Supporting Information

A Highly Sensitive Protocol (FRET/SIMNSEF) for Determination of Mercury Ions: Unity of Fluorescence Quenching of Graphene and Enhancement of Nanogold

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1. Experimental Details

1.1 Materials

$HAuCl_4 \cdot 4H_2O$ (99.9%), $NaBH_4$ (99%), Vitamin C (99.9%), CTAB (99%) and $AgNO_3$ (99.9%), tri-sodium citrate (99.9%), 1-Ethyl-3-(3-dimethyl aminopropyl) carbodiimide hydrochloride (EDC), N-hydroxy succinimide (NHS), 2-(N-Morpholino) ethanesulfonic acid (MES), tetraethoxy silane (TEOS) and 3-N-morpholinopropanesulfonic acid (MOPS) were purchased from Aldrich. DNA oligomers were purchased from Sangon (Shanghai, China) and used without further purification. All experiments were carried out in aqueous MOPs buffer (10 mM MOPs with 50 mM $NaNO_3$, pH 7.2) unless stated otherwise. Graphite powder was purchased from China National Pharmaceutical Group Corporation (Shanghai, China). Other chemicals were of analytical grade. The double distilled water that was used throughout the experiments was purified using a Milli-Q system.

1.2 Synthesis of gold nanorods (GNRs):

Tunable GNRs were synthesized by seed-mediated approach, which could be referred to our previous report. Preparation of the GNRs could be divided into three steps. As an initial step, preparation of seed solution could be described as follows in detail. 10 mL 0.5 mM $HAuCl_4$ was mixed with 10 mL 0.2 M CTAB. Subsequently, 600 μL 0.02 M ice-cold $NaBH_4$ was added to the mixed solution. Vigorous stirring of the seed solution could be kept for 120 s; furthermore, the seed solution has been settled at room temperature for 1h. As the second step, the growth solution of the GNRs was prepared. 10 mL 0.2 M CTAB was mixed with 1 mL 4 mM $AgNO_3$ solution at ambient temperature. Then, 10 mL 1 mM $HAuCl_4$ was added to the growth solution followed by addition of 140 μL 0.08 M vitamin C. Finally, the GNRs were prepared by addition of 24 μL seed

solution to the growth solution. The formation of GNRs with different aspect ratios can be resorted to addition of amount of $AgNO_3$.

1.3 Triangular gold nanoplates (TGNs) and pseudo-spherical gold nanoparticles (PSGNs)

The TGNs have been synthesized by seed-mediated approach in our previous work. 0.5 ml 10 mM sodium citrate was diluted with 18 ml fresh redistilled water and 0.5 ml 10 mM $HAuCl_4$ was added into the solution. Subsequently, 0.5 ml 100 mM $NaBH_4$ was quickly added to the mixture and stirred for 120 s so as to yield gold nanoparticle seeds. The resulting mixture was aged for 2 - 6 hours in order to allow the hydrolysis of unreacted $NaBH_4$. After the aging period, three growth solutions were prepared for the seed-mediated growth step. The first two solutions (labeled a and b) contained 0.25 ml 10 mM $HAuCl_4$, 0.05 ml 100 mM NaOH, 0.05 ml 100 mM vitamin C, and 9 ml the purified CTAB solution by re-crystallized process. The final growth solution (designated c), contained 2.5 ml 10 mM $HAuCl_4$, 0.50 ml 100 mM NaOH, 0.50 ml 100 mM vitamin C, and 90 ml the as-prepared CTAB solution. Controlled amounts of KI, which were added to the original CTAB solutions labeled as a, b and c**,** were at the same iodide concentration. Final concentrations of 50 μM iodide were prepared by adding aliquots of a 0.1 M solution of KI to the as-prepared 250 ml CTAB solutions. Optimization based on addition amount of KI can form TGNs with different aspect ratios. On the other hand, the PSGNs could be formed without addition amounts of KI at the same reaction conditions.

1.4 Synthesis of anisotropic silica shell-insulated gold nanoparticles

GNRs@SiO_2: The representative GNRs with aspect ratio 2.5 have been choosen for the silica coating. Before performing silica coating, the as-prepared GNRs were centrifuged for once at a speed of 10000 rpm to remove excess CTAB. The PH value of 10 mL GNRs solution was tuned to approximately 9 via addition of ammonia aqueous solution. Subsequently, 0.2 mL, 0.56 ml and 0.9 mL 10 mM TEOS ethanol solution was added to the GNRs solution, respectively, so as to yield silica coating with different thickness, which corresponds to ca 7nm, 11 nm and 20 nm. After addition of TEOS, the solution has been vigorously vibrated for 1h so as to promote hydrolysis and condensation of TEOS on the surface of GNRs and efficiently inhibit formation of mesoporous silica. The solution was kept immobile for overnight until the reactions were finished. Silica-coated GNRs with different thickness should be repeated rinsed by water and ethanol for a couple of times and collected by centrifugation so as to form well-dispersed silica coated GNRs for subsequent characterization and experiments.

