

Tunable PET process by the intercalation of cationic styryl dye in DNA base pairs and its application as turn-on fluorescent sensor for Ag⁺

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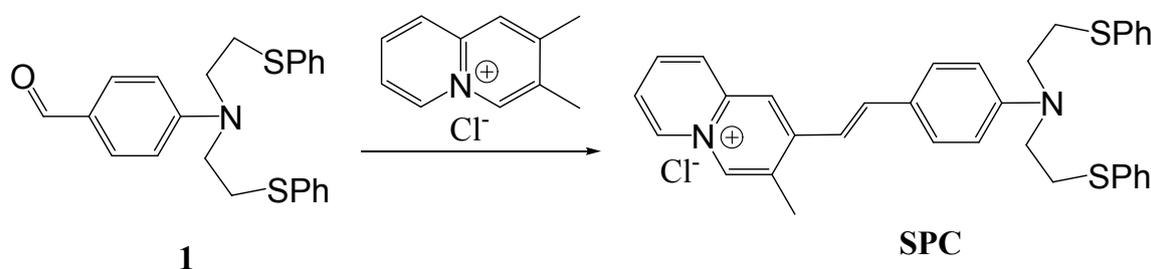
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Materials and methods

1. Instruments

All solvents and reagents (analytical grade and spectroscopic grade) were obtained commercially and used as received unless otherwise mentioned. Calf thymus DNA and fish sperm DNA were purchased from Beijing DingGuo Biotech Co. Ltd. The DNA concentration per nucleotide was determined by absorption spectroscopy by using the molar absorption coefficient ($\epsilon = 6600 \text{ M}^{-1} \text{ cm}^{-1}$) at 260 nm. NMR spectra were recorded on a Varian Mercury Vx-300 at 300 (¹H NMR), Bruker spectrometer at 400 (¹H NMR) MHz and 100 (¹³C NMR) MHz. Chemical shifts (δ values) were reported in ppm down field from internal Me₄Si (¹H and ¹³C NMR). High-resolution mass spectra (HRMS) were acquired on an Agilent 6510 Q-TOF LC/MS instrument (Agilent Technologies, Palo Alto, CA) equipped with an electrospray ionization (ESI) source. Elemental analyses were performed on a Vanio-EL elemental analyzer (Analysensystem GmbH, Germany). UV absorption spectra were recorded on a UV-2550 UV-VIS spectrophotometer (Shimadzu, Japan). Fluorescence measurements were performed using an F-4600 fluorescence spectrophotometer (Hitachi, Japan) equipped with a quartz cell (1 cm × 1 cm). Melting points were recorded on a RY-2 Melting Point Analyzer (Analytical Instrument Factory, Tianjin) and are uncorrected.

2. Synthesis of 2-(4-*N,N*-bis(2'-phenylthioethyl)aminostyryl)-3-methyldehydropyridocolinium chloride SPC



Synthesis of the cationic styryl dye SPC.

4-*N,N*-bis(2'-phenylthioethyl)aminobenzaldehyde **1** was prepared according to a reported procedure.^{S1}

To a 25 mL flask, was charged 2,3-dimethyldehydroquinolinium chloride (200 mg, 0.1 mmol), 4-*N,N*-bis(2'-phenylthioethyl)aminobenzoaldehyde **1** (390 mg, 0.1 mmol), acetonitrile (5 mL), piperidine (10 μ L). The reaction mixture was stirred for 72 h at 85 $^{\circ}$ C. After cooling to room temperature, **SPC** was crystallized from the solution. The product was filtered and washed with acetonitrile (2 mL \times 2). The product was recrystallized from methanol to afforded pure **SPC** as red powder in 38% yield (217 mg); mp: 242 $^{\circ}$ C-244 $^{\circ}$ C. HRMS: m/z $[M-Cl]^+$ = 533.2086; Calcd: 533.2085; 1H NMR (300 MHz, DMSO- d_6 , ppm): 9.17 (s, 1H), 9.03 (d, J = 6.9 Hz, 1H), 8.79 (s, 1H), 8.27 (d, J = 8.7 Hz, 1H), 8.12 (t, J = 7.8 Hz, 1H), 7.82 (d, J = 6.6 Hz, 1H), 7.75 (d, J = 16.2 Hz, 1H), 7.57 (d, J = 8.4 Hz, 2H), 7.38-7.23 (m, 10H), 7.18 (d, 16.2 Hz, 1H), 6.59 (d, J = 8.4 Hz, 2H), 3.58 (t, J = 6.9 Hz, 4H), 3.17 (t, J = 6.9 Hz, 4H), 2.60 (s, 3H); ^{13}C NMR (100 MHz, DMSO- d_6 , ppm): 149.1, 147.5, 142.0, 140.4, 136.2, 135.8, 132.7, 130.9, 130.0, 129.9, 129.8, 129.7, 127.1, 127.0, 124.7, 122.7, 119.4, 116.2, 112.6, 50.8, 30.8, 17.7. Anal. Calcd for $C_{34}H_{33}ClN_2S_2 \cdot 2H_2O$: C 67.47; H 6.16; N 4.63; Found: C 67.28; H 6.29; N 4.57.

