Observation of a dynamic crossover in RNA hydration water which triggers a dynamic transition in the biopolymer

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High-resolution quasielastic neutron scattering spectroscopy was used to measure H_2O and D_2O hydrated RNA samples. The contribution of scattering from RNA was subtracted out by taking the difference of the signals between the two samples. The measurements were made at a series of temperatures from 270 K down to 180 K. The relaxing-cage model was used to analyze the difference quasielastic spectra. We observed clear evidence of a fragile-to-strong dynamic crossover (FSC) at $T_L=220$ K in RNA hydration water. We further show that the mean-square displacements of the hydrogen atoms in both RNA and its hydration water exhibit a sharp change in slope at approximately the same temperature 220 K. This latter fact suggests that the dynamic transition in RNA is triggered by the abrupt change of mobility of the hydration water at its FSC temperature.

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I. INTRODUCTION

It is known that many proteins exhibit sharp slowing down of their functions (kinetics of biochemical reactions) at temperatures within the interval $T \sim 240-200$ K [1,2]. It has also been shown experimentally [3–5] and computationally [6,7] that there is a sharp increase of the mean-squared atomic displacement $\langle x^2 \rangle$ in protein at about $T_C=220$ K, suggesting a dynamic transition (the so-called glass transition) in proteins at this temperature. There is strong evidence that this dynamic transition of protein is solvent induced, since the hydration water of the protein also shows some kind of dynamic transition at a similar temperature [8–10]. In our previous research [11], it was demonstrated that this dynamic transition of hydration water on lysozyme protein was, in fact, a fragile-to-strong dynamic crossover (FSC) at 220 K, where the FSC is the consequence of the existence of the liquid-liquid transition critical point (which is also called the second critical point) of water at a higher pressure and is the result of crossing the Widom line emanating from the second critical point [12].

More recently, we also showed that hydration water on B-DNA exhibits a similar FSC at $T_L=222$ K [13]. To assess whether this dynamic transition temperature T_C of proteins is universal for other kinds of biopolymers, experiments [14,15] and computations [16] were performed on these other biopolymers. However, in these experimental works, there were no explicit demonstrations of the FSC phenomenon in the hydration water of the biomolecules.

This paper presents evidence for a dynamical transition in a biopolymer (RNA) that is induced by hydration water on its surface. RNA (ribonucleic acid) is a nucleic acid polymer consisting of nucleotide monomers. This biopolymer is actively involved in several important steps during the translation of genetic information from DNA into protein products. RNA can act as a messenger (*m*RNA) carrying the genetic information that will be expressed ultimately as proteins, as transfer (*t*-RNA) recognizing the codons of the *m*RNA and bonding to the appropriate amino acid, and in general can effectively work as an enzyme (L19 RNA and *r*RNA). It is worth noting that in very simple organisms like viruses, RNA can be the only constituent of genes. RNA and DNA differ both chemically and from the structural point of view. While DNA is double-stranded, RNA is a single-stranded molecule in most of its biological forms and has a much shorter chain of nucleotides. Like DNA, most biologically active RNA species are extensively, but not fully, base paired to form double helices. In RNA, the base pair of adenine is not thymine, as it is in DNA, but rather uracil. While DNA contains deoxyribonucleic acid, RNA contains ribonucleic acid (i.e., there is no hydroxyl group attached to the pentose ring in the 2′ position in DNA, hence RNA possesses an extra two hydroxyl group). RNA has a greater extent of hydration than DNA due to its extra oxygen atoms (that is, ribose O2′) and unpaired base sites. Molecular dynamics simulations [17] and osmotic stressing experiments [18,19] showed that, in the cases of short RNA and DNA oligonucleotides duplexes (6 ± 12 bp), hydration sites around the *r*(*G*-*C*) account for two extra molecules in respect to the *d*(*G*-*C*) while around *r*(*A*-*U*) the biopolymer can bond one molecule more than *d*(*A*-*T*).