TGNs@SiO_2: The as-synthesized gold nanoplates were centrifuged 10 mL aliquots at 4500 rpm so as to remove excess CTAB surfactant. After that, the collected precipitate was redispersed in 10 mL of DI water. 1 mL of 20mM NaOH solution was added upon stirring in order to adjust PH value to ca. 10. Subsequently, addition of 12 μL 20% TEOS in ethanol has been

performed for every 30 minute intervals under vigorous stirring. Following this step, the reaction mixture was continuously reacted for 3 days to form silica coating with thickness 10 nm. The following rince process was the same as it did on GNRs.

PSGNs@ SiO_2: The pesudo-spherical nanoparticles were centrifuged 10 mL aliquots at 8000 rpm so as to remove excess CTAB surfactant. The PH value of 10 mL PSGNs solution was tuned to approximately 9. Then, 0.56 mL 10 mM TEOS ethanol solution was added to the PSGNs solution. The reaction mixture was continuously reacted for overnight to form 10 nm silica shell thickness. The following rince process was identical with the case of GNRs.

1.5 Synthesis of graphene oxide

GO was synthesized via a modified Hummers and Offeman's method from graphite flakes. In a typical reaction, 1 g of graphite and 1 g of $NaNO_3$ were taken in 500 mL round bottom flask and 45 mL of 98% H_2SO_4 was added. Then, 6 g of $KMnO_4$ was added slowly to the mixture at ice bath. After 1 h, the flask was sifted to oil bath and reaction mixture was allowed to stir at 35 °C for 2 h; subsequently, temperature is slowly increased up to 60 °C and kept stirring for 2 h. Finally, the reaction mixture was added to 200 mL of water, which was allowed to stir at 90 °C for 5 h and the reaction was ended via the addition of 10 mL of 30% H_2O_2, which results in change of color from yellow to brown. The warm solution was filtered and washed with 5% HCl and then with deionize water. The filter cake was dissolved in water, and sonicated to exfoliate oxidized graphene. The obtained mixture was centrifuged firstly at 2000 rpm for 5 min to remove all visible particles, and then centrifuged at 10000 rpm 30 min. The final sediment was redispersed in water with mild sonication, giving a solution of exfoliated GO.

1.6 MSO conjugated anisotropic SIMN

The anisotropic silica shell-insulated gold nanoparticles were first ammonified with 1% aminopropyltriethoxy silane (APTES) ethanol solution for one hour at room temperature. Ammonified SIAMN@SiO_2 nanostructures had been collected by centrifugation at 6000 rpm for 30 min, and were washed three times with water and twice with ethanol. MSO functionalized anisotropic SIMN were finished through the following process. The obtained ammonified anisotropic SIMNs were dispersed in 5 mL 100 mM MES buffer (pH 6.0) at room temperature. And they were mixed with 20 mM EDC and 20 mM NHS for stirring 1h. Following the activation step, the carboxylic group-modified DNA was added to a final concentration of 2 μM. The reaction mixture was stirred for about 16 h at room temperature. The samples were then centrifuged for 30 min at 7000 rpm and were washed three times with 100 mM MES buffer and twice with water, and obtained MSO functionalized SIMN.

1.7 Mercury fluorescence assays:

Mercury fluorescence assays were performed through two approaches. Process 1 (Fig 3A): 10 nmol of FAM-tagged mercury specific oligonucleotide was incubated with Hg^{2+} of a series of concentrations in a MOPs buffer (10 mM with 50 mM $NaNO_3$, pH 7.2) for 10 min. A GO solution (0.02 mg/mL) of 2 mL was added to this mixture. Fluorescence measurements were performed after 1 min. Process 2 (Fig 3B): the GO-SIMN-MSO complex was prepared by incubating the DNA (15 nM) with 2 mL 0.2 mg/mL GO solution for 5 min at room temperature. An addition of increasing concentrations of Hg^{2+} ions in a MOPs buffer was added to this mixture. Fluorescence measurements were performed after incubating for 20 min.

1.8 Measurements

Ultraviolet vision near infrared (UV-Vis-NIR) absorption spectra was recorded by using a Solidspec-3700 spectrophotometer. Transmission electron microscopy (TEM) image was obtained from a JEOL JEM-2010 instrument operated at 100 kV. Raman spectra were collected from DXR Smart Raman Spectrometer using 25 mW of 514 nm radiation from a helium neon laser. Integration times were provided as 30 s. The fluorescence spectra were recorded on spectrofluorometer (JY Fluorolog-3-Tou) with 3 cm quartz cell.

