A high sensitive and specific QDs FRET bioprobe for MNase†

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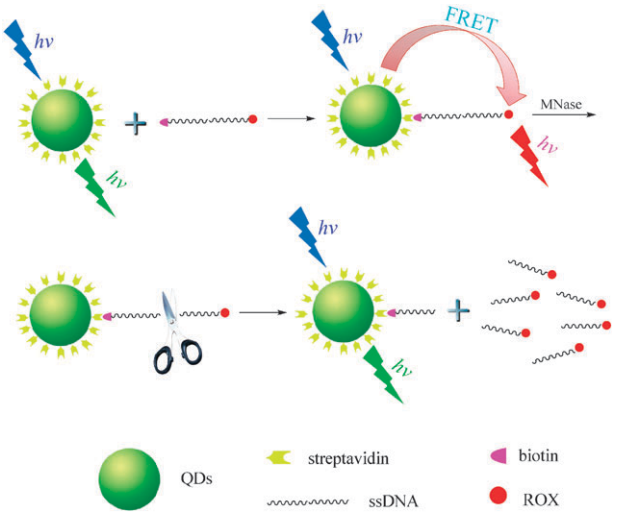
We used CdSe/ZnS quantum dots–ssDNA–fluorescent dye conjugates as bioprobes to detect micrococcal nuclease with high specificity and sensitivity, and further utilized the bioprobe to monitor the micrococcal nuclease activity in the culture medium of *Staphylococcus aureus* by fluorescence microscopy.

Luminescent semiconductor quantum dots (QDs) have attracted widespread attention in diverse research areas in the past two decades.[1] Compared to traditional organic dyes and fluorescent proteins, QDs have some advantages such as high emission quantum yield, narrow and symmetric emission and so on, which make them excellent donors in fluorescence resonance energy transfer (FRET) processes.[2] Luminescent QD-FRET bioprobes have been used to investigate the interactions between enzymes and substrates,[3] for example, nuclease and nucleic acid.[4] FRET between CdSe/ZnS QDs and dyes have been used to probe the activity of DNase and DNA polymerase.[4b,c] These results have indicated that QD-FRET probes could be effective nuclease sensors. Here we show that such probes can even be used for quantitative determination of nuclease providing a well-defined limit of detection. We further applied these probes in a real sample to show its application under realistic diagnostic assay conditions.

The micrococcal nuclease (MNase) is a nonspecific endo–exonuclease that digests single- and double-stranded DNA and RNA, but it preferentially digests single-stranded DNA (ssDNA). The cleavage of DNA or RNA occurs preferentially at AT or AU-rich regions to yield mononucleotides and oligonucleotides with terminal 3′-phosphates.[5] MNase is a thermostable nuclease, and its activity is strictly dependent on Ca^{2+}, which should be present in the nuclease digestion reaction buffer.[6] It has been reported that MNase is the extracellular nuclease of *Staphylococcus aureus* (*S. aureus*),[7] which is a spheric gram-positive bacterium and causes both community acquired and hospital acquired infections.[8] The existence of MNase can be the standard to identify *S. aureus* and the content of MNase can be used to evaluate the pathogenicity of *S. aureus*.[9] Conventional methods, for example, culture techniques and polymerase chain reaction (PCR) analyses,[10] have been applied to differentiate *S. aureus* from other staphylococci by MNase testing. However, these methods were time consuming (2–4 h or 8 h) and lack the sensitivity and specificity needed for rapid diagnosis. Here we introduce a simple and rapid method for the quantitative detection of MNase with high sensitivity and specificity using QD-FRET bioprobe for the first time, and further monitor MNase activity with this bioprobe in the culture medium of *S. aureus* by fluorescence microscopy.

Scheme 1 depicts the principle of MNase detection using the QD-FRET bioprobe. The bioprobe was composed of two sections: the streptavidin-conjugated QDs (SA-QDs) and the modified ssDNA. The 3′-end of the ssDNA (5′-TAT ATG GAT GAT GTG GTA TT-3′) was modified with biotin while the 5′-end was modified with 6-carboxy-X-rhodamine (ROX), which acted as an acceptor. FRET between QDs and ROX resulted in a color change of the system from green to orange-red. After the ssDNA was cleaved by MNase into small fragments, the color changed back to green.

The reaction between streptavidin and biotin was finished within 10 min in the nuclease digestion buffer, which contains 20 mM Tris-HCl, pH 8.0, 5 mM NaCl and 2.5 mM $CaCl_2$. The



Scheme 1 The principle of MNase detection using our QDs–ssDNA–fluorescent dye conjugate bioprobes.