From the measurement of mean-squared atomic displacement (MSD) of H-atoms in RNA and its hydration water, the similarity of the dynamic transition in RNA and its hydration water suggests that the dynamic transition in RNA is also induced by its hydration water, despite differences in the architecture and chemical backbones of proteins, DNA and RNA.

Using high-resolution quasielastic neutron scattering (QENS) spectroscopy, we demonstrate clearly that there is a sharp FSC temperature of the hydration water on RNA at $T_L=220$ K. The change of mobility of the hydration water molecules across T_L drives the dynamic transition in RNA which happens at the same temperature. The coincidence of

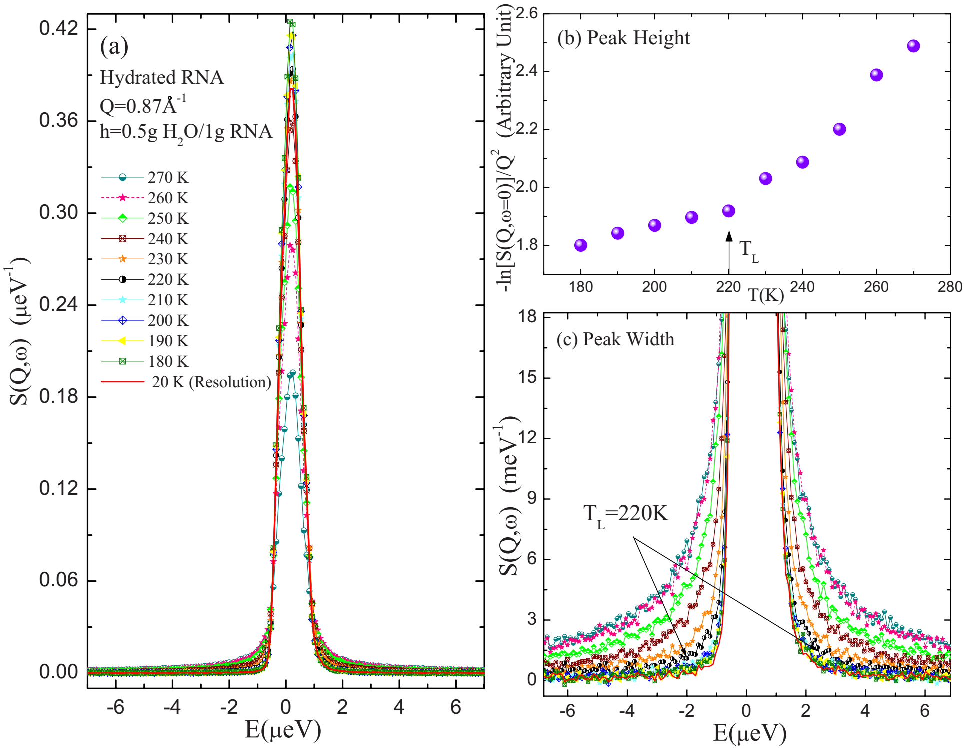

FIG. 1. (Color online) Measured difference QENS spectra at various temperatures T. (a) The normalized QENS spectra at $Q=0.87$ Å^{-1}. (b) The T dependence of the logarithm of the peak heights, which is linearly related to the MSD of the H atoms of the hydration water. (c) The wing of the peaks, from which we extract the average relaxation time $\langle\tau_T\rangle$. Both peak heights and widths indicate qualitatively the existence of a crossover temperature T_L for the hydration water.

the transition temperature in proteins [11], DNA [13], *t*RNA [15], and RNA reinforce the plausibility that the dynamic transitions are not the intrinsic properties of the biomolecules themselves but are imposed by the hydration water on their surfaces.

