Supplementary Material (ESI) for Chemical Communications

Electronic Supporting Information

A Selective G-quadruplex-Based Luminescent Switch-on Probe for the Detection of Nanomolar Silver(I) Ion in Aqueous Solution

Bradley Man,‡[a] Daniel Shiu-Hin Chan,‡[ab] Si-Wo Ang,[b] Fang Yang,[b] Hui Yang,[a] Siu-Cheong Yan,[b] Chung-Hang Leung*[b] and Dik-Lung Ma*[a]

[a]Department of Chemistry, Hong Kong Baptist University, Kowloon Tong, Hong Kong (China). E-mail: edmondma@hkbu.edu.hk
[b]Department of Chemistry and Open Laboratory of Chemical Biology of the Institute of Molecular Technology for Drug Discovery and Synthesis, The University of Hong Kong, Pokfulam Road, Hong Kong. Email: duncanl@hku.hk.
‡These authors contributed equally to this work.
**D.-L. Ma and C.-H. Leung contributed equally to this work.*

Experimental section

Materials. Calf thymus DNA (ct DNA) was purchased from Sigma Aldrich and purified by literature methods.[1a] The DNA per base pair concentration was determined by UV/Vis absorption spectroscopy using the following molar extinction coefficients at the indicated wavelengths: calf thymus DNA ε_{260} 13200 bp cm^{-1} M^{-1}.[1b] DNA oligomers were obtained from Tech Dragon Limited (Hong Kong). The sequences of the oligomers are:

C_{32} = [5′- CTCTCTTCTCTTCATTTTTCAACACAACACAC-3′]
C_{42} = [5′- AGGGACGGGACCCCCCCCCAAAACCGCCCGCCTGTGGAGGGT -3′]

The G-quadruplex structure was prepared by incubating C_{42} in Tris/KCl buffer, which was heated to 95 °C for 10 min and slowly cooled to room temperature. Unless otherwise stated, DNA binding experiments were performed in Tris buffer solution (10 mM Tris, 100 mM KCl, pH 7.5) at 20 °C. All chemicals used for the synthesis of complex **1** were purchased from Sigma Aldrich and used as received. 2-Phenyl-1,10-phenanthroline was synthesized using literature methods.[2] Stock solutions (10 mM) of **1** were prepared in DMF and further dilutions to designated concentrations were made using deionized water.

Physical measurement. Absorption spectra were recorded on a Perkin-Elmer Lambda 19 UV/Vis spectrometer or a Cary 300 UV/Vis spectrometer. Emission spectra were recorded on a SPEX Fluorolog-2 Model fluorescence spectrophotometer. Emission spectra and lifetime measurements for complex **1** were performed on a PTI TimeMaster C720 Spectrometer (Nitrogen laser: pulse output 337 nm) fitted with a 380 nm filter. Error limits were estimated: λ (±1 nm); τ (±10%); ϕ (±10%). All solvents used for the emission and lifetime measurements were degassed using three cycles of freeze-vac-thaw.

^{1}H NMR spectra were recorded on a Bruker DPX400 NMR spectrometer operating at 400 MHz (^{1}H) and 100 MHz (^{13}C). ^{1}H and ^{13}C chemical shifts were internally referenced to solvent shifts (^{1}H DMSO-d_6 δ 2.50 ppm; ^{13}C DMSO-d_6, δ 39.5 ppm). High resolution mass spectra were recorded using a QSTAR XL Hybrid LC/MS/MS System. Circular dichroism (CD) spectra were measured on a JASCO-815 spectrometer.

