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Supporting Information

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METHODS

Materials.

Sodium borohydride ($NaBH_4$), cetyltrimethylammonium bromide (CTAB), chlorauric acid ($HAuCl_4 \cdot 3H_2O$), silver nitrate($AgNO_3$), L-ascorbic acid (AA), bicinchoninic acid (BCA), and 2,6-pyridinedicarboxylicacid (PDCA) were purchased from Alfa Aesar and used as received. Copper (II) chloride dehydrate ($CuCl_2 \cdot 2H_2O$) and other reagents and chemicals were at least analytical reagent grade and purchased from Beijing Chemical Reagent Company (Beijing, China). Milli-Q water (18 M Ω cm) was used for all solution preparations.

Instruments.

UV−vis −NIR absorption spectra were recorded on a Varian Cary 50. Transmission electron microscopy (TEM) was obtained with a TecnaiG2 20 S-TWIN operating at an acceleration voltage of 200 kV. Zeta potential of the NRs dispersions was measured by Zetasizer Nano ZS.

Synthesis of Au NRs.

Au NRs were synthesized through the seed-mediated growth method. CTAB-capped Au seeds were prepared as the following procedure: 7.5 mL of 0.1 M CTAB solution was mixed with 100 μL of 25 mM $HAuCl_4$ and deionized water to 9.4 mL. Then, ice-cold $NaBH_4$ (0.6 mL, 0.01 M) was added under vigorous stirring. The seed solution was then kept at room temperature and used within 2–5 h. Then, Au NRs were synthesized by reduction of $HAuCl_4$ with AA on Au seeds: 1mL seed solution was added to the growth solution consisted of CTAB (100 mL, 0.1 M), $HAuCl_4$ (2 mL, 25 mM), $AgNO_3$ (1.1 mL, 10 mM), H_2SO_4 (1 mL, 1 M) and AA (800 μL, 0.1 M) to initiate the growth. After about 3h, the Au NRs were separated from the growth solution by centrifugation (12000 rpm for 5 min) twice. The precipitation was collected and redispersed in 100mL of deionized water.

Synthesis of Au@Ag NRs with Ag/Au ratio of 1.75.

A 3.5 mL of purified Au NRs solution was mixed with 15 mL of 0.1 M CTAB and 26.5 ml of deionized water. Then, 300 μL of 10 mM $AgNO_3$, 300 μL of 0.1 M AA, and 450 μL of 0.2 M NaOH were added to the solution. The mixture was kept in a 30 °C water bath for about half an hour to initiate the overgrowth. After preparation, the Au@Ag nanorods were separated from the growth solution by centrifugation (12000 rpm for 5 min) once. The precipitation was collected and re-dispersed in 50 ml of CTAB (0.1 M) for further use.

Protein detection using SPR-BCA assay.

Step 1 is the oxidation of protein by Cu^{2+}: 100 μl of 10 mM Cu^{2+} aqueous solution, 100 μl of 0.1 M BCA, and 100 μl of 0, 0.5, 1, 5, 20, 50, 200, 500, 1000, and 2000 μg/ml protein standards (stepwise dilution using 0.1 M PBS (pH = 7.4) buffer solution) were mixed together and reacted at 60 °C for 30 min. Then, the solutions were cooled down to room temperature for further use. The amount of Cu^{2+} is referred to the conventional BCA assay (ref.16).

Step 2 is the etching of Au@Ag rods by Cu^{2+}/BCA pair: Pipette 10 μL of above reaction solution into 200 μL prepared Au@Ag suspension and incubate at 40 °C for 7 min. Then, 10 μL of 10 mM PDCA was added to stop the etching reaction. The obtained solutions were used for spectroscopic measurements. Notes that Cu^{2+} concentration is 167μM in etching solution, lower than 4.5 mM in BCA assay.

