Mechanical-energy-based amplifiers for probing interactions of DNA with metal ions

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We report our development of a simple and cost-effective method to amplify and probe the interactions of DNA with metal ions, which are important for various fundamental processes in living systems. This method is based on perturbing energy landscapes using mechanical energy stored in bent DNA molecules. In this proof-of-principle study, the mechanical-energy-based amplifiers were applied to examine the interactions between DNA and Mg^{2+} ions or Ag^{+} ions. We demonstrated that interactions between DNA and Mg^{2+} or Ag^{+} ions, which are not detectable using gel electrophoresis without amplification, can be easily measured using our molecular amplifiers. In addition, we showed that quantitative details about the DNA-metal interactions can be estimated using our method. Our method is simple, sensitive, and cost-effective. We expect that the developed method will be useful for various applications.

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I. INTRODUCTION

A fascinating concept in physics is that many properties of a system (including the equilibrium and dynamics) are governed by the system's Hamiltonian H, or the potential energy V, which has been commonly referred to as the "energy landscape" of the system and has been increasingly useful in other fields, such as chemistry, biochemistry, and biology [1]. An interesting direction rising from this concept is to perturb the energy landscape to possibly modulate and/or bias chemical and biochemical systems and reactions by various means [2]. Among the available means, mechanical methods are particularly appealing because mechanical methods are *universal* in the sense that they do not depend on the exact type and details of the involved chemical and biochemical systems and reactions. Therefore, it is of great interest to make use of mechanical energies and forces for controlling chemical, biochemical, and biological reactions, with significant progresses in the past three decades [1]. For example, mechanical forces induced by ultrasound have been applied to polymer solutions to accelerate and alter the course of the related chemical reactions [3]. In addition, mechanical tensions have been introduced to enzymes using DNA molecular springs to control their enzymatic activities [4–6].

In this article, we report our development of a new concept of exploiting mechanical energies and forces to amplify the interactions between DNA and metal ions, which are important for life [7]. On one hand, DNA-metal interactions are essential for various fundamental processes in cells. For example, the formation of secondary and higher-order structures of nucleotides, DNA repair, and genomic stability require the presence, mediation, and/or participation of metal ions such as magnesium ions (Mg^{2+}) [8–10]. On the other hand, many metal ions could be toxic, resulting in DNA damage and cell death, which can accumulate and possibly lead to diseases such as cancers and other diseases [11]. For example, many studies showed that Ag^{+}, Cu^{2+}, and Al^{3+} ions induce DNA damage and have genotoxicity [12]. Therefore, it is important to understand the interactions between DNA and metal ions in solutions, which however is not straightforward to measure directly. First, most chemical and biochemical methods are not sensitive enough: the most well studied DNA-metal interactions using biochemical methods are DNA cleavages [7], but most DNA-metal interactions are much milder. In addition to biochemical assays, many spectroscopic methods have been used to study DNA-metal interactions. However, while some of them are not sensitive enough (e.g., x-ray absorption spectroscopy), some require samples in solid phase and thus are not suitable for studies in solutions (e.g., electron paramagnetic resonance) [7]. Furthermore, sensitive techniques such as infrared and Raman spectroscopy and nuclear magnetic resonance spectroscopy typically require expensive equipment [7]. Therefore, there is an urgent need for developing simple, sensitive, and cost-effective methods to study the interactions between DNA and metal ions.

In this work, we took advantage of mechanical energy stored in bent DNA molecules and developed a simple, cost-effective method to amplify and probe the interactions between DNA with metal ions. The strategy of this method is illustrated in Figs. 1(a) and 1(b), where hypothetic energy landscapes along the DNA-metal "reaction" coordinate are shown, assuming one of the local minima in the energy landscape (indicated by the open magenta arrow) gives the detectable signal of the DNA-metal interaction. Without amplification (i.e., normal linear DNA), the signal from the interaction at equilibrium might be too low to detect; however, by perturbing the energy landscape using the bending energy stored in bent DNA molecules, more molecules might be distributed in the detectable state (open magenta arrow), resulting in an amplification of the detectable signals. It is noted that the mechanical energy stored in the bent DNA does not

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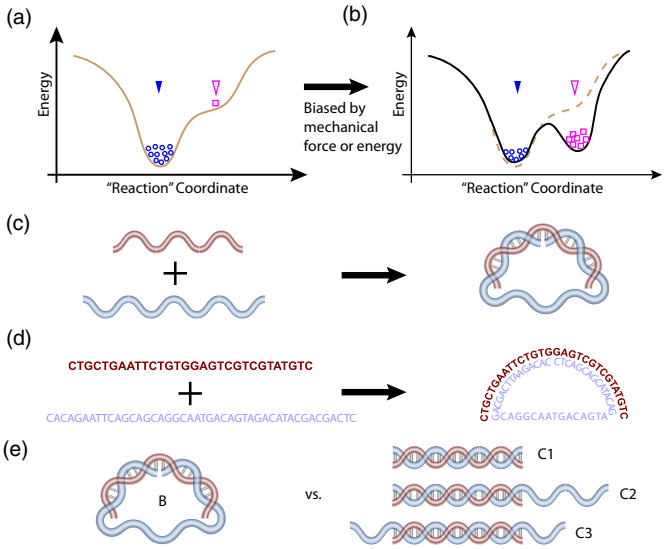

