Supplementary material (ESI) for Chemical Communications

Electronic Supporting Information

for

Ultra-sensitive detection of prion protein with a long range resonance energy transfer strategy**

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Experimental Details

Apparatus

The fluorescence of QDs was measured with an F-2500 fluorescence spectrophotometer (Hitachi, Tokyo, Japan) equipped with a 200μL microquartz fluorescence cell, while the plasmon resonance absorption (PRA) of AuNPs with a Hitachi U-3010 spectrophotometer (Tokyo, Japan). High-resolution transmission electron micrographs (HRTEM) were taken with a JEM-2100F field emission electron microscope, using an accelerating voltage of 200 kV. Samples were cast from water solutions onto copper grids by placing a droplet of a aqueous sample solution on grids. Fluorescence life time was carried out with FL-TCSPC fluorescence spectrophotometer (Horiba Jobin Yvon Inc., France). A pHS-3C digital pH meter (Leici, China) was used to detect the pH values. Fluorescence Imaging was performed with a DSU live-cell confocal microscope (Olympus, Japan) system, running the software Invivo 3.2/3D Analyzer 6.2 that coupled to a Olympus IX81 microscope with an objective (×40, 0.9 NA). A JC12V100W Halogen bulb was used to excite the preparation (510-560 nm), and emission of fluorescence was selected with Barrier Filter BA595-615 nm for QDs 605. Image analysis and processing were performed with

Image-Pro® Plus Version 6.3 for Windows ™.

Reagents.

Carbonyl-modified CdSe/ZnS quantum dots (HOOC-QD605) was commercially purchased from Wuhan Jiayuan Quantum Dots CO. LTD (Wuhan, China). HS-aptamer of anti-prion protein (HS-GTT TTG TTA CAG TTC GTT TCT TTT CCC TGT CTT GTT TTG TTG TCT[1,2]) was synthesized by Beijing Sunbio-Technology Co. (Beijing, China) and the DNA was used without further purification. Nα, Nα-Bis(carboxymethyl)-L-lysine hydrate (NTA-lysine) was purchased from Sigma-Aldrich (Missouri, U.S.A.), while *N*-hydroxysuccinimide (NHS) and 1-ethyl-3-(3-(dimethylamino) propyl)-carbodiimide (EDC) were obtained from Sigma. 2-(*N*-morpholino) ethanesulfonic acid (MES) was from Sino-American Biotec. (U.S.A). Pepsin and bovine serum albumin (BSA) were purchased from Shanghai Biology Products Institute (Shanghai, China), and all of the amino acids were from Sigma-Aldrich (Missouri, U.S.A.). Other commercial reagents such as sodium chloride and nickel chloride were analytical reagent grade without further purification. 0.02 mol L^{-1} MES buffer was prepared by dissolving 0.3905 g MES in 100 milliliter ultra-pure water (18.2 MΩ) and adjusted pH to 6.0 by NaOH. All water used is ultra-purified with LD-50G-E Ultra-Pure Water System (Lidi Modern Waters Equipments Co., Chongqing, China).

Expression and Purification of Recombinant Human Prion Protein.

In this experiment, the plasmid encoding recombinant human ($rhPrP^C$) (23–231) was a generous gift of Professor G. F. Xiao (Key Laboratory of Virology, School of Life Science, Wuhan University), and it was transformed to competent bacteria of strain *Escherichia coli* BL21-DE3. The bacteria were cultured in lysogeny broth (LB) medium with kanamycin (Sigma) over a night, transferred into 2×YT medium with 1% inoculation volume, and induced by isopropyl *β*-D-1-thiogalactopyranoside (IPTG) (Sigma). After expression for 6 h, the cells were harvested and purified with the nickel–nitrilotriacetic acid (NTA) agarose resin (Invitrogen, Germany). Concentration of the prion protein was determined with the Bradford Protein excretion Kit (TianGen, Beijing).

Preparation of QD-NTA-Ni and Aptamer-Gold Conjugates.

The QDs–NTA-Ni conjugates were prepared as reported previously.[3,4] Shortly, carbonyl-modified QDs reacted with NTA-lysine to give the QDs-NTA conjugates after ultrafiltration to remove the redundant NTA-lysine. And then, excess $NiCl_2$ was added to the solution of the above product to react for about 1h.

