SUPPORTING INFORMATION

Distance Mediated Electrochemiluminescence Enhancement of CdS Thin Film Induced by the Plasmon Coupling of Gold Nanoparticle Dimer

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ESI 1. Experiment section

Reagents

The oligonucleotides used in this work were purchased from Sangon Biological Engineering Technology & Co. Ltd (Shanghai, China) and the sequences are shown in Table S1. Tris (2-carboxyethyl) phosphine hydrochloride (TCEP), 6-Mercapto-1-hexanol (MCH), cloroauric acid ($HAuCl_4$), sodium borohydride ($NaBH_4$), sodium citrate, (3-aminopropyl)triethoxysilane (APTES, 99%) and O-(3-carboxypropyl)-O'-[2-(3-mercaptopropionylamino)ethyl]-polyethylene glycol (Mw 3000, SH-PEG-COOH) were provided by Sigma-Aldrich (St. Louis, MO). AuNPs with an average diameter of 5 nm, 10 nm, 15 nm and 20 nm were purchased from Ted Pella, Inc. Sodium sulfide ($Na_2S{\cdot}9H_2O$) and potassium peroxydisulfate ($K_2S_2O_8$) were obtained from Nanjing Chemical Co. Ltd. Cadmium nitrate tetrahydrate ($Cd(NO_3)_2{\cdot}4H_2O$) were supplied by Sinopharm Chemical Reagent Co. Ltd. Phosphate buffer solution (PBS, 0.1 M, KH_2PO_4-K_2HPO_4-NaCl) containing 50 mM $K_2S_2O_8$ (pH 8.3) as a coreactant was used for ECL detection. All other regents were of analytic grade and used without further purification. The Millipore (model milli-Q) ultrapure water (resistivity of 18.2 MΩ cm) was used throughout the experiment.

Table S1. Sequences of oligonucleotides used in this work

label		oligonucleotide sequences
9-mer	pDNA-a	5'-SH-$(CH_2)_6$-TTACCTCGT-3'
15-mer	ssDNA1-a	5'-ACTTGACCTACTGCA-$(CH_2)_6$-3'
24-mer	ssDNA2-a	5'-SH-$(CH_2)_6$-TGCAGTAGGTCAAGTACGAGGTAA-3'
18-mer	pDNA-b	5'-SH-$(CH_2)_6$-TTACCTCGTCAGTACCTA-3'
18-mer	ssDNA1-b	5' -GTCACTTGACCTACTGCA-SH-$(CH_2)_6$-3'
36-mer	ssDNA2-b	5'-SH-$(CH_2)_6$-TGCAGTAGGTCAAGTGACTAGGTACTGACGAGGTAA-3'
27-mer	pDNA-c	5'-SH-$(CH_2)_6$-TTACCTCGTCAGTACCTATAGTCCGAT-3'
27-mer	ssDNA1-c	5'-CTAGTCCGAGTCACTTGACCTACTGCA-$(CH_2)_6$-3'
54-mer	sDNA2-c	5'-SH-$(CH_2)_6$-TGCAGTAGGTCAAGTGACTCGGACTAGATCGGACTA TAGGTACTGACGAGGTAA-3'

Apparatus

The ECL measurements were conducted on a MPI-E multifunctional electrochemical and chemiluminescent analytical system (Xi'An Remax Electronic Science & Technology Co. Ltd., Xi'An, China, 350-650 nm) at room temperature. The voltage of the photomultiplier tube (PMT) was set at -500V, and the scan rate was set at 0.1 V s^{-1} in the detection process. The experiments were carried out with a conventional three-electrode system. The working electrode was a 3 mm diameter glassy carbon electrode (GCE), meanwhile, a Pt wire and saturated calomel electrode (SCE) served as the counter and reference electrodes, respectively. The ECL spectra were measured with optical filters (20 nm spaced). (Scheme S1) Electrochemical impedance spectroscopy (EIS) experiments were conducted on a PGSTAT30/FRA2 system (Autolab, Netherland). Transmission electron microscopy (TEM) was performed with a JEM-2100 transmission electron microscope (JEOL Ltd., Japan). The UV-vis absorption spectra were obtained on a Shimadzu UV-3600 UV-vis-NIR photospectrometer (Shimadzu Co., Japan) at room temperature.

