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A Dual-Round Signal Amplification Strategy for Colorimetric/ Photoacoustic/ Fluorescence Triple Read-Out Detection of Prostate Specific Antigen

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Contents

1.Experimental section

Chemicals and Materials. Chloroauric acid ($HAuCl_4$▪$3H_2O$), cetyltrimethylammonium bromide (CTAB), sodium borohydride ($NaBH_4$), L-ascorbic acid (AA), silver nitrate ($AgNO_3$), methanol, anhydrous ethanol, ammonia (NH_3▪H_2O), tetraethyloryhosilicate (TEOS), 3-Mercaptopropyltriethoxysilane (MPTS), ethylene glycol (EG), rhodamine B, 2-aminoethanol, ethanolamine, acetone, glucose, N-hydroxysuccinimide (NHS), carbodiimides (EDC), methanesulfonyl chloride (MsCl), sodium iodide (NaI) and trisodium citrate were obtained from JK Chemical. PSA, DBCO-PEG4-NHS ester (NHS-DBCO) and Azido-PEG4-NHS ester (NHS-azide) were purchased from Sigma-Aldrich. N-hydroxysuccinimide-activated MBs (NHS-MBs) was purchased from BEAVER (Suzhou, China). The monoclonal primary antihuman PSA antibody (PSA-Ab1) and secondary antihuman PSA antibody (PSA-Ab2) were obtained from R&D Systems (Beijing, China). ELISA antibody dilution, ELISA coating buffer and ELISA washing solution were obtained from Sangon Biotech (Shanghai, China).

Instrumentation. Mass spectra were taken by using an Agilent liquid chromatography-mass spectrometry (LC-MS) instrument (Agilent, U.S.A.). Nuclear magnetic resonance (NMR) spectra were measured on a Bruker Avance III 600 MHz superconducting Fourier NMR spectrometer (Germany). UV-vis spectra were acquired on a Cary 60 UV-vis spectrophotometer (Agilent Technologies, Santa Clara, CA) and a SYNERGY H1 microplate reader (BioTEK, U.S.A.). Fluorescence spectra were obtained on a Thermo Scientific Lumina fluorescence spectrophotometer (Thermo Fisher Scientific Co.) Transmission electron microscopy (TEM) images were acquired on a JEM-F200 instrument (JEOL, Tokyo, Japan). PA imaging was taken by a Vevo 2100 LAZR system (FUJIFILM VisualSonics, U.S.A.).

Synthesis. *Silica coated Au@Ag core-shell nanorods (denoted as Au@Ag@SiO_2)* 10 mL of as-prepared silica coated Au nanorods (Au@SiO_2) was washed by centrifugation (10000 rpm for 10 min) to remove the excess CTAB and redispersed with 10 mL of ultrapure water. Then above solution was added to 45 mL of 1.5% w/w of MPS in ethanol solution and stirred for 20 h. After being washed with ethanol for twice to remove the excess MPS, the precipitations were diluted to 30 mL of $AgNO_3$ (1.2 mM) in ethylene glycol solution, and 600 μL of ethanolamine (10 mM) in ethylene glycol solution were added into above solution under gentle stirring at 37 °C until the color turned from pink to green. Then the product was washed by acetone, ethanol and ultrapure water, and redispersed in ultrapure water for further use.

DBCO-Ab2 1.0 mg/mL of Ab2 was dissolved in 100 μL of ELISA antibody dilution (final concentration 0.1 mg/mL). Then, NHS-DBCO (6.5 μL, 1 mM) was mixed with Ab2 and incubated for 30 min at room temperature. After quenching buffer (50 mM Tris-HCl, pH 8) was added and incubated for 5 min to stop the reaction, the products were purified by centrifugation (6000 g, 10 min) with a 30 K ultrafiltration centrifuge tube.

