Supporting Information

AgNPs/DNA/TPdye Conjugate-based Two-photon Nanoprobe for GSH Imaging in Cell Apoptosis of Cancer Tissue

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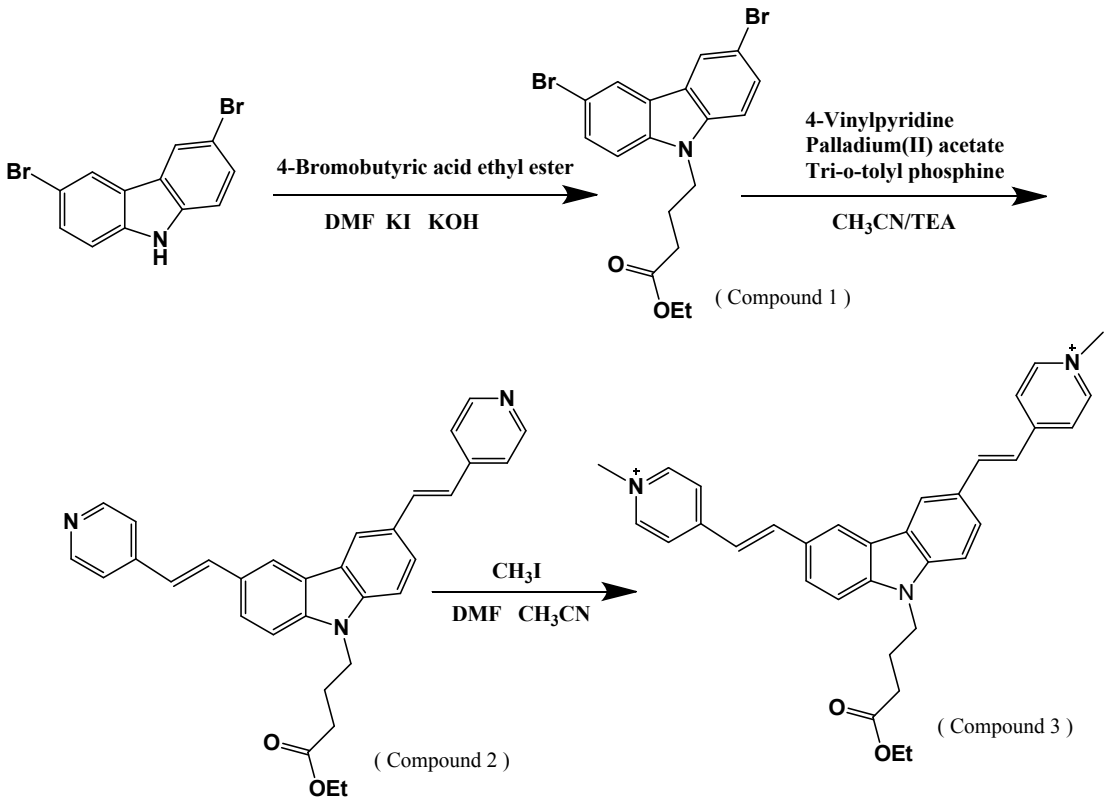
Chemicals and Instruments

N-methylmaleimide (NMM), beta-phenylethyl isothiocyanate (PEITC), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylte-trazolium bromide (MTT), $AgNO_3$ and $NaBH_4$ were purchased from Sigma-Aldrich. The TPdye of Ethyl-4-[3,6-Bis(1-methyl-4-vinylpyridium iodine)-9H-carbazol-9-yl)] butanoate (EBMVC-B) used in this work

was synthesized as described in the following, and the hairpin-structured DNA sequence (HPDNA, 5′- GGTTAATCC AAGAATCAATAACTACATAA GGATTAACC-3′) was synthesized by Sangon Biotech. Co., Ltd. (ShangHai, China). It was dissolved in ultrapure water as stock solutions, and the concentration of oligonucleotide was accurately identified according to UV absorption at 260 nm. The HeLa (cervical cancer) cell lines and cervical cancer tissue slices obtained from mouse were provided by the Biomedical Engineering Center of Hunan University (China). All other chemicals obtained from commercial suppliers were analytical grade and used without further purification. All solutions were prepared using ultrapure water, which was obtained through a Millipore Milli-Q water purification system (Billerica, MA) and had an electric resistance of 18 MΩ. All experiments were carried out at room temperature.

Transmission electron microscopy (TEM) was performed on a JEOL JEM-3010. Energy-dispersive X-ray (EDX) spectra were obtained using the TEM microscope. The pH values were calibrated with a model 868 pH meter (Orion). UV-vis absorption spectra were measured on a Hitachi U-4100 UV/Vis spectrometer (Kyoto, Japan) using a quartz cuvette having 1 cm path length. One-photon excitation (OPE) fluorescence spectra were performed on a PTI ASOC-10 Fluorescence System (Photo Technology International, Birmingham, NJ, USA). Two-photon excitation (TPE) fluorescence spectra were obtained with a mode-locked Ti: sapphire pulsed laser (Chameleon Ultra II, Coherent Inc.) and then recording with a DCS200PC single photon counting (Beijing Zolix Instruments Co., Ltd.). For MTT assay, the spectrophotometrical absorbance of each well was measured by using a Tecan microplate reader (Bio-Rad ELISA reader, Hercules, CA). Two-photon excitation fluorescence images (TPFI) of cells or tissue slices were obtained using an Olympus FV1000-MPE multiphoton laser scanning confocal microscope (Japan).

Preparation of the Two-photon Dye (TPdye: Ethyl-4-[3,6-Bis(1-methyl-4 -vinylpyridium iodine)-9H-carbazol-9-yl)] butanoate, EBMVC-B)

