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An ultrasensitive biosensor for DNA detection based on hybridization chain reaction coupled with the efficient quenching of a ruthenium complex to CdTe quantum dots†

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A highly sensitive and selective DNA biosensor based on hybridization chain reaction is described, which combines CdTe quantum dots (QDs) and a ruthenium complex. Based on the variation of fluorescence signals of the CdTe QDs, the target DNA is determined.

It is important to detect a specific sequence DNA for clinical diagnosis, pathology, gene expression analysis, molecular biology research and biomedical studies.[1] Due to the low-abundant DNA biomarkers linked to the diseases in biological samples, it is highly desirable to develop ultrasensitive and specific methods for DNA detection.[2] To meet these requirements, great efforts have been devoted to signal amplification such as rolling circle amplification,[3] exonuclease III-assisted amplification[4] and strand displacement amplification,[5] *etc.* Despite high sensitivity and selectivity, many of these enzyme-based DNA biosensors greatly increased the complexity.

Recently, Tan and co-workers reported an ingenious biosensor for DNA detection without enzymes or label processes. They describe a DNA detection system which combines the amplification capability of hybridization chain reaction (HCR) with the spatially sensitive fluorescence signal of pyrene molecules conjugated to hairpin probes.[6a] In HCR, there are two auxiliary probes, H1 and H2, which are dual-labeled with pyrene moieties at each end. When the target is present in the reaction solution, auxiliary probes H1 and H2 can be assembled into nicked double-stranded DNA (dsDNA) structures, which is similar to alternate copolymers from a cascade of hybridization events triggered by a single target nucleic acid molecule. This method is an excellent isothermal signal amplification technique without the involvement of enzymes. Because of the advantages of HCR, it is widely applied in DNA detection as a signal amplification strategy.[6]

Nucleic acid molecular "light switches" are a kind of polypyridyl ruthenium complexes that are not photoluminescent in water, as the triplet metal-to-charge transfer ligand excited state is effectively quenched by hydrogen bonds between water and the phenazine nitrogen of the ligand. When it binds to dsDNA, the interaction between the ligand and the base pairs of the duplex nucleic acid protects the phenazine nitrogen from water, leading to intense fluorescence emission.[7] Luminescent semiconductor quantum dots (QDs) have attracted widespread attention in diverse research areas.[8] In recent years, our group reported a new fluorescent ensemble probe comprising an ionic conjugate between water-soluble CdTe QDs and $Ru(bpy)_2(dppx)^{2+}$ (bpy = 2.2′-bipyridine, dppx = 7,8-dimethyldipyrido[3.2-*a*:2′,3′-*c*]-phenanthroline). The ruthenium complex served both as an effective quencher for QDs *via* the photoinduced electron transfer process and as a reporter for dsDNA. In the presence of dsDNA, the green fluorescent QDs were set free and concomitantly red fluorescence was emitted by the $Ru(bpy)_2(dppx)^{2+}$ intercalated DNA complex. Based on this principle, we developed a new sensor for the detection of complementary dsDNA in dual-color fluorescence[9] