Support Information

Plasmonic resonance energy transfer from Au nanosphere to quantum dot at single particle level and its homogenous immunoassay

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Chemical and Material

Capture antibody, detection antibody, and prostate specific antigen (PSA) were purchased from Shanghai Linc-Bio Science Co., LTD (Shanghai, China). Au nanosphere (AuNS) with a diameter of 70 nm was purchased from Nanopartz Inc. (Loveland CO, US). Albumin from chicken egg, bovine serum albumin, human serum albumin, trypsin inhibitor from chicken egg white, phytohemagglutinin, transferrin, fibrinogen, poly (diallyldmethylammonium chloride), and N-(3-(dimethylamino)propyl)-N-ethylcarbodiimide hydrochloride (EDC) were ordered from Sigma-Aldrich (St. Louis, MO, USA). QD 800 (cat. no. Q21371MP), phosphate buffer saline (PBS, containing 4.0 mM phosphoric acid and 155.2 mM NaCl), and coverslips were obtained from Thermo Scientific (Waltham, MA, USA). The single-strand DNAs (ssDNAs) were purchased from Sangon Biotech Co., LTD (Shanghai, China). The sequences of ssDNAs were summarized in Table S1. The ssDNA 1-8 and the ssDNA 1a-8a were used to modify AuNS and QD 800, respectively. A transmission grating with 70 lines/mm was purchased from Edmund Scientific (Barrington, NJ). Other chemicals were purchased from local vendors.

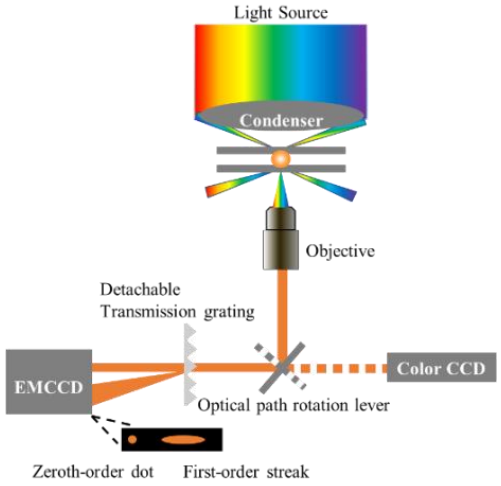
name	sequence (5'to 3')	Modification
DNA 1	$(A)_{20}$CGCCTACTACCGAATTCGATAGTCATCAGC	
DNA 1a	GCTGATGACTATCGAATTCGGTAGTAGGCG	5'-NH_2
DNA 2	$(A)_{20}$CGTTCCGTGTGCATACTGAATTCCGTGTTACTCTTGC	
DNA 2a	GCAAGAGTAACACGGAATTCAGTATGCACACGGAACG	5'-NH_2
DNA 3	$(A)_{20}$CGTTCCGTGTGCATACTGAATTCCGTGTTACTCTTGCCAACCTCG	
DNA 3a	CGAGGTTGGCAAGAGTAACACGGAATTCAGTATGCACACGGAACG	5'-NH_2
DNA 4	$(A)_{20}$CGTTCCGTGTGCATACTGAATTCCGTGTTACTCTTGCCCCCGTCGACCTTTA	
DNA 4a	TAAAGGTCGACGGGGGCAAGAGTAACACGGAATTCAGTATGCACACGGAACG	5'-NH_2
DNA 5	$(A)_{20}$CGTTCCGTGTGCATACTGAATTCCGTGTTACTCTTGCCCCCGTCGACCTTTAGCTCTATA	
DNA 5a	TATAGAGCTAAAGGTCGACGGGGGCAAGAGTAACACGGAATTCAGTATGCACACGGAACG	5'-NH_2
DNA 6	$(A)_{20}$CGTTCCGTGTGCATACTGAATTCCGTGTTACTCTTGCCCCCGTCGACCTTTAGCTCTAGTGTATTTC	
DNA 6a	GAAATACACTAGAGCTAAAGGTCGACGGGGGCAAGAGTAACACGGAATTCAGTATGCACACGGAACG	5'-NH_2
DNA 7	$(A)_{20}$CGTTCCGTGTGCATACTGAATTCCGTGTTACTCTTGCCCCCGTCGACCTTTAGCTCTAGTGTATTTCATGATTGC	
DNA 7a	GCAATCATGAAATACACTAGAGCTAAAGGTCGACGGGGGCAAGAGTAACACGGAATTCAGTATGCACACGGAACG	5'-NH_2
DNA 8	$(A)_{20}$CGTTCCGTGTGCATACTGAATTCCGTGTTACTCTTGCCCCCGTCGACCTTTAGCTCTAGTGTATTTCATGATTGCTACGTAGC	
DNA 8a	GCTACGTAGCAATCATGAAATACACTAGAGCTAAAGGTCGACGGGGGCAAGAGTAACACGGAATTCAGTATGCACACGGAACG	5'-NH_2