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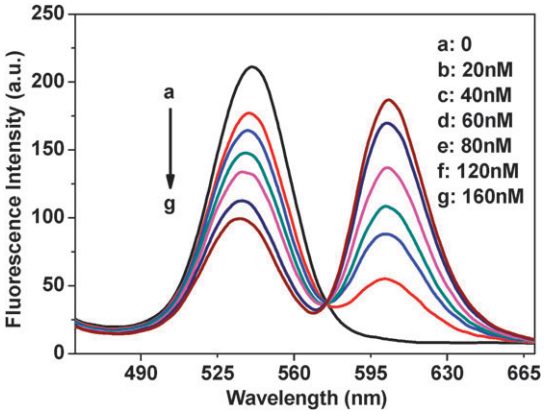

Fig. 1 The fluorescence spectra of 65 nM SA-QDs at different concentrations of modified ssDNA.

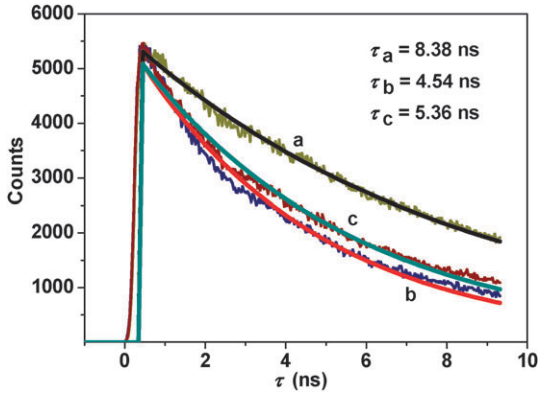

Fig. 3 The fluorescence decay traces of the SA-QDs (a), the bioprobe (b) and the bioprobe reacted with 2.0 units mL^{-1} MNase (c). All measurements were made at λ = 550 nm.

fluorescence intensity of QDs at 547 nm (F_d) decreased dramatically, meanwhile, that of ROX at 603 nm (F_a) increased significantly when excitated at 388 nm (Fig. 1). FRET efficiency between QDs and ROX is significantly improved due to the flexibility of ssDNA, which puts the ROX spatially closer to the QDs.[11a] Alteration of the SA-QDs : modified ssDNA ratio from 1 : 0.3 to 1 : 2.46, the quenching efficiency increased from 16% to 54%. Higher quenching efficiency did not occur with the increase of modified ssDNA concentration because of the saturated interaction between SA-QDs and the modified ssDNA.[11b] In order to enhance the sensitivity of MNase determination, the molar ratio of SA-QDs : modified ssDNA of 1 : 2.46 was chosen to develop the QD-FRET bioprobe.

The QD-FRET bioprobe was foremost applied to detect MNase in aqueous solution. Initial experiments demonstrated that the reaction of bioprobe with MNase was expeditious and completed in about 10 min at 37 °C (see ESI†). After the system was incubated for 15 min at 37 °C, F_d and F_a were recorded to measure MNase. Fig. 2 shows the fluorescence spectra of the QD-FRET bioprobe in 0 ~ 3.6 units mL^{-1} MNase. A linear response was observed in this range (insert in Fig. 2). The relative fluorescence intensity ratio (F_d/F_a) was normalized to $(F_d/F_a)_0$, which was the value of the bioprobe in the absence of MNase. Based on the 3 times standard deviation of 8 measurements of bioprobe solution containing 0.8 units mL^{-1} MNase, the limit of detection for MNase was up to 0.06 units mL^{-1}.

Furthermore, the fluorescence decay curves were measured to characterize the photophysical properties of the bioprobe–MNase system.[4a,12] As shown in Fig. 3, SA-QDs exhibited a lifetime of 8.38 ns. When the interaction occured between SA-QDs and modified ssDNA, SA-QDs lifetime was shortened to 4.54 ns. The results confirmed the occurrence of FRET between QDs and ROX. After the treatment of the bioprobe with 2.0 units mL^{-1} MNase, SA-QDs recovered its original lifetime partially, 5.36 ns. This partial recovery of SA-QDs lifetime was based on the incomplete digestion of ssDNA by MNase.

In order to investigate the specific detection of MNase with our bioprobe, another phosphodiesterase nuclease (S1 nuclease)[13] was chosen as the contrast to cleave ssDNA under the same condition. The S1 nuclease is ssDNA-specific nuclease,

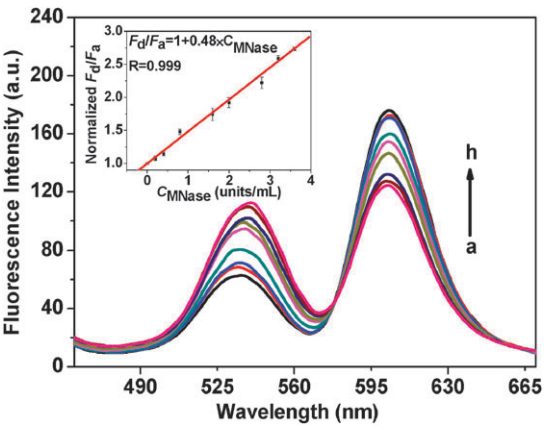

Fig. 2 The fluorescence spectra of bioprobe in different concentrations of MNase: (a) 0, (b) 0.2, (c) 0.4, (d) 0.8, (e) 1.6, (f) 2.0, (g) 2.8, (h) 3.2 units mL^{-1}. The insert shows the linear relationship between the normalized ratio F_d/F_a and MNase concentration. F_d and F_a were the emission peaks of the QDs at 547 nm, and the ROX at 603 nm, respectively. F_d/F_a values were normalized to $(F_d/F_a)_0$, which is the ratio F_d/F_a prior to adding MNase to the bioprobe solution.

