Supporting Information

A luminescent cyclometalated gold(III)-avidin conjugate with a long-lived emissive excited state that binds to proteins and DNA and possesses anti-proliferation capacity

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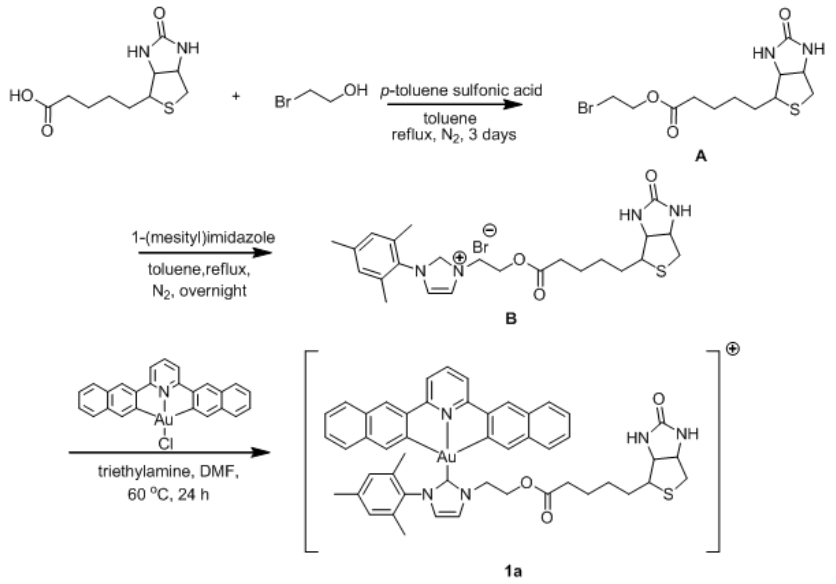
Materials and Methods

Chemicals, proteins, and double-stranded DNAs were purchased from Sigma Chemical Co. Ltd. Single-stranded DNAs were purchased from Integrated DNA Technologies. All solvents for reaction and photophysical studies were of HPLC grade. Flash column chromatography was performed using silica gel 60 (230-400 mesh ASTM) with chloroform/acetonitrile as eluent. ^{1}H NMR spectra were recorded on a Avance DPX300 or AV400 spectrometer. Chemical shifts (ppm) were referenced to tetramethylsilane (for $CDCl_3$) or non-deuterated solvent residual. Mass spectra were measured using a DFS High Resolution Magnetic Sector MS, Finnigan LCQ Classic mass spectrometers or Bruker ultraflex II MALDI-TOF/TOF MS. Absorption spectra were recorded on a Perkin-Elmer Lambda 900 UV-visible spectrophotometer. Emission spectra were recorded on a SPEX Fluorolog-3 Model fluorescence spectrophotometer. Solutions for photophysical studies were degassed by using a high vacuum line in a two-compartment cell with five freeze-pump-thaw cycles. The emission quantum yield was measured with $[Ru(bpy)_3](PF_6)_2$ (Φ = 0.062) as reference. Emission lifetime measurements were performed with a Quanta Ray DCR-3 pulsed Nd:YAG laser system (pulse output 355 nm, 8 ns). Error limits were estimated: λ(± 1 nm); τ (± 10%); Φ (± 10%). The emission spectra of **1a** or conjugate **1** in the presence of proteins or DNAs were recorded on an LP920-KS Laser Flash Photolysis Spectrometer (Edinburgh Instruments Ltd., Livingston, UK) with 500 ns time delay. The excitation source was the 355 nm output (third harmonic) of a Nd:YAG laser (Spectra-Physics Quanta-Ray Lab-130 Pulsed Nd:YAG Laser). The emission of samples was detected by a Hamamatsu R928 photomultiplier tube and the signals were processed by a PC plug-in controller with L900 software. Dynamic light scattering experiments were conducted using Zetasizer 3000HSA. Elemental analyses were performed at the Institute of Chemistry of the Chinese Academy of Sciences, Beijing. Optical and fluorescence micrographs of cells were taken on a Zeiss Axiovert 200M inverted fluorescence microscopy. Confocal fluorescence micrographs were examined in a Carl Zeiss LSM510 META (Germany) laser scanning confocal microscope with a Plan Apochromat 63x 1.4NA oil immersion objective. Transmission electron microscopy (TEM) experiment was taken on a CM 100 transmission electron microscope.

Compound Characterization and Experimental Procedure

The synthetic route of the biotin-linked N-heterocyclic carbene ligand (**B**) and **1a** is as follows:

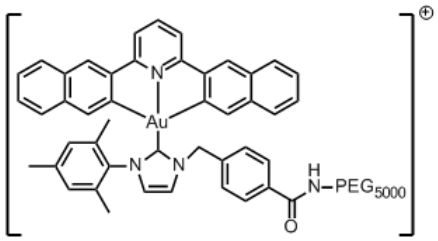


Synthesis of A. To a mixture of biotin (1.03 g, 4.22 mmol) and 2-bromoethanol (5.23 g, 0.042 mol) in 25 mL toluene, p-toluene sulfonic acid (72.6 mg, 0.42 mmol) was added and the reaction mixture was refluxed under nitrogen for 72 h. After cooling to room temperature, solvent was removed by evaporation and the crude product collected was purified by column chromatography with a mixture of dichloromethane/methanol (9:1) as eluent. Yield: 1 g (68 %). MS (+ESI) *m*/*z*: 373.1 [M+Na$^+$]. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K): δ = 5.87 (s, 1 H), 5.29 (s, 1 H), 4.51 (m, 1 H), 4.38 (t, 2 H, J = 6.0 Hz), 4.32 (m, 1 H), 3.52 (t, 2 H, J = 6.0), 3.16 (m, 1 H), 2.92 (dd, 1 H), 2.75 (d, 1 H, J = 12.8), 2.39, (t, 2 H, J = 7.4), 1.72 – 1.42 (m, 6H).

Synthesis of B. A (0.644 g, 1.84 mmol) and 1-(mesityl)imidazole[1] (0.285 g, 1.53 mmol) was reflux in 35 mL toluene for 24h. The resulting reaction mixture was filtered and the residue collected was washed with excess toluene and diethyl ether. Yield: 0.588 g (60 %). MS (+ESI) *m*/*z*: 457.3 [M$^+$]. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K): δ = 9.42 (s, 1 H), 8.04 (s, 1 H), 7.76 (s, 1 H), 7.13 (s, 2 H), 4.58 (m, 2 H), 4.51 (m, 1 H), 4.32 (m, 1 H), 3.21 (m, 1 H), 2.93 (m, 1 H), 2.70 (d, 2 H, J = 12.5 Hz), 2.40 – 2.36 (m, 5 H), 2.10 (s, 6 H), 1.72 – 1.40 (m, 6H).

