Supplementary Information

DNA methylation induces subtle mechanical alteration but significant chiral selectivity

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1. Experimental details and the instrument

Materials:

The DNA oligomers were purchased from IDT (Integrated DNA Technologies, Coralville, USA). Magnetic beads M280 was purchased from Fisher Scientific (10 mg/mL, Cat. # 11205D). The drug molecules, *d*-tetrahydropalmatine (THP) (ab142555, Abcam Biochemicals, purity >98%), *l*-THP (1614, AvaChem Scientific, 98%), *l*-stepholidine (FS74167, Biosynth International, purity >95%) were purchased and used directly. All other chemicals were purchased from Sigma Aldrich unless specified otherwise.

DNA sequences for calibration are:

5′Bio/G TAC GTA AAT CTA CTG CTG AA (Strand 0)

3′-G CAT TTA GAT GAC GA/Bio (15 bp with Stran 0)

3′- CAT TTA GAT GAC GA/Bio (14 bp with Stran 0)

3′-AT TTA GAT GAC GA/Bio (13 bp with Stran 0)

3′-T TTA GAT GAC GA/Bio (12 bp with Stran 0)

3′-TTA GAT GAC GA/Bio (11 bp with Stran 0)

DNA sequences for methylation study are:

5′Bio/CCC AAT CGA CCC (none methylated)

3′Bio/GGG TTA GCT GGG (complementary to the previous strand)

5′Bio/CCC AAT m^5CGA CCC (one methylated)

3′Bio/GGG TTA Gm^5CT GGG (for two methylated duplex)

Instrument:

A home-built atomic magnetometer was used to measure the magnetic signal of the samples, with a sensitivity of ~150 fT√Hz. The ^{133}Cs atomic sensor, which was cubic with 6 mm side length, was housed in a four-layer magnetic shield. Linear polarized laser at 894.5952 nm was used to excite the Cs atoms (TLB-6818, Newport). A linear motor was used to scan the sample (Ultra Motion, Cutchogue). For force generation, a piezo disk of 15 mm diameter and 2 mm thick was used. Ultrasound at 1.00 MHz was generated by a function generator (DS345, Stanford Research System) and amplified by an AR amplifier (75A250A, AR).

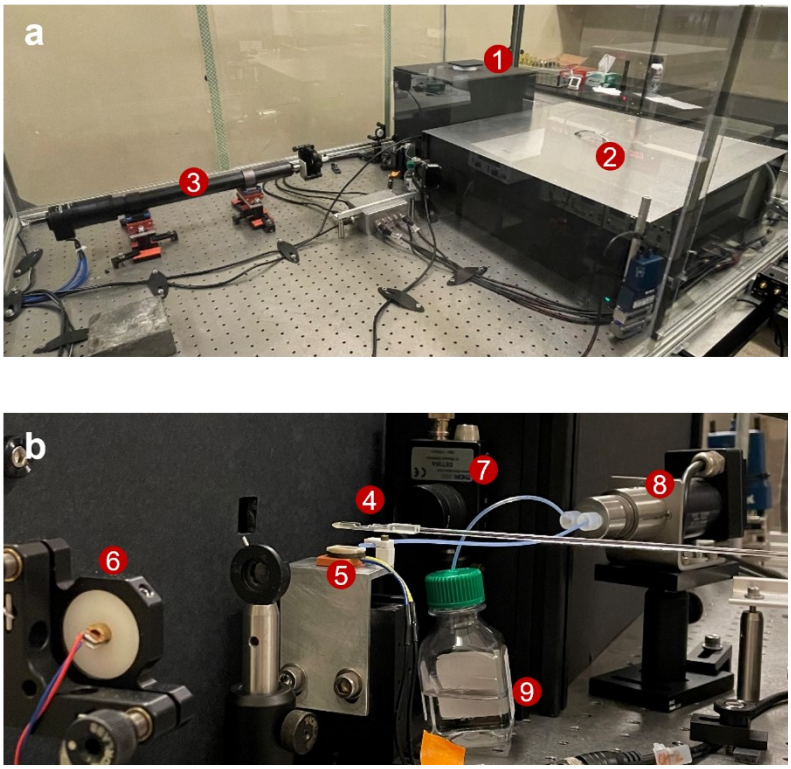


Figure S1. Photos of the instrument. a. Overview of the apparatus. 1, atomic sensor housed inside a four-layer magnetic shield; 2, laser and optics; 3, motor for sample scanning. b. details of the sample region. 4, sample holder; 5, piezo on a translation stage; 6, laser diode; 7, photo detector; 8, pump; 9, water reservoir.