Table S1. Single strand DNA sequences

Instrument

The experiments of single particle imaging were conducted on an Olympus IX71 equipped with an Evolve 512 EMCCD and a QIClickTM colorful CCD. To obtain scattering spectrum, a detachable transmission grating was inserted before the EMCCD as shown in Scheme S1. The scattering light divides into a zeroth-order beam and a first-order beam after passing through the transmission grating. The wavelength (λ) conforms to the formula: $\lambda = L \times d / S$, in which S, d, and L represent

the distance from the grating to the EMCCD sensor, the grating constant, and the distance from the zeroth-order dot to the corresponding first-order streak, respectively. The d/S value in our system was measured to be 2.7 nm/pixel. Image J was used to process the images.



Scheme S1. Transmission grating-based dark-field spectral imaging.

The TEM image, the fluorescence intensity of QD 800 and the scattering spectrum of AuNS (Figure S1), and the absorbance spectra of QD (Figure S1) were measured on an FEI Tecnai G2 T12, Hitachi F-4500 spectrophotometer, and a Tu-1901 UV-VIS spectrophotometer, respectively.

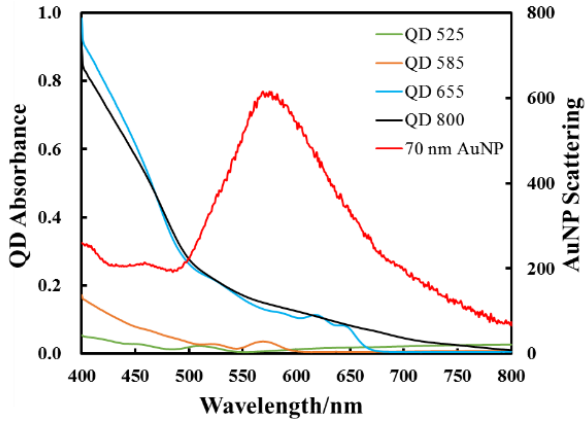


Figure S1. Absorbance spectra of QDs and scattering spectrum of AuNS with a diameter of 70 nm. The concentrations of QD and AuNS were 100.0 pM and 10.0 pM, respectively.

Plasma Samples

Plasma sample was handled according to the approved protocols by the Institutional Review Board of Jiangsu Normal University. The samples were provided by Xuzhou Center Hospital. All subjects signed written informed consents.

Positively charged glass slide preparation

After it was immersed in a chromic acid solution for 12 hr, the slide was ultrasonically washed 3 times with distilled water. The clean slide was sonicated in the bath of 1.0% poly (diallyldmethylammonium chloride) (V/V) for 30 min, soaked overnight, and dried at 85 ℃ for use later.

ssDNA modified AuNS (ssDNA-AuNS) preparation

ssDNA-AuNSs were prepared according to the method described by Liu et al[1]. In brief, AuNS was

mixed with ssDNA. The concentrations of AuNS and ssDNA in the mixture were 196.0 pM, and 1.7 μM, respectively. Immediately, the mixture was placed in -20.0 ℃ refrigerator. After overnight freezing, the mixture was naturally thawed to room temperature. To remove excessive ssDNA, the mixture was centrifuged at 13,000 rpm for 1 min, and the ssDNA-AuNS was resuspended with PBS. The set of wash was repeated twice. To remove AuNS aggregation, the ssDNA-AuNS suspension was centrifuged at 2500 rpm for 2 min. Then, the supernatant was recovered and stored in 4 ℃. Before use, the AuNS concentration was measured according to Lambert-Beer's law. According to datasheet provided by manufacture, extinction coefficient of 70 nm AuNS is $3.64*10^{10}$ M^{-1} cm^{-1} at a wavelength of 538.0 nm.

