Terminal-Specific Interaction between Double-Stranded DNA Layers: Colloidal Dispersion Behavior and Surface Force

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S-1. Experimental Details

Materials. All reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan) unless otherwise mentioned. Chemically-synthesized DNAs (OPC-purified grade) were purchased from Tsukuba Oligo Service (Ibaraki, Japan). The DNA concentration was determined by measuring the absorbance at 260 nm. *N*-Succinimidyl *S*-acetylthioacetate (SATA), tris(2-carboxyethyl) phosphine (TCEP), and hydroxylamine hydrochloride ($NH_2OH \cdot HCl$) were obtained from Pierce (Rockford, IL). Colloidal dispersions of gold nanoparticles (GNPs) with a diameter of 40 nm were purchased from BBInternational (Cardiff, UK). Ultra-pure water (> 18.1 $M\Omega \cdot cm^{-1}$) purified with a Milli-Q pure water purification system (Millipore, Billerica, MA) was used for all of the experiments.

Formation of dsDNA Layers on the GNP Surface by the hpDNA-Anchoring Method. The hpDNA-functionalized GNPs (hpDNA-GNPs) were prepared according to our previous report[S1] with a slight modification. First, the thiol group of hpDNA-SH was activated by TCEP treatment. Then, 5 nmol of hpDNA-SH was incubated with 1 ml of GNP dispersion overnight at 30°C. The dispersion medium was then exchanged for 10 mM Tris-HCl buffer (pH 7.4) containing 50 mM NaCl by adding the corresponding salts into the dispersion to reduce electrostatic repulsion between the hpDNA-SH strands and to facilitate surface immobilization. The mixture was further incubated at 30°C for 48 h. To remove the excess hpDNA-SH, the mixture was centrifuged at 15,000 g for 30 min, and the supernatant was replaced with 1 ml of 10 mM phosphate buffer (pH 7.4). The precipitate was re-dispersed by vortex mixing for several seconds. This process was repeated three times. Finally, the precipitate was re-dispersed into 10 mM phosphate buffer (pH 7.4) to make a stock solution for the various experiments in this study. The hydrodynamic diameter of hpDNA-GNPs was determined at 25°C by dynamic light scattering (DLS) measurements with a Zeta-sizer Nano ZS (Malvern, U.K.) equipped with a 4 mW He-Ne laser (633 nm).

Quantification of the Amount of hpDNA-SH Immobilized on the GNP Surface. The number of hpDNA-SH strands immobilized on the GNP surface was estimated by DTT displacement assay.[S2] In this experiment, fluorophore-labeled hpDNA-SHs (5'-Cy3-hpDNA-SH) were used. To

eliminate surface-immobilized 5'-Cy3-hpDNA-SH from the GNP surface, dithiothreitol (DTT) was added to the dispersion. The final concentrations were 100 mM for DTT and 0.2 nM for GNP, respectively. The mixture was incubated at 50°C overnight. After removal of the GNPs by centrifugation at 15,000 g for 30 min, the fluorescence of the supernatant (Ex: 540 nm; Em: 580 nm) was measured on a microplate fluorometer (ALVO X; Perkin Elmer, MA) and converted to a concentration by comparison to a standard curve. The results are expressed as the average of three samples (± S.D.).

Preparation of Gold Substrates and Gold-Coated Colloidal Probes. Silicon wafers with a size of 10×10 mm (n-type; Shin-etsu Chemicals, Japan) were used as substrates. Colloidal probes were prepared by attaching a silica bead (diameter 18 μm; Duke Sci. Corp., Palo Alto, CA) at the end of the tipless cantilever (NP-OW; normal spring constant: 0.06 $N \cdot m^{-1}$; Veeco, Santa Barbara, CA) using a small amount of epoxy resin (Araldite; Ciba-Geigy, Basel, Switzerland). The silicon substrates and colloidal probes were subjected to the Ge and Au vacuum deposition process after surface cleaning by UV ozone treatment (UV-300; SUMCO, Tokyo, Japan). A layer of 2.0-nm-thick Ge was deposited as a wetting promoter, followed by Au deposition at a thickness of 100 nm. The deposition rates of Ge and Au were 0.01 and 0.1 $nm \cdot s^{-1}$, respectively. The RMS roughness of the Au substrates was approximately 0.93 nm. In this study, we assumed the same surface roughness for the gold-coated colloidal probe.