3. The fluorescence quantum yield

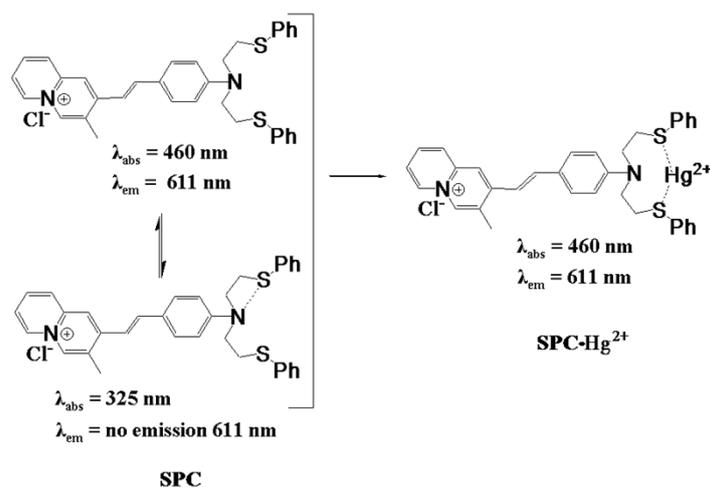
The fluorescence quantum yield of the sample (Φ_1) can be calculated according to the following equation:

$$\Phi_1 = \Phi_B \times \frac{Abs_B \times F_1 \times \lambda_{exB} \times \eta_1^2}{Abs_1 \times F_B \times \lambda_{ex1} \times \eta_B^2}$$

Where Φ_1 , Φ_B are the quantum yield of the sample and the standard; Abs_B , Abs_1 stand for the absorption in the excited wavelength; F_1 , F_B are the integration area; λ_{exB} , λ_{ex1} are the excited wavelength; η_1 , η_B are the refractive index.

When the wavelength at the intersection of two absorption curves of the standard and sample is chosen as the excitation wavelength of the standard and the sample. Here, we selected rhodamine B as the standard, and 507 nm as the excitation wavelength, so the equation is

$$\Phi_1 = \Phi_B \times \frac{F_1 \times \eta_1^2}{F_B \times \eta_B^2}$$



Scheme S1. Schematic representation of the intramolecular N...S interaction interconversions of **SPC** and **SPC·Hg²⁺** complex. Their UV/vis absorption and fluorescence emission characters are listed under the structures.

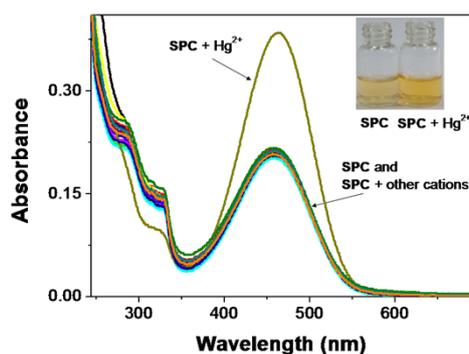


Figure S1. UV-vis spectra of sensor **SPC** (10 μ M) upon the addition of the nitrate salts (10.0 equivalents) of Ag^+ , Al^{3+} , Ca^{2+} , Cd^{2+} , Co^{2+} , Cr^{3+} , Cu^{2+} , Fe^{3+} , Hg^{2+} , K^+ , Mg^{2+} , Na^+ , NH_4^+ , Ni^{2+} , Pb^{2+} , and Zn^{2+} in EtOH/H₂O (1:1, v/v). Inset: photograph of the colour change of **SPC** upon the addition of Hg^{2+} (10 equivalents).

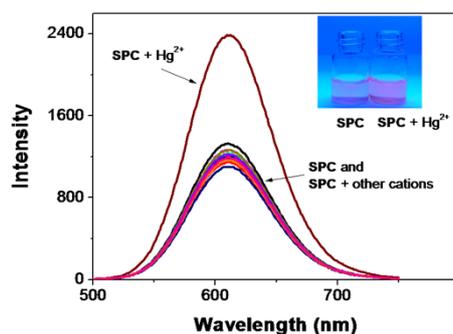


Figure S2. Fluorescence spectra of **SPC** upon the addition of the nitrate salts (10.0 equivalent) of Ag^+ , Al^{3+} , Ca^{2+} , Cd^{2+} , Co^{2+} , Cr^{3+} , Cu^{2+} , Fe^{3+} , Hg^{2+} , K^+ , Mg^{2+} , Na^+ , NH_4^+ , Ni^{2+} , Pb^{2+} , and Zn^{2+} in EtOH/H₂O (1:1, v/v). Excitation wavelength $\lambda_{\text{ex}} = 450$ nm, slit 2.5, 5.0. Inset: photograph of the fluorescence change under UV 365 nm excitation of **SPC** upon the addition of Hg^{2+} (10 equivalents).

