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Luminescent Quantum Dots Fluorescence Resonance Energy Transfer-Based Probes for Enzymatic Activity and Enzyme Inhibitors

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The paper describes the development and characterization of analytical properties of quantum dot-based probes for enzymatic activity and for screening enzyme inhibitors. The luminescent probes are based on fluorescence resonance energy transfer (FRET) between luminescent quantum dots that serve as donors and rhodamine acceptors that are immobilized to the surface of the quantum dots through peptide linkers. Peptide-coated CdSe/ZnS quantum dots were prepared using a one-step ligand exchange process in which RGDC peptide molecules replace trioctylphosphine oxide (TOPO) molecules as the capping ligands of the quantum dots. The peptide molecules were bound to the surface of the CdSe/ZnS quantum dots through the thiol group of the peptide cysteine residue. The peptide-coated quantum dots were labeled with rhodamine to form the FRET probes. The emission quantum yield of the quantum dot FRET probes was 4-fold lower than the emission quantum yield of TOPO-capped quantum dots. However, the quantum dot FRET probes were sufficiently bright to enable quantitative enzyme and enzyme inhibition assays. The probes were used first to test the enzymatic activity of trypsin in solution based on FRET signal changes of the quantum dot-based enzymatic probes in the presence of proteolytic enzymes. For example, exposure of the quantum dot FRET probes to 500 μg/mL trypsin for 15 min resulted in 60% increase in the photoluminescence of the quantum dots and a corresponding decrease in the emission of the rhodamine molecules. These changes resulted from the release of rhodamine molecules from the surface of the quantum dots due to enzymatic cleavage of the peptide molecules. The quantum dot FRET-based probes were used to monitor the enzymatic activity of trypsin and to screen trypsin inhibitors for their inhibition efficiency.

Luminescent quantum dots have emerged as a viable alternative to organic fluorophores in the analysis of biological samples due to their high photostability, high emission quantum yield, narrow emission peaks, and size-dependent wavelength tunability.[1–5] To facilitate their application in aqueous biological systems, the commonly used hydrophobic capping ligands of luminescent quantum dots such as trioctylphosphine oxide (TOPO) molecules must be replaced with hydrophilic capping ligands. Various ligand exchange methods were developed in recent years to form stable water-soluble quantum dots. The TOPO ligands are often exchanged with thiol-functionalized compounds like mercaptoacetic acid,[6] dihydrolipoic acid,[7] Dithiothreitol,[8] and cysteine-containing peptides.[9] It is also possible to coat the quantum dots with a protective polymer layer.[10] However, such coating significantly increases the size of these luminescent particles and separates the quantum dots from their environment. This restricts the use of luminescent quantum dots in biological systems to imaging applications.

Several research groups including ours have recently explored employing quantum dots in fluorescence resonance energy transfer (FRET)-based assays.[11–20] For example, Medintz and coworkers reported the conjugation of maltose binding protein

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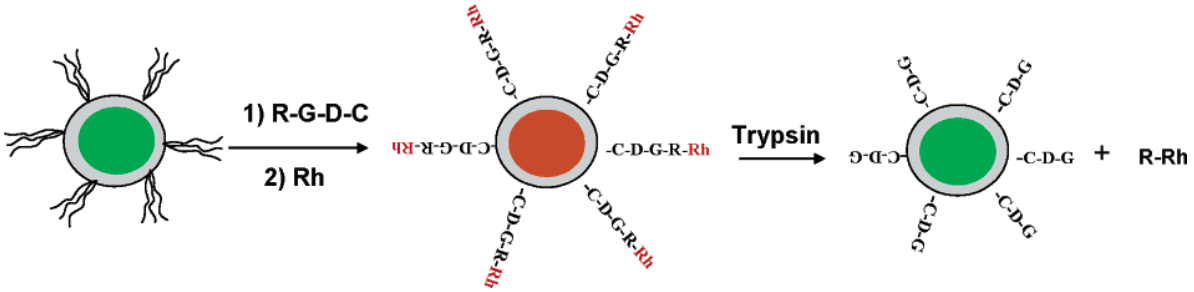
Scheme 1. Diagram Describing the Principle of Quantum Dot FRET-Based Enzymatic Activity Probes[a]



[a] The peptide molecules bind to the ZnS shell of CdSe/ZnS quantum dots through the thiol group of cysteine (C) in a ligand-exchange process in which the peptide molecules replace the trioctylphosphine oxide (TOPO) molecules on the surface of the quantum dots. Rhodamine is used to label the peptide-coated quantum dots to form the FRET-based enzymatic activity probes. The probes are used to monitor the enzymatic activity of trypsin, which cleaves the peptide between glycine (G) and arginine (R) to release the attached rhodamine molecules to the solution.

