Supporting Information

An Artificial Sandwich Base for Monitoring Single Nucleobase Changes and Charge Transfer Rates in DNA

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1. Experimental Section

1.1 General instruments and information

Oligonucleotides were prepared with an Applied Biosystems (ABI) 394 DNA/RNA synthesizer. MALDI-TOF analyses of oligonucleotides were performed by the Shanghai Sangon Mass Spectrometry Facility. Melting curve measurements were carried out on a Real-Time PCR instrument (Model 7500, Applied Biosystems) equipped with a SYBR green filter and Cary 100 UV-Vis spectrophotometer.

All reagents were used as received from commercial sources or prepared as described in references. Anhydrous solvent pyridine, CH_3CN, CH_2Cl_2 and DMF were distilled under a nitrogen atmosphere and stored with 4 Å molecular sieves. ^1H spectra were recorded on a Bruker AM300 spectrometer. ^{13}C NMR spectra were recorded on a Bruker AM400 spectrometer. Chemical shifts (δ) are reported in ppm, and coupling constants (J) are in Hertz (Hz). The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad.

1.2 DNA Synthesis

All DNA synthesis reagents were purchased from Glen Research. The completed sequences were then deprotected in saturated ammonium hydroxide at room temperature for 12 hours and further purified by reversed-phase HPLC (ProStar; Varian) on a C-18 column using 0.1 M triethylamine acetate (TEAA) buffer (Glen Research) and acetonitrile (SigmaAldrich) as the eluents. The collected DNA products were dried and detritylated by dissolving and incubating DNA products in 200 μL of 80% acetic acid for 20 min. The

detritylated DNA product was precipitated with NaCl (3 M, 25 µL) and ethanol (600 µL) and then desalted on a Glen-Pak DNA Purification Cartridge.

1.3 Electrochemistry[1]

All electrochemical measurements were performed on a CHI760B electrochemical workstation (Chenhua Instrument Company of Shanghai, China). A conventional three-electrode glass cell was used with a platinum wire (0.5 mm diameter) as the counter electrode, Silver/silver chloride was used as the reference electrode and glassy carbon (3.0 mm diameter) was used as the working electrode for all the potentials reported herein. All electrodes were purchased from Tianjin Aida Hengsheng Technology Development CO., LTD (Tianjin, China). Milli-Q water (resistance of >18 MΩ cm) was used throughout, for both cleaning and buffer preparation. Electrode processing protocols and measurement setup followed the previous report[1]. 50 mM NaCl, 50 mM $MgCl_2$, 50 mM Tris-HCl buffer, pH 6.8 solution was used for DNA solution preparation and purged with nitrogen for 10 minutes before measurements.

1.4 Thermodynamic studies[2]

For the determination of melting temperatures, Fe-DNAs (1 µM) and the SYBR green I dye (1X, SigmaAldrich) were dissolved in a PIPES buffer (100 mM NaCl, 10 mM $MgCl_2$, 10 mM PIPES, pH 7.0), and the solutions were heated at 95 ˚C for 5 min. Then the samples were transferred into Real Time tubes and placed in a Real-Time PCR machine (7500, Applied Biosystems) equipped with an SYBR greenfilter. The samples were cooled to 15 ˚C, and then the temperature was increased from 15 to 95 ˚C at a rate of 0.5 ˚C/min. Fluorescence data from melting curves were converted into Tm by plotting the negative derivative of fluorescence vs temperature (dF/dT vs.T). The concentrations of Fe-DNAs and SYBR green I dye were optimized with Oligo1 and Oligo2 prior to the study.

2. Synthesis of Fe-phosphoramidite

Synthesis of compound 2

Ferrocene carboxylic acid **1** (1 g, 4.34 mmol) was coupled with S-aminopropanediol (0.39 g, 4.34 mmol) in the presence of diisopropylethylamine (0.84 g, 6.51 mmol) and N,N,N',N'-Tetramethyl-O-(1H-benzotriazol-1-yl)uronium hexafluorophosphate, O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (1.64 g, 4.34 mmol) in dry DMF. After the reaction mixture was stirred at room temperature for 3 h, the solvent was removed and the crude residue was purified with silica gel column chromatography (Dichloromethane: Methanol = 95 : 5, Rf = 0.3) to offer the desired compound **2** in 95% yield. 1H NMR (400 MHz, DMSO-d6) δ 7.84 (s, 1H), 4.83 (d, J = 2.3 Hz, 3H), 4.34 (d, J = 2.4 Hz, 2H), 4.18 (s, 5H), 3.64 (m, 1H), 3.39 (d, J = 5.3 Hz, 2H), 3.18 (d, J = 6.0 Hz, 2H), 1.26 (t, J = 9.0 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d6): 170.27 (C=O), 76.94 (ipso Cp), 71.27, 70.42, 69.85, 68.70, 64.31, 53.89, 42.89. MS (ESI^-) m/z 304 $[M + H]^+$.

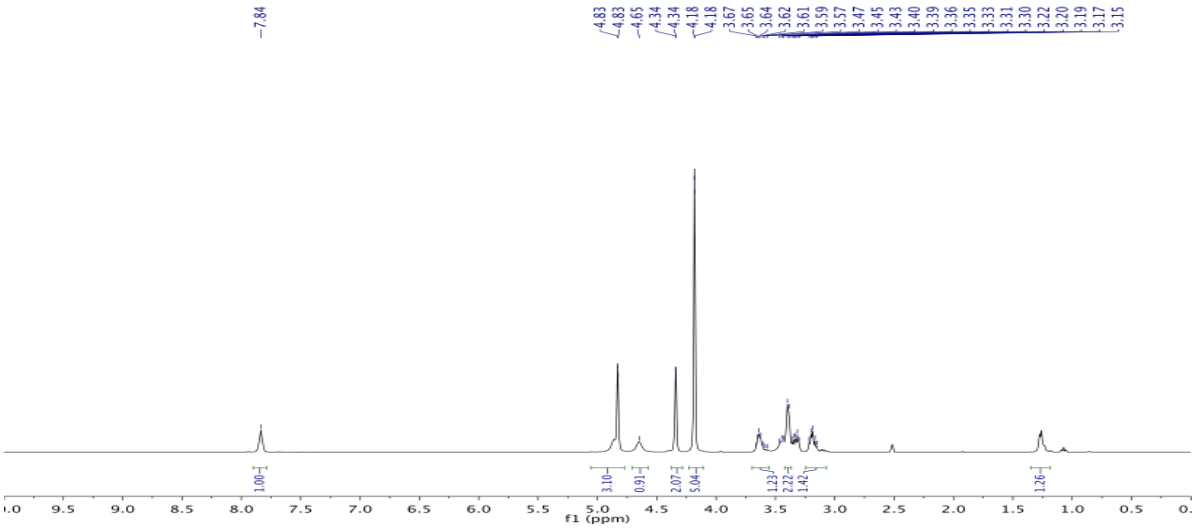

Figure S1. ^{1}H NMR spectrum of compound **2**.

