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Direct measurement of the mechanism by which magnesium specifically modifies the mechanical properties of DNA

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We examine the effect of physiological cations Na^+ , K^+ , Mg^{2+} , and Ca^{2+} on the mechanical properties of bundles of λ -phage DNA using silicon nanotweezers (SNTs). Integrating SNTs with a microfluidic device allows us to perform titration experiments while measuring the effect in real-time. The results show that only for Mg^{2+} and in particular, at the intra-nuclear concentration (100 mM), the interaction occurs. *Published by AIP Publishing.* <https://doi.org/10.1063/1.5008622>

The direct observation of the mechanical properties of biopolymers is clearly a considerable task. Simple modifications of proteins or other biopolymers may change interaction constants by one or more orders of magnitude, even for such seemingly trivial modifications such as fluorescence labeling.¹ Thus, modification for surface attachment, as in various force measurements, is not necessarily totally apt under physiological situations.²

Another key point is that any measurements of mechanical properties must be carried out under the correct biological conditions. Too often, we assume that phosphate-buffered saline (PBS) is a universal medium. Unfortunately, this is not true. Cation concentrations vary depending on localization. Extracellular concentrations are quite different from intracellular concentrations, which again differ from ion concentrations in the nucleus (Table I).³ To further complicate matters, ion concentrations may vary under external stress. Thus, there exists a need for a method capable of detecting effects of local specific ion concentrations on the mechanical properties of biopolymers.

Besides various force spectroscopy methods, *e.g.*, optical tweezers and magnetic tweezers suffering from the use of chemical modification and molecular stressing while measuring the properties of DNA,^{2,4} the development of microelectromechanical systems (MEMS) technology-based tweezers targeting biological samples opened up methods for handling biopolymers,⁵ cells, and even small animals.⁶ Although silicon nanotweezers (SNTs) showed successful real-time measurements on the mechanical properties of biopolymers,⁷ such methods were either performed in air or remained “static” not allowing titration experiments and thus seriously restricting our ability to study the effects of molecular interactions on the mechanical properties of biopolymers in liquid.⁸ Therefore, adapting microfluidics to the direct measurement of mechanical properties of biopolymers is a major step forward.⁹ Here, we describe how the adaptation of a microfluidic system to the SNT [Fig. 1(a)] allows us to observe the effects of the common physiological cations Na^+ , K^+ , Mg^{2+} , and Ca^{2+} on the mechanical properties of DNA, using the non-coordinating NO_3^- anion as the counterion.

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TABLE I. Concentrations of the major physiological cation and the corresponding resonance frequency shift. In the case of Ca^{2+} , only free ion concentrations are given. For certain localisations, a range of concentrations are known.

Cation	Concentration (mM)			Resonance frequency shift (Hz)		
	Extracellular	Intracellular	Nuclear	Extracellular	Intracellular	Nuclear
Na^+	140	10	2	-0.03	-0.02	-0.01
K^+	3.6–5	150	230	-0.02 ^a	-0.04	0.00
Mg^{2+}	0.6–1	5–20	100	0.15 ^b	0.17 ^c	0.22
Ca^{2+}	2	0.0001	0.001	0.07	0.08	0.08

^a K^+ at 4 mM.

^b Mg^{2+} at 1 mM.

^c Mg^{2+} at 10 mM.

Three major points are to be emphasized: first, the tips of the SNT, one of which is fixed and the other can be oscillated in the horizontal plane, are covered with aluminum, increasing the strength of the DNA binding by strong Al-Phosphate coordination bonding.³ This allows mechanical force to be applied to the bridging DNA bundles. By means of a lock-in amplifier, the SNT can be set to move at the resonant frequency (while monitoring the amplitude value in real-time) with the biopolymers attached between the two arms (Fig. 1 in the [supplementary material](#)). Second, a fluidic device allows the exchange of the solution in which the SNTs are immersed [Fig. 1(b)]. In this way, variable concentrations of solutes can be studied and titration experiments are now possible without the need subjecting DNA bundles to the large mechanical stresses associated with passage across the water meniscus. Third, the real-time measurements allow the direct calculation of changes in the biopolymers' mechanical characteristics, *e.g.*, stiffness and viscous losses, using the resonance frequency and amplitude values ([supplementary material](#)).