(MBP) to the surface of the quantum dots, which served as luminescent donors. The MBP molecules were then labeled with molecular fluorescence quenchers or fluorescent acceptors such as QSY9, BHQ-10, rhodmaine red, and Cy3.[15−18] The resulting quantum dot FRET-based probes were used to detect maltose[15] and TNT[18] in solution. Recently, quantum dots linked to peptide molecules that were labeled with quenchers or molecular acceptors were used as probes for proteolytic activity.[21−23] West and co-workers conjugated gold nanoparticles to CdSe/ZnS quantum dots through peptide linkers.[21] Enzymatic cleavage of the peptide by collagenase resulted in the desorption of gold nanoparticles from the surface of the quantum dots, which in turn increased the initially quenched emission of the quantum dots. However, the response time of these assays appears to be long possibly due to aggregation of the quantum dots−gold nanocrystal clusters, strong interactions between the quantum dots and gold nanoparticles that do not involve peptide linking and steric hindrances that decrease the rate of enzymatic cleavage of molecular peptides trapped between the larger nanoparticles. Mattoussi and co-workers recently developed a modular peptide structure that allowed the attachment of dye-labeled substrates for proteases to the surface of luminescent quantum dots. The researchers used these dye-labeled quantum dots in proteolytic assays that were carried out under both excess enzyme and excess substrate conditions. The assays provided quantitative data including enzymatic velocity, Michaelis−Menten kinetic parameters, and mechanisms of enzymatic inhibition.[22] Mattoussi and co-workers also reported a detailed mechanistic study that aimed to understand the mechanism of fluorescence resonance energy transfer between quantum dots and linked molecular acceptors.[23] The researchers concluded that FRET between quantum dots and linked molecular acceptors could be described using the Forester FRET theory. It seems that due to their nanometric dimensions quantum dots could be described at first approximation as point dipoles. Therefore, the distance between quantum dots and linked molecular acceptors could be measured from the center of the quantum dots to the molecular acceptors adsorbed to the quantum dot surface. It should be mentioned however that other factors like the shape of the quantum dots and the nature of the shell separating between the quantum dots and molecular acceptors could affect the distance dependence of the FRET efficiency in this system. More studies are needed to fully understand the mechanism of FRET between nanoparticle donors like quantum dots and molecular acceptors. In a recent communication, we reported the synthesis of quantum dot FRET-based enzymatic activity probes that are based on the attachment of rhodamine molecules to the surface of 3-nm-sized CdSe/ZnS quantum dots through the use of RGDC peptide linkers.[24] A diagram of the principle of quantum dot-based enzymatic activity probes is shown in Scheme 1. First, the TOPO ligands were exchanged with the tetrapeptide RGDC (arginine-glycine-aspartic acid-cysteine). The peptide molecules were then attached to the CdSe/ZnS quantum dots through the thiol group of cysteine (C). The resulting peptide-coated quantum dots were labeled with Rhodamine Red-X through the formation of amide bonds with the amino terminals of the coating peptides. When excited at 445 nm, the rhodamine-labeled quantum dots exhibited two clearly separated emission peaks at 545 nm of the quantum dots and 590 nm of the attached rhodamine molecules. The emission of the quantum dots was quenched and the emission of the attached rhodamine molecules increased due to FRET between the quantum dots and the rhodamine acceptors. Upon enzymatic cleavage by extracellular metalloproteinases (MMPs), the rhodamine molecules were removed from the surface of the quantum dots. This resulted in an increase in the emission peak of the quantum dots and a corresponding decrease in the emission of the displaced rhodamine molecules due to a decrease in FRET efficiency between the quantum dots and rhodamine molecules. The simultaneous change in the two emission peaks enabled quantitative measurement of MMP activity. The high monodispersity of the quantum dot solutions enabled completion of the assay in 15 min. The quantum dot FRET-based probes were used to monitor the

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proteolytic activity of MMPs in cell cultures and effectively discriminated between normal and breast cancer cells based on distinct difference in their expression of MMPs. In this paper, we describe the synthesis, characterization, and analytical properties of quantum dot FRET-based enzymatic activity probes for the enzyme trypsin and demonstrate their use for rapid real-time monitoring of trypsin activity and inhibition efficiency of trypsin inhibitors.

