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Supporting Information

Reconstruction of Nano-Flares Based on Au-Se Bands for High-Fidelity Detection of RNA in Living Cells

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Table of Content

1. Materials and instruments

Hydrogen tetrachloroaurate(III) ($HAuCl_4 \cdot 4H_2O$, 99.99%), Trisodium citrate ($C_6H_5Na_3O_7 \cdot 2H_2O$), sodium dodecylsulfate (SDS) and dimethyl sulfoxide (DMSO) were purchased from China National Pharmaceutical (Shanghai, China); L-buthioninesulfoximine (BSO), selenocystine, □-mercaptoethanol (□-ME), glutathione (GSH), 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and human EGF were purchased from Sigma-Aldrich (St. Louis, MO, USA). The DNA sequences were synthesized and further purified by Takara Bio (Dalian, China). T47D and MDA-MB-231 were purchased from Procell (Wuhan, China). MCF-7 cells (human breast cancer cell line) were purchased from KeyGEN biotechnology (Nanjing, China). Cell culture products, unless mentioned otherwise, were purchased from Invitrogen Company, Ltd. (USA). All chemicals were analytical grade and used without further purification. Sartorius ultrapure water of 18.2 MΩ·cm was used throughout the experiments.

All pH values were measured by a pH-3c digital pH meter (LeiCi, China) with a combined glass-calomel electrode. Transmission electron microscopy (TEM) was carried out on a JEM-1400 electron microscope (Japan), and the samples were prepared by carbon-coated copper grids. Fluorescence spectra were acquired with fluorescence spectrometer (RF-6000, Shimadzu, Japan). DLS was measured in Zetasizer Nano ZS (Malvern, UK). Absorption spectra were collected on a UV–Vis spectrometer (TU-1900, Purkinje General, China). Centrifugation was performed on an Eppendorf 5417R Centrifuge (Germany). Absorbance in the MTT assay was measured by SpectraMax M2 multiscan spectrum (MD, USA). Confocal fluorescence images were accomplished with a confocal laser scanning microscopy (ZEISS LSM 880, Germany).

2. Experiments

Preparation of the Au NPs

The 13 nm Au NPs were synthesized by the classical sodium citrate reduction method reported before.[1] Typically, 70 mL $HAuCl_4$ (0.01% w.p.) aquous solution was heated to boiling with vigorous stirring, and 3.5 mL of trisodium citrate solution (1% w.p.) was rapidly added under stirring. The color of the solution changed from pale yellow to colorless and finally to burgundy. The solution was kept boiling for additional 20 min, and then cooled down to room temperature under stirring. Afterward, the solutionwas filtered by using a 0.45 μm Millipore membrane filter and stored in refrigerator at 4 °C. The concentration of AuNPs was determined by the intensity of their extinction at 524 nm (ε = 2.7 ×108 $L \cdot mol^{-1} \cdot cm^{-1}$).

Synthesis of the Au-Se NFs

In order to couple the carboxyl groups of selenocystine and the amino groups of recognition sequence by forming the CONH amide bonds. 3.6 nmol selenocystine and 7.2 nmol amino-modified recognition sequence were mixed in 200 μL H_2O. Afterwards, the mixture were added to 100 μg EDC·HCl and 150 μg NHS and reacted for 24 h at 37 °C. After the reaction is completed, the connection products were purified by Amicon Ultra 3000 centrifugal filter devices and then added to 6 mL Au NPs solution (3 nM). The mixed solution was irradiated with visible light for 6 h and stirred overnight. For the next day, 10% SDS solution was further added to the mixture to achieve a final concentration of 0.1%. After 12 hours, 0.1 M PBS solution (pH 7.4) was added to the mixture to yield 0.01 M phosphate final concentration, then aqueous 0.2 M NaCl was slowly added to the solution to bring the total NaCl concentration of the probe solution to 0.1 M over an eight-hour period. This mixed solution was further aged for 24 h at room temperature. Then the resulting solution was centrifuged at 13,000 rpm for 30 min at 4 °C, and the precipitate was washed with H_2O for two times and dispersed in 1 mL PBS (0.01 M, pH 7.4). Then, 14.4 nmol Cy5 dye labeled flare strands were added to the solution above and well mixed. The mixture were heated to 85 °C for 5 min, and slowly cooled to room temperature and shaked for at least 24