Formation of dsDNA Layers on the Surface of Gold Substrates and Gold-Coated Colloidal Probes. First, the thiol group of hpDNA-SH was activated by incubating with 100 μM TCEP in 10 mM Tris-HCl (pH 7.5) containing 1 M NaCl at 25°C overnight. The hpDNA-SH concentration was set at 1 μM. Then, the activated hpDNA-SH solution was dropped onto the freshly prepared gold substrates and colloidal probes, and incubated overnight at room temperature. The resulting gold substrates and colloidal probes were rinsed with 10 mM sodium phosphate buffer (pH 7.4) containing 100 mM NaCl to remove excess molecules before use.

S-2. Melting curves and melting temperature (T_m) of hpDNA

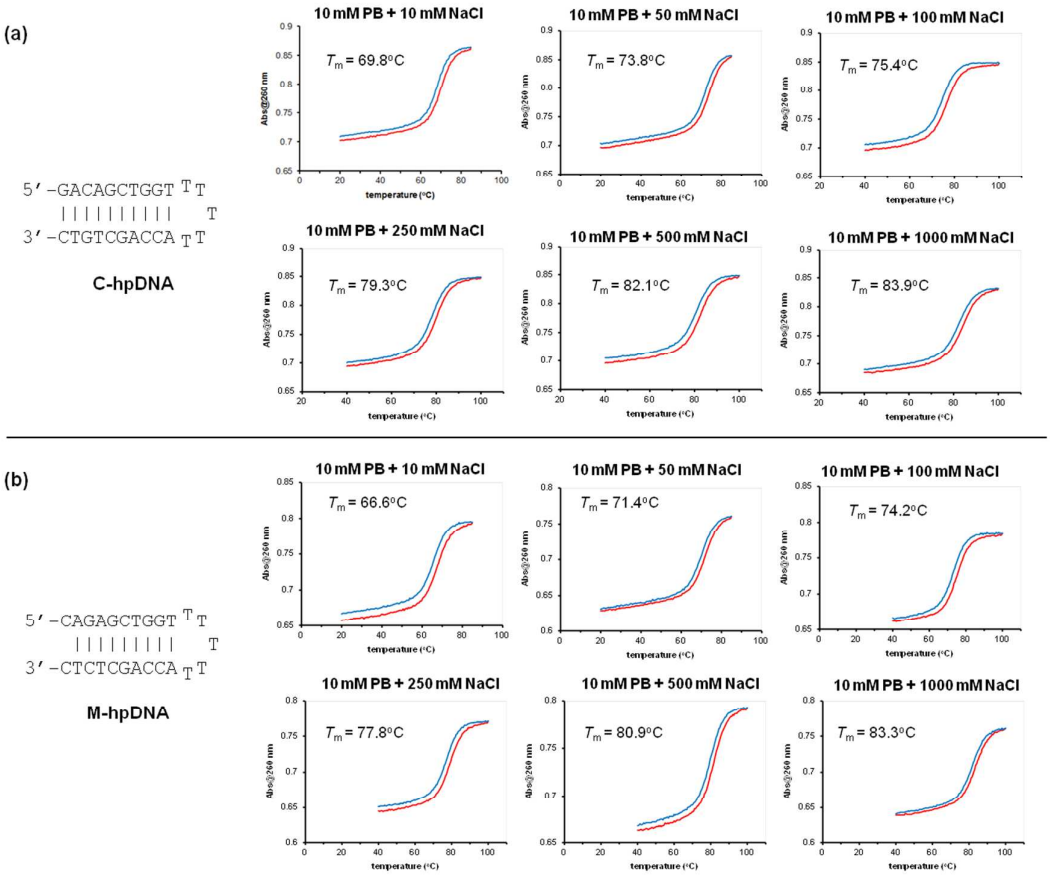

Figure S1. Base sequences, melting curves at 260 nm, and estimated T_m values for (a) C-hpDNA and (b) M-hpDNA in 10 mM sodium phosphate buffer (pH 7.4) containing various concentrations of NaCl. Red line: heating process. Blue line: cooling process. [C-hpDNA] = [M-hpDNA] = 3 μM. The heating and cooling ramp was 1 $^{o}C \cdot min^{-1}$. The melting temperature (T_m) was determined as an average of the maximum values in the first derivative of the melting curves obtained from the heating and cooling processes.