EXPERIMENTAL METHODS

Materials. The tetrapeptide RGDC used for ligand exchange was purchased from American Peptide Co. (Sunnyvale, CA) with a purity of 99.0%. The composition and purity of the peptide was confirmed by mass spectrometry and reversed-phase high-pressure liquid chromatography. Rhodamine Red-X, succinimidyl ester 5-isomer was obtained from Molecular Probes, Inc. (Eugene, OR). Dulbecco's PBS buffer solution at pH 7.4 (1×) (2.67 mM KCl, 1.47 mM KH_2PO4, 137.93 mM NaCl, and 8.06 mM $Na_2HPO4 \cdot 7H_20$) was purchased from Invitrogen Corp. (Carlsbad, CA). Chemicals for preparing for TOPO-coated CdSe/ZnS, trypsin from porcine pancreas, 1,10-phenantroline, 4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride, and 4-amidinophenylmethanesulfonyl fluoride hydrochloride were purchased from Sigma. All reagents were used as received.

Synthesis of TOPO-Capped and Peptide-Coated Luminescent Quantum Dots. TOPO-capped CdSe/ZnS quantum dots were prepared following a method first proposed by Peng[25] with slight modifications.[26] The ligand exchange reaction used to replace the TOPO ligands with our tetrapeptide was carried out in a mixture of pyridine and dimethyl sulfoxide (DMSO) following a method that was reported by Pinaud and co-workers.[9] A 1-mL aliquot of 1 μM TOPO-coated CdSe/ZnS quantum dots was precipitated with methanol and redissolved in 2 mL of 9:1(v/v) pyridine/DMSO cosolvent. The use of this cosolvent effectively prevented aggregation of quantum dots during the reaction. A 200-μL aliquot of 5 mg/mL peptide in DMSO was added to the reaction mixture. The pH was adjusted to 10 by adding tetramethylammonium hydroxide (TAMOH; 20% (w/v) in methanol) to the reaction mixture. The TAMOH molecules were used to form anionic cysteine thiolates to facilitate binding of the peptide to the CdSe/ZnS quantum dots through the cysteine residues. The peptide-coated quantum dots were vortexed for 30 min at 3000 rpm, centrifuged (3000 rpm for 10 min), and resuspended in 2 mL of DMSO. Then the sample was centrifuged and resuspended in 2 mL of Dulbecco's PBS buffer solution at pH 7.4. Unbound peptide molecules were removed by two repeated cycles of spin dialysis using Amicon Centricon spin dialysis tubes with a cutoff molecular mass of 30 kDa (Microcon YM30, Millipore Corp.). In each spin dialysis cycle, the sample was precipitated by centrifugation at 2000 rpm for 20 min and washed with the Dulbecco's PBS buffer. The peptide-coated quantum dots were finally suspended in 2 mL of Dulbecco's PBS buffer solution at pH 7.4, and the solution was kept at 4 °C until used.

Conjugation of Rhodamine to the Peptide-Coated Quantum Dots. Rhodamine-labeled, peptide-coated quantum dots were prepared by adding 150 μL of 0.1 μM peptide-coated quantum dots and varying volumes (0−150 μL) of 4.8 μM Rhodamine Red-X, succinimidyl ester to a PBS buffer solution of pH 7.4 to a final volume of 1.5 mL. The reaction mixture was incubated for 1 h at room temperature. The rhodium-labeled peptide-coated quantum dots were washed of free rhodamine by spin dialysis as described above.

Enzyme Activity and Inhibition Assays. Rhodamine-labeled, peptide-coated quantum dots that were prepared in solution containing a rhodamine/quantum dots ratio of 48:1 were used as our FRET-based enzymatic activity probes. A 500-μL aliquot of 10 nM quantum dot FRET-based probes and varying volumes ranging from 0−500 μL of 1 mg/mL trypsin were added to Dulbecco's PBS buffer solution at pH 7.4 to a final volume of 1.0 mL. Time-dependent spectral measurements were carried out following the addition of trypsin using a fluorescence spectrometer or a digital fluorescence spectral imaging system. Inhibition assays were performed in a chambered cover glass (LabTek) and were monitored using digital fluorescence imaging microscopy and spectroscopy. To carry out the inhibition assays, 250 μL of 1 mg/mL trypsin was added to Dulbecco's PBS buffer solution at pH 7.4 that contained inhibitors of various concentrations to a total volume of 500 μL. Following 30-min incubation, 500 μL of 10 nM quantum dot FRET-based probes was added to the mixture and FRET measurements were carried out to monitor to enzymatic reaction.