After separation from the Ni^{2+} solution and washed by boiled ddH_2O, QD-NTA-Ni can be used directly or stored for later use. AuNPs, were synthesized by the reduction of $HAuCl_4$ with citrate sodium, [5,6] where the negative citrate ion acts as a stabilizer capped on the AuNPs surface. The AuNPs suspension was stored in darkroom in order to guarantee its stability for several weeks. The procedure of AuNPs functionalized with aptamer was adapted from ref, [5] and the plasmon absorption band was found to have a red shift of 4 nm after functionalizing with SH-Aptamer (for the details, please see Figure S2 in supporting information).

Cell Culture and Cellular Incubation with QD-NTA-Ni and Aptamer-Gold Conjugates.

SK-N-SH cells (human bone marrow neuroblastoma), which were kindly provided also by Professor G. F. Xiao (the State Key Laboratory of Virology, School of Life Science, Wuhan University), were cultured in medium supplemented with 10% fetal bovine serum at 37 ^oC under 5% CO_2. The cells were cleaved by trypsin and replaced onto 18 mm glass coverslips in a 24-well tissue culture plate and allowed to grow for 24 hours. For the incubation, nanoparticles were added into the medium. The cells were grown for another 3 h, washed thrice in PBS buffer, fixed with 4% p-formaldehyde for 30 min, and mounted on microscope slides.

Procedures.

A 20.0 μl MES buffer, 20.0 μl NaCl solution (2M), 40μl QDs–NTA-Ni conjugates (1.0×10^{-8} M) and an appropriate volume of PrP^C solution were added to a 1.5 mL microtube, and then the mixture was diluted to 180.0 μl with water. After the mixture was incubated for 1h at 25 °C, 20.0 μl of AuNPs-aptamer was added to the above solution and it was vortex-mixed thoroughly before the fluorescence or fluorescence life time measurements.

References

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Figures

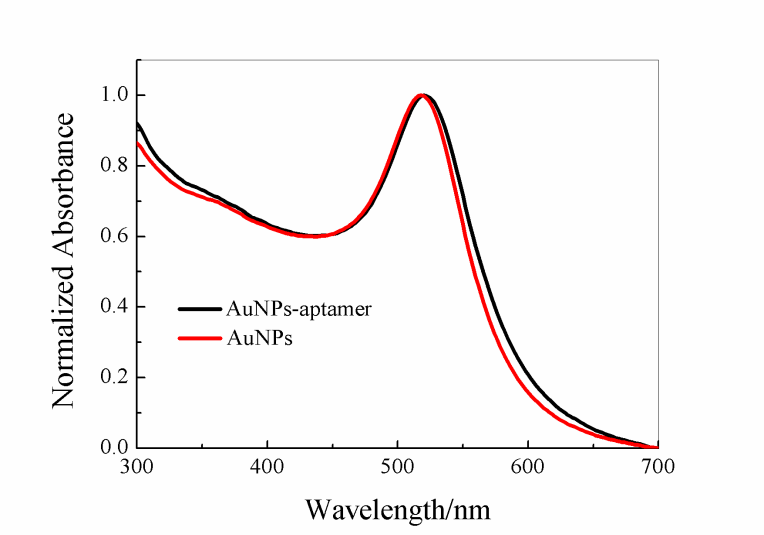

Fig. S1 Normalized absorption spectra of AuNPs before (Dark) and after functionalized with aptamer (Red). The absorbance peak red shifted 4nm after functionalizing with SH-Aptamer.

