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# A self-assembled quantum dot probe for detecting β-lactamase activity

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**Abstract**

This communication describes a quantum dot probe that can be activated by a reporter enzyme, β-lactamase. Our design is based on the principle of fluorescence resonance energy transfer (FRET). A biotinylated β-lactamase substrate was labeled with a carbocyanine dye, Cy5, and immobilized on the surface of quantum dots through the binding of biotin to streptavidin pre-coated on the quantum dots. In assembling this nanoprobe, we have found that both the distance between substrates and the quantum dot surface, and the density of substrates are important for its function. The fluorescence emission from quantum dots can be efficiently quenched (up to 95%) by Cy5 due to FRET. Our final quantum dot probe, assembled with QD605 and 1:1 mixture of biotin and a Cy5-labeled lactam, can be activated by 32 μg/mL of β-lactamase with 4-fold increase in the fluorescence emission.


*Keywords:* Quantum dots; Fluorescence resonance energy transfer; Nanoparticle probe; β-Lactamase; Enzyme-activated probe; Self-assembly

Nanosize semiconductor crystallites (also known as quantum dots, QDots) are robust and stable light emitters. Recent wide interest in quantum dots arises from the unique optical and electrical properties of these semiconductor nanocrystals, including broad absorption and narrow emission spectra, large extinct coefficients, resistance to photobleaching, long fluorescence lifetime, and size-tunable emission. Quantum dots usually are functionalized with coating molecules (e.g., small molecules, peptides, antibodies, and DNAs) for biological applications such as in vitro assay detection and in vivo cell labeling and imaging [1–12]. Similar to organic fluorophores, quantum dots can serve as energy donors with organic dyes as acceptors in fluorescence resonance energy transfer (FRET) assays [9]. A number of FRET-based QDot biosensors have been reported so far, for examples, for detecting maltose [9], 2,4,6-trinitrotoluene [10], toxins [11], and protease activity [12]. Quantum dots can also serve as an energy acceptor in bioluminescence resonance energy transfer, and emit light both in vitro and in vivo with no need for excitation light [13]. Here we report a design of a QDot-based nanosensor that can detect the activity of β-lactamase, an important bacterial enzyme.

β-Lactamases are a family of bacterial enzymes that are able to efficiently cleave penicillins and cephalosporins [14]. Detection of β-lactamase activities in biological samples is of clinical importance since they are responsible for the bacterial resistance to β-lactam antibiotics. β-Lactamases can also serve as a biological reporter for biological processes such as the activation of promoter/regulatory elements in living mammalian cells [15], protein–protein interactions [16,17], RNA splicing [18,19], and protease activity [20]. Several fluorogenic substrates based on small organic fluorophores have been reported for detecting activity in vitro and in vivo [15,21,22]. In this study, we explored whether a QDot-based nanoprobe could be designed for reporting β-lactamase activity. Such a nanosensor should take advantage of the unique optical properties of QDots, such as photostability, large stoke shift, and size-tunable emission, and facilitate the detection of β-lactamases.

Our designing strategy for a QDot-based nanosensor is illustrated in Fig. 1, based on the principle of FRET. In designing our probe, we chose QDots with long

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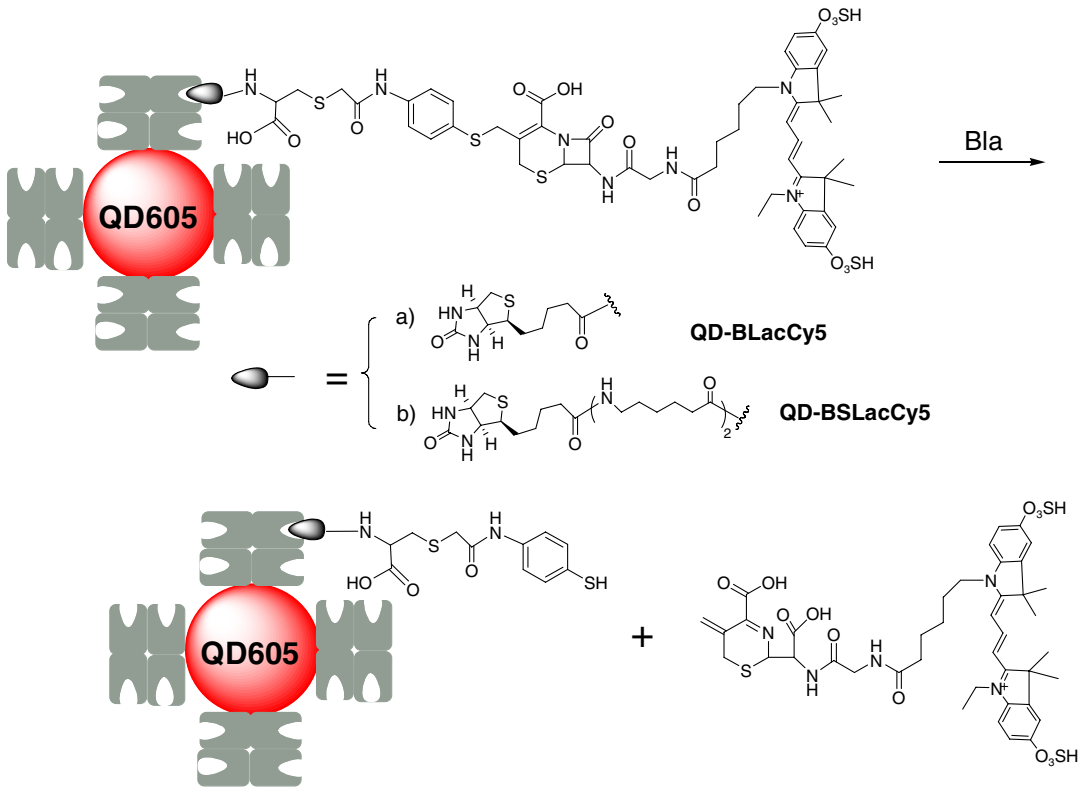



Fig. 1. Schematic presentation of the design of the QDot-based nanosensors for detecting Bla. The Bla substrate is labeled with the FRET acceptor Cy5, and immobilized to QDots via the biotin and streptavidin binding. Bla activity cleaves the lactam ring and releases Cy5 to restore the QDot fluorescence emission.

wavelength emission—QD605 as the FRET donor, and a carbocyanine dye—Cy5 as the FRET energy acceptor. The QDot surface is coated with streptavidin, and a biotinylated lactam is labeled with Cy5. Binding of biotin to streptavidin will immobilize the lactam-tethered Cy5 at the surface of QD605. The fluorescnece emission from QD605 will thus be quenched by Cy5 due to FRET. Cleavage of the lactam ring by the enzyme will release Cy5 from the QD605 surface and thus restore the QD605 fluorescence emission.

## Materials and methods

*General methods and chemicals.* Cy5-NHS was purchased from Amersham Biosciences. Sulfo-NHS-LC-LC-Biotin was purchased from Pierce Biotechnology Inc. QD605 was purchased from Quantum Dot Corporation (Hayward, California), and has typical CdSe/ZnS core-shell structures with the quantum yield (determined in 50 mM, pH 9 borate buffer) of 65%. Other chemicals and solvents unless other stated were purchased from Sigma–Aldrich Chemical Co. Water was deionized by passing through a Milli-Q water filtration system. Reversed-phase HPLC separation was achieved using a C18 column (250 × 10 mm), eluted with a linear gradient of 20–80% acetonitrile in 0.1% trifluoroacetic acid over 30 min at a flow rate of 3.0 mL/min. Mass Spectrometry was characterized on a MALDI-TOF (matrix-assisted laser desorption ionization-time of flight) mass spectrometer. β-Lactamase was customarily prepared by Biologics Process Development, Inc. (San Diego, California). Concentration of β-lactamase was determined by the absorption at 280 nm. Boc-Gly-Lac-Cys (Scheme 1) was prepared according to published procedure [22].