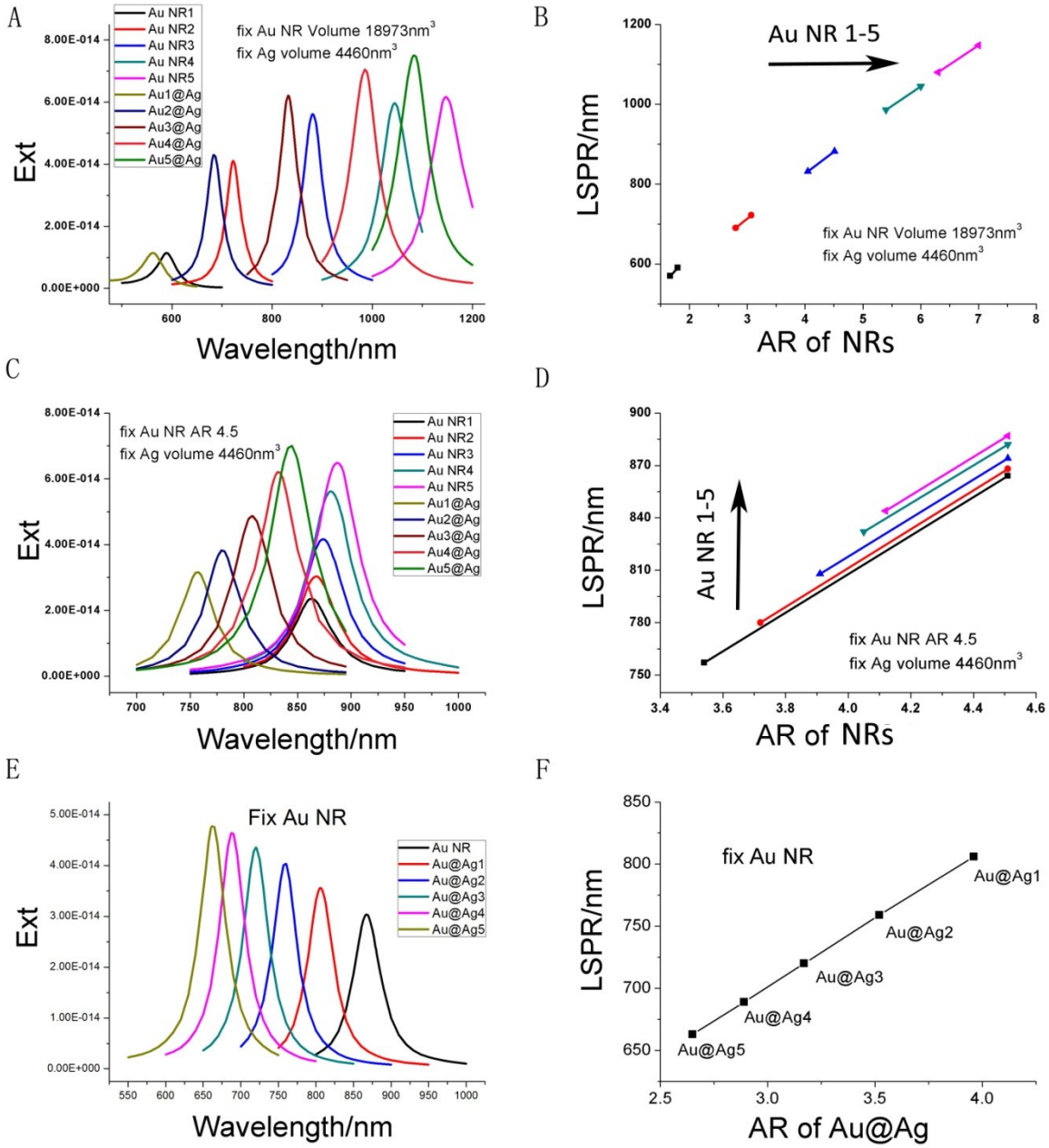
Protein detection using conventional BCA assay.

Conventional BCA assay is done according to published procedure (ref.16). The working reagents include reagent A and reagent B. Reagent A is prepared by dissolving 0.25 g $CuSO_4 \cdot 5H_2O$, 1g sodium tartrate, 0.1g KI and 3.5 g NaOH in 100 ml deionized water. Reagent B is 0.1 M BCA. Then mixing 1ml of reagent A, 200μL of reagent B and 1ml of 0, 0.5, 1, 5, 20, 50, 200, 500, 1000, and 2000 μg/ml protein standards (stepwise dilution using 0.1 M PBS (pH =7.4) buffer solution). The mixture was reacted at 60°C for 30 min. Then, the solutions were cooled down to room temperature for spectroscopic measurements.

Finite element method (FEM) calculations.

FEM calculations were performed using the RF module of COMSOL Multiphysics. The model of Au NR was a cylinder with two hemispherical caps. The surrounding medium was water (refractive index was 1.33) and the dielectric function of gold was adopted from the experimental data by Johnson and Christy (1).

(1) P. B. Johnson and R. W. Christy, Phys. Rev. B: Solid State, 1972, 6, 4370-4379.



New Figure S1. FEM simulated extinction spectra of the Au cores and Au@Ag probes: (A) Effect of aspect ratio of the Au core. (B) The relationship between LSPR and AR of the NRs in (A). (C) Effect of volume of the Au core. (D) The relationship between LSPR and AR of the NRs in (C). (E) Effect of size of the Ag shell. (F) The relationship between LSPR and AR of the NRs in (E).