S-3. Synthesis of Thiol group incorporated hairpin DNAs (hpDNA-SHs)

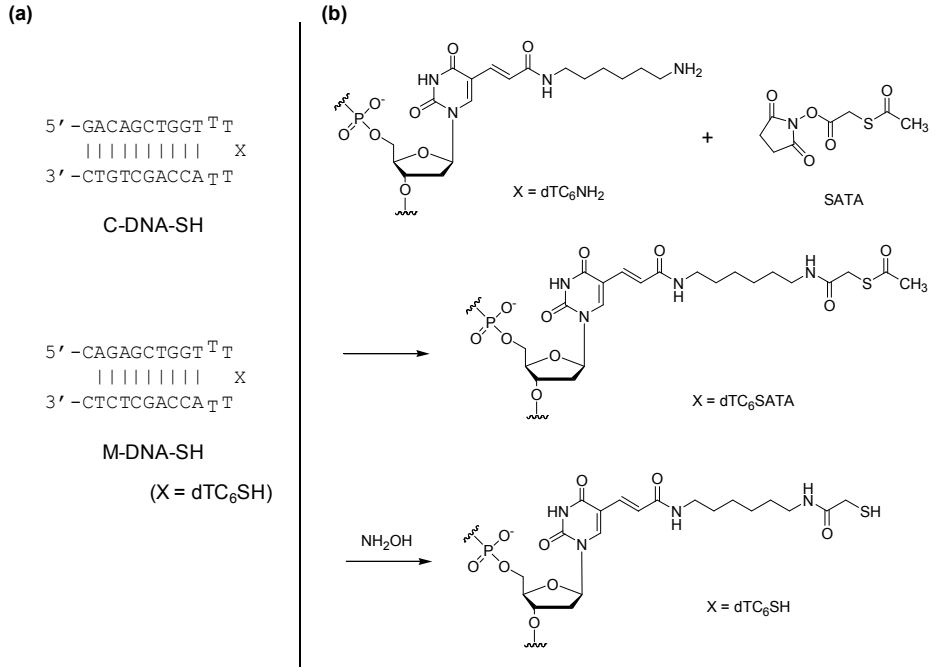

Figure S2. (a) Base sequences and (b) schematic representation of the synthesis of hpDNA-SH.

The synthetic route of hpDNA-SH is shown in Fig.S2. To a solution of hpDNA-NH_2 (X= dTC_6NH_2, 40 nmol) in 50 mM sodium phosphate (pH 8, 200 μl) was added a solution of SATA in DMF (2 mg/ 40 μl). The obtained mixture was allowed to react overnight at 25°C. The reaction progress was checked by the reverse-phase high performance liquid chromatography (RP-HPLC, C_{18} column). The HPLC system consisted of the following components from Shimadzu Scientific Instruments (Kyoto, Japan): an LC10AT HPLC pump, a CTO-10AS column oven, and an SPD-10A UV-vis detector controlled by LC solution software. For an analytical column, an Inertsil ODS-3 column (radius of silica gel particle: 5 μm, 4.6×250 mm) from GL science (Tokyo, Japan) was used.

RP-HPLC analyses were performed as follows: flow rate 1.0 ml·min^{-1}, mobile phase A: 50 mM triethylammonium acetate (TEAA, pH 7), mobile phase B: acetonitrile, gradient condition: 0-3 min 5 % B in A, 3-33 min 5-20 % B in A, 33-40 min 20-100 % B in A. The wavelength used for the detection was 260 nm. The product was purified using RP-HPLC under similar conditions and confirmed on the basis of the matrix-assisted laser-desorption ionization time-of-flight (MALDI-TOF) mass spectra. For C-DNA-SATA: M_{found} = 7909.4 g·mol^{-1} (M_{theory} = 7909.2 g·mol^{-1}), For M-DNA-SATA: M_{found} = 7869.4 g·mol^{-1} (M_{theory} = 7869.1 g·mol^{-1}).