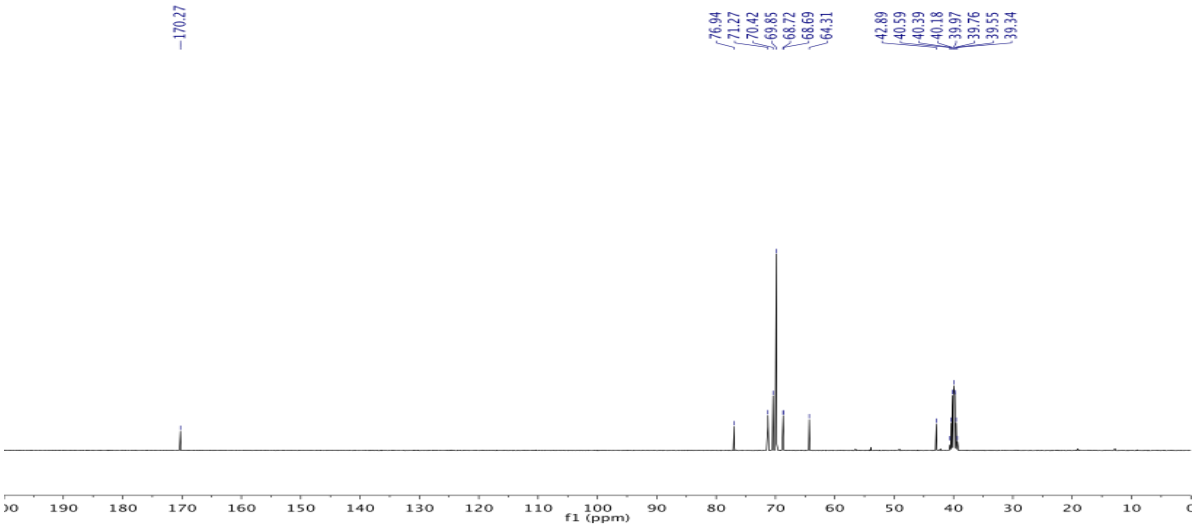

Figure S2. ^{13}C NMR spectrum of compound **2**.

Synthesis of compound 3

Compound 2 (1 g, 3.30 mmol) was dissolved in dry pyridine in an ice bath under nitrogen. Then 4,4'-dimethoxytrityl chloride (1.34 g, 3.96 mmol) in CH2Cl2 was added to the above mixture. After 30 min of vigorous stirring, the reaction mixture became transparent. Then the ice bath was removed and the reaction mixture was further stirred at room temperature. After 12 h, the solvent was removed by evaporation, and the obtained solid was subjected to silica gel column chromatography (AcOEt: hexane : Et3N = 35 : 65 : 1, Rf = 0.5) to offer the desired compound3 in 60% yield.1H NMR (400 MHz, CD3CN) δ 7.48 (d, J = 7.8 Hz, 2H), 7.35 (d, J = 8.4 Hz, 4H), 7.29 (t, J = 7.1 Hz, 2H), 7.23 – 7.16 (m, 1H), 6.84 (d, J = 8Hz, 4H), 6.72 (t, J = 6.0 Hz, 1H), 4.63 (dd, J = 2.2 Hz, 2H), 4.31 (d, J = 2.2 Hz, 2H), 4.13 (s, 1H), 4.09(s, 5H), 3.89 (t, J = 5.7 Hz, 1H),3.73(s, 6H) 3.51-3.42 (m, 1H), 3.38 – 3.27 (m, 1H), 3.12 – 3.01 (m, 2H). 13C NMR (101 MHz, DMSO-d6) δ 169.92 (C=O), 158.49(ipso dmt), 145.68 (ipso Ar), 136.37 (ipso Ar), 136.31 (ipso Ar), 130.28 (CH-Ar), 130.25 (CH-Ar), 128.27(CH-Ar), 128.23(CH-Ar), 127.03 (CH-Ar), 113.61 (CH-Ar), 85.68 (ipso-cp), 77.07, 70.29, 69.79, 68.69, 68.63, 55.49, 45.94, 9.35. MS (ESI) m/z 628.2 [M + Na]+.