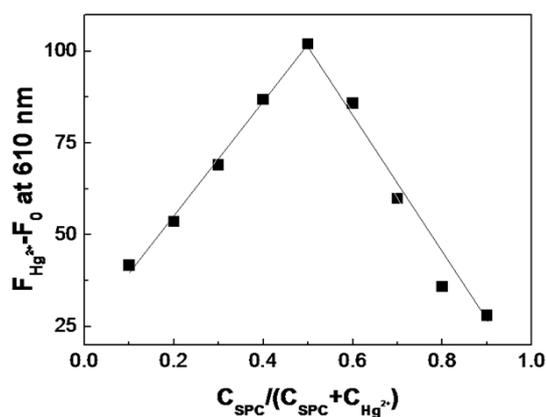


Figure S3. Job's plot of SPC in EtOH/H₂O (1:1, v/v) showing the 1:1 stoichiometry of the complex between Hg(NO₃)₂ and SPC. The total of the sensor SPC and Hg(NO₃)₂ is 10 μM. $\lambda_{\text{ex}} = 450$ nm, slit 2.5, 5.0.

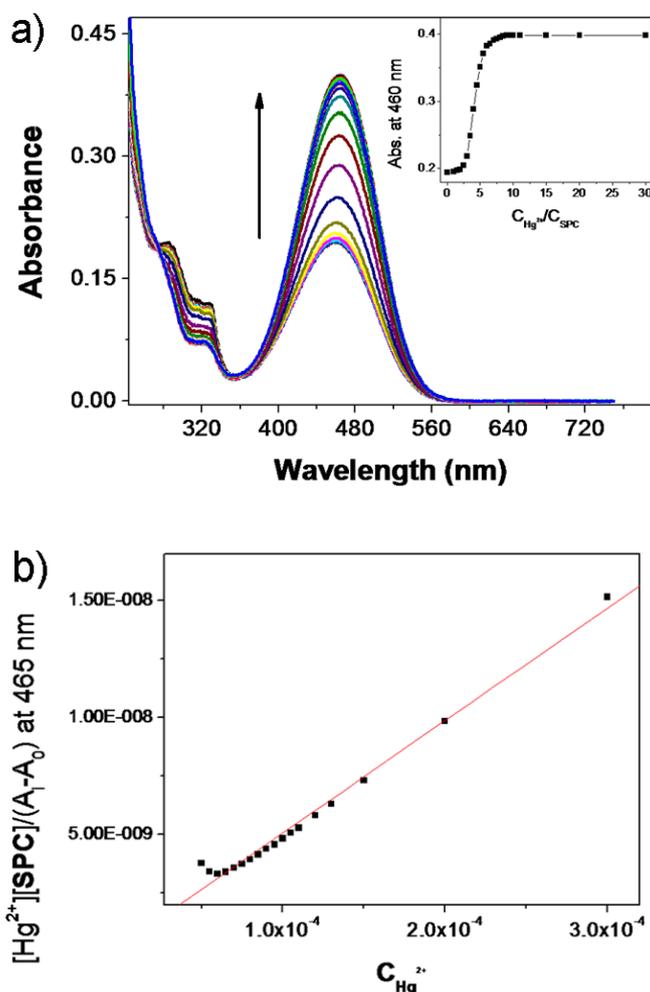


Figure S4. a) UV-vis titration spectra of SPC (10 μM) with increasing amounts of Hg(NO₃)₂ (0-30 equiv) in EtOH/H₂O (1:1, v/v). Inset: the absorbance changes of SPC at 465 nm as a function of the Hg²⁺ concentration. b) Benesi-Hildebrand data from UV/vis titrations (5-30 equivalents) for the SPC and Hg²⁺ complex. The bonding association constant is calculated as $1.83 \times 10^5 \text{ M}^{-1}$ ($R = 0.992$).