Synthesis of 1a. A mixture of gold precursor[2] (0.1 g, 0.178 mmol), **B** (0.115 g, 0.214 mmol), and triethylamine (0.036 g, 0.356 mmol) were stirred at 60 °C in 50 mL degassed DMF under argon for 24 h. After cooling to room temperature, lithium

trifluoromethanesulfonate (0.278 g, 1.78 mmol) was added and stirred for 1 h. The reaction mixture was concentrated to 5 mL by rotary evaporation and upon addition of 20 mL water into the mixture, crude product was precipitated out. The precipitated crude product was collected by filtration and purified by column chromatography with a mixture of chloroform/acetonitrile (1:1) as eluent. To ensure the counter anion to be a triflate ion, the collected product was re-dissolved in 20 mL of chloroform and extracted with 20 mL of aqueous LiOTf. The collected organic layer was dried and concentrated by rotary evaporation and pumping. Yield: 0.091 g (45%). MS (+FAB) *m*/*z*: 982.1 [M^+]. ^{1}H NMR (400 MHz, CD_3OD, 298 K): δ = 8.44 (s, 2 H), 8.26 – 8.11 (m, 4 H), 7.95 (m, 3 H), 7.79 (m, 4 H), 7.62 – 7.52 (m, 4 H), 6.85 (s, 2 H), 4.62 (m, 4 H), 4.49 (m, 1H), 4.22 (m, 1H), 3.05 (m, 1H), 2.89 (m, 1H), 2.70 (d, 1 H, J = 13.0 Hz), 2.23 (s, 6 H), 2.12 – 1.95 (m, 5 H), 1.65 – 1.35 (m, 6 H).Elemental analysis Calcd for $C_{50}H_{48}N_5O_6F_3S_2Au \cdot 3CHCl_3 \cdot 0.5H_2O$: C, 42.43; H, 3.49; N, 4.67. Found: C, 42.27; H, 3.55; N, 5.03.



1b

Synthesis of 1b. A mixture of gold precursor (0.036 g, 0.064 mmol), 1-(4-{methoxy poly(ethylene glycol) carbamoyl}-benzyl)-3-(2,4,6-trimethyl-phenyl)-3*H*-imidazol-1-ium; idodide[3] (molecular weight ~5200 Da, 0.4 g, 0.077 mmol), and potassium *tert*-butoxide (0.009 g, 0.096 mmol) were refluxed in 10 mL degassed DMF under argon for 24 h. After cooling to room temperature, lithium trifluoromethanesulfonate (0.1 g, 0.64 mmol) was added and stirred for 1 h. The reaction mixture was concentrated by rotary evaporation and purified by column chromatography with a mixture of chloroform/acetonitrile as eluent. Yield: 0.10 g (28%). MS (MALDI-TOF) *m*/*z*: 5669 [M^+]. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K): δ = 8.16 (s, 2 H), 7.88 – 7.73 (m, 8 H), 7.65 – 7.41 (m, 12 H), 6.71 (s, 2 H), 5.89 (s, 2 H), 3.80 – 3.45 (broad m, polymeric), 2.11 (s, 3 H), 2.10 (s, 6 H).

HABA assay

5 µM aliquots of **1a** (0.8 mM) were added to a mixture of HABA (160 µM) and avidin (4 µM) in 2 mL of PBS buffer solution at pH 7.4. Absorption spectra were recorded after equilibrating for 1 min per aliquot. The binding stoichiometry of **1a** to

avidin was determined by plotting $-\Delta abs_{500\ nm}$ versus [**1a**]:[avidin].

Emission titration experiment

3.75 μM aliquots of **1a** (0.8 mM) were added to a solution of avidin (4 μM) in 2 mL of PBS buffer at pH 7.4. Emission spectra were recorded after equilibrating for 1 min per aliquot.

Emission responses of 1a or conjugate 1 towards the presence of different analytes

The aliquots of the stock solutions of different analytes (refer to table S3) were added into 2 ml of PBS/DMSO (9:1) solutions (pH = 7.4) of **1a** or conjugate **1** (20 μM). The emission spectra were recorded after equilibrating for 5 min.

Emission responses of 1a or conjugate 1 at different pH

Solution of **1a** or conjugate **1** (20 μM) in 2 ml of PBS/DMSO (9:1) solutions (pH = 4, 6, 7.4, 8 or 10). The emission spectra of the solutions were recorded after equilibrating for 5 min.

Emission responses of a solution mixture of 1a and BSA towards the presence of different analytes

The aliquots of the stock solutions of different analytes (refer to table S3) were added into 2 ml of PBS/DMSO (9:1) solutions (pH 7.4) of **1a** (20 μM) and BSA (20 μM). The emission spectra were recorded after equilibrating for 5 min.

Emission responses of 1a and conjugate 1 towards proteins or DNAs

A solution of 20 μM of **1a** or conjugate **1** in PBS/DMSO (9:1) (pH 7.4) was added with aliquots of stock solutions of different proteins or DNAs and allowed for equilibration of 1 min before recording.

Absorption titration experiment[4]

A solution of 10 μM of **1a** in PBS/DMSO (9:1) was added with aliquots of stock solution of ctDNA (10 mM) and the absorption spectra were recorded after equilibrating for 1 min per aliquot. The binding constant was determined by applying the Scatchard equation:

$$[DNA]/\Delta\varepsilon_{ap} = [DNA]/\Delta\varepsilon + 1/(\Delta\varepsilon \times K_b),$$

where $\Delta\varepsilon_{ap} = |\varepsilon_A - \varepsilon_F|$ where $\varepsilon_A = A_{obs}$ /[complex], and $\Delta\varepsilon = |\varepsilon_B - \varepsilon_F|$ where ε_B and ε_F correspond to the extinction coefficients of the DNA-bound and –unbound complex, respectively. Plot of [DNA]/ $\Delta\varepsilon_{ap}$ versus [DNA] gave a slop equalling to $1/\Delta\varepsilon$ and a y

intercept equalling to $1/(\Delta\varepsilon \times K_b)$, and K was obtained from the ratio of the slop to the y-intercept.

Fluorescence quenching experiment[5]

A solution of 10 μM of BSA in PBS/DMSO (9:1) was added with aliquots of stock solution of **1a** (10 mM) and the emission spectra were recorded after equilibrating for 1 min per aliquot. The binding constant was determined by applying following equation:

$$\log[(I_0-I)/I] = \log K + n\log[Q]$$

where I_0 and I are the fluorescence intensity of BSA without and with **1a**, respectively; [Q] is **1a** concentration. The binding constant can be determined by plotting $\log[(I_0-I)/I]$ versus log[Q] which gave the y-intercept equalling to log K.