h at room temperature for hybridization. After that, the solution was centrifuged at 13,000 rpm for 30 min and washed by H_2O twice to remove unhybridized flare strands. Finally, the nanoprobe was sterilized through 0.22 μm pore size filters and dispersed in0.01 M PBS solution with a final concentration of 3 nM as stock solution kept at 4 °C. The nanoprobe was diluted to certain concentration for use throughout subsequent experiments.

Quantitation of DNA Chains Loaded on the Au NPs

The number of flare strands loaded on Au NPs was determined according to the previous protocols. In brief, for the first method, β-mercaptoethanol was added (final concentration of 144 mM) to the nanoprobe (1 nM), and the mixture was incubated overnight at room temperature in the dark with shaking to completely release the DNA duplexes. The released duplexes and Au NPs were then separated *via* centrifugation at 13,000 rpm for 30 min and the fluorescence of the duplexes in the supernatant was detected with a fluorescence spectrometer. For the second method, the nanoprobe (1 nM) were heated to 85 °C for 10 min to separate the duplexes, and then rapidly centrifuged at 13,000 rpm for 30 min. The fluorescence of flare strands in the supernatant was detected with a fluorescence spectrometer. The fluorescence intensities in the supernatant of the two methods were both obtained at 648 nm excitation and 667 nm emission, and converted to molar concentrations of the flare strands by interpolation from a standard linear calibration curve, which was prepared with known concentrations of Cy5-labeled flare strands with identical buffer, pH, and ionic strength. With dividing the molar concentrations of flare strands by the original nanoprobe concentration, the number of the flare strands per nanoparticle was calculated.

Response experiment

The nanoprobe (1 nM) was incubated with the complementary vimentin mRNA target with increasing DNA targets concentrations (0, 62.5, 125, 250, 500, and 1000 nM). After incubation at 37 °C for 1 h in PBS buffer (0.01 M, pH 7.4), the fluorescence

was excited at 648 nm and measured at 667 nm. All experiments were repeated at least three times.

Kinetics study

The nanoprobe (1 nM) was incubated with the complementary vimentin mRNA target (500nM) at 37 °C in PBS buffer (0.01 M, pH 7.4). The fluorescence intensity was scanned from 0 to 40 min at 648 nm excitation and 667 nm emission. The experiments were repeated at least three times.

GSH interference study *in vitro*

1. The nanoprobe (1 nM) was incubated with 5 mM GSH at 37 °C for 12 h. At time points of 0, 2, 4, 6, 8, and 12 h,the fluorescence intensity of the solution was excited at 648 nm and measured at 667nm. Afterwards, the same solution was incubated with the complementary vimentin mRNA target (500 nM) at 37 °C for 1 h. The fluorescence intensity of the solution was excited at 648 nm and measured at 667 nm again.

2. The nanoprobe (1 nM) were incubated with increasing concentrations of GSH (0, 100, 200, 500, 1000, 2000, and 5000 μM) at 37 °C for 12 h. The fluorescence intensity of the solution was excited at 648 nm and measured at 667 nm.

pH Influence Study

For the study of pH influence on the nanoprobe detection, the experiment was designed with two groups. Groups I: the nanoprobe (1 nM) under the pH values ranging from 6.2 to 8.0 serving as the control; Group II: the nanoprobe (1 nM) incubated with the complementary vimentin mRNA target (500 nM) under the pH values ranging from 6.2 to 8.0. After incubation for 1 h at 37 °C, The fluorescence intensities of the two groups were excited at 648 nm and measured at 667 nm, respectively. All experiments were performed at least three times.