Parameters of calculated models in Figure S1A (Au core with fixed volume of 18973 nm^3 and fixed Ag shell volume of 4460 nm^3)

	Length(nm)	Width(nm)	AR
Au NR1	45.81	25.45	1.80
Au1@Ag	45.81	28.28	1.62
Au NR2	63.51	20.66	3.07
Au2@Ag	63.51	22.96	2.77

Au NR3	80.91	17.96	4.51
Au3@Ag	80.91	19.96	4.05
Au NR4	97.31	16.22	6.00
Au4@Ag	97.31	18.03	5.40
Au NR5	107.53	15.36	7.00
Au5@Ag	107.53	17.07	6.30

Parameters of calculated models in Figure S1C (Au core with fixed aspect ratio of 4.51 and fixed Ag shell volume of 4460 nm^3)

	Length(nm)	Width(nm)	AR	Volume(nm^3)
Au NR1	58.63	13.00	4.51	7203
Au1@Ag	58.63	16.54	3.54	11663
Au NR2	64.24	14.24	4.51	9470
Au2@Ag	64.24	17.27	3.72	13930
Au NR3	72.16	16.00	4.51	13430
Au3@Ag	72.16	18.47	3.91	17890
Au NR4	80.91	17.96	4.51	18973
Au4@Ag	80.91	19.96	4.05	23433
Au NR5	85.69	19.00	4.51	22489
Au5@Ag	85.69	20.80	4.12	26949

Parameters of calculated models in Figure S1E (Fixed Au core AR = 4.5 and volume = 9470 nm^3)

	Length(nm)	Width(nm)	AR
Au NR	64.24	14.24	4.51
Au@Ag1	64.24	16.24	3.96
Au@Ag2	64.24	18.24	3.52
Au@Ag3	64.24	20.24	3.17
Au@Ag4	64.24	22.24	2.89
Au@Ag5	64.24	24.24	2.65

New Table S1. Geometric parameters of the Au@Ag nanorods used for simulation.

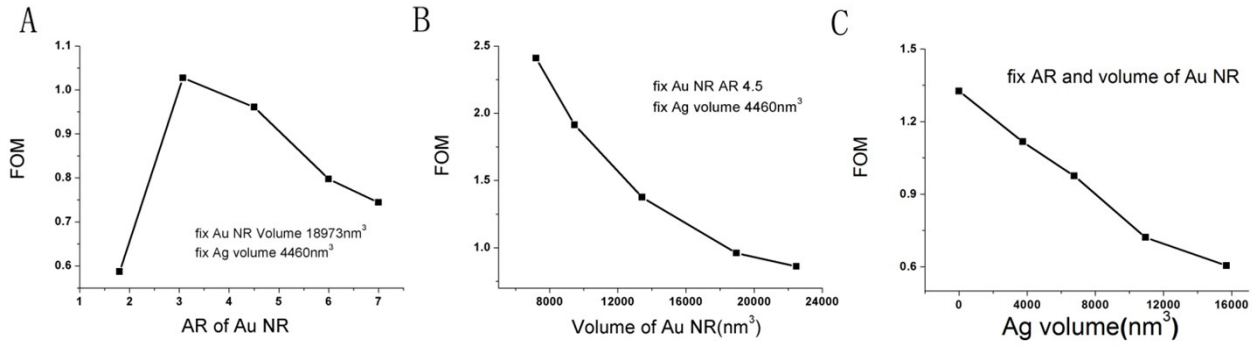
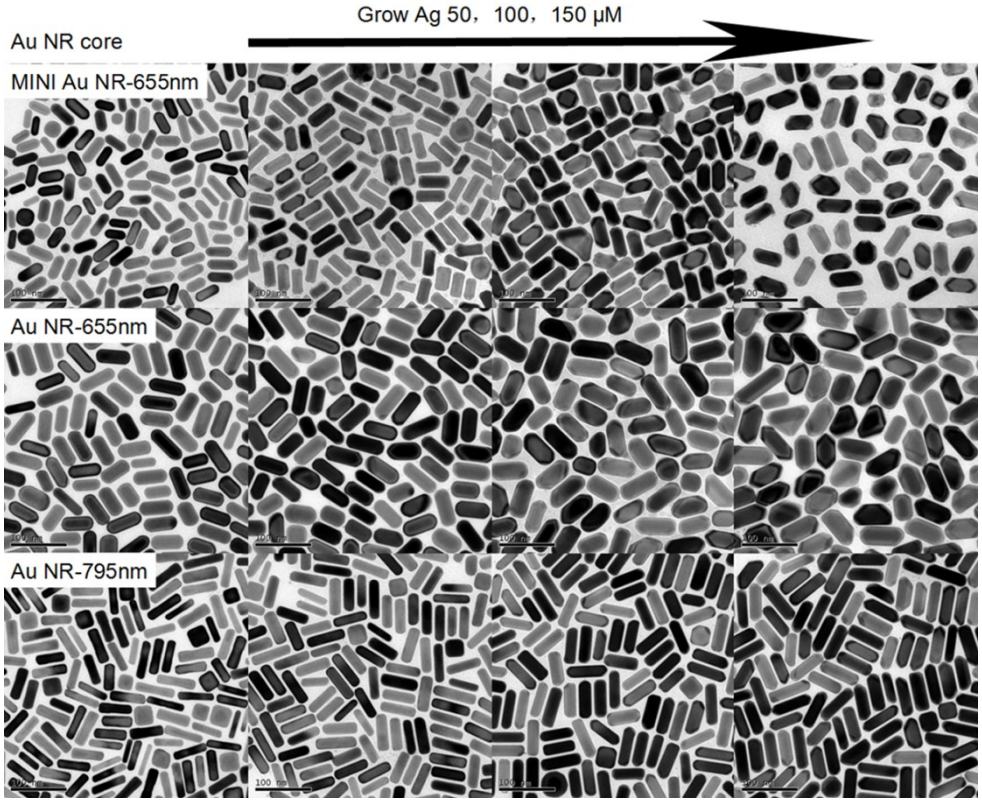


Figure S2. Relationships between FOM and (A) AR, (B) volume of Au NR core, and (C) volume of Ag shell.