Spectroscopic titration. A solution of the platinum(II) complex **1** (50 μM) was prepared in Tris buffer solution (10 mM Tris-HCl, pH 7.5). Aliquots of a millimolar ct DNA

solution were added. Absorption spectra were recorded in the 260–580 nm range, after equilibrium at 25 °C for 10 min. The intrinsic binding constant, K, was determined from a plot of $D/\Delta\varepsilon_{ap}$ vs D according to equation (1):[2]

$$D/\Delta\varepsilon_{ap} = D/\Delta\varepsilon + 1/(\Delta\varepsilon \times K) \qquad (1)$$

where D is defined as the concentration of DNA, $\Delta\varepsilon_{ap} = |\varepsilon_A - \varepsilon_F|$, $\varepsilon_A = A_{obs}/[\text{complex}]$, and $\Delta\varepsilon = |\varepsilon_B - \varepsilon_F|$; ε_B and ε_F correspond to the extinction coefficients of DNA–complex adduct and unbound complex, respectively.

Gel mobility shift assay. A 123-bp DNA ladder (50 μM) was incubated with platinum complex **1** (50, 250 μM), ethidium bromide (250 μM) or Hoechst 33342 (250 μM) at 37 °C for 30 min, and the mixtures were analysed by gel electrophoresis (Pharmacia Biotech GNA-200 submarine unit with Power Pac 300 power supply, Bio-Rad) using a 1% (w/v) agarose gel and 1× Tris-acetate-EDTA (TAE) buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0).

Cytotoxicity test (MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-tetrazolium bromide) assay). CCD cells were seeded in a 96-well flat-bottomed microplate at 8000 cells/well in 100 μL of minimal essential medium (MEM-Eagle, Sigma) containing 10% fetal bovine serum (Invitrogen) and 1% antibiotic and antimycotic Solution (Sigma). Complex **1** and cisplatin (positive control) were dissolved in DMSO and mixed with the growth medium (final DMSO concentration ≤ 4%). Serial dilutions of each complex were added to each well. The microplate was incubated at 37 °C, 5% CO_2, 95% air in a humidified incubator for 72 h. After incubation, 10 μL MTT reagent (5 mg/mL) was added to each well. The microplate was re-incubated at 37 °C in 5% CO_2 for 4 h. Solubilization solution (10% SDS, 0.01 M HCl) (100 μL) was added to each well. The microplate was further incubated for 18 h. The absorbance at 570 nm was measured using a microplate reader. The IC_{50} values of **1** (concentration required to reduce the absorbance by 50% compared to the controls) were determined by the dose-dependence of surviving cells after exposure to the metal complex for 72 h.

Emission measurement. Solutions of the platinum(II) complex **1** (1.0 μM) with C_{32} or C_{42} oligonucleotide (0.1 μM) were prepared in Tris buffer (10 mM Tris-HCl, 2 mM KCl, pH 7.5). Aliquots of a millimolar stock $AgNO_3$ solution (0–20.0 μM) or various metal ions (2 μM) were then added. Emission spectra were recorded in the 350−700 nm range, after equilibration at 20.0 °C for 1 h. Excitation wavelength = 330 nm.

Luminescence quantum yields were determined using the method of Demas and Crosby[5] $[Ru(bpy)_3][PF_6]_2$ in degassed acetonitrile as a standard reference solution (Φ_r = 0.062) and calculated according to the following equation:

$$\Phi_s = \Phi_r(B_r/B_s)(n_s/n_r)^2(D_s/D_r)$$

where the subscripts s and r refer to sample and reference standard solution respectively, n is the refractive index of the solvents, D is the integrated intensity, and Φ is the luminescence quantum yield. The quantity B was calculated by $B = 1 - 10^{-AL}$, where A is the absorbance at the excitation wavelength and L is the optical path length.