Cell imaging

HeLa cells (2×10^5) were grown on a 35 mm tissue culture dish with 2 mL culture medium and incubated at 37 ^oC under an atmosphere of 5% CO_2 for 24 h. The culture medium was removed and cells were washed with PBS for 3 times, and incubated with medium containing the conjugate **1** or **1a** at a concentration of 10 μM for 4 h. The cells were then washed with PBS for 3 times and imaged using a Carl Zeiss LSM510 META (Germany) laser scanning confocal microscope. For co-localization study of **1a** or conjugate **1** with Hoechst 33342, 10 μM of **1a**- or conjugate **1**-treated HeLa cells (4 h) were washed with PBS for 3 times and treated with medium 1 μM of Hoechst 33342 at 37 ^oC under an atmosphere of 5% CO_2 for 15 min. The medium was removed and cells were washed with PBS for 3 times before confocal microscopic examination.

Cytotoxicity evaluation

In general, the cancerous cells (cell density = 4000 cells/100 μL) were seeded in a 96-well, flat-bottomed microtiter plate in growth medium (100 μL) and incubated at 37 ℃ under a 5% CO_2 atmosphere for 24 h. Different concentrations of **1a** or conjugate **1** (prepared by pre-mixing **1a** and avidin for 30 min) were added and the plates were incubated for 48 h. After a total incubation time of 72 h, MTT in PBS solution (5 mg/mL, 10 μL) was added to each well. The plates were incubated at 37℃ under a 5% CO_2 atmosphere for another 4 h, followed by adding 100 μL of sodium dodecyl sulfate (SDS, 10 %) in diluted hydrochloric acid to each well. After placing the plates in dark at 37℃ for overnight, the percentage of survival cells were determined with a microtitre plate reader at 590 nm.

Hoechst 33342 nuclear staining assay

HeLa cells (1.5 x 10^5) were grown on a 35 mm tissue culture dish with 2 mL culture medium and incubated at 37 ^oC under an atmosphere of 5% CO_2 for 24 h. The culture medium was removed and cells were washed with PBS for 3 times, and incubated with medium containing the conjugate **1** or **1a** at a concentration of 4 μM for 24 h. After incubation, cells were stained with 1 μM of Hoechst for 15 min. The stained cells were observed with a Zeiss Axiovert 200M inverted fluorescence microscope.

Intracellular reactive oxygen species (ROS) measurement

Intracellular ROS triggered by conjugate **1** or **1a** in HeLa cells was examined using 5-(and-6-)-chloromethyl-2',7'-dichlorodihydrofluorescei diacetate (CM-H_2DCFDA) which will be oxidized by ROS to fluorescent oxidized form of CM-H_2DCFDA. HeLa cells (5 x 10^4) were grown on a 24-well plate with 500 μL culture medium and incubated at 37 ^oC under an atmosphere of 5% CO_2 for 24 h. The cells were then treated with various concentrations of conjugate **1** or **1a** (1, 2, 5, or 10 μM) for 4 h or 1.5 mM of hydrogen peroxide for 0.5 h (positive control). After incubation, the cells were washed with PBS for 3 times and labeled with CM-H_2DCFDA (10 μM) in medium for 45 min. Cells were then washed with PBS and lysed with 0.1% Triton X-100 (100 μL). The cell lysates were transferred to a dark-bottomed 96-well plate. The fluorescent intensity of the oxidized CM-H_2DCFDA was measured by a microtitre plate reader using excitation and emission wavelength at 485 nm and 530 nm, respectively. After eliminating the background signal from conjugate **1** or **1a**, intracellular production of ROS was determined by the relative fluorescence intensity of CM-H_2DCFDA compared with the untreated control.

Supporting Figures/Tables

Table S1. Electronic absorption spectral data of **1a** at 298 K.

Solvent	λ_{abs}/nm (ε/dm^3 mol^{-1} cm^{-1})
CH_2Cl_2	250 (65,850), 274 (55,420), 302 (39,370), 383 (17,545), 403 (27,545)
CH_3CN	249 (67,535), 272 (58,080), 299 (37,265), 378 (16,315), 396 (22,425)
MeOH	249 (66,585), 272 (56,370), 300 (37,155), 380 (15,945), 398 (22,355)

Figure S1. Electronic absorption spectrum of **1a** in CH_2Cl_2, CH_3CN, and MeOH at 298 K.

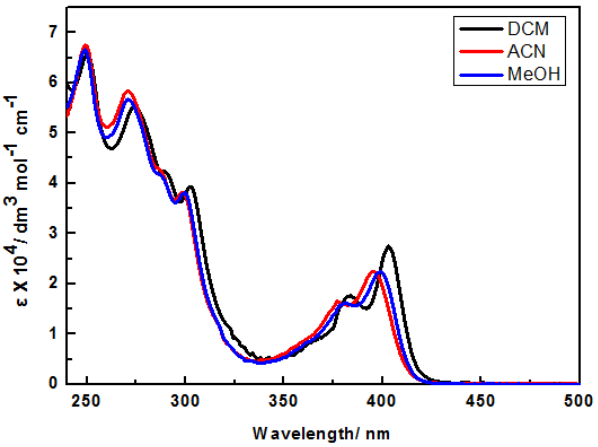
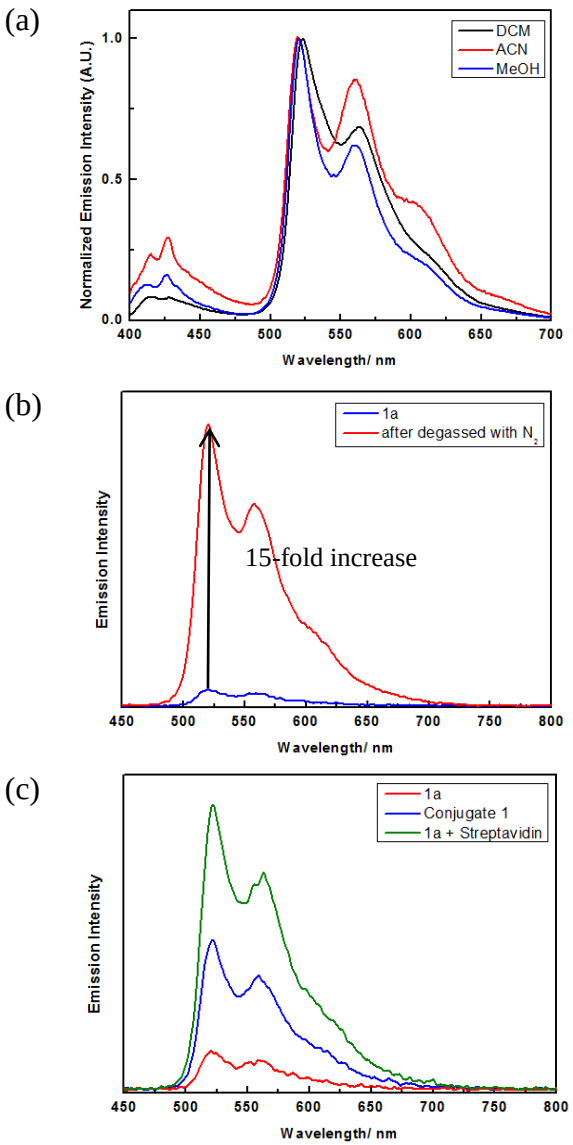


Table S2. Photophysical data of **1a** at 298 K.