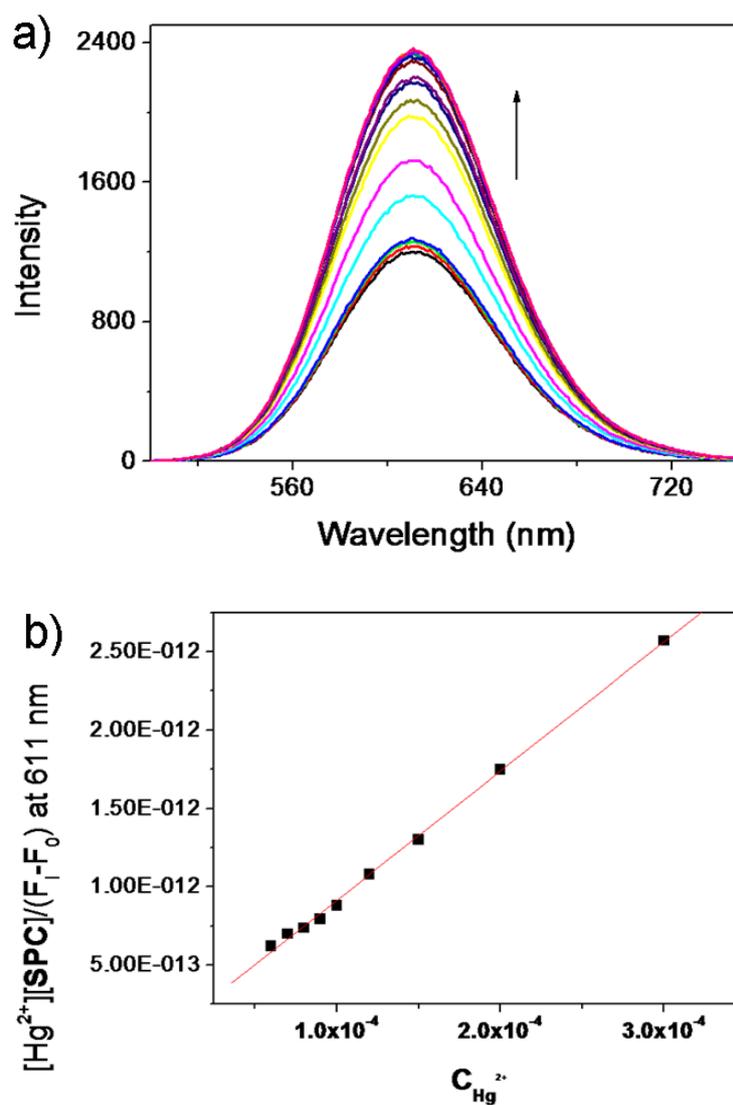


Figure S5. a) Fluorimetric titration of $\text{Hg}(\text{NO}_3)_2$ (0-30 equiv.) to SPC in EtOH/ H_2O (1:1, v/v). Excitation wavelength $\lambda_{\text{ex}} = 450$ nm, slit 2.5, 5.0. b) Benesi-Hildebrand data from fluorescence titrations (6-30 equivalents) for the SPC and Hg^{2+} complex. The bonding association constant is calculated as $9.6 \times 10^4 \text{ M}^{-1}$ ($R = 0.999$).

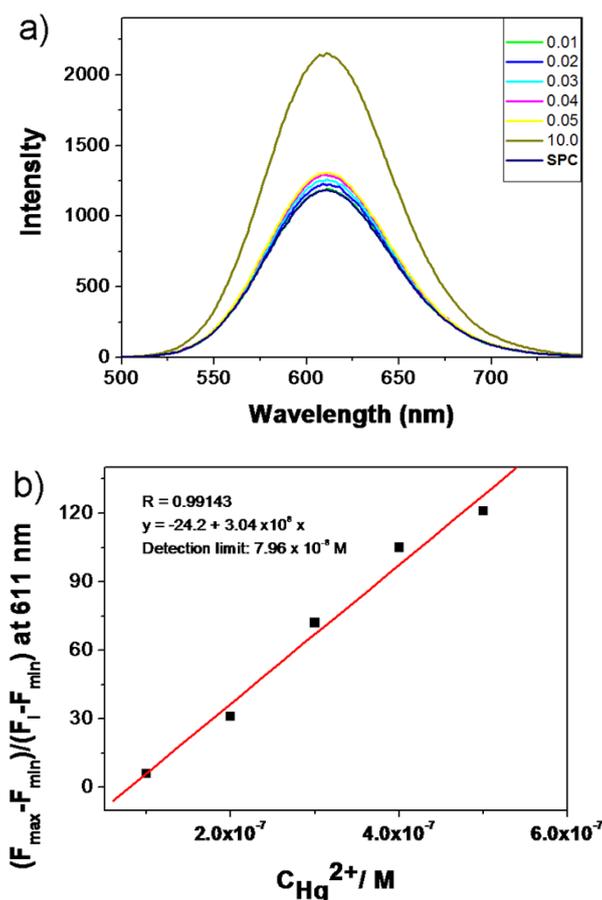


Figure S6. a) Emission of SPC at different concentrations of Hg^{2+} (0, 0.01, 0.02, 0.03, 0.04, 0.05 and 10 μM) added, $\lambda_{\text{ex}} = 450 \text{ nm}$, slit: 2.5, 5.0. b) Normalization of the emission intensities at 611 nm between the minimum emission (0.0 μM Hg^{2+}) and the emission at 10 μM Hg^{2+} . The detection limit was determined to be $7.96 \times 10^{-8} \text{ M}$ ($R = 0.991$).