II. SAMPLE DESCRIPTION AND NEUTRON SCATTERING EXPERIMENTS

Ribonucleic acid from torula yeast (RNA) was obtained from Sigma (R6875, batch number 021k7063) and used without further purification. The sample was extensively lyophilized to remove any water left. The dried RNA powder was then hydrated isopiestically at 5 °C by exposing it to water vapor in equilibrium with a $NaClO_3$ 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. A second sample was then prepared using D_2O in order to subtract out the incoherent signal from hydrogen atoms of the RNA. Both hydrated samples had the same water or heavy water-to-dry RNA 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 bulklike water.

Neutron scattering measurements were done on the high-flux back-scattering spectrometer at NIST Center for Neutron Research (NCNR) with a resolution of 0.8 μeV and a dynamic range of ± 11 μeV. The elastic scans were measured at a heating-cooling rate of 0.75 K/min.

III. RELAXING CAGE MODEL ANALYSIS

Taking the difference of the QENS measurements of the two samples gives the Fourier transform of the intermediate scattering function (ISF) of the hydrogen atoms, $F_H(Q,t)$, of water molecules on the surface of RNA [20,21]. The Q-independent average translational relaxation time $\langle\tau_T\rangle$ is obtained from the difference QENS data by using the relaxing cage model (RCM) for the hydration water [22]. The RCM describes the translational dynamics of water at supercooled temperature in terms of the product of two functions:

$$F_H(Q,t) = F^S(Q,t)\exp\{-[t/\tau_T(Q)]^{\beta}\}, \tag{1}$$

$$\tau_T(Q) \cong \tau_0(0.5Q)^{-\gamma}, \tag{2}$$

where the first factor, $F^S(Q,t)$, represents the short-time vibrational dynamics of the water molecule in the cage. This short time intermediate scattering function is calculated in the Gaussian approximation using the known density of states of hydrogen atoms in water. This function is fairly insensitive to temperature variation, and thus can be calculated from a molecular dynamics (MD) simulation. The second factor, the α-relaxation term, contains the stretch exponent β, and the Q-dependent translational relaxation time $\tau_T(Q)$, which is a strong function of temperature. The latter quantity is further specified by two phenomenological parameters τ_0 and γ, the prefactor and the exponent controlling the power-law Q-dependence of $\tau_T(Q)$ respectively. The average translational relaxation time, which is a Q-independent quantity we use in this paper, is defined as $\langle\tau_T\rangle = \tau_0\Gamma(1/\beta)/\beta$, where $\Gamma(x)$ is the gamma function. The temperature dependence of the translational relaxation time is calculated from three fitted parameters, τ_0, β, and γ, by analyzing a group of quasielastic peaks at different Q values simultaneously. For this analysis, we chose seven spectra from data taken at HFBS at each temperature, as described in detail in Ref. [11].