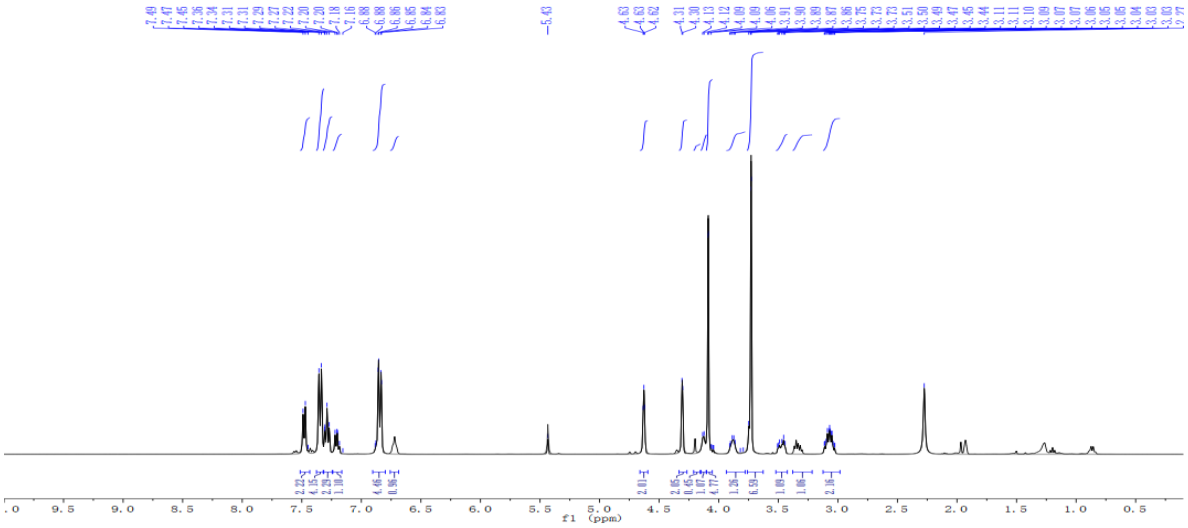

Figure S3. ^{1}H NMR spectrum of compound **3**.

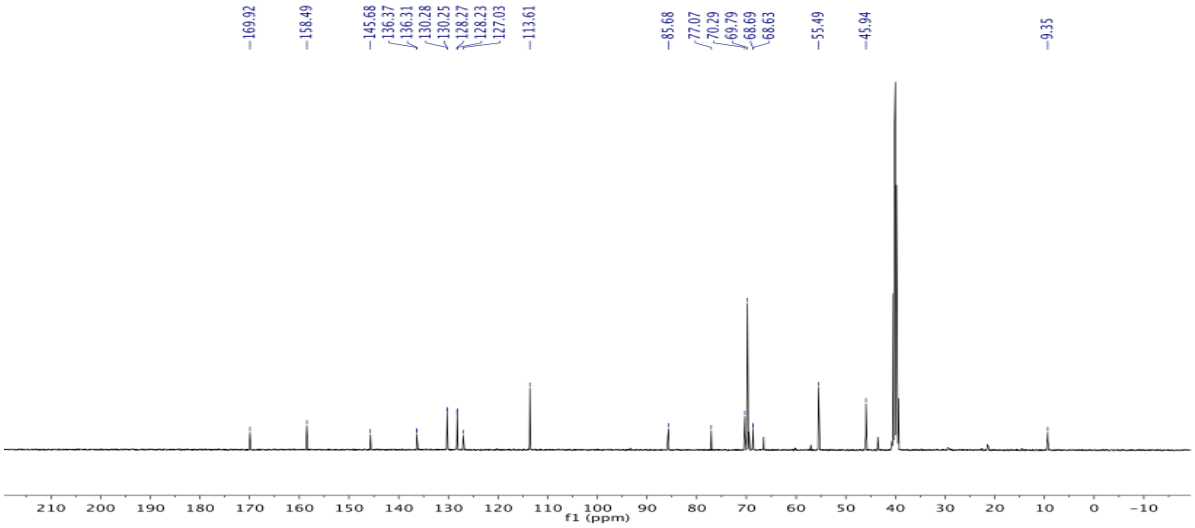

Figure S4. ^{13}C NMR spectrum of compound **3**.

Synthesis of Fe-phosphoramidite

To a solution of compound 3 (1 g, 1.65 mmol) in anhydrous DCM (15 mL) was added diisopropylethylamine (0.43 g, 3.30 mmol), followed by chlorophosphoramidite (0.47 g, 1.98 mmol) at 0 °C. The mixture was allowed to warm to RT and stirred for 1 h. Then the reaction mixture was diluted with 5 mL DCM and washed with saturated $NaHCO_3$ solution and saturated saline solution. The organic phase was dried over Na_2SO_4 and then concentrated in vacuo. The residue was subjected to silica gel column chromatography (AcOEt : hexane: Et_3N = 20 : 80 : 1, Rf = 0.5) to offer the desired Fe-phosphoramidite as a yellow foam in 75% yield (mixture of diastereoisomers).^{1}H NMR(400 MHz, CDCl3): 7.48 (d, J = 7.8 Hz, 2H), 7.36 (d, J = 8.3 Hz, 4H), 7.32 – 7.24 (m, 3H), 7.21 (t, J = 6.6 Hz, 1H), 6.83 (t, J = 7.0 Hz, 4H), 6.23 (t, J = 5.0 Hz, 0.86H), 6.03 (s, 0.23H), 4.61 (s, 0.63H), 4.53 (d, J = 7.2 Hz, 1.41H), 4.29 (s, 1.36H), 4.27 (s, 0.8H), 4.11 (s, 3.7H), 4.09 (d, J = 6.3 Hz, 2.7H), 3.89 (d, J = 7.1 Hz, 0.59H), 3.77 (s, 6.28H), 3.74 – 3.56 (m, 4.40H), 3.56 – 3.48 (m, 0.67H), 3.31 (d, J = 5.2 Hz, 0.85H), 3.30 – 3.25 (m, 0.61H), 3.16 (dd, J = 9.5, 5.3 Hz, 0.76H), 2.59 (t, J = 6.2 Hz, 1.32H), 2.45 (t, J = 6.3 Hz, 0.70H), 1.20 (d, J = 6.4 Hz, 7.58H), 1.15 (d, J = 6.7 Hz, 3.94H).^{13}C NMR (101 MHz, $CDCl_3$): 170.09, 158.51, 144.74, 135.86, 130.10, 128.01, 127.48, 126.40, 117.69, 113.17, 86.24, 72.51, 72.36, 70.29,

70.13, 69.66, 68.50, 68.27, 67.87, 67.55, 64.88, 64.57, 60.35, 58.45, 58.28, 58.10, 55.18, 24.68, 21.02, 20.40, 20.25, 14.19. MS (ESI) m/z 828.30 $[M + Na]^+$. ^{31}P NMR (162 MHz, $CDCl_3$) δ 149.68, 148.72.