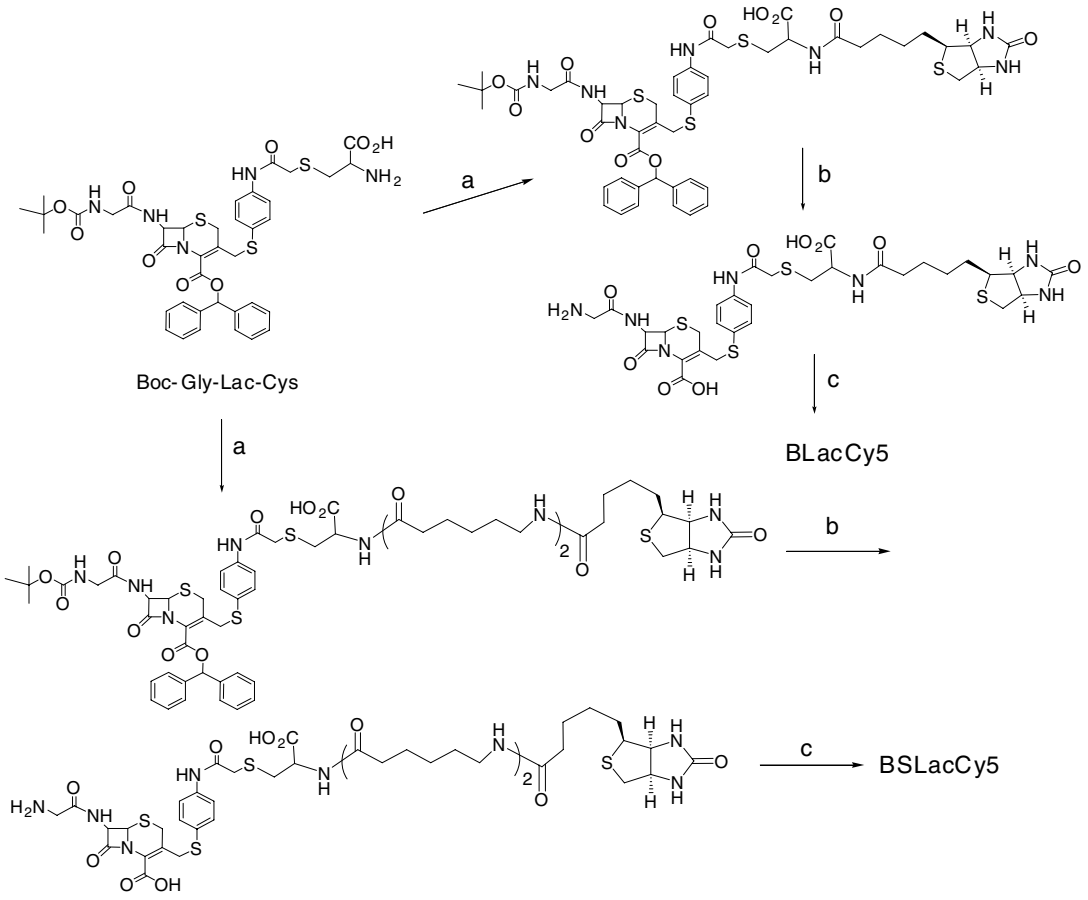
*Synthesis of Boc-Gly-Lac-Cys-LCLC-Biotin.* To 23.2 mg (28 μmol) of Boc-Gly-Lac-Cys in 0.1 mL of dried DMF was added 20 mg (31 μmol) of Sulfo-NHS-LC-LC-Biotin in 50 μL dried DMF under Argon. After the mixture was cooled in ice bath, 9.8 μL (80 μmol) of 2,6-lutidine was injected via syringe. The reaction was monitored and purified by HPLC to afford 17 mg of the desired product (yield = 47.7%). Calculated MS ($C_{61}H_{79}N_9O_{13}S_4$): 1273.47; found: 1296.96 ($M + Na^+$).

