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ABSTRACT

Melting or thermal denaturation of a DNA molecule and the different bubble-rich, premelting DNA states that serve as a precursor for DNA thermal denaturation are vital events in DNA thermophysics. In this study, we employ cantilever-based sensing to firstly pinpoint the occurrence of DNA melting and identify the temperature T_m characterizing the melting. Very importantly, this sensing is carried out with an extremely small volume (∼picoliters) of DNA sample with the cantilever demonstrating an extremely high sensitivity on the order of $\mathrm{mJ/g \cdot K}$ corresponding to pico-Joules of energy input. Secondly, this same large sensitivity of the cantilever is used to quantify the hitherto unknown thermophysical properties of the bubble-rich DNA premelting states. In fact, for both the melting and premelting states, the cantilever provides a framework to calculate the specific heat capacity and the storage and loss moduli of the cantilever-DNA-solution system, thereby establishing a platform for quantifying DNAs' thermo-mechanical behavior.

Melting or thermal denaturation of a DNA molecule in which the A-T and C-G hydrogen bonds are broken and a double-stranded DNA (ds-DNA) molecule separates into two single-stranded DNA (ss-DNA) molecules is one of the most fundamental processes in DNA thermophysics.[1–4] This melting process, which takes place across a temperature interval of 3°–20° (depending on the size and the ATCG composition of the DNA molecule), has been extensively probed using a host of methods such as differential scanning calorimetry (DSC),[5,6] UV (ultra-violet)-spectrophotometry,[7] Raman spectroscopy,[5,8] thermal fluctuation spectroscopy (TFS),[9] *etc.* Depending on the method by which the DNA thermal denaturation is probed, different kinds of information are available: For example, the DSC technique quantifies the total enthalpy change and the change in the heat capacity, while the TFS method is appropriate for quantifying the unzipping events that lead to denaturation. On the other hand, a single temperature T_m, which is typically the middle point of the temperature range across which the DNA melting occurs, is considered as the characteristic representative temperature for the DNA melting process.[1] In fact, the influence of different factors (e.g., fraction of A-T and C-G pairs,[10–12] presence of ions like Na^+, Mg^{2+}, Ca^{2+},[8,13,14] *etc.*) on the melting process has been quantified by how they affect T_m. While substantial research has been conducted in precisely determining this T_m for different combinations of system parameters (DNA length, composition, nature of ions in the fluid bath, *etc.*), significantly less is known about the properties of the premelting, bubble states that precede the DNA melting process.[3,9,15,16] These bubble states originate due to the local breaking of the A-T and C-G bonds (A-T bonds break with relatively lower energy than the C-G bonds). The complete melting would necessitate the breaking of all the bonds. As long as some of the bonds are intact, the nondenatured DNA is in a state rich with bubbles. These bubbles develop and collapse spontaneously, and are manifested as fluctuations in the temperature-vs-time profiles.[9] Also, recent experiments have confirmed via implicit approaches (such as the change in the DNA end-to-end distance) the presence of such

bubbles in the DNA architecture at premelting temperatures.[17] However, there exist very few studies that have tried to quantify the thermophysical properties of such premelting bubble-rich DNA molecules.

In this study, we employ a hollow microfluidic cantilever system filled with DNA solution to conduct thermomechanical quantification of the occurrence of DNA melting as well as quantification of the properties of the DNA premelting, bubble-rich states. There are two key facets to this study. Firstly, the use of the hollow microfluidic cantilever system allows the detection of DNA melting with an extremely small volume (∼picoliters) of DNA solution. This study, therefore, is an example of detection of DNA melting with one of the smallest volumes of the DNA solution relying on the extremely high sensitivity of the cantilever system on the order of $\mathrm{mJ/g \cdot K}$ corresponding to picoJoules of energy input at 300 K.[18] Previous studies on DNA melting have employed much larger volumes (∼microliters or more[9]). The second important facet of this study is the utilization of this ultrahigh sensitivity of the cantilever-DNA-solution system for quantifying the properties of the premelting, bubble-rich DNA states through the temperature and dissipation responses of the cantilever-DNA-solution system. These properties include the specific heat capacity of the solution of bubble-rich DNA molecules as well as the loss and storage moduli (in a form where these moduli are related to each other) of the cantilever-DNA-solution system. The present research group has conducted extensive research on detecting a variety of species and processes using the extremely high sensitivity of the hollow microfluidic cantilevers;[18–20] in this study, the same set up is employed to quantify the DNA melting phenomenon using an ultralow volume DNA solution as well as to make predictions on the hitherto unknown properties of the premelting, bubble-rich DNA states.

