Supporting information

Synthesis of N-(aminobutyl)-N-(ethylisoluminol) Functionalized Gold Nanomaterials for Chemiluminescent Bio-probe
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**Experimental Section**

*Chemicals and solutions:* A HAuCl₄ stock solution (2% HAuCl₄, w/w) was prepared by dissolving 1.0 g of HAuCl₄·4H₂O (Shanghai Reagent, China) in 412 mL of purified water and stored at 4 ºC. N-(aminobutyl)-N-(ethylisoluminol) (ABEI) was purchased from Sigma-Aldrich (USA). Antibody (goat-anti-human IgG) and streptavidin (SA) were obtained from Solarbio (Beijing, China). DNA used in this work was synthesized and HPLC-purified by sangon Inc (shanghai, China). The sequence of the modified oligonucleotide used is 5′-biotin-AAAAAAAAAAGACCTAGTCCTTCCAACAGC-3′. A 4.0 mmol/L stock solution of ABEI was prepared by dissolving ABEI in 0.1 mol/L NaOH solution and was kept at 4 ºC. All other reagents were of analytical grade. Ultrapure water was prepared by a Milli-Q system (Millipore, France) and used throughout.

*Synthesis and Characterization of CF-AuNMs:* All glassware used in the following procedures was cleaned in a bath of freshly prepared HNO₃-HCl (3:1, v/v), rinsed thoroughly in ultrapure water, and dried prior to use. Gold colloids with various morphologies were prepared by the reduction of HAuCl₄ with ABEI in aqueous solution at room temperature. A 9 mL portion of HAuCl₄ stock solution was mixed with 45 mL ultrapure water. While stirring vigorously, various amounts of ABEI stock solution were added rapidly, the solution was maintained at room temperature for 2 hours. Then another 6 mL portion of HAuCl₄ stock solution was added and the reaction was kept on for another 2 hours, during which time a color change from yellow to black to purple was observed before a deep-pink, wine-red or purple color
was reached. All the prepared colloidal gold dispersions were stored at 4 °C for future use. ABEI volumes of 4.5, 5.0, 5.5 and 6.0 mL were selected for synthesizing CF-AuNMs with various morphologies.

As-synthesized colloidal gold dispersions were subsequently characterized by TEM (Jeol Ltd., JEOL-2010, Japan), EDS (Jeol Ltd., JEOL-2010, Japan), FT-IR (Bruker Instruments, Billerica, MA), and UV-visible spectroscopy (Agilent 8453 UV-visible spectrophotometer, USA). The histograms were obtained by measuring the diameters of nanoparticles (n > 100) in TEM photomicrographs. For XRD measurement, proper amount of colloids was centrifuged and dried under vacuum at room temperature. The obtained dry powders were characterized by using a Philips X’Pet Pro Super diffractometer with Cu Kα radiation (λ = 1.54 Å). Colloidal gold dispersions (chain-shaped of CF-AuNMs) prepared with 5.0 mL of ABEI were chosen for the UV-visible spectroscopy and XPS characterization. First, the dialysis procedure was used to remove unreacted reagents and resulted products (small molecules) in chain-shaped of CF-AuNMs. A 3500 molecular weight cutoff dialysis membrane was used, and ultrapure water was refreshed about six times under stirring until no absorption was observed in the UV-visible spectrum of the dialyzed-out solution. A typical dialysis operation needed about 2 days, and finally the dialyzed CF-AuNMs solution was obtained. Second, a salt-out procedure was introduced to further exclude the effect of excess ABEI and coexisting free molecules in the dialyzed CF-AuNMs for characterizing the surface state of CF-AuNMs. In a typical salt-out procedure, a suitable amount of NaCl was added to the dialyzed CF-AuNMs
and the precipitates were washed with ultrapure water through a centrifugation-ultrasonic dispersion process to removing inorganic or organic impurities completely. Finally the purified CF-AuNMs were dried under vacuum at room temperature for XPS detection. XPS was performed on an ESCALABMK II electron spectrograph (VG Scientific, UK) with Al Kα radiation as the X-ray source. The XPS data were fitted by XPSPeak and further plotted by OriginPro 7.0.

CL detections of CF-AuNMs: The static injection CL detection was conducted on a BPCL Luminescence Analyze (Beijing, China) with a fixed voltage of -900 V. For a typically CL measurement, 400 µL of 0.1 mol/L H₂O₂ was added to a cylindrical cell, then 500 µL of the centrifuged CF-AuNMs with various morphologies (dissolved in pH=13 0.1 mol/L NaOH solution) was injected into the cell to initiate the CL reaction. The CL intensity during the reaction along with time was recorded by the Luminescence Analyzer.