New Figure S3. TEM images of the three Au NR cores and the corresponding Au@Ag nanorods after Ag overgrowth. ([Au NRs] = 0.167 nM, [Ag^+] = 50,100, and 150 μM)

Ag / shell / Au core		Ag 0	Ag 50 μM	Ag 100 μM	Ag 150 μM
Mini Au NR-655nm	Length /nm	42.55 ± 5.11	47.26 ± 5.43	51.21 ± 6.14	54.07 ± 5.94
	Width /nm	17.39 ± 2.01	21.56 ± 1.97	25.10 ± 2.10	29.21 ± 3.58
	AR	2.46 ± 0.24	2.20 ± 0.23	2.04 ± 0.22	1.88 ± 0.31
Au NR-655nm	Length /nm	57.49 ± 7.36	60.04 ± 8.56	63.36 ± 7.62	65.98 ± 8.38
	Width /nm	24.72 ± 3.39	29.49 ± 3.03	32.81 ± 2.96	36.53 ± 5.11
	AR	2.36 ± 0.41	2.06 ± 0.39	1.95 ± 0.30	1.82 ± 0.24
Au NR-795nm	Length /nm	60.58 ± 7.40	61.27 ± 8.36	62.17 ± 6.83	65.59 ± 7.90
	Width /nm	17.06 ± 2.87	18.51 ± 2.40	19.88 ± 1.77	22.84 ± 2.34
	AR	3.73 ± 0.54	3.35 ± 0.34	3.11 ± 0.36	2.90 ± 0.45

New Table S2. The aspect ratio and size of the three Au NR cores and the corresponding Au@Ag nanorods after Ag overgrowth. At least 100 nanorods for each sample are counted.

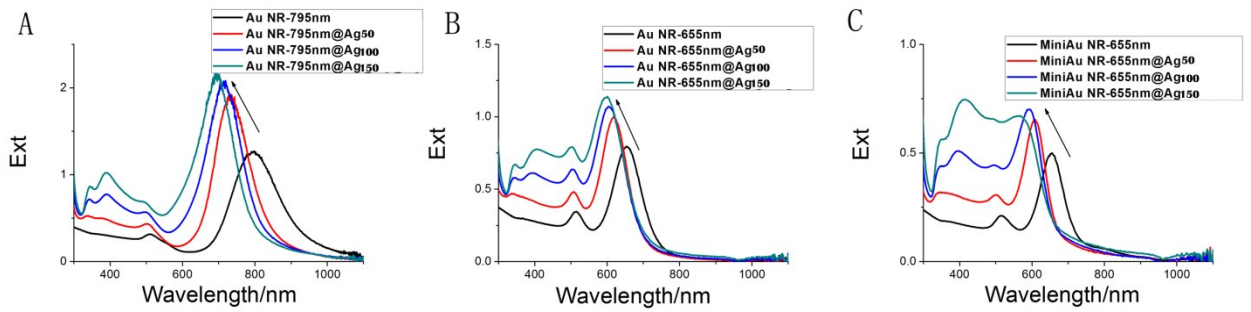


Figure S4. Experimental extinction spectra of three different Au NRs and Au@Ag nanorods grown from them: A, B and C for AuNR-795 nm, AuNR-655 nm and mini-AuNR-795 nm, respectively. ([Au NRs] = 0.167 nM, [Ag^+] = 50,100, and 150 μM)

As shown by theoretical simulation, the Au core with a large AR and a small size exhibits a high sensitivity. And control of Ag shell can be used to tailor dynamic range. Experimentally, we employed three different Au NR cores to verify the above results. Au NR-655nm (meaning the LSPR maximum at 655 nm) has the largest core volume whereas the mini Au NR-655nm has the smallest core volume. Au NR-795nm has the largest aspect ratio but medium core volume. For 50 μM Ag deposition, the AuNR-795 exhibits the larger LSPR shift (61 nm) than the mini Au NR-655nm (47 nm). The Au NR-655nm shows less LSPR shift (37 nm) than the mini Au NR-655nm due to larger core size. The experimental results are in agreement with our simulations.

