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Short communication

Near infrared sensing based on fluorescence resonance energy transfer between Mn:CdTe quantum dots and Au nanorods

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ABSTRACT

A novel sensing system based on the near infrared (NIR) fluorescence resonance energy transfer (FRET) between Mn:CdTe quantum dots (Qdots) and Au nanorods (AuNRs) was established for the detection of human IgG. The NIR-emitting Qdots linked with goat anti-human IgG (Mn:CdTe-Ab1) and AuNRs linked with rabbit anti-human IgG (AuNRs-Ab2) acted as fluorescence donors and acceptors, respectively. FRET occurred by human IgG with the specific antigen–antibody interaction. And human IgG was detected based on the modulation in FRET efficiency. The calibration graph was linear over the range of 0.05–2.5 μM of human IgG under optimal conditions. The proposed sensing system can decrease the interference of biomolecules in NIR region and increase FRET efficiency in optimizing the spectral overlap of AuNRs with Mn:CdTe Qdots. This method has great potential for multiplex assay with different donor–acceptor pairs.

1. Introduction

Fluorescence resonance energy transfer (FRET) is a powerful technique for characterizing and probing short distance-dependent interactions between the donor and the acceptor (Lakowicz, 1999; Clapp et al., 2006). Recently, luminescent semiconductor nanocrystals or quantum dots (Qdots) are proved to be effective FRET donors due to their broad excitation spectra and tunable, narrow and symmetric photoemission. These unique characteristics could enhance FRET as an effective technique for the application in molecular binding event studies, protein conformation change, and biological assays (Clapp et al., 2006; Roda et al., 2009; Sapsford et al., 2006; Algar and Ulrich, 2008; Li et al., 2008).

In most of the Qdots-based FRET approaches, organic dyes are usually used as the acceptors (Medintz et al., 2003; Shi et al., 2007; Chong et al., 2007). However, the dyes commonly suffer from photo-bleaching problem. In recent years, several researches have demonstrated that Au nanoparticles (AuNPs) are promising candidates as the acceptors for FRET system, for their capacity of inducing strong fluorescence quenching and without photobleaching (Wargnier et al., 2004; Oh et al., 2005; Pons et al., 2007; Shan et al., 2009). However, the absorption spectra of AuNPs with different sizes cannot be easily resolved because the surface plasmon resonance peaks broaden with the increase of diameter at longer wavelength (above 650 nm). This disadvantage limits AuNPs application in FRET for multiple assays.

Compared with AuNPs or organic dyes, Au nanorods (AuNRs) have attracted much attention in biomedical applications due to their attractive features such as two peaks assignable to the transverse and longitudinal surface plasmon resonance bands, high extinction coefficient and broad absorption range that can be tuned by changing their aspect ratio (Link et al., 1999; Thomas et al., 2004; Gole and Murphy, 2005; Huang et al., 2006; Li et al., 2009). AuNRs are good quenchers for Qdots fluorescence (Nikoobakht et al., 2002). Moreover, the strong characteristic absorption bands in the visible to near infrared (NIR) region make AuNRs suitable acceptor for FRET system especially in NIR region (650–1100 nm).

Nowadays, biosensing in NIR region has attracted considerable attention because of the low absorption coefficient for most biomolecules (Klostranec and Chan, 2006; Weissleder, 2001; Huang et al., 2006). Although many Qdots-based FRET systems have been reported and widely used, most of them were in visible region. The main challenge of moving Qdots-based FRET system from visible to NIR window is to find a good matching donor–acceptor pair. AuNRs with tunable absorption peaks in NIR region could offer excellent overlap with Qdots emission for multiplexed biological detection. However, to the best of our knowledge, there was no report about FRET sensing in NIR region with Qdots–AuNRs bioconjugates.

Herein, we demonstrate a near infrared sensing system for the detection of human IgG based on the FRET between Mn:CdTe Qdots and AuNRs. The NIR-emitting Qdots linked with goat anti-human IgG (Mn:CdTe-Ab1) acted as fluorescence donors. The NIR-absorption AuNRs linked with rabbit anti-human IgG (AuNRs-Ab2) acted as acceptors. Then FRET occurred by the conjugation between Mn:CdTe-Ab1 and AuNRs-Ab2 in the presence of human IgG. The calibration graph for human IgG detection is linear over the

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range of 0.05–2.5 μM. The combination of AuNRs with Qdots had the advantage in optimizing the spectral overlap for the increase of FRET efficiency and could conduct multiplex sensing with different donor–acceptor pairs.