Medium	λ_{em}/nm	τ_o/ μs	Φ_{em}
CH_2Cl_2	415, 523, 564, 614 sh	115	5.0 %
CH_3CN	413, 520, 560, 608 sh	25	1.3 %
MeOH	413, 520, 561, 609 sh	91	1.9 %

Figure S2. (a) Emission spectrum of **1a** in CH_2Cl_2, CH_3CN, and MeOH at 298 K in degassed conditions. (b) Emission spectrum of **1a** in 9:1 PBS/DMSO solution of pH 7.4 after degassing with N_2 gas for 3 min in open air. (c) Emission spectrum of **1a** in the presence of streptavidin in 9:1 PBS/DMSO solution of pH 7.4 in open air.



In the replacement of avidin with streptavidin, a 7-fold increase in the emission intensity at 520 nm was observed. Since streptavidin and avidin share similar tertiary and quaternary structure and related amino acids in the biotin binding site, the difference in emissive enhancement may be due to the difference in isoelectronic point (pI) of these proteins. The pI value of streptavidin is ~6 while that of avidin is ~10. We suggest that at pH 7.4, streptavidin carried an overall negative charge and could have a better interaction with the cationic gold complex and thus a better protection of gold centre from oxygen to give a higher enhancement in emission intensity.

Figure S3. (a) Absorption titration curve of the reaction between **1a** and avidin in PBS solution at 298 K. (b) Emission titration curves for titration of **1a** with (1) avidin (■) and (2) a blank in PBS solution (●) at 298 K.

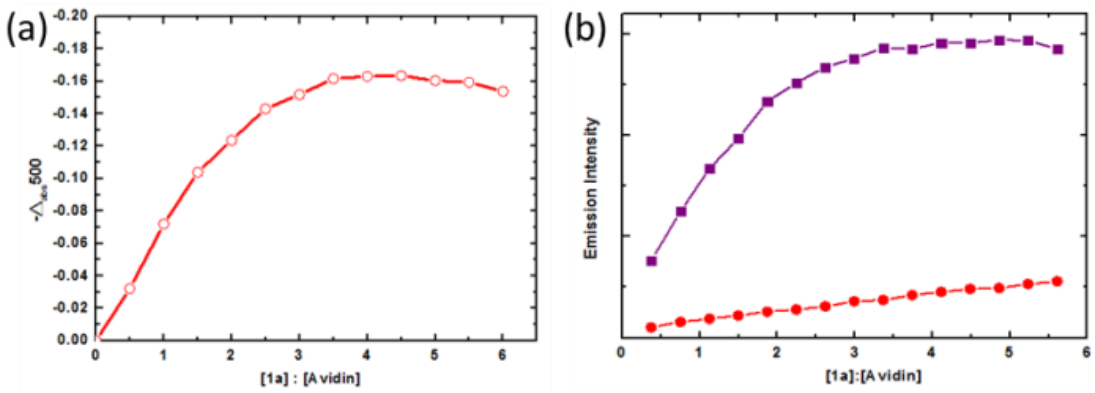
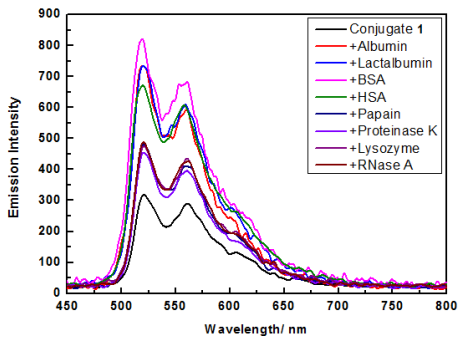


Figure S4. (a) Emission spectrum of conjugate **1** with different proteins (3 equiv.) and (b) emission spectrum of **1a** with different proteins (3 equiv.) in PBS/DMSO (9:1) solution in open air at 298 K.

(a)



(b)

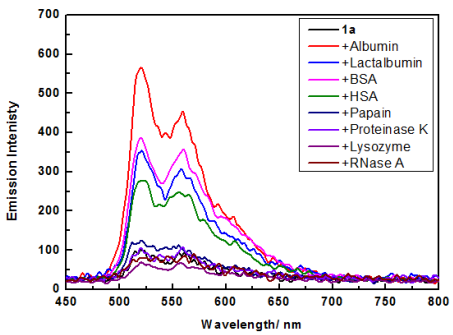


Figure S5. (a) Emission spectrum of **1a** with HSA (6 equiv.) and (b) emission spectrum of conjugate **1** with HSA (3 equiv.) in PBS/DMSO (9:1) solution in open air at 298 K.

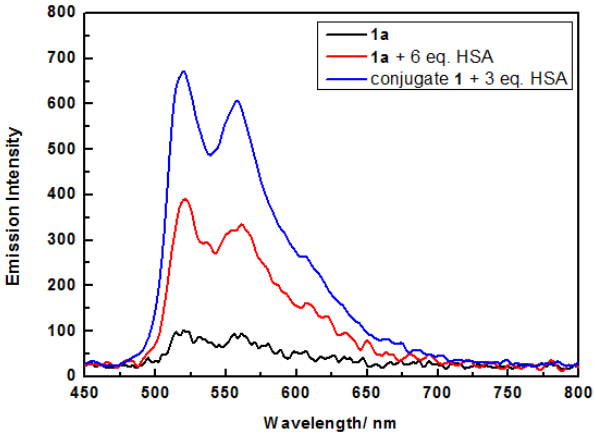


Figure S6. (ai) Analysts added to a solution of **1a** or conjugate **1** in PBS buffer with 10 % DMSO of pH 7.4 for examining the effect of the presence of different analysts towards the emission intensity of **1a** or conjugate **1**. (aii) Solutions of **1a** or conjugate **1** in PBS buffer of different pH (4, 6, 7.4, 8, or 10) with 10 % DMSO for examining the effect of the pH towards the emission intensity of **1a** or conjugate **1**. (b) Analysts added to a solution mixture of **1a** and BSA in PBS buffer with 10 % DMSO of pH 7.4 for examining the effect of the presence of different analysts towards the emission intensity of the solution mixture. (c) Emission spectrum of **1a** (20 μM) in the presence of 20 or 60 μM ascorbic acid in 9:1 PBS/DMSO of pH 7.4.

