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Synthesis and Application of Quantum Dots FRET-Based Protease Sensors

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Water-soluble CdSe/ZnS luminescent semiconductor quantum dots have been widely used in immunoassays,[1−3] in situ hybridization,[4] cell biology,[5−7] and in vivo imaging[8,9] studies. These quantum dots are typically synthesized in organic solvents using organometallic precursors. To facilitate their biological applications, the hydrophobic trioctylphosphine oxide (TOPO) ligands of quantum dots are replaced with hydrophilic capping ligands to form water-soluble quantum dots.[10−12] In recent years, several research groups have utilized luminescent quantum dots as donors in fluorescence resonance energy transfer (FRET) measurements.[13−19] For example, Mauro and co-workers have developed FRET-based maltose-binding assays by coating CdSe/ZnS quantum dots capped with dihydrolipoic acid with maltose binding protein molecules. The FRET assay for maltose was based on the interaction between the quantum dots and the acceptors QSY9,[14] rhodamine red,[15] or Cy3.[16] FRET between streptavidin conjugated quantum dots and biotinylated gold nanoparticles have also been reported.[17] A hybrid quantum dots antibody fragment FRET based TNT sensor was developed as well.[18] West and colleagues recently reported the use of gold nanoparticles-coated quantum dots as collagenase activity probes.[19] This paper describes the development of quantum dots FRET-based protease sensors and their application for the measurement of extracellular matrix metalloproteinases (MMPs) activity in normal and cancerous breast cells.

The quantum dots based probes were prepared by exchanging the TOPO capping ligands of CdSe/ZnS quantum dots with tetra peptide RGDC molecules. The peptide molecules were labeled with rhodamine that served as acceptors. Upon enzymatic cleavage of the peptide molecules, the rhodamine (acceptor) molecules no longer provided an efficient energy transfer channel to the quantum dots. As a result, the emission color of the quantum dots changed back to green (Scheme 1).

Peptide-coated quantum dots were labeled with rhodamine Red-X, succinimidyl ester. When excited at 445 nm, the emission spectra of the rhodamine-labeled quantum dots showed two clearly separated emission peaks of the quantum dots at 545 nm and of the rhodamine molecules at 590 nm. The emission peak of the quantum dots decreased with increasing rhodamine concentration, indicating the occurrence of FRET between the quantum dots and the rhodamine molecules. Control experiments (see Figure 1B of Supporting Information) showed that the emission intensity of solutions of similar rhodamine concentration in the absence of peptide-coated quantum dots was 10 times lower than the intensity of the red emission peak at 590 nm of the rhodamine-labeled quantum dots. It must be noted that FRET interactions between quantum dots and fluorescent acceptor molecules are not fully understood. Unlike in FRET between donor and acceptor molecules, the distance between quantum dots and molecular acceptors is not as well-defined. Additionally, recent studies have shown that electron transfer between quantum dots and attached fluorophores increases the FRET efficiency in this system.[20] Our study shows

Scheme 1. Principle of Quantum Dots-Based Enzymatic Activity Probes

that a short tetra-peptide that links between the quantum dots and the acceptor molecules is sufficient to observe measurable FRET signals between quantum dots and molecular acceptors. This enables real-time monitoring of enzymatic activity of peptide cleaving enzymes. It is possible that the accumulative interaction between a single quantum dot and multiple acceptor molecules compensates for the reduced FRET efficiency between quantum dots and individual acceptor molecules when these are bound through a short linker. The use of molecular acceptors rather than gold nanoparticles, as described by West et al.,[19] is advantageous because it enables real-time monitoring of the enzymatic activity and comparative analysis between the enzymatic activity of peptide cleaving enzymes. The faster response time of FRET systems composed of quantum dots and molecular acceptors may be attributed to the high monodispersity of the rhodamine-labeled quantum dots. Another advantage of quantum dots−molecular acceptor systems is the simultaneous emission increase and decrease of the quantum dots and molecular acceptors, respectively, which enables a ratiometric analysis method and results in high quantitative power.

The quantum dots FRET-based enzymatic activity probes were first used to determine the activity of collagenase in solution to test the analytical capabilities of the quantum dots FRET-based probes in a model system. Collagenase is an MMP. FRET measurements of the quantum dots at increasing levels of collagenase in solution are shown in Figure 1. The fluorescence intensity of the quantum dots at 545 nm increased while the fluorescence intensity of the rhodamine molecules decreased due to the enzymatic cleavage of the RGDC peptide by collagenase. The temporal dependence of the ratio F_d/F_a of the quantum dots at increasing collagenase concentration ranging from 0 to 5 μg/mL (λex = 445

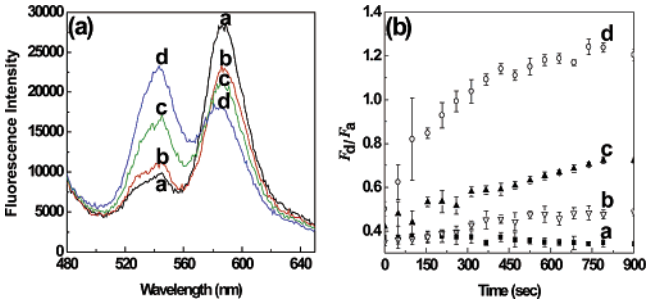

Figure 1. (a) Emission spectra of rhodamine-labeled peptide-coated quantum dots 15 min following the addition of collagenase of increasing concentration. (b) Time dependence of the ratio F_d/F_a (see text) of the rhodamine-labeled peptide-coated quantum dots at increasing collagenase concentration. (a) 0 μg/mL (black), (b) 0.5 μg/mL (red), (c) 2.5 μg/mL (green), and (d) 5.0 μg/mL (blue).