A microfluidic *pL* (∼100 pl) channel cantilever (see Fig. 1) is filled with the DNA solution and the response of the DNA molecules to the changes in temperature at premelting and melting temperature ranges is probed. The dissipation response and temperature variation of the cantilever-DNA-solution system are noted in the presence of constant heat input, and the system exhibits a sensitivity on the order of $\mathrm{mJ/g \cdot K}$ corresponding to ∼picoJoules of energy input at 300 K.[18] More details on the experimental procedure are provided in the *Materials and Methods* section in the supplementary material.

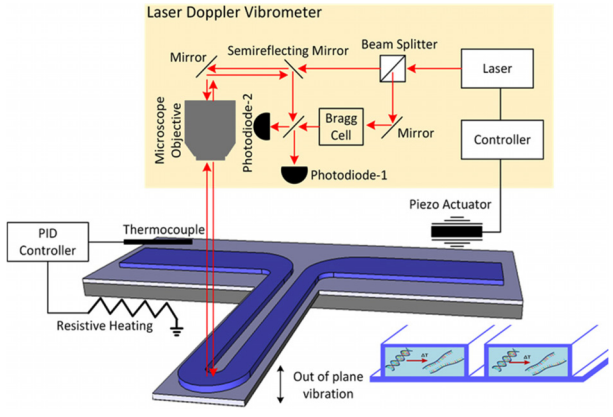

FIG. 1. Schematic of the cantilever and a pictorial summary of the technique that it used for the dissipation measurements.

Figures 2(a)–2(c) provide the time variation of the temperature (T) of the cantilever-DNA-solution system for three different types of DNA molecules subjected to a constant heat supply. The T-vs-ln(t) variation studied in Figs. 2(a)–2(c) is fitted by a curve. The time derivative of that curve [see the insets of Figs. 2(a)–2(c)] invariably shows an extremum at the characteristic melting temperature $T{=}T_m$ (see the caption of Fig. 2 for more details). *We hypothesize that this response characterizes the melting process and confirms that the specific heat capacity at the melting temperature for a DNA molecule with a particular A-T-C-G combination is a constant.* Such an inference can be attributed to the fact that Fig. 2 shows $\frac{d^2T}{d\alpha^2} = 0$ (where $\alpha = \ln\{t\}$), yielding (see the supplementary material for the derivation)

$$C_P(T = T_m) = \frac{Q_m}{A_1}, \tag{1}$$

where $C_P(T = T_m)$ is the DNA specific heat at the melting temperature T_m, Q_m is the total per unit mass heat added to cause the melting, and A_1 is a constant. Obviously, for a given DNA molecule, Q_m will be