Fluorescence Spectroscopy Measurements. Emission spectra of TOPO-coated quantum dots, peptide-coated quantum dots, and quantum dots−peptide−rhodamine conjugates were measured using a spectrofluorometer (PTI International, model QM-1), equipped with a 75-W continuous Xe arc lamp as a light source. All samples were excited at 445 nm, which is near the minimum of the rhodamine absorption spectrum, in order to reduce interference from direct excitation of rhodamine. Emission scans measured from 480 to 700 nm in a 1-cm cell.

Digital Fluorescence Imaging Microscopy and Spectroscopy. Emission spectra and images of quantum dots−peptide−rhodamine conjugates were obtained using a digital luminescence imaging microscopy and spectroscopy system. An Olympus (IX-71) inverted fluorescence microscope equipped with a 250-mm spectrograph and a high-performance, 16-bit, back-illuminated CCD camera (Roper Scientific) was used for these measurements. A filter cube containing a 440 ± 20 nm band-pass excitation filter, a 465-nm dichroic mirror, and a 475-nm long-pass emission filter was used to ensure spectral purity. The Roper Scientific software Win Spec/32 was used for image and spectral analysis. The spectra and images were taken through 10× and 20× objectives with numerical apertures of 0.3 and 0.5, respectively. An exposure time of 100 ms was typically used to acquire the fluorescence spectra and images of the quantum dot-based probes.

RESULTS AND DISCUSSION

Peptide-Coated Quantum Dots. Peptide-coated quantum dots were synthesized as described in the Experimental Section. Figure 1a shows the emission spectra of 100 nM solutions of TOPO and peptide-coated quantum dots when excited at 445 nm. It can be seen that the emission intensity of the peptide-coated quantum dots was four times lower than the emission intensity of TOPO-coated quantum dots. Normalized emission spectra of TOPO- and peptide-coated quantum dots are shown in Figure 1b.

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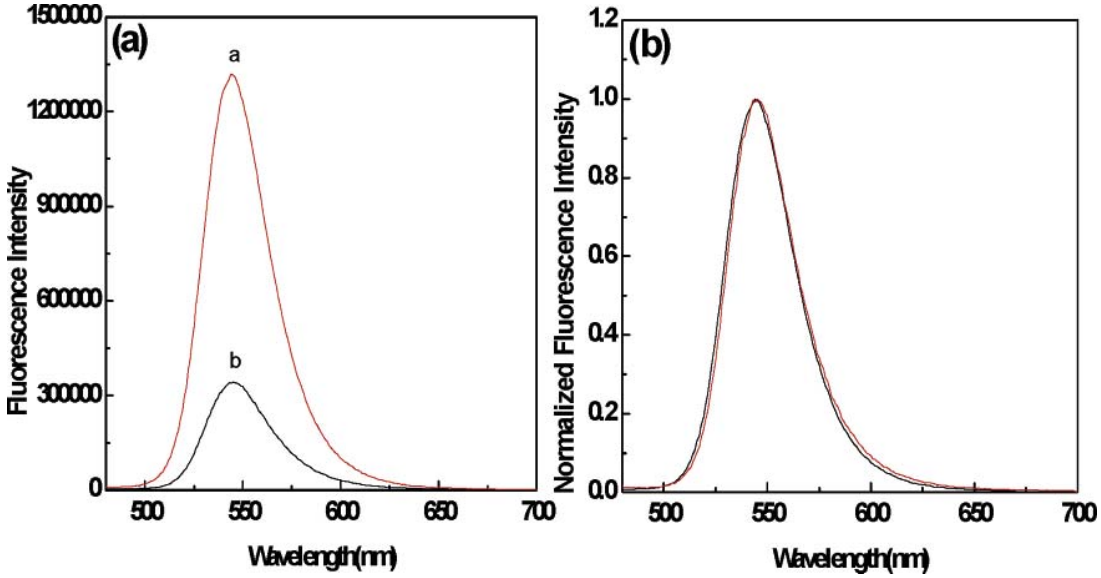

Figure 1. (a) Emission spectra and (b) normalized emission spectra of TOPO-coated quantum dots (red) and peptide-coated quantum dots (black) (λ_{ex} = 445 nm).