FIG. 1. Overall strategy of the mechanical-energy-based amplifiers for probing interactions of DNA with metal ions. (a, b) Perturbing a hypothetic energy landscape to redistribute molecules so that higher signals are detected. With the original, unperturbed energy landscape (a), fewer molecules are distributed on the detectable state (open magenta arrow), producing lower signals. In contrast, after biasing the energy landscape by mechanical forces or energies (b), more "molecules" are redistributed on the detectable state (open magenta arrow), "amplifying" the signals for detections. (c) Self-assembly of a bent double-stranded DNA. (d) Self-assembly of a bent double-stranded DNA with sequences shown. (e) Bent DNA molecules (construct B) as amplifiers *vs* linear DNA molecules (constructs C1, C2 and C3) as negative controls.

necessarily introduces additional interactions of DNA with metal ions; instead, the mechanical energy improves the sensitivity for observing the interactions. A good analog to illustrate this idea is throwing marble balls onto wooden sticks. If the collisions are weak enough, the sticks rarely crack, producing "low signals." In contrast, after applying stress and prebending the sticks so that they are close to break down, collisions at the same strength would result in higher number of cracked sticks, generating "higher signals." The mechanical energy stored in the pre-bent sticks does not change their interactions with the balls; instead, it makes the signals much easier to be observed. In other words, the mechanical energy "amplifies" the signals.

The bent DNA molecules are achieved following the pioneer work by the Zocchi group [13–15]. Briefly, as shown in Figs. 1(c) and 1(d), two single-stranded DNA sequences are designed. The left 1/3 of the long sequence (light blue) hybridizes to the left half of the short sequence (dark red), while the right 1/3 of the long sequence hybridizes to the right half of the short sequence, leaving the middle 1/3 of the long sequence unhybridized. This design will produce, upon hybridization, a bent double-stranded DNA (containing a nick), while the single-stranded part is stretched. In contrast to previous work focusing on understanding the mechanical properties and bending energy of the bent DNA molecules (with or without nicks) [13–16], the goal of the current study is to explore applications of the bent DNA molecules.

As a proof-of-concept, these mechanical-energy-based amplifiers were applied to examine the interactions between DNA and Mg^{2+} ions or Ag^{+} ions. We demonstrated that interactions between DNA and Mg^{2+} or Ag^{+} ions, which are not detectable using gel electrophoresis without amplification, can be easily measured using our molecular amplifiers. In addition, we showed that our method is capable of obtaining quantitative details about the DNA-metal interactions. Our method is simple, sensitive, and cost-effective, without requiring sophisticated and/or expensive equipment. We expect that the developed method will be useful broadly for various applications involving interactions of DNA with ions, molecules, reagents and drugs.

II. METHODS AND MATERIALS

Synthesized single-stranded DNA molecules were purchased from Integrated DNA Technologies (IL, USA), and resuspended in distilled water to a final concentration of 100 μM. The sequences of DNA strands for constructing bent DNA molecules and the controls [Fig. 1(e)] are listed in Table I. The long strand of the bent molecule [construct B in Fig. 1(e)] has 45 bases, while the length of the short strand is 30. Upon hybridization, a circular construct is formed, with a double-stranded portion of 30 basepairs (with a nick) and a single-stranded portion of 15 bases [Figs. 1(c) and 1(d)]. Three linear constructs [C1, C2, and C3 in Fig. 1(e)] were used as negative controls. Upon hybridization, C1 is double-stranded completely, while C2 and C3 have overhangs of single strands at one or two sides, respectively. The long strands for C2 and C3 are the same as the long one in the bent molecule.

Single strands were mixed at equal molar amount in background buffer (0.4 mM Tris HCl with pH adjusted to 7.5 using NaOH, 0.5 mM NaCl; the ionic strength is ~1 mM) to reach a final concentration of 2 μM with Mg^{2+} or Ag^{+} ions at various concentrations ($[Mg^{2+}] = 0, 1, 2, 3, 4, 5, 6, 7$ mM; $[Ag^{+}]$=0, 10, 20, $\cdots$, 80, 90 μM). Mg^{2+} and Ag^{+} ions were provided from aqueous solutions of $MgCl_2$ and $AgNO_3$, respectively. The mixtures were heated to 75°C for 2 min, and gradually cooled down to 22°C (room temperature) in 5 h. The mixtures were incubated at 22°C for overnight to allow full equilibrium, followed by gel electrophoresis on the second day.

Polyacrylamide gels (12%) were prepared in the laboratory. Briefly, 3 ml of acrylamide/bis solutions (40%, Bio-Rad Laboratories, CA, USA), 1 ml of 10X tris-borate-EDTA (TBE) buffer (Bio-Rad Laboratories), 20 μl of freshly made ammonium persulfate (APS, 10% in water, Thermo Fisher Scientific, MA, USA) and 6 ml of distilled water were mixed thoroughly and degassed for 10 min in vacuum. The mixture was poured into gel cast cassette immediately after adding 8 μl of tetramethylethylenediamine (TEMED) (Thermo Fisher Scientific, MA, USA), followed by incubation at room temperature for one to two hours to allow full gelation before use.

