

## SUPPORTING INFORMATION

### **Silver metallization engineered conformational switch of G-quadruplex for fluorescence turn-on detection of biothiols**

#### **EXPERIMENTAL SECTION**

**Materials and Instrumentation:** The DNA oligomer was synthesized by Shanghai Sangon Biological Engineering Technology & Services (Shanghai, China). The sequence was as follows: G-quadruplex DNA: 5'-GGG-TTT-TGG-GTT-TTG-GGT-TTT-GGG-3'. NMM was purchased from Porphyrin Products (Logan, UT), and its concentration was measured by using absorbance spectroscopy on a JASCO V-550 and found to be  $\lambda = 379$  nm, assuming an extinction coefficient of  $1.45 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ . Silver nitrate ( $\text{AgNO}_3$ ) and sodium borohydride ( $\text{NaBH}_4$ ) were purchased from Alfa Aesar. Other chemicals including amino acids and biothiols were purchased from Sigma–Aldrich and used without further purification. All water used to prepare buffer solutions was obtained by using a Milli-Q water system. **All measurements were performed in 10 mM Tris- $\text{HNO}_3$ , 100 mM  $\text{NaNO}_3$ , 10 mM  $\text{KNO}_3$ , pH 7.5 buffer.**

The fluorescence spectra were recorded using a JASCO FP6500 spectrophotometer (JASCO International Co. Ltd., Tokyo, Japan). The concentration of DNA was determined using a JASCO V-550 UV/Vis spectrophotometer, equipped with a temperature-controlled cuvette holder. TEM images were recorded using a FEI TECNAI G2 20 high-resolution transmission electron microscope operating at 200 kV. CD spectra were determined using a Jasco 810

(Jasco International Co., LTD., Tokyo, Japan)

**Synthesis of DNA–Silver Nanohybrids:** The DNA–silver nanohybrids were synthesized by reduction of  $\text{AgNO}_3$  with  $\text{NaBH}_4$ . Briefly, DNA was mixed excess  $\text{AgNO}_3$  (30:1  $\text{AgNO}_3/\text{DNA}$  molar ratio). After 5 min of incubation, freshly prepared  $\text{NaBH}_4$  solution ( $\text{AgNO}_3$  and  $\text{NaBH}_4$  were mixed in a 1:5 molar ratio) was dropped into the above aqueous solution under vigorous stirring. After mixing, the resulting yellow colloidal silver solution was stirred for another 30 min. As shown in Fig. S4, the UV–vis absorption spectra of the silver metalized DNA exhibited the DNA absorption peak at 260 nm and the surface plasmon response band centered at 445 nm. Due to the stronger electrostatic repulsion than Vander Waals attraction between DNA stabilized nanoparticles, DNA-templated silver deposition was monodisperse as shown in the TEM image (Fig. S5).

**Circular dichroism (CD) measurements:** CD spectra were carried out on a JASCO J-810 spectropolarimeter equipped with a temperature controlled water bath. The optical chamber of CD spectrometer was deoxygenated with dry purified nitrogen (99.99 %) for 45 min before use and kept the nitrogen atmosphere during experiments. Three scans were accumulated and automatically averaged. All of the CD experiments were taken at room temperature (25°C), in pH 7.5 Tris- $\text{HNO}_3$  buffer,  $[\text{DNA}] = 3 \times 10^{-6}$  M,  $[\text{Metalized DNA}] = 3 \times 10^{-6}$  M,  $[\text{biothiols}] = 0.6 \times 10^{-6}$  M.

**Simply Mixture of Silver Nanoparticles and G-quadruplex DNA:** Silver nanoparticles were synthesized by reduction of  $\text{AgNO}_3$  by  $\text{NaBH}_4$  in the presence of sodium citrate.<sup>[1]</sup> Then silver nanoparticles at the molar ratio of Ag : DNA equal to 30: 1 were added to the solution of G-quadruplex. After incubation for 30 min, NMM was added to the solution. The final

fluorescence value of NMM was close to the emission intensity of the G-quadruplex/NMM complex, which was mainly due to the fact that the G-quadruplex could not effectively adsorb onto gold nanoparticles or silver nanoparticles.<sup>[2]</sup> Therefore, the results of the experiments confirmed the homogeneity of the AgNP-DNA hybrid synthesized by DNA metallization method.