(ai)

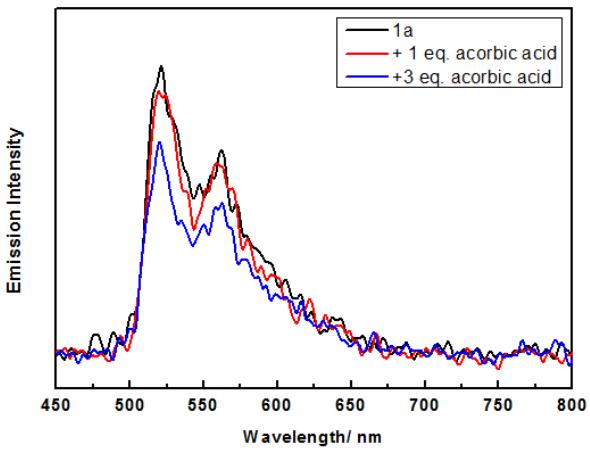
Analyst	Concentration
NaCl	50 mM
KCl	
$NaHCO_3$	
Sodium citrate	
$MgCl_2$	
$MgSO_4$	
$(NH_4)_2SO_4$	
glutathione	1 mM

(aii) pH 4,6,7.4, 8, or 10 of PBS

(b)

Analyst	Concentration
NaCl	50 mM
NaOAc	
$NaHCO_3$	
Sodium phosphate	
Sodium citrate	
EDTA	
Glucose	
Urea	

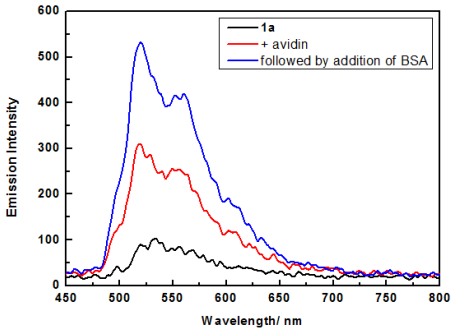
(c)



Emission spectrum shown that a ~8 % emission intensity drop at 520 nm of **1a** was observed in the presence of 20 μM of ascorbic acid. Further increasing the concentration of ascorbic acid to 60 μM resulted in a ~20% drop in emission intensity.

Figure S7. (a) Emission spectrum of **1a** binds with 0.25 equivalence of avidin, followed by addition of 1 equivalence BSA. (b) Emission spectrum of reversing the addition order of avidin and BSA.

(a)



(b)

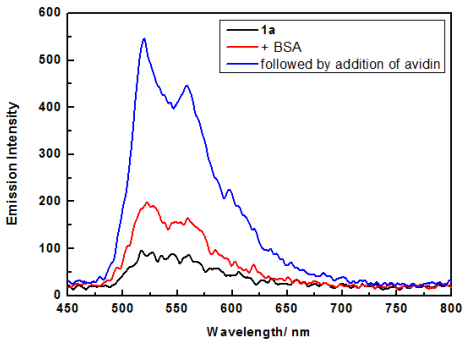
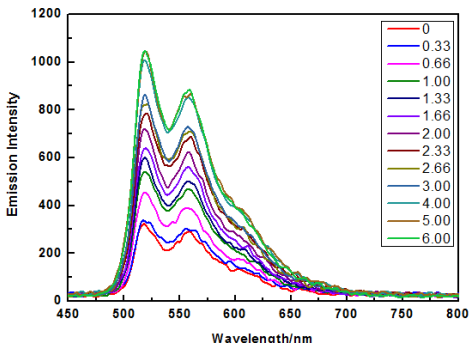


Figure S8. (a) Emission spectrum of conjugate **1** with different ratio in equivalence of BSA to **1** in PBS/DMSO (9:1) solution in open air at 298 K. (b) Plots of emission intensity at 520 nm versus ratio of [BSA]/[Conjugate **1**] from 0 – 3.

(a)



(b)

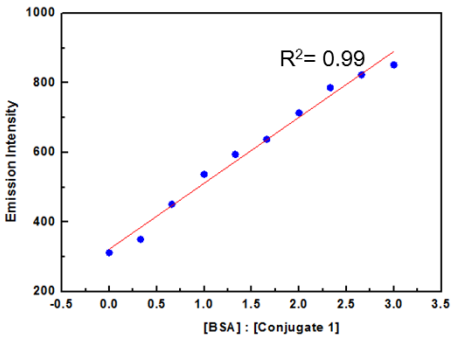
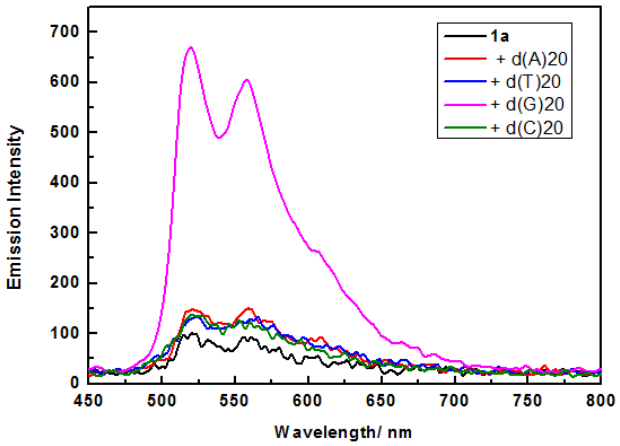


Figure S9. Emission spectrum of **1a** with ssDNA oligonucleotides (1 equiv.) in PBS/DMSO (9:1) solution in open air at 298 K.



G-rich DNA strands are reported to form G-quadruplex by self-aggregation.[6] It is proposed that π-conjugated metal complex such as platinum(II) Schiff base complexes could interact with the G-quadruplex structure.[7] It is thus possible that similar interaction between $d(G)_{20}$ and **1a** could occur. Such interaction may pose protection from oxygen quenching of **1a** and lead to enhancement in emission intensity. To support the assumption, **1a** was used to stain a G-rich DNA, G4A1 oligomer (5'-TGGGGAGGGTGGGGAGGGTGGGGAAGG-3') which is reported to generate intramolecular G-quadruplex in the presence of 100 mM KCl.[7] The reaction was conducted by mixing **1a** with G4A1 (1:1) in a solution of 10 mM Tris/HCl containing 100 mM KCl and 10 % DMSO. As shown in the figure below, a 6-fold enhancement in the emission intensity at 520 nm was observed. Notably, no emissive enhancement was observed in mixing **1a** with other non-G-rich DNAs ($d(A)_{20}$, $d(T)_{20}$, $d(C)_{20}$ and ctDNA).