Five microliters of the prepared DNA samples were mixed thoroughly with 5 μl of water and 2 μl of 6× DNA loading buffer (Bio-Rad Laboratories, CA, USA). The mixtures were loaded into the wells of the prepared gel. The gel electrophoresis (apparatus purchased from Edvotek Inc., DC, USA) was

TABLE I. DNA sequences used in this study. The labels of the constructs refer to their schematic sketches shown in Fig. 1(e).

Construct	Sequences (5′-3′)
B	**CTG CTG AAT TCT GTG GAG TCG TCG TAT GTC**
	CAC AGA ATT CAG CAG CAG GCA ATG ACA GTA GAC ATA CGA CGA CTC
C1	**GAG ATG TCA AGA ATT CCG TCA GCA C**
	GTG CTG ACG GAA TTC TTG ACA TCT C
C2	**TAC TGT CAT TGC CTG CTG CTG AAT TCT GTG**
	CAC AGA ATT CAG CAG CAG GCA ATG ACA GTA GAC ATA CGA CGA CTC
C3	**GTA TGT CTA CTG TCA TTG CCT GCT GCT GAA**
	CAC AGA ATT CAG CAG CAG GCA ATG ACA GTA GAC ATA CGA CGA CTC

run at 100V for 45–60 min in 1× TBE buffer, followed by staining the gel with 1× SYBR Safe solution (Thermo Fisher Scientific, MA, USA) for 15–30 min with gentle shaking. The stained gel was then imaged with a typical exposure time of 2–5 s using a gel documentation system (UVP LLC., CA, USA). The acquired gel images were analyzed using ImageJ [17,18].

III. RESULTS AND DISCUSSIONS

A. DNA-Mg^{2+} Interactions

We first examined the well-known interaction between DNA and Mg^{2+} ions using our method (Fig. 2). As DNA molecules are negatively charged, electrostatic interactions are expected between Mg^{2+} ions and DNA. In addition, electrostatic screening effects due to Mg^{2+} ions stabilize double-stranded DNA molecules, which has been measured by magnetic tweezers, optical tweezers and atomic force microscopy [19–22]. However, such interactions between DNA and Mg^{2+} ions cannot be easily observed with standard chemical or biochemical assays such as gel electrophoresis. For example, short linear double-stranded DNA molecules treated with Mg^{2+} from 0 mM (control) to 7 mM did not show any difference in gel electrophoresis (Fig. 2 a, indicated by red squares). To quantify this observation, we measured the band intensities using ImageJ [17,18] and compared them with the control (i.e., $[Mg^{2+}] = 0$ mM), and observed a flat curve [red squares in Fig. 2(e)]. In contrast, when amplifying the signal of DNA-Mg^{2+} interactions using the bent DNA molecules, the effect of Mg^{2+} at the same concentrations (0–7 mM) is quite obvious [Fig. 2(d)]: The intensity of the bent DNA band [indicated by blue circles in Fig. 2(d)] decreased as the concentration of Mg^{2+} increased. In addition, we found that the dependence on Mg^{2+} concentration of the intensity of the bent DNA band is roughly linear [blue circles in Fig. 2(e)]. We note that a change was observed for $[Mg^{2+}] = 1$ mM with the bent DNA amplifiers, while such a change was absent with $[Mg^{2+}] = 7$ mM without amplification, indicating that the "amplification gain" of our bent DNA amplifiers for probing DNA-Mg^{2+} interactions is at least 7. To exclude the possibility that the observed change in the gel electrophoretic pattern is due to the single-stranded portion of the bent molecules, we performed control experiments with linear DNA molecules that contains both double-stranded and single-stranded parts [constructs C2 and C3 in Fig. 1(e)]. We observed little changes for constructs C2 and C3 in the presence of 1–7 mM Mg^{2+} as shown in Figs. 2(b), 2(c), and 2(e) (orange triangles and magenta ×). This observation suggests that the bent double-stranded DNA and the stored elastic energy are critical to detecting the DNA-Mg^{2+} interactions.

In addition, our mechanical-energy-based amplifiers are capable of reporting quantitatively the interaction between