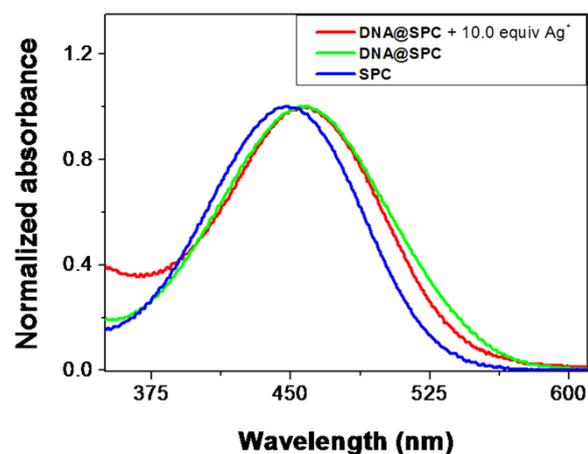


Figure S7. Normalized absorption spectra of SPC, DNA@SPC and DNA@SPC with 10.0 equivalents of Ag^+ in water. From photoelectric equation $E_g = h\nu = 1240/\lambda$, the electric energy level of SPC ($\lambda_{\text{max}} = 458 \text{ nm}$) is 2.8118 eV; the electric energy level of DNA@SPC ($\lambda_{\text{max}} = 460 \text{ nm}$) is 2.6956 eV and the electric energy level of DNA@SPC with 10.0 equivalents of Ag^+ ($\lambda_{\text{max}} = 460 \text{ nm}$) is 2.6956 eV. Therefore, the electric energy level of SPC is decreased by 0.1162 eV upon intercalation of SPC in DNA.

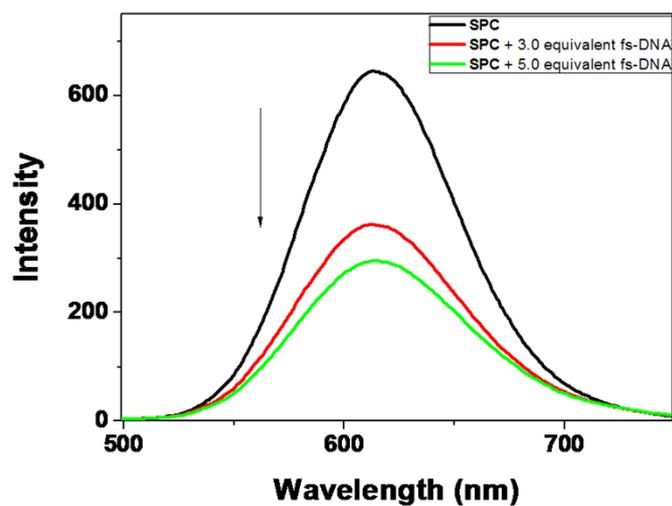


Figure S8. Fluorescence spectra of **SPC** (1.0×10^{-5} M) upon the addition of fish sperm DNA (fs-DNA) in water, $[\text{DNA}]/[\text{SPC}] = 0, 3.0, 5.0$. Excitation wavelength $\lambda_{\text{ex}} = 455$ nm, slit 5.0, 5.0 nm.

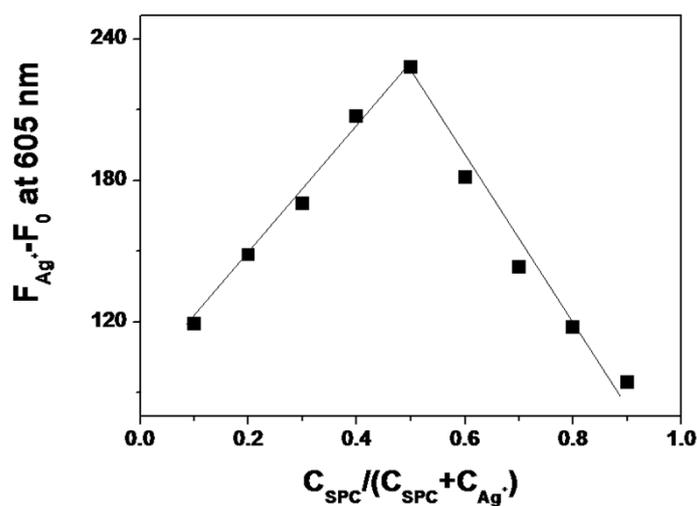


Figure S9. Job's plot of the **DNA@SPC** intercalates formed by **SPC** (1.0×10^{-5} M) and **ct-DNA** (5.0×10^{-5} M) in H_2O showing the 1:1 stoichiometry of the complex between AgNO_3 and **SPC**. The total of the sensor **SPC** and AgNO_3 is $10 \mu\text{M}$. $\lambda_{\text{ex}} = 455$ nm, slit 5.0, 5.0.