Preparation of Au nanoparticles

Au NPs with average diameter 5±1 nm were prepared through the reduction of $HAuCl_4$ by $NaBH_4$ according to the reported methods[1]. Briefly, 0.6 mL of ice cold $NaBH_4$ (0.1 M) was added to 20 mL of aqueous solution containing 2.5×10^{-4} M $HAuCl_4$ under stirring. The mixture immediately turned to orange-red color, indicating the formation of gold nanoparticles. Keep on stirring in ice bath for 10 min. Then, the solution reacted at room temperature with continuous stirring for another 3h till the color changed from orange-red to wine red. The prepared colloid Au NPs were stored in brown glass bottles at 4 ℃ for further use.

Preparation of ssDNA functionalized AuNPs

0.1mM thiolated ssDNA was first activated with 10 mM TCEP in 10 mM PBS buffer (pH 7.4) for 1 h at room temperature. Then 50 μL of 1 μM DNA was added into 300 μL gold colloidal solution with gentle shaking. Followed by addition of 100μL 1mM SH-PEG-COOH in PBS buffer. After incubated under mild shaking for 8 h, the mixture was then aging for another 16 h with NaCl (2.0 M) added to the solution to bring its total NaCl concentration to 0.1 M. Then NPs were centrifuged and washed three times with PBS buffer, and resuspended in buffer solution (100 μL). Then the ssDNA-AuNP probes were obtained.

Synthesis of CdS NCs

CdS NCs were prepared according to the literature[2] with slight modification. In brief, $Cd(NO_3)_2 \cdot 4H_2O$ (0.1683 g) was dissolved in 30mL ultra-pure water, and heated to 70℃ under stirring, then a freshly prepared solution of Na_2S (0.596 g) in 30 mL ultra-pure water was slowly injected and instantly orange-yellow solution was obtained. The reaction was held at 70℃ for 3 h with continuous refluxing. The final reaction precipitates were centrifugated and washed thoroughly with absolute ethanol two times and ultrapure water two times. Then the obtained precipitate was redispersed into water for centrifugation to collect the upper yellow solution of CdS NCs. The average size of synthesized CdS NCs was about 5 nm, as indicated by transmission electron microscopy and UV-vis spectrum. The final solution was stored at 4℃ for further use.

Preparation of CdS NCs modified GCE

The GCE was pretreated by polishing with sand papers of 2000# and 5000# sequentially and then with 0.05 μm alumina powder to obtain a mirror-like surface. Then, GCE was thoroughly rinsed with water and sonicated in ethanol and ultrapure water in turn. 10 μL of the CdS NCs colloidal solution were dropped onto the pretreated GCE and dried in air at room temperature to produce the uniform CdS NCs film. At last, the CdS NCs modified GCE was stored in 0.1M PBS buffer (pH 7.4) containing 0.1M NaCl for characterization and further modification.

In situ fabrication of AuNP dimer on the electrode surface

The synthesis of AuNP dimer on the surface of GCE was carried out through the hybridization of DNA complementary. Briefly, 50 μL of 0.1 μM pDNA in 10 mM PBS (pH 7.4) containing 0.1 M NaCl were activated with 2 μL 10 mM TCEP for reduction of disulfide bonds. And the CdS NCs modified GCE was immersed in the pDNA solution for 16 h at 4 ℃. Then the electrode was rinsed with PBS and immersed in 100 μM MCH to remove nonspecifically adsorbed DNA and obtain a MCH monolayer to help to improve subsequent hybridization efficiency[3]. After rinsed with 10 mM PBS, the electrode was immersed in ssDNA1 functionalized AuNPs containing 0.1 M Na^+ and 5 mM Mg^{2+} at 37℃ for 90 min, and then incubated with ssDNA2 functionalized AuNPs in the same condition for the hybridization of ssDNA1 and ssDNA2 to obtain AuNPs dimers. For the TEM characterization, the ssDNA1 and ssDNA2 functionalized AuNPs were pre-hybridized at 37℃ and centrifugated separation.