When working with biomacromolecules under conditions approximating to physiological conditions, the four main physiological cations sodium, potassium, magnesium, and calcium with varying cation concentrations (in different physiological locations; Table I) should be taken into account. Each of these cations is introduced in a microfluidic device (placing a PDMS slab with a 100- μm channel on a cover slip) to access by SNT tips via a side opening [Figs. 1(c) and 1(d)]. The dimensions of the side opening (105 μm \times 90 μm) have to be small enough to provide a stable air-liquid interface and large enough to allow the insertion of SNT tips (with a gap of 11 μm) without suffering from the capillary effect. Concentration titration of each cation is performed using a pressure pump connected to the outlet. A negative pressure is

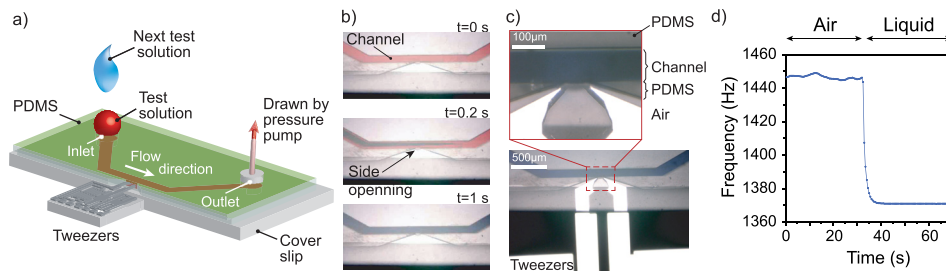


FIG. 1. (a) A microfluidic device with a side opening allows SNTs to access the channel. The solution inside the channel can be exchanged (by the pressure pump connected to the outlet of the channel) owing to the highly stable air-liquid interface. As a result, titration experiments are possible with high sensitivity, after inserting only the tips of SNTs with a DNA bundle in between. (b) Successive images demonstrate exchanging solutions inside the channel within seconds. The air-liquid interface at the side opening was extremely stable due to the surface tension. (c) A DNA bundle between the tweezer tips enters into a microfluidic channel via the side opening. (d) Using a phase-lock-loop amplifier, the resonance frequency of the system can be monitored in real-time. SNTs with a DNA bundle between the tips were inserted in liquid at around $t = 35$ s. Inserting the drier, thus stiffer, the DNA bundle in liquid caused a sudden decrease in the resonance frequency as DNA became softer due to hydration.

applied to withdraw the liquid inside the channel to exchange with a new one fed from the inlet. The pressure (~ -20 mbar) is controlled to provide a constant liquid flow of $45 \mu\text{l min}^{-1}$ for 20 s.

The experiments were repeated multiple times using the same DNA bundle for each cation to verify the reproducibility of the effects on the same sample. Initially, a DNA bundle was captured between the SNT tips⁹ and added to a Tris buffer solution (pH 6.8) to yield the base resonance frequency level (for 3 min). Before exposing the bundles to the cation solutions, the pH was lowered (3 min) to validate the existence of the bundle and returned back to the Tris buffer solution (3 min). This pH test was performed between each cycle of cation solution addition to compare the condition of the DNA bundle. The bundle was incubated in each cation solution for 6 min. The average resonance frequency values were calculated based on the last 30 s of incubation. The pH values of each cation solution were between 7.0 and 7.5, and thus, any observed change in resonance frequency was not due to the pH effect. Control experiments (the same solution sequences without DNA between tips) were also performed to examine the effect of this procedure on the detection mechanism (red curves, Fig. 2).