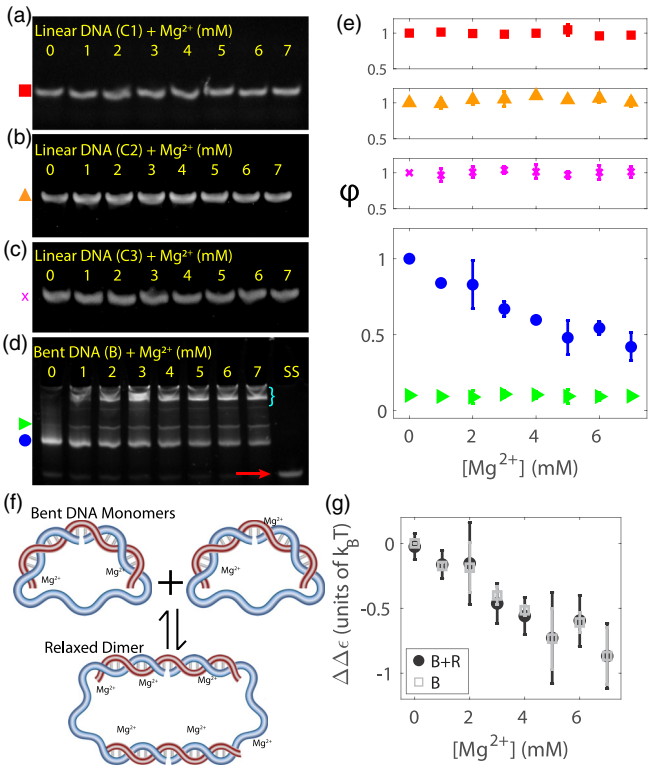

FIG. 2. Probing DNA-Mg^{2+} interactions using bent DNA amplifiers. (a–c) Gel electrophoresis for linear DNA controls in the presence of Mg^{2+} ions of 0–7 mM. (a) Construct C1, (b) construct C2, (c) construct C3. (d) Gel electrophoresis for bent DNA in the presence of Mg^{2+} ions of 0–7 mM. Lane SS: the long single-stranded DNA (45 bases) in the absence of Mg^{2+} ions. (e) Dependence on Mg^{2+} concentration of the intensities of the bands indicated by the corresponding markers in panels (a)–(d). Error bars stand for standard deviation of replicates. (f) Conversion ("reaction") between bent DNA monomers (blue circles in panel d) and relaxed dimers [green triangles in panel (d)]. (g) Estimated change in the difference of free energy between the relax dimers and bent monomers as a function of Mg^{2+} concentration. Estimations were carried out using either the bent monomer band only (B, gray squares) or both the bent monomer and relaxed dimer bands (B+R, black circles).

Mg^{2+} and DNA molecules. Figure 2(d) shows that bands with heavier molecular weights appeared in the presence of Mg^{2+} ions [indicated by the green triangle and the cyan "}" in Fig. 2(d)]. Previous studies by Qu *et al.* showed that these bands correspond to higher-order multimers [13–15]: for example, two monomers form a dimer; one monomer and one dimer (or three monomers) form a trimer; one monomer and one trimer (or four monomers) form a tetramer. Although the heavier multimers (i.e., tetramers and above) were not resolved in our experiments, it is clear that the intensities of the bent monomer bands (blue circle) decreased in the presence of Mg^{2+} ions, while the intensities of the bands with heavier molecular weight increased. This observation suggests that Mg^{2+} ions lead to a conversion from the bent DNA monomers to the relaxed DNA dimers and multimers [Fig. 2(f)]. A complete quantitative understanding of this observation requires taking into account all the possible reactions; however, for simplicity, here we focus only on the conversion ("reaction") between monomers and dimers [Fig. 2(f)]. The conversion between the monomers and dimers can be understood by starting with the chemical potential of solute molecules μ_s in water,

$$\mu_s = \epsilon_s + k_B T \ln\left(\frac{N_s}{N_w}\right) = \epsilon_s + k_B T \ln(x_s), \tag{1}$$

where ϵ_s is the energy of each solute molecule, k_B the Boltzmann constant, T the temperature, N_s the number of solute molecules, N_w the number of water molecules, and $x_s = N_s/(N_w + N_s) \approx N_s/N_w$ the molar fraction of the solute molecules [23]. At equilibrium, we have $\mu_r = 2\,\mu_b$, where μ_r is the chemical potential of a relaxed DNA dimer and μ_b the chemical potential of a bent DNA monomer. Therefore, we have [13]

$$\epsilon_r - 2\epsilon_b = k_B T \ln\left(\frac{x_b^2}{x_r}\right). \tag{2}$$

The difference in the free energy between half a dimer and a single bent DNA molecule is then

$$\Delta\epsilon = \frac{\epsilon_r}{2} - \epsilon_b = k_B T \ln(x_b) - \frac{1}{2} k_B T \ln(x_r). \tag{3}$$

As a result, this difference $\Delta\epsilon$ can be estimated from the molar fractions of the bent DNA monomers and the relaxed dimers, which are proportional to the band intensities, $x_b = \beta I_b$ and $x_r = \frac{1}{2}\beta I_r$, where β is a constant. Note that, as the length of the relaxed dimers are twice that of the monomers, each dimer contributes twice the intensity of a monomer. Since the intensity of the dimer bands remains almost constant [green triangles in Fig. 2(d) and 2(e)], the observed decrease in the band intensity of the bent DNA monomers [blue circles in Fig. 2(e)] in the presence of $[Mg^{2+}]$ suggests that $\Delta\epsilon$ decreased as $[Mg^{2+}]$ increased.