Fabrication of bio-probe and its CL measurements: The chain-shaped CF-AuNMs was used as tags to label antibody and DNA to obtain bio-probe. The bio-probe of the chain-shaped CF-AuNMs labeled antibody was prepared as follow: 1 mL (pH-adjusted pH 8.0) of the dialyzed colloidal gold solution was added to 25 µL of antibody (1.0 mg/mL). After incubated at room temperature for 0.5 h, 5 % bovine serum albumin (BSA) solution was added to a final concentration of 1 % with stirring for 5 min. The conjugate was centrifuged at 12500 rpm for 45 min (Universal 320, Hettich, Germany), and the red precipitates were dispersed with 250 µL of PBS (0.1 mol/L PBS, pH 7.4) containing 1 % BSA to obtain chain-shaped CF-AuNMs.
CF-AuNMs labeled antibody bio-probe. Fabrication of the chain-shaped CF-AuNMs labeled DNA bio-probe was as follow: 1 mL (pH-adjusted pH 8.0) of the dialyzed chain-shaped of CF-AuNMs solution was added to 25 µL of SA (1.0 mg/mL). After incubated at room temperature for 0.5 h, the solution was further added with 5 % BSA solution to the final concentration of 1 % BSA, then stirred for 5 min. The conjugate of chain-shaped of CF-AuNMs with SA was centrifuged at 12500 rpm for 20 min, and the red precipitates were dispersed with 1 mL of 0.05 mol/L pH 8.0 Tris-HCl containing 0.3 mol/L NaCl. Biotinylated DNA (1×10^{-6} M) was added to the red solution and incubated at 37°C for 1 h. Thermo Shaker (Biosan, Latvia) was used to control the temperature of the reactions. The 1 mL of the as-prepared mixture was centrifuged at 12500 rpm for 10 min, and the red precipitates were resuspended with 250 µL of 0.05 mol/L Tris-HCl (pH 8.0, containing 0.3 mol/L NaCl ) to obtain chain-shaped of CF-AuNMs labeled DNA bio-probe.

The static injection CL measurement of the chain-shaped of CF-AuNMs labeled antibody or DNA bio-probe was conducted on Luminescence Analyze with a fixed voltage of -900 V. For a typically CL measurement, 400 µL of 0.1 mol/L H_2O_2 containing 0.1 mol/L NaOH was added to a cylindrical cell, then 500 µL of the centrifuged CF-AuNMs labeled antibody or DNA bio-probe was injected into the cell to initiate the CL reaction. The CL intensity during the reaction along with time was recorded by the Luminescence Analyzer.
Figure S1. Typical EDS spectra of ABEI functionalized AuNPs.

The EDS results (Figure. S1)) indicated the nanoparticles were gold. All the Cu peaks in the EDS spectra were attributed to the copper grids.
Figure S2. Typical XRD pattern of ABEI functionalized AuNPs.

From Figure S2, five diffraction peaks of the AuNPs ((111), (200), (220), (311), (222)) indicated that as-prepared AuNPs were very consistent with the gold crystal of face-centered cubic (fcc) structure.
Figure S3. UV-visible absorption spectra of A) pure ABEI, B) original chain-shaped of CF-AuNMs, C) dialyzed chain-shaped of CF-AuNMs, D) secondary redispersed chain-shaped of CF-AuNMs precipitated during the dialyzing, centrifuging, redispersing and salt-out procedures, E) supernatant after dialyzing, centrifuge, redispersing and salt-out of chain-shaped of CF-AuNMs. Inset: magnification of curve E.

The dialyzing, centrifuging, redispersing and salt-out procedures were carried out to remove free ABEI and coexisting free molecules from colloids, so that true
information about the surface of the CF-AuNMs could be obtained by instrumental characterization. UV-visible absorption spectra of CF-AuNMs were examined as shown in Figure S1. From Figure S3A, it could be seen that the characteristic absorption peaks of pure ABEI appeared around at 290 nm and 320 nm. After dialysis, the ABEI dual absorption peaks could be detected (Figure S3C) but the intensity of the peak decreased compared with that of the original colloids (Figure S3B), and the maximal surface plasmon absorption wavelength remained at 525 nm but the intensity of the peak also decreased. The former result indicated that the dialysis operation was feasible and effective in removing free ABEI and coexisting free molecules from colloids, and the latter result was attributed to the dilution effect during the dialysis procedure. Subsequently, a further salt-out operation was executed on the dialyzed colloid and observable coagulation appeared after the addition of salts. The dual peak absorption of ABEI appeared again in the supernatant after centrifugation (Figure S3E). The precipitates were collected and redissolved in NaCO₃ solution. From Figure S3D, it could be obviously seen the maximal surface plasmon absorption wavelength increased from 525 nm to 535 nm and the peak became wide. These results demonstrated that the protective molecules were partially dissociated from the surface of the CF-AuNMs and the repulsive interaction became weak during the salt-out process, which was the reason why the ABEI molecules could be detected again in the supernatant. Furthermore, these results also provided evidence for the existence of ABEI on the surface of AuNPs.
Figure S4. X-ray photoelectron spectra of A) Au 4f and B) C 1s and D) N 1s of pure ABEI and C) C 1s and E) N 1s of ABEI-reduced chain-shaped of CF-AuNMs. The inset shows the molecular structure of ABEI.