2. Additional Data

2.1 Raman Spectra of gaphene oxide

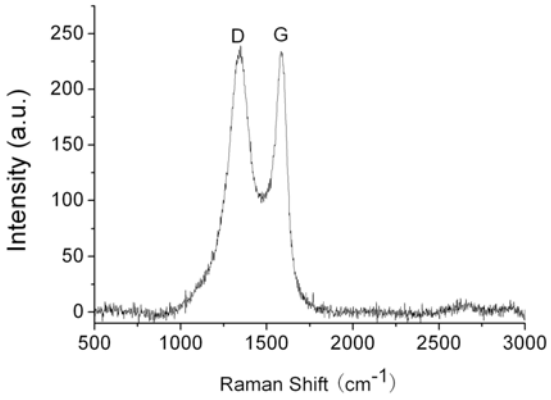

Fig. S1 Raman spectra (514 nm excitation) of GO

2.2 UV-Vis-NIR spectra of silica functionalized gold nanoparticles

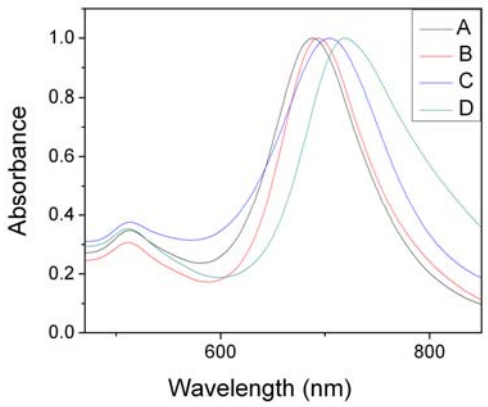

Fig. S2 LSPR of pristine (A) and GNRs@$SiO_2$with different SiO_2 thickness, 7 nm (B), 11 nm (C) and 20 nm (D), respectively.

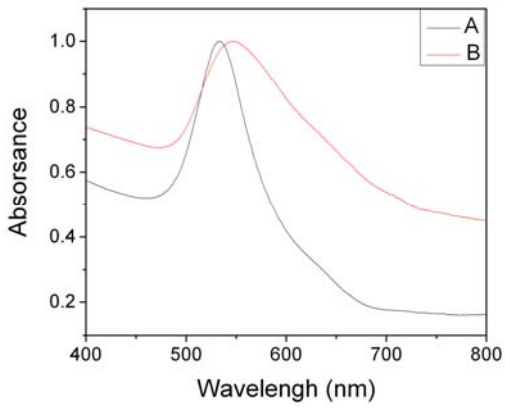

Fig. S3 LSPR of pristine (A) and PSGNs@SiO_2 with 11 nm SiO_2 thickness (B).

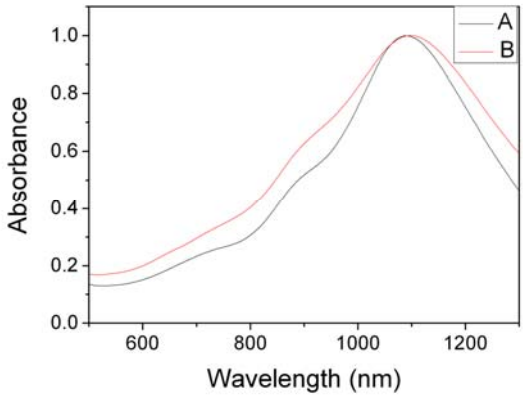

Fig. S4 LSPR of pristine (A) and TGNs@SiO_2 with 11 nm SiO_2 thickness (B).

2.3 Silica functionalized gold nanoparticles conjugated with MSO

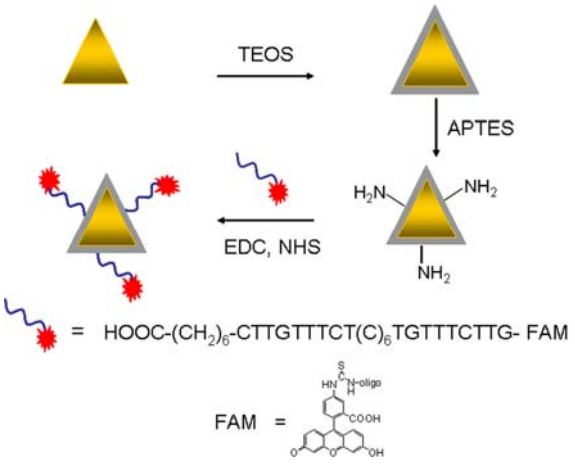

Fig. S5 Schematic graph of silica functionalized gold nanoparticles conjugated with MSO.

