Supporting Information for:

**Z-DNA stabilization is dominated by the Hofmeister effect**

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**Materials and methods:**

**DNA preparation**

For the single molecule FRET experiment, modified oligonucleotides were purchased from Integrated DNA Technologies (Coralville, IA). DNA duplexes were annealed by mixing the biotinylated and non-biotinylated strands at a ratio of 1:2 in a 50 μM concentration in a buffer containing 10mM Tris (pH 8.0) and 50mM NaCl. The non-biotinylated strands were added in excess to minimize the chance of having un-annealed biotinylated strand in a single-molecule measurement. The annealing mixture was heated to 95 ºC for 3 minutes and allowed to cool slowly for 1.5 hours in a heating block.

For the bulk DNA denaturing FRET experiment, DNA duplexes were annealed by mixing Cy5-labeled and Cy3-labeled strands at a 1.5:1 ratio in a 50 μM concentration in a buffer containing 10mM Tris (pH 8.0) and 50mM NaCl. The Cy5-labeled strands were added in excess to minimize the chance of having a free Cy3-labeled single strand in the measurement. For CD experiments, non-modified oligonucleotides were purchased from Bioneer (South Korea). DNA duplexes were annealed by mixing stands at a 1:1 ratio in a 500 μM concentration in a buffer containing 10mM Tris (pH 8.0) and 50mM NaCl.

**Single-molecule experiments**

A sample chamber with a narrow width of less than 1 cm was created by assembling a quartz slide and a glass cover-slip using double-sided adhesive tape and epoxy. Biotinylated DNA molecules (80pM) were immobilized on the quartz slide coated with polyethylene glycol (PEG) and streptavidin. Images were then obtained using a wide-field total-internal-reflection fluorescence microscope with a 100-ms time resolution using an electron multiplying charge-coupled device (EM-CCD) camera (iXon DV887ECS-BV, Andor Technology) and a custom-made C++ program. As an oxygen scavenger system to slow photobleaching, an imaging buffer was injected that contained 10mM Tris-HCl (pH 8.0, 1% (v/v) Trolox (Sigma-Aldrich), 0.4% D-glucose (Sigma-Aldrich), 1 mg/ml glucose oxidase (Sigma-Aldrich) and 0.04 mg/ml catalase (Roche, Nutley, NJ) with designated ions. All single-molecule experiments were done at 25 ºC after at least 1 hour incubation time. The B-Z transition times are in the range of several
minutes for various salt conditions (Optics Express, Vol. 20, Issue 28, pp. 29353-29360 (2012), Figure S14).

**Fluorescence spectroscopy**

The bulk FRET efficiency was determined using Varian Eclipse fluorescence spectrophotometer. The same sample prepared for the smFRET trial was used in this experiment. DNA duplex (10nM) was incubated in buffer containing 10mM Tris-HCl (pH 8.0) with the designated ions. Cy3 was excited by light with a wavelength of 532nm. The emission wavelength peaks for Cy3 and Cy5 were set to 565nm and 665nm, respectively. The FRET efficiency was calculated by $I_{665\text{nm}} / (I_{565\text{nm}}+I_{665\text{nm}})$. Here, $I_{\text{peak}}$ denotes the fluorescence intensity of the peak. All bulk experiments were done at 28 ºC after at least 1 hour incubation time.

**Circular Dichroism**

CD spectra were recorded using a Jasco-815 spectropolarimeter with a quartz cell with an optical path length of 1 mm in the wavelength range of 240~310 nm; the band width was 1 nm, the scanning speed was 50 nm/min and the response time was 8s. The DNA duplex (5 μM) was incubated in 10 mM Tris (pH 8.0) buffer containing the designated concentration of salts. All CD experiments were performed at 25 ºC after at least 1 hour incubation time.
Supplementary Figures:

**Figure S1.** CD spectra of (CG)\textsubscript{6} at various concentrations of (a) CaCl\textsubscript{2}, (b) Mg(ClO\textsubscript{4})\textsubscript{2}, and (c) Ca(ClO\textsubscript{4})\textsubscript{2}.
Figure S2. Z-DNA fractions at varying concentrations of (a) Mg(ClO$_4$)$_2$, (b) Ca(ClO$_4$)$_2$, (c) MgCl$_2$, and (d) CaCl$_2$. The Z-DNA fractions were obtained from the relative change of the CD signal at 290-nm (red lines) or 250-nm (black lines) by assuming that B-to-Z was complete at saturating salt concentrations.
Figure S3. Z-DNA fractions at varying concentrations of various salts. The Z-DNA fractions were obtained from the relative change of the CD signal at 255-nm by assuming that B-to-Z was complete at saturating salt concentrations.
**Figure S4.** Quantitation of Z-DNA embedded in B-DNAs via CD spectra. (a) DNA structure used for the measurements. (b) CD spectra at varying NaClO₄ concentrations. (c) Z-DNA fractions at varying NaClO₄ concentrations. The Z-DNA fractions were obtained from the relative increase of the CD signal at 255-nm (lower triangles) or at 290-nm (upper triangles). It was assumed that B-to-Z was complete at saturating salt concentrations.
Figure S5. Z-DNA formation in B(CG)$_6$ B by other salts. (a) DNA structure used for single-molecule FRET measurements. FRET histograms (b) FRET distributions at varying Ca(ClO$_4$)$_2$ concentration. (c) FRET distributions at varying MgCl$_2$ concentration.
Figure S6. CD spectra of B(CG)$_6$B at various salts. (a) DNA structure used for the measurements. CD spectra at various concentrations of (b) Mg(ClO$_4$)$_2$, (c) Ca(ClO$_4$)$_2$, (d) MgCl$_2$, and (e) CaCl$_2$. 
Figure S7. Quantitation of Z-DNA in B(CG)_6B via CD spectra. (a) DNA structure used for the measurements. (b) Z-DNA fractions at varying concentrations of Mg(ClO_4)_2 (black) and Ca(ClO_4)_2 (red). Z-DNA fractions were obtained from the relative increase of the CD signal at 255-nm by assuming that B-to-Z was complete at saturating salt concentrations. (c) the same as (b) except that Z-DNA fractions were obtained from the relative increase of the CD signal at 290-nm by assuming that B-to-Z was complete at saturating salt concentrations.
Figure S8. DNA denaturation by various salts. (a) DNA structure (B$_{12bp}$) used for the measurements. Fluorescence spectra of the B$_{12bp}$ at varying concentration of (b) MgCl$_2$, (c) Mg(ClO$_4$)$_2$, and (d) Ca(ClO$_4$)$_2$. Excitation wavelength: 532-nm.
Figure S9. DNA denaturation by high salts: (a) DNA structure (B\textsubscript{12bp}) used for the experiments. (b) Single-molecule images of B\textsubscript{12bp} immobilized on a quartz surface. Cy3 images excited 532-nm (green), and Cy5 images excited at 640-nm (red) are shown for 50 mM NaClO\textsubscript{4} (left), and 10 minutes after an injection of 3 M NaClO\textsubscript{4} (right). Disappearance of the green spots (i.e., denaturation of the DNA duplexes) at 3M NaClO\textsubscript{4} is clear.
Figure S10. DNA denaturation by various salts: (a) DNA structure ($B_{12bp}$) used for the measurements. (b) Bulk FRET efficiencies at varying concentrations of various salts. The FRET efficiency was calculated as $I_{665nm}/(I_{565nm}+I_{665nm})$ from Bulk FRET spectra like Figure 3b, where $I_{\text{wavelength}}$ represents the fluorescence intensity at the designated wavelength.
Figure S11. B-Z transition of (CG)$_6$ by various salts. (a) DNA construct ((CG)$_6$) used for the measurements. (b) Z-DNA fractions at varying concentrations of various salts. The Z-DNA fractions were obtained from the relative change of the CD signal at 290-nm by assuming that B-to-Z was complete at saturating salt concentrations.
Figure S12. B-to-Z transition of B(CG)$_6$ by various salts. (a) DNA construct used for the single-molecule FRET (top) and CD (bottom) measurements. FRET histograms (top) and CD measurements (bottom) at varying (b) NaCl, (c) MgCl$_2$, (d) NaClO$_4$, and (e) Mg(ClO$_4$)$_2$ concentrations. B-to-Z transition was not observed when an anion was Cl$^-$.
Figure S13. B-to-Z transition of B(CG)$_6$ B by NaClO$_4$. (a) DNA construct used for single-molecule FRET (top) and CD measurements (bottom). (b) FRET histograms and (c) CD measurements at varying NaClO$_4$ concentrations.
Figure S14. Z-DNA stabilization kinetics. (a) Z-DNA stabilization kinetics of (CG)$_6$, B(CG)$_6$, and B(CG)$_6$B at various concentrations of NaClO$_4$. The half times were measured by either bulk FRET or CD measurement. (b) Z-DNA stabilization kinetics of (CG)$_6$ for various salt conditions. The half times were measure by CD measurement.