Figure S4 showed the Au 4f, C 1s and N 1s X-ray photoelectron spectra of pure ABEI and the as-prepared ABEI-reduced chain-shaped of CF-AuNMs after further treatments. All binding energies (BEs) were calibrated with respect to the C 1s BEs at 284.6 e V. The spin-orbit splitting of doublet components for Au 4f_{7/2} and Au 4f_{5/2}
(Figure S4A) were measured to be 3.7 eV, which was in good agreement with previous reports. This result was supporting evidence for the presence of Au$^0$ in as-prepared CF-AuNMs. As can be seen from Figure S4B, the C 1s spectrum of pure ABEI was curve-fitted into three components at 284.9, 286.0 and 287.3 eV with the same full width at half-maximum (fwhm, 1.25 eV), which might correspond to carbon atoms from 1 to 3 in the molecular structure of ABEI. The area ratio of the three curves was ca. 3.91:1.96:1, which matched the ratio of carbon atoms (C1:C2:C3) in the molecular structure of ABEI. The maximal peak centered at 284.9 (C1 in the molecular structure of ABEI) was attributed to the carbon atom in the –CH– group. The component at 286.0 eV (C2) was associated with the carbon atom in the –C–NH– group. The component at 287.3 eV (C3) was due to the carbon atom in the –CO–NH– group. The C 1s spectrum of ABEI-reduced chain-shaped of CF-AuNMs is shown in Figure S4C. Compared with the C 1s of pure ABEI, peaks of C1, C2 and C3 shifted -0.1, -0.3 and -0.3 eV with the same fwhm 1.50 eV, respectively, toward lower binding energies. Moreover, owing to part of –CO–NH– group in ABEI molecule was oxidized to carboxylic group, a new component centered at 288.8 eV (C4) appeared, which was attributed to the carbon atom in the carboxylic group (–COO–) of the oxidation product N-(aminobutyl)-N-(ethylphthalate) of ABEI, the area ratio of C1:C2:(C3+C4) (4:2:1) matched the theoretical value well. The result not only supported the notion that the oxidation product N-(aminobutyl)-N-(ethylphthalate) of ABEI was N-(aminobutyl)-N-(ethylphthalate), but also strongly supported that ABEI and its oxidation product coexisted on the
surface of the AuNPs.

The analysis of the N 1s spectra was consistent with the above discussion. The N 1s spectra of pure ABEI (Figure S4D) was curve-fitted into two components at 399.5 and 400.8 eV, attributed to the nitrogen atoms in the –N–C– group and –N–CO– group, respectively. The area ratio (1.04:1) matched the theoretical value very well with the same fwhm 1.60 eV. The N 1s spectrum of CF-AuNMs is shown in Figure S4E, peak of the nitrogen atoms in the –N–C– group had a negative shift (-0.3 eV), and peak of the nitrogen atoms in the –N–CO– group shifted about -0.5 eV, respectively. The area ratio (2.06:1) did not match the theoretical value with the same fwhm 1.60 eV. This was due to the –N–CO– group of ABEI was oxidized to the –COO– group of N-(aminobutyl)-N-(ethylphthalate), and the relative content of the –N–CO– group decreased. Notably, the N 1s spectrum of CF-AuNMs also confirmed the absence of the protonated state (–NH$_3^+$) reported to be at approximately 402.3 eV. Consequently, the electrostatic interaction between the protonated amino group and negatively charged AuNPs did not exist, which was reported to occur in the other fatty-amine-protected CF-AuNMs in aqueous solution. In addition, the Au-N covalent interaction was the only force between the gold core and the ABEI/its oxidation product capping molecules.

The shifts of binding energies in C 1s and N 1s implied an interaction between ABEI/its oxidation product and Au atoms at the interface. The electron clouds inclined to transfer from the Au surface to ABEI/its oxidation product.
Figure S5. FT-IR spectrum of A) pure ABEI and B) ABEI functionalized AuNPs.

From Figure S5, the typical doublets of the amino group in the ABEI standard IR spectrum around 3400 cm\(^{-1}\) (\(\nu_{\text{N-H}}\)) disappeared (Fig S5A) and only one broad band (Fig S5B) appeared in the range of 3300-3700 cm\(^{-1}\), which was a reflection of the weak covalent interaction between the amino group and the AuNPS.
Figure S6. UV-visible absorption spectra of various morphologies of CF-AuNMs synthesis with varying amounts of ABEI solution: a) 4.5, b) 5.0, c) 5.5, d) 6.0 mL.
Figure S7. CL behaviors of ABEI functionalized AuNPs and luminol functionalized AuNPs.

As shown in Figure S7, 400 µL of 0.1 mol/L H₂O₂ was added to a cylindrical cell, then 500 µL of the centrifuged CF-AuNM (dissolved in pH=13 0.1 mol/L NaOH solution) was injected into the cell to initiate the CL reaction. In synthesis of chemiluminescent functionalized AuNPs, the amount of ABEI and luminol are the same.