Synthesis of chloro(2-phenyl-1,10-phenanthroline)platinum(II) (1). A mixture of K_2PtCl_4 (207 mg, 0.50 mmol) and 2-phenyl-1,10-phenanthroline (128 mg, 0.50 mmol) in MeCN–H_2O (1:1, 20 mL) was heated at 100 °C allowing the MeCN to slowly evaporate. The precipitate was collected by filtration and washed with water to yield complex **1** as an orange solid (79 mg, 0.16 mmol, 32 %). MS (FAB): *m/z* 485 $[M]^+$, 450 $[M - Cl]^+$. HRMS (ESI) for

$[PtL(CH_3CN)]^+$: calcd. 491.0835, found 491.0954. ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ 8.85 (d, J = 4.8 Hz, 1H), 8.71 (d, J = 8.2 Hz, 1H), 8.50 (d, J = 8.6 Hz, 1H), 8.04–7.93 (m, 4H), 7.56 (d, J = 7.1 Hz, 1H), 7.39 (d, J = 7.4 Hz, 1H), 7.09–6.87 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 8.85 (d, J = 4.8 Hz, 1H), 8.71 (d, J = 8.2 Hz, 1H), 8.50 (d, J = 8.6 Hz, 1H), 8.04–7.93 (m, 4H), 7.56 (d, J = 7.1 Hz, 1H), 7.39 (d, J = 7.4 Hz, 1H), 7.09–6.87 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 164.9, 148.3, 147.2, 142.2, 138.7, 138.3, 137.8, 134.8, 131.4, 131.2, 129.3, 127.8, 127.4, 126.9, 125.8, 126.3, 124.3, 119.3. UV-vis (CH_3CN, 298 K): [λ/nm (ε_{max}/dm^3 mol^{-1} cm^{-1}): 249 (1.5×10^4) and 304 (1.1×10^4)].

References

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Fig. S1 UV-vis spectra of **1** (50 μM) in Tris buffer solution with ct DNA at 20 ^{o}C. Inset: plot of $D/\Delta\varepsilon_{ap}$ vs D. Absorbance was monitored at 330 nm.