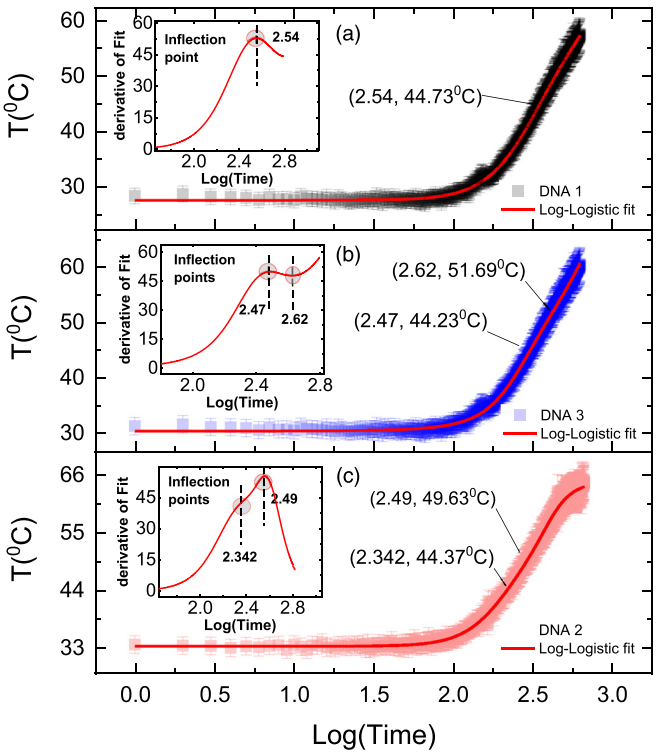

FIG. 2. Dynamic temperature vs time evolution for the cantilever-DNA-solution system subjected to constant heat input. The corresponding error bars indicate temperature inflections of the bath at definite times (see the insets). The results are shown for three different DNA molecules at 1 μM concentrations. In the insets, we identify the inflection points, which provide the corresponding T_m by noting the time when this inflection occurs. For DNA 2 and DNA 3, there are two time values where inflections occur; T_m and T_n are the temperatures corresponding to the larger and smaller times where the inflection occurs. (a) DNA 1: 5′-AAAAAATTTTTAAATTTAAATTTAAATTTAAATTTAAATTTAAA-3′; (T_m=44.73 °C), (b) DNA 3: 5′-CCCAAATTCAAATTAATAAAAACCC-3′; (T_m=51.69 °C, T_n=44.23 °C), and (c) DNA 2: 5′-GGAGGGAAGAAGAAATTGGGGTTGA-3′; (T_m=49.63 °C, T_n=44.37 °C).

a constant. Thus, the right hand side of Eq. (1) is a constant that depends entirely on the nature (size, A-T-C-G combination, *etc.*) of the DNA. Consequently, the left hand side, i.e., $C_P(T = T_m)$, must also be a constant. Several experimental studies have established that the DNA melting process is characterized by a large change in the DNA heat capacity at the melting temperature.[21–23] Given that prior to the melting process the DNA heat capacity is significantly small,[21] this large and fixed change in the heat capacity is equivalent to the attainment of constant heat capacity at the melting temperature, i.e., a result established by our experiments. It is also important to mention here that Eq. (1) assumes that the heat added is primarily causing an enthalpy increase in the entire cantilever-DNA-solution system, i.e., we are treating the cantilever-DNA-solution system as a lumped mass system for our analysis.

It is worthwhile to discuss here why we identify this temperature T_m as the melting temperature. We carry out separate OD_{260} measurements (i.e., UV spectrophotometry experiments) using a Varian Cary 50 UV-vis spectrometer (please see the *Materials and Methods section* in the supplementary material for more details). Figures 3(a)–3(c) depict UV-Vis OD_{260} measurements of the DNA samples from 40 to 55 °C. The corresponding transition curve fits (for the absorption-vs-temperature profiles) and their derivatives [see Fig. 3(d)] reveal the transition maxima for different samples. The temperatures of these transition maxima are noted, and are very close to T_m (see Fig. 2) for the corresponding DNA samples [see Fig. 3(e)]. Given the fact that the UV spectrophotometry measurement is often considered a most reliable approach for quantifying the DNA melting temperature,[9,24,25] this comparison allows us to convincingly infer that indeed the temperature-vs-time measurements using the cantilever provide a definite confirmation of the occurrence of the DNA melting event.

For all experiments, we use 1 μM DNA samples in pH 7.4 1× PBS (10 mM Na_2HPO_4, 2.7 mM KCl, 137 mM NaCl, and 1.8 mM KH_2PO_4).