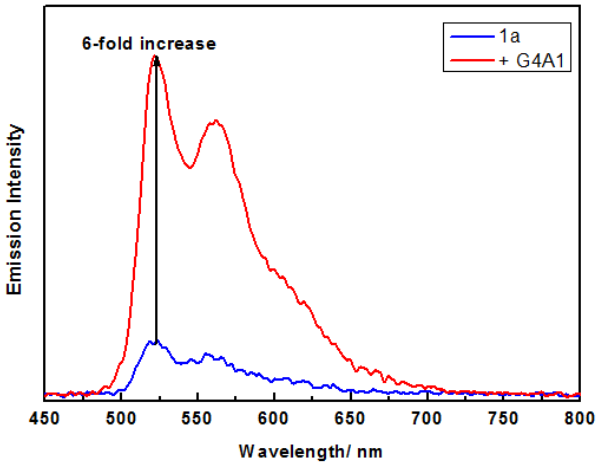


Figure S10. Emission spectrum of **1a** or conjugate **1** with ssDNA oligonucleotides $d(A)_x$ (x = 5, 20 or 50) (1 equiv.) in PBS/DMSO (9:1) solution in open air at 298 K.

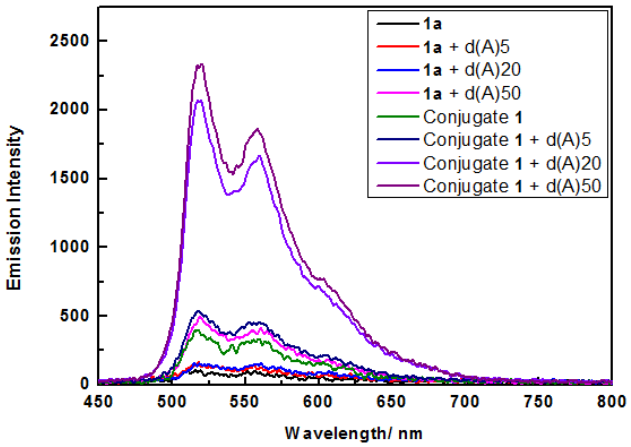
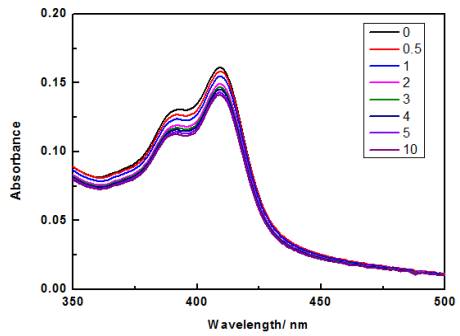


Figure S11. (a) Absorption spectrum of **1a** with different ratio in equivalence of ctDNA to **1a** in PBS/DMSO (9:1) solution in open air at 298 K. (b) Plots of [ctDNA]/$\Delta\varepsilon_{ap}$ versus [ctDNA].

(a)



(b)

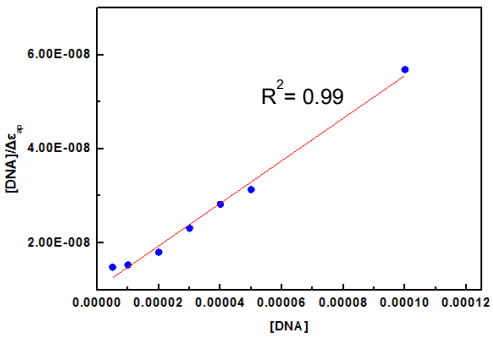
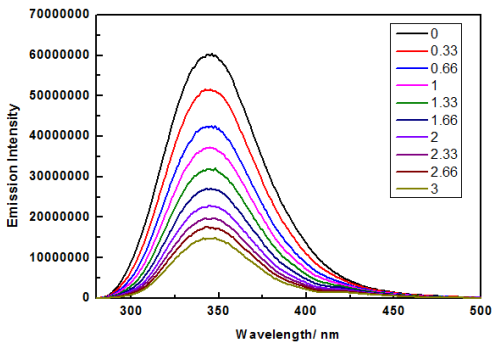


Figure S12. (a) Emission spectrum of BSA with different ratio in equivalence of **1a** to BSA in PBS/DMSO (9:1) solution in open air at 298 K. (b) Plots of $\log[(I_0-I)/I]$ versus log[**1a**] from ratio of 0.33 to 3.

(a)



(b)

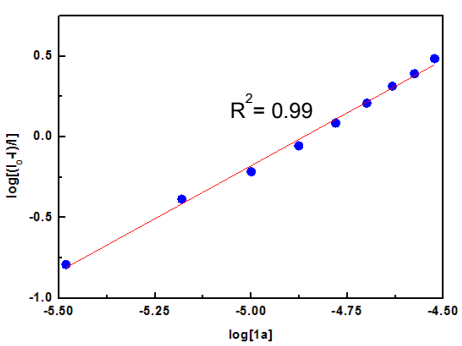
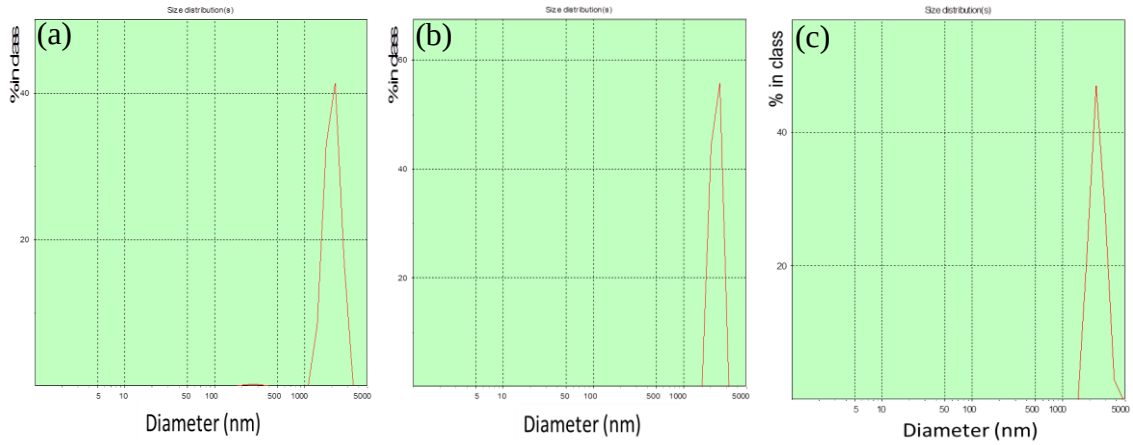
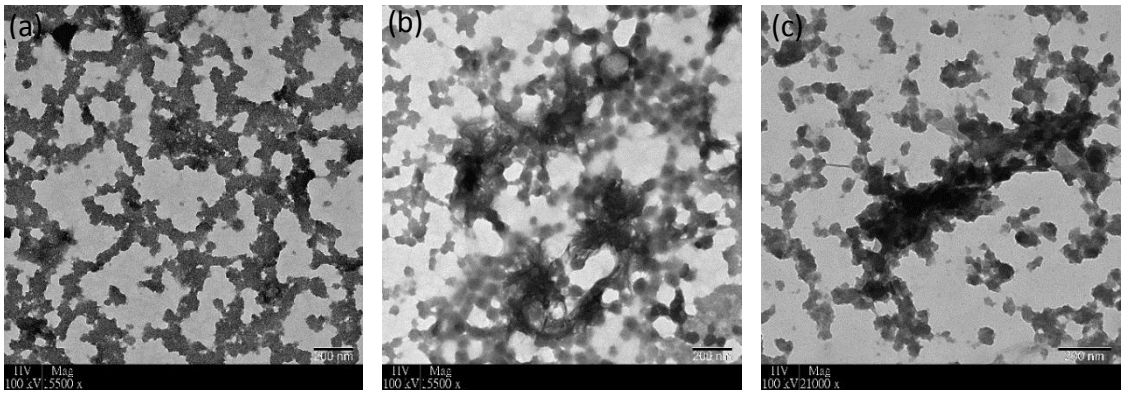