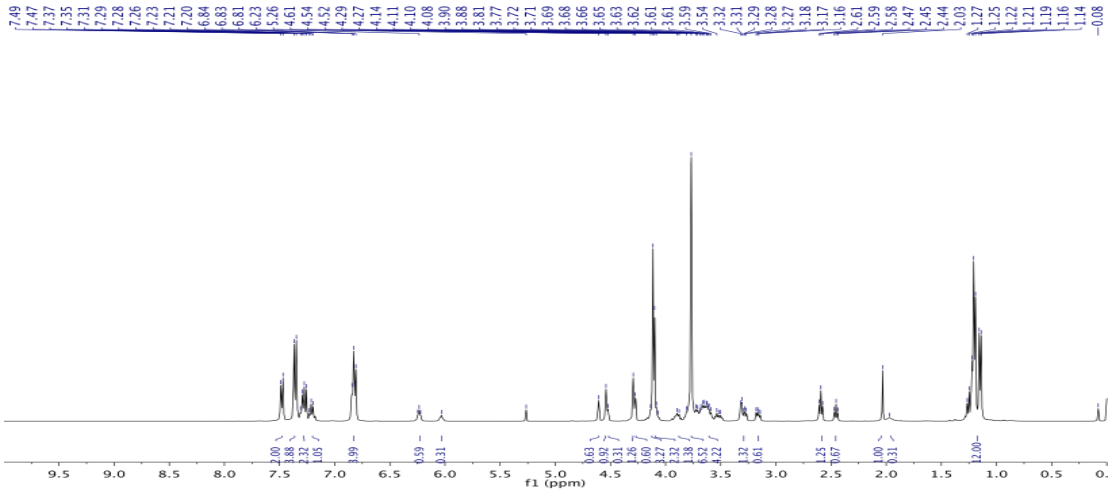

Figure S5. ^{1}H NMR spectrum of Fc-phosphoramidite.

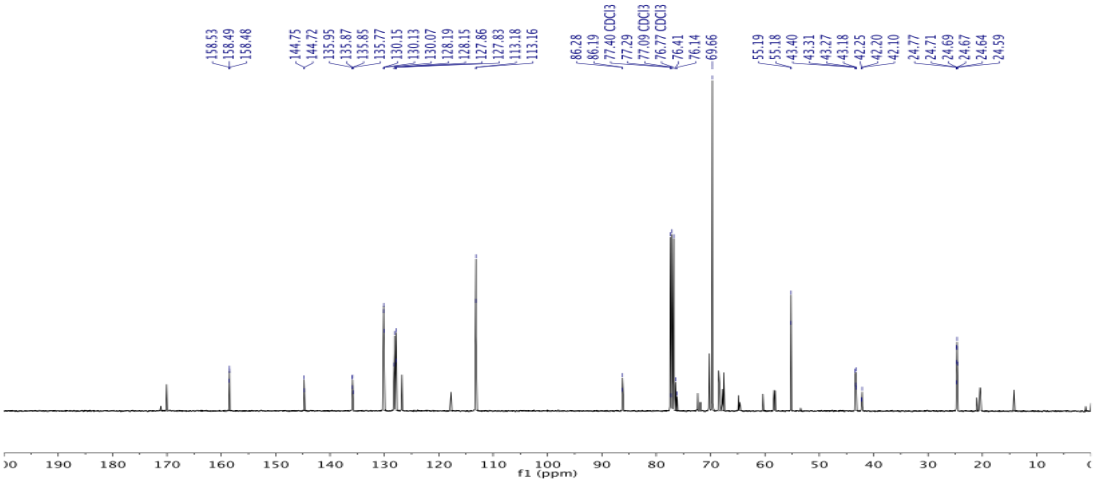

Figure S6. ^{13}C NMR spectrum of Fc-phosphoramidite.

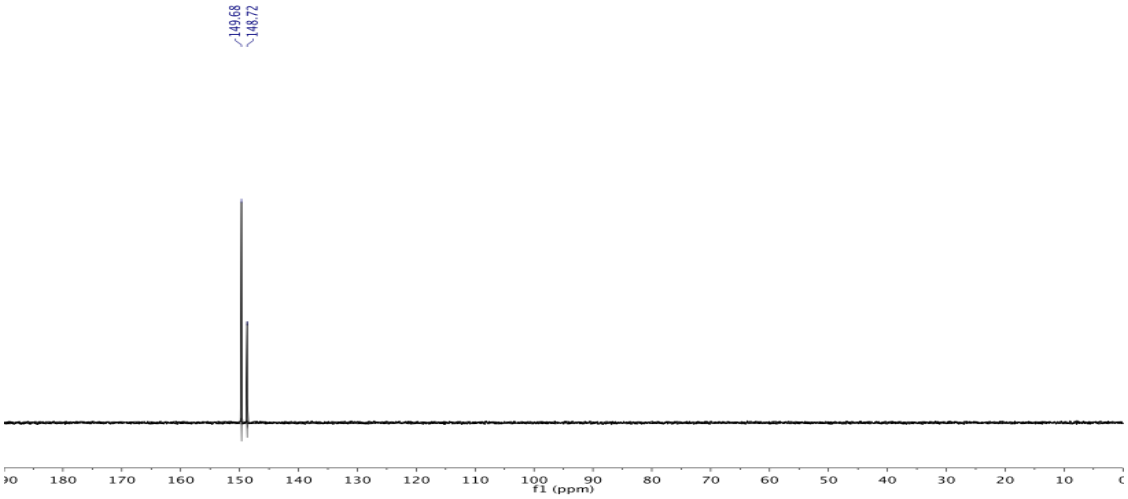

Figure S7. ^{31}P NMR spectrum of Fc-phosphoramidite.

3. Results and Discussion

Table S1. DNA complementary sequences (CS) designed in this article.

Name	Sequences (from 5' to 3')
a1-CS-A	CAT TGA GAG AGT CCA
a1-CS-T	CAT TGA GAG AGT CCT
a1-CS-G	CAT TGA GAG AGT CCG
a1-CS-C	CAT TGA GAG AGT CCC
a2-CS-A	CAT TGA GAG AGT ACA
a2-CS-T	CAT TGA GAG AGT TCA
a2-CS-G	CAT TGA GAG AGT GCA
a2-CS-C	CAT TGA GAG AGT CCA
a3-CS-A	CAT TGA GAG AGT CCA
a3-CS-T	CAT TGA GTG AGT CCA
a3-CS-G	CAT TGA GGG AGT CCA
a3-CS-C	CAT TGA GCG AGT CCA
a4-CS-A	CAA TGA GAG AGT CCA
a4-CS-T	CAT TGA GAG AGT CCA
a4-CS-G	CAG TGA GAG AGT CCA
a4-CS-C	CAC TGA GAG AGT CCA

Table S2.Redox properties of the Ferrocene base incorporated into DNA strand.

