without further purification. The sample was extensively lyophilized to remove any water left. The dry DNA fibres were then hydrated isopiestically at 5°C by exposing them to water vapor in equilibrium with a NaClO\textsubscript{3} saturated water solution placed in a closed chamber (relative humidity, RH =75%). The final hydration level was determined by thermogravimetric analysis and also confirmed by directly measuring the weight of absorbed water. This hydration level corresponding to about 15 water molecules per base pairs was chosen to have the primary hydration sites almost completely filled (i.e. one monolayer of water). This latter condition corresponds to equilibration against RH=80% \textsuperscript{10} and about 20 water molecules per base pairs \textsuperscript{11}. A second sample was then prepared using D\textsubscript{2}O in order to subtract out the incoherent signal from the DNA hydrogen atoms. Both hydrated samples had the same water or heavy water/dry DNA molar ratio. Differential scanning calorimetry analysis was performed in order to detect the absence of any feature that could be associated with the presence of bulk-like water.

High-resolution incoherent QENS spectroscopy method is used to determine the temperature dependence of the average translational relaxation time $\langle \tau_T \rangle$ for the hydration water. Because neutrons are predominantly scattered by an incoherent process from the hydrogen atoms in water, high-resolution QENS technique is an appropriate tool for the study of diffusional process of water molecules. Using the High-Flux Backscattering Spectrometer (HFBS) in NIST Center for Neutron Research (NCNR), we were able to measure the $Q$-dependent relaxation time $\tau_Q(Q)$ (in Eq. 1) from $\approx 400$ ps to $\approx 5$ ns over the temperature range of 270 K to 185 K, spanning both below and above the FSC temperature. For the chosen experimental setup, the spectrometer has an energy resolution of 0.8 $\mu$eV and a dynamic range of $\pm 11 \mu$eV \textsuperscript{12}, in order to be able to extract the broad range of relaxation times covering both the fragile and the strong regimes of the average relaxation times $\langle \tau_T \rangle$ from measured spectra.

QENS experiments measure the Fourier transform of the Intermediate Scattering Function (ISF) of the hydrogen atoms, $S_{H}(Q,t)$, of water molecules on the surface of DNA. Molecular Dynamics (MD) simulations have shown that the ISF of both bulk \textsuperscript{13} and confined \textsuperscript{14} supercooled water can be accurately described as a two-step relaxation: a short-time Gaussian-like (in-cage vibrational) relaxation followed by a plateau and then a long-time (time $> 1.0$ ps) stretched exponential relaxation of the cage. The so-called Relaxing Cage Model (RCM) \textsuperscript{15}, which we use for data analysis, models closely this two-step relaxation and has been tested extensively against bulk and confined supercooled water through MD and experimental data \textsuperscript{14, 15, 16}. By considering only the spectra with wave vector transfer $Q < 1.1$ Å$^{-1}$, we can safely neglect the contribution from the rotational motion of water molecule \textsuperscript{16}. The RCM describes the translational dynamics of water at supercooled temperature in terms of the product of two functions:

$$F_H(Q,t) \approx F_T(Q,t) = F^S(Q,t) \exp \left(-\frac{t}{\tau_T(Q)}\right)^\beta,$$

$$\tau_T(Q) = \frac{\langle \tau_H \rangle}{\langle \tau_D \rangle}, \quad \tau_T(Q) = \frac{\tau_0}{\gamma - 1}, \quad \tau_0 = \tau_0 \Gamma (1/\beta)/\gamma,$$ (1)

where the first factor, $F^S(Q,t)$, represents the short-time vibrational dynamics of the water molecule in the cage. This function is fairly insensitive to temperature variation, and thus can be calculated from MD simulation. The second factor, the $\alpha$-relaxation term, contains the stretch exponent $\beta$, and the $Q$-dependent translational relaxation time $\tau_T(Q)$, which strongly depends on temperature. The latter quantity is further specified by two phenomenological parameters $\tau_0$ and $\gamma$, the exponent controlling the power-law $Q$-dependence of $\tau_T(Q)$. $\langle \tau_T \rangle$ is a $Q$-independent quantity where $\Gamma$ is the gamma function. It essentially gives a measure of the structural relaxation time of the hydrogen-bond cage surrounding a typical water molecule. The temperature dependence of the translational relaxation time is then calculated from three fitted parameters, $\tau_0$, $\beta$, and $\gamma$, by analyzing a group of nine quasi-elastic peaks at different $Q$ values simultaneously.

Fig. 1 shows the mean-squared hydrogen atom displacements obtained by a method of elastic scan for hydrogen atoms in hydration water ($\langle x_{H,O}^2 \rangle$ ) and in DNA molecules ($\langle x_{DNA}^2 \rangle$), respectively. One sees that at low temperatures up to their respective crossover temperatures, both curves have a gentle linear temperature dependence. But above the crossover temperatures, they both rise sharply with different slopes. We call the crossover temperature of the former $T_L$, and that of the latter $T_C$, both have values approximately 220 K. This shows that the dynamic crossover phenomenon of DNA and its hydration water is highly correlated, and occurs at the same temperature. As we shall see, this temperature can be defined much better for the hydration water in a dynamic measurement.