GOx-MBs 200 μL of NH_2-MBs were added to a 2 mL of EP tube and purified with a magnetic stand, then washed with 400 μL of PBS solution (50 mM, pH 7.4) for three times. After that 200 μL of freshly prepared glutaraldehyde solution (15%) was added to the tube, vortex and mix to make MBs fully suspend. Then the tube was put on a vertical mixer with foil wrapped and the whole reaction was kept at 25 °C for 1 h. After MBs were activated, the supernatant was magnetically removed. After being washed with a buffer (50 mM, pH 7.4) for three times, 100 μL of GOx (30 mg/mL) in PBS was added into the tube, and the mixture was incubated for 2 h in a shaker (100 rpm). After being purified with a magnetic stand, the products were redispersed into 200 μL of PBS (10 mM, pH 7.4) for further use.

Ab2-GOx-MBs GOx-MBs were modified with an azide group by introducing 20 μL of NHS activated azide (NHS-azide, 1 mM) to 200 μL of GOx-MBs solution. The mixture was incubated at room temperature for 30 min, and purified with a magnetic stand, then dispersed into 200 μL of PBS (10 mM, pH 7.4). The obtained azide-modified GOx-MBs was mixed with 20 μL of DBCO-Ab2 and incubated at room temperature for 2 h. After the incubation, the resulting solution was purified with a magnetic stand and dispersed in 2 mL of PBS (10 mM, pH 7.4) for further use.

Verification of the Au@Ag@SiO_2-based assay. Various concentrations of H_2O_2 solutions (0-20 mM) were added into 96-well plates, and the Au@Ag@SiO_2 solution was subsequently added to each well. After being incubated for 1 h, a photograph, the corresponding UV-vis spectra and PA imaging were collected. Various concentrations of GOx solutions (0-1000 μg/mL) were added into 96-well plates and incubated with Glu (20 mM) in water at 37 °C for 30 min, the Au @Ag@SiO_2 solution was added to above solutions. After being incubated for 1 h, a photograph, the corresponding UV-vis spectra and PA imaging were collected.

PSA Detection. First, Ab1 (4 μg/mL, 50 μL) dispersed in an ELISA coating solution was added to 96-well plates and incubated at 4 °C overnight. After being washed with wash buffer for three times, 200 μL of blocking solution including 1% BSA was added and incubated at 37 °C for 1 h. The plates were subsequently washed with wash buffer for three times, and then 50 μL of different concentrations of PSA were respectively added to each well. After incubating for 1 h at 37 °C, the plate was washed three times with washing buffer, and 50 μL of the prepared Ab2-GOx-MBs were added at 37 °C for 1 h. Then, 50 μL of 20 mM glucose solution was added and incubated for 30 minutes. After the incubation, the resulting solution was purified with a magnetic stand and incubated with 50 μL of Au@Ag@SiO_2 for 1 h. Then a photograph was taken and the corresponding UV-vis spectrum was collected by a SYNERGY H1 microplate reader. The fluorescence spectrum (λex = 530 nm, λem = 585 nm) was collected after being incubated with 100 μL of Ag^+-fluorescent probe (10 μM) for 2 h. Finally, the PA imaging was taken.

2. Characterizations of Au@Ag@SiO_2 before and after etching.

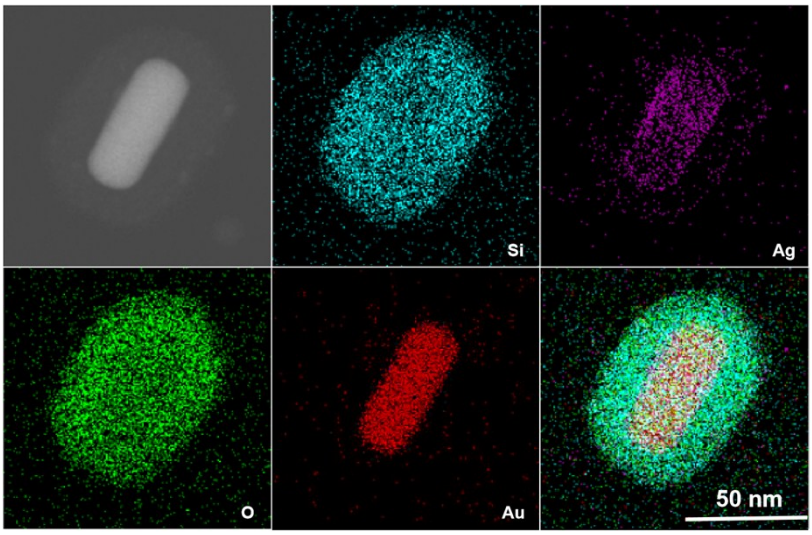

Figure S1. EDS of Au@Ag@SiO_2 after etching.