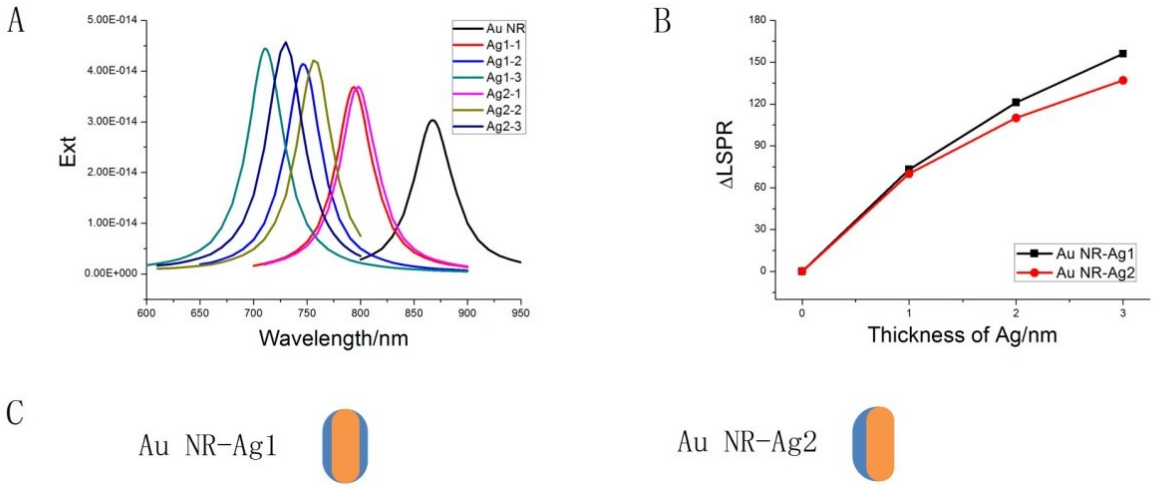


Figure S5. FEM simulated extinction spectra: (A) Au core and Au@Ag NRs with different Ag coatings for homogeneous shell (Ag1) and inhomogeneous shell (Ag2). (B) ΔLSPR variation vs. Ag thickness. (C) The cartoons of corresponding Au@Ag rods.

	Au@Ag	Au@Ag-i	Au NR-core
Length/nm	62.8 ± 9.0	60.1 ± 7.7	61.3± 8.7
Width/nm	24.8 ± 4.4	18.9 ± 3.9	13.3 ± 2.1
AR	2.53	3.18	4.61

New Table S3. Measured dimensions of Au@Ag and Au NRs in Figure 2.