Strand	E_{pc}	E_{pa}	$E_{1/2}$	ΔE_p
a1	0.479	0.394	0.4365	0.085
a2	0.472	0.401	0.4365	0.071
a3	0.486	0.398	0.442	0.088
a4	0.479	0.398	0.4385	0.081

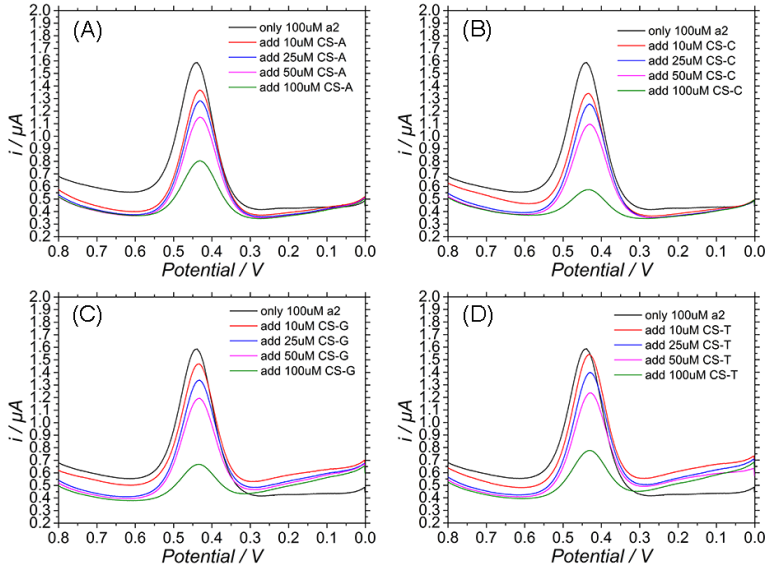

Figure S8.Squarewave voltammograms of Fc-DNA (a2) alone and after addition of cDNA with 10-100 μM CS-A (A), CS-C (B), CS-G (C) and CS-T (D).

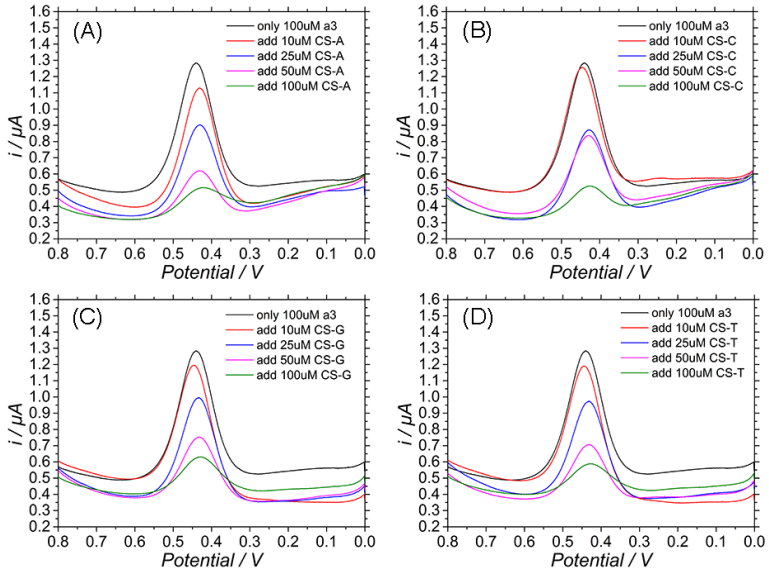

Figure S9.Squarewave voltammograms of Fc-DNA (a3) alone and after addition of cDNA with 10-100 μM CS-A (A), CS-C (B), CS-G (C) and CS-T (D).

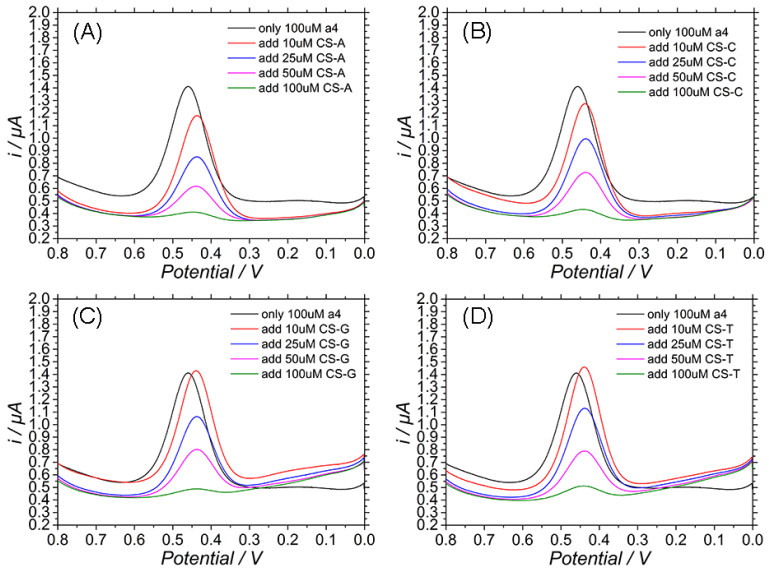

Figure S10.Squarewave voltammograms of Fc-DNA (a4) alone and after addition of cDNA with 10-100 μM CS-A (A), CS-C (B), CS-G (C) and CS-T (D).