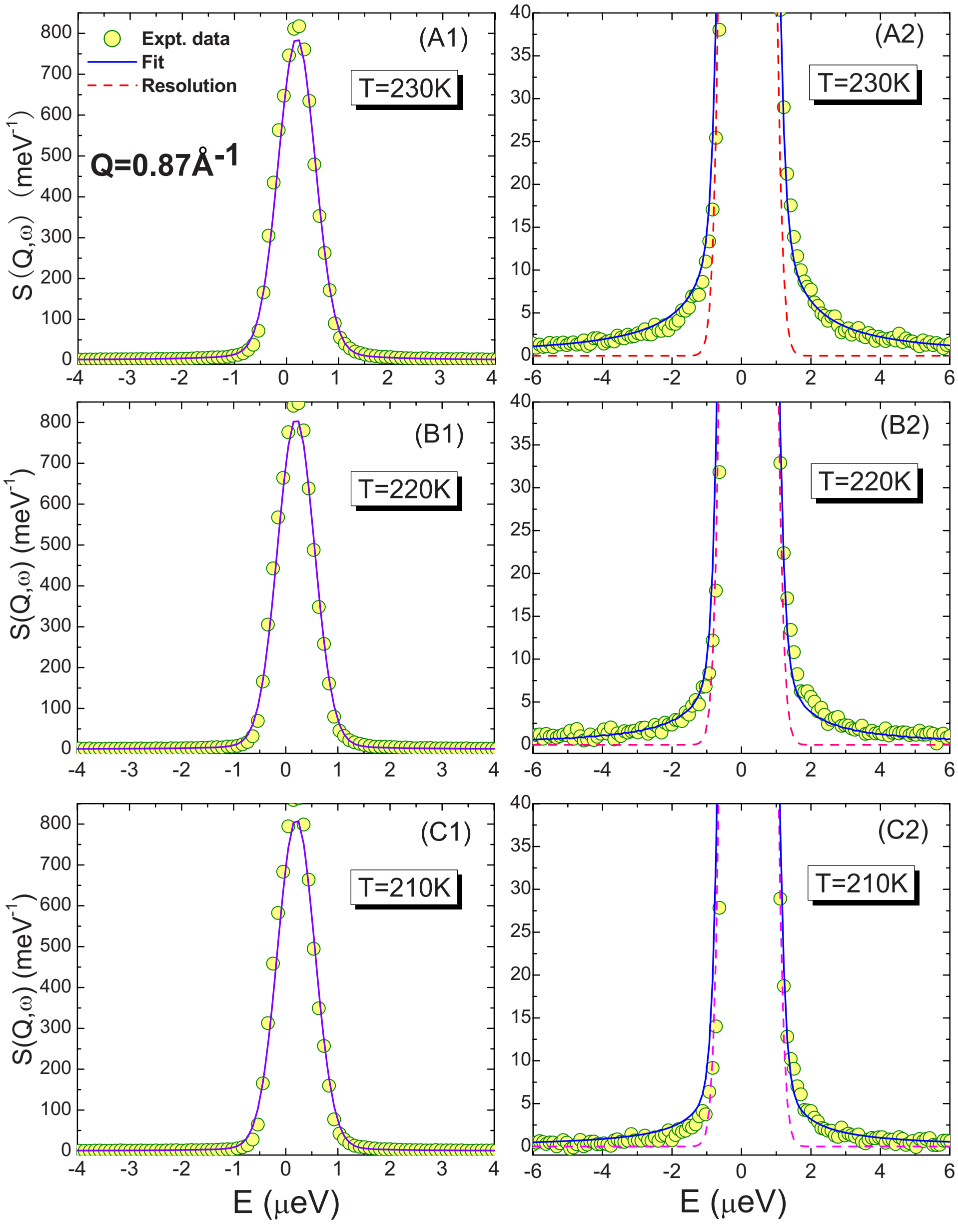

FIG. 2. (Color online) RCM analyses of the normalized difference spectra. Symbols (empty circles) are QENS data and the solid lines are the RCM fits. (a)–(c) Spectra taken 10 K above, at, and 10 K below, respectively, the crossover temperature.

IV. RESULTS AND DISCUSSIONS

We plot in Fig. 1 the QENS spectra directly obtained from the experiments. The normalized QENS peaks of the hydration water as a function of temperature qualitatively show that there is some kind of crossover temperature existing at around 220 K, judging from the variation of their peak height and peak width. The logarithm of the peak height is proportional to the mean-squared displacement (MSD) of the hydrogen atoms $\langle x^2_{H_2O}\rangle$, which is shown in Fig. 5(a).

However, a much sharper definition of this dynamic crossover temperature T_L can be obtained from RCM analysis of the difference spectra of the two hydrated samples. Figure 2 shows the comparison of the RCM analyses results with the experimental data. It can be seen that the RCM analyses are satisfactory. The panels on the left-hand side show the overall agreement of the RCM analyses and the experimental data. The panels on the right-hand side show the comparison of the instrument resolution with the quasielastic scattering data. The difference between the resolution function and the experimental data is the dynamic part which we can extract information from. We can see that the lower the temperature, the closer the experimental spectrum is to the resolution function.