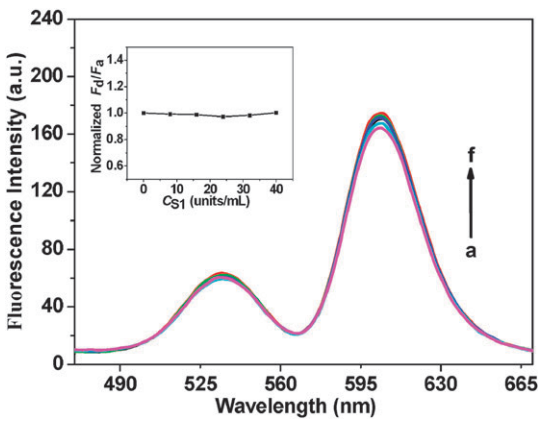

Fig. 4 The fluorescence spectra of the bioprobe in different concentrations of S1 nuclease: (a) 0, (b) 8, (c) 16, (d) 24, (e) 32, (f) 40 units mL^{-1}. The insert shows the relationship between the normalized ratio F_d/F_a and S1 nuclease concentration. F_d and F_a were the emission peaks of the QDs at 547 nm, and the ROX at 603 nm, respectively. F_d/F_a values were normalized to $(F_d/F_a)_0$, which is the ratio F_d/F_a prior to adding S1nuclease to the bioprobe solution.

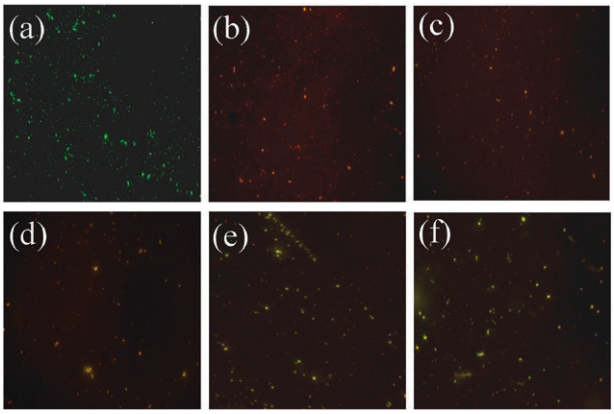

Fig. 5 The digital fluorescence microscopy images of the SA-QDs (a) and the bioprobe (b) for the monitoring of MNase activity in the culture medium of *S. aureus* inoculated and shaked at 37 °C for 0 (c), 2 (d), 6 (e) and 10 h (f).

which exhibits endo- and exolytic hydrolytic activity for the phosphodiester bonds of ssDNA or RNA and produces mono- or oligonucleotide fragments. The excision of ssDNA by S1 nuclease have occurred in AT rich regions, which is the same with MNase cleavage site.[13a] The fluorescence spectra of the bioprobe in different concentrations of S1 nuclease were shown in Fig. 4. F_d and F_a were not affected obviously with S1 nuclease, and the normalized F_d/F_a ratio was also not changed with the change of S1 nuclease concentration (insert in Fig. 4). These results validated the high specificity of bioprobe for MNase testing.

The bioprobe was first applied to monitor MNase activity in the culture medium of *S. aureus* by fluorescence microscopy. The emission color of SA-QDs (Fig. 5a) was green while that of the bioprobe (Fig. 5b) was orange-red in the culture medium. When the activation time of *S. aureus* was prolonged, the emission color of bioprobe changed back to green gradually (Fig. 5c–f), which indicated the increase of MNase concentration in the culture medium. The emission color of Fig. 5e was almost the same with that of Fig. 5f, because MNase concentration did not change markedly in the culture medium of *S. aureus* activated for 6 h and 10 h. It has been reported that the synthesis and secretion of MNase began in the early growth period of *S. aureus* and finished at the end of the logarithmic growth phase (about 8 h), and then the concentration of MNase kept invariant.[14] The experimental phenomena were consistent with the results reported. These experimental results showed the feasibility of our bioprobe for the identification of *S. aureus* and evaluation of *S. aureus* pathogenicity by MNase testing in the culture medium.

In summary, a simple and rapid method to detect MNase with high sensitivity and specificity based on the new QD-FRET bioprobe has been developed. We have further utilized the bioprobe to monitor MNase activity in the culture medium of *S. aureus* and evaluate the pathogenicity of *S. aureus*. This method could be extended to other applications such as measuring the activity of other specific nuclease, nuclease inhibitors and activators in organism body.

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