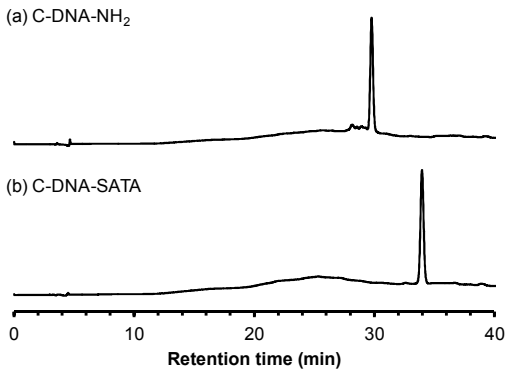

Figure S3. RP-HPLC charts (Abs. at 260 nm trace) of (a) C-DNA-NH_2 and (b) C-DNA-SATA.

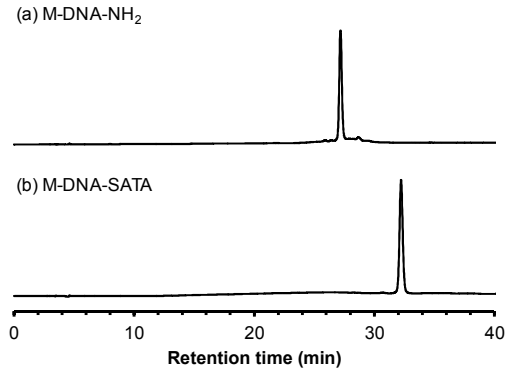

Figure S4. RP-HPLC charts (Abs. at 260 nm trace) of (a) M-DNA-NH_2 and (b) M-DNA-SATA.

Next, hpDNA-SATA was dissolved in a freshly prepared hydroxylamine solution (50 mM, the pH was adjusted to 7.2 with NaOH) containing 2.5 mM EDTA and incubated at 35^{o}C for 2 h. The mixture was ultra-filtrated (VIVACON, MW cutoff 2,000) and then lyophilized. The product was characterized by MALDI-TOF mass spectroscopy. For C-DNA-SH: M_{found} = 7867.2 g·mol^{-1} (M_{theory} = 7868.2 g·mol^{-1}), For M-DNA-SH: M_{found} = 7828.0 g·mol^{-1} (M_{theory} = 7827.4 g·mol^{-1}).

S-4. Colloidal dispersion stability of hpDNA/GNP mixtures

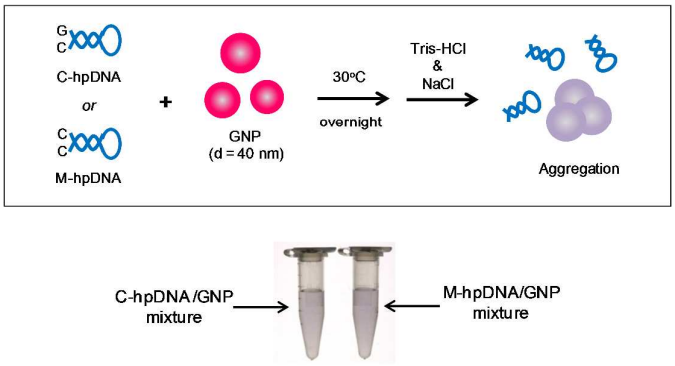

Figure S5. Schematic illustration and photographs of C-hpDNA/GNP and M-hpDNA/GNP mixtures after the addition of NaCl. C-hpDNA or M-hpDNA (5 nmol) was incubated with 1 ml of GNP dispersion overnight at 30°C. Then, the dispersion medium was exchanged into 10 mM Tris-HCl (pH 7.4) containing 50 mM NaCl by adding the corresponding salts into the mixture. Addition of 50 mM NaCl resulted in the immediate aggregation of GNPs, and solution color was rapidly changed from red to light purple as shown Fig.S5. Base sequences of C-hpDNA and M-hpDNA are shown in Fig.S1.