*Synthesis of Boc-Gly-Lac-Cys-Biotin.* To 10 mg (12 μmol) of Boc-Gly-Lac-Cys in 0.1 mL of dried DMF was added 12.3 mg (36 μmol) of NHS-Biotin in 50 μL of dried DMF under Argon. After the mixture was cooled in ice bath, 3.0 μL (25 μmol) of 2,6-lutidine was injected via syringe. The reaction was monitored and purified by HPLC to afford 4.1 mg of the desired product (yield = 32.6%). Calculated MS ($C_{49}H_{57}N_7O_{11}S_4$): 1047.30; found: 1048.50 ($M + H^+$).

*Synthesis of BSLacCy5.* Seventeen milligrams (13 μmol) of Boc-Gly-Lac-Cys-LCLC-Biotin was dissolved under Argon at 0 °C in 1 mL of TFA with 2.5% $H_2O$ and 2.5% anisole. Two hours later, the mixture was poured into cold ether (10 mL) and incubated in −20 °C for 10 min. After centrifuge, the precipitate was vacuum dried and ready for the next step. To 2.7 mg (2.4 μmol) deprotected Gly-Lac-Cys-LC-LC-Biotin in 1 mL of dried DMF was added 0.9 mg (1.1 μmol) Cy5-NHS (dissolved in 0.5 mL DMF). Then 1.25 μL (7.2 μmol) of DIPEA and 1.1 μL (9.6 μmol) of 2,6-lutidine were added under Argon. The reaction was monitored and purified by HPLC to afford the title product 0.5 mg (yield = 28%). Calculated MS ($C_{76}H_{100}N_{11}O_{18}S_6$): 1646.56; found: 1647.53 ($M + H^+$).

*Synthesis of BLacCy5.* To 1.9 mg (2.12 μmol) deprotected Gly-Lac-Cys-Biotin in 1 mL dried DMF was added 2 mg (2.53 μmol) Cy5-NHS (dissolved in 0.5 mL DMF). Then 0.85 μL (4.89 μmol) of DIPEA and 0.86 μL (7.38 μmol) of 2,6-lutidine were added under Argon. The reaction was monitored and purified by HPLC to afford the title product 1.38 mg (yield = 46%). Calculated MS ($C_{64}H_{78}N_9O_{16}S_6$): 1420.39; found: 1424.23.

*Detection of Bla by the assembled probes.* QD605 (1.5 μM) was diluted to 2–5 nM in PBS for titration experiments. The concentrations of




Scheme 1. Syntheses of BLacCy5 and BSLacCy5 (a) Biotin-NHS or Sulfo-NHS-LC-LC-Biotin, DIPEA, rt, HPLC purification; (b) TFA, anisole, DCM; (c) Cy5-NHS, 2,6-lutidine, DIPEA.

BSLacCy5 and BLacCy5 were determined by UV absorption at 650 nm ($\varepsilon = 250{,}000\ M^{-1}cm^{-1}$) and kept at 3–5 μM in $H_2O$. Five microliters of Bla (1 mg/mL) solution was added each well in the evaluation of Bla activity. Control experiments were done by adding PBS to QDots solution. The gain numbers and Z-positions were optimized in each measurement. The measurement was performed on the SAFIRE microplate reader (TECAN, Research Triangle Park).

## Results and discussion

To test our design, we prepared the first biotinylated Bla substrate, Biotin-Lac-Cy5 (BLacCy5 in Fig. 1), and examined whether it can bind QD605 and quench the QDots emission. Fig. 2a shows that addition of BLacCy5 (1.76 μM) to a solution of QD605 (2.2 nM) led to immediate decreases in the fluorescence emission of QD605. As the molar ratio between BLacCy5 (at a final concentration of 67 nM) and QD605 (at a final concentration of 2.1 nM) reached around 32, the emission of QD605 decreased by ~95%. Further addition of BLacCy5 induced little decrease on the QD605 emission. Controls with addition of Cy5 without the conjugated β-lactamase substrate showed little effect on the emission of QD605. This result indicates that the binding of BLacCy5 to the immobilized streptavidin of QD605 leads to the quenching of QD605 fluorescence emission.