From the results of RCM analysis of experimental $S_H(Q,\ \omega)$, we obtain three parameters, τ_0, β, and γ, and are able to calculate the theoretical intermediate scattering function (ISF) from Eq. (1). In Fig. 3(a), we draw the ISF of the hydrogen atoms in RNA hydration water for each different temperature. They show clearly the two-step relaxation process (beta relaxation for the short time process and alpha relaxation for the long time one) described by the RCM. Furthermore, we can directly extract the alpha relaxation time from the ISF by taking $1/e$ points for each temperature, which is plotted in Fig. 3(b). One can clearly see that there is a dynamic crossover at $T_L=220$ K.

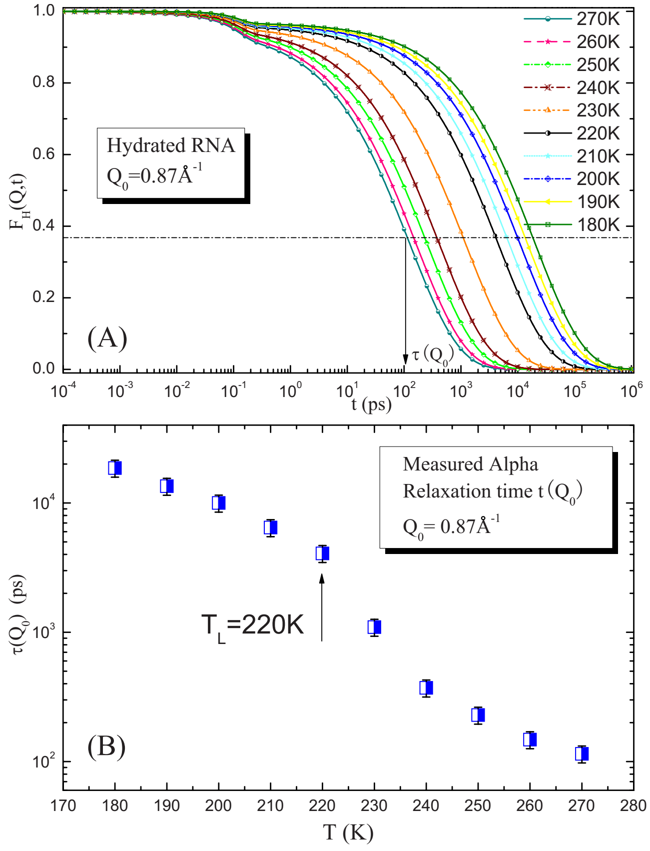

FIG. 3. (Color online) (a) Intermediate scattering functions (ISF) at Q_0 of hydrogen atoms in RNA hydration water, as a function of temperature. They are extracted from analysis of the quasielastic spectra by using the relaxing-cage model. It is seen that the ISF exhibits a two-step decay consisting of the beta and the alpha relaxation processes. (b) The alpha-relaxation times at Q_0, as a function of T, extracted from $1/e$ points of the alpha decays.

The average translational relaxation time $\langle\tau_T\rangle$ can also be calculated from the fitted parameters τ_0, β, and γ. Figure 4 shows the log$\langle\tau_T\rangle$ vs $1/T$ plot. At high temperatures, above 220 K, $\langle\tau_T\rangle$ obeys a Vogel-Fulcher-Tammann (VFT) law, namely, $\langle\tau_T\rangle=\tau_0\exp[DT_0/(T-T_0)]$, where D is a dimensionless parameter providing the measure of fragility and T_0 is the ideal glass transition temperature. Below 220 K, the $\langle\tau_T\rangle$ switches over to an Arrhenius behavior, which is $\langle\tau_T\rangle=\tau_0\exp(E_A/RT)$, where E_A is the activation energy for the relaxation process and R is the gas constant. This dynamic crossover from the super-Arrhenius (the VFT law) to the Arrhenius behaviors is cusplike and thus it sharply defines the crossover temperature to be T_L=220 K, much more accurately than that indicated by the MSD $\langle x^2_{\mathrm{H_2O}}\rangle$, shown in Fig. 5(a). Figure 4(a) shows the FSC phenomenon of the hydration water in RNA. Figure 4(b) shows the same plot for the DNA hydration water. It can be seen that the crossover temperature in both RNA and DNA hydration water are approximately the same, within the experimental error.