Measurement of the densities of AuNPs monomers and dimers on the electrode surface

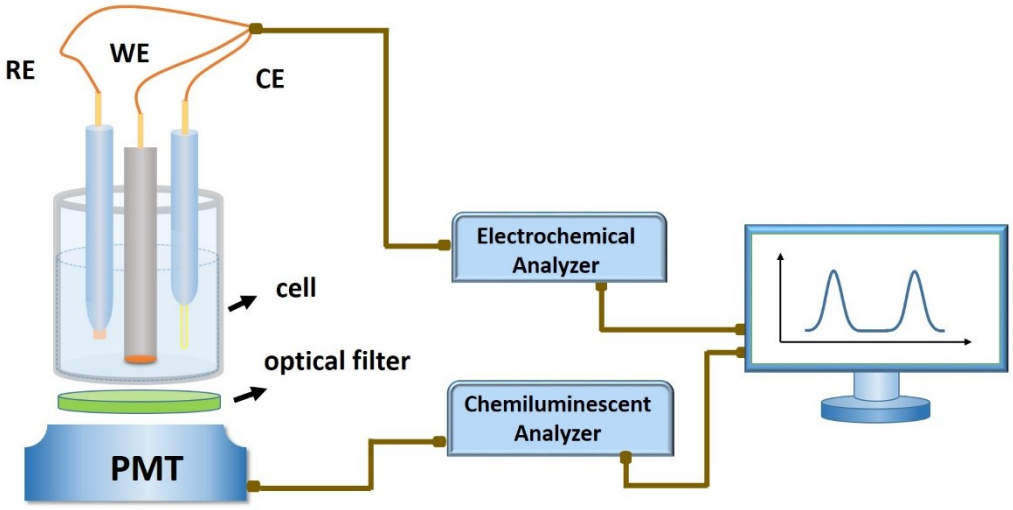
To measure the densities of AuNPs, ITO glass with fixed area of 1cm×2cm was used as the substrate. All the modification processes including CdS film construction, incubation of probe DNA and fabrication of AuNPs monomers and dimers were according to the modification on GCE. UV-Vis spectrum of the ssDNA1 and ssDNA2 functionalized AuNPs bulk solutions were recorded before and after the incubations. The differences were calculated and the densities of AuNPs modified on the surface of electrode were estimated according to the standard curves of UV-Vis spectrum of AuNPs solutions with different conditions.

Finite-difference time domain (FDTD) calculation

The finite-difference time domain (FDTD) (the package of Lumerical FDTD Solutions 8.15) method was used to perform the simulations about total extinction and electromagnetic response of gold monomer and dimer with different size. In the simulations, gold nanosphere monomer and dimer with the interparticle distance of 2 nm were used as model. The refractive index of surrounding medium was set as 1.33. A total-field scatted-field source with circular polarization by averaging over two orthogonal polarizations was used.

Fluorescence lifetime measurement of CdS NCs, CdS/Au monomer and CdS/Au dimer

The fluorescence lifetimes were measured to verify the resonance energy transfer between AuNPs and CdS NCs. First, thiolated pDNA was activated with 10 mM TCEP buffer (pH 7.4) for 1 h at room temperature. Then 50 μL of 1 μM DNA was added in to 400 μL newly synthesized CdS NCs solution with gentle shaking to obtain the pDNA modified CdS complex. After incubating with ssDNA2 functionalized AuNP monomer and pre-synthesized AuNP dimer respectively, the samples of CdS/Au monomer and CdS/Au dimer were obtained. The relative molar ratio was about 1:1 (pDNA to ssDNA). Fluorescence lifetime measurements were carried out using a time-correlated single photon couting (SPC) spectrometer (FLS 980). The exciting wavelength was set at 405 nm, and 520 nm was chosen to monitor the fluorescence decays.



