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Electronic Supplementary Information for

Boosting Chiral Amplification in Plasmon-Coupled Circular Dichroism Using Discrete Silver Nanorods as Amplifiers

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EXPERIMENTAL SECTION

1. Reagents and Chemicals.

Silver nitrate ($AgNO_3$, 99.8%) and ascorbic acid (AA, 99.7%) were purchased from Sinopharm Chemical Reagent Company. Trisodium citrate (99%) was purchased from Shanghai Chemical Reagent Company. L-arginine (98%), polyvinylpyrrolidone (PVP, average M_w~40k), and hexadecyltrimethylammonium chloride (CTAC, 25 wt.% in H_2O) were purchased from Sigma-Aldrich. Hexadecyltrimethylammonium bromide (CTAB, 99%) was obtained from Acros. Sodium borohydride ($NaBH_4$, 98%) and hydrogen peroxide (H_2O_2, 30%) were purchased from Aladdin. Cetylpyridinium chloride monohydrate (CPC, 98%), L-cysteine hydrochloride monohydrate (L-Cys, 99%), and D-cysteine hydrochloride monohydrate (D-Cys, 99%) were purchased from TCI. Thiol Detection Assay Kit was purchased from Cayman Chemical (item No. 700340). The assay utilizes a proprietary fluorometric detector that reacts with thiol groups to emit a strong fluorescent signal. All chemicals were used as received without further purification. Ultrapure water was used in all experiments.

2. Synthesis of Au@Ag Nanocubes.

Au@Ag nanocubes were synthesized according to a previously reported procedure.[1] Typically, a precursor solution of Au seeds was first prepared via reduction of $HAuCl_4$ by freshly prepared $NaBH_4$ solution in the presence of CTAB solution.[1] After the Au seed solution was stirred for 2 min and kept undisturbed for 2 h, 390 mL of water was added to prepare a seed solution for future use. For the growth of octahedral Au NPs, 19.4 mL of water, 4 mL of 0.1 M CTAB solution, 0.1 mL of 10 mM $HAuCl_4$ solution, and 1.5 mL of 0.1 M AA solution were mixed. Subsequently, 0.3 mL of the seed solution was added to the mixture immediately. The reaction products were centrifuged at 15000 rpm for 10 min after 8 h and redispersed in water with the same volume.

Au@Ag nanocubes were synthesized by using the octahedral Au NPs as seeds.[1] In a typical procedure, 20 mL of the above seed solution and 3.2 mL of 0.1 M CPC aqueous solution were mixed and heated to 60°C in an oil bath. Under magnetic stirring, 0.4 mL of 10 mM $AgNO_3$ solution and 1.6 mL of 0.1 M AA solution were added. After 1 h, the solution was cooled to room temperature and centrifuged at 12000rpm for 3min. The sediment was re-dispersed in ultrapure water with the same volume for future use.

3. Synthesis of Ag Decahedral Nanoparticles.

Ag decahedral NPs were synthesized through a photochemical method according to previous report of Kitaev et al.[2] In a typical procedure, 14 mL of ultrapure water, 0.52 mL of 50 mM sodium citrate solution, 15 μL of 50 mM PVP solution, 50 μL of 5 mM L-arginine solution, 0.4 mL of 5 mM $AgNO_3$ solution, and 0.2 mL of 0.1 M freshly prepared $NaBH_4$ solution were subsequently added in a 20 mL via. After stirring for 1 h, 0.3 mL of H_2O_2 solution was added rapidly under magnetic stirring until bubble evolution vanishes. Finally, the resultant mixture was exposed to a blue LED array (λ_{max}: 450-455 nm) with power of 90 watts for 24 h at room temperature.