The emission spectra show negligible changes in the peak emission wavelength and bandwidth following the ligand exchange process. Despite the significant reduction in emission quantum yield, which is typical to quantum dots when dispersed in aqueous media, the quantum dots were sufficiently luminescent to successfully prepare quantum dot-based enzymatic activity probes. The ligand exchange method that was used in our experiments to coat the quantum dots with peptide molecules is a simple one-step reaction that resulted in high-quality, peptide-coated quantum dots. As seen in Figure 1b, the photophysical properties of the quantum dots were not affected by the peptide coating. Additionally, the attachment of peptide molecules to the quantum dots enabled further conjugation of rhodamine to the amino terminals of the peptides. In previously described optimization studies (see supportive information of ref 24), we found that the maximum FRET efficiency between the quantum dots and rhodamine acceptors was at a 48:1 rhodamine/quantum dots molar ratio in the labeling solution. Increasing the ratio beyond this level did not result in increased FRET efficiency. Additionally, control measurements that involved direct excitation of the rhodamine acceptors at 590 nm revealed that the fluorescence of the rhodamine molecules increased at increasing rhodamine concentration up to a 100:1 rhodamine/quantum dots ratio. This indicated that the maximum FRET efficiency was obtained at a 48:1 rhodamine/quantum dots ratio due to saturation of binding sites of the peptide molecules and not due to fluorescence quenching of the rhodamine molecules. Based on these results, we concluded that there were between 50 and 100 peptide residues on each quantum dot. When stored at room temperature in the dark, solutions of the peptide-coated quantum dots were stable in PBS buffer solutions at pH 7.2 for one week. To prevent irreproducible results in the enzyme assays, samples of quantum dot FRET-based probes were used within 48 h of their preparation.

Enzyme Activity Measurements. The quantum dots FRET-based enzymatic probes were used to determine the activity of the enzyme trypsin. Trypsin is a proteolytic enzyme with a molecular weight of 23 800 that cleaves proteins and peptides at the carboxyl end of lysine (K) and arginine (R). Emission spectra showing the effect of trypsin at increasing concentration on the emission of the quantum dot FRET probes are shown in Figure 2a (λ_{ex} = 445 nm). The spectra were recorded 15 min after adding trypsin to the quantum dots solution. An increase in the quantum dots emission peak at 545 nm and a decrease in the rhodamine emission peak at 590 nm are clearly seen, indicating a significant decrease in the FRET efficiency. Figure 2b describes the trypsin concentration dependence of the ratio F_d/F_a. F_d and F_a were the peak emission intensities of the quantum dot FRET-based probes when excited at 445 at 545 (quantum dot donors) and 590 nm (rhodamine acceptors), respectively. The F_d/F_a ratio was normalized to $(F_d/F_a)_0$, which is the value of F_d/F_a prior to the addition of trypsin to the quantum dot FRET-based solutions. The trypsin concentration-dependent FRET signal changes were attributed to the enzymatic cleavage of the peptide molecules, which led to the expected release of rhodamine molecules from the quantum dots to the solution. Control experiments were carried out with CdSe/ZnS quantum dots that were labeled with nonfluorescent peptides. Adding trypsin at concentrations used in our enzymatic assays did not result in a significant change of the quantum dots luminescence signal. These experiments precluded the possibility that nonspecific adsorption of enzyme molecules to the surface of the quantum dots contributed to changes in their luminescence intensity. Similar experiments were carried out with rhodamine-labeled peptide molecules in the absence of CdSe/ZnS quantum dots. Adding trypsin to solutions containing the rhodamine-labeled peptides did not result in a significant change in the rhodamine fluorescence intensity despite the trypsin-initiated peptide cleavage. These measurements precluded the possibility that the decrease in the fluorescence intensity of rhodamine during FRET enzymatic assays in which CdSe/ZnS quantum dots linked to rhodamine-labeled peptide molecules were used as the substrate resulted from a change in the chemical environment of the rhodamine molecules during the enzymatic cleavage of the peptide molecules.