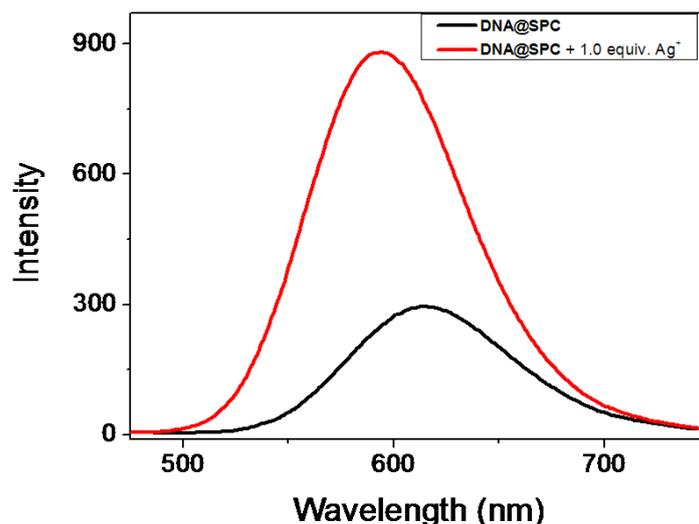


Figure S10. Fluorescence spectra of the DNA@SPC intercalates formed by fish sperm DNA and SPC upon the addition of 1.0 equivalents of Ag⁺ in H₂O. Excitation wavelength $\lambda_{\text{ex}} = 455$ nm, slit 5.0, 5.0 nm.

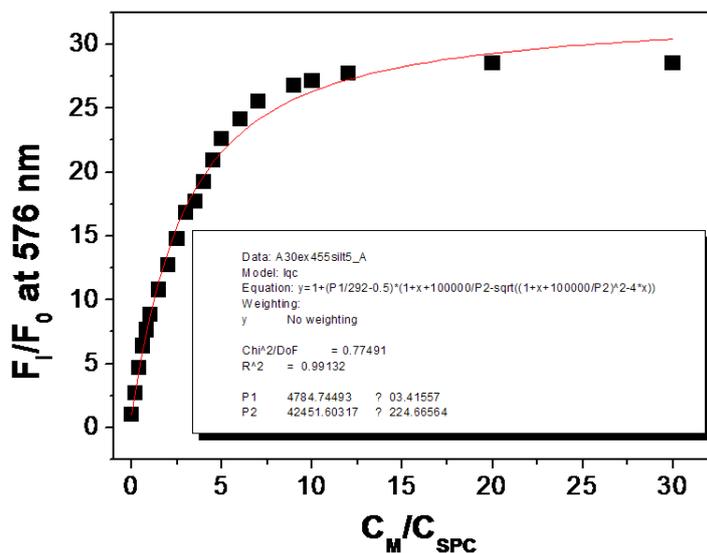


Figure S11. A nonlinear least-square analysis of DNA@SPC and Ag⁺ cation based on the SPC and Ag⁺ concentrations in a 1:1 complex. The nonlinear curve fitness based on 1:1 complex expression:^{S2}

$$F/F_0 = 1 + (F_{\text{max}}/2F_0 - 1/2) \{1 + C_M/C_L + 1/K_S C_L - [(1 + C_M/C_L + 1/K_S C_L)^2 - 4C_M/C_L]^{1/2}\}$$

where F and F₀ are the fluorescence intensity of SPC in the presence and absence of Ag⁺, C_M and C_L are the concentrations of Ag⁺ and SPC (10 μM); K_S is the stability constant.

^{S2} (a) K. A. Connors, *Binding Constants, the Measurement of Molecular Complex Stability*; John Wiley & Sons: New York, 1987. (b) B. Valeur, *Molecular Fluorescence Principles and Applications*; Wiley-VCH Verlag GmbH: New York, 2001.