2. Materials and methods

2.1. Reagent

The human IgG (Ag), goat anti-human IgG (Ab1) and rabbit anti-human IgG (Ab2) were purchased from Beijing Biodee Biotechnology Co., Ltd (Beijing, China). N-hydroxysuccinimide (NHS) and bovine serum albumin (BSA, 96–99%) were obtained from Sigma (St. Louis, MO, USA). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) was purchased from Pierce (Rockford, IL). $AuCl_3{\cdot}HCl{\cdot}4H_2O$ and hexadecyltrimethylammonium bromide (CTAB) were the products of Shanghai Chemical Reagent Co., Ltd. (Shanghai, China). Tellurium powder (Te, 99.8%, 200 mesh) and mercaptopropionic acid (MPA, 99%) were purchased from Acros Organics (New Jersey, USA). Cadmium chloride, manganese chloride, silver nitrate and sodium borohydride were purchased from Tianjin Chemical Research Institute (Tianjin, China). Ascorbic acid was purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). All reagents were of analytical grade and used without further purification. The ultrapure water with 18.2 MΩ cm^{-1} (Millipore Simplicity, USA) was used throughout the experiments.

2.2. Preparation of Mn doped CdTe Qdots and AuNRs

The NIR-emitting Mn:CdTe Qdots were synthesized according to the reported methods in the synthesis of CdTe with some modifications (Qian and Ren, 2006). In brief, 0.375 mmol of freshly prepared oxygen-free NaHTe solution was added to 200 mL of nitrogen saturated $CdCl_2$ (0.25 mmol) and $MnCl_2$ (0.05 mmol) solution at pH 8.5, with 35 μL of mercaptopropionic acid (MPA) as stabilizer. Then it was refluxed at 95 °C. The Qdots with different fluorescence were synthesized under different refluxing time separately.

The AuNRs were synthesized by a seed-mediated growth procedure (Nikoobakht and El-Sayed, 2003; Gou and Murphy, 2005). The synthesized AuNRs were collected by centrifugation and suspended in 0.005 M hexadecyltrimethylammonium bromide (CTAB) solution.

2.3. Bioconjugation of Mn:CdTe or AuNRs with antibody

The functionalization strategy for Mn:CdTe Qdots and AuNRs were schematically represented in Fig. 1. The AuNRs were stabilized with alkanethiol compounds, mercaptopropionic acid, to form an alkanethiol monolayer on the {1 1 1} side surface. The chemical modification was achieved as follows: 0.5 mL of 20 mM MPA was added into 5 mL of the AuNRs solution and stirred mildly for 10 h. AuNRs were then collected by centrifugation and suspended in a 0.005 M CTAB solution to yield a final concentration of 1 μM.

The synthesized aqueous solution of MPA-capped Mn:CdTe Qdots were purified with Milipore filtration tube (CMW = 5000) and suspended in phosphate buffer solution (PBS, 10 mM, pH 7.4). Then, the MPA-capped Mn:CdTe Qdots and MPA-modified AuNRs could conveniently conjugate to goat anti-human IgG (Mn:CdTe-Ab1) and rabbit anti-human IgG (AuNRs-Ab2), respectively. 10 mg of EDC and 5 mg of NHS were added to 5 mL of the Mn:CdTe Qdots or AuNRs PBS solution, and stirred for 30 min to activate the carboxylate groups. Then 1 mg of the Ab1 or Ab2 was added to the activated solution. To reduce nonspecific interaction, the rest active sites were blocked with 1% BSA solution. After reaction overnight, the antibody conjugated Mn:CdTe Qdots or AuNRs were separated from the solution by centrifugation and redispersed in PBS solution. And the solution was kept at 4 °C before being used.

2.4. Fluorescence measurement

The fluorescence measurements were carried out at room temperature using an Edinburgh FLS920P fluorescence spectrometer (Edinburgh Instruments Ltd., UK). For every series of measurements, the mixture of Mn:CdTe-Ab1 and AuNRs-Ab2 was first treated as blank sample, and fluorescence spectrum was recorded. Various concentrations of the human IgG were then added to the blank and incubated for 1 h at 37 °C in darkness, and quenching spectra were taken.