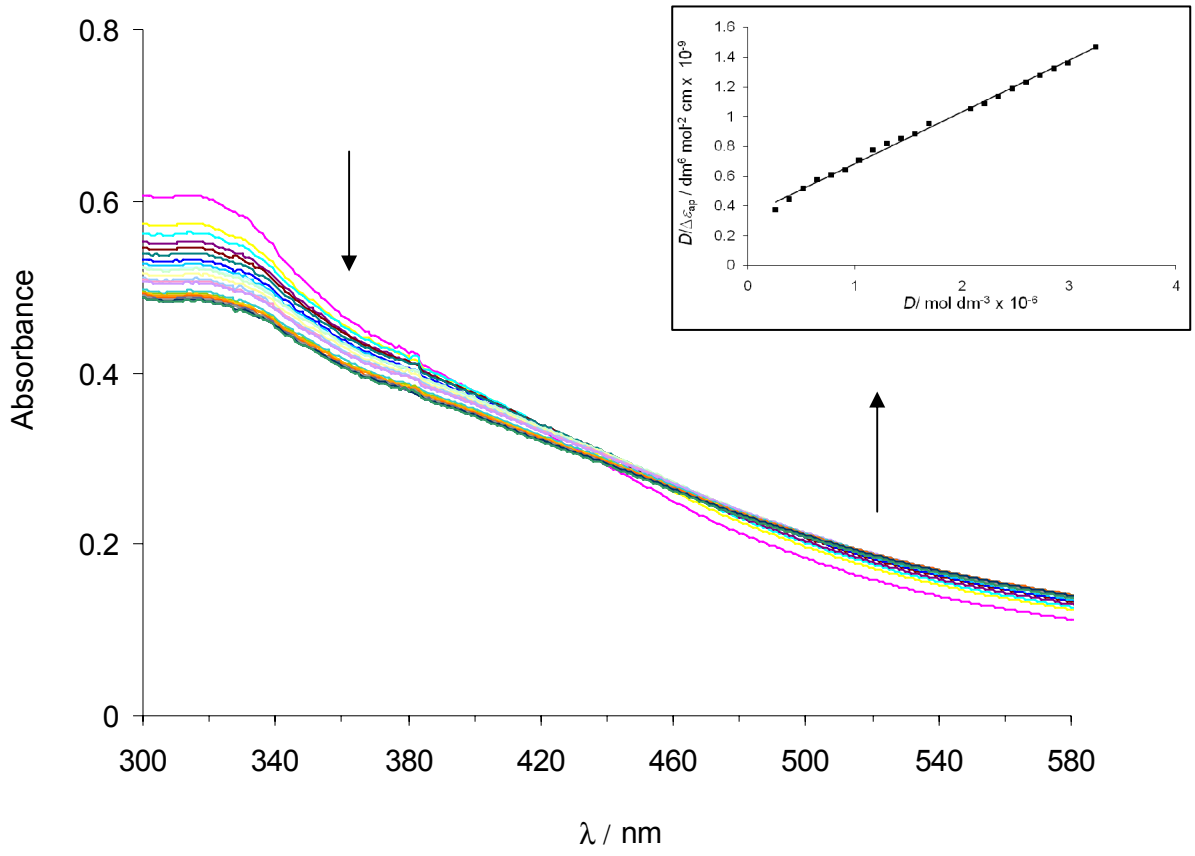


Fig. S2 Gel electrophoresis of a 123-bp DNA ladder in 1% (w/v) agarose gel showing the mobility of DNA. Lanes A and F contain DNA (50 μM) only. Lanes B–E contain DNA (50 μM) in the presence of ethidium bromide (250 μM, lane B), Hoechst 33342 (250 μM, lane C), or platinum(II) complex **1** (250 μM, lane D or 50 μM, lane E).

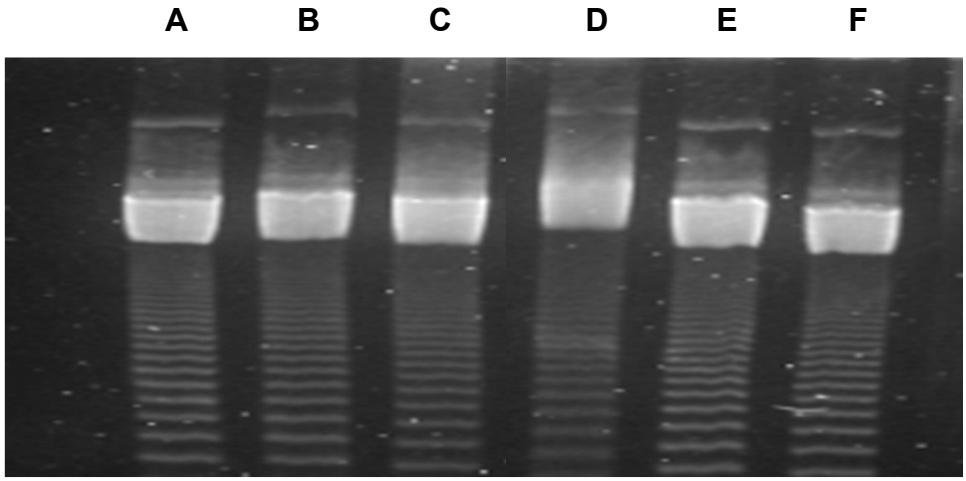


Fig. S3 Emission spectral traces of **1** (1.0 μM) and C_{32} (0.1 μM) upon addition of Ag^+ (0–5.0 μM) in Tris-HCl buffer (10 mM, pH 7.5) containing KCl (2 mM). Inset: Phosphorescence intensity at 560 nm *vs.* Ag^+ concentration.