4. Synthesis of Ag Nanorods.

Ag nanorods were synthesized through thermal regrowth method using Ag decahedral NPs as seeds, following a previously reported method.[2] In a typical procedure, 8 mL of the above Ag decahedral seed solution was centrifuged at 15000 rpm for 10 min and re-dispersed in 1 mL ultrapure water. Subsequently, 6 mL of ultrapure water, 0.72 mL of 50 mM sodium citrate solution, and 0.135 mL of 50 mM PVP aqueous solution were added to a flask and heated for 10 min in a 100 °C oil bath. Then 1 mL of the seed solution and 0.4 mL of 5 mM $AgNO_3$ solution were added subsequently under magnetic stirring. After heated for 10 min, another 0.4 mL 5 mM $AgNO_3$ solution was added and the mixture was heated for another 10 min. Finally, the vial was cooled in an ice-bath and store in dark at room temperature.

5. Functionalization of Ag NPs with Cysteine.

Before functionalization, 15 mL of Ag nanocubes solution, 20 mL of Ag decahedral NPs solution, and 10 mL of Ag nanorods solution were centrifuged at 12000 rpm for 5 min and re-dispersed in 10 mL of CTAC (5 mM) and PVP (5 mM) solution, respectively. For the functionalization process, 0.3 mL freshly prepared cysteine solution with different concentration was added to 2.7 mL of the solution at room temperature and gently mixed. After 40 min, the mixture was directly diluted five times for the measurement of CD spectrum. To compare the enhancement effect of three types of NPs, the intensity of extinction spectra of each NP is set at about 0.6.

6. Instrumentation.

CD spectra and the corresponding UV−visible spectra were recorded at room temperature using a Jasco J-820 spectropolarimeter with a high-performance quartz glass cuvette of 1 cm light path length. The digital integration time is 0.5 sec. The date pitch and band width are 1.00 nm. Scanning mode is continuous and scanning speed is 500 nm/min. Transmission electron microscopy (TEM) images were acquired using a Japan's Hitachi H-600 TEM operating at 100 kV. Fluorescence spectra were collected on a SpectraMax M5 microplate reader (Molecular Devices Corp, Sunnyvale, CA, USA). The fluorescence spectra are measured by using an excitation wavelength of 395 nm and an emission wavelength of 510 nm. To simulate the distribution of electromagnetic fields around different Ag NPs, finite-difference time-domain (FDTD) methods were performed by using the Lumerical FDTD Solutions 8.0 software.

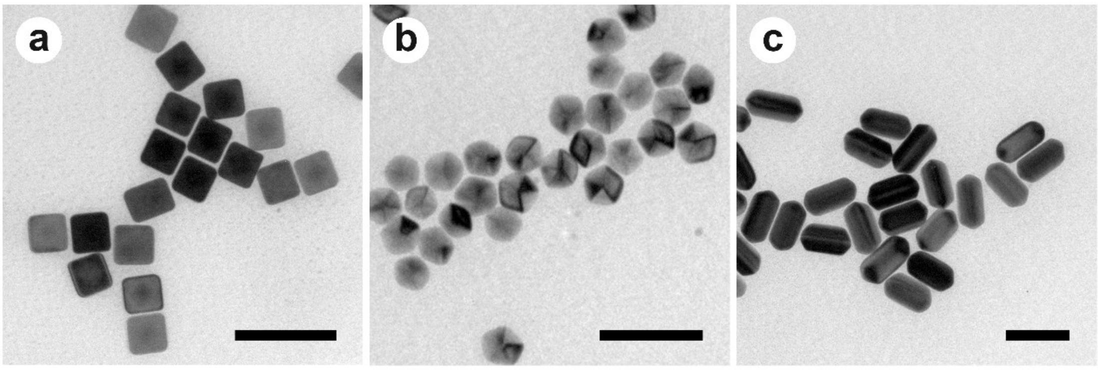

Fig. S1 TEM images of L-Cys-functionalized (a) Au@Ag nanocubes, (b) Ag decahedral NPs, and (c) Ag nanorods. Scale bars: 100 nm

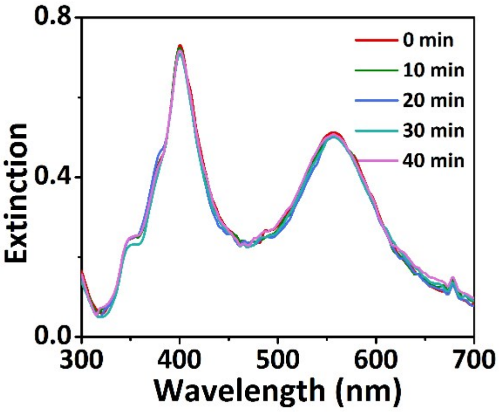

Fig. S2 Extinction spectra of Ag nanorods in the presence of 0.5 μM L-Cys obtained at different time intervals during the 40-minute incubation period.