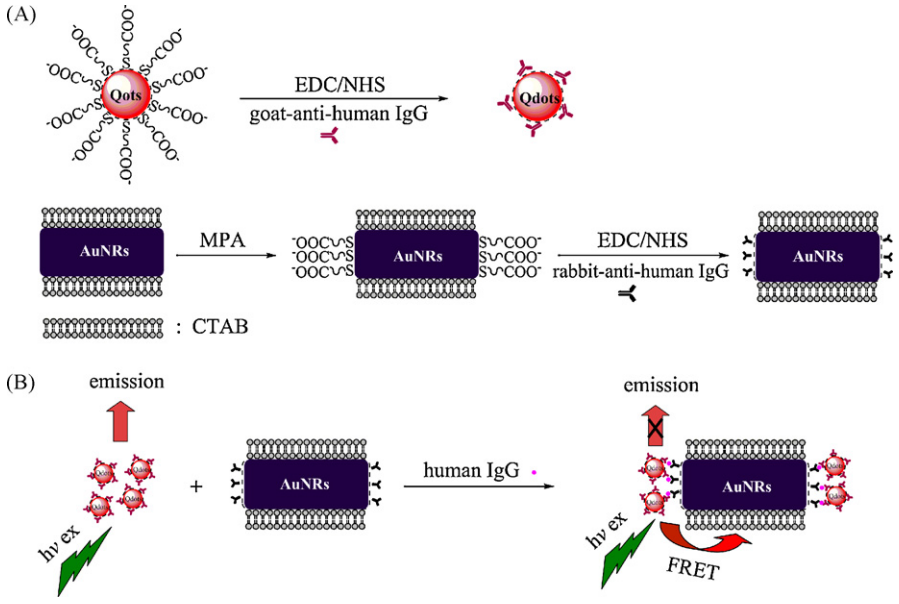

Fig. 1. (A) Schematic illustration of antibody immobilized on the surface of Mn:CdTe Qdots and AuNRs with EDC/NHS. (B) Schematic illustration of the FRET system between Qdots and AuNRs. See text for details.

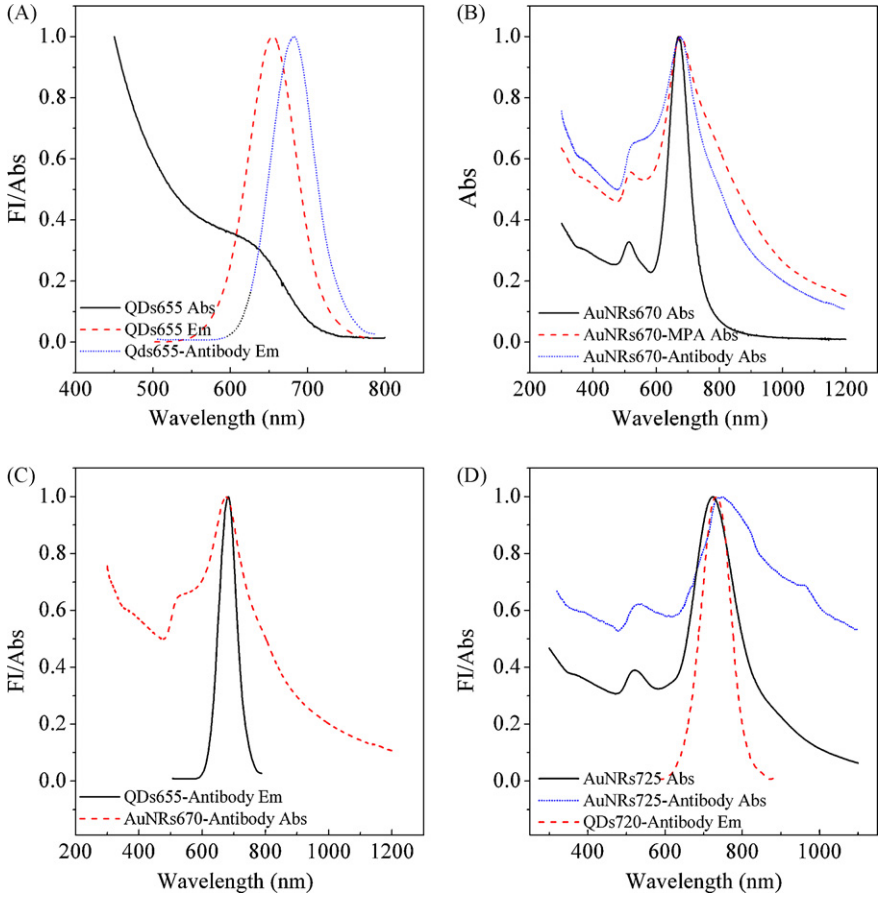

Fig. 2. Normalized fluorescence spectra of Mn:CdTe Qdots and absorption spectra of AuNRs. (A) Mn:CdTe655 before and after labeled with goat anti-human IgG. (B) AuNRs670 before and after SAM formation, AuNRs670 after labeled with rabbit anti-human IgG. (C) Overlap spectra of the Mn:CdTe655-Ab1 and AuNRs670-Ab2. (D) Mn:CdTe720 labeled with goat anti-human IgG, AuNRs725 before and after labeled with rabbit anti-human IgG.