**Fluorescence Assay for Biothiols Detection:** In a typical procedure, DNA–silver nano hybrid (DNA=0.5×10<sup>-6</sup> M, 200 μL) was mixed with different concentrations of Biothiols or other analytes (2.5 μL). The mixture was equilibrated for 3 h at room temperature. Finally, NMM (0.1×10<sup>-3</sup> M, 3 μL) was added and equilibrated for 5 min before spectral measurements. The solutions were excited at 399 nm, and emission spectra were collected from 550 to 750 nm.

**Visual Detection of Biothiols:** Biothiols or other interfering substances (2.5 μL, 0.24× 10<sup>-3</sup> M) were added to DNA–silver nano hybrids (DNA = 1.5 × 10<sup>-6</sup> M, 200 μL). After 3 h of incubation, NMM (0.1×10<sup>-3</sup> M, 9 μL) was added to react for another 5 min. Photographs of the solutions were taken using a UV transilluminator.

**Determination of Binding Constants:**<sup>[2]</sup> Binding constants were measured by fluorescence titration methods, in which fixed concentrations of DNA-silver hybrid titrated with increasing biothiols concentrations.

According to the 1 : n binding mode, the binding reaction can be described as:



Where P denotes DNA-silver hybrid, M denotes biothiols, and PM denotes silver-thiols conjugates.

So, the binding constant can be described as:

$$K_b = [PM_n]/[P][M]^n \quad (2)$$

With

$$[P] = [P_0] - [PM_n] \quad (3)$$

$$[M] = [M_0] - n[PM_n] \quad (4)$$

Where  $[P_0]$  is the concentration of DAN-silver hybrids and  $[M_0]$  is the concentration of biothiols

So,

$$K_b = [PM_n] / \{ [P_0] - [PM_n] \} \{ [M_0] - n[PM_n] \}^n \quad (5)$$

The formation of silver-thiol conjugates  $PM_n$  can be quantitated by the fluorescence signal that satisfies the following equation:

$$F = F_0 + (F_\infty - F_0) [PM_n] / [P_0]$$

So

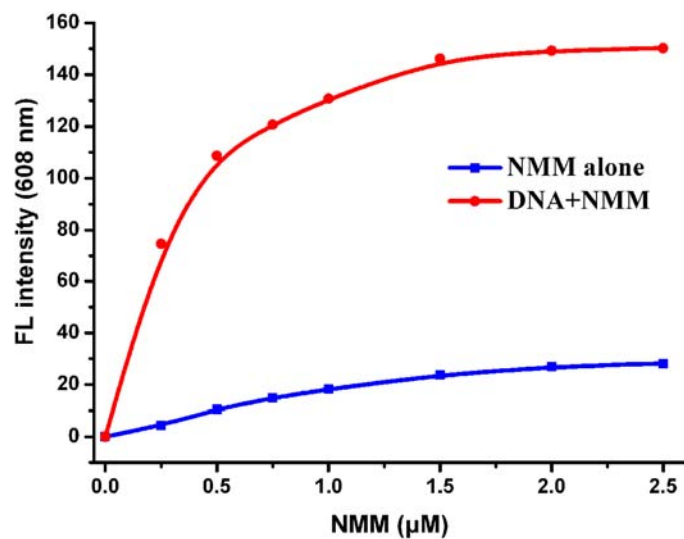
$$K_b = \{ \Delta F \times [P_0] / \Delta F_{\max} \} / \{ [P_0] - \Delta F \times [P_0] / \Delta F_{\max} \} \{ [M_0] - n \Delta F \times [P_0] / \Delta F_{\max} \}^n \quad (7)$$