To obtain the mean-squared atomic displacement $\langle x^2\rangle$, we make elastic scans through the temperature range from 5 to 300 K, which covers completely the supposed crossover temperature T_L and our QENS measurement temperatures. Since the system is in a stationary metastable state at temperatures below and above T_L, we make measurements by heating and cooling, respectively, and observe exactly the same results. We calculate the MSD from the Debye-Waller factor, $S_H(Q,\ \omega=0)=\exp[-Q^2\langle x^2\rangle]$, by linearly fitting the logarithm of $S_H(Q,\ \omega=0)$ with Q^2. Figure 5(a) shows the MSD of the hydration water molecule, $\langle x^2_{\mathrm{H_2O}}\rangle$, in the observational time interval of about 2 ns (corresponding to the energy resolution of 0.8 μeV). Figure 5(b) shows the MSD of hydrogen atoms in the RNA molecule. From these two figures, one can conclude that the dynamic transition temperature of the hydration water (denoted as T_L) and the dynamic transition temperature of the RNA molecule (denoted as T_C) are approximately the same (within the error bars of the kink positions). Note that in Fig. 5(b), the transition of slope in MSD for RNA happens at a temperature $T_C\approx 240$ K, slightly higher than $T_L\approx 220$ K, of hydration water shown in Fig. 5(a). This suggests that there may be a "delay" in the induced transition of RNA to a more flexible form after the sharp

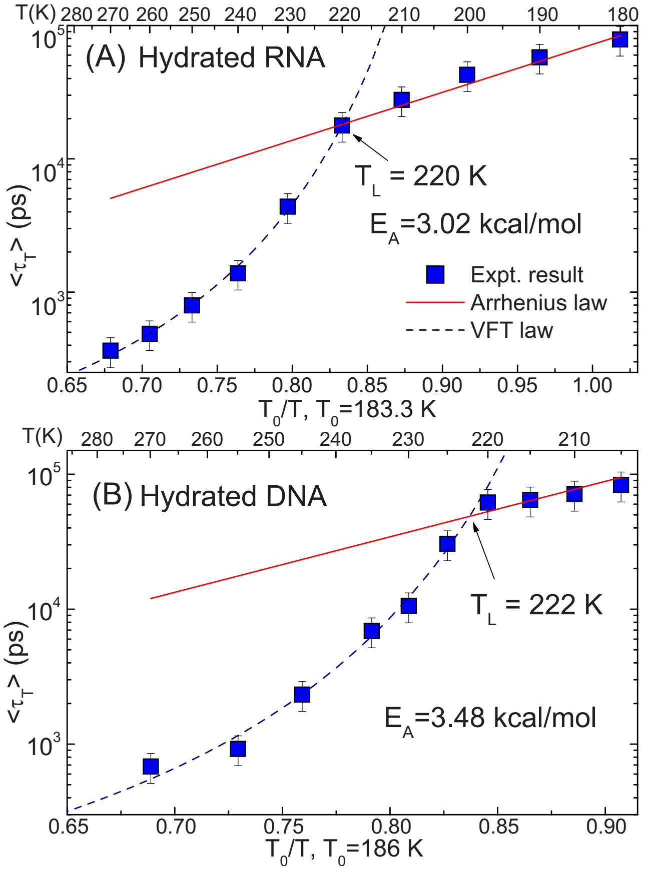

FIG. 4. (Color online) (a) The extracted average translational relaxation time $\langle\tau_T\rangle$ from fitting of the quasielastic spectra by the relaxing cage model plotted in a log scale against $1/T$. It shows clearly a well-defined cusplike dynamic crossover behavior occurring at T_L=220 K. The dashed line represents fitted curves using the VFT law, while the solid line is the fitting according to the Arrhenius law. (b) Result of a similar analysis for a hydrated DNA case for the purpose of comparison [12]. Note the crossover temperature, T_L=222 K in this case.