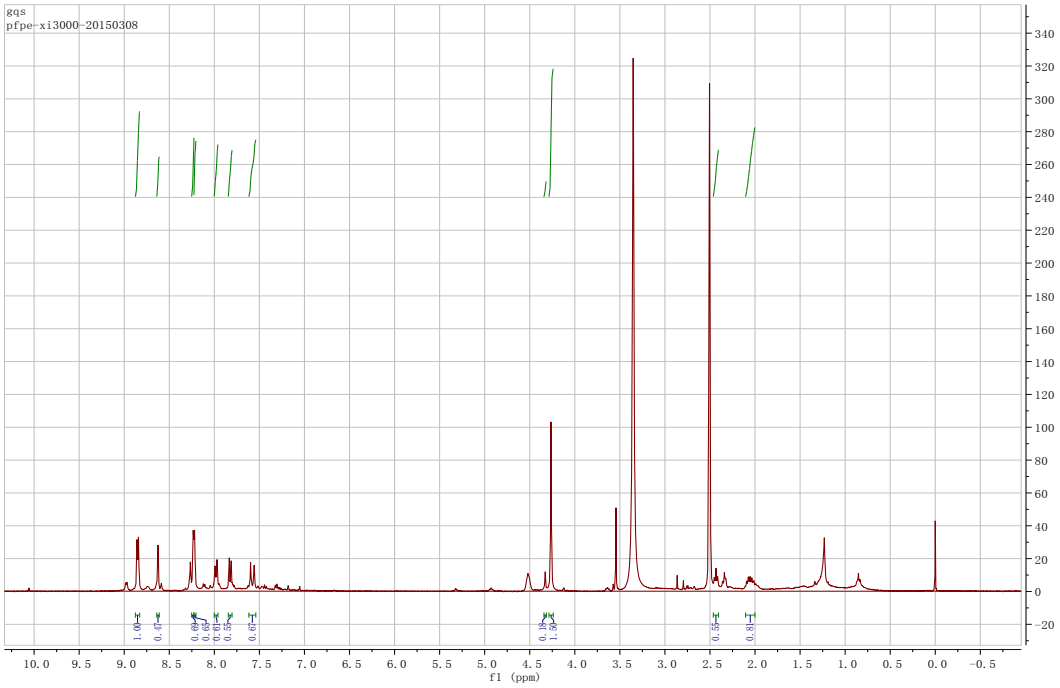


Synthesis of Ethyl-4-(3,6-dibromo-9H-carbazol-9-yl) butanoate (compound 1). To a mixture of KOH (1.12 g, 20 mmol) and KI (80 mg, 0.48 mmol) dissolved in dry DMF (20 mL) was added 3,6-dibromocarbazole (compound 0) (0.65 g, 2 mmol), ethyl-4-bromobutanoate (1.15 mL, 8 mmol). The mixture was stirred at 60 ^{0}C under argon atmosphere overnight. After the addition of 100 mL H_2O to the final mixture, the mixture was extracted with ethyl acetate and then the organic layer was washed twice with water and once with brine, and dried over anhydrous Na_2SO4. After filtration, the solution was concentrated under reduced pressure to give crude product The final white powder compound 1 was obtained by chromatography using petroleum/ethyl acetate(5:1, V/V) as an eluent.[1]

Synthesis of Ethyl-4-[3,6-Bis(4-vinylpyridium iodine)-9H-carbazol-9-yl)] butanoate (compound 2).[2] compound 1 (731.7 mg, 1.7 mmol) was added into a mixture containing 4-vinylpyridine (667 mg), Palladium(II) acetate (5 mg) and tri-o-tolyl phosphine (50 mg) under the triethylamine (3 mL)/acetonitrile(9 mL) solvent pairs in a high pressure bottle. The mixture was stayed at 105 ^{0}C for 48 h. After the reaction, the mixture was transferred to a flask and the solvent was removed under reduced

pressure to give a yellow crude product, which was purified by chromatography on silica gel using CH_2Cl_2/CH_3OH (5:1,V/V) as an eluent to give compound 2 as earth yellow solid.

Synthesis of Ethyl-4-[3,6-Bis(1-methyl-4-vinylpyridium iodine)-9H-carbazol-9-yl)] butanoate (EBMVC-B, compound 3). Excess CH_3I and compound 2 (487.0 mg, 1 mmol) in acetonitrile /DMF was refluxed for 4h, then the orange red powder, compound 3 was obtained with a 90% yield after recrystallization twice using methanol. ^{1}HNMR (d6-DMSO, 400 MHz, δ): 8.83 (d, 4H), 8.63 (s, 2H), 8.24 (d, 4H), 8.22 (d, 2H), 7.95 (d, 2H), 7,80 (d, 2H), 7.60 (d, 2H),4.39(m,2H), 4.35 (t, 2H), 4.25 (s, 3H), 4.24 (s, 3H), 2.38 (t, 2H), 2.10 (m, 2H),1.35(t,3H). MS (ESI) *m/z* for $C_{34}H_{35}N_3O_2$ 258.88 found, 258.88 (M^{2+}). Anal. calcd. for $C_{34}H_{35}I_2N_3O_2$: C, 52.93; H, 4.57; N, 5.45. Found: C, 52.67; H, 4.50; N, 5.43.



Preparation of the DNA/AgNPs/TPdye Nanoprobe

Generally, 1.5 μM HPDNA in 10 mM HEPES (2-[4-(2-Hydroxyethyl)-1-piperazinyl] ethanesulfonic acid) solution (PH 7.4, 100 mM KNO_3) containing 7.5 μM EBMVC-B was previously heated to 95 ^{0}C for 5 min and subsequently incubated

for 2 h at room temperature. Then 300 μL of the above solution was transferred into a 1.5 mL volumetric pipe and cooled in ice water, and then 300 μL $AgNO_3$ (300 μM) was added. The obtained solution was incubated at 0 °C for 20 min to form the DNA/Ag^+ complex. To synthesize DNA-templated AgNPs, 300 μL freshly prepared $NaBH_4$ solution (375 μM) was added into the above obtained mixture solution. After 30 min incubation in ice-water for complete reaction, the DNA/AgNPs/TPdye nanoprobe was formed and stored at 4 °C before use.

Gel Imaging

The AgNPs/DNA/TPdye nanoprobe was prepared according to the experimental part, the reaction mixture was incubated for 1 h at room temperature. Finally, all the mixtures were centrifuged at 12000 rpm and 15 μL of the obtained supernatants was used for gel running. Electrophoresis condition: 15% polyacrylamide gel and the gel was run at 200 V for 1 hrs, gel image was carried out with a ChemiDoc XRS^+ Imaging System (Bio-RAD).

Cytotoxicity Assay with the DNA/AgNPs/TPdye Nanoprobe.

For cytotoxicity assay, HeLa cells were grown in RPMI 1640 medium (Thermo Scientific HyClone) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Invitrogen), 100 U/mL penicillin, and 100 U/mL gentamicin. When in the proliferative period, HeLa cells were dispersed within replicate 96-well microliter plates to a total volume of 100 μL/each well and maintained at 37 ^{0}C in a 5% CO_2/95% air incubator for 24 h. Then, the culture media was removed and the cells were incubated in culture medium containing the as-prepared DNA/AgNPs/Pdye Nanoprobe with different concentrations (concentration of the nanoprobe refers to the concentration of HPDNA) for 48 h, and then washed with the culture medium. An amount of 100 μL of the fresh culture medium containing MTT (0.5 mg/mL) was then added, followed by incubating for 4 h to allow the formation of formazan dye. After removing the medium, 150 μL DMSO was added to each well to dissolve the formazan crystals. Absorbance was measured at 490 nm in a microplate reader. Relative cell viability was expressed as: ([OD]test/[OD]control) ×100%. Each

experiment was repeated at least three times.

Live Cell Imaging with the DNA/AgNPs/TPdye Nanoprobe

For cell imaging experiments, HeLa cells were seeded in culture plate and grown overnight on glass coverslips at the bottom of the plate. When the cells were ~90% confluent, the coverslips were washed three times with phosphate buffered saline (PBS) (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.4). Before incubation with the prepared nanoprobes, the HeLa cells were incubated with/without 1 mM NMM for 1 h or 15 μM PEITC for 3 h. Then, 1.0 mL of fresh cell growth medium supplemented with 35 μL of the prepared DNA/AgNPs/TPdye nanoprobe was added to the culture plate. After incubation for 45 min, the cells were washed with Dulbecco's phosphate buffered saline (DPBS) three times. Two-photon confocal fluorescence imaging of HeLa cells was observed under an Olympus FV1000-MPE multiphoton laser scanning confocal microscope, with a mode-locked titanium-sapphire laser source (120 fs pulse width, 80 MHz repetition rate) set at wavelength 800 nm. Three dimensional images were taken every 2 μm by scanning the samples across a defined section along the z-axis.

GSH Imaging in Cancer Tissue Using the DNA/AgNPs/TPdye Nanoprobe

1.0 mm-thick cervical tumor tissue slices were obtained from the mouse which was pretreated with/without PEITC for 24 h. Then the slices were incubated with 500 nM DNA/AgNPs/TPdye Nanoprobe (concentration of the nanoprobe refers to the concentration of HPDNA) in 10% bovine serum-containing PBS for 1 h at 37 ^{0}C. After washing with PBS to remove the remaining nanoprobes, two-photon confocal fluorescence imaging, Z-scan imaging and the 3D two-photon confocal fluorescence images accumulated along the Z-direction at depth of 0−300 μm (20× magnification) of this treated tumor tissue slices were observed under an Olympus FV1000-MPE multiphoton laser scanning confocal microscope, with a mode-locked titanium-sapphire laser source set at wavelength 800 nm.