Where  $\Delta F = F - F_0$ ,  $\Delta F_{\max} = F_{\max} - F_0$

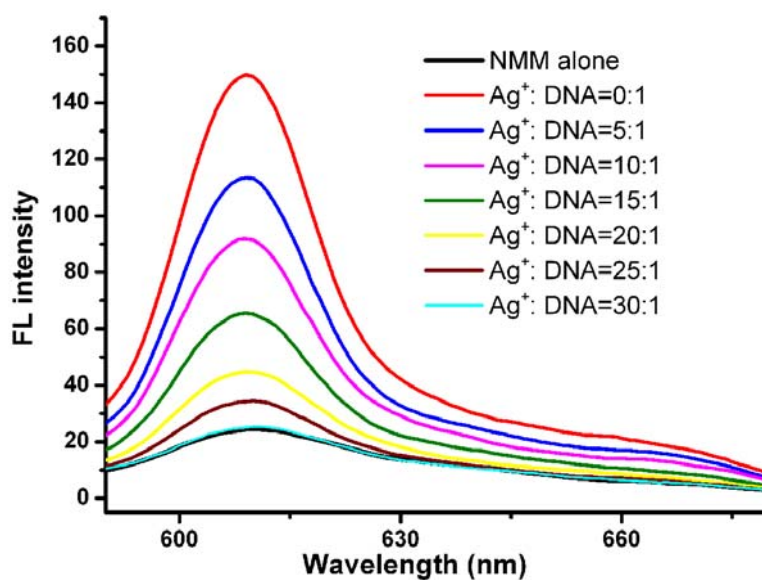
Nonlinear least-squares analysis of the fluorescence titration data (Fig 2, Fig S7 and Fig S8) yielded the binding constants of Cys, Hcy and GSH were  $2.734 \times 10^{11} \text{ M}^{-1}$ ,  $2.824 \times 10^{11} \text{ M}^{-1}$ ,  $2.656 \times 10^{11} \text{ M}^{-1}$ , respectively.

**Preparation of Real Samples:** Fresh human blood samples were collected in tubes containing EDTA, and centrifuged at 5000 rpm for 20 min. The supernatant solution, which contains proteins and amino acids, was used as the source of plasma. Since most of the thiol compounds in plasma were linked to other thiols and proteins by disulfide bonds, it was necessary to reduce disulfide bonds before analysis and the following procedure according to literatures was carried out. Firstly, 40  $\mu\text{l}$  of HCl (0.2 M) and 20  $\mu\text{l}$  of  $\text{PPh}_3$  (400 mM in  $\text{H}_2\text{O}-\text{CH}_3\text{CN}$  20:80 v/v and 2.0 M HCl) were added to 500  $\mu\text{l}$  of plasma and incubated for 15 minutes at room temperature to hydrolyze the disulfide bonds. After that, 500  $\mu\text{l}$  of  $\text{CH}_3\text{CN}$  was mixed with 500  $\mu\text{l}$  of hydrolyzed plasma to precipitate plasma proteins followed by centrifugation at 3000g for 20 minutes twice. The supernate containing thiol compounds was collected for further analysis. **Since the thiols content of plasma is beyond the dynamic range**