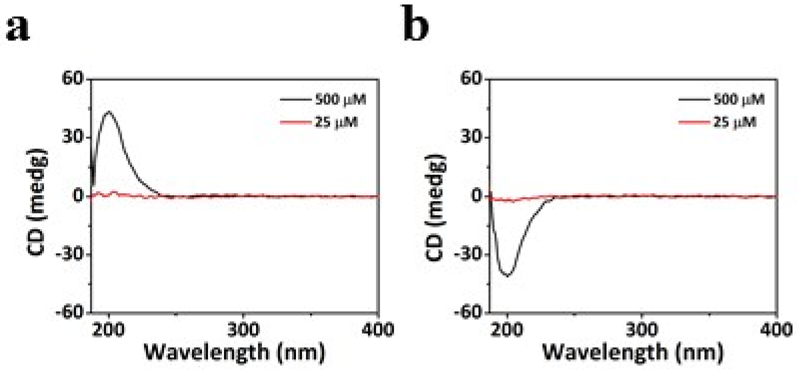

Fig. S3 CD spectra of (a) L-Cys and (b) D-Cys.

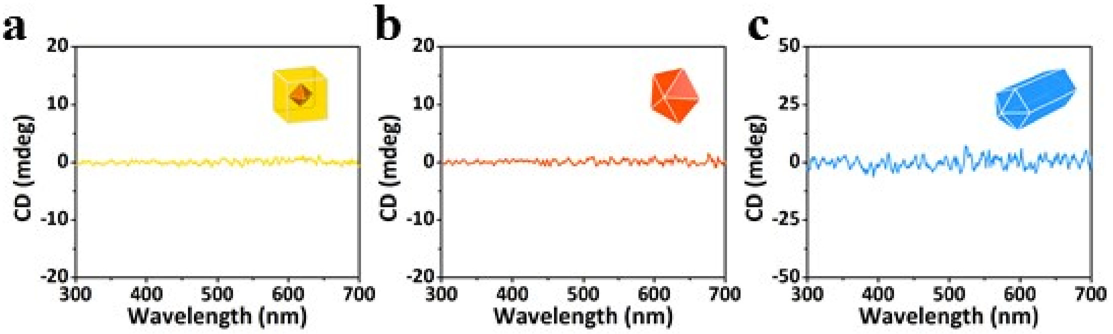

Fig. S4 CD spectra of (a) Au@Ag nanocubes, (b) Ag decahedral NPs, and (c) Ag nanorods.

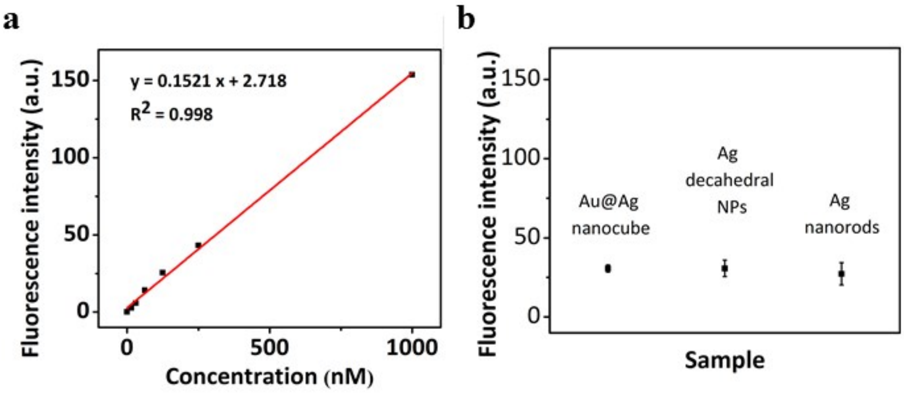

Fig. S5 (a) Concentration curve from 0 nM to 1000 nM for the thiol detection fluorometric assay kit. There is a good linear relationship between the fluorescence intensity and concentration of cysteine within the measured range. (b) The original data of determining the concentration of free Cys in the supernatant using the fluorometric assay kit.