Measurement of One-photon Quantum Yields and TPA Cross Sections of

EBMVC-B and EBMVC-B/DNA

The one-photon quantum yields (QY) of samples were estimated using Rhodamine B (literature quantum yield: Φ_F = 0.95 in ultrapure water) as a reference standard, which was freshly prepared to reduce the measurement error.[3] The quantum yield Φ as a function solvent polarity is calculated using the following equation:

$$\Phi_F = \Phi_{F,\mathrm{cal}} \cdot \frac{S}{S_{cal}} \cdot \frac{A_{cal}}{A} \cdot \frac{n^2}{n^2_{cal}} \qquad (1)$$

Where Φ_F is the quantum yield, S is the areas' integral values of the corrected fluorescence spectra, A stands for the absorbance and n is refractive index. The subscript *cal* and no denote the standard and sample, respectively.

TPA cross sections have been measured using the two-photon induced fluorescence method, and thus cross section can be calculated by means of equation (2),[4]

$$\delta_s = \delta_r \frac{\phi_r}{\phi_s} \frac{c_r}{c_s} \frac{n_r}{n_s} \frac{F_s}{F_r} \qquad (2)$$

where the subscripts s and r refer to the sample and the reference compound, respectively. The terms c and n are the concentration and refractive index of the applied solution. F is two-photon excited fluorescence integral intensity. Φ refers to the fluorescence quantum yield. Rhodamine B in methanol (σ_{800} = 42 GM) was used as the reference.

Measurement of the Binding Constant of EBMVC-B to DNA

One-photon fluorescence titration method was used to measure the binding constants (k) by means of equation (3) and equation (4) (Figure S3).[4]

$$c_b = c_t[(F - F_0) / (F_{\max} - F_0)] \qquad (3)$$

$$r / c_f = k_n - k_r \qquad (4)$$

For equation (3), c_t is the total concentration of EBMVC-B, c_b is the concentration of EBMVC-B binding to DNA, F is the observed fluorescence intensity at given DNA concentration, F_0 is the fluorescence intensity without addition of DNA, and F_{max} is the fluorescence intensity of the totally binding compound. For equation (4), k is

binding constant, *n* is the number of dye sites of per phosphate, *r* is the ratio of the concentration of the binding dye to the concentration of DNA (in phosphate) and C_f is the concentration of free dye. The concentration of the binding compound was calculated using equation (3).

Characterization of the Synthesized TPdye and TPdye/DNA Complex

The optical properties of the synthesized TPdye (EBMVC-B) and the TPdye/DNA complex were first examined. The UV/Vis absorption spectra of the EBMVC-B dye and the EBMVC-B/DNA complex (sequence of the used DNA and its optimization can be found in Table S1 and Figure S4, SI) were shown in Figure S5A, and they both exhibited a peak absorption at around 450 nm. The maximal molar extinction coefficient (ε) of the EBMVC-B/DNA complex was estimated to be 3.46×10^4 M^{-1} cm^{-1} at 450 nm in aqueous, also very close to that of EBMVC-B (3.37×10^4 M^{-1} cm^{-1}) (Table S2, SI). The emission spectra of EBMVC-B and the EBMVC-B/DNA complex upon one-photon excitation (OPE) at 450 nm were displayed in Figure S5B and Figure S6 (SI). The emission intensity at 550 nm of the TPdye/DNA complex was enhanced almost up to 23 times compared to that emitted by EBMVC-B directly dissolved in aqueous solution. The fluorescence quantum yield (Φ) of the EBMVC-B/DNA complex in aqueous solution was determined to be 0.22 with Rhodamine B as the reference (Φ=0.95)[3], much higher than that of 0.0024 for EBMVC-B. This result indicates that by forming a complex with and being protected by the HPDNA, the EBMVC-B dye experienced less twisted intra-molecular charge transfer (TICT). The binding constant of EBMVC-B with HPDNA was calculated to be 1.87×10^5 M^{-1} through DNA titration; and each HPDNA was bound with at least 3 dye molecules (Figure S3, SI). The luminescent brightness of the EBMVC-B/DNA (defined as ε×Φ) was found to be 1.69×10^6 M^{-1} cm^{-1} with the excitation wavelength of 450 nm at room temperature. These measurements reveal the remarkable signal amplification capability of the TPdye/DNA complex.

We also measured the TPA action cross section (δ×Φ, δ is the TPA cross section) and the TPE emission spectra of the EBMVC-B/DNA complex. As shown in Figure

S5C, the maximal TPA action cross section of EBMVC-B in its DNA complex was measured to be 429 Goeppert-Mayer (GM) at room temperature (λ_{ex} = 800 nm, 1 GM = 10^{-50} cm^4 $sphoton^{-1}$ $molecule^{-1}$) with Rhodamine B as the reference (δ = 140 GM),[5] which was much higher than that of EBMVC-B in aqueous solution (2.0 GM). The TPE emission spectra of EBMVC-B and the EBMVC-B/DNA complex are shown in Figure S5D. The samples were excited with femtosecond laser pulses with a central wavelength at 800 nm and pulse duration of 120 fs. Similar to the OPE experiments, very weak two photon-induced emission was observed for EBMVC-B owing to its small TPA action cross section in aqueous solution. The observed large enhancement of TPE emission intensity for the EBMVC-B/DNA complex is the result of its larger TPA action cross section obtained from the protective microenvironment by the HPDNA. Figure S5D also shows that, the corresponding maximal emission peak of this TPE emission spectrum locates at 550 nm and the profile of this TPE emission spectrum matches exactly to that of the OPE emission spectrum, indicating that the fluorescence emission excited at the near-infrared region is the fluorescence induced by two-photon excitation. Furthermore, the photostability of this TPdye/DNA complex was also measured by using a 150 W xenon lamp as an excitation source. The emission intensity almost did not decrease after irradiation for 1 h, indicating the good photostability of the EBMVC-B/DNA complex (Figure S7, SI). The good photostability, high $\delta \times \Phi$ value and the characteristic of oligonucleotide-based probe enables the EBMVC-B/DNA complex to be a promising tool for TPE imaging.

Long-term stability of the TPdye/DNA complex is a crucial requirement for biomedical imaging. Thus, the stability of the formed EBMVC-B/DNA complex under various environmental conditions was investigated through testing its fluorescence intensity. Effect of pH on the EBMVC-B/DNA complex was examined under pH values ranging from 4.0 to 10.0, a pH range covering diverse physiological conditions found inside cells and tissues. Little changes in the fluorescence intensity were observed (Figure S8, SI). Additionally, there was no significant variation in fluorescence intensity of the EBMVC-B/DNA complex with increasing concentrations of the physiological relevant metal ions, including Na^+, K^+, Ca^{2+}, and

Mg^{2+} (Figure S9, SI). Both phenomena indicates that the TPdye/DNA complex is stable under a wide physiological pH range and can sustain high ion strength. Then, effects of the physiological relevant thiol-containing amino acids (Cys, Hcy, Met) or peptides (GSH) and the hydrogen sulfide on the stability of this EBMVC-B/DNA complex were investigated (Figure S10, SI). No significant changes in fluorescence intensity were noticed with the addition of any of the thiol-containing compound, indicating that the EBMVC-B/DNA complex itself does not respond to these thiol-containing molecules and can be a reliable fluorescence reporter for GSH. Furthermore, stability of this EBMVC-B/DNA complex in human serum or human cervix carcinoma (HeLa) cell lysate was also investigated (Figure S11, SI). The results show that the EBMVC-B/DNA complex can be stable for tens of hours. The results from all stability tests support that the formed EBMVC-B/DNA complex could be used for sensing assay in complicated biological conditions.