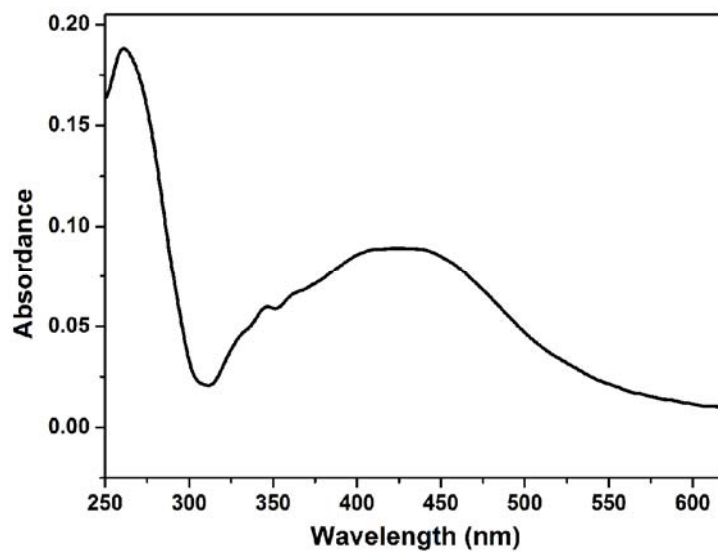
of the proposed method, the plasma sample has been appropriately diluted with Tris-HNO<sub>3</sub> buffer before measurement. For recovery studies known concentrations of Cys were added to plasma samples and the total thiol concentrations were then determined.<sup>[3-5]</sup>



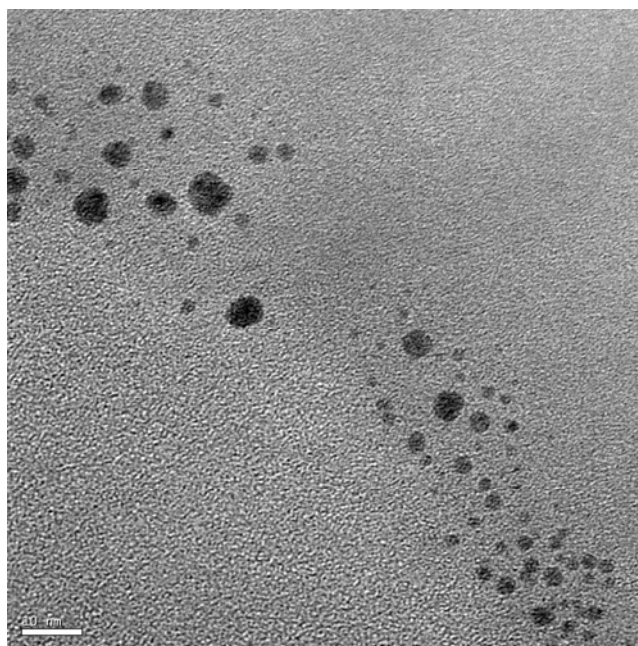
**Figure S1.** Fluorescence titration of the G-quadruplex DNA with NMM. Experiments were carried out in a buffer containing 10 mM Tris-HNO<sub>3</sub>, 100 mM NaNO<sub>3</sub>, 10 mM KNO<sub>3</sub>, pH 7.5, [DNA]=  $0.5 \times 10^{-6}$  M.



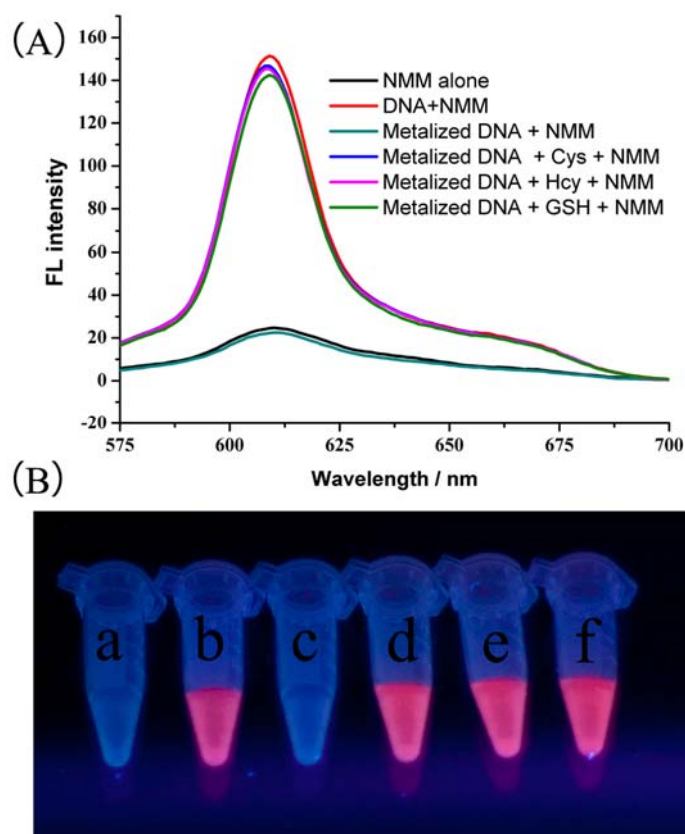
**Figure S2.** Fluorescence emission response profiles of NMM to the metalized DNA in different molar ratios of Ag (I) to G-quadruplex DNA. [NMM]=  $1.5 \times 10^{-6}$  M, [DNA] =  $0.5 \times 10^{-6}$  M.