Element	Line Type	K Factor	K Factor type	Absorption Correction	Wt%	Wt% Sigma
O	K series	1.480	Theoretical	1.00	16.95	0.35
Si	K series	1.000	Theoretical	1.00	13.62	0.27
Ag	L series	1.774	Theoretical	1.00	27.92	0.52
Au	L series	2.673	Theoretical	1.00	41.51	0.60
Total:					100.00	

Table S1. Elemental quantification results of Au@Ag@SiO_2 before etching.

Table S2. Elemental quantification results of Au@Ag@SiO_2 after etching.

Element	Line Type	K Factor	K Factor type	Absorption Correction	Wt%	Wt% Sigma
O	K series	1.480	Theoretical	1.00	25.87	0.47
Si	K series	1.000	Theoretical	1.00	17.14	0.34
Ag	L series	1.774	Theoretical	1.00	7.88	0.42
Au	L series	2.673	Theoretical	1.00	49.11	0.64
Total:					100.00	

3. Characterization of Ab2-GOx-MBs

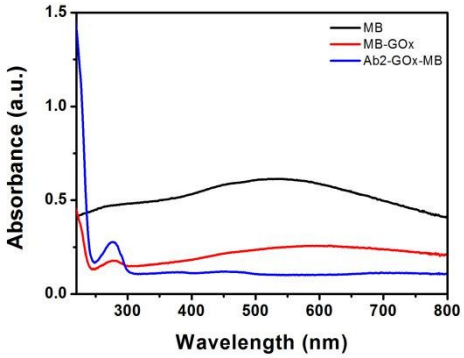

Figure S2. UV-vis-NIR spectrum of MBs, GOx-MBs and Ab2-GOx-MBs.

4. Synthesis of Rhodamine B-Based Ag^+ Fluorescence Probe and the effect with Ag^+.

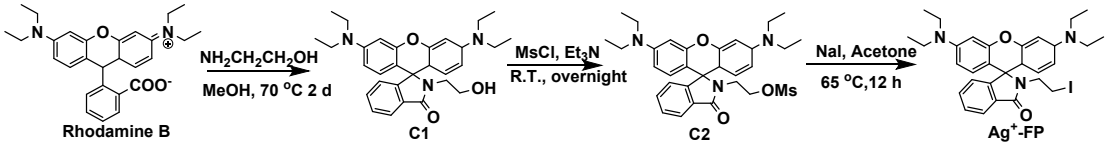

Figure S3. Synthesis route of Ag^+ fluorescence probe (Ag^+-FP).