More quantitatively, we estimated the effect of Mg^{2+} ions on DNA [i.e., the change of $\Delta\epsilon$ in the presence (+) and absence (−) of Mg^{2+} ions] by

$$\Delta\Delta\epsilon = \Delta\epsilon^+ - \Delta\epsilon^- = k_B T\left[\ln\left(\frac{x_b^+}{x_b^-}\right) - \frac{1}{2}\ln\left(\frac{x_r^+}{x_r^-}\right)\right]. \tag{4}$$

If we normalize the molar fractions to the control (i.e., $[Mg^{2+}] = 0$ mM), $\varphi_b^- = \frac{x_b^-}{x_b^-} = 1$, $\varphi_b^+ = \frac{x_b^+}{x_b^-}$, $\varphi_r^- = \frac{x_r^-}{x_b^-}$, and $\varphi_r^+ = \frac{x_r^+}{x_b^-}$, we have

$$\Delta\Delta\epsilon = k_B T\left[\ln\left(\varphi_b^+\right) - \frac{1}{2}\ln\left(\frac{\varphi_r^+}{\varphi_r^-}\right)\right]. \tag{5}$$

Using the data in Fig. 2(e) (both blue circles and green triangles), it was found that $\Delta\Delta\epsilon$ decreases linearly as the concentration of Mg^{2+} increases, as shown in Fig. 2(g) (black circles). Furthermore, we examined the possibility of using the dependence of $\Delta\Delta\epsilon$ on the molar fraction of the bent DNA monomer φ_b to capture the main feature of $\Delta\Delta\epsilon$ in the presence of Mg^{2+} ions (i.e., $\Delta\Delta\epsilon$ decreases as $[Mg^{2+}]$ increases). For this purpose, we estimated $\Delta\Delta\epsilon$ by considering the first term and ignoring the other bands,

$$\Delta\Delta\epsilon \sim k_B T \ln(\varphi_b^+). \tag{6}$$

It turns out that the estimations from the bent monomer only [gray squares in Fig. 2(g)] are very close to the calculations using both the bent monomer and the relaxed dimer [black circles in Fig. 2(g)]. A caveat to emphasize here is that the heavier multimers have been ignored in the current analysis [Fig. 2(g)]. As a result, we have underestimated φ_r^+/φ_r^- and thus $\Delta\Delta\epsilon$ in Eq. (5).

To better understand the physics of how Mg^{2+} ions promote the conversion from the bent monomers to the relaxed dimers and/or multimers, we examined qualitatively several possible contributions to $\Delta\Delta\epsilon = \frac{1}{2}(\epsilon_r^+ - \epsilon_r^-) - (\epsilon_b^+ - \epsilon_b^-)$. The purpose of the discussions below is to assess the *order of magnitude* of various potential contributions; further quantitative investigations are needed to determine their exact values. These discussions are based on the well-known electrostatic screening effects of Mg^{2+} ions, including (a) stabilization of base-pairing and base-stacking [24,25] and (b) contribution to electrostatic interactions.

The stabilization of base-pairing and base-stacking in DNA due to Mg^{2+} ions is expected to affect the behavior of the nick in our bent DNA monomers, the persistence length of double-stranded DNA, and the hybridization between two DNA strands.

(a1) Effects on the nick-behavior. It has been shown that sharply bending a double-stranded DNA with a nick leads to kink-formation (i.e., disruption of base-stacking) and even strand-peeling (disruption of base-pairing) [16]. The disruptions of base-stacking and base-pairing will then reduce the hybridization energy in the bent monomers. As Mg^{2+} ions stabilize the base-pairing and base-stacking [24,25], we would have less disruption and thus less reduction in the hybridization energy in the presence of Mg^{2+}, i.e., $(\frac{1}{2}\epsilon_{r,nh}^+ - \epsilon_{b,nh}^+) < (\frac{1}{2}\epsilon_{r,nh}^- - \epsilon_{b,nh}^-)$. Therefore, we obtain $\Delta\Delta\epsilon_{nh} < 0$, which has the same sign with the measurement [Fig. 2(g)]. In addition, the stabilization of base-stacking and base-pairing due to Mg^{2+} ions is likely to render a higher bending elastic energy in the bent monomers, $\epsilon_{b,ne}^+ > \epsilon_{b,ne}^-$, which gives $\Delta\Delta\epsilon_{ne} = -(\epsilon_{b,ne}^+ - \epsilon_{b,ne}^-) < 0$. Therefore, we expect that the effect of the nick in the DNA, $\Delta\Delta\epsilon_n = \Delta\Delta\epsilon_{nh} + \Delta\epsilon_{ne}$, is < 0, showing the same sign as our experimental results [$\Delta\Delta\epsilon < 0$ as shown Fig. 2(g)]. We

note that the order of magnitude of $\Delta\Delta\epsilon_n$ can be estimated from the computational work by Cong *et al.* [16]. If we assume that the Mg^{2+}-stabilized nicked DNA is similar to a nick-free one (an overestimation), we expect that $\Delta\Delta\epsilon_n$ is between 0 and $-5\,k_BT$ if only base-unstacking is present, or between $-5\,k_BT$ and $-15\,k_BT$ if strand-peeling occurs [16].