S-5. Zeta potential of C-DNA-GNPs and M-DNA-GNPs at various NaCl concentrations

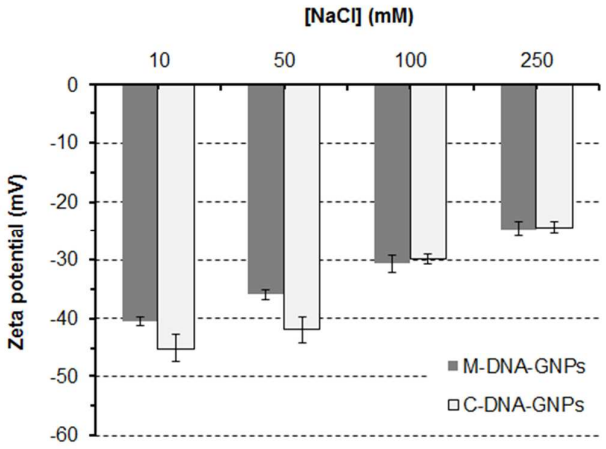

Figure S6. Zeta potential of hpDNA-GNPs (C-DNA-GNPs, M-DNA-GNPs) at various NaCl concentrations. Sample temperature was hold at 25^{o}C. The measured electrophoretic mobility (η) was converted into zeta potential (ζ) applying the Smoluchowski equation: $\zeta = 4\pi\eta\upsilon/\varepsilon$ in which υ is the viscosity and ε is the dielectric constant of the solvent, respectively. The zeta potential values shown in figure are expressed as the average of five measurements (± S.D.).

S-6. Histogram of minimum force value (maximum attraction force) observed on approaching M-DNA layers

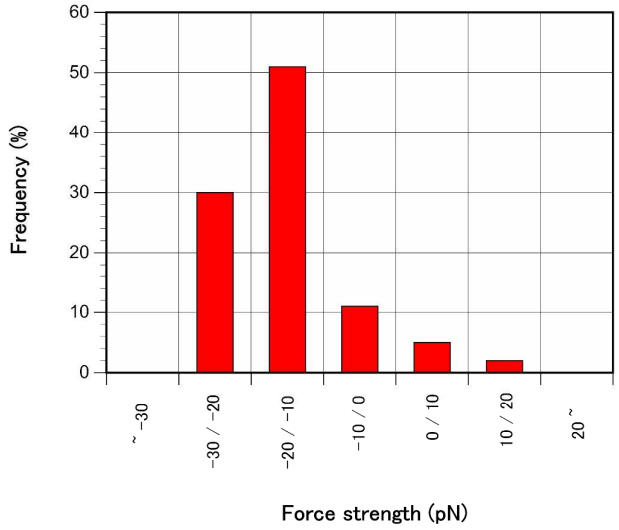

Figure S7. Histogram of minimum force value (maximum attraction force) observed on approaching M-DNA layers in sodium phosphate buffer (10 mM, pH 7.4) containing 1 M NaCl. Data was derivative from more than 100 of force-distance curves. Positive force value means repulsive force and negative force value means attraction force, respectively. All the observed attractive force was less than 30 pN.