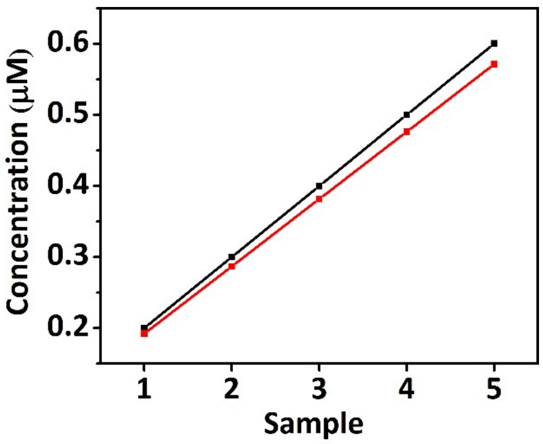

Fig. S6 Adsorption study of L-Cys on nanorods. Different concentrations of L-Cys (black line) are added and the amount of adsorbate (red line) is calculated by subtracting the L-Cys concentrations in the supernatant.

Calculation of enhancement factor (A_{CD}).

The enhancement factor A_{CD} is defined as the ratio of the CD signal of chiral molecule-NPs complexes taken at the corresponding frequencies of plasmon resonance to the native chiral molecule CD signal in the UV region normalized by L-Cys concentrations, respectively.

$$A_{CD} = \frac{CD_{(\lambda plasmon)}/c_{(L-Cys)}}{CD_{0(\lambda uv)}/c_{0(L-Cys)}}$$

where $CD_{(\lambda plasmon)}$ is the maximum of the CD signal of L-cys functionalized Ag NPs, $c_{(L\text{-}Cys)}$ is the concentration of L-Cys in the Cys-NPs complexes. $CD_{0(\lambda uv)}$ and $c_{0(L\text{-}Cys)}$ are the maximum of the CD signal of pure L-Cys and the concentration of L-Cys on this condition, respectively. In this work, $CD_{0(\lambda uv)}$ is equal to 43.32 and $c_{0(L\text{-}Cys)}$ is 500 μM.

Table. S1 Comparison on A_{CD} of different NPs functionalized with 0.5 μM L-Cys.

NPs	**$CD_{(\lambda plasmon)}$**	**Initial $c_{(L\text{-}Cys)}$**	**Apparent A_{CD}**	**adsorbed $c_{(L\text{-}Cys)}$**	**Corrected A_{CD}**
Au@Ag nanocubes	0	0.5 μM	0	0.4632 μM	0
Ag decahedral NPs	5.25	0.5 μM	121	0.4632 μM	131
Ag nanorods	127.41	0.5 μM	2941	0.4678 μM	3143

Calculation of anisotropy factor (g-factor).

The optical activity of the sample is often measured by the absorption anisotropy factor, which is calculated by the following formula,

$$g = \frac{\Delta A}{A} = \frac{\theta}{32.98 * A}$$

The unit of θ in the formula is the degree (deg), A is the corresponding ultraviolet absorption. The measurement unit of ellipticity in our experiment is mdeg. Therefore, g-factor can be calculated by the following formula,

$$g = \frac{\Delta A}{A} = \frac{\theta}{32980 * A}$$

Table. S2 Comparison on g-factor of different NPs functionalized with 0.5 μM L-Cys.