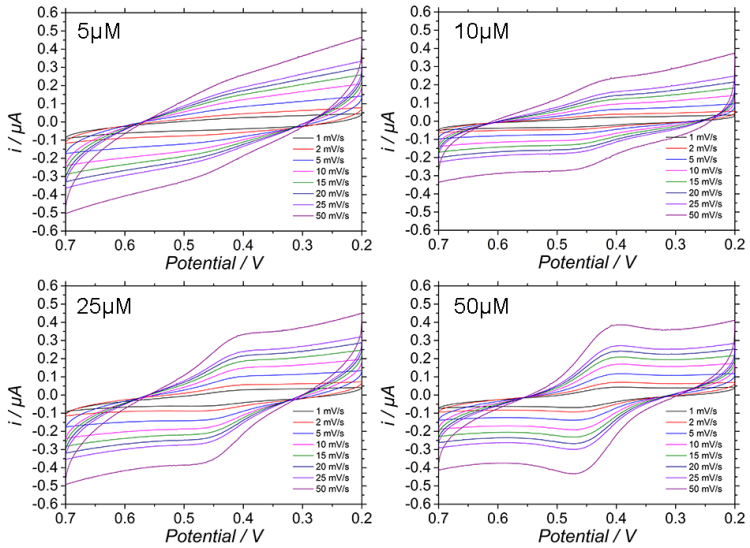

Figure S11.Cyclic voltammograms (1−50 mVs^{-1}) of Fc-DNA (a1) strand with different concentration.

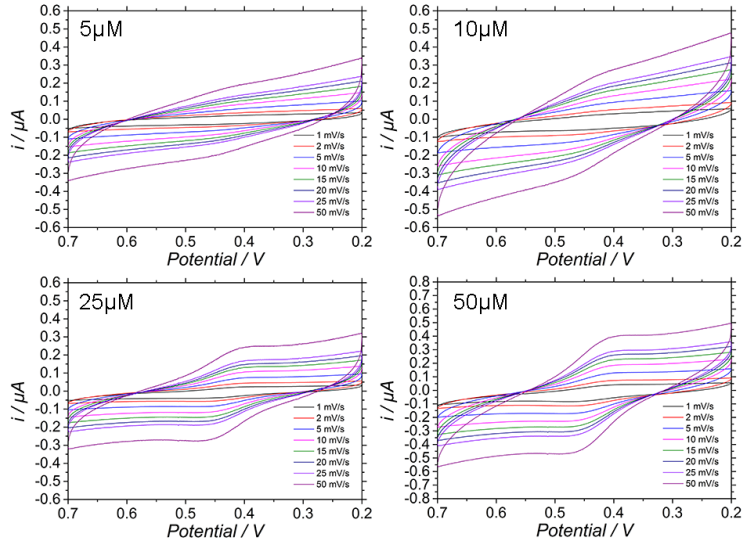

Figure S12.Cyclic voltammograms (1−50 mVs^{-1}) of Fc-DNA (a2) strand with different concentration.

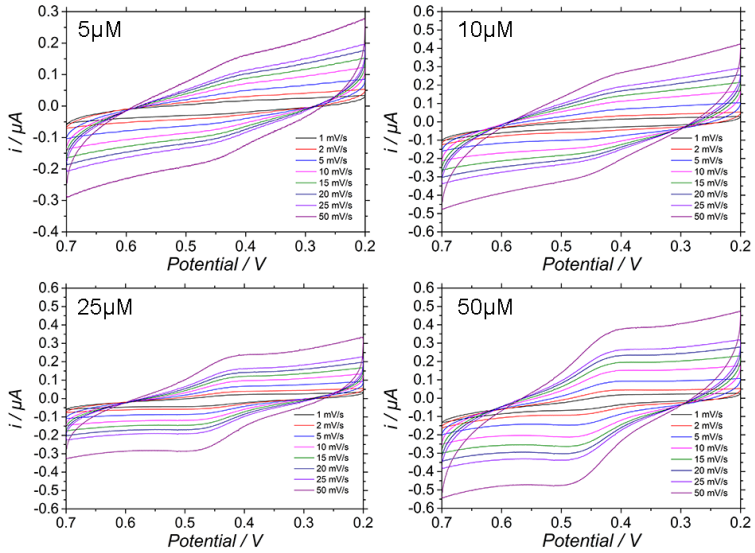

Figure S13.Cyclic voltammograms (1−50 mVs^{-1}) of Fc-DNA (a3) strand with different concentration.

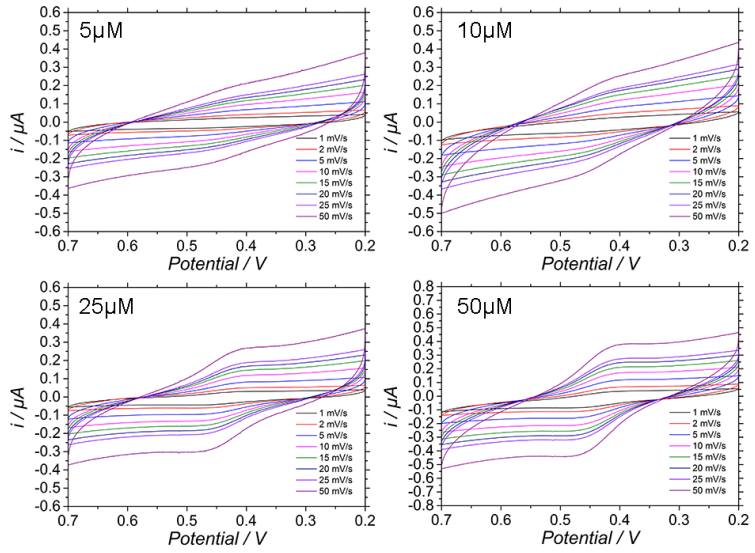

Figure S14.Cyclic voltammograms (1−50 mVs^{-1}) of Fc-DNA (a4) strand with different concentration.