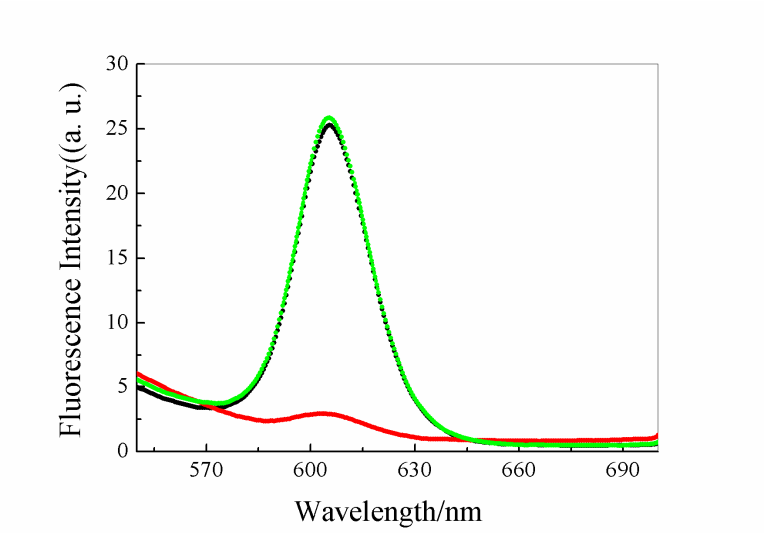

Fig.S2 Optimal fluorescence quenching efficiency of QDs was achieved with the addition of AuNPs at the concentration of 4.38×10^{-11}M, QDs (black),QDs+PrP^C(Green), QDs+PrP^C+AuNPs-Apt(red).

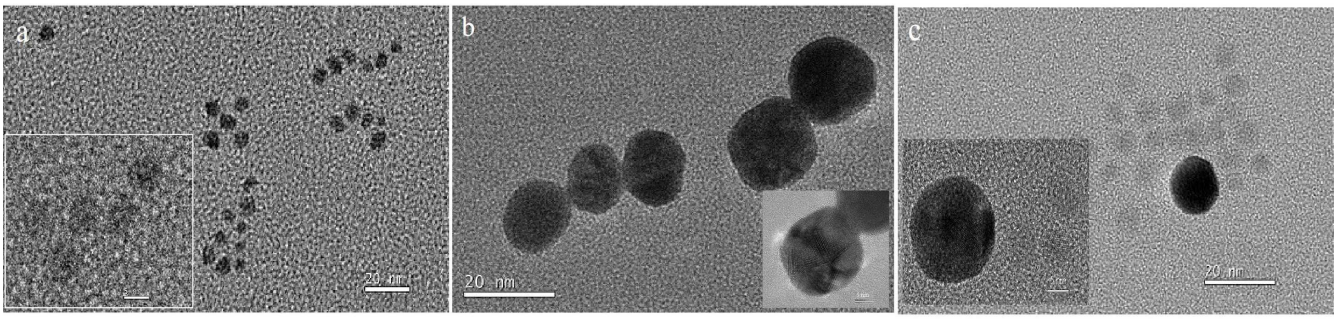

Fig. S3 HRTEM images of individual CdSe/ZnS nanocrystals (a), Au nanoparticles (b), and corresponding complexes of QDs-PrP^C-AuNPs (c). The insets show the nanocrystals have several lattice planes with perfect crystallinity.

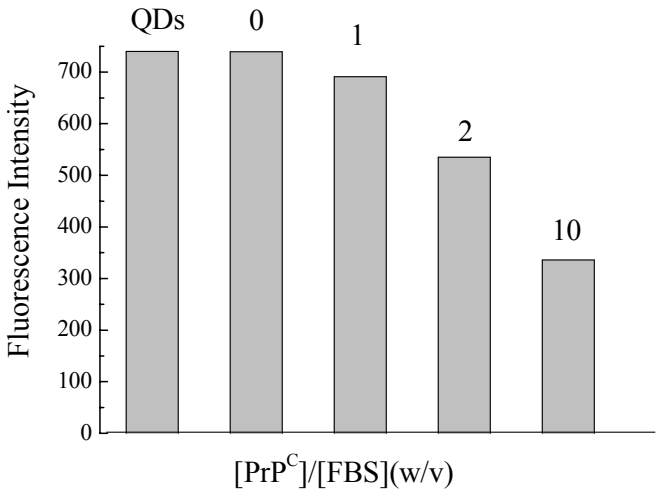

Fig. S4 Dose-response curves assay in the presence of fetal bovine serum (FBS). The fluorescence intensities are plotted as functions of PrP^C-to-FBS ratio (w/v). A ratio of 1 (w/v) is equivalent to a PrP^C concentration of 5 fg mL^{-1}.