The QD605 used here has a coating of polymer and streptavidin (about 10–12 copies) at a thickness of about 5 nm [23]. The biotin group is approximately 2 nm apart from Cy5 on BLacCy5 based on a simple CPK modeling [24]. Thus the distance of Cy5 on BLacCy5 to the core of QD605 is between 5 and 7 nm depending on the geometry of immobilized streptavidin, which should be within the range for FRET to occur [9]. The FRET emission of Cy5 was rather weak with just more than 3-fold stronger than the direct emission of the same amount of Cy5 under the same excitation wavelength (420 nm). The weak Cy5 emission is due to the self-quenching among a large number of Cy5 immobilized through binding to streptavidin [25,26].

Next, we examined whether β-lactamase can hydrolyze the immobilized BLacCy5 and restore the QD605 emission. We used TEM-1 β-lactamase (Bla), the 29 kDa isoform product of the ampicillin resistance gene, in our test. After incubation of 32 μg/mL of Bla with the self-assembled QD/BLacCy5 complex for 100 min, we observed a small increase of the QD605 emission. On the other hand, a pre-incubation of BLacCy5 with Bla produced much less quenching of the QD605 emission, confirming that BLacCy5 itself can be hydrolyzed by Bla in the absence of QD605. The low activation efficiency by Bla observed

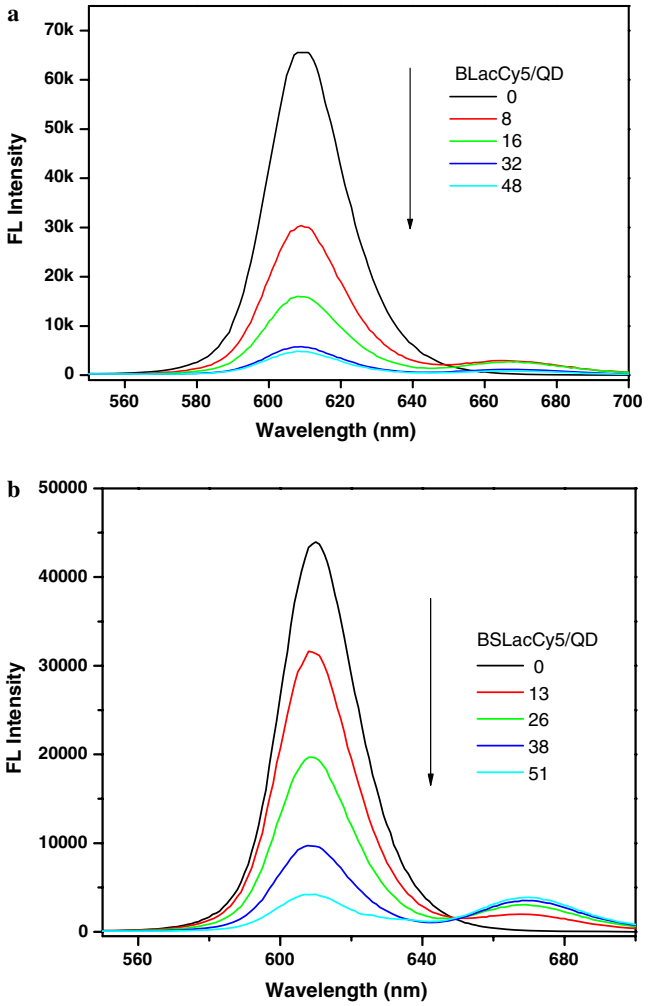



Fig. 2. Binding of Cy5-labeled β-lactamase substrates induces FRET quenching of the quantum dot emission ($\lambda$ex = 420 nm). The ratios of (a) BLacCy5/QD605 and (b) BSLacCy5/QD605 are as indicated.

with this self-assembled quantum dot probe indicates that there was significant steric hindrance for Bla to access the immobilized BLacCy5. This steric hindrance may come from the self-blocking effect of immobilized BLacCy5 and from streptavidin on QDots.