2.4 Fluorescence spectra of GO/ SIMN@SiO_2/MSO with different shell thickness

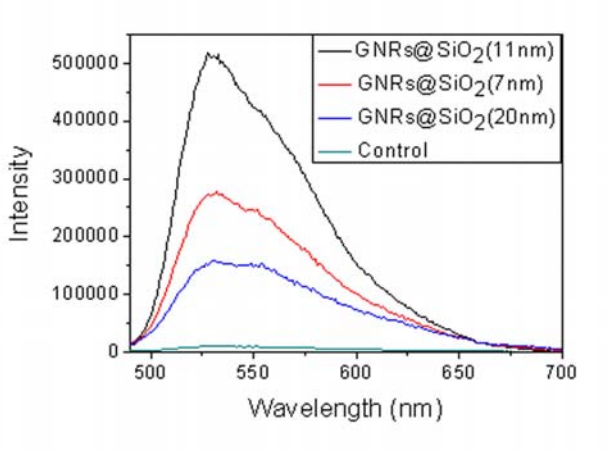

Fig. S6 Fluorescence spectra of GO/GNRs@SiO_2/MSO with different shell thickness in the presence of Hg^{2+} (10 nM): A) 7 nm, B) 11 nm, C) 20 nm and D) emission spectrum of control.

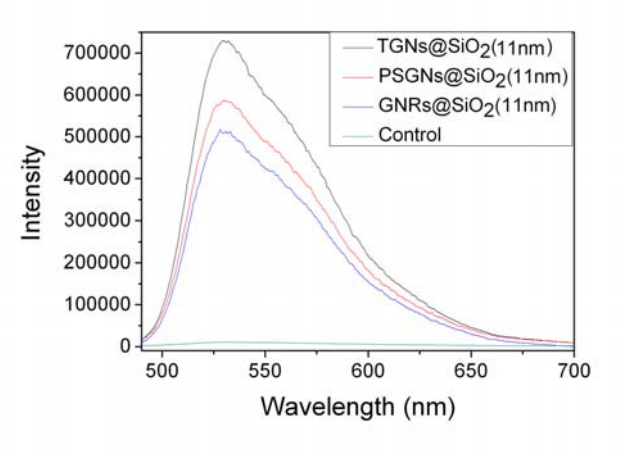

Fig. S7 Fluorescence spectra of GO/SIAMN@SiO_2/MSO with different shapes (TGNs, PSGNs, GNRs) in the presence of Hg^{2+} (10 nM): A) TGNs, B) PSGNs, C) GNRs and D) emission spectrum of control.

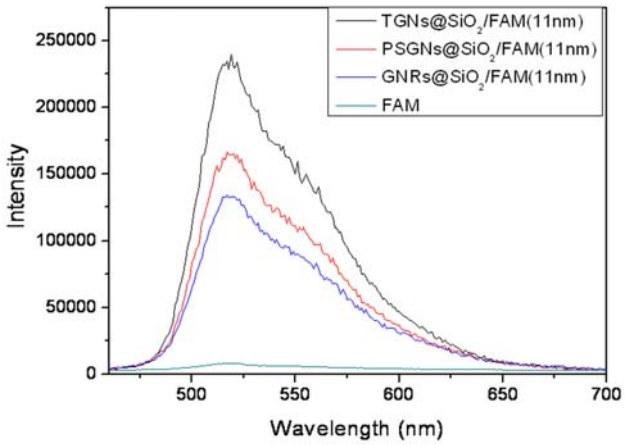

Fig. S8 Fluorescence spectra of 11 nm anisotropic SIMN@SiO_2/FAM with different shapes (TGNs, PSGNs, GNRs) in the absence of Hg^{2+} and GO, with 20 nM concentration based on the FAM; EFs of TGNs, PSGNs and GNRs are ca. 46, 33 and 26, respectively.

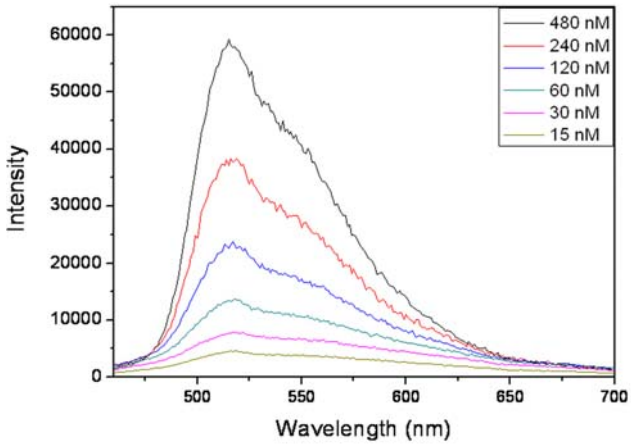

Fig. S9 Fluorescence spectra of the FAM labelled DNA and GO in the presence of various concentrations of Hg^{2+} (15, 30, 60, 120, 240, 480 nM).