DNA premelting states are typically characterized by the formation of unstable bubbles by the local breaking of the A-T and C-G bonds. These bubbles appear and disappear randomly, and such events are expected to cause fluctuations in the temperature-vs-time profile of a DNA solution.[9] We witness $\frac{d^2T}{d\alpha^2} = 0$ (where $\alpha = \ln\{t\}$) at a temperature (T_n) much smaller than T_m (see the insets and the caption of Fig. 2). Therefore,

$$C_P(T = T_n) = \frac{Q_n}{A_2}, \tag{2}$$

where $C_P(T = T_n)$ is the DNA specific heat at its premelting state at $T{=}T_n$, Q_n is the total per unit mass heat added to ensure that the DNA attains the premelting state at T_n, and A_2 is a constant. A given DNA premelting state with localized bubbles is characterized by a corresponding fixed value of Q_n necessary to cause the local breakage of the A-T and C-G bonds that lead to this particular premelting stage. Consequently, $C_P(T = T_n)$ will be a constant and a characteristic of the premelting, bubble-filled DNA state. *Therefore, our cantilever-based measurements provide a thermal signature for characterizing a particular bubble-rich premelting DNA state.* There has been prior research on identifying such bubble-rich premelting states of the DNA.[3,9,15–17] However, this paper describes a premelting state in terms of definite thermophysical properties of that premelting state.

Figures 4(a)–4(c) demonstrate the temperature-dependent dissipation (D) response of the cantilever-DNA-solution system at melting and premelting temperature ranges for three different DNA solutions. The cantilever system, consisting of the hollow microchannel and the

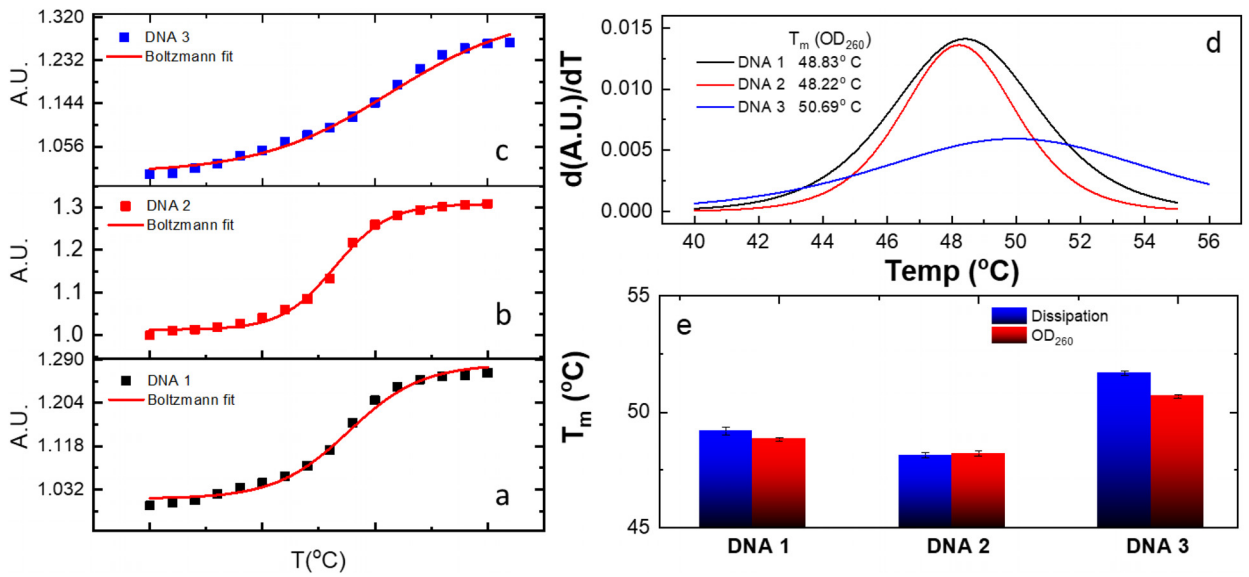

FIG. 3. (a)–(c) Absorption-vs-temperature profiles for the three DNA solutions obtained from the OD_{260} measurement (i.e., UV spectrophotometry experiments). The absorption is quantified by Absorption Units (A.U.). (d) Variation of the temperature-derivative of the absorption (the derivative is obtained from the mathematical fits of the absorption-vs-temperature profiles) with temperature. The maxima peaks in this variation provide the T_m from the OD_{260} measurement (i.e., UV spectrophotometry experiments). (e) Comparison of T_m values obtained from the cantilever with those obtained from the results of the OD_{260} measurement (i.e., UV spectrophotometry experiments). Respective error bars are also shown.