Formation and Characterization of the DNA/AgNPs/TPdye Nanoprobe

Because ssDNA sequences have been used as the template for synthesis of silver nanoparticles (AgNPs) and the A/T bases-riched dsDNA has been employed to load the TPdye EBMVC-B,[2,6] the TP nanoprobe of AgNPs/DNA/TPdye was prepared by using a hairpin-structured DNA fragment with a 25-base loop and a 12-base stem (HPDNA) as the template while reducing the EBMVC-B/HPDNA/Ag^+ complex with $NaBH_4$. Figure S12A displays that, the fluorescence intensity of the EBMVC-B/HPDNA complex decreased proportionally with the increasing amounts of Ag^+; and at the same time the concomitant absorption peak of AgNPs centered at 410 nm was observed (Figure S12B). The quenching of the TPdye fluorescence is resulted from the energy or electron transfer processes between Ag and the dye as more and more AgNPs/DNA/TPdye nanoprobe formed.[7] When the concentration of Ag^+ reached 10 μM, the fluorescence of the EBMVC-B/HPDNA complex (100 nM of HPDNA and 500 nM of EBMVC-B) was nearly completely quenched. However, in contrast to the above phenomenon, significant fluorescence polarity change in EBMVC-B only cannot be observed upon formation of AgNPs (Figure S13, SI),

indicating that the EBMVC-B molecule itself cannot adsorbed on the AgNPs's surface, and thus further indicating that the immobilization of TPdye EBMVC-B on the AgNPs's surface is realized via the HPDNA mediator. The AgNPs/DNA/TPdye nanoprobe was then further characterized by transmission electron microscopy (TEM) and energy-dispersive X-ray (EDX) spectroscopy to obtain its morphological profile and structural information. We can see from the TEM that the AgNPs/DNA/TPdye nanoprobe shows uniform and monodispersed spherical shape with a diameter distribution from 8 to 10 nm (Figure S12C). EDX analysis of the AgNPs/DNA/TPdye nanoprobe shows the coexistence of Ag, C, O, N and P elements which belongs to AgNPs, EBMVC-B and oligonucleotides (Figure S12D), respectively, further indicating that the coverage of EBMVC-B/HPDNA on the AgNPs surface and the successful formation of TP AgNPs/DNA/TPdye nanoprobe. It is worthy of noting that for the non-template-synthesized AgNPs/DNA/TPdye conjugate prepared by directly mixing AgNPs with EBMVC-B/HPDNA, the maximal quenching efficiency is only about 20% (Figure S14, SI)), much lower than that of templated-synthesized AgNPs/DNA/TPdye conjugate under the same condition.

For practical biomedical applications, a fluorescent nanoprobe should not interfere with the metabolism of the living system. So, cytotoxicity of the AgNPs/DNA/TPdye nanoprobe was evaluated before its application in biomedical imaging. The cytotoxicity tests were performed with HeLa cells as the model cell line using the standard cell viability assay — the MTT assay.[8] Much high cell viability was observed (survival rate was higher than 85% in 1.0×10^4 cells/well) even after the cells were treated with AgNPs/DNA/TPdye nanoprobe at concentration up to 100 nM for 24 h (concentration of the AgNPs/DNA/TPdye nanoprobe refers to the concentration of HPDNA) (Figure S15, SI). The results show that no significant influence on HeLa cells can be observed under a certain amount of AgNPs/DNA/TPdye nanoprobes (for example 100 nM) and a certain incubation time (for example 24 h).

In Vitro Detection of GSH by the DNA/AgNPs/TPdye Nanoprobe

In a typical assay, 50 μL of the DNA/AgNPs/TPdye nanoprobe prepared as described in the experimental section was first transferred into a 1.0 mL volumetric pipe and 400 μL of 20 mM HEPES buffer (pH 7.4) was added, then 50 μL of the freshly prepared GSH with different concentrations or other biomolecules was added and incubated at room temperature for 10 min. After reaction, the fluorescence spectra of the resulting solutions were recorded. For the OPE measurement, the fluorescence spectra were recorded in a quartz cuvette on PTI QM4 Fluorescence System with the excitation wavelength of 450 nm and the emission wavelengths in the range from 480 to 650 nm with both excitation and emission slits of 10 nm. For the TPE measurement, the two-photon emission fluorescence spectra in the range from 500 to 650 nm were obtained by exciting all samples at 800 nm with a mode-locked Ti:sapphire pulsed laser (Output laser pulses were centered at 800 nm and an average power of 100 mW was as the excitation source. The laser pulses have pulse duration of 120 fs and repetition rate of 80 MHz.), followed by recording with a DCS200PC single photon counting.

To evaluate the performance of this DNA/AgNPs/TPdye nanoprobe in complex conditions, we carried out the fluorescence emission measurement of EBMVC-B/HPDNA complex in RPMI 1640 cell media with 10% FBS (Thermo Scientific HyClone) using the OPE and TPE techniques. Figure S1A shows that the cell growth media had a high autofluorescence and dominated the fluorescence spectra from 500 to 650 nm under OPE. Furthermore, the fluorescence emission intensity inversely increased when the EBMVC-B/HPDNA complex was dispersed into the cell growth media, which might be a result from the higher autofluorescence of the cell growth media. However, in contrast to the OPE measurements, the TPE fluorescence emission intensity and its emission spectrum in the cell growth media were almost the same as in the HEPES buffer (Figure S1B). Then the fluorescence emission spectra of the DNA/AgNPs/TPdye nanoprobe in cell media with/without GSH by using the OPE and TPE method were carried out respectively. For the OPE method (Figure S1C), the fluorescence emission of the cell media was very strong even without the DNA/AgNPs/TPdye nanoprobe and a significant fluorescence emission increase

cannot be obtained when DNA/AgNPs/TPdye nanoprobe was mixed with the cell media containing GSH. However, the TPE fluorescence emission of the cell media was very weak without the DNA/AgNPs/TPdye nanoprobe, and remarkable fluorescence enhancement was attained from the nanoprobe upon addition of GSH (Figure S1D).

References

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Table S1. DNA sequences used in this work.

Name	Sequences (5'-3')
G-quadruplex 1	GGGTAGGGCGGGTTGGGT
G-quadruplex 2	GGGTAGGGCGGGTTGGGTAAGAATCAATAACTACATAA
Random ssDNA	AAGAATCAATAACTACATAA
HPDNA	GGTTAATCCAAGAATCAATAACTACATAAGGATTAACC

Table S2. Photophysical Data for EBMVC-B and EBMVC-B/HPDNA in buffer.

Probe	A	C (mol L^{-1})	ε (M^{-1} cm^{-1})
EBMVC-B	0.0337	1.00×10^{-6}	3.37×10^{4}
EBMVC-B/ HPDNA	0.0346	1.00×10^{-6}	3.46×10^{4}

A: Absorption of the sample; C: Concentration of the tested sample (mol L^{-1}); ε : Molar extinction coefficient (M^{-1} cm^{-1}).