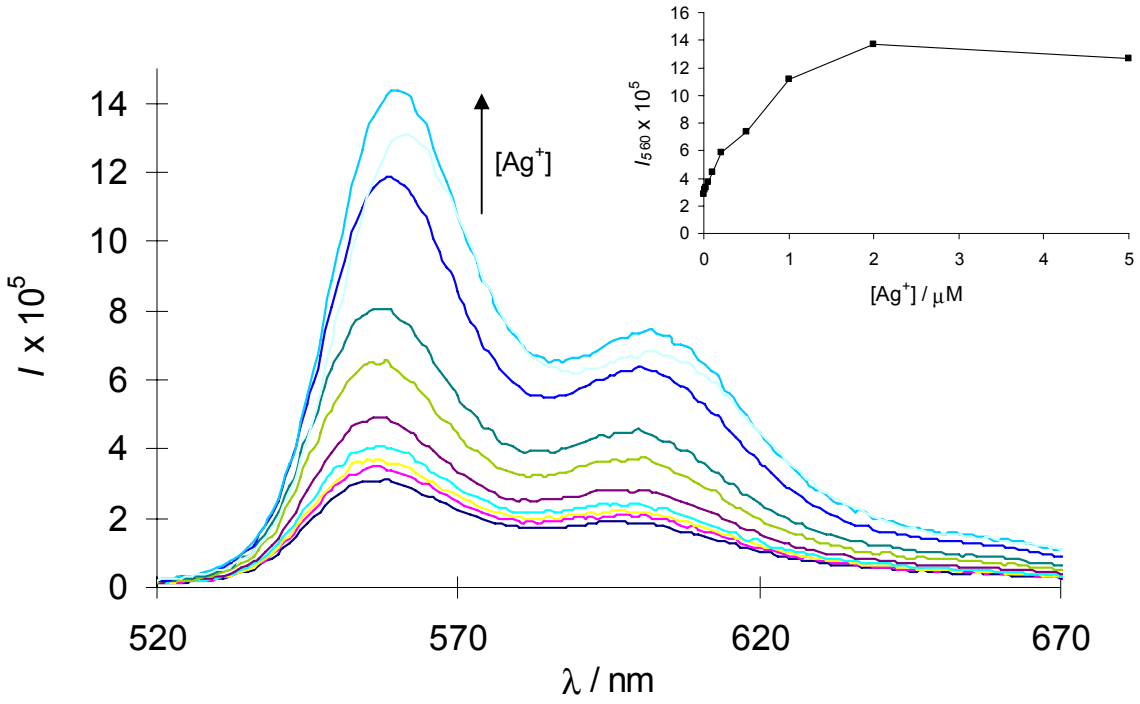


Fig. S4 CD spectrum of C_{42} (5 μM) at 25 °C at $[Ag^+]$/[DNA] ratios of 0, 0.2, 4, 20 and 40 in in Tris buffer (10 mM, pH 7.5) containing KCl (2 mM).

