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Gold Nanoparticle−Quantum Dot−Polystyrene Microspheres as Fluorescence Resonance Energy Transfer Probes for Bioassays

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Supporting Information

ABSTRACT: The paper describes the development of highly sensitive particle-based fluorescence resonance energy transfer (FRET) probes that do not use molecular fluorophores as donors and acceptors. In these probes, CdSe/ZnS luminescent quantum dots (QDs) were capped with multiple histidine-containing peptides to increase their aqueous solubility while maintaining their high emission quantum yield and spectral properties. The peptide-modified QDs (QD−His) were covalently attached to carboxyl-modified polystyrene (PS) microspheres to form highly emitting PS microspheres (QD−PS). Gold nanoparticles (AuNPs) were then covalently attached to the QD−PS surface to form AuNP−QD−PS composite microspheres that were used as FRET probes. Attachment of AuNPs to QD−PS completely quenched the QD emission through FRET interactions. The emission of QD−PS was restored when the AuNPs were removed from the surface by thiol ligand displacement. The new AuNP−QD−PS FRET platform is simple to prepare and highly stable, and it opens many new possibilities for carrying out FRET assays on microparticle-based platforms and in microarrays. The versatility of these assays could be greatly increased by replacing the linkers between the QDs and AuNPs with ones that selectively respond to specific cleaving agents or enzymes.

The desire to thoroughly investigate biochemical systems with high sensitivity has driven tremendous research interest in the bioanalytical research community. In the past decade, it has led to the development of a new class of fluorescent probes, semiconductor luminescent quantum dots (QDs). Because of their unique electronic properties, QDs are characterized by broad absorption spectra, narrow and symmetrical emission spectra, tunable and size-dependent emission wavelengths, and high photochemical stability.[1,2] When capped with hydrophilic ligands, luminescent QDs are a viable alternative to molecular fluorophores in luminescence bioassays.[1,2]

Luminescent CdSe/ZnS QDs are often synthesized in organic solvents and capped with hydrophobic ligands such as trioctylphosphine oxide (TOPO). To enable QD solubility in aqueous solutions, the hydrophobic capping ligands must be replaced with hydrophilic ligands. TOPO capping ligands are usually exchanged with thiol-functionalized ligands such as dihydrolipoic acid (DHLA), 16-mercaptohexadecanoic acid (MHDA), mercaptoacetic acid (MMA), or organic dendrons.[1,2] Cross-linking of QDs with a thin shell of silanols or a thick shell of amphiphilic diblock or triblock copolymers has also been used to improve their solubility and stability in aqueous solutions.[3] Hydrophilic QDs have been applied in immunoassays,[3,4] fluorescence *in situ* hybridization (FISH),[5] cellular assays,[6] and *in vivo* studies.[7] However, the stability of QDs in aqueous solutions has remained a challenge since most QD-capping strategies involve binding of thiol groups to their surface. Unfortunately, thiol bonds are easily photo-oxidized, which leads to removal of the ligands from the surface of the QDs and their precipitation. While polymer-coated QDs are more stable in aqueous solutions, the polymer coating substantially increases the particle size in comparison with the size of TOPO−QDs (<10 nm).[8] The goal of our study was to design new and improved QD-based probes based on fluorescence resonance energy transfer (FRET). This precluded the use of a polymer coating to stabilize the QDs in aqueous solutions, since the thick polymer coatings would significantly decrease the FRET interactions between the polymer-coated QDs and any linked acceptors.

The first objective of the current study was to increase the aqueous stability and emission quantum yield of water-soluble QDs without using polymer coatings. To realize this objective, we initially replaced the hydrophobic TOPO ligands on the surface of CdSe/ZnS QDs with MHDA.[9] This procedure was followed by binding of a polyhistidine peptide (H_2N-K-K-H-H-H-H-H-E-E-CO_2H) to the QD−MHDA surface, which did not replace the thiol capping.[10] This produced stably luminescent, water-soluble polyhistidine peptide-coated QDs (QD−His). The binding affinity of multiple histidines to ZnS is much stronger than the binding affinity of a single thiol bond to the ZnS surface. Therefore, modifying the QD surface with multiple histidine-containing peptides greatly increased stability of the QDs in aqueous solution. Furthermore, the multiple carboxyl and amino groups from lysine (K) and glutamic acid (E) provided chemical accessibility of the QDs to subsequent surface modifications. Most importantly, capping the QDs with histidine-containing peptides enabled the development of a new generation of QD-based FRET probes, the second objective of this project.