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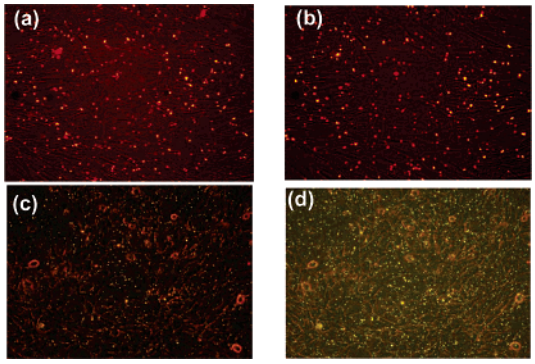

Figure 2. Digital fluorescence microscopy images of rhodamine-labeled peptide-coated quantum dots in cell culture. (a) Incubated in HTB 126 cell line for 0 min, (b) incubated in HTB 126 cell line for 15 min, (c) incubated in HTB 125 cell lines for 0 min, and (d) incubated in HTB 125 cell lines for 15 min.

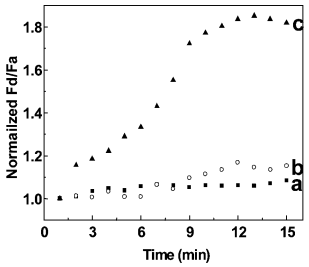

Figure 3. Temporal dependence of FRET signal (F_d/F_a) of quantum dot FRET-based protease sensors in the absence of cells (a) and when attached to the extracellular matrix of normal breast cells (b) and cancerous breast cells (c). The quantum dots FRET-based probes discriminate between normal and cancerous cells in a 10 min assay.

nm) provided information on the rate of the enzymatic reaction. F_d and F_a were the emission peaks of the quantum dots at 545 nm (donor), and the rhodamine molecules at 590 nm (acceptor), respectively. The ratio F_d/F_a was collagenase-concentration dependent. For a concentration of 5 μg/mL collagnase, the enzymatic reaction was completed in 15 min.

Following the successful demonstration of using quantum dot FRET-based probes to monitor the activity of collagenase, we measured in real time the activity of MMPs in cell cultures. Digital fluorescence microscopy images were used to measure the FRET between quantum dots and rhodamine and the effect of MMPs in normal and cancerous breast cells on the FRET signal. The experiments were carried out using the cell lines HTB 125 (normal breast cells) and HTB 126 (breast cancer cells). The cells were maintained following protocols provided by the American Type Tissue Culture Collection. An amount equal to 10^6 cells/mL were seeded and cultured on glass slides for 48 h to reach 90% confluence and realize a fully developed extracellular matrix. The quantum dot FRET probes were added to the cultures and covered with cover slips to enable close proximity of the probes to the extracellular matrix. Figure 2 shows images of the quantum dot FRET probes in normal (images a,b) and cancerous (images c,d) breast cells when taken at $t = 0$ and $t = 15$ min following the addition of the probes.

Figure 3 describes the temporal dependence of the FRET signal of the quantum dot FRET probes in normal and cancerous breast cultures. FRET signals were obtained by acquiring the emission spectra of multiple fields of view using a digital fluorescence imaging spectroscopy system (see Supporting Information for details). Curve (a) shows the results of a control experiment in which quantum dot FRET probes were observed in the absence of cells. No change in the emission properties of the quantum dots was detected. Curves (b) and (c) follow the response of the quantum dot FRET probes in normal (b) and cancerous (c) breast cells. In the fluorescence images shown in Figure 2 and the kinetic curves shown in Figure 3 it can be seen that the MMPs activity in breast cancer cultures is significantly higher compared to normal cells. The change in FRET signal is completed in about 10 min.

In conclusion, we have successfully synthesized rhodamine-labeled peptide-coated CdSe/ZnS quantum dots and use them as FRET probes to monitor the proteolytic activity of MMPs in normal and cancerous cell cultures. We were able to discriminate between a normal and cancerous tissue in less than 15 min. The method can be extended to other applications involving overexpression of proteolytic activity. Changing the peptide sequence would enable measuring the activity of specific proteolytric enzymes. It could also enable high throughput screening of protease inhibitors and activators in an array format.

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Supporting Information Available: The protocol for the synthesis of rhodamine-labeled peptide-coated quantum dots and optimization of the rhodamine/quantum dots ratio. This material is available free of charge via the Internet at http://pubs.acs.org.

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