Procedure:

The sample well with dimensions of 4×3×2 mm^3 was coated with biotin (Biotin-PEG-SVA-5000 and MPEG-SVA-5000, 1:30 ratio, Laysan Bio Inc). Streptavidin (ThermoFisher Scientific) with a concentration of 0.25 mg/mL was introduced onto the surface and incubated for 1 h. After rinsing off excess streptavidin, the first DNA (1 μM) with a biotin linker was added into the sample well and immobilized on the surface via biotin-streptavidin interaction. The incubation process was for 1 hr. Excess DNA was also rinsed off afterwards; then 1 μM of probing DNA was added and incubated overnight. Next, streptavidin-coated magnetic beads M280 were diluted to approximately 0.5 mg/mL and introduced into the sample well. After 2 hr incubation, each sample was magnetized for 2 min on a permanent magnet (~0.5 T), and centrifuged (5427R, Eppendorf) at 1500 rpm (revolution per minute) for 20 min to remove nonspecifically bound magnetic beads. For the DNA-drug interaction experiments, the drug

(*d*-THP, *l*-THP, or *l*-stepholidine) was introduced to the sample well after centrifugation and incubated for 1 hr, with final concentration of 100 μM. All the mixtures were in TAM_{10} buffer, which contains 20 mM tris-HCl (15568025, ThermoFisher Scientific), 30 mM NH_4Cl (213330, Sigma Aldrich), 70 mM KCl (P3911, Sigma Aldrich), 5 mM EDTA (15575, Invitrogen), 7 mM BME (2-mercaptoethanol) (M3148, Sigma Aldrich), 10 mM $MgCl_2$ (M1028, Sigma Aldrich).

2. Force calibration

Force calibration was carried out using a series of DNA duplexes with 11-15 bp respectively. The dissociation forces in pN were obtained using centrifugal force, in which $F = m\omega^2 r$. Here m is the buoyant mass of the magnetic beads, ω is the angular speed of the centrifuge for duplex dissociation, r is the radius of the centrifuge (7.5 cm for Eppendorf 5470R) (Refs. 24, 25). Linear fitting yields $F = 1024.7V^2 + 16.0$, in which V is in volts and F in pN.

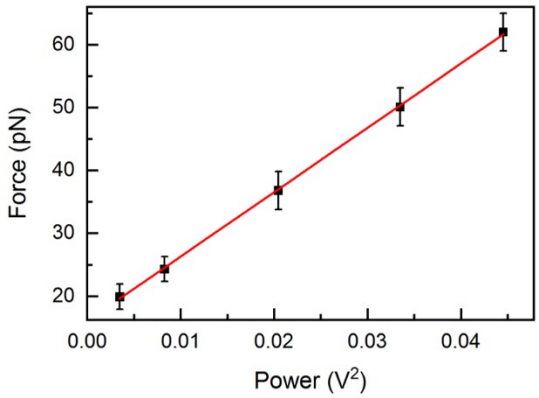


Figure S2. Calibration of ultrasound force using DNA duplexes with different lengths

3. Force dependence on sample distance calibration

Using the 13-bp DNA duplex, we have measured the dissociation voltage at several sample distances. The variation of dissociation voltages shows the necessity of maintaining a constant sample distance for the duration of the experiment.

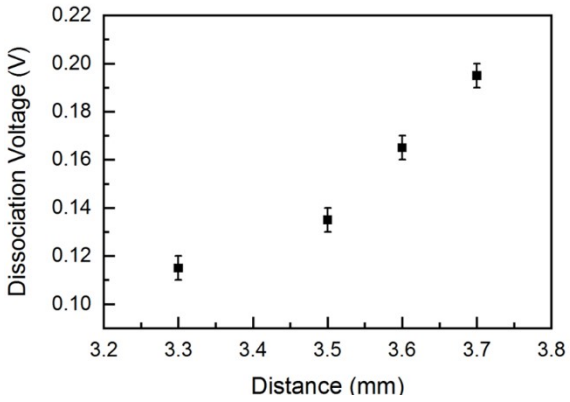


Figure S3. Dissociation voltage vs. sample distance.

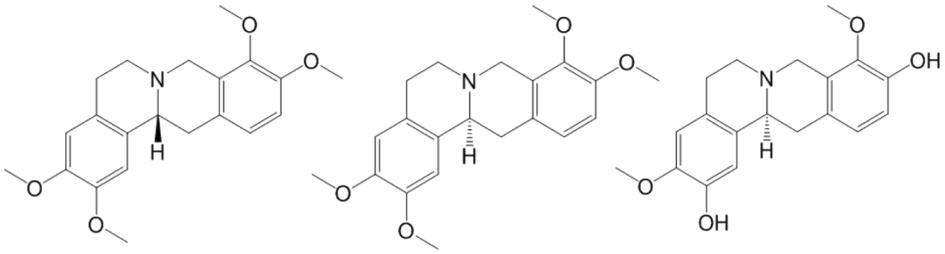
4. Standard deviations of the dissociation voltages*

	#1	#2	#3	#4	#5	Standard deviation*
0 CH_3	0.155	0.155	0.155	0.154	0.153	0.00089
1 CH_3	0.161	0.161	0.161	0.160	0.162	0.00071
2 CH_3	0.171	0.171	0.171	0.170	0.169	0.00089

*Unit: V.

All the standard deviations were less than the 0.001 V variation of the ultrasound amplitude.

5. Structures of the drug molecules



d-THP *l*-THP *l*-SPD

Figure S4. Structures of the three drug molecules. THP: tetrahydropalmatine; SPD: stepholidine.