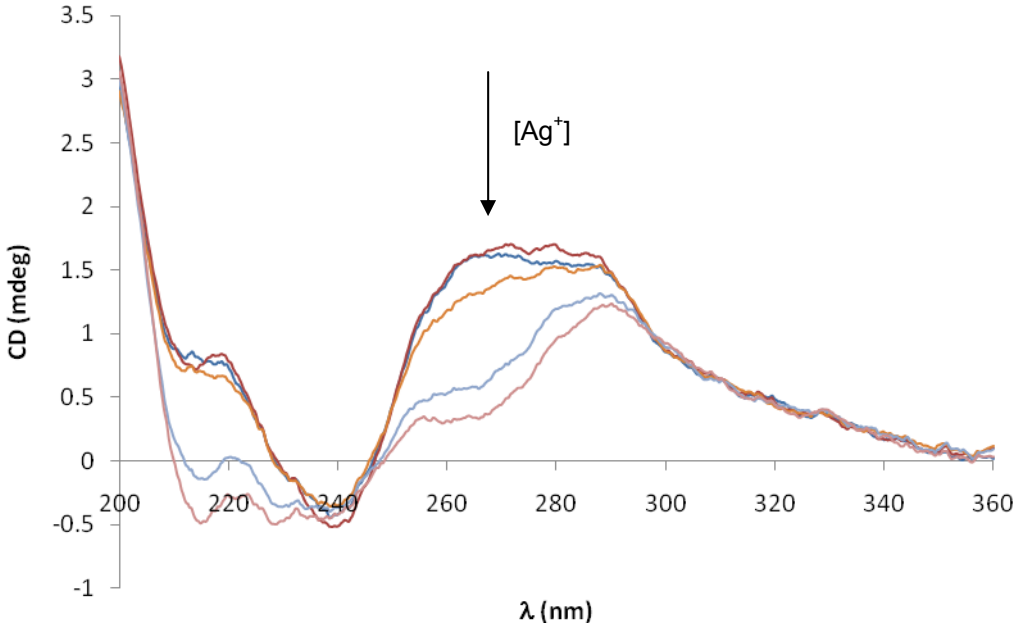


Fig. S5 Emission spectral traces of **1** (1.0 μM) and C_{42} (0.1 μM) upon addition of Ag^+ (20 nM) in Tris buffer (10 mM, pH 7.5) containing KCl (2 mM), showing a signal-to-noise ratio greater than 3.

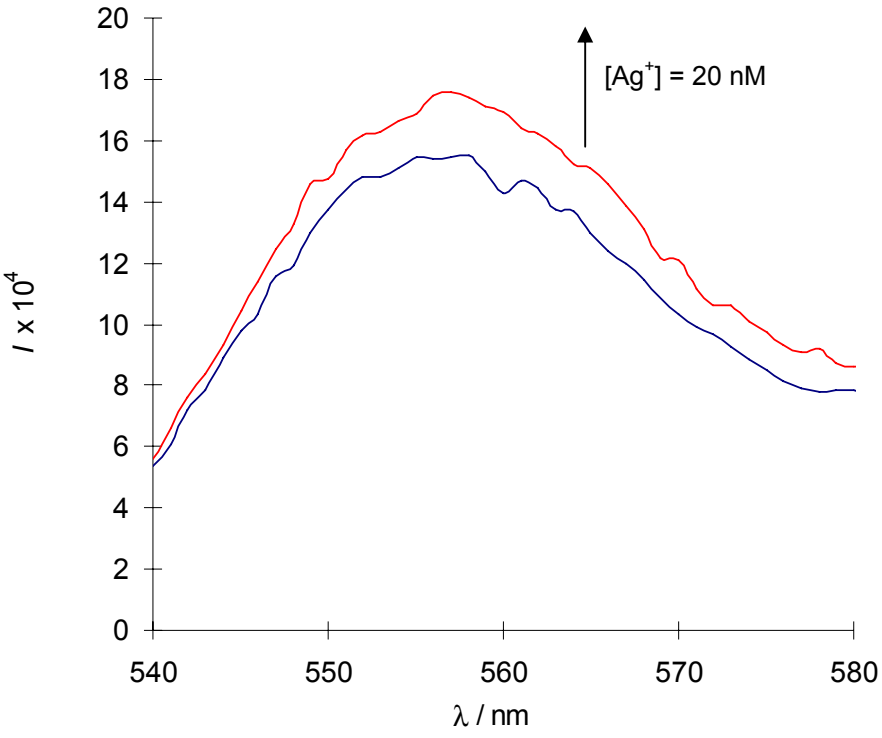
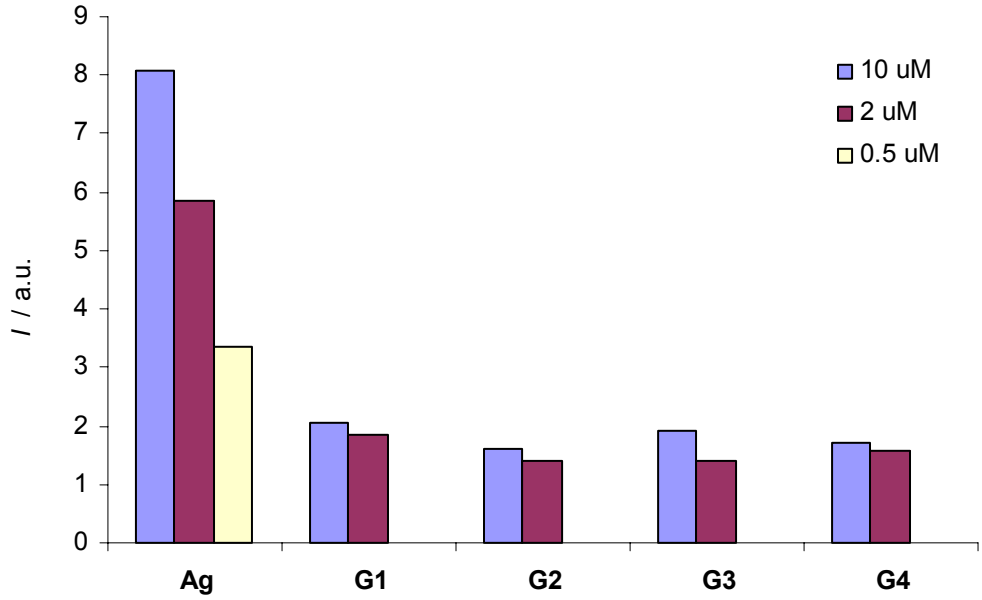