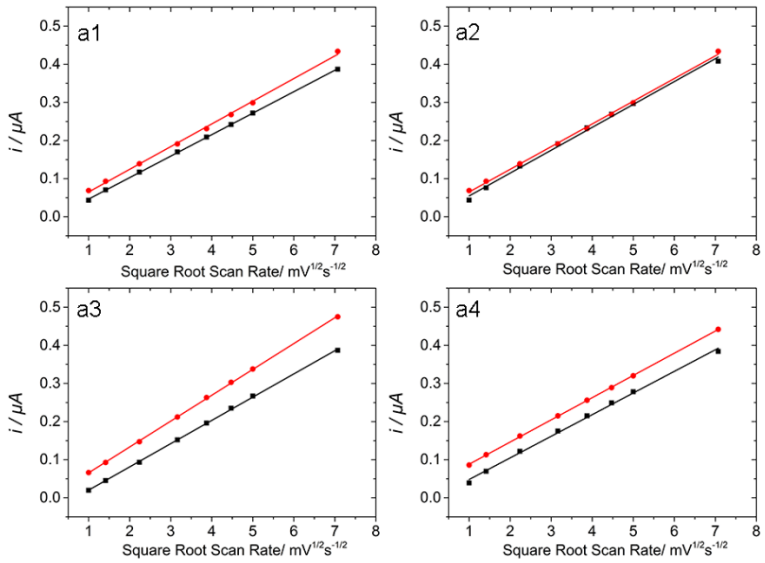

Figure S15.Scan rate dependence of anodic (black) and cathodic (red) peaks of cyclic voltammograms for Fc-DNAs (50 μM). The linear relationship between the current and square root of the scan rate indicates a reversible process where the redox species remains in solution.

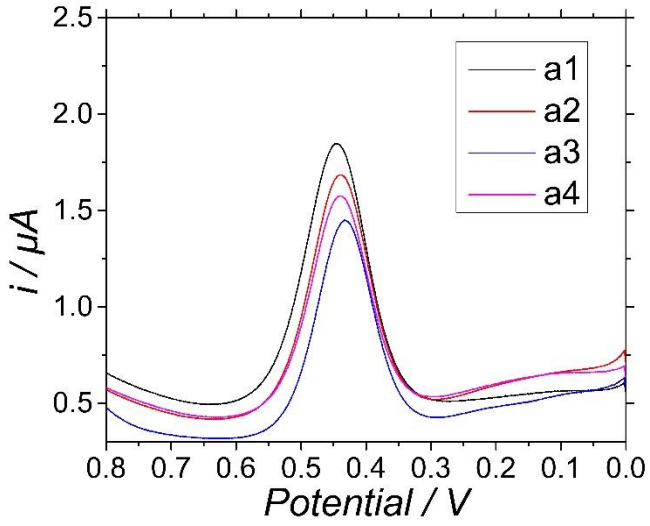

Figure S16.Squarewave voltammograms for four Fe-DNA strands. [DNA]=100 μM at 20mVs−1 in pH-6.8 buffer (50mM Tris-HCl, 50mM NaCl, 50mM MgCl2).

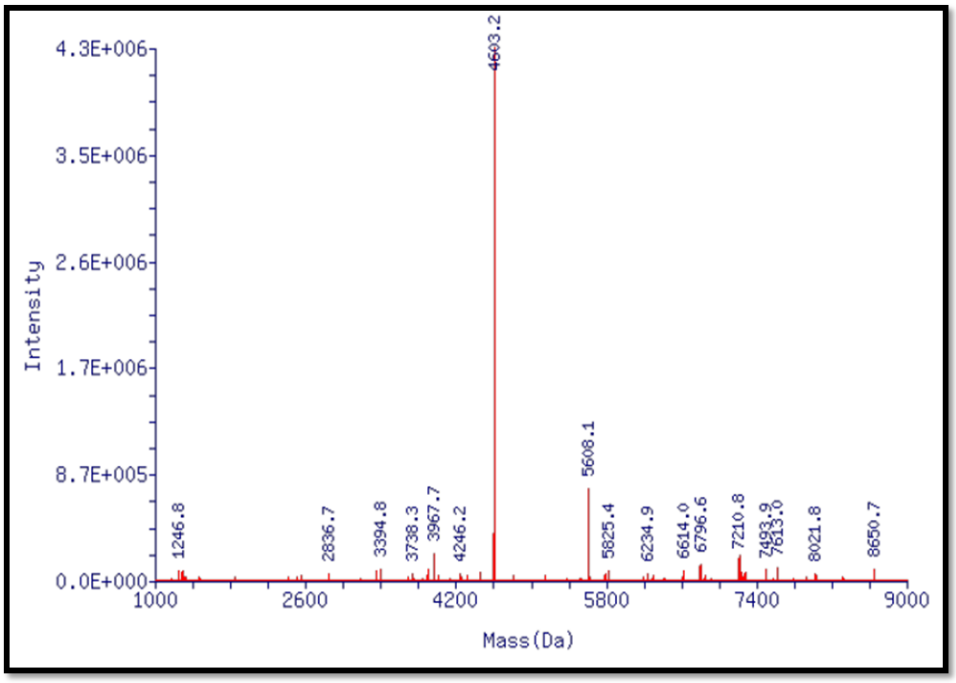

Figure S17. MALDITOF Analysis of a1 . Calculated molecular weight was 4603.0, and observed DNA peak was 4603.2 .