Figure S13. Dynamic light scattering experiment of (a) reaction mixture of avidin with ctDNA, (b) avidin with ctDNA in the presence of biotin and (c) avidin with ctDNA in the presence of **1a**.[8]



Dynamic light scattering experiments obtained from reaction mixture of avidin and 1 equiv. of ctDNA afforded aggregates with average diameter of 1.9 µm. Addition of 4 equiv. of free biotin to the mixture afforded aggregates with average diameter of 2.4 µm. Addition of **1a** instead of free biotin to the mixture resulted in aggregates with comparable average diameter of 2.5 µm. These results suggested that DNAs would bind with conjugate **1** at the avidin and resulted in the formation of aggregates.

Figure S14. Transmission electron microscopy images of (a) reaction mixture of avidin with ctDNA, (b) avidin with ctDNA in the presence of biotin and (c) avidin with ctDNA in the presence of **1a**.



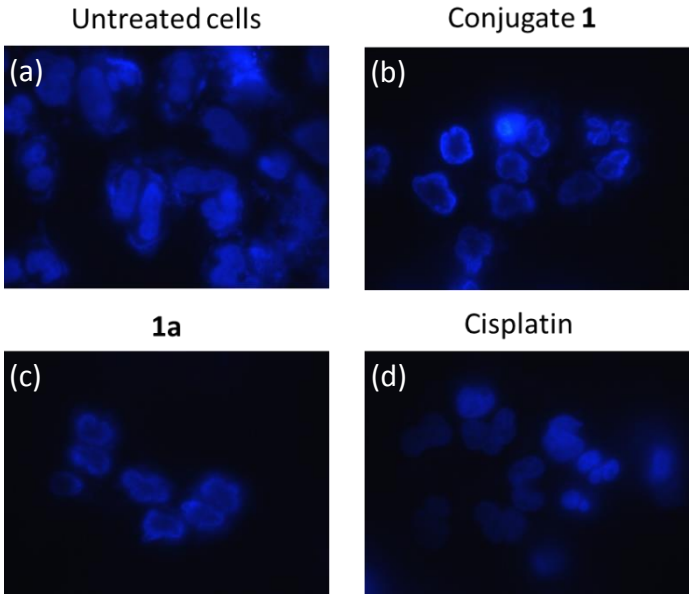
TEM examination of reaction mixture of ctDNA and avidin resulted in toroidal or rod-like conformation with an average size of 50-100 nm. Addition of biotin afforded larger aggregates of diameters of around 50-400 nm. Addition of **1a** instead of biotin gave aggregates of diameters of around 50-1200 nm.

Table S3. Comparison of cytotoxicity towards different cell lines after treatment with **1a**, conjugate **1**, **1b**, or cisplatin for 72 h.

	HeLa	HepG2	MDA
	IC_{50}/μM		
1a	1.2±0.1	2.8±0.6	2.2±0.5
Conjugate **1**	1.1±0.3	6.9±1.2	2.1±0.3
1b	>100	>100	>100
Cisplatin	12.5±3.6	97.1±20.2	24.3±8.4

The IC_{50} values are determined by at least three independent assays.

Figure S15. Fluorescence images of Hoechst 33342-stained HeLa cells in the (a) absence, or (b) presence of conjugate **1** or (c) **1a** (4 µM, 24 h) or (d) cisplatin (20 µM, 24 h).



Nuclear shrinkage and fragmentation was observed in the presence of conjugate **1**, **1a** or cisplatin which implied the occurrence of apoptosis.

Figure S16. Alternations in ROS level of HeLa cells after treating with various concentrations of **1a** or conjugate **1** (1, 2, 5, or 10 μM) for 4 h. For positive control, incubation of HeLa cells with 1.5 mM hydrogen peroxide for 30 min afforded 1.8-fold increase in ROS level.

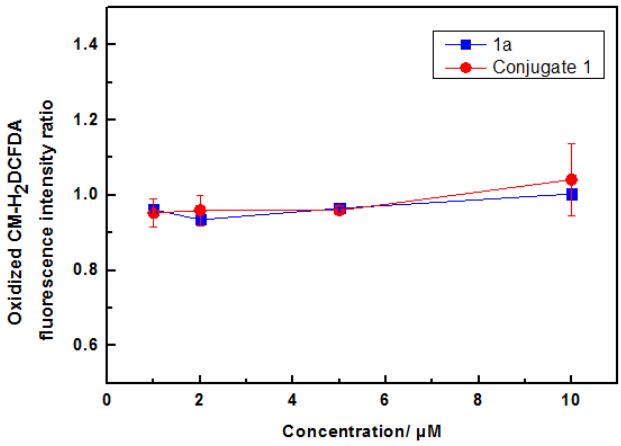


Figure S17. Bright-field (left), fluorescence (middle), and merged images (right) of HeLa cells treated with conjugate **1** or **1a** (10 μM) for 4-hour.