(a2) Effects on persistence length (L_p). When *ignoring kink-formation or strand-peeling* due to the nick, Mg^{2+} ions' electrostatic screening effects will shorten the persistence length L_p of double-stranded DNA [19–22]. For example, Baumann *et al.* measured that the persistence length of DNA reduced to 42%–54% in the presence of 100 μM Mg^{2+} ions [19]. In addition, Brunet *et al.* proposed an interpolation formula in a recent work [21], which fitted their experimental data very well [21] and predicted that the persistence length of our DNA would be reduced to ~80% when the ionic strength increased from ~1 mM ($[Mg^{2+}] = 0$ mM) to ~22 mM ($[Mg^{2+}] = 7$ mM) in our experiments. These experimental results [19–22] suggested that the decrease in the persistence length of DNA due to Mg^{2+} ions is in the order of ~0.5. The persistence length of a polymer is tightly related to the bending elastic energy (ϵ_e, as the bending stiffness B is proportional to L_p), which is expected to contribute to $\Delta\Delta\epsilon$. For relaxed molecules, as they are not bent, the bending energy is negligible; therefore, changes in the persistence length due to Mg^{2+} ions do not contribute: $\epsilon^+_{r,e} - \epsilon^-_{r,e} \sim 0$. In contrast, for the bent monomers, a shorter persistence length resulted in a lower bending elastic energy, $\epsilon^+_{b,e} - \epsilon^-_{b,e} < 0$. Therefore, we have $\Delta\Delta\epsilon_e > 0$, which shows the opposite sign compared to the measurement [$\Delta\Delta\epsilon < 0$ as shown Fig. 2(g)]. Using the elastic bending energy measured by Qu *et al.* [13], 8.6–9.7 k_BT (or in the order of ~10 k_BT) for a bent monomer with 30 bp of the double-stranded segment and 15 bases of the single-stranded segment, we estimate that $\Delta\Delta\epsilon_e$ is in the order of ~5 k_BT for 7 mM Mg^{2+} ions in our experiments.

(a3) Effects on hybridization energy (ϵ_h). Even if *ignoring kink-formation or strand-peeling* due to the nick, it has been reported that Mg^{2+} ions stabilize the hydrogen bonds for the base-pairing of double-stranded DNA [24,25]. Therefore, the hybridization energy (ϵ_h) could be a potential contribution to $\Delta\Delta\epsilon$. However, in the absence of strand-peeling (or unzipping), the hybridization energy is expected to be proportional to the number of base-pairs. As the length of the relax dimers is twice of the length of bent monomers, we have $(\epsilon^+_{r,h} - \epsilon^-_{r,h}) = 2 \times (\epsilon^+_{b,h} - \epsilon^-_{b,h})$. Therefore, the effect of Mg^{2+} on the hybridization energy cancels out, resulting in $\Delta\Delta\epsilon_h \approx 0$.

The presence of Mg^{2+} ions is also likely to affect electrostatic interactions inside DNA molecules and that between DNA and Mg^{2+} ions.

(b1) Effects on electrostatic interactions inside DNA molecules. For double-stranded DNA segments, it is likely that the electrostatic interactions $\epsilon_{\rm esn}$ are reduced for both bent monomers and relaxed molecules, $\Delta\Delta\epsilon_{r,\rm esn} < 0$ and $\Delta\Delta\epsilon_{b,\rm esn} < 0$, due to the screening effect of Mg^{2+} ions. For the single-stranded segments, we expect a shorter persistence length ($L_{p,\rm ss}$), which results in higher entropic elastic energy ($\epsilon_{\rm ss} \propto k_BT/N_sL^2_{p,\rm ss}$ where N_s is the length of the single-stranded segment). Therefore, we have $\Delta\Delta\epsilon_{r,\rm ss} > 0$ and $\Delta\Delta\epsilon_{b,\rm ss} > 0$. Qu et al. showed that the combined contribution from the electrostatic interactions inside DNA molecules and entropic elastic energy of the single-stranded segments is $9.7 - 8.6 = 1.1\,k_BT$ [13], or in the order of ~1 k_BT. Therefore, we expect that $|\Delta\Delta\epsilon_{\rm esn} + \Delta\Delta\epsilon_{\rm ss}|$ is also in the order of ~1–2 k_BT.

(b2) Effects on electrostatic interactions between DNA and Mg^{2+} ions ($\epsilon_{\rm esi}$). Because these electrostatic interactions do not depend on the conformation of the DNA, and that the length of the relax dimers is twice of the length of bent monomers, we expect that $(\epsilon^+_{r,\rm esi} - \epsilon^-_{r,\rm esi}) = 2 \times (\epsilon^+_{b,\rm esi} - \epsilon^-_{b,\rm esi})$. Therefore, we have $\Delta\Delta\epsilon_{\rm esi} \sim 0$, which is negligible.

By comparing the signs of the various contributions ($\Delta\Delta\epsilon_e$, $\Delta\Delta\epsilon_h$, $\Delta\Delta\epsilon_n$, $\cdots$) with that of the experimental results [$\Delta\Delta\epsilon < 0$ as shown in Fig. 2(g)], we concluded that, although Mg^{2+} ions play a role in most of these terms, the stabilization of Mg^{2+} on base-stacking and base-pairing in the nicked DNA is likely the main driving "force" for the monomer-to-multimer conversion [Fig. 2(f)]. Therefore, both the bending of the DNA molecules and the nick are important for perturbing the energy landscape and amplifying the DNA-Mg^{2+} interactions.

B. DNA-Ag^+ Interactions

With the successful application of our bent DNA amplifiers to study DNA-Mg^{2+} interactions, we exploited them to investigate the interactions of DNA with Ag^+ ions. The significance of DNA-Ag^+ interactions includes their genotoxicity and potential uses as antibiotic alternatives. For example, it has been reported that Ag^+ ions at < 100 μM concentrations show significant antibiotic activities against bacteria [26,27]. More importantly, it has been argued that it is more difficult for bacteria to develop resistance to Ag^+ ions compared to commonly prescribed antibiotics [28]. Therefore, it is of great interest to understand the antibiotic mechanism of Ag^+ ions, which includes DNA-Ag^+ interactions. It was measured that Ag^+ ions caused DNA condensation in bacteria [29]; however, this result could not be verified previously by *in vitro* experiments such as gel electrophoresis [30].