We show in Fig. 2 as an example, a complete set (temperature series) of QENS area-normalized spectra. The broadening of the quasi-elastic peaks at the wing becomes more and more noticeable as temperature increases. At the same time, the peak height decreases accordingly because the area is normalized to unity. In panel B, we plot the peak height as a function of temperature. It is noticeable that the rate of increase as a function of temperature is different across the temperature 225 K. From panel C, we may notice, from the wings of these spectral lines, that two groups of curves, 270–250 K and 220–185 K, are separated by the curve at a temperature 220 K. This visual information, obtained from the spectra before data analysis, reinforces the results of the detailed
FIG. 1: Mean-squared atomic displacement $\langle x^2 \rangle$ of all the hydrogen atoms extracted from the Debye-waller factor measured by an elastic scan, as a function of temperature for H$_2$O hydrated and D$_2$O hydrated DNA samples. The solid circles represent $\langle x^2 \rangle$ dominated by contributions from H-atoms in hydration water, while the empty circles are those dominated by H-atoms contained in DNA molecules. One can clearly see that both curves have a sharp transition of slope around 220 K indicating that the dynamic crossover temperatures of the DNA ($T_C$) and the hydration water ($T_L$) are approximately the same.

In Fig. 2, we present the temperature dependence of the average translational relaxation time, $\langle \tau_T \rangle$, for the hydrogen atom in a water molecule calculated by Eq. 1. It is seen that, in the temperature range from 270 to 230 K, $\langle \tau_T \rangle$ obeys Vogel-Fulcher-Tammann (VFT) law, a signature of fragile liquid, quite closely. But at $T = 222$ K it suddenly switches to an Arrhenius law, a signature of a strong liquid. So we have a clear evidence of FSC in a cusp form. The $T_0$ for the fragile liquid turns out to be 180 K, and the activation energy for the strong liquid, $E_A = 3.48$ kcal/mol. As a comparison, we plot the same quantity in panel B for hydration water in lysozyme protein [3]. It is to be noted that the crossover temperature is sharply defined at $T_L = 220$ K, slightly lower than in the DNA case.

FIG. 2: Measured neutron spectra. Panel A shows normalized QENS spectra at $Q = 0.87$ Å$^{-1}$ at a series of temperatures. Panels (B) and (C) display respectively the heights of the peak (B) and the wings of the peak (C), at those temperatures. One notes from panel (B) a cusp-like transition signaling the rate of change of peak height from a steep high temperature region to a slower low temperature region at a crossover temperature of about 225 K. The error bars are of the size of the data points. Panel (C) indicates a similar change of the rate of increase of the width at a similar crossover temperature. In this panel, the scatter of the experimental points gives an idea of the error bars.

FIG. 3: RCM analyses of the QENS spectra at two temperatures, above and below the crossover temperature. Panels (A1) and (B1) show the results of the full analyses of the spectra at two temperatures. Panels (A2) and (B2) indicate the detail fittings of the wings. The resolution function is also indicated in the figure. One can see a sharpening of the quasi-elastic peak as temperature goes below the crossover temperature $T_L$ at 220 K. The scatter of the experimental points gives an idea of the error bars.
to an extensive hydrogen-bond network, which is less-dominantly low-density water structure, corresponding the crossover temperature, it evolves into locally pre-not fully developed hydrogen-bond network; and below dominantly high-density water structure [16, 17], with a hydration water is more fluid, implying having locally pre-

In summary, we present unequivocal evidence that there is a fragile-to-strong dynamic crossover phenomenon observable in both DNA and protein hydration water. Above the crossover temperature, the hydration water is more fluid, implying having locally predominantly high-density water structure [16, 17], with a not fully developed hydrogen-bond network; and below the crossover temperature, it evolves into locally predominantly low-density water structure, corresponding to an extensive hydrogen-bond network, which is less-

fluid. This mobility change across $T_L$ can be seen from Fig. 4 which shows the power law $Q$-dependence of ISF, $\beta\gamma$, as a function of temperature (calculated by Eq. 1). One sees from the figure that $\beta\gamma$ decreases steadily as the temperature decreases, reaching the lowest value 0.2 at the crossover temperature 220 K. It should be noted that for a freely diffusing water molecule, $\beta\gamma = 2$, therefore the very low value of $\beta\gamma = 0.2$ signifies a restricted mobility of the hydration water. There is a strong evidence from MD simulations of protein hydration water that this drastic change of mobility across the FSC triggers the so-called glass transition in protein molecules [18, 19, 20].

This paper supplies an experimental evidence which re-

FIG. 4: The extracted $Q$-independent average translational relaxation time $\langle \tau_L \rangle$ from fitting of the quasielastic spectra plotted in log scale against temperature. Panel (A) is a result from hydrated DNA, whereas Panel (B) is the same quantity measured in hydrated lysozyme shown for comparison [3]. There is a clear evidence in both cases a well-defined cusp-like dynamic crossover behavior occurring at $T_L$ indicated in the respective figures. The dashed lines represent fitted curves using VFT law, while the solid lines the fitting according to Arrhenius law. $T_L$ in both cases occurs at $222 \pm 2$ K.

![Graph showing $\langle \tau_L \rangle$ against temperature for hydrated DNA and lysozyme](image)

FIG. 5: Temperature dependence of the exponent $\beta\gamma$ of the power law $Q$-dependence of ISF.

![Graph showing $\beta\gamma$ against temperature](image)

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