NPs	**CD (mdeg)**	**A**	**g-factor**
Au@Ag nanocubes	0	0.49	0
Ag decahedral NPs	5.25	0.37	0.000426
Ag nanorods	127.41	0.36	0.0106

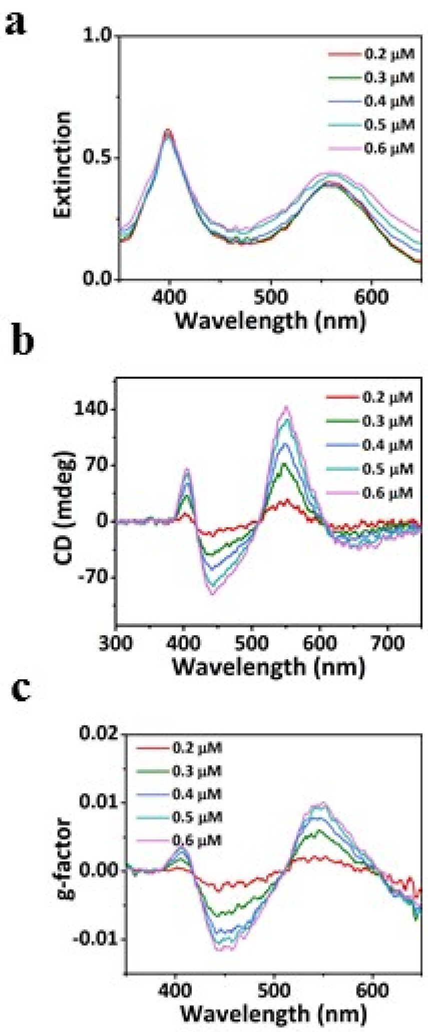

Fig. S7 (a) Optical extinction spectra, (b) CD spectra, and (c) g-factor of Ag nanorods after mixing with L-Cys solution of varying concentrations as marked in the figure legend.

Table. S3 Comparison on A_{CD} of nanorods mixing with L-Cys solution having varying concentrations

Sample	**$CD_{(\lambda plasmon)}$**	**Initial $c_{(L\text{-}Cys)}$**	**Apparent A_{CD}**	**adsorbed $c_{(L\text{-}Cys)}$**	**Corrected A_{CD}**
1	28.00	0.2 μM	1615	0.1996 μM	1618
2	72.17	0.3 μM	2776	0.2856 μM	2916
3	97.08	0.4 μM	2801	0.3714 μM	3016
4	127.78	0.5 μM	2949	0.4678 μM	3152
5	144.11	0.6 μM	2772	0.5578 μM	2981

Table. S4 Summary of enhancement factor in literatures

	NPs	**State**	**Chiral Molecule**	**A_{CD}**	**References**
1	Ag NPs	Aggregates	L-GS–bimane	~100	[3]
2	Au NPs	Discrete NPs	Cysteine	~4	[4]
3	Au NRs	Aggregates	Cysteine	~3	[5]
4	Au NRs	Discrete NPs	CdTe QDs	3.25	[6]
5	Au@Ag NCs	Discrete NPs	ssDNA	85~103	[1]
6	Au NSs	Clusters	Cysteine	99~119	[7]
7	Au NRs	Dimers	DNA	~300	[8]
8	Au@Ag NCs	Discrete NPs	Cysteine	0	This work
9	Ag decahedral NPs	Discrete NPs	Cysteine	131	This work
10	**Ag nanorods**	**Discrete NPs**	**Cysteine**	**3143**	**This work**

Finite-difference time-domain (FDTD) method.

FDTD is an algorithm that discretizes both time and space domain of Maxwell's equations. FDTD simulations carried out by FDTD Solutions software were performed to simulate the electromagnetic near-field distribution of an individual Ag NP. Structural parameters used in simulations were chosen to match the TEM images of NPs that are shown in Fig. 1. The distribution of electromagnetic field surrounding NPs was computed numerically to explain Ag NPs impact on plasmon-coupled CD enhancement.