3. Results and discussion

3.1. Characterization of Mn:CdTe Qdots and AuNRs

Under optimized conditions, the fluorescence spectra of Mn:CdTe Qdots were tunable in the range of 560–742 nm by changing the refluxing time. It was found that the fluorescence peaks were prone to reaching NIR region compared with CdTe Qdots. This may be the reason that doping Mn^{2+} act as recombination centers for the excited electron–hole pairs (Erwin et al., 2005; Bhattacharjee and Perez-Conde, 2003). Two different Mn:CdTe Qdots with emission maximum at 655 nm and 720 nm, respectively, were used in the present study. And their quantum yields (QYs) were measured to be 15–20%.

The aspect ratio of the prepared AuNRs was tunable between 1.9 and 4, corresponding to the strong characteristics absorption bands in the range of 540–825 nm. The AuNRs with absorption at 670 nm and 725 nm were used in this work. The modification of AuNRs with MPA could retain the CTAB capping at the {1 1 0}/{1 0 0} side surface and could improve the dispersion and stability of the modified AuNRs in aqueous solution (Pierrat et al., 2007; Yu and Irudayaraj, 2007). The prepared MPA-functionalized AuNRs were stable even after 2 weeks in water without significant aggregation or changes of optical characteristics. The prepared Mn:CdTe Qdots emission at 655 nm (Mn:CdTe655), AuNRs absorption at 670 nm (AuNRs670) and AuNRs670 after MPA modification were characterized by Fourier transform infrared spectroscopy (FTIR). The FTIR spectrum was used to identify the functional groups present in the Mn:CdTe Qdots and AuNRs. The shift of the asymmetric vibration of carboxyl group from 1720 cm^{-1} (MPA) to 1640 cm^{-1} resulted from the covalent bonds between thiols and carboxyls with the Cd atom or Au atom of the surface of Qdots or AuNRs (Liu et al., 2007; Chen et al., 2006). The characteristic peak at 1640 cm^{-1} corresponds to C=O vibrations, which represents the carboxyl group on the Mn:CdTe Qdots or AuNRs surface. The presented —COOH groups can be used to link protein through NH–CO bonds.

In Fig. 2A, the goat anti-human IgG labeled Mn:CdTe655 showed the same narrow emission as non-labeled Mn:CdTe655 Qdots, but an obvious 20 nm red-shift was observed. This may be due to the decrease of confinement in one dimension (Tang et al., 2002; Heilemann et al., 2004). Fig. 2B depicted the absorption spectra of AuNRs670 suspensions with MPA functionalization. A broader and red-shift of 5–7 nm in the longitudinal peaks was clearly identified, indicating the formation of alkanethiol self-assembled monolayers. The emission of the antibody labeled Mn:CdTe Qdots overlaps well with the absorption of the antibody labeled AuNRs, suggesting an efficient FRET between the Qdots donor and the AuNRs acceptor (seen Fig. 2C and D).

3.2. The sensing for human IgG

The optical quenching mechanism of the Qdots fluorophores by proximal AuNRs can be described as the Forster dipole–dipole interaction model (Pons et al., 2007). And the strong overlap between the AuNRs absorption and the Qdots photoluminescence favors the non-radiative energy transfer within the donor–acceptor assay. In the present method, FRET occurred in the presence of human IgG, which allowed detecting the antigen by monitoring the changes in