Here, we demonstrate that our method can be used to sensitively measure the interactions between DNA and Ag^+ ions. First, we examined the effect of Ag^+ ions (0–90 μM) on linear double-stranded DNA (construct C1), and observed no changes with gel electrophoresis [Fig. 3(a), and red squares in Fig. 3(e)], consistent with previous reports [30]. In addition, similar to the experiments with Mg^{2+} ions, two other controls with both double-stranded segments and single-stranded overhangs (constructs C2 and C3) were tested [Figs. 3(b) and 3(c)]. Again, little changes were observed [orange triangles and magenta × in Fig. 3(e)]. In contrast, using the bent DNA amplifiers, the interactions between DNA and Ag^+ ions were easily observed at 10 μM of Ag^+ ions, as shown in Fig. 3(d). We note that our method can detect changes at $[Ag^+] = 10\,\mu$M, while, without amplification, no such changes were observed with even $[Ag^+] = 90\,\mu$M. The "amplification gain" of our method for probing DNA-Ag^+ interactions is at least 9.

It was observed that Ag^+ ions caused the intensity of the bent DNA band to decrease [blue circles in Figs. 3(d) and Fig. 3(e)], similar to the apparent effect of Mg^{2+} ions.

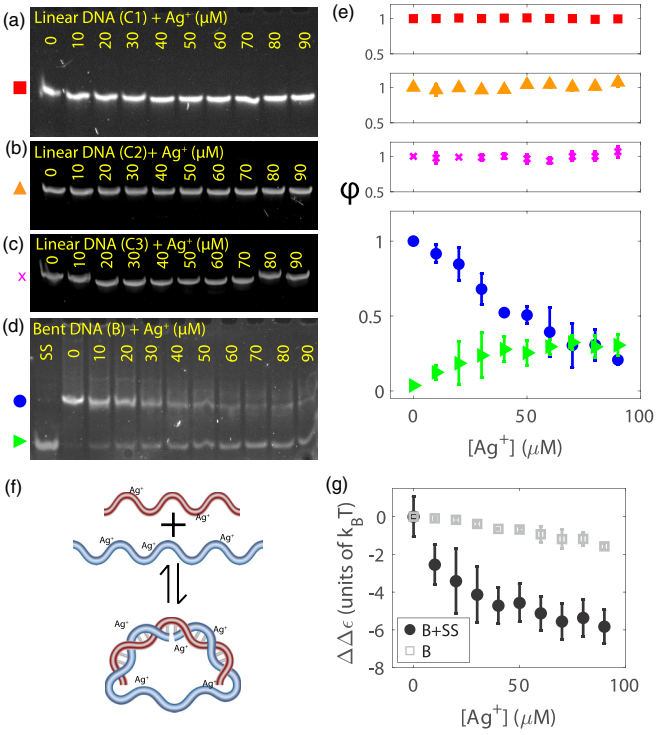

FIG. 3. Probing DNA-Ag^+ interactions using bent DNA amplifiers. (a–c) Gel electrophoresis for linear DNA controls in the presence of Ag^+ ions of 0–90 μM. (a) construct C1, (b) construct C2, (c) construct C3. (d) Gel electrophoresis for bent DNA in the presence of Ag^+ ions of 0–90 μM. Lane SS: the long single-stranded DNA (45 bases) in the absence of Ag^+ ions. (e) Dependence on Ag^+ concentration of the intensities of the bands indicated by the corresponding markers in panels (a)–(d). Error bars stand for standard deviation of replicates. (f) Conversion ("reaction") between bent DNA monomers (blue circles in panel d) and unhybridized single strands (green triangles in panel d). (g) Estimated change in the difference of free energy between the unhybridized single-strands and bent monomers as a function of Ag^+ concentration. Estimations were carried out using either the bent monomer band only (B, gray squares) or both the bent monomer and unhybridized single-stranded bands (B+SS, black circles).

However, different from Mg^{2+}, DNA dimers and higher-order multimers did not appear significantly in the presence of Ag^+ ions. Instead, the band of the single-stranded DNA showed up in the presence of Ag^+ ions [indicated by the green triangle in Fig. 3(d)], suggesting that the DNA-Ag^+ interactions are different from the DNA-Mg^{2+} interactions. In addition, the emergence of the single-stranded DNA band indicates that Ag^+ ions likely affect DNA hybridization, which is not surprising as Ag^+ ions have been found to interact with DNA bases, especially cytosine [31,32], and possibly induce chain-slippage [33].