Scheme S1 Experimental setup of ECL spectra measurement

ESI 2. Particle size distribution analysis

The histogram of the diameter of Au NPs was measured using Nano Measure 1.2. The standard deviation was calculated as 0.369.

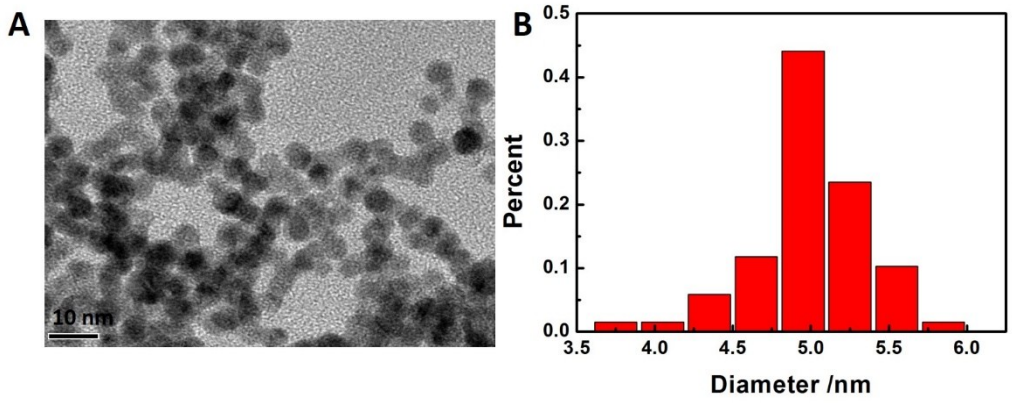


Fig. S1 (A) The TEM image and (B) histogram of the diameter of Au NPs.

ESI 3. The UV-Vis absorption spectra and ECL signal response related to AuNPs modified with different blocking agents.

Au nanoparticles were modified with different blocking agents to occupy the active site, the UV-Vis absorbance spectroscopy would change greatly with apparent shift on the LSPR peak of Au NPs. From Fig. S2A (ESI†), the absorbance of Au nanoparticles was red shifted after modified with MCH or PEG via Au-S bonding. And the peak shift of MCH (Fig. S2A, curve c, ESI†) modified Au was greater than PEG (Fig. S2A, curve b, ESI†), which caused lower degree of

overlapping in spectroscopy and energy transfer efficiency, resulting in the different responses of ECL intensity of CdS NCs film. We found that while AuNP monomers fabricated to the surface of GCE, PEG modified AuNPs induced higher enhancement of ECL intensity of CdS film for about 4.3 fold (Fig. 2A, curve c), and the MCH modified AuNPs induced 3.8 fold (Fig. S2B, curve c, ESI†) ECL enhancement. After the dimers were constructed, both showed higher increase. The PEG modified AuNP dimers resulted in 6.8 fold ECL enhancement (Fig. 2A, curve d), which was higher than that based on MCH (4.5 fold) (Fig. S2B, curve d, ESI†). Therefore, PEG was chosen for AuNPs modification.