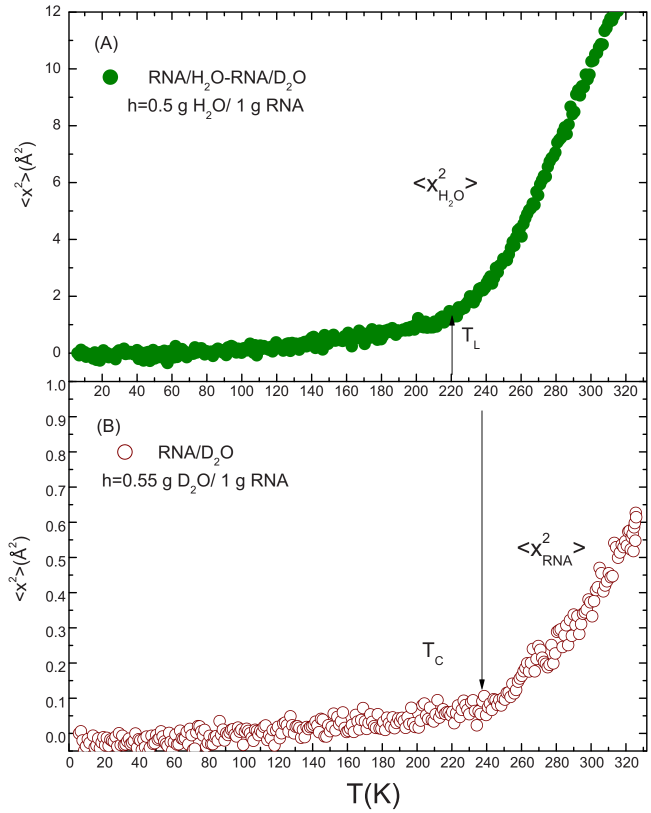

FIG. 5. (Color online) The mean-squared atomic displacement (MSD) averaged over all the hydrogen atoms, $\langle x^2 \rangle$, extracted from the Debye-Waller factor measured by an elastic scan with resolution of 0.8 μeV, as a function of temperature for the H_2O hydrated and D_2O hydrated RNA samples. (a) Data processed from the difference between the two, which gives MSD of the H atoms in the hydration water. (b) Data processed from the latter, which gives MSD of H atoms in RNA.

FSC dynamic transition in its hydration water. At FSC (happens at the crossing of the Widom line) the relative proportion of low-density water (LDL) to high-density water (HDL) is about 50:50 [23]. One may need to have a high enough proportion of HDL to LDL which happens 20 K after crossing the Widom line to generate enough mobility of hydration water to trigger the glass transition in RNA (see more discussion in the Conclusion section).

V. CONCLUSION

We have shown in this paper experimental evidence for the existence of a super-Arrhenius to Arrhenius dynamic crossover (T_L) in RNA hydration water at 220 K and proposed a plausible reason that the dynamic crossover of the hydration water triggers the onset of the dynamic transition at the same temperature (T_C) in RNA. There is a controversy in the literature [24–26] whether this crossover can be called a fragile-to-strong dynamic crossover, defined by Angell and co-workers [27]. We, however, like to emphasize here the physical significance of the dynamic crossover we have observed regardless of what we call it. We have shown before [28] that above T_L the structure of hydration water is predominately in its high-density form (HDL), which is more fluid; but below T_L, it transforms to predominately the low-density form (LDL), which is less fluid. The concepts of the existence of HDL and LDL in deeply supercooled water have been extensively discussed in the recent literature [29,30]. Thus this abrupt change in the mobility of hydration water apparently induces the change in the energy landscape of RNA, which causes the dynamic transition (or the glass transition) in the biopolymer.

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