Effect of BCA/Cu^{2+} ratio on Au@Ag etching

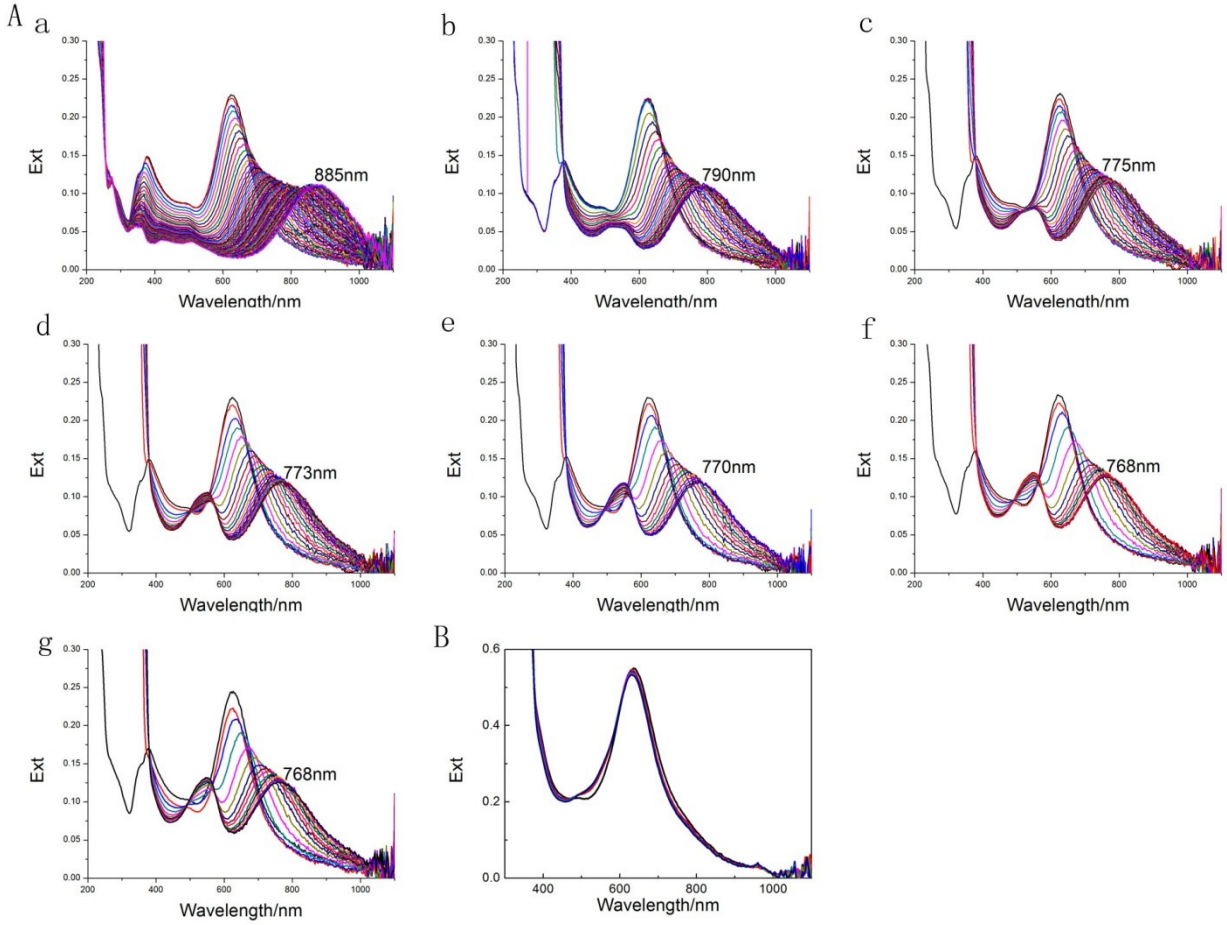


Figure S6. Effect of BCA amount on Au@Ag etching: (A) Extinction spectra evolution during etching by 30 μM Cu^{2+} ions and different concentrations of BCA (a) 0, (b) 60, (c) 100, (d) 200, (e) 300, (f) 400, (g) 500 μM. (B) Extinction spectra evolution of Au@Ag before and after adding 50 μM Cu^{+}-BCA complex. For all reactions, [CTAB] = 0.1M.

Effect of Cu^{2+} concentration on the LSPR wavelength

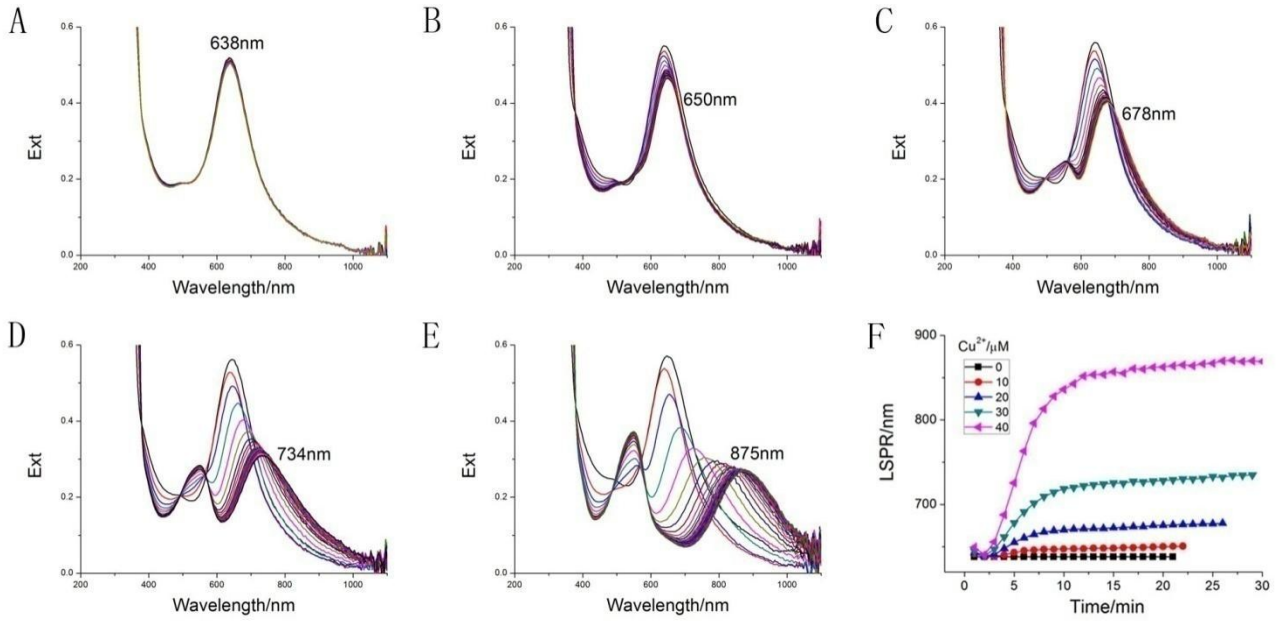


Figure S7. Extinction spectra evolution of Au@Ag etching by (A) 0, (B) 10, (C) 20, (D) 30, (E) 40 μM Cu^{2+} ions. (F) LSPR wavelength vs. etching time at different concentration of Cu^{2+} ions. For all reactions, [BCA /Cu^{2+}] = 10, [CTAB] = 0.1M.