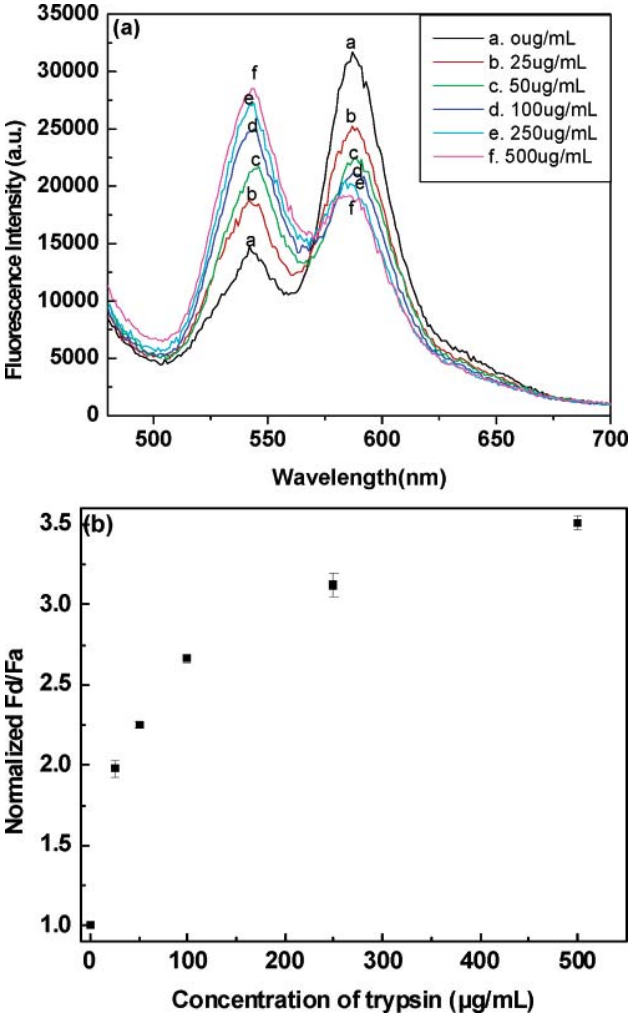

Figure 2. (a) Emission spectra of the quantum dot FRET-based probes at increasing trypsin concentrations: (a) 0, (b) 25, (c) 50, (d) 100, (e) 250, and (f) 500 μg/mL (λ_{ex} = 445 nm). (b) Trypsin concentration dependence of F_d/F_a, 15 min following the addition of trypsin to solutions of quantum dot-based probes. F_d/F_a values were normalized to $(F_d/F_a)_0$, which is the ratio F_d/F_a prior to adding trypsin to the quantum dot probes solutions.

Time-dependent measurements of the FRET signal at increasing trypsin levels were carried out to demonstrate the ability of the quantum dot-based probes to provide dynamic information over long observation times. Figure 3 shows the temporal dependence of the ratio F_d/F_a at increasing trypsin concentration ranging from 0 to 500 μg/mL (λ_{ex} = 445 nm). The F_d/F_a ratio was normalized to $(F_d/F_a)_0$, which is the value of F_d/F_a prior to the addition of trypsin to the quantum dot FRET-based solutions. It can be seen that the ratio F_d/F_a increased faster at higher trypsin concentrations. For a concentration of 250 μg/mL trypsin, the enzymatic reaction was completed in less than 15 min. As expected, the duration of the enzymatic assays increased with decreasing enzyme concentrations. It was possible to detect as low as 0.1 μg/mL (0.4 pmol) in a 2-h assay. The short assay time is a significant advantage over previously reported FRET-based quantum dot assays in which longer reaction times were reported.[20] Control experiments using solutions of quantum dot-based probes in the absence of trypsin indicated insignificant changes in the FRET signal (F_d/F_a) over several hours. This

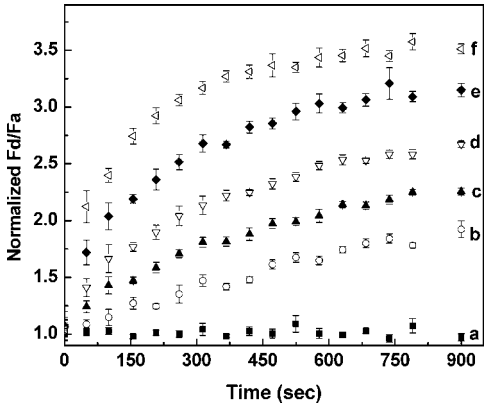

Figure 3. Temporal dependence of the rhodamine-labeled, peptide-coated quantum dots at increasing trypsin concentrations: (a) 0, (b) 25, (c) 50, (d) 100, (e) 250, and (f) 500 μg/mL. λ_{ex} = 445 nm. The ratio F_d/F_a was normalized to $(F_d/F_a)_0$, which is the ratio F_d/F_a prior to adding trypsin to the quantum dot probes solutions.

supported our conclusion that the quantum dot FRET-based probes only responded to the proteolytic activity of trypsin. Digital fluorescence images shown in Figure 4 provided clear visual evidence of FRET between the quantum dots and the attached rhodamine and of the effect of trypsin on the FRET signal. Image 4a shows a digital fluorescence image of peptide-coated quantum dots with their characteristic green emission. Image 4b shows the fluorescence image of the peptide-coated quantum dots when labeled with rhodamine. The quantum dots emit yellow-orange light due to FRET between the quantum dots and rhodamine molecules. Image 4c shows the fluorescence image of the quantum dot FRET-based probes 15 min following the addition of 250 μL of 1 mg/mL trypsin. It can be seen that the emission color of the quantum dots turned green due to enzymatic cleavage of the RGDC peptide linker, which released rhodamine molecules to the solution and restored the green emission color of the quantum dots. It must be noted that the rate of enzymatic cleavage of a substrate consisting of a target peptide linking a quantum dot as a donor and a molecular acceptor would be lower than the rate of enzymatic cleavage of a target peptide linking between a molecular donor and a molecular acceptor. This is due to steric hindrances in the quantum dot/molecular acceptor system. However, the application of luminescent quantum dots as donors in FRET assays is advantageous due to the ability to link multiple acceptors to a single quantum dot, which in turn leads to larger FRET signal changes in enzymatic assays. Additionally, the high photostability of quantum dots enables highly sensitive real-time enzymatic assays in volume-limited samples and eventually in single cells.