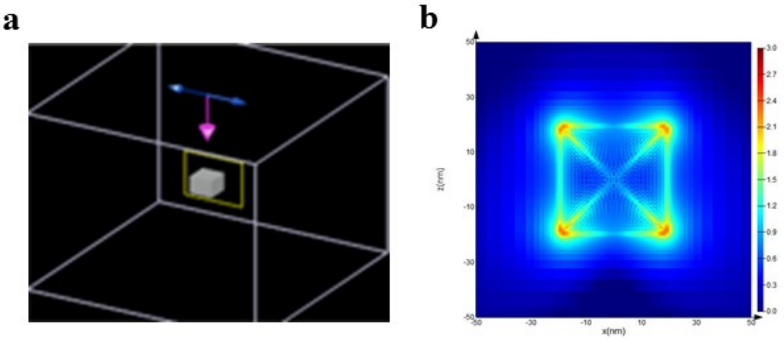

Fig. S8 (a) The perspective view of electromagnetic near-filed distribution simulation model for an individual Ag nanocube. (b) The electromagnetic near-filed distribution simulation results.

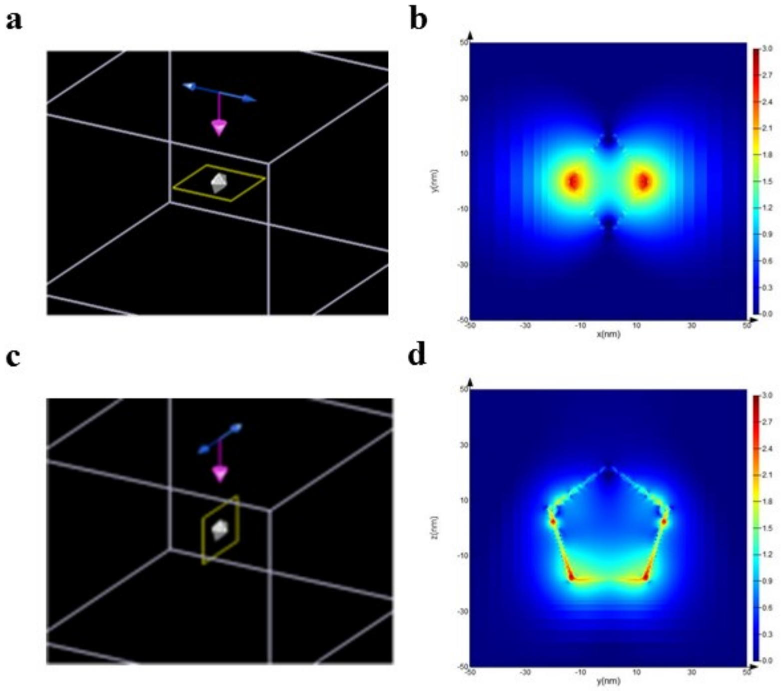

Fig. S9 (a) and (c) The perspective view of electromagnetic near-filed distribution simulation model for an individual Ag decahedral NP. (b) and (d) The electromagnetic near-filed distribution simulation results from different cross sections.