In Figs. 2(a) and 2(b), the response curves for interactions are given, respectively: sodium and magnesium cations. The curves for potassium and calcium are given in Fig. 2 in the [supplementary material](#). The observed frequency changes at the first cation injection cycle are summarized in Table I. For Na^+ , K^+ , and Ca^{2+} , no effects are observed under any of the physiological conditions. The subsequent cycles for these cations show little or no change with respect to the first cycle. For Mg^{2+} , the situation is quite different. A non-reversible effect is seen in each cycle of Mg^{2+} injection (starting at $t = 1$ min, $t = 28$ min, and $t = 55$ min) as an increase in the resonance frequency (more dominant at the intra-nuclear concentration). A similar effect is seen also in the amplitude. Mg^{2+} causes a decrease in the amplitude, while Na^+ does not show any effect (Fig. 3 in the [supplementary material](#)). Monitoring resonance frequency provides the necessary information related to stiffness, and together with the amplitude value, viscous losses are obtained. Calculated stiffness and viscous losses throughout the experiment are shown in Fig. 4 in the [supplementary material](#). This non-reversible effect tends to plateau by the third cycle. To sum up, in the presence of Mg^{2+} , the DNA bundle has increased stiffness and higher viscous losses. The non-reversible characteristic of this increase suggests that Mg^{2+} is bound directly to phosphate groups of the DNA bundle. We should note that Na^+ , K^+ , and Ca^{2+} are water structure breakers, whereas Mg^{2+} is a structure former. Also, the first

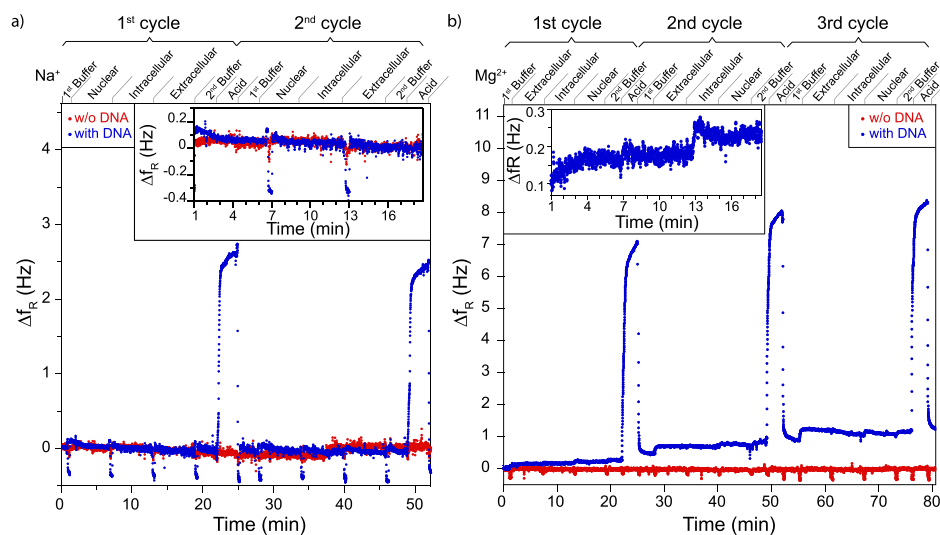


FIG. 2. Real-time resonance frequency changes of the SNT with (blue) and without (red) DNA bundles in (a) Na^+ solutions and (b) Mg^{2+} solutions. Before injecting each set of the cation solutions, a buffer-acid-buffer cycle was performed to monitor the condition of the DNA bundle. Insets correspond to a close-up at the given time periods. To aid the reader, the solutions used are shown at the top.

three show non-ordered rapidly changing water coordination, whereas Mg^{2+} is a slow exchanging octahedrally water coordinated cation.¹⁰

In conclusion, the use of micro-fluidic coupled SNTs has allowed the *in-situ* detection of the binding of Mg^{2+} cations to DNA more dominantly at the intra-nuclear concentration. These findings demonstrate the power of this method for the real-time determination of biorelevant interactions.

See [supplementary material](#) for more information on the experimental procedures and supplementary figures referred in the text.

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