**Figure S3.** UV-Vis absorption spectrum of DNA template silver deposition.

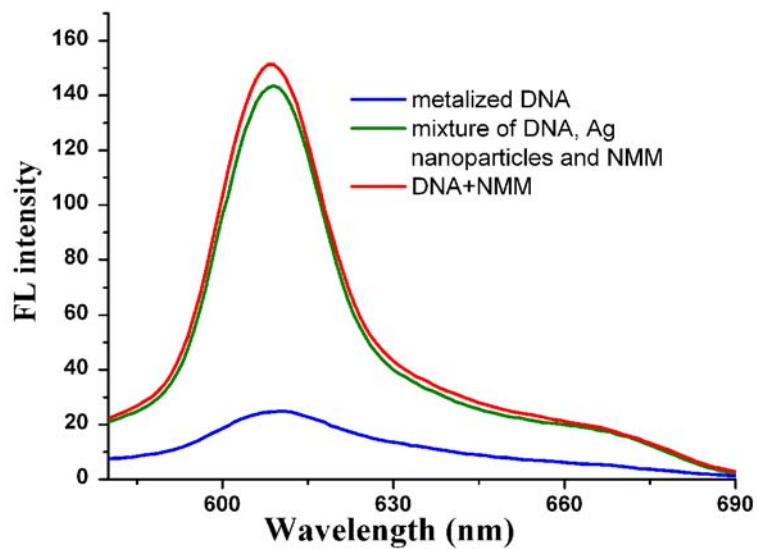


**Figure. S4** TEM image of DNA template silver deposition.

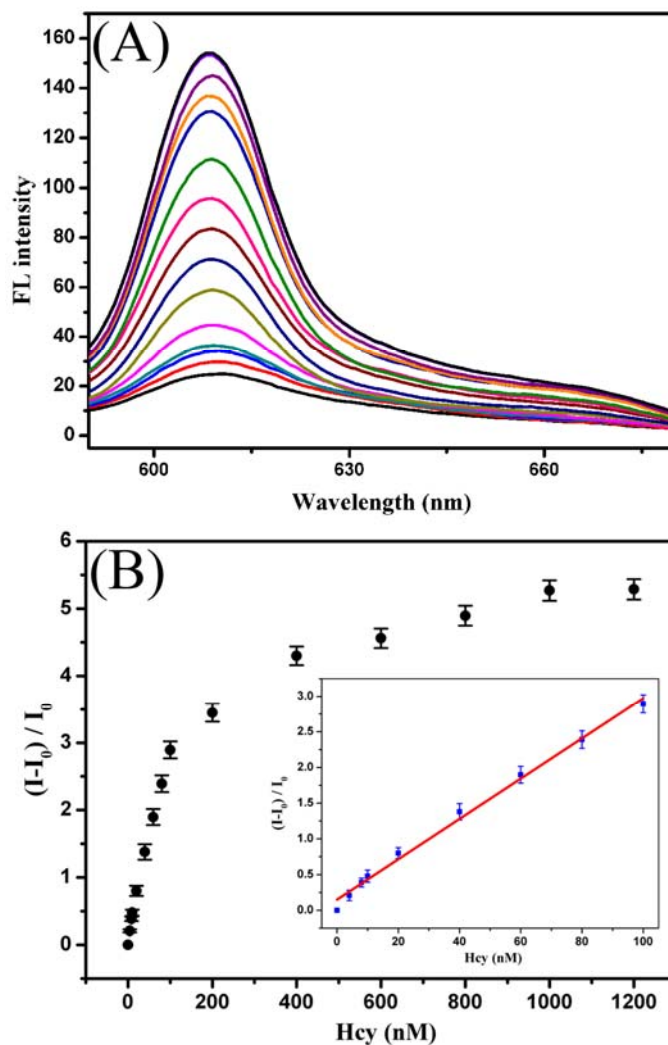


**Figure S5.** A) Fluorescence spectra of solutions containing NMM, NMM/metalized DNA, NMM/G-quadruplex DNA, and NMM/metalized DNA in the presence of biothiols, upon excitation at  $\lambda = 399$  nm,  $[\text{NMM}] = 1.5 \times 10^{-6}$  M,  $[\text{DNA}] = 0.5 \times 10^{-6}$  M,  $[\text{biothiols}] = 1 \times 10^{-6}$  M. FL = fluorescence. B) Photographs of solutions containing a) NMM, b) NMM/metalized DNA, c) NMM/G-quadruplex DNA, and d), e), f) NMM/metalized DNA in the presence of Cys, Hcy, GSH taken using a UV transilluminator.  $[\text{NMM}] = 4.5 \times 10^{-6}$  M,  $[\text{DNA}] = 1.5 \times 10^{-6}$  M,  $[\text{biothiols}] = 3 \times 10^{-6}$  M.