QD−His particles with an average diameter of 5 nm were covalently attached to 6−10 μm carboxyl-modified polystyrene (PS) microspheres using a standard carbodiimide (EDC) coupling

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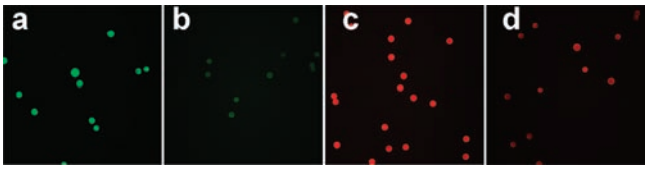


Figure 1. Digital fluorescence images of green-emitting (a) QD−PS and (b) Alexa 488−PS (λ_{ex} = 488 nm, λ_{em} = 535 nm) and red-emitting (c) QD−PS and (d) rhodamine−PS (λ_{ex} = 540 nm, λ_{em} = 605 nm). Exposure times for all images were 150 ms.

protocol, forming highly luminescent QD-encoded polystyrene microspheres (QD−PS).[11] Figure 1 shows a comparison of (a) green- and (c) red-emitting QD−PS microspheres with PS microspheres that were coated with (b) green- and (d) red-emitting molecular fluorophores (FL−PS) under the same illumination conditions. Digital imaging analysis showed that the emission of QD−PS was ~10-fold higher than the emission of FL−PS. The low fluorescence intensity of FL−PS is attributed to self-quenching of dye molecules in close proximity to one another on the surface of the PS microspheres. The greater fluorescence intensity of QD−PS represents a major advantage of QDs over molecular fluorescent dyes. Unlike fluorescent dyes, QDs can be coated or embedded in polymer particles at high concentration to produce brighter particles and more effective FRET probes.

QD-based FRET probes were previously fabricated in our laboratory[12−14] and by Medintz and co-workers[15,16] by linking organic dye molecules or quenchers to the surface of luminescent QDs. The QD-based FRET probes were used in homogeneous assays and were limited by the chemical and photostability of the linked organic molecules. Chang and co-workers[17] previously developed solution FRET assays for proteolytic activity that made use of QDs as donors and gold nanoparticles (AuNPs) as acceptors. Aggregation of QDs and AuNPs created steric hindrances that increased the assay time. Furthermore, Wang and co-workers have previously shown that aggregation of QDs can alter their luminescence properties.[18] In our study, we covalently attached AuNPs with an average diameter of 5 nm to the surface of QD−PS to form highly bright and nonaggregating particle-based FRET probes in which the QDs served as luminescent donors and the AuNPs as acceptors. The synthesis and operating principle of AuNP−QD−PS FRET probes is shown in Scheme 1. Cystamine linkers ($H_2N-CH_2-CH_2-S-S-CH_2-CH_2-NH_2$) were attached to the AuNPs, and an EDC coupling protocol was used to link the AuNPs to the QD−PS surface to form AuNP−QD−PS conjugates. The close proximity between the coattached AuNPs and QDs on the microsphere surface enabled FRET interactions between the QD donors and AuNP acceptors.

Figure 2 shows digital fluorescence images of (a) QD−PS microspheres prior to the attachment of AuNPs, (b) AuNP−QD−PS microspheres, and (c) the AuNPs−QD−PS microspheres following incubation with 5 mg/mL dithiothreitol (DTT) for 1 h at room temperature. DTT induces thiol displacement of the AuNPs from the surface of the AuNP−QD−PS microspheres, which restores the emission of the QDs because FRET between the QDs and AuNPs no longer occurs.

Figure 3 shows the fluorescence spectra of AuNP−QD−PS microspheres incubated with 5 mg/mL DTT for different time intervals at room temperature. The luminescence intensity of the PS microspheres increased with incubation time because of the removal of AuNPs from the surface.