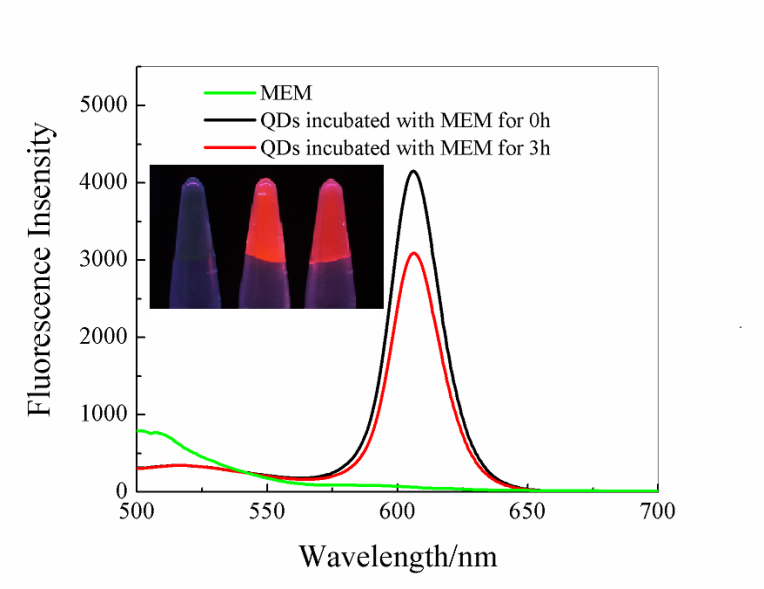

Fig.S5 Fluorescence spectra of QDs (6.25×10^{-9} M) incubated with cell growth medium (MEM) for about 3h. Inset shows the color changes of MEM, MEM+QDs (0 h), MEM+QDs (3 h) respectively from left to right. λexc = 360 nm.

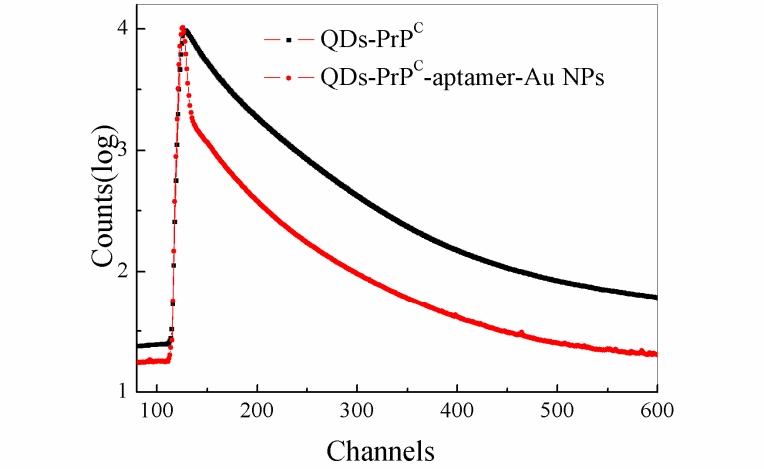

Fig.S6 Fluorescence decay curves at 605 nm of QDs-PrP^C (Black) and the QDs-PrP^C-AuNPs-Apt conjugates (Red). The excitation wavelength was 360 nm and the fluorescence was monitored at 605 nm.

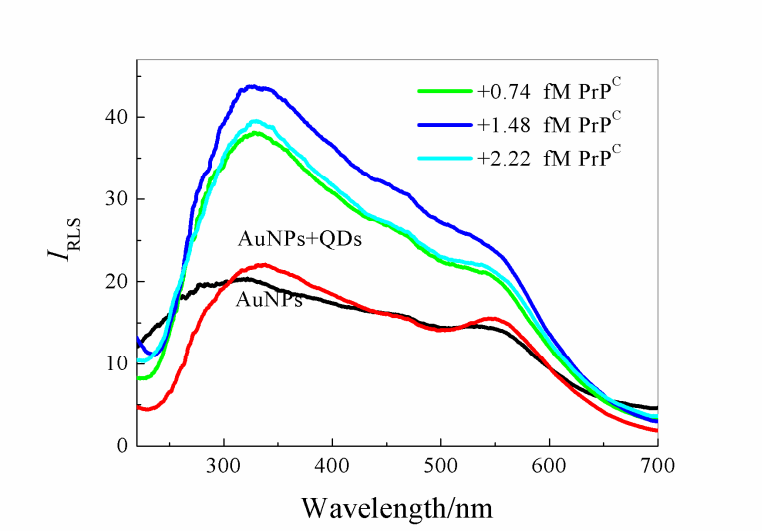

Fig. S7 Resonance light scattering (RLS) spectra of AuNPs with QDs-PrP^C conjugates. AuNPs-Apt (2.16×10^{-11} M) and MES buffer solution (20 mM, pH 6.25) containing 0.2 mol L^{-1} NaCl were employed.