Nuclease Stability Study

For the study of nuclease stability on the nanoprobe detection, the experiment was designed with three groups. Groups I: the nanoprobe (1 nM) was incubated with DNase I (2 U/L) for 1h at 37 °C in PBS buffer (0.01 M, pH 7.4).Groups II:The nanoprobe

(1 nM) was incubated with DNase I (2 U/L) and the complementary vimentin mRNA target (500 nM) for 1 h at 37 °C in PBS buffer (0.01 M, pH 7.4). Groups III: untreated nanoprobe (1 nM) was used as control at 37 °C for 1 h. The fluorescence intensities of the three groups were excited at 648 nm and measured at 667 nm, respectively. All experiments were performed at least three times.

Cell culture

All the cells were cultured in Dulbecco's modified Eagle's medium (DMEM) which was supplemented with 10% fetal bovine serum and 100 U/mL 1% antibiotics (penicillin/streptomycin) and kept in a 100% humidified atmosphere containing 5% CO_2 at 37°C.

MTT assay

To evaluate the cytotoxicity of the nanoprobe, a tetrazolium-based colorimetric MTT assay was performed. Cells were seeded in replicate 96-well microtiter plates (1×10^6 cells/well in 200 μL DMEM). The culture was kept in 5% CO_2/95% air incubator at 37 °C. After cell attachment for 24 h, the initial medium was removed and the cells were respectively cultured with 1 nM naked-AuNPs and 1 nM nanoprobe for different time periods (24, 48, and 72 h). Then MTT solution (0.5 mg /mL in PBS, 100 μL) were added to each well after being washed with PBS and further incubated for 4 h. After discarding the remaining MTT solution, DMSO (150 μL) was added to dissolve the purple formazan. The absorbance at 490 nm was recorded with a microtiter plate reader.

Confocal fluorescence image assay

1. MCF-7, T47D and MDA-MB-231 cells were seeded in confocal dishes for 24 h in an incubator at 37 °C and 5% CO_2. The nanoprobe (1 nM) was added into the dishes and incubated for 2 h, then the dishes were washed three times with PBS buffer. After that, the cells were examined with a ZEISS LSM 880 confocal laser scanning microscope with an objective lens (×20). The Cy5 fluorescence was recorded in the red channel with 633 nm excitation. The intensities of the fluorescence in certain regions of images were measured by ImageJ software.

2. MCF-7 were seeded in confocal dishes for 24 h in an incubator at 37 °C and 5% CO_2, then treated with or without EGF (50 ng/mL) for 24h. Next, the cells were washed three times with PBS buffer and incubated with the nanoprobe (1 nM) for 2 h, then the dishes were washed three times with PBS buffer. The fluorescent signals in these cells were examined as above.

Intracellular biothiols influence on the Au-Se NFs

To test the influence of biothiols on the Au-Se NFs' detection in living cells, MDA-MB-231 cells were divided into two groups and plated in chamber slides for 24 h. One group of MDA-MB-231 cells were incubated with 5 mM BSO for 3 h at 37°C and the other group served as control without treatment. The nanoprobe (1 nM) were added to the above two groups of MDA-MB-231 cells for 4 h at 37°C, respectively. Then the cells were imaged by CLSM as above and the intensities of the fluorescence in certain regions of images were measured by ImageJ software.

RT-PCR

The cells were harvested, and total RNA was extracted using phenol, guanidine isothiocyanate and chloroform (TRIzol reagent, Invitrogen) followed by treatment with DNase according to the manufacturer's protocol. RNA was reverse transcribed using 1st Strand cDNA Synthesis Kit (Takara Bio, Japan). Real-PCR was performed on cDNA with SYBR Green PCR Kit (Roche, Switzerland) on a Roche LightCycler® 480 System. The standard deviation for this data was calculated from three independent experiments. Primer sequences used were as follows: vimentin forward, 5'-TGT CCA AAT CGA TGT GGA TGT TTC-3'; vimentin reverse, 5'-TTG TAC CAT TCT TCT GCC TCC TG-3'; GAPDH forward, 5'-GGG AAA CTG TGG CGT GAT-3'; and GAPDH reverse, 5'-GAG TGG GTG TCG CTG TTG A-3'.