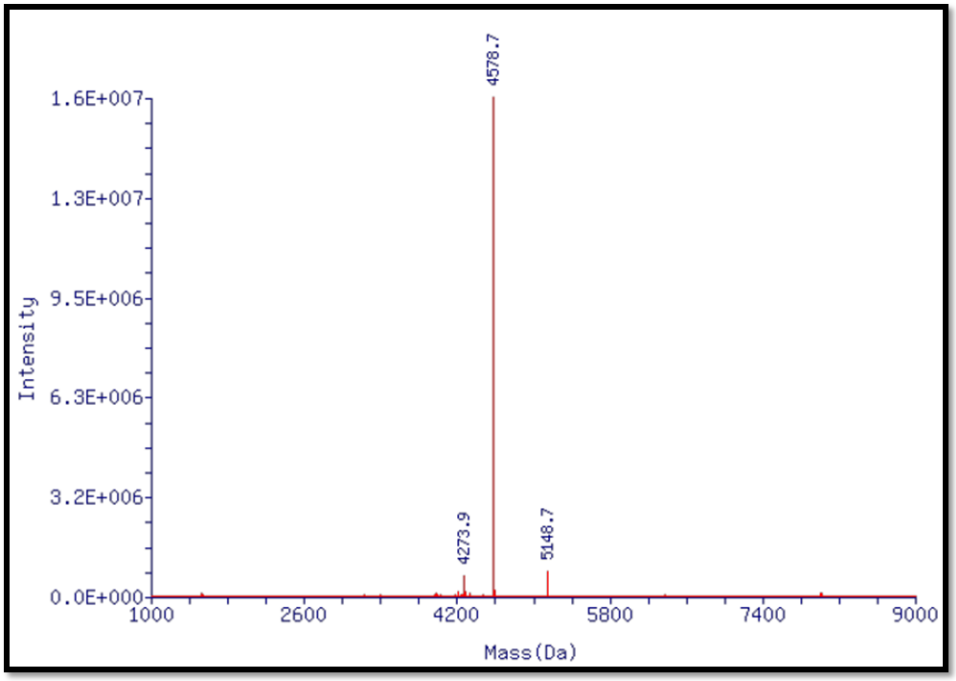

Figure S18. MALDITOF Analysis of a2. Calculated molecular weight was 4578.0, and observed DNA peak was 4578.7.

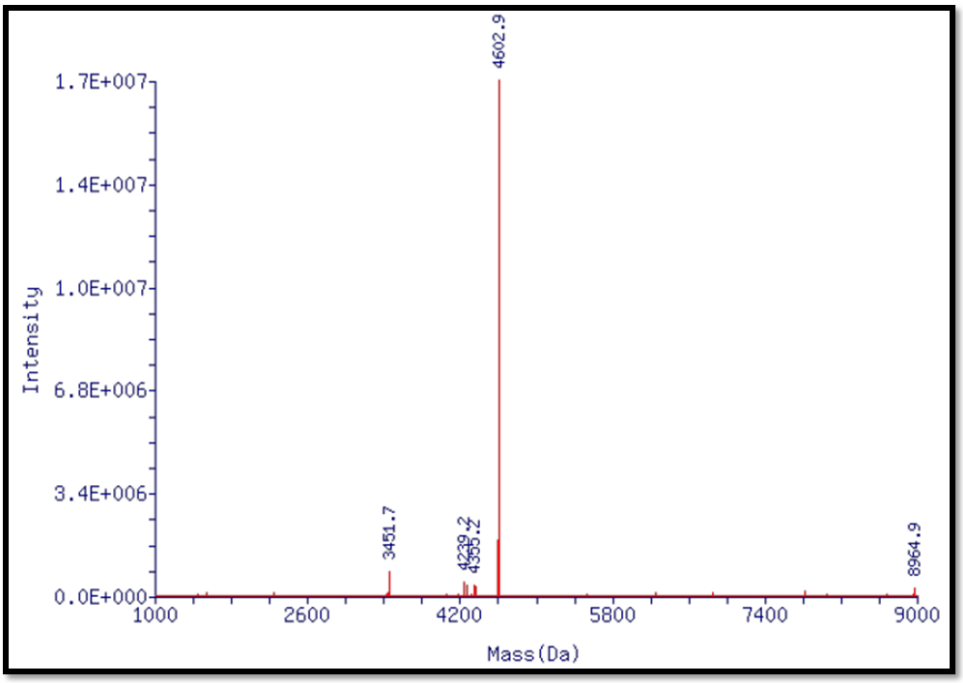

Figure S19. MALDITOF Analysis of a3. Calculated molecular weight was 4603.0, and observed DNA peak was 4602.9 .

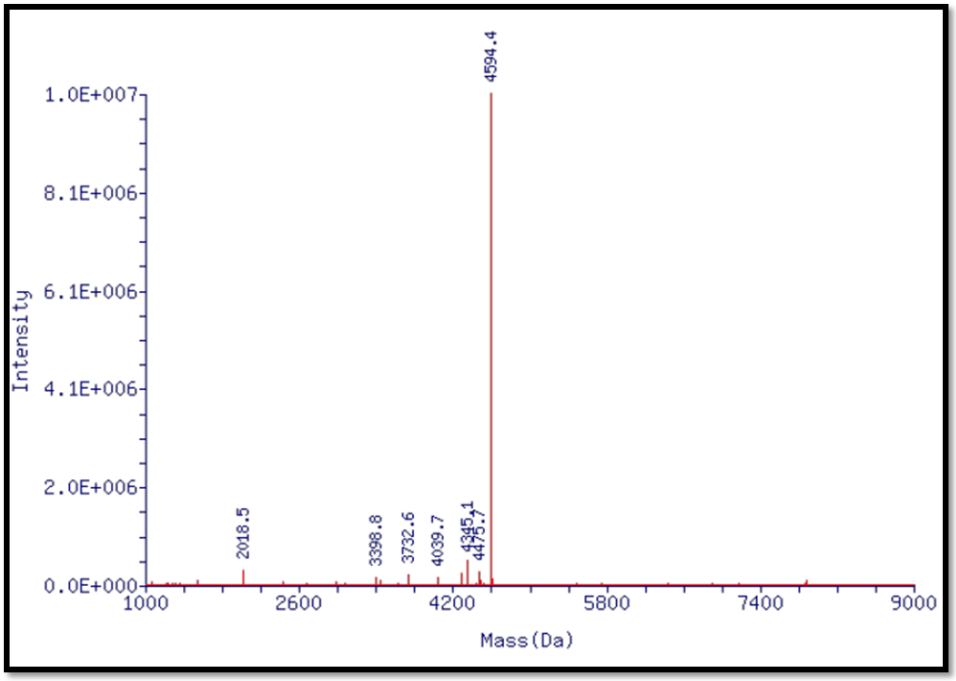

Figure S20. MALDITOF Analysis of a4. Calculated molecular weight was 4594.0, and observed DNA peak was 4594.4 .

References

[1] Jean-Louis H. A. Duprey.; James Carr-Smith,; Sarah L. Horswell,; Jarosław Kowalski,; James H. R. Tucker. J. Am. Chem. Soc. 2016, 138, 746−749

[2] Ruowen Wang.; Cheng Jin.; Xiaoyan Zhu.; Liyi Zhou.; Wenjing Xuan.; Yuan Liu.; Qiaoling Liu.; Weihong Tan. J. Am. Chem. Soc., 2017, 139, 9104–9107.