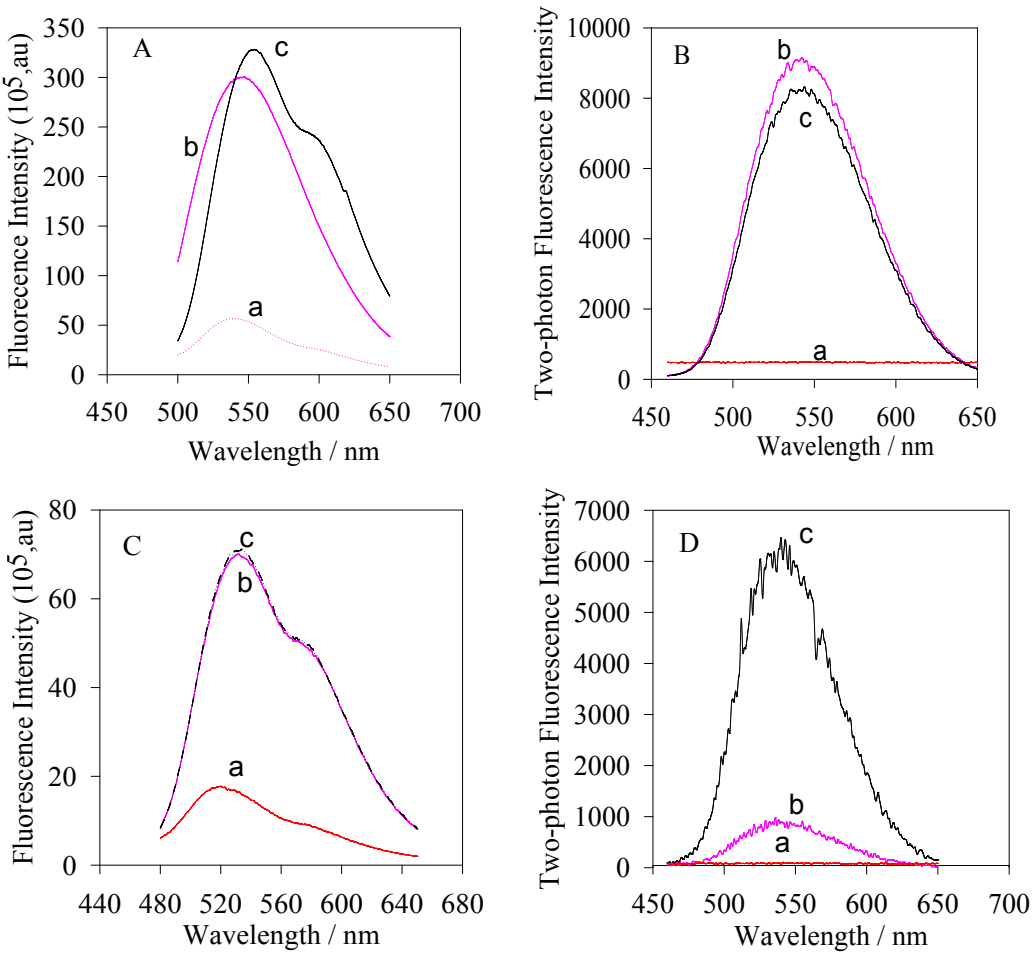

Figure S1. OPE (A) and TPE (B) fluorescence emission spectra of the only cell growth media (curve a), EBMVC-B/HPDNA (5.0 μM/1.0 μM) in HEPES buffer solution (curve b), and EBMVC-B/HPDNA (5.0 μM/1.0 μM) in cell growth media (curve c). (C) OPE and (D) TPE fluorescence emission spectra of the AgNPs/DNA/TPdye nanoprobe (1.0 μM, concentration of the nanoprobe refers to the concentration of HPDNA) under the absence (curve b) or presence (curve c, the final concentration of GSH is 100 μM) of GSH in cell growth medium. Only the cell growth medium was taken as the control experiment (curve a). The experimental steps were described in experimental section.

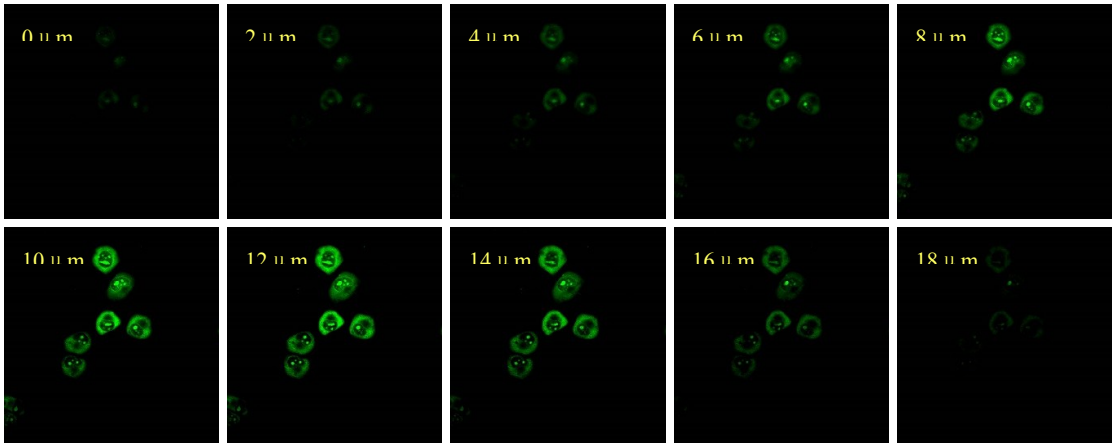

Figure S2. Z-scanning confocal fluorescence microscopy images of HeLa cells incubated with AgNPs/DNA/TPdye nanoprobe.

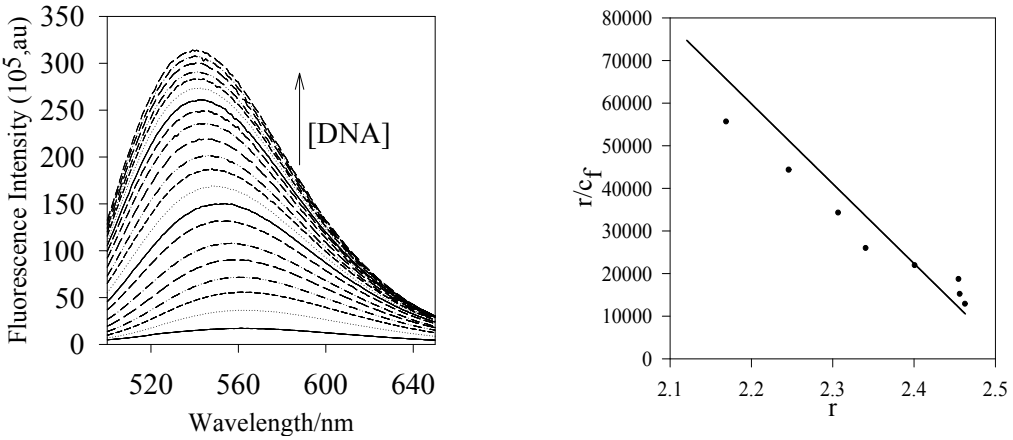

Figure S3. One-photon fluorescence titration of EBMVC-B with HPDNA in 20 mM HEPES buffer solution (PH 7.4) (left) and their fitted curve according to Scatchard equation (right). λ_{ex} = 450 nm; [EBMVC-B] = 500 nM; [HPDNA] = 0 ~ 250 nM.

Figure S4 Fluorescence emission intensities of the TPdye (EBMVC-B) under the presence of different DNA sequences in 20 mM HEPES buffer solution. All the DNA sequence's concentration is 100 nM. [EBMVC-B] = 500 nM. $\lambda_{ex}/\lambda_{em}$ = 450 nm/550 nm.