To quantify the DNA-Ag^+ interactions, we focused on the hybridization "reaction" of DNA as shown in Fig. 3(f). With Eq. (1) and the equilibrium condition $\mu_b = 2\mu_{ss}$, we have

$$\Delta\epsilon = 2\epsilon_{ss} - \epsilon_b = k_B T \ln\left(\frac{x_b}{x_{ss}^2}\right) \tag{7}$$

and

$$\Delta\Delta\epsilon = \Delta\epsilon^+ - \Delta\epsilon^- = k_B T\left[\ln\left(\frac{x_b^+}{x_b^-}\right) - 2\ln\left(\frac{x_{ss}^+}{x_{ss}^-}\right)\right]. \tag{8}$$

If we normalize the molar fractions to the control (i.e., $[Ag^+] = 0\,\mu$M), $\varphi_b^- = \frac{x_b^-}{x_b^-} = 1$, $\varphi_b^+ = \frac{x_b^+}{x_b^-}$, $\varphi_{ss}^- = \frac{x_{ss}^-}{x_b^-}$, and $\varphi_{ss}^+ = \frac{x_{ss}^+}{x_b^-}$, we obtain

$$\Delta\Delta\epsilon = k_B T\left[\ln\left(\frac{\varphi_b^+}{\varphi_b^-}\right) - 2\ln\left(\frac{\varphi_{ss}^+}{\varphi_{ss}^-}\right)\right]. \tag{9}$$

We estimated $\Delta\Delta\epsilon$ from the experimental data [blue circles and green triangles in Fig. 3(e)] and found that $\Delta\Delta\epsilon$ decreased with increasing $[Ag^+]$ as shown in Fig. 3(g) (black circles).

We note that the dependence of $\Delta\Delta\epsilon \sim k_B T \ln(\varphi_b^+)$ (i.e., using the monomer band only) is also able to capture the main feature of $\Delta\Delta\epsilon$ in the presence of Ag^+ ions (i.e., $\Delta\Delta\epsilon$ decreases as $[Ag^+]$ increases), as shown in Fig. 3(g) (gray squares). However, unlike the result for Mg^{2+} ions, the estimations based on the $\Delta\Delta\epsilon \sim \ln(\varphi_b^+)$ dependence are quantitatively off. The reason for this deviation is that the intensities of the dimer bands stay constant in the presence of Mg^{2+} ions [green triangles in Fig. 2(d)] but the intensities of the single-stranded bands increase steadily in the presence of Ag^+ ions [green triangles in Fig. 3(d)].

IV. CONCLUSION

To conclude, we developed a simple and cost-effective method to amplify and probe the interactions between DNA and metal ions by taking advantage of mechanical energy stored in bent DNA molecules. We demonstrated these mechanical-energy-based amplifiers by applying them to examine the interactions between DNA and Mg^{2+} ions, or Ag^+ ions. In addition, we showed that quantitative details about the DNA-metal interactions can be obtained with our method. This method is simple and convenient as the bent DNA molecules were self-assembled. Our method is cost-effective because it uses gel electrophoresis, a standard and commonly used biochemical technique. By perturbing the energy landscape, our method amplifies the DNA-metal interactions, making it sensitive and capable of detecting the effect of metal ions on DNA that are not detectable using the same biochemical assay.

As a proof-of-concept, we have focused on our study on Mg^{2+} and Ag^+ ions. However, we expect that our method is readily applicable to other metal ions. As the concentrations of metal ions are important indicators of water quality, we expect that our method could be used for monitoring water quality. One advantage of our DNA-based method is biocompatibility. In addition to metal ions, it is likely that our bent DNA amplifiers can be used to investigate the interactions of DNA with other chemicals, including organic molecules and reagents. In principle, it is even possible to develop our method into a convenient technique for screening DNA-targeting drugs. Furthermore, our method can be used for improving existing assays and techniques in various applications, such as isolation of aptamers for metal ions [34].

The goal of this work is to demonstrate the principle and feasibility of the developed method. However, it would be interesting to examine the method in more details and to push the sensitivity of the method for further applications. For example, as it has been reported that nicks promote DNA base-pair disruption in bent double-stranded DNA molecules [16], an immediate question is how the metal ions affect the stability of the nicks, which could possibly be answered using our method with appropriate designs (i.e., by varying the length and sequence) of the bent DNA. In addition, in combination with other techniques (such as fluorescence resonance energy transfer), the mechanical-energy-based amplifiers might be capable of examining the dynamics of the conversion between smoothly bent DNA and sharply kinked DNA in the presence of nicks, as well as how the dynamics depends on the metal ions. Furthermore, we point out that our method is versatile to control the sensitivity, as the mechanical energy in the bent DNA can be modulated conveniently by changing the length of the single-stranded part of the self-assembled DNA [13–15].

Finally, we point out that there are several ways to use our mechanical-energy-based amplifiers to examine interactions of DNA with metal ions, and likely other molecules. For example, DNA-metal interactions can be qualitatively reported by the visual changes in the gel electrophoretic patterns [Figs. 2(a)–2(e) and 3(a)–3(e)]. In addition, the quantitative information about the DNA-metal interactions can be extracted [black circles in Figs. 2(g) and 3(g)], especially when the underlying "reactions" caused by the metal ions are clear [Figs. 2(f) and 3(f)]. Furthermore, the dependence of $\Delta\Delta\epsilon$ on the molar fractions of the bent monomers alone can semi-quantitatively report the interactions of DNA and metal ions, which potentially provides a convenient way in practice for looking at the interactions but without knowing the details or mechanisms. As a result, our method is expected to be versatile for various applications at different levels and complexity.

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