ssDNA modified QD 800 (ssDNAa-QD 800) preparation

To activate the carboxyl group of QD 800, 5.0 μL of QD 800 was mixed with 10.0 μL of freshly prepared 1 mg/mL EDC in 50.0 mM $NaHCO_3$ (pH 9.0) and 3.0 μL of the same $NaHCO_3$, and shaken in the dark for 20 min. Then 200.0 μl of ssDNA was added into the activated QD 800. The reaction was allowed to continue for 4 hr in the dark. To remove excessive ssDNA, ssDNAa-QD 800 was purified through centrifugation at 14,000 rpm for 5 min with 100 KD ultrafiltration tube. PBS was added into the remaining solution until the volume was up to 500 μL. The set of wash was repeated twice. The purified ssDNA-AuNS was stored in 4 ℃. The concentration of the ssDNAa-QD 800 was determined based on QD 800 fluorescence intensity (y=950.24x+54.486, correlation coefficient 0.9998).

Capture antibody modified AuNS (CA-AuNS) preparation

Capture antibody was immobilized on AuNS through two steps, namely preparing double strand DNA duplex modified AuNS (dsDNA-AuNS) and conjugating capture antibody to dsDNA-AuNS. dsDNA-AuNSs were prepared according to the method described by Liu et al[1]. In brief, AuNS was mixed with ssDNA 1 and its complementary partner, ssDNA 1a. The concentrations of AuNS, ssDNA 1, and ssDNA 1a in the mixture were 196.0 pM, 1.7 μM, and 1.7 μM, respectively. Immediately, the mixture was stored in -20.0 ℃ refrigerator. After overnight freezing, the mixture was naturally thawed to room temperature. After removing excessive DNA and AuNS aggregation by multi-centrifugations, the dsDNA-AuNS was resuspended with PBS. To activate the carboxyl group of dsDNA, 2.0 μL of freshly prepared 10.0 mg/mL EDC in 155.0 mM NaCl was added to the activated dsDNA-AuNS and the mixture was shaken at room temperature for 20 min. After removing excess EDC by centrifugation, the dsDNA-AuNS was immediately mixed with capture antibody and bovine serum albumin in a ratio of 1:96:384. The coupling reaction was allowed to continue for 3 hr at room temperature, and was terminated with tris-HCl. After removing the excessive proteins and AuNS aggregation by multi-centrifugations, the CA-AuNSs were stored in 4 ℃. Before use, the AuNS concentration was measured according to Lambert-Beer's law. According to datasheet provided by manufacture, extinction coefficient of 70 nm AuNS is $3.64*10^{10}$ M^{-1} cm^{-1} at a wavelength of 538.0 nm.

Detection antibody modified QD 800 (DA-QD 800) preparation

The activation of QD 800 was conducted following the above mentioned procedure. 20.0 μL of detection antibody was mixed with 10.0 μL of activated QD 800, and incubated for 4 hr in the darks. A 250.0 mm*4.6 mm size exclusion chromatography column packed with Superose 6 was used to purify DA-QD 800. 50.0 mM $NaHCO_3$ (pH 9.0) was used as mobile phase. Flow rate, injection volume, and detection wavelength were set as 0.1 mL/min, 15.0 μL, and 224 nm, respectively. The collected DA-QD 800s were store in 4 ℃. The concentration of the DA-QD 800s was determined

based on QD 800 fluorescence intensity (y=950.24x+54.486, correlation coefficient 0.9998).

Hybridization reaction between ssDNA-AuNS and ssDNAa-QD 800

ssDNA-AuNS was mixed with ssDNAa-QD 800, PBS, and NaCl. The concentrations of NaCl, phosphoric acid, and ssDNA-AuNS in the reaction solution (pH 7.4) were 300.0 mM, 4.2 mM, and 5.0 pM, respectively. The concentrations of ssDNAa-QD 800 was set in the range of 2.5 pM to 75.0 pM. In the dark, the solution was shaken at 95 ℃ for 5 min. After it was slowly cooled to room temperature, the solution was immediately used for single particle imaging.