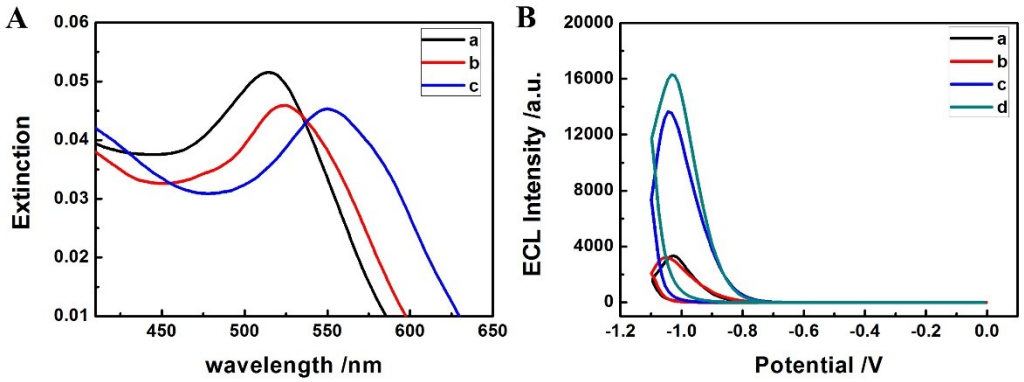


Fig. S2 (A) The extinction of (a) AuNPs and that modified with (b) PEG or (c) MCH; (B) ECL intensity curve of GCE with MCH modified Au nanoparticles at different stage. (a) GCE/CdS, (b) GCE/CdS/pDNA/MCH, (c) GCE/CdS/pDNA/MCH/AuNP monomers, (d) GCE/CdS/pDNA/MCH/ AuNP dimers.

ESI 4. Densities of self-assembled AuNP monomers and dimers

To measure the densities of AuNP monomers and dimers on CdS film, we recorded the UV-Vis spectrum of the ssDNA1 and ssDNA2 functionalized AuNPs bulk solutions before and after self-assemblies of AuNPs on the substrate surfaces (Fig. S3). The differences were calculated and the densities of AuNPs modified on the surface of electrode were estimated according to the standard curves of UV-Vis spectrum of AuNPs solutions with different concentrations. According to the experimental results, the density of AuNP monomers was $1.50\times10^{11}/cm^2$. After the incubation of ssDNA1-PEG-AuNPs, 44% monomers were linked with another particle to form AuNP dimers and the density of dimers was calculated as $6.57\times10^{10}/cm^2$.

It should be noted that we optimized the incubation time to ensure the saturated assembly of

AuNPs, which guaranteed the good repeatability of the experiment.

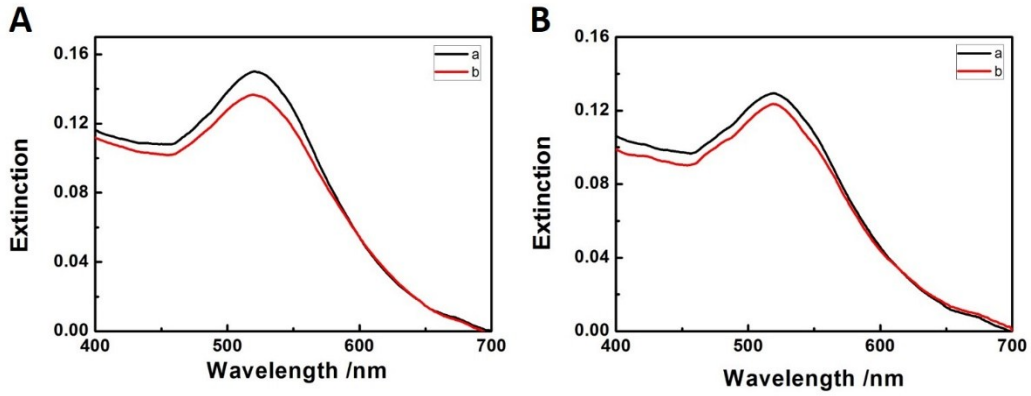


Fig. S3 The UV-Vis absorbance spectra of (A) ssDNA2-PEG-AuNPs and (B) ssDNA1-PEG-AuNPs before (a) and after (b) self-assemblies of AuNPs on the surfaces of modified ITO.

ESI 5. The EIS characterization of GCE at different stages.