Since streptavidin has a deep biotin binding pocket [27], we inserted a spacer between the biotin and lactam to minimize the steric interference of the biotin-streptavidin complex with the Bla hydrolysis. We synthesized the Biotin-Spacer-Lac-Cy5 (BSLacCy5) (Fig. 1), which contained two 6-amino-hexanoic acids as the spacer, and examined its efficiency in quenching the emission of QD605. Since the distance between Cy5 and the QD605 increased, the quenching efficiency of BSLacCy5 decreased as well, and it took 60% more of BSLacCy5 than BLacCy5 to reach 90% of quenching (Fig. 2b).

To decrease the steric interference among immobilized BSLacCy5, we incubated QD605 with a mixture of biotin and BSLacCy5 (at a ratio of 1:1), which presumably would decrease the density of BSLacCy5 at the surface by 50%. The QD605 emission decreased by 5-fold after incubated with the 1:1 mixture of biotin and BSLacCy5, shown as 20% of the QD605 emission at time = 0 in Fig. 3. Bla was added to the self-assembled quantum dot probe, and within 80 min, nearly 4-fold increase in the fluorescence emission of QD605 was observed, which was about 70% of its original emission of QD605 (Fig. 3).

In contrast, when a mixture of biotin and BLacCy5 (at a ratio of 1:1) was complexed with QD605, although the QD605 emission was quenched effectively by almost 80%, addition of Bla did not restore the QD605 emission (Fig. 3). This result further confirmed the importance of the spacer, two 6-amino-hexanoic acids, in BSLacCy5 for efficient Bla hydrolysis. On the other hand, when streptavidin at the surface of QD605 was all bound by BSLacCy5, the cleavage rate by Bla was still very slow, suggesting that the appropriate density of substrates on the surface of QDots is equally critical for the assembled nanoprobe. In order for the QDot nanoprobe to function, both the distance between the substrates and the QDots and the density of the substrate on the surface have to be optimal so that the background fluorescence is low and the substrate is accessible to the enzyme.

We report here a design for fluorogenic nanoprobes based on quantum dots for detecting an enzymatic reaction. The nanoprobe is self-assembled through the binding of streptavidin immobilized on QDots to biotin linked to the β-lactamase substrate. Our study has indicated that two parameters: the distance between substrates and the QDot surface, and the density of substrates at the QDot surface, are important for the function of the self-assembled nanosensor. Here we used QD605 in our design, but other QDots with different emission wavelengths can be simlarly assembled, especially QDots with infrared

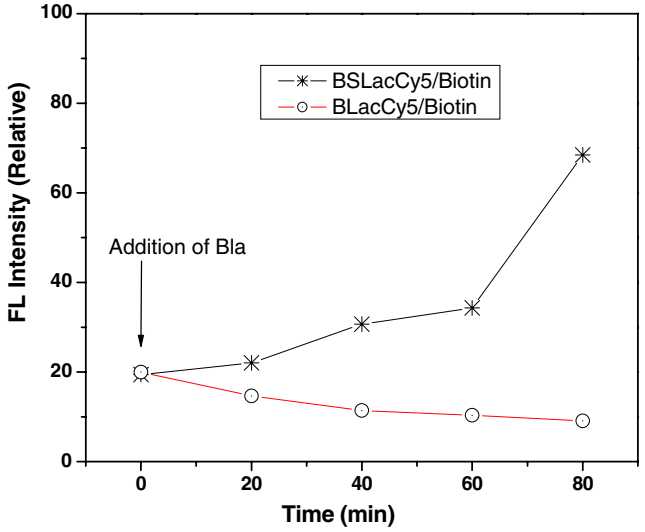



Fig. 3. Activation of the self-assembled quantum dot probes by Bla (0.03 mg/mL) over time. The quantum dot probes were assembled from QD605 and BSLacCy5/Biotin (1:1) (-*-), and from QD605 and BLacCy5/Biotin (1:1) (-○-). The fluorescence emission was measured with excitation at 420 nm.

emission, which will offer advantages in detecting β-lactamase gene expression in vivo [15,20,22].

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