Screening Enzyme Inhibitors. To further demonstrate the utility of the quantum dot FRET-based enzymatic probes we measured the inhibition efficiency of the trypsin inhibitors 4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride, 4-amidinophenylmethanesulfonyl fluoride hydrochloride, and 1,10-phenanthroline. 4-(2-Aminoethyl)benzenesulfonyl fluoride hydrochloride and 4-amidinophenylmethanesulfonyl fluoride hydrochloride are water-soluble, relatively nontoxic, irreversible inhibitors of serine proteases.[27,28] 1,10-Phenanthroline is a reversible inhibitor of metal-

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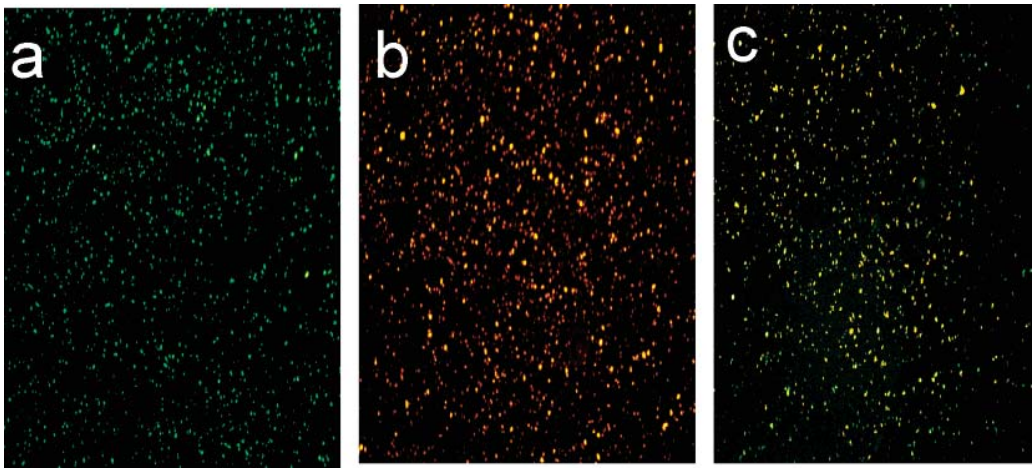

Figure 4. Digital fluorescence images of (a) peptide-coated quantum dots showing green emission, (b) rhodamine-labeled, peptide-coated quantum dots showing yellow-orange emission due to FRET between the quantum dots and rhodamine molecules, and (c) quantum dot FRET-based probes when incubated for 15 min in a solution of 250 μg/mL trypsin showing green emission. The emission color change was attributed to enzymatic cleavage of the peptide molecules, which resulted in marked reduction in FRET efficiency between the quantum dots and rhodamine molecules when released to the solution.

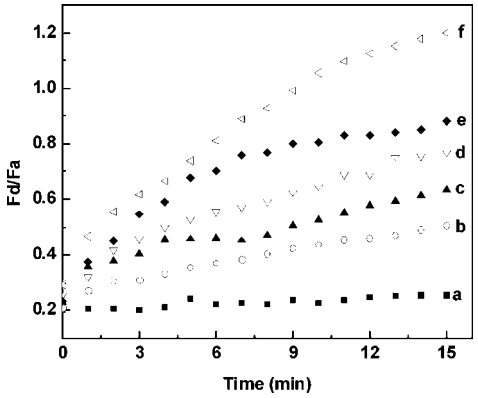

Figure 5. Temporal dependence of F_d/F_a in the presence of 250 μg/mL trypsin and increasing concentrations of the trypsin inhibitor 4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride. (a) A control experiment in the absence of trypsin and trypsin inhibitor, (b) 2.50 mg/mL, (c) 1.25 mg/mL, (d) 250 μg/mL, (e) 50 μg/mL, and (f) 0 μg/mL.