References

1 W. Pan, T. Zhang, H. Yang, W. Diao, N. Li, B. Tang, *Anal. Chem*., 2013, **85***,* 10581-10588

oligonucleotides	Sequence (N ->C) (5'-3')
The amino-modified recognition sequences	CTTTGCTCGAATGTGCGGACTTAAAAAAAA-NH_2
The fluorescent dye labeled flare strands	Cy5-AAGTCCGCACA
The perfectly matched target DNA sequence	AAGTCCGCACATTCGAGCAAAG

Table S1 Sequences of oligonucleotides used in this work.

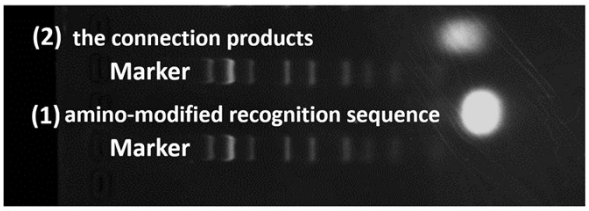

Fig. S1 The connection products were verified by agarose gel electrophoresis. (1) represented for amino-modified recognition sequence; (2) represented for the connection products of amino-modified recognition sequence with selenocystine.

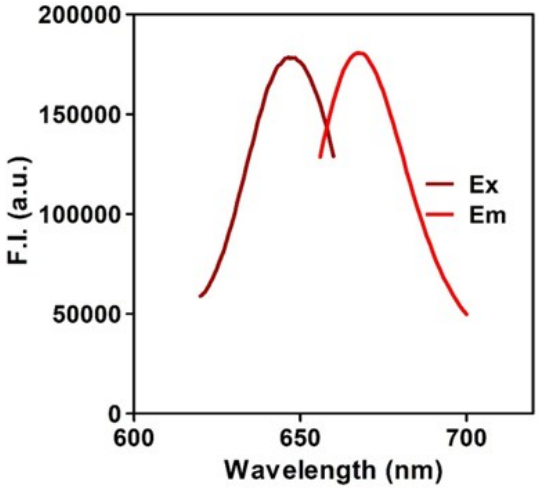

Fig. S2 The excitation and emission spectra of the Cy5-labeled flare strand.

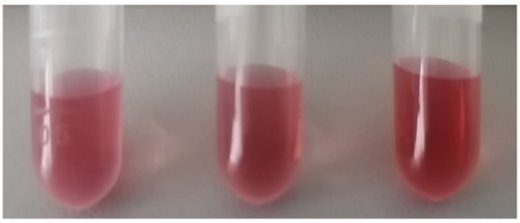

Fig. S3 Digital photographs of the Au-Se NFs (1 nM) in various aqueous media: H_2O, PBS buffer (0.01 M, pH 7.4) and dulbecco's modified eagle medium (DMEM) cell medium with 10% fetal bovine serum (FBS).

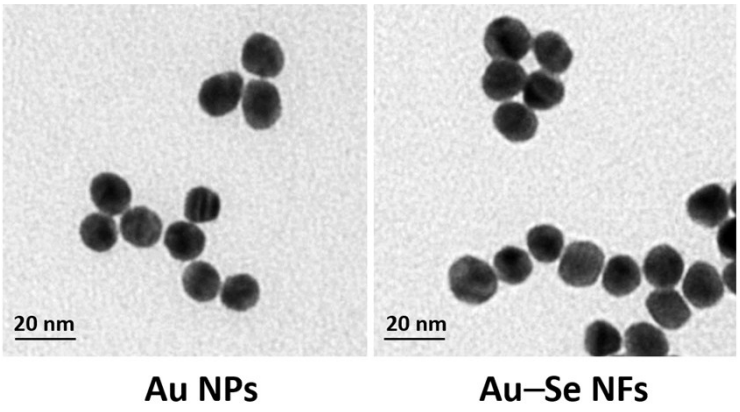

Fig. S4 TEM images of Au NPs (left) and the Au-Se NFs (right).