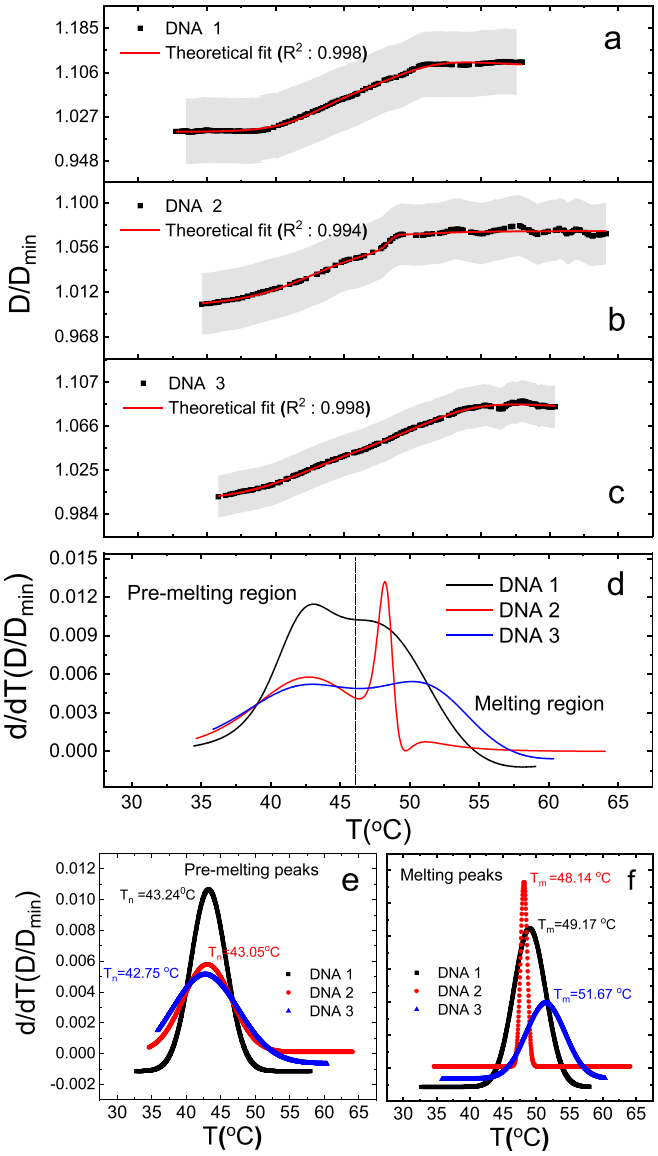

FIG. 4. (a)–(c) Results of dynamic dissipation changes with temperature for the microfluidic-cantilever-DNA-solution system for three different DNAs (identified in the caption of Fig. 2). Theoretical fits are obtained for the dissipation-vs-temperature profile. (d) Derivative of the dissipation with respect to the temperature, i.e., the d(D/D$_{min}$)/dT-vs-T profile (please see the figure on the top right panel). The d(D/D$_{min}$)/dT-vs-T profile demonstrates two separate peaks: One at the melting temperature zone [magnified in (e)] and another at the premelting temperature zone [magnified in (f)].