S-7. Circular dichroism (CD) spectra of hpDNA

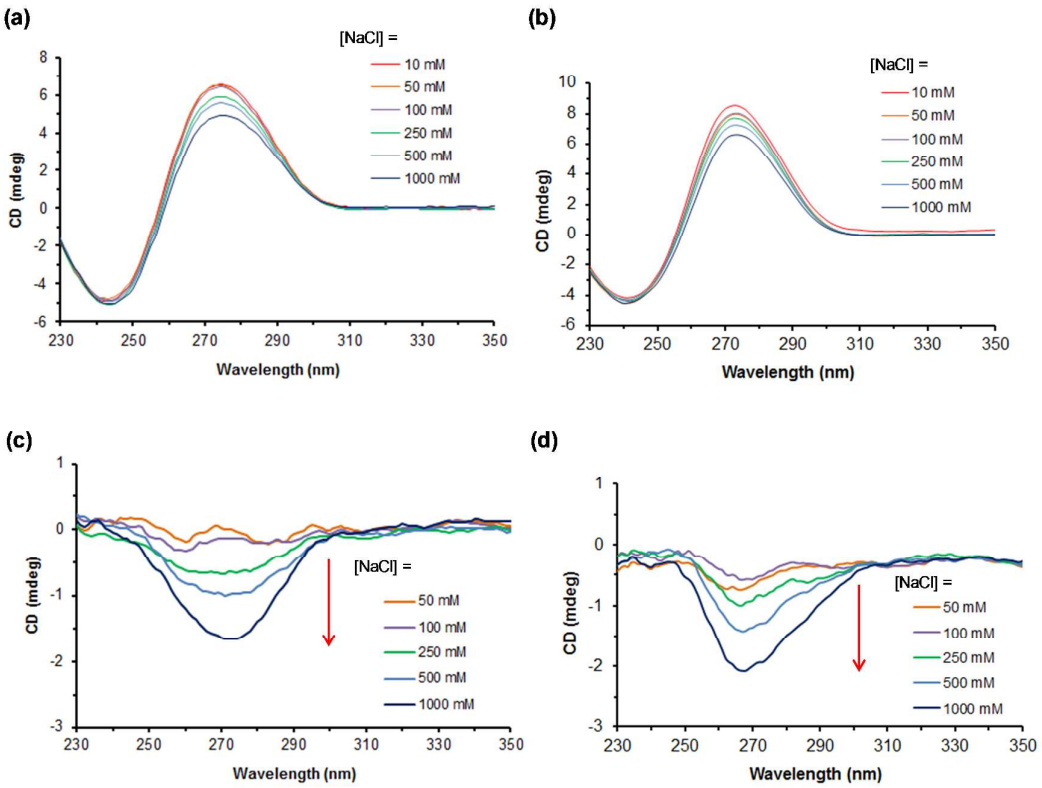

Figure S8. CD spectra of 20 μM of (a) C-hpDNA, (b) M-hpDNA in 10 mM sodium phosphate buffer (pH 7.4) with various concentration of NaCl. Sample temperature was set at 25°C. Scanning rate was 100 nm/min. Four spectrum scans were accumulated over the wavelength range of 230 to 350 nm. The scan of the buffer solution was subtracted from the accumulated scan of each sample. (c) CD difference spectra of C-hpDNA derived from (a) by subtracting the spectrum at 10 mM NaCl from that at corresponding NaCl concentration. (d) CD difference spectra of M-hpDNA derived from (c) by subtracting the spectrum at 10 mM NaCl from that at corresponding NaCl concentration. Along the increase of NaCl concentration, the positive CD band observed around 275 nm gradually decreased while the negative bang around 245 nm was practically unchanged. This characteristic change on CD spectra means a conformational change of DNA duplex (in this case, stem moiety) from B-form to C-form, i.e., reduction of DNA-bound water molecules with the increase of NaCl concentration.[S3]

S-8. Force-distance dependence observed for approaching C-DNA layer and M-DNA layer