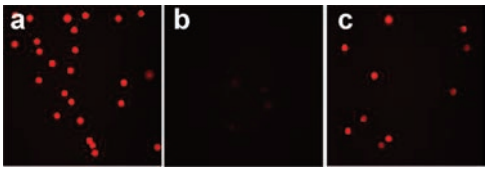
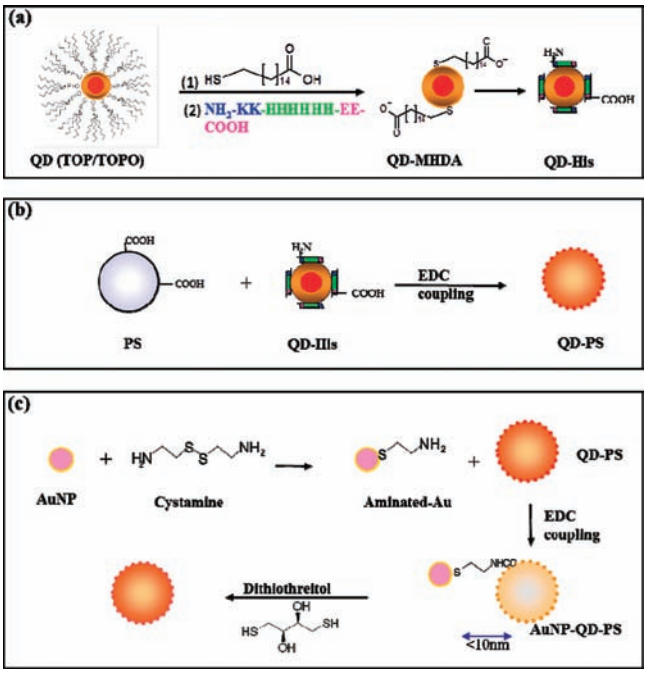


Figure 2. Digital fluorescence images of red-emitting (λ_{ex} = 450 nm, λ_{em} = 605 nm) (a) QD−PS, (b) AuNP−QD−PS, and (c) AuNP−QD−PS microspheres following incubation with 5 mg/mL DTT for 1 h at room temperature. Treatment of the AuNP−QD−PS microspheres with DTT restores the emission of the QDs by removing the AuNPs from the microsphere surface.

Scheme 1. (a) Use of Ligand Exchange Reactions To Form MHDA-Modified CdSe/ZnS QDs, Which Are Then Capped with Multiple Histidine-Containing Peptides (QD-His); (b) Conjugation of Excess QD-His to Carboxylated Polystyrene Microspheres (PS) Using EDC Coupling To Form Strongly Emitting QD−PS Microspheres; (c) Conjugation of Amino-Modified Gold Nanoparticles (AuNPs) to QD−PS Microspheres Using EDC Coupling To Form AuNP−QD−PS Composite Particles[a]



[a] In part (c), FRET interactions between the QDs and AuNPs quench the emission of the composite particles, which is restored once the AuNPs are removed from the PS microsphere surface with DTT. The arrow indicates that the distance between the AuNPs and QDs is less than 10 nm.

In summary, we have successfully prepared QD-based FRET probes by the covalent coattachment of CdSe/ZnS QDs and AuNPs to the surface of PS microspheres. Capping the QDs with histidine-containing peptides greatly increased their aqueous stability and photoluminescence properties. FRET interactions between the QDs and AuNPs completely quenched the emission of the QDs. The emission of the QDs was restored when the AuNPs were removed from the PS surface by treatment with DTT. The new AuNP−QD−PS FRET platform opens many

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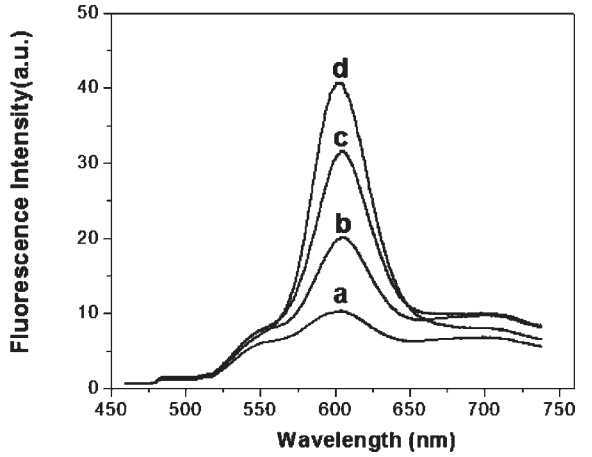

Figure 3. Luminescence intensity of AuNP−QD−PS microspheres incubated with 5 mg/mL DTT for (a) 0, (b) 10, (c) 60, and (d) 120 min.

new possibilities for carrying out FRET assays in microparticle-based microarrays. The versatility of these assays could be greatly increased by using linkers between the QDs and AuNPs that selectively respond to specific cleaving or displacement agents.

■ ASSOCIATED CONTENT

Supporting Information. Experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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