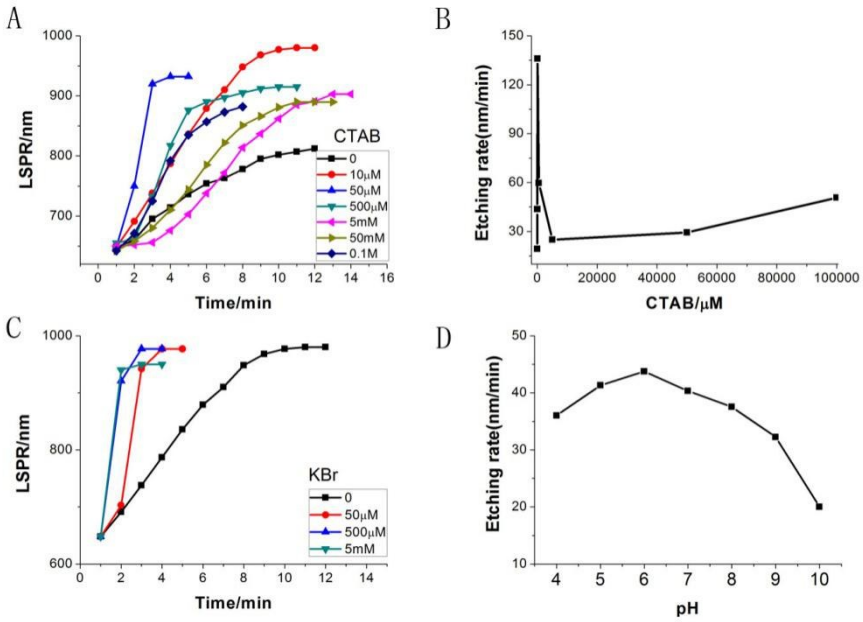
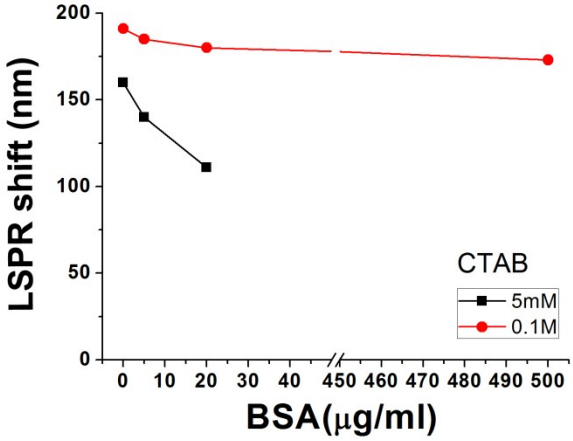


Figure S8. Effects of CTAB, Br^-, and pH on Au@Ag etching: (A) LSPR wavelength vs. etching time at different concentration of CTAB; (B) etching rate vs. CTAB concentration; (C) LSPR wavelength vs. KBr concentration ([CTAB] = 10 μM); (D) etching rate vs. pH value. For all reactions, [Cu^{2+}] = 50 μM and [BCA] = 10 [Cu^{2+}].

BSA standard	0	500 μg/ml	1000 μg/ml
5 mM CTAB	55.5 ± 0.5mV	23.9 ± 1.3 mV	25.8 ± 0.9 mV
0.1 M CTAB	47.1 ± 0.2 mV	42.2 ± 0.5 mV	41.3 ± 1.0 mV

Table S4. Zeta potentials of Au@Ag dispersed in 5 mM or 0.1 M CTAB aqueous solutions adding different amounts of BSA. Pipette 50 μL of each standard BSA sample into 1000 μL prepared Au@Ag suspensions.



New Figure S9. LSPR shift vs. BSA amount in the case of 5 mM and 0.1M CTAB, respectively. ([Cu^{2+}] = 50 μM and [BCA] = 10 [Cu^{2+}], detected at the 6th minute)

Increasing BSA concentration, we observed the slightly reduced LSPR shift for the case of 0.1 M CTAB. It indicates that without step 1 (mixture of Cu^{2+}/BCA and protein is incubated at 60°C for 30 min), there is small amount of BSA oxidation by Cu^{2+} at room temperature. In contrast, for 5 mM CTAB, the LSPR shift is obviously decreased even at low BSA amount, suggesting that protein adsorption inhibits the Ag shell etching by Cu^{2+}/BCA pair.