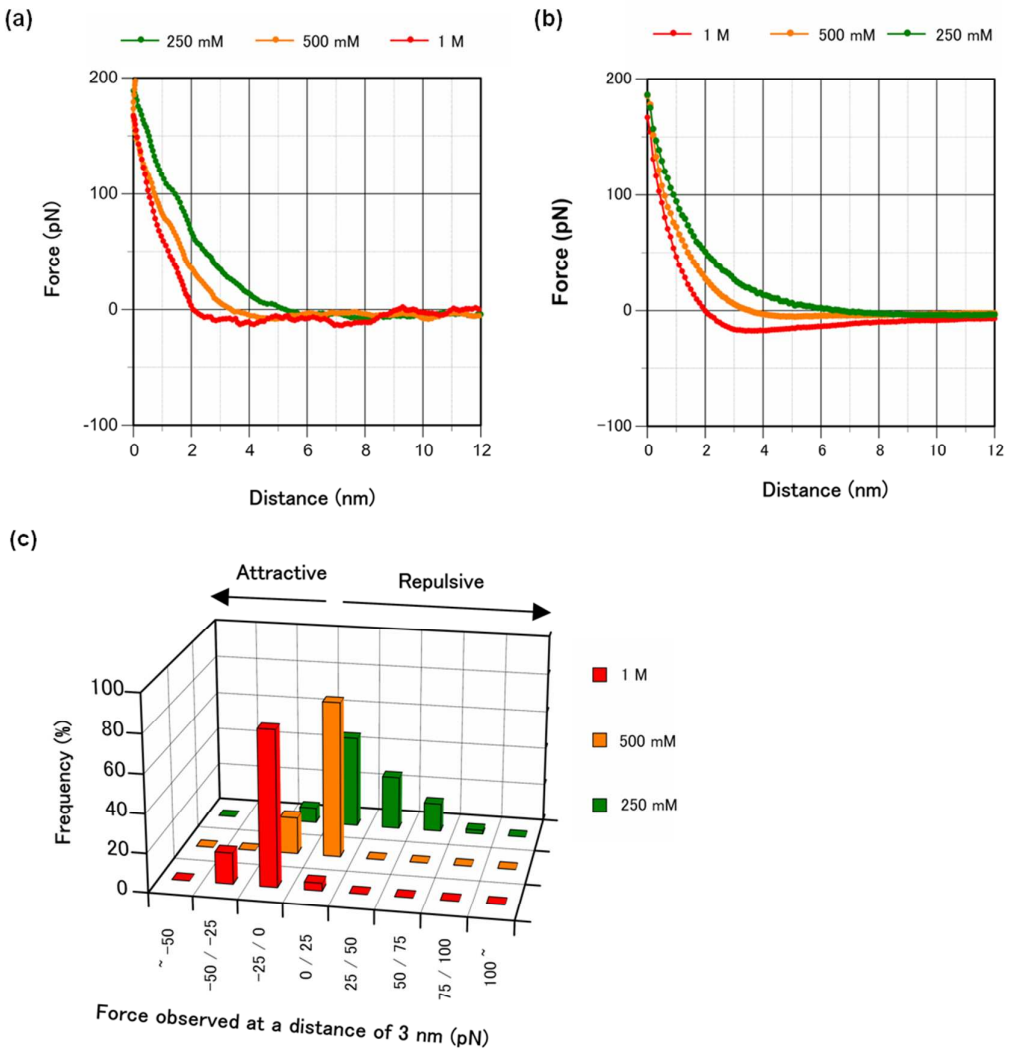

Figure S9. Representative single force-distance curves (a) and averaged curves (b) obtained for approaching C-DNA layer and M-DNA layers in sodium phosphate buffer (10 mM, pH 7.4) containing 250 mM, 500 mM, and 1 M of NaCl. (c) Histograms for force observed at a distance of 3.0 nm for C-DNA layer and M-DNA layer. Positive force value means repulsion and negative force value means attraction, respectively. The observed attractive forces were larger than those for M-DNA-to-M-DNA layer case, but far below those observed for C-DNA-to-C-DNA case.

S-9. Potential binding site of water molecules to the pairing/unpairing terminal base pairs

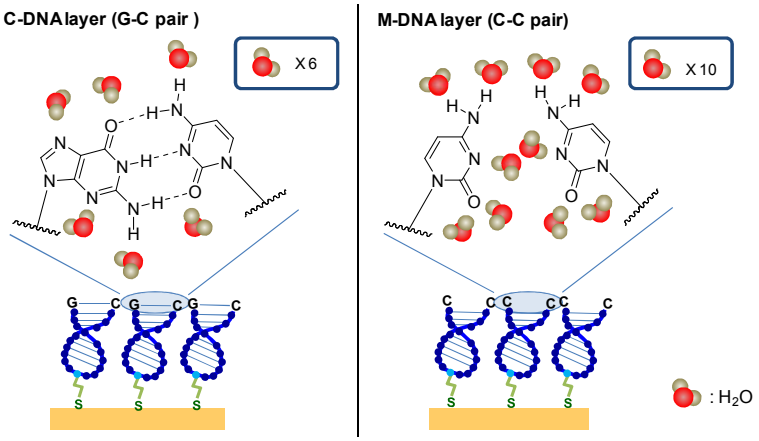

Figure S10. Schematic illustration of potential binding sites of water molecules to the (a) G–C pair and (b) C–C mismatch terminal base pair.

References

S1. Kanayama, N.; Takarada, T.; Fujita, M.; Maeda, M. *Chem.-Eur. J.* **2013**, *19*, 10794-10798.

S2. Hurst, S. J.; Lytton-Jean, A. K. R.; Mirkin, C. A. *Anal. Chem.* **2006**, *78*, 8313-8318.

S3. Saenger, W. *Principles of Nucleic Acid Structure*, Springer **1984**.