Immunoreaction between CA-AuNS, PSA standard (serum), and DA-QD 800

CA-AuNS, DA-QD 800, PBS, and PSA standard solution (serum) were mixed. The concentrations of NaCl, phosphoric acid, DA-QD 800, and CA-AuNS were 140 mM, 4.2 mM, 143 pM, and 5.0 pM, respectively. The immunoreaction was allowed to continue for 2 hr in the dark. Immediately, the solution was used for single particle imaging.

TEM imaging

The TEM samples were prepared following the protocol for hybridization reaction between ssDNA-AuNS and ssDNAa-QD 800 with small modification. After the hybridization reaction was finished, the QD 800s unconjugated to AuNS were centrifuged centrifugation at 8000 rpm (each for 8 min) twice. The QDs unlinked to AuNS were in the supernatant and discarded, and the QDs linked to AuNS deposited with AuNS and were resuspended with NaCl (10.0 mM, pH 4.5). 5.0 μL of the obtained solution was dropped onto a positively charged copper grid. Figure S2 showed the TEM images of the hybridization solutions after removing the unconjugated QD 800. There was no QD 800 in the reaction solution of ssDNA-AuNS and QD 800 in Figure S2A because QD 800 could not be linked to ssDNA-AuNS. There were QD 800s in the reaction solution of ssDNA-AuNS and ssDNAa-QD 800 in Figure S2B because ssDNAa-QD were linked to ssDNA-AuNS through hybridization. Therefore, QDs in Figure S2B had been coupled to AuNS before they were dried on a TEM grid.

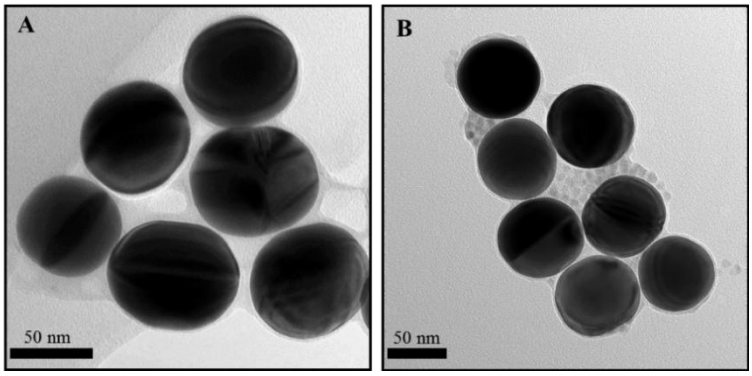


Figure S2. (A) Typical TEM images of the hybridization solution of ssDNA and QD 800 after removing QD 800 unconjugated to AuNS (A); and typical TEM images of the hybridization solution of ssDNA-AuNS and ssDNAa-QD 800 after removing QD 800 unconjugated to AuNS.

Single particle scattering imaging

1.5 μL of the test solution was dropped onto the positive charged slide, immediately covered with a coverslip, and sealed with nail polishing oil. To ensure the scattering dot was obtained from single particle, two measures were used. One was the concentration of AuNS was set in the range in which the AuNS concentration was in a linear relationship with the numbers of the imaged dots. The linear relationship between the numbers of the imaged dots and the particle concentration was a widely

used criterion for single particle imaging[2]. For ssDNA-AuNS and positively charged glass slide, the ssDNA-AuNS concentration was in a linear relationship with the numbers of the imaged dots when the concentrations of ssDNA-AuNS were in the range of 1.0 pM and 10.0 pM. The ssDNA-AuNS concentration used in this study was 5.0 pM. Figure S3 presented the scattering images of ssDNA-AuNSs in the presence of QD 800 or ssDNAa-QD 800. The AuNS density did not change no matter whether QD 800 coupling to AuNS or not. Another was that the single AuNS was distinguished from AuNS aggregation and processed later. Under dark-field microscopy, the color of a single AuNS with a diameter of 70 nm was green while that of AuNS aggregation was yellow even red, depending aggregation degree[3]. In this research, the same spot was imaged both by EMCCD and colorful CCD. The dots in yellow and red in colorful images were from aggregations judged by color, and then the corresponding dots in the black-and-white images were excluded from the subsequent data analysis.