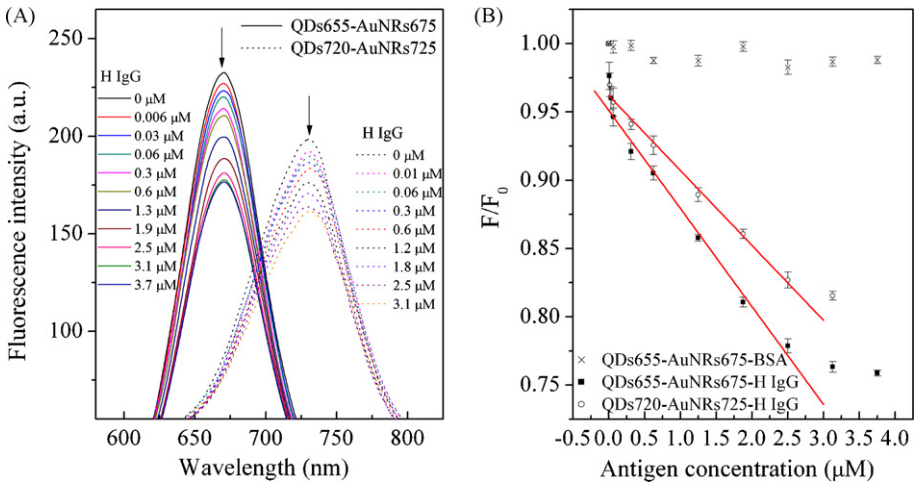

Fig. 3. FRET-based sensing of human IgG. (A) Fluorescence spectra of Mn:CdTe-Ab1 in the presence of AuNRs-Ab2 with different concentrations of human IgG; (B) Relative FL intensity (F/F_0) of the system under the same experimental conditions as in (A).

the Mn:CdTe Qdots fluorescence (see Fig. 1B). To examine whether the FRET-based sensing was applicable to detect the human IgG, the changes of Qdots fluorescence spectra in the presence of modified AuNRs were monitored after addition of human IgG. The results revealed that the fluorescence intensity of the Mn:CdTe Qdots was quenched after the addition of human IgG. The estimated FRET efficiency, defined as $E = 1 - F_{DA}/F_D$ where F_{DA} and F_D are the fluorescence intensity of the presence and absence of human IgG, respectively, was about 13% after adding 2 nmol of human IgG (final concentration of 1.25 μM). It was obvious that Mn:CdTe Qdots and AuNRs were within a short distance by the antigen–antibody specific interaction, which induced an efficient energy transfer and consequently resulted in the fluorescence quenching of the Mn:CdTe Qdots.

In order to confirm that the observed FRET signal is due to specific antigen–antibody interaction and not from nonspecific adsorption, a control experiment was carried out. Instead of human IgG, BSA was introduced into this system. As imagined, there was no significant fluorescence intensity changes in the presence of BSA, because BSA could not lead to the conjugation of Mn:CdTe-AuNRs. It suggests that this FRET system we established is suitable for specific detection of human IgG.

On the basis of the above results, biosensing system based on Mn:CdTe655 Qdots and AuNRs670 nanoparticles was established for the detection of human IgG. Different concentrations of human IgG were added into the Mn:CdTe655 Qdots and AuNRs670 mixed solutions, and incubated for 1 h at 37 °C. The concentration of added human IgG was determined by measuring the Mn:CdTe655 Qdots fluorescence spectra (see Fig. 3). As shown, the fluorescence intensity of the Qdots was decreased gradually with the increase of human IgG concentration, but it showed sign of saturation when the concentration exceeded 3 μM. To examine the feasibility of the presented FRET system, another donor–acceptor pair with Mn:CdTe720 and AuNRs725 was studied. The functionalization and labeling procedures were the same as mentioned above. As shown in Fig. 3, the quenching of the Mn:CdTe Qdots fluorescence occurred in the presence of externally added human IgG. Fig. 3B shows the changes in the relative fluorescence intensity (F/F_0, RFI) in the different concentrations of external added human IgG, where F_0 and F represented the maximum fluorescence intensity in the absence and presence of human IgG, respectively. The calibration curve was $F/F_0 = 0.9463 - 0.06914C_{\text{H-IgG}}$ ($r = 0.9976$) and $F/F_0 = 0.9584 - 0.05285C_{\text{H-IgG}}$ ($r = 0.9994$) for QDs655-AuNRs675-H IgG and QDs720-AuNRs725-H IgG sensing system, respectively. The RFI decreased linearly with the increasing human IgG concentration over the range of 0.05–2.5 μM human IgG.

4. Conclusion

In summary, FRET between the Mn:CdTe Qdots and AuNRs with near infrared optical characteristics were successfully applied in sensing human IgG. It has been demonstrated that the FRET system is capable of sensing a molecule with specific interaction between biomolecules. The combination of AuNRs with Qdots could take the advantage of minimizing the interference of biomolecules, optimizing the spectral overlap to increase FRET efficiency and conducting multiplex sensing with different donor–acceptor pairs. It is anticipated that the proposed FRET system based on the fluorescence quenching of Qdots with AuNRs would become an attractive method for bioassay and molecules screen.

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