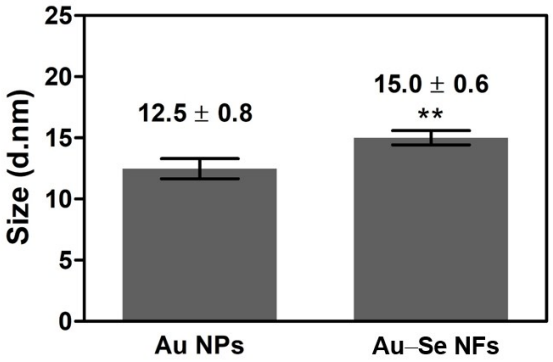

Fig. S5 The average sizes of the Au NPs and the Au-Se NFs (n = 4). Error bars represent standard errors of the mean. (**) Statistically significant difference (**$p \leq 0.01$) as compared to the Au NPs group.

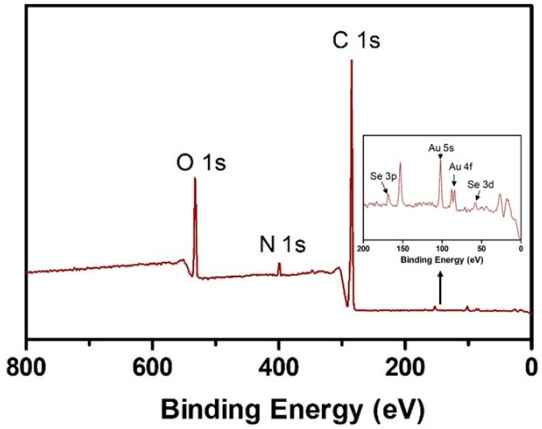

Fig. S6 XPS spectrum of the Au-Se NFs.

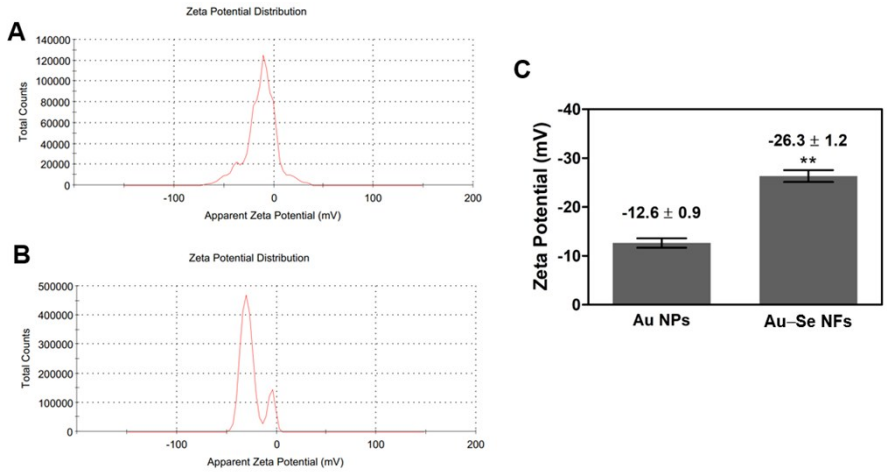

Fig. S7 The zeta potential values of the (A) Au NPs and (B) the Au-Se NFs. (C) The statistics of the two nanoparticles (n = 4). Error bars represent standard errors of the mean. (**) Statistically significant difference (**$p \leq 0.01$) as compared to the Au NPs group.