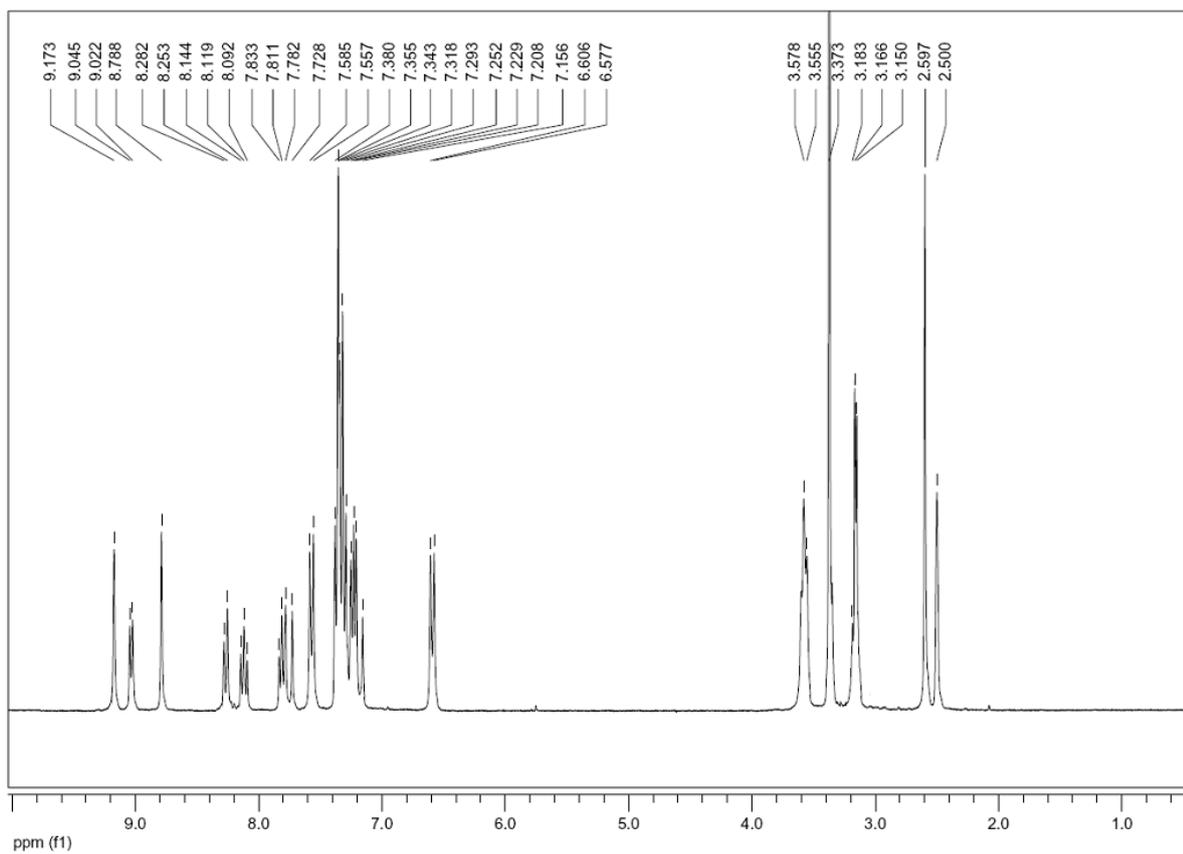


Figure S12. ^1H NMR of SPC (300 MHz, DMSO-d_6).