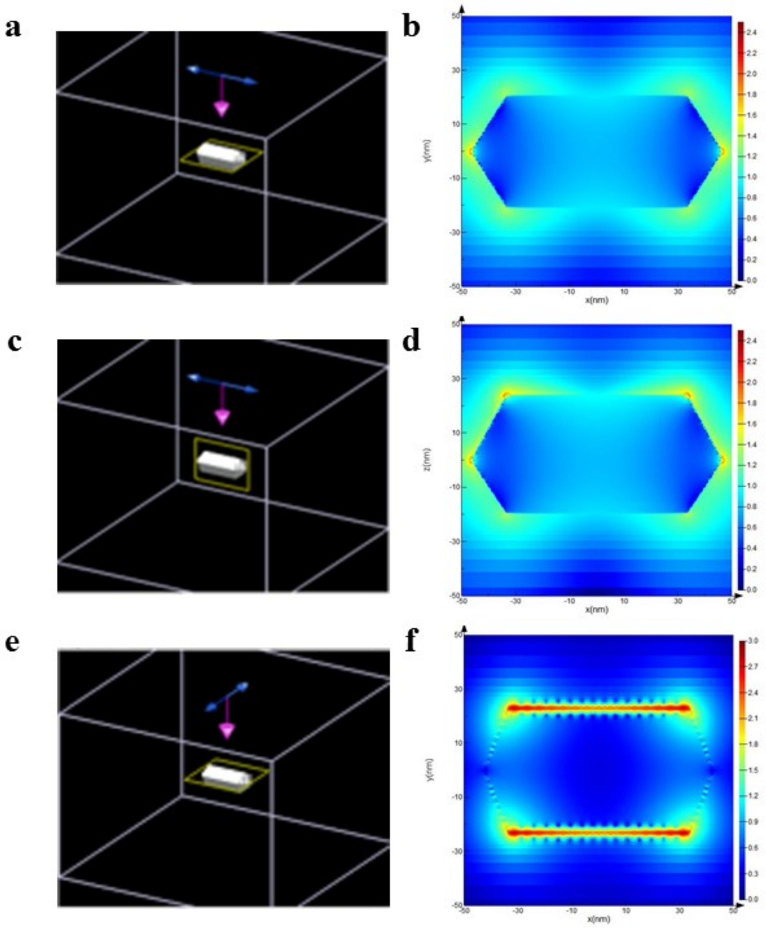

Fig. S10 (a), (c), and (e) Three different perspective views of electromagnetic near-filed distribution simulation model for an individual Ag nanorod. (b) and (d) The electromagnetic near-filed distribution simulation results in longitudinal direction from different cross sections. (f) The electromagnetic near-filed distribution simulation results in transverse direction

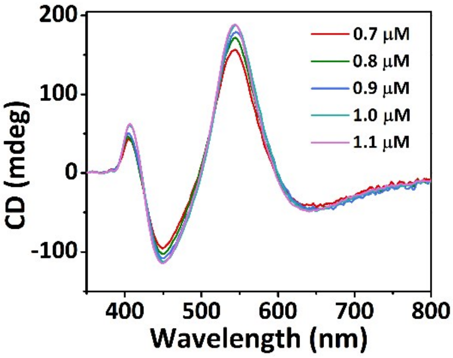

Fig. S11 CD spectra of Ag nanorods after mixing with L-Cys solution of varying concentrations. The CD intensity reaches the saturation when the concentration of cysteine increases to 1.0 μM.

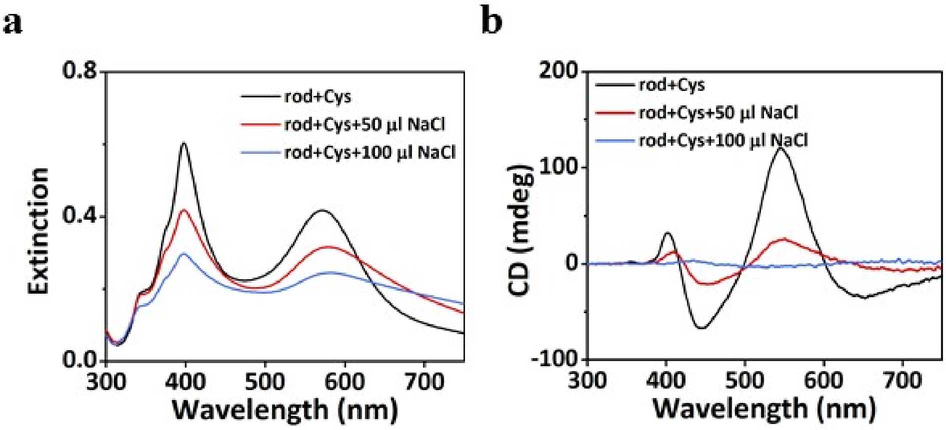

Fig. S12 The impact of aggregation of Ag nanorods on PCCD by introducing NaCl solution. (a) Extinction spectra and (b) CD spectra of L-Cys-functionalized Ag nanorods after adding 50 μl and 100 μl of 1 M NaCl, respectively.

References

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