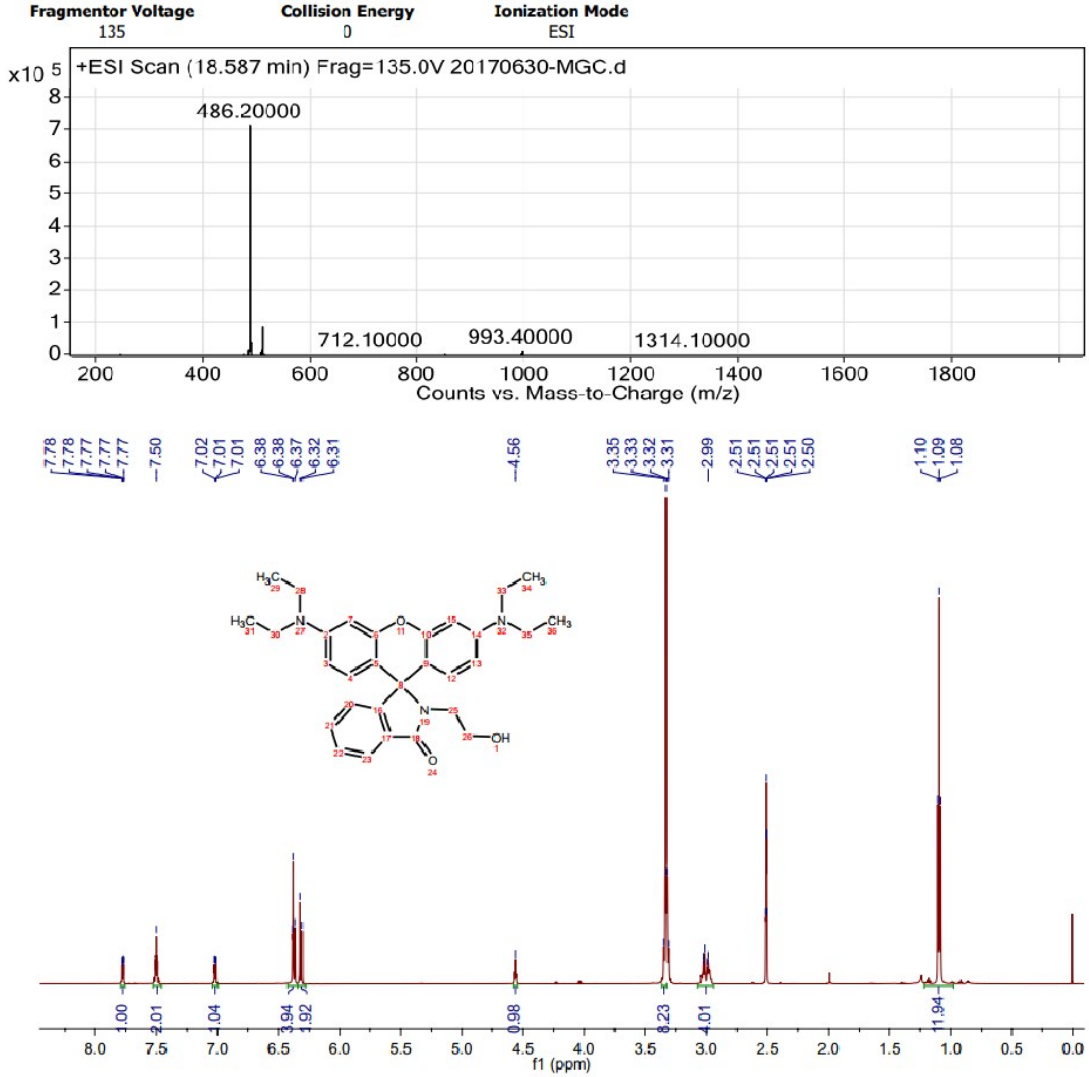

Figure S4. ESI-MS and ^{1}H NMR spectra of C1.