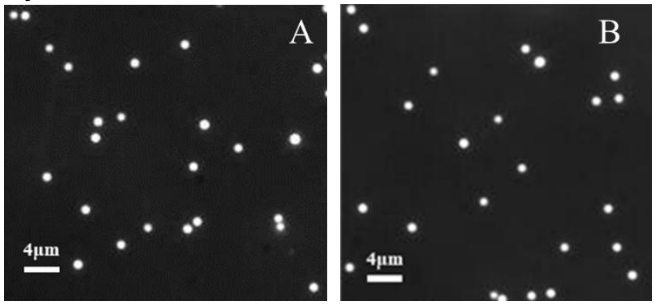


Figure S3 Typical single particle scattering image of ssDNA-AuNS in the presence of QD 800 (A) and in the presence of ssDNAa-QD 800 (B).

The influence of distance between ssDNA-AuNS and ssDNAa-QD 800 in PRET efficiency (PE)

dsDNA duplex was used as distance regulator. The reasons that ds DNA was chosen were as following: 1) dsDNA within 100 base pairs was rigid, and 2) the distance between two neighbouring base pairs was known to be 0.34 nm. The distance between AuNS and QD 800 was set in the range of 10.0 nm to 27.5 nm by controlling the number of dsDNA base pairs (Table S1). Figure S4 described the influence of distance between AuNS and QD 800 in PE. The PE value gradually increased with distance, reaching the maximum at a distance of 15.0 nm, then gradually decreased.

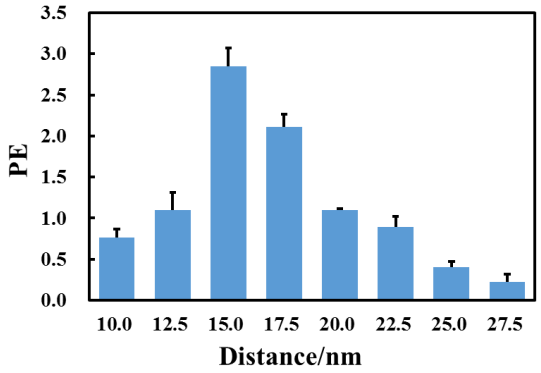


Figure S4. Influence of distance between AuNS and QD 800 in PE. The concentrations of ssDNA-AuNS and ssDNAa-QD 800 were 50.0 pM and 5.0 pM, respectively.

The influence of ssDNAa-QD 800 concentration in PE

In a series of reaction solutions, the concentration of ssDNA-AuNS was fixed at 5.0 pM, and the concentrations of ssDNAa-QD 800 varied from 2.5 pM to 75.0 pM. Figure S5 showed the effect of ssDNAa-QD 800 concentration on PE. With ssDNAa-QD 800 concentration increasing, PE increased and was saturated at the point of 50.0 pM.

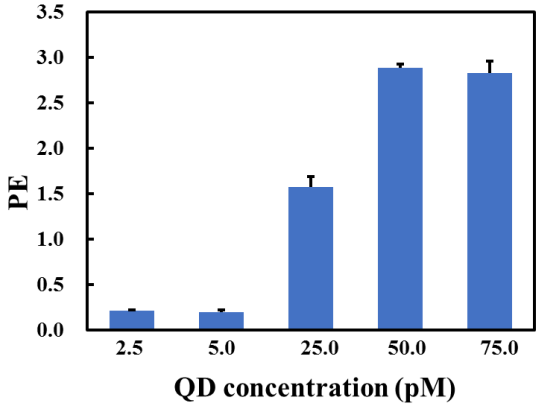


Figure S5. Influence of ssDNAa-QD 800 concentration in PE. The concentration of ssDNA-AuNS was kept at 5.0 pM.

Reference

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2. H. Gai, Q. Wang, Y. Ma, B. Lin, *Angew. Chem. Int. Edit.* 2005, 44, 5107.

3. X. Liu, Z. Wu, Q. Zhang, W. Zhao, C. Zong, H. Gai, *Anal. Chem.* 2016, 88, 2119.