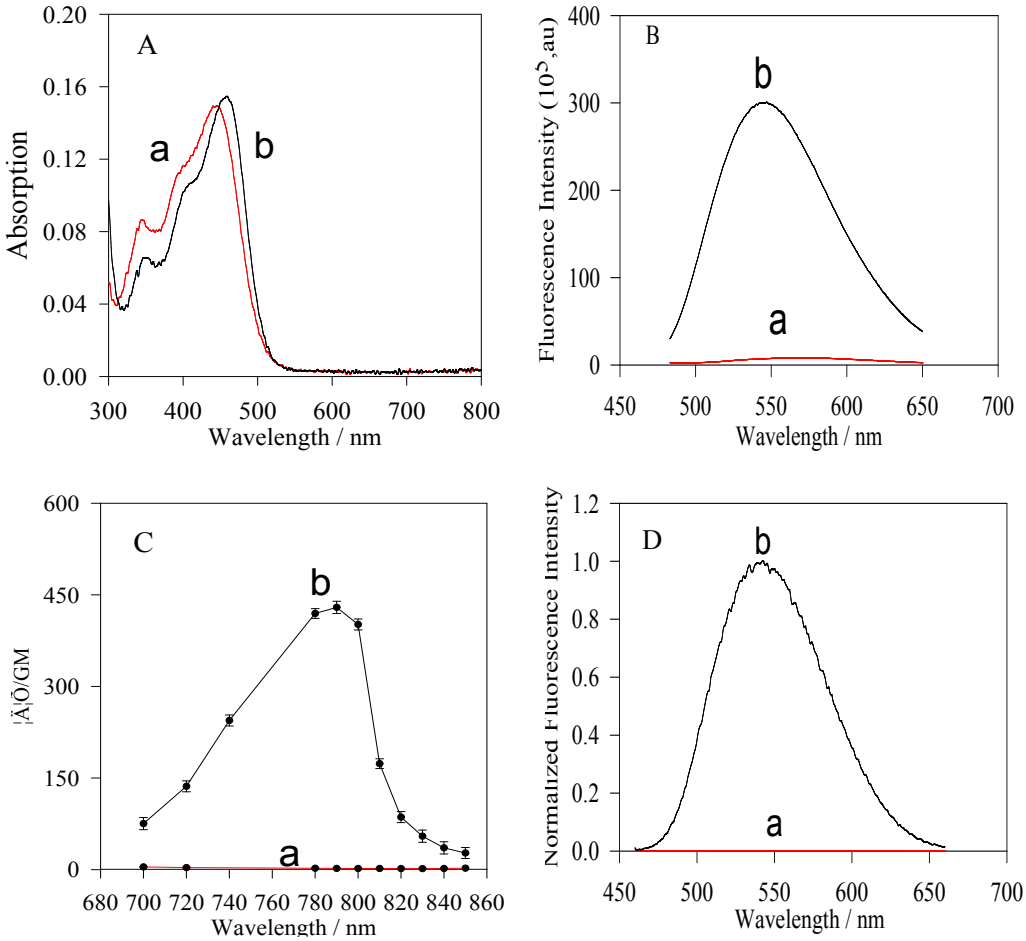

Figure S5. The absorbance spectra (A) and the OPE fluorescence emission spectra (B) of EBMVC-B (1.0 μM, curve a) and EBMVC-B/HPDNA complex (1.0 μM/200 nM,

curve b) in 20 mM HEPES buffer solution (pH 7.4). TPE action cross sections (C) and TPE fluorescence emission spectra (D) of EBMVC-B (5.0 μM, curve a) and EBMVC-B/HPDNA complex (5.0 μM/1.0 μM, curve b) in 20 mM HEPES buffer solution (pH 7.4). For 'B', λ_{ex} = 450 nm; for 'D', λ_{ex} = 800 nm.

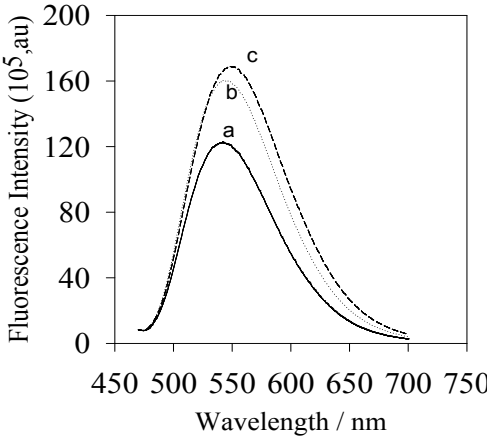

Figure S6. Fluorescence emission spectra of the EBMVC-B/HPDNA complex in 20 mM HEPES buffer solution under the different concentrations of EBMVC-B (a: 200 nM; b: 500 nM; c: 1000 nM). [HPDNA] = 100 nM. λ_{ex} = 450 nm.

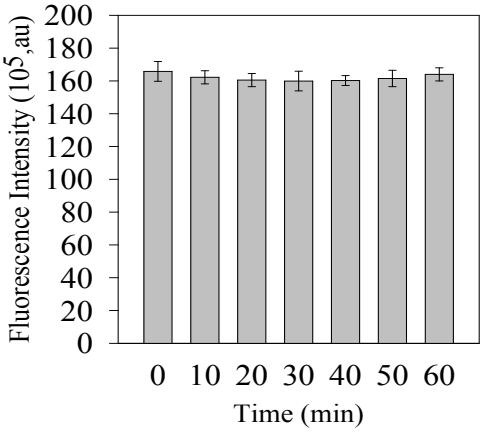

Figure S7. Fluorescence emission intensity changes of EBMVC-B/HPDNA complex as a function of time in 20 mM HEPES buffer solution under xenon lamp as an excitation. $\lambda_{ex}/\lambda_{em}$ = 450 nm/550 nm, [HPDNA]= 100 nM, [EBMVC-B] = 500 nM.

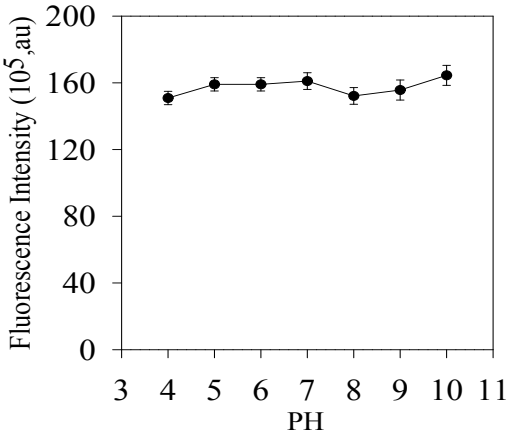

Figure S8. Effect of pH on the one-photon excited fluorescence intensity of EBMVC-B/HPDNA complex in 20 mM HEPES buffer solution. $\lambda_{ex}/\lambda_{em}$ = 450 nm/550 nm, [HPDNA] = 100 nM, [EBMVC-B] = 500 nM.