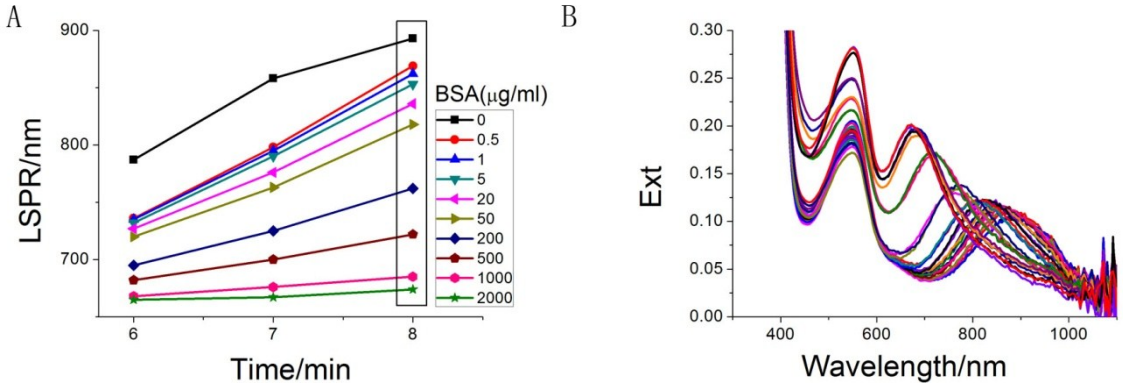


Figure S10. (A) LSPR maximum vs. time at different concentrations of BSA (CTAB = 0.1 M, Cu^{2+} = 167μM, BCA = 10 Cu^{2+}); (B) Extinction spectra of all the standard samples (repeated 3 times for each sample) at the 8th minute.

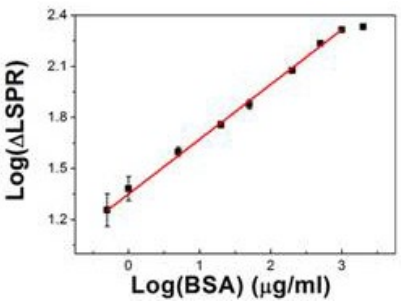


Figure S11. The log–log plots of ΔLSPR versus the concentration of the standard BSA. ($\Delta LSPR = LSPR_{Au} - LSPR_{Au@Ag}$)

Comparison of SPR-BCA assay using extinction intensity at 780 nm and conventional BCA assay using absorbance intensity at 562 nm

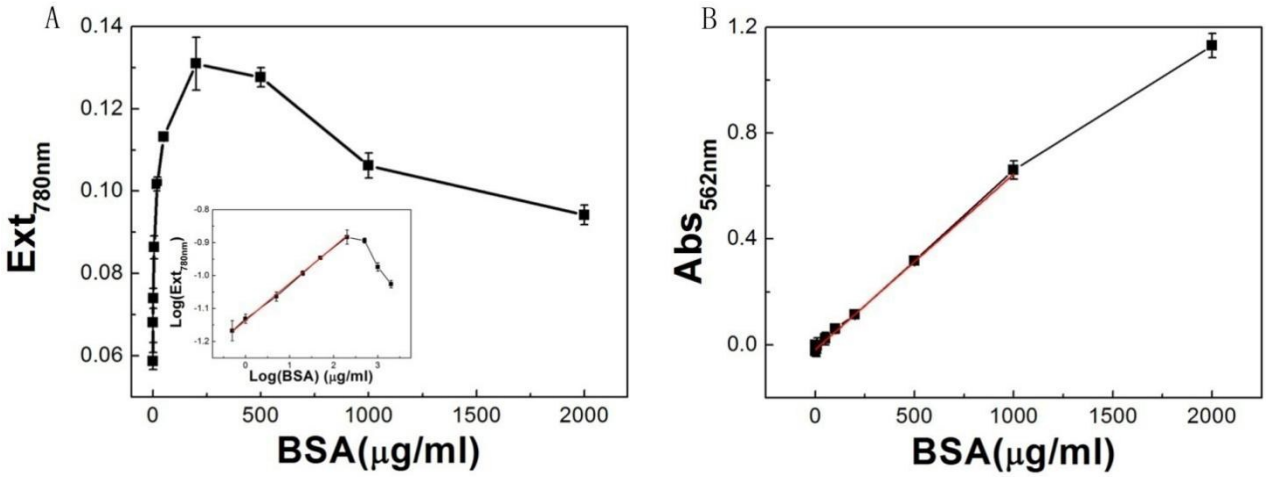


Figure S12. (A) Standard curve of SPR-BCA assay for the detection of BSA using the extinction intensity at 780 nm, the inset is the log–log plots of Ext_{780nm} versus the concentration of the standard BSA, the regression equation is y= 0.11x-1.14 (R^2 = 0.99837), LOD= 80.7 ng/ml; (B) Standard curve of conventional BCA assay using absorbance intensity at 562 nm for the detection of BSA.

	Pep	OVA	BSA	Hb	IgG	TRY	LYZ	AVG	SD
pI	1.0	4.6	4.7	7.1	8.0	10.3	11.0	---	---
Mw(kDa)	35.0	43.0	68.5	64.5	149.9	23.3	14.6	---	---
SPR-BCA	0.840	0.994	1.000	0.767	1.052	0.948	0.680	0.900	0.138
BCA	0.754	0.845	1.000	0.882	1.012	1.017	0.845	0.910	0.103

Table S5. Parameters of various proteins and response characteristics for different proteins, ΔLSPR of SPR-BCA assay and absorbance ratios (562 nm) of BCA assay for proteins relative to BSA.
Pep: pepsin; OVA: ovalbumin; HSA: human serum albumin; BSA: bovine serum albumin; Hb: hematoglobin; IgG: immunoglobulin G; TRY: trypsin; LYZ: lysozyme.