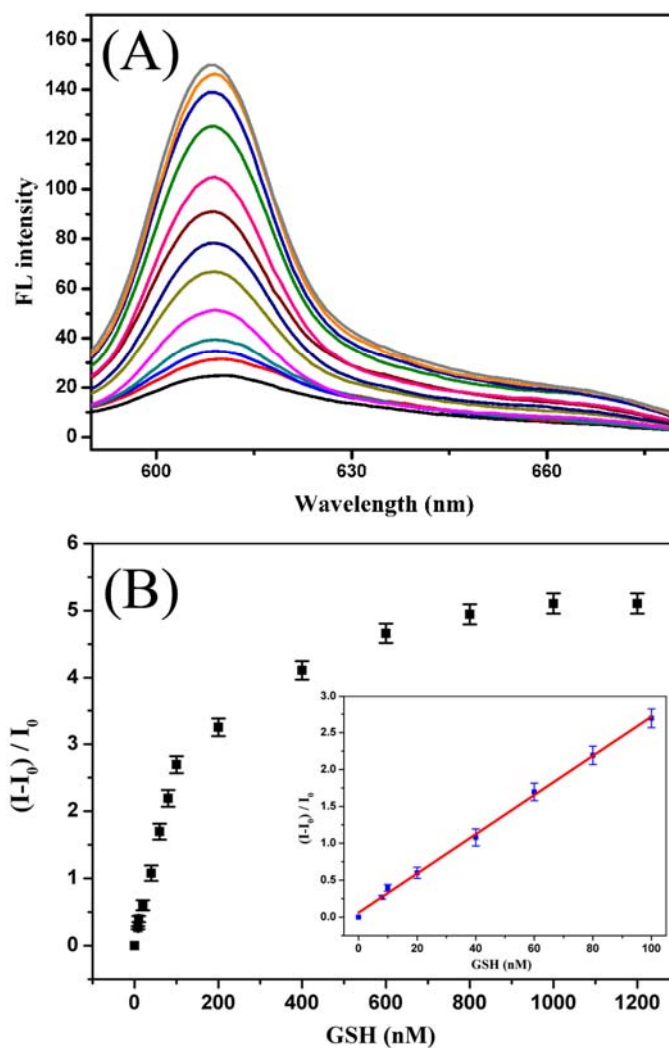




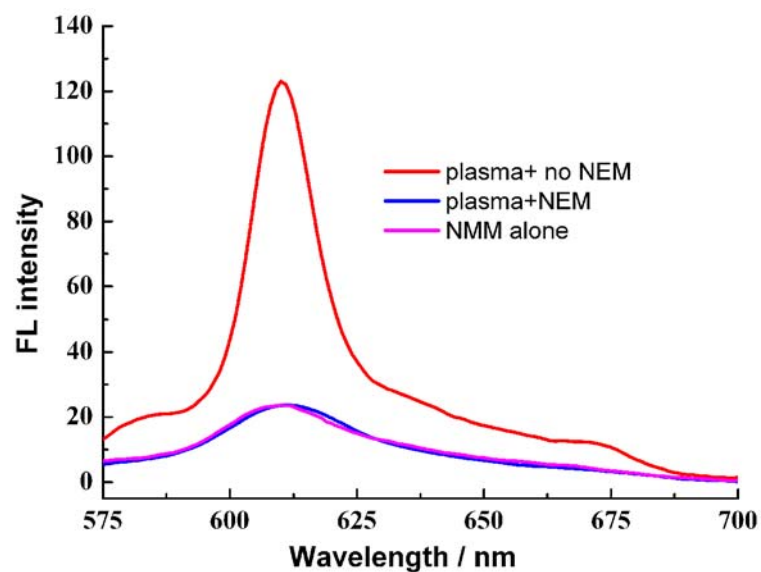
**Figure S6.** Fluorescence emission spectra of solutions containing DNA-silver hybrid/NMM, DNA/silver nanoparticles/NMM, and DNA/NMM, respectively. [NMM] =  $1.5 \times 10^{-6}$  M, [DNA] =  $0.5 \times 10^{-6}$  M, [biothiols] =  $1 \times 10^{-6}$  M.



**Figure S7.** (A) Fluorescence emission spectra of NMM/DNA-silver hybrids in the presence of increasing Hcy concentrations ( $0$ – $1.2 \times 10^{-6}$  M).  $[\text{NMM}] = 1.5 \times 10^{-6}$  M,  $[\text{DNA}] = 0.5 \times 10^{-6}$  M. (B) Plots of the fluorescence intensity measured at 609 nm as a function of the Hcy concentration.  $[\text{NMM}] = 1.5 \times 10^{-6}$  M,  $[\text{DNA}] = 0.5 \times 10^{-6}$  M. Inset: the linear plot. Error bars were estimated from at least three independent measurements.



**Figure S8.** (A) Fluorescence emission spectra of NMM/DNA–silver hybrids in the presence of increasing GSH concentrations ( $0$ – $1.2 \times 10^{-6}$  M).  $[\text{NMM}] = 1.5 \times 10^{-6}$  M,  $[\text{DNA}] = 0.5 \times 10^{-6}$  M. (B) Plots of the fluorescence intensity measured at 609 nm as a function of the GSH concentration.  $[\text{NMM}] = 1.5 \times 10^{-6}$  M,  $[\text{DNA}] = 0.5 \times 10^{-6}$  M. Inset: the linear plot. Error bars were estimated from at least three independent measurements.



**Figure S9.** Fluorescence changes induced by prepared human plasma with or without pretreatment by thiol blocking agent, NEM.

**Table S1.** Determination of thiol compounds in human plasma

Sample	Determined thiol compounds ( $\mu\text{M}$ )	Added Cysteine ( $\mu\text{M}$ )	Found ( $\mu\text{M}$ )	Recovery (%)	RSD (n=3, %)
1	230.6	100	332.5	102.5	3.69
		100	428.7	99.8	1.82
2	275.2	100	373.9	97.5	5.17
		100	477.1	103.8	7.33

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