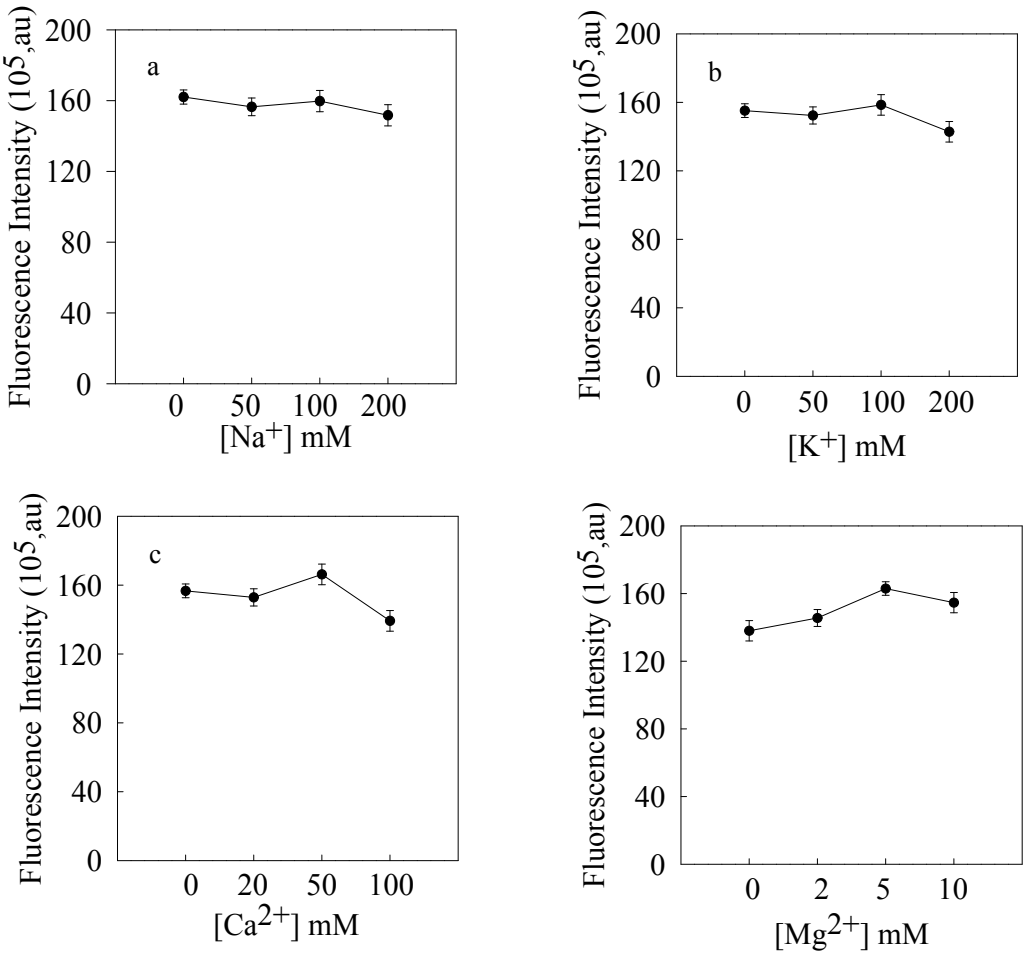

Figure S9. Effect of physiological relevant metal ions including Na^+, K^+, Ca^{2+} and Mg^{2+} on the one-photon excited fluorescence intensity of EBMVC-B/HPDNA complex in 20 mM HEPES buffer solution. $\lambda_{ex}/\lambda_{em}$ = 450 nm/550 nm, [HPDNA] = 100 nM, [EBMVC-B] = 500 nM.

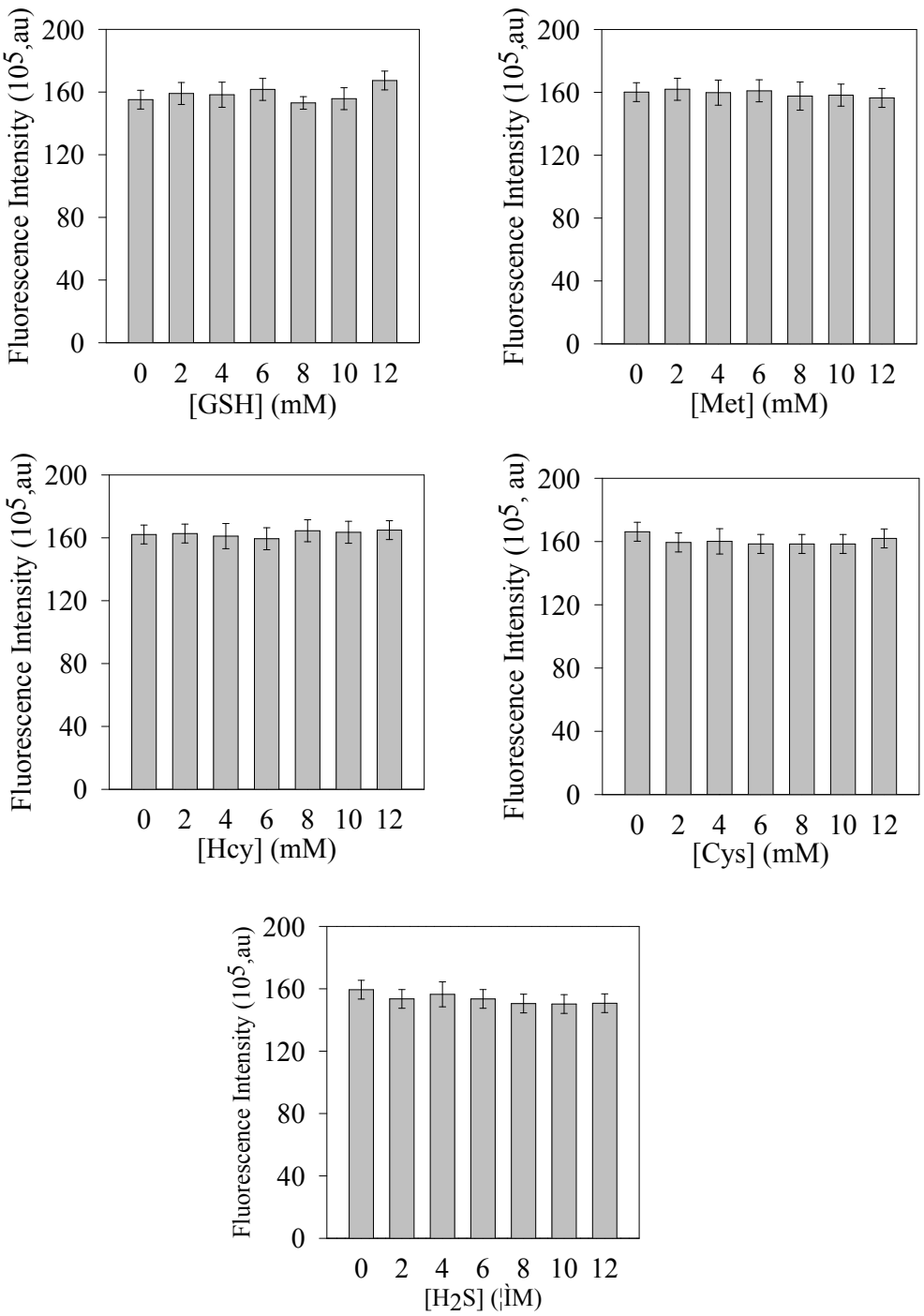

Figure S10. Effect of sulfur-containing bioactive compounds including GSH, Cys, Hcy, Met, H_2S on the one-photon excited fluorescence intensity of EBMVC-B/HPDNA in 20 mM HEPES buffer solution. $\lambda_{ex}/\lambda_{em}$ = 450 nm/550 nm, [HPDNA] = 100 nM, [EBMVC-B] = 500 nM.

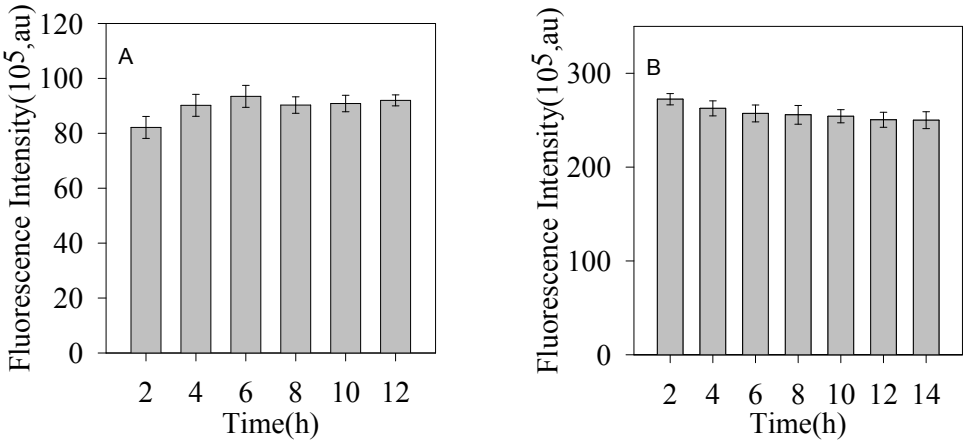

Figure S11. Fluorescence emission intensity changes of EBMVC-B/HPDNA complex as a function of time in cell lysate (A) or human serum (B). $\lambda_{ex}/\lambda_{em}$ = 450 nm/550 nm, [HPDNA] = 100 nM, [EBMVC-B] = 500 nM.