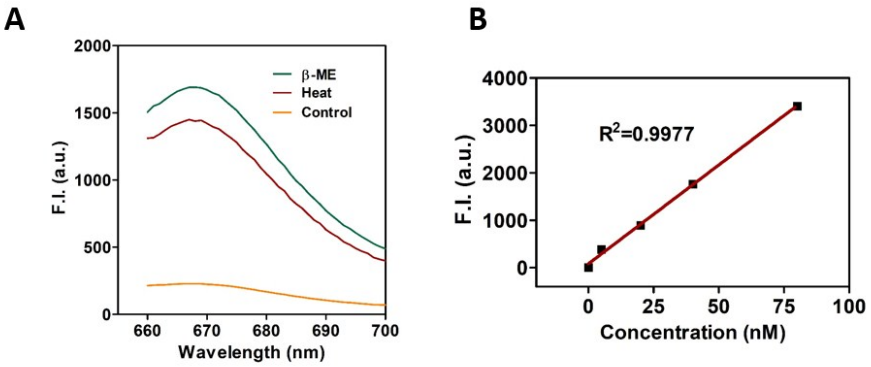

Fig. S8 (A) Fluorescence spectra of the Au-Se NFs after heated to 85 °C for 20 min or reacted with 144 mM β-ME for 12h. (B) Standard linear calibration curves of the Cy5-labeled flare strand.

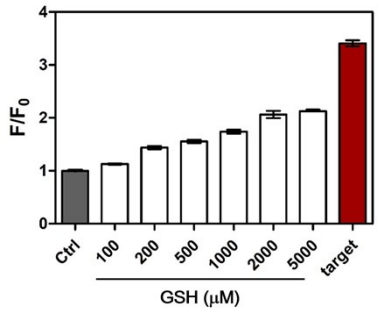

Fig. S9 Fluorescence intensity changes in the background signals of the Au-Se NFs (1 nM) incubated with different concentration of GSH (0, 100, 200, 500, 1000, 2000, and 5000 μM) and target DNA (500 nM) for 12h.

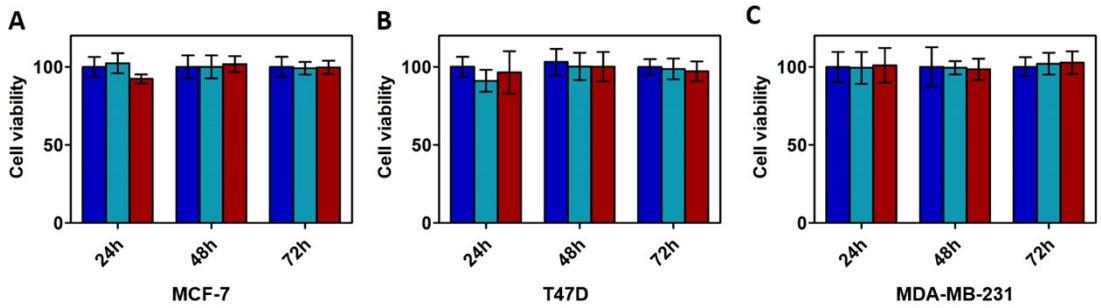

Fig. S10 MTT assay of (A) MCF-7, (B) T47D and(C) MDA-MB-231 cells. The cells were incubated with naked Au NPs (1 nM) and the Au–Se nano-flares (1 nM) for 24, 48 and 72 h. Dark blue bars represented for untreated control cells, light blue bars represented for the naked Au NPs, and red bars represented for the Au-Se NFs.

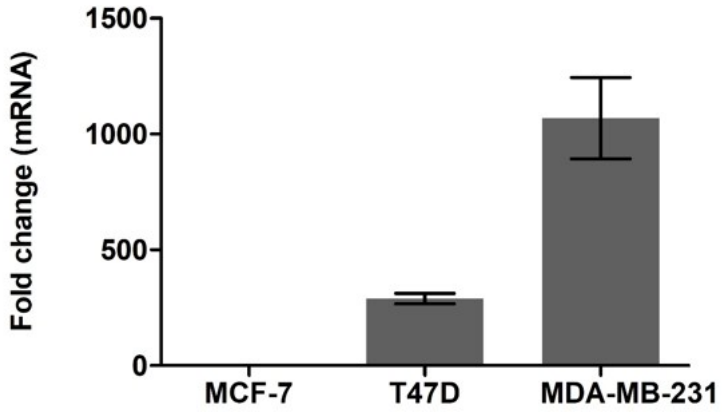

Fig. S11 RT-PCR showed the relative expression of vimentin mRNA in MCF-7, T47D and MDA-MB-231 cells.