Electrochemical impedance spectroscopy (EIS) is an effective method for probing the interfacial properties of modified electrodes. Here the EIS were measured to characterize the surface status of electrode at different stage which employing $[Fe(CN)_6]^{3-/4-}$ as the redox probe in the supporting electrolyte solution. And the electron-transfer resistance (Ret) value can be estimated from the semicircle diameter of EIS Nyquist plot. As Fig. S4 showed, the bare GCE had a small semicircle diameter of EIS (Fig. S4, curve a). While the GCE was modified with CdS NCs, pDNA and MCH respectively, the Ret increased step by step. After the ssDNA2-AuNP probes were characterized to the surface of GCE, the Ret value decreased apparently, which means that AuNPs could accelerate the reaction of $[Fe(CN)_6]^{3-/4-}$ redox system. And the further reduced Ret (Fig. S4, curve f) also certificated the formation of AuNP dimers. These phenomena were in consistent with the ECL and CV results.

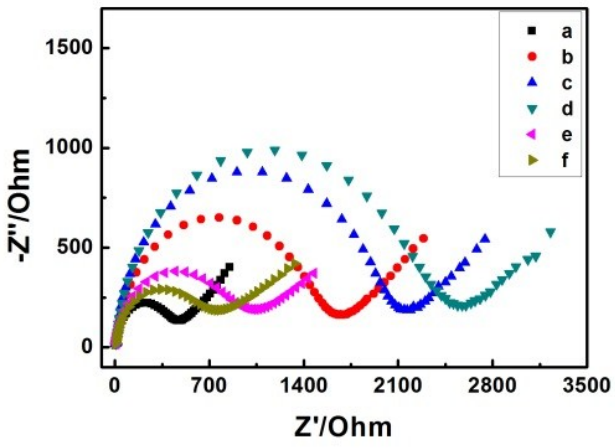


Fig. S4 The EIS characterize of the GCE at different stages. (a) GCE, (b) GCE/CdS NCs, (c) GCE/CdS NCs/pDNA, (d) GCE/CdS NCs/pDNA/MCH, (e) GCE/CdS NCs/pDNA/MCH/AuNP monomers, (f) GCE/CdS NCs/pDNA/MCH/AuNP dimers. EIS were measured in 5.0 mM $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ containing 0.1 M KCl.

ESI 6. FDTD simulation of EM field of AuNP dimer with different widths of gaps.

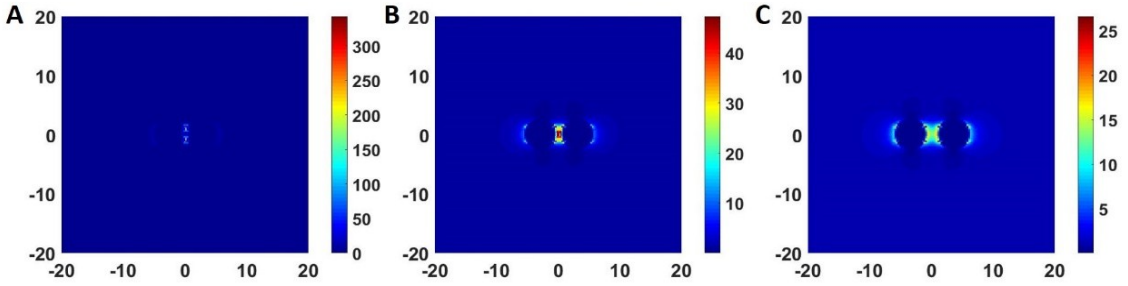


Fig. S5 The electromagnetic field enhancement of AuNP dimer with different gap widths of (A) 0 nm, (B) 1 nm and (C) 2 nm.

Reference

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2. Y. Y. Zhang, H. Zhou, P. Wu, H. R. Zhang, J. J. Xu and H. Y. Chen, *Analytical chemistry*, 2014, **86**, 8657-8664.
3. R. Levicky, T. M. Herne, M. J. Tarlov and S. K. Satija, *Journal of the American Chemical Society*, 1998, **120**, 9787-9792.