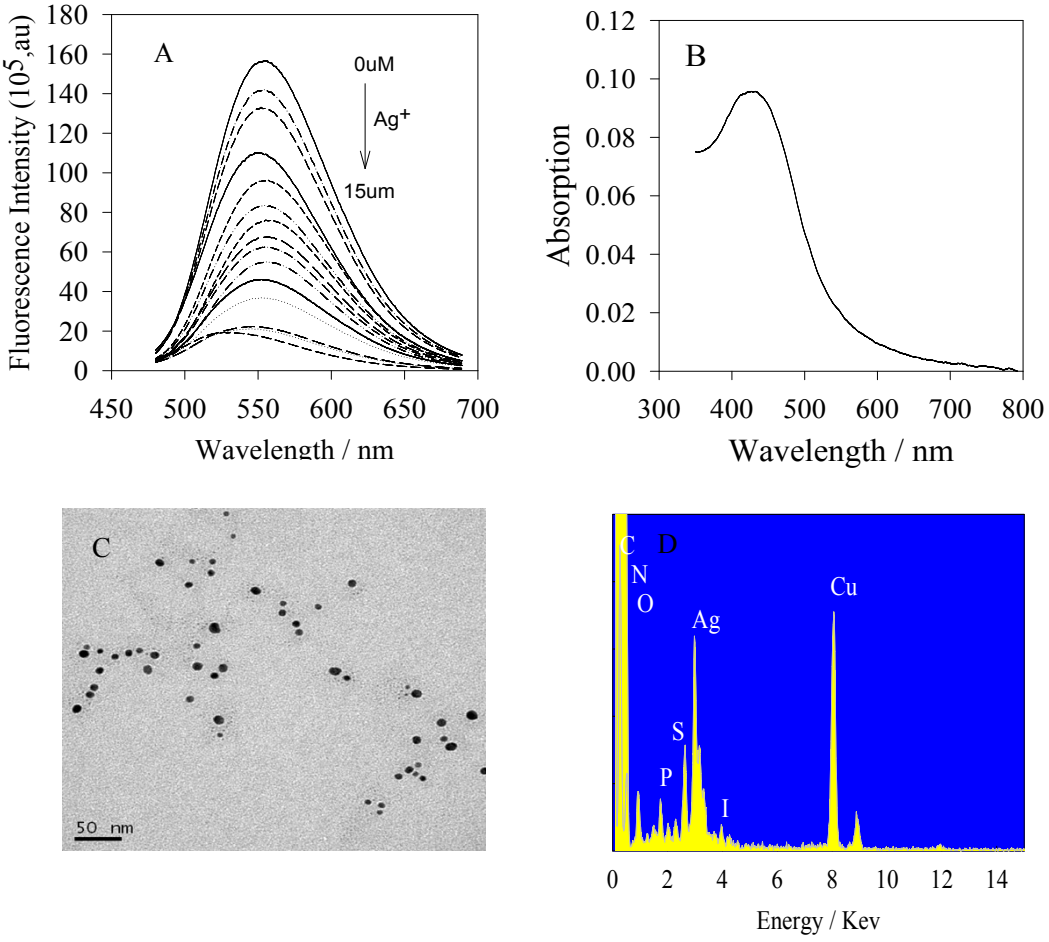

Figure S12. (A) Fluorescence emission spectra (λ_{ex} = 450 nm) of EBMVC-

B/HPDNA complex (500 nM/100 nM) under the different concentrations of Ag^+, the arrow indicates fluorescence signal changes as the Ag^+ concentrations increase from 0 to 12 μM. The used $NaBH_4$ concentration is same as Ag^+. The experiment was carried out as described in experimental section. (B) The corresponding UV-vis absorption spectra of (A, the concentration of Ag^+ is 10 μM), showing formation of the DNA-templated AgNPs. (C) TEM image and (D) EDX spectroscopy analysis of the prepared AgNPs/DNA/TPdye nanoprobe.

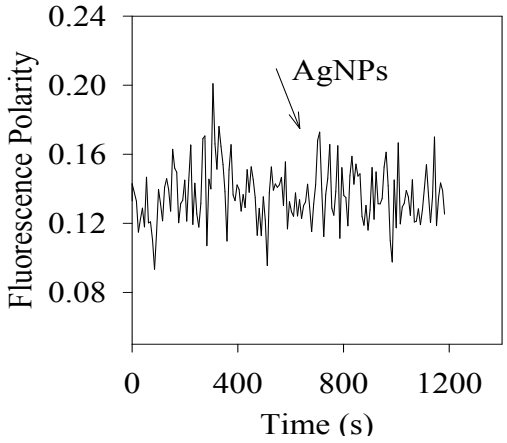

Figure S13. Real-time fluorescence polarization changes of EBMVC-B upon the formation of AgNPs. The formation time of AgNPs is marked with an arrow. $\lambda_{ex}/\lambda_{em}$ = 450 nm/550 nm, [EBMVC-B] = 500 nM, [Ag^+/$NaBH_4$] = 10 μM.

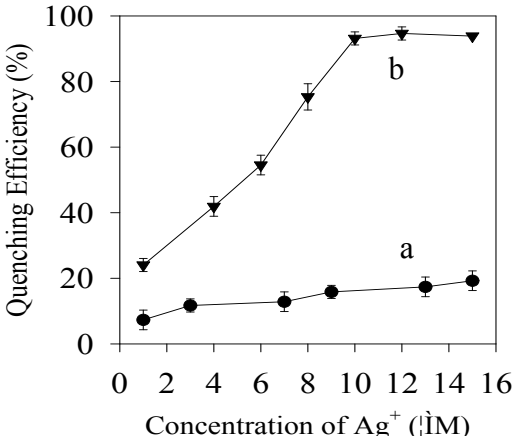

Figure S14. Quenching efficiency of the fluorescence emission at 550 nm (QE%) of the EBMVC-B/HPDNA complex with various concentrations of AgNPs (a, the concentration of AgNPs refers to the concentration of Ag^+) or Ag^+ (b). λ_{ex} = 450 nm, [HPDNA] = 100 nM, [EBMVC-B] = 500 nM.

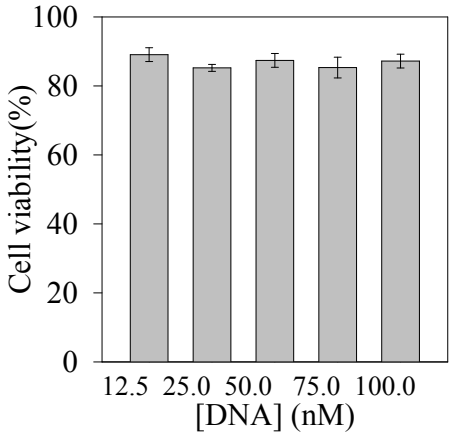

Figure S15. Cell viability of HeLa treated with different concentrations of AgNPs/DNA/TPdye nanoprobe for 24 h in fresh medium. Note: The concentration of the nanoprobe refers to the concentration of HPDNA.