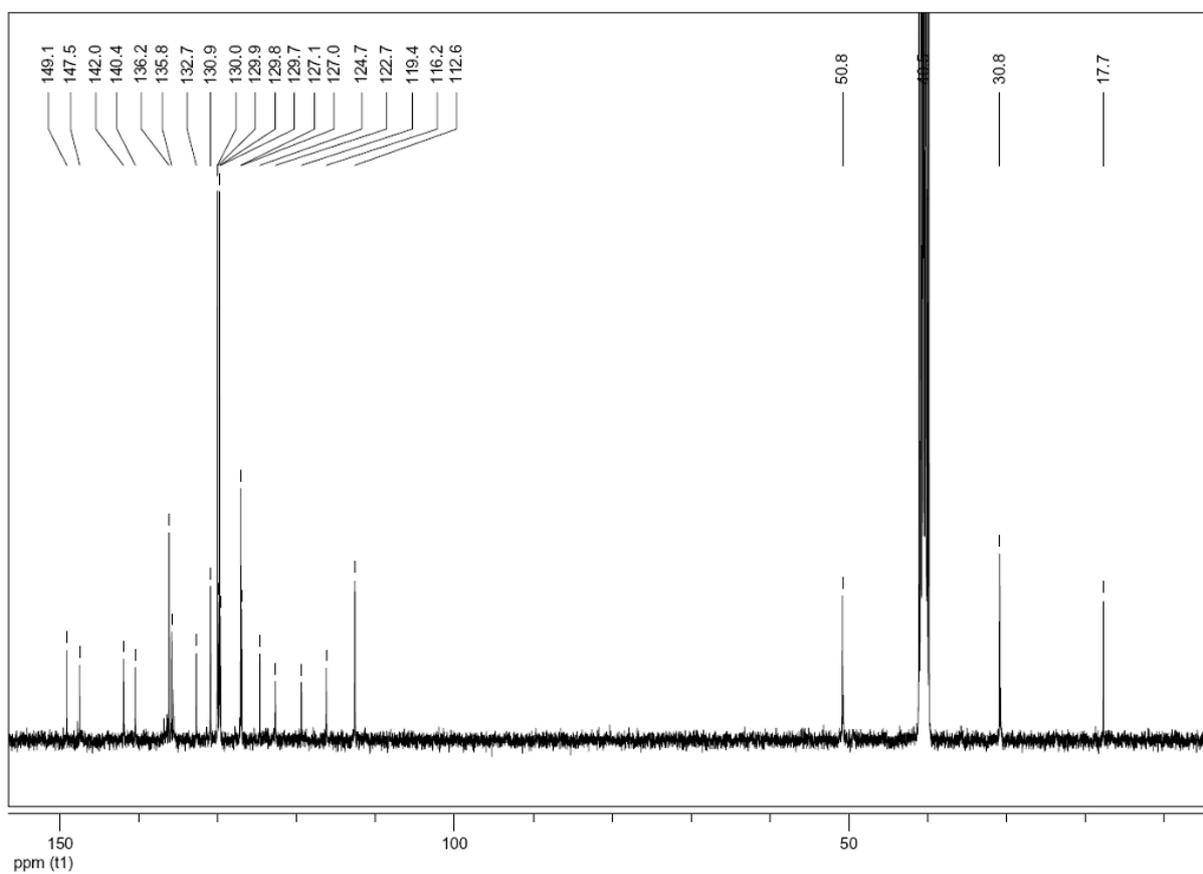


Figure S13. ^{13}C NMR of SPC (100 MHz, DMSO-d_6).

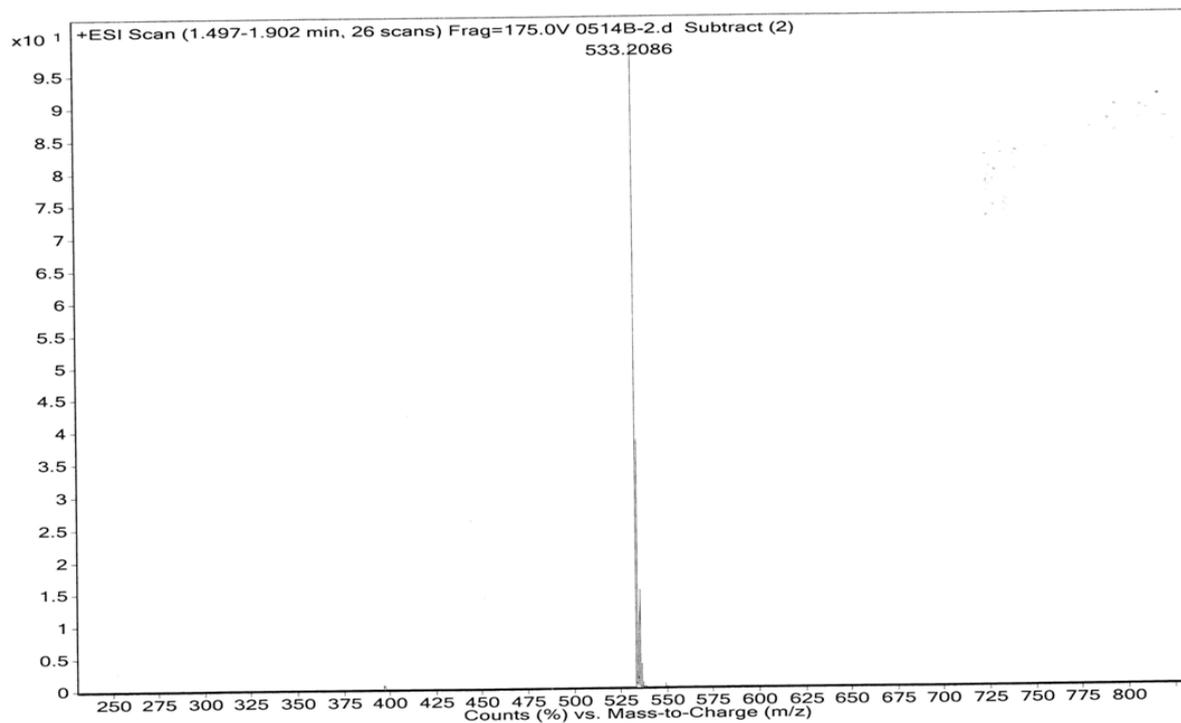


Figure S14. HRMS (LC/MS) spectra of SPC. The peak at $m/z = 533.2086$ was assigned to the mass of $[\text{SPC}-\text{Cl}]^+$.