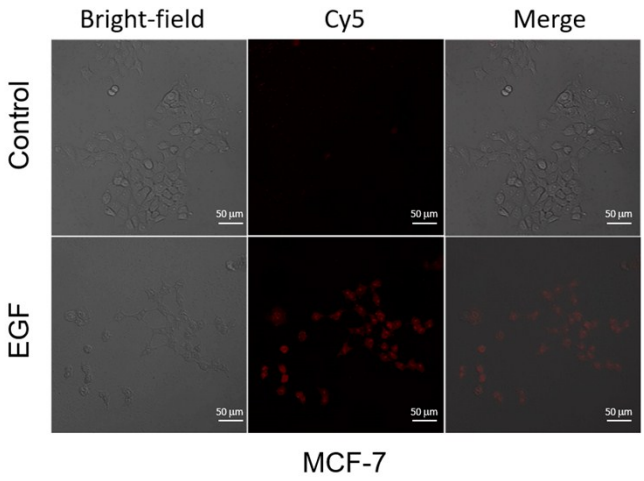

Fig. S12 CLSM images of the expression changes of vimentin mRNA in MCF-7 cells treated with or without EGF (50 ng/ml) for 24h by the Au-Se NFs (1 nM). Scale bars: 50 μm.

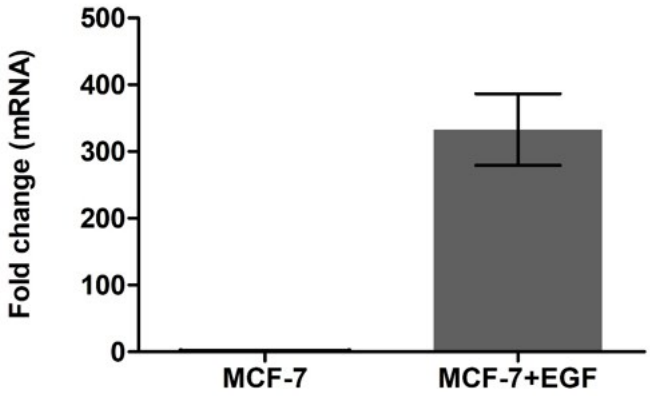

Fig. S13 RT-PCR result of the relative expression of vimentin mRNA in MCF-7 cells treated with or without EGF (50 ng/mL) for 24 h.

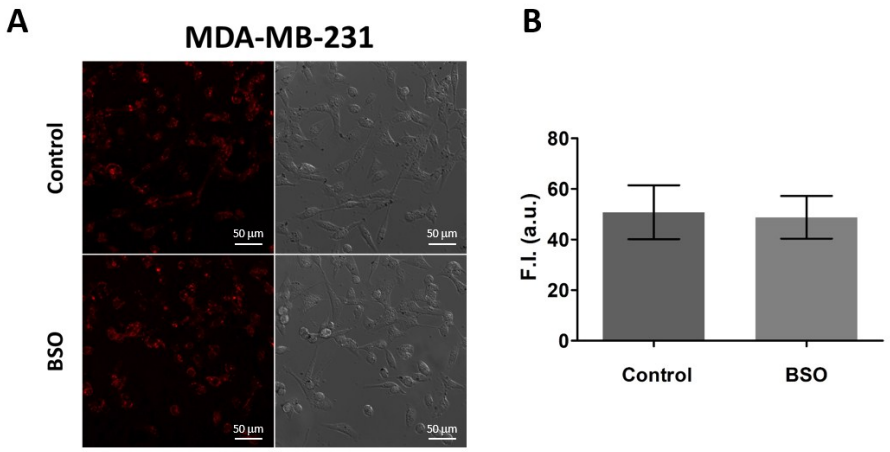

Fig. S14 (A) CLSM images of the differences of fluorescence signal in MDA-MB-231 cells pretreated with or without BSO (5 mM) for 3 h by the Au-Se NFs (1 nM). (B) Quantitative analysis of the relative fluorescence intensities in (A) by ImageJ software.