dilute DNA solution, is likely to behave as a viscoelastic system stemming from a combination of the elastic behavior of the hollow-channel cantilever and a non-Newtonian viscous-liquid behavior of the dilute DNA solution. D is inversely proportional to the well-known *Q-factor* that characterizes the response of a cantilever system. The temperature of the system increases with continuous heat input (see Fig. 2), and this temperature-response is manifested by the changes in the dynamic dissipation of the microfluidic channel cantilever as a function of the temperature. The significance of the dissipation-*versus*-temperature variation is better elucidated in the corresponding variation of the temperature gradient of dissipation [$d(D/D_{min})/dT$] with temperature [see Fig. 4(d)]. In melting temperature ranges, one witnesses an expected peak in the $d(D/D_{min})/dT$-*vs*-T profile establishing the occurrence of the DNA melting. Such peaks occurring at $T=T_m$ are representative of the occurrence of the melting that leads to a peak in the rate of change of absorption of energy by the fluid bath (see Fig. 3). The most important observation from these figures is however not these peaks at $T=T_m$; rather the observation of a second peak in the $d(D/D_{min})/dT$-*vs*-T profile at the premelting temperature $T=T_n$.

These peaks at $T=T_m$ and $T=T_n$, while confirming the occurrence of the melting state and a premelting bubble-rich state, also help to relate the storage and loss moduli of the cantilever-DNA-solution system as [using the condition $(dD/dT)_{T_m,T_n} = 0$ and the relatively weak variation of storage and loss moduli of the cantilever-DNA-solution system with temperature T (please see the supplementary material for derivation)]

$$\left(\frac{1}{E''}\frac{d^2E''}{dT^2} - \frac{1}{E'}\frac{d^2E'}{dT^2}\right)_{T=T_m,T_n} = 0, \tag{3}$$

where E' and E'' are the storage and loss moduli of the cantilever-DNA-solution system. Therefore, our experiments provide a unique relationship connecting the storage and loss moduli of the DNA-solution-containing cantilever in the melting and premelting states of the DNA, and in the process provide a platform to better quantify the DNA melting and premelting states by using only picoliters of DNA solution.

There are several critical points that beg discussion. Firstly, in the present study, the nonmelted, bubble-free state is the B-DNA, while the melted phase is of two single-stranded DNA molecules. The thermal denaturation induced bubble-rich, premelted state will lead to a reduction in the DNA end-to-end distance due to a reduced persistence length,[17] and is therefore different from the S-DNA or M-DNA states that are witnessed when the B-DNA is subjected to an overstretching transition in the presence of a large external stretching force.[26–28] Second, we also calculate T$_m$ for different DNA samples using an online tool,[29] which are found to be higher than that obtained from our cantilever-based measurement as well as OD$_{260}$ measurement (see Table S1 in the supplementary material). This can be attributed to possible imperfect hybridization events triggered by the specific base sequences. Third, it is possible that in addition to the formation of transient bubbles, there can be fraying or transient opening[30] at the ends of DNA-1 consisting of only A and T bases. Finally, it should be noted that in order to identify the phases of the premelted states, it is critical to accurately quantify the latent heats for the melted and premelted states, which in turn will necessitate a significant improvement of our measurement framework providing precise temperature control near the transition temperature.

To summarize, we have conducted cantilever-based experiments to study DNA melting and the properties of the DNA premelting, bubble-rich states using extremely small volumes (~picoliters) of DNA solution. Supported by separate UV-spectrophotometry experiments, we establish that the cantilever provides an excellent quantification of the melting temperature T_m. Furthermore, using this same

cantilever platform, we develop a framework for quantifying the physical properties (e.g., specific heat capacity and storage and loss moduli) of the DNA-solution-cantilever system, which aids in characterizing the properties of the DNA at both melting and premelting, bubble-rich states. While such bubble-rich states have been previously hypothesized/identified in a number of publications, development of such a framework to quantify these states has been missing. Overall, we anticipate that the present study will open a window for probing the thermal response of DNA and other polymeric systems in a set-up that utilizes an extremely small volume of chemical species utilizing the ultrahigh sensitivity of the hollow, microfluidic cantilever system.

See supplementary material for (a) Materials and Methods and (b) derivations of Eqs. (1)–(3).

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