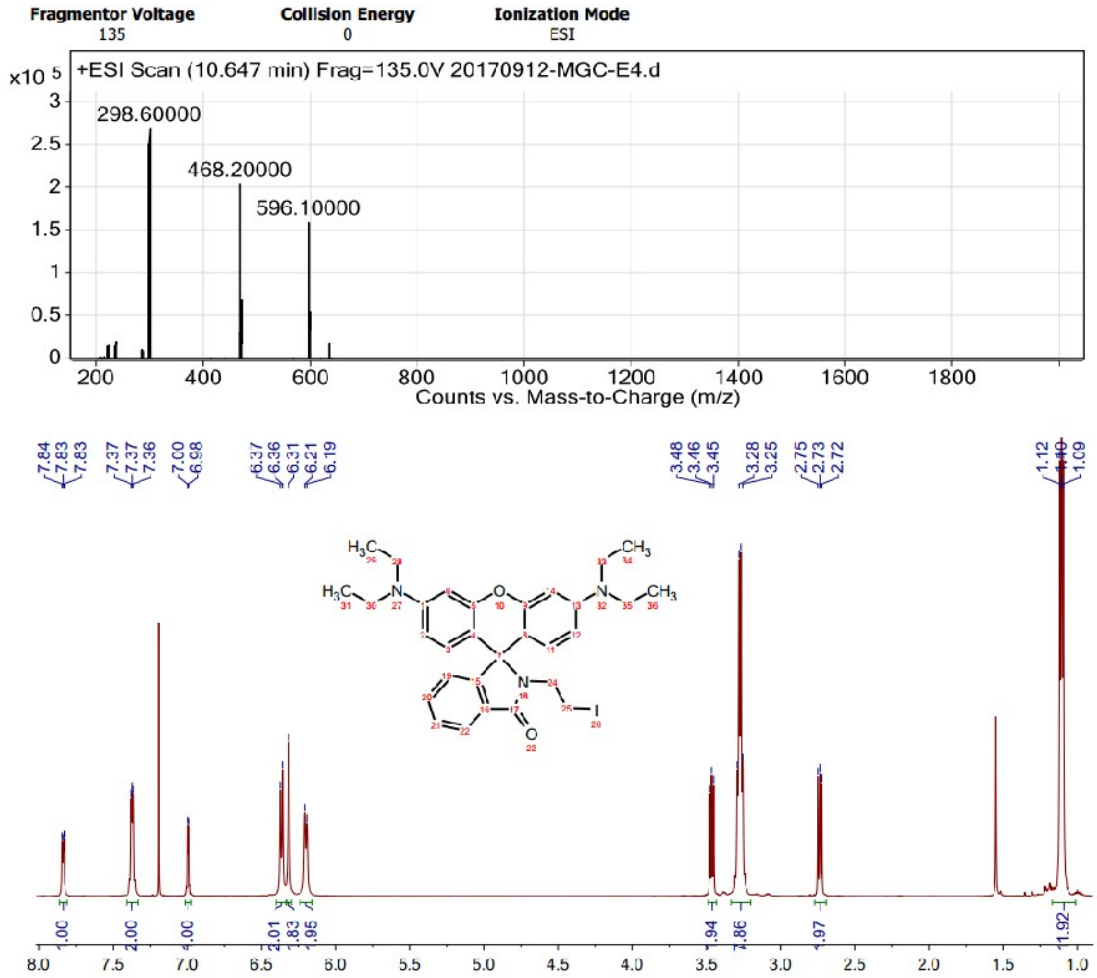

Figure S5. ESI-MS and ^{1}H NMR spectra of Ag^{+} fluorescence probe (Ag^{+}-FP).

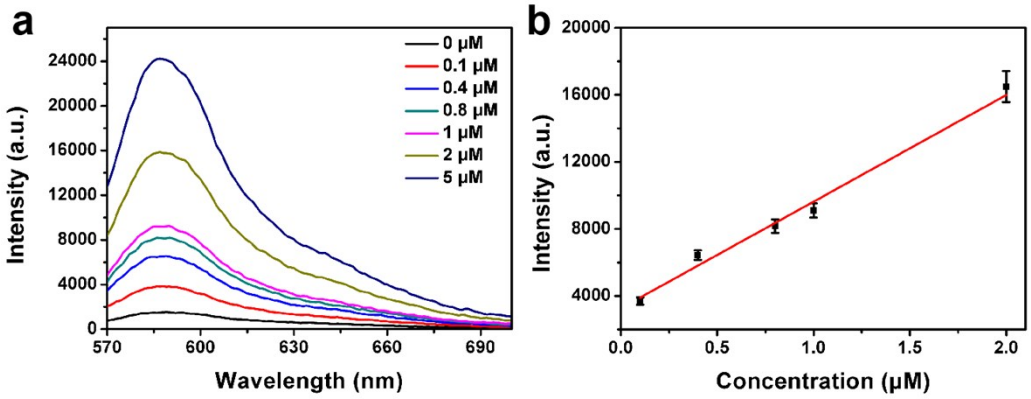

Figure S6. (a) Fluorescence spectra of Ag^{+}-FP with the addition of various concentration of Ag^{+} (0~10 μM). (b) Calibration curves of fluorescence intensity at 588 nm versus the concentrations of PSA. n = 3, mean ± SD.