Fig. S6 Comparative emission response of **1** (1.0 μM) and C_{42} (0.1 μM) upon addition of Ag^+ or various other metal ions in Tris buffer (10 mM, pH 7.5) containing KCl (2 mM), demonstrating selectivity for Ag^+. Group 1: Hg^{2+}, Cu^{2+}, Mn^{2+}; group 2: Na^+, NH_4^+; group 3: Ca^{2+}, Mg^{2+}, Ba^{2+}; group 4: Cd^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}.



Scheme S1. Schematic representation of the function of an Ag^+ probe that operates based on modulation of the secondary structure of oligonucleotide C_{32} by Ag^+ ions allowing intercalation of **1,** resulting in a strong luminescence emission.

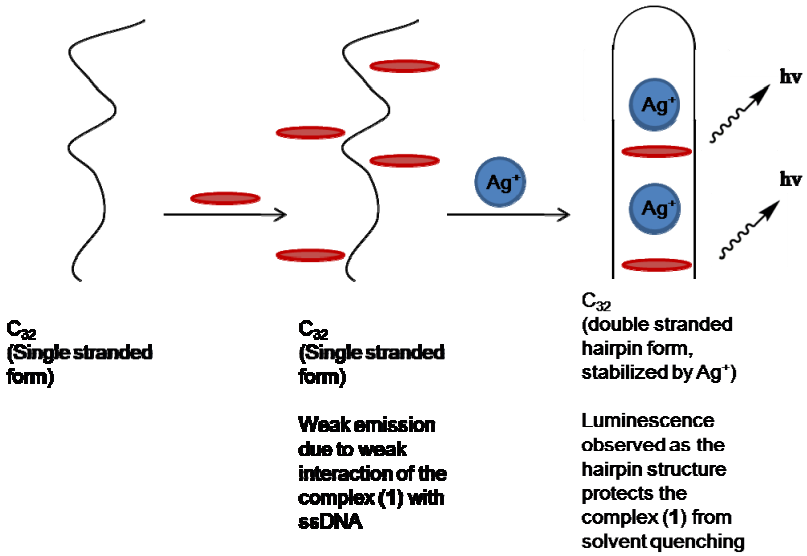


Table S1. Spectral properties of the chloro(2-phenyl-1,10-phenanthroline)platinum(II) (**1**) recorded in CH_3CN at 25 °C.

Complex	UV-vis λ / nm ($dm^3\ mol^{-1}\ cm^{-1}$)	Emission λ / nm ($\tau/\mu s$, ϕ_{em})
1	249 (1.5×10^4) 304 (1.1×10^4)	570 (6.9, 0.074)