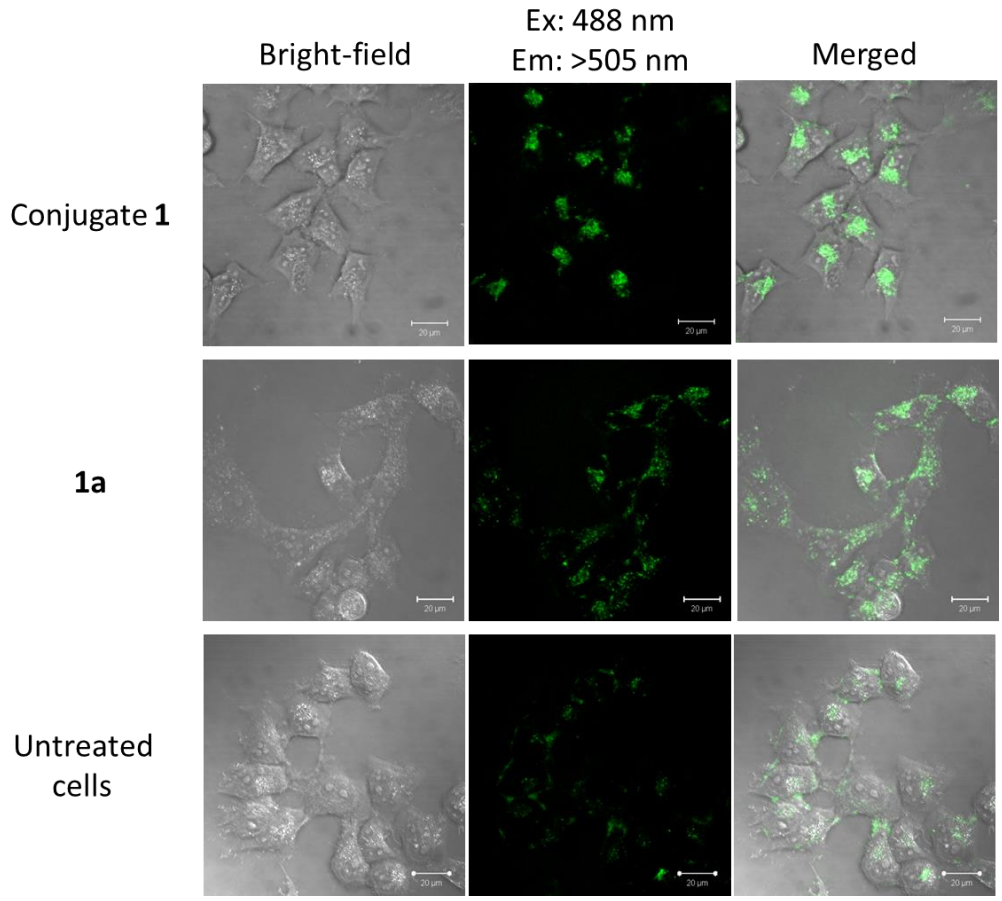
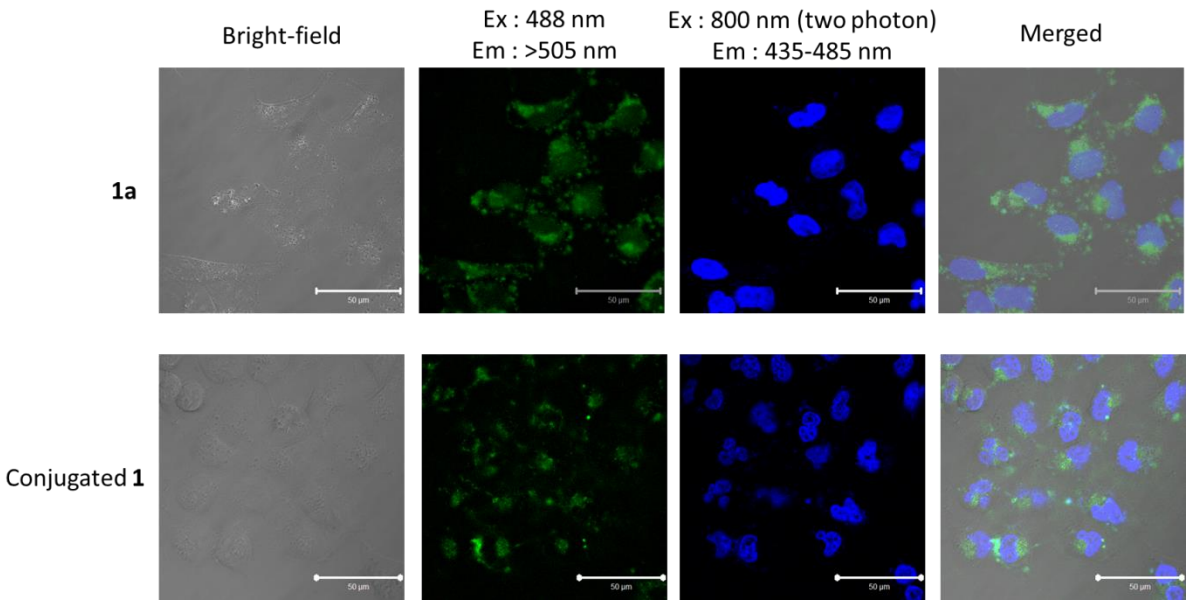
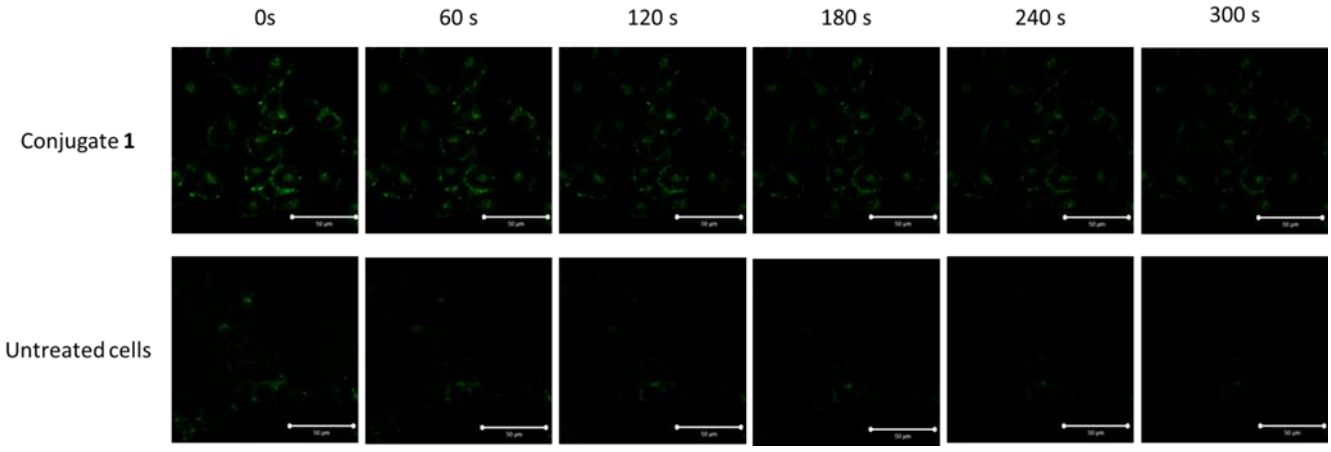


Figure S18. Fluorescence microscopic analyses of **1a** (10μM) and conjugate **1** (10μM) in HeLa cells co-stained with Hoechst 33342 (1 μM) for 15 min.



Both **1a** and conjugate **1** were excluded from the nucleus.

Figure S19. Fluorescence images of HeLa cells treated with conjugate **1** (10 µM) continually irradiated at 488 nm at different time periods.



HeLa cells were treated with 10 µM of conjugate **1** at 37 ^{o}C under an atmosphere of 5% CO_2 for 4 h. After that, the cells were continually irradiated at 488 nm and the cell images were recorded every 60 second. The emission of conjugate **1** was significantly dropped after irradiated for 120s and constantly decreased until 300s. The cell imaging of untreated cells was also performed under the same incubation and irradiation conditions as control.

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