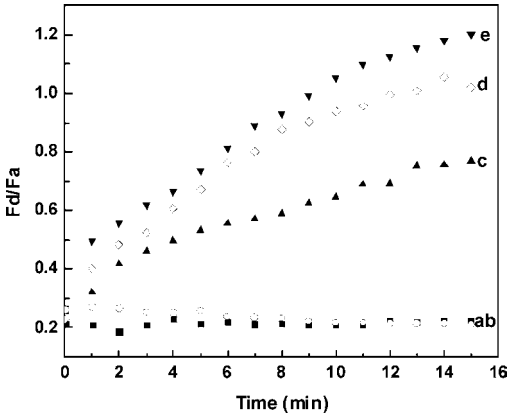

Figure 6. Real-time monitoring of the inhibition efficiency of 250 μg/mL trypsin inhibitor in the presence of 250 μg/mL trypsin. (a) A control experiment in the absence of trypsin and trypsin inhibitor (■), (b) 1,10-phenantroline (○), (c) 4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride (▲), (d) 4-amidinophenylmethanesulfonyl fluoride hydrochloride (◇), and (e) in the absence of trypsin inhibitor (▼).

loproteinases and metal-activated proteinases.[29] Figure 5 shows the temporal dependence of the ratio F_d/F_a of quantum dot FRET-based enzymatic probes in the presence of 250 μg/mL trypsin and 4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride at increasing concentrations from 0 to 2.5 mg/mL. In the inhibition assays, the trypsin inhibitor was first incubated with 250 μL of 1 mg/mL trypsin for 30 min at room temperature. A 500-μL aliquot of 10 nM quantum dot FRET-based probes was added to the solution, and the fluorescence of the quantum dots was monitored using digital fluorescence spectroscopy. The rate of increase of the ratio F_d/F_a was found to be dependent on the concentration of the enzyme inhibitor. A level of 2.5 mg/mL 4-(2-aminoethyl)-benzenesulfonyl fluoride hydrochloride led to 75% inhibition of trypsin activity. A comparison between the inhibition efficiency of 4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride, 4-amidinophenylmethanesulfonyl fluoride hydrochloride, and 1,10-phenantroline at 250 μg/mL is shown in Figure 6, which describes the temporal dependence of the FRET efficiency (F_d/F_a) of the quantum dot FRET-based probes in the presence of the tested inhibitors. It can be seen that at this level 1,10-phenanthroline completely inhibits trypsin activity. It can also be seen that the inhibition efficiency of 4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride is 2.3-fold higher than the inhibition efficiency of 4-amidinophenylmethanesulfonyl fluoride hydrochloride.

SUMMARY AND CONCLUSIONS

We have successfully synthesized peptide-coated CdSe/ZnS quantum dots. The peptide-coated quantum dots were water-soluble, biocompatible, and maintained their original photophysical properties. We designed and developed new quantum dot-based FRET probes in which rhodamine molecules were bound to the quantum dots through a peptide linker that contained selective cleavage sites to proteolytic enzymes like trypsin. The quantum dot FRET-based enzymatic probes were used to test the enzymatic

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activity of trypsin in solution. The FRET signal changes were found to be trypsin concentration dependent. The rapid response time of the probes enabled real-time monitoring of trypsin activity in assay times that lasted less than 15 min. The enzymatic assays could be performed at subpicomole trypsin level. However, the assay times increased with decreasing trypsin concentration. The probes were used to determine the inhibition efficiency of the three organic compounds 4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride, 4-amidinophenylmethanesulfonyl fluoride hydrochloride, and 1,10-phenanthroline. In the current form of the quantum dots FRET-based probes it was difficult to determine the inhibition efficiency of larger trypsin inhibitors, for example, protein molecules. It is possible that protein molecules could displace the peptide molecules from the quantum dots. This would release rhodamine molecules to the solution even in the absence of trypsin. To overcome this difficulty, we are currently synthesizing CdSe/ZnS quantum dots that are capped with the protein metallothionin (MT) following a method recently developed by Benson and co-workers.[30] This would improve the stability of the quantum dots in biological media due to MT binding to the quantum dots through multiple cysteine residues. Additionally, it would be possible to link rhodamine molecules to the MT-capped quantum dots, which in turn would result in more stable quantum dot FRET-based probes. The effect of MT coating on the FRET efficiency and on the ability to monitor enzymatic cleavage of peptide molecules using FRET between the quantum dots and rhodamine residues remained to be determined. We are also developing a lithographic technique to fabricate quantum dot FRET-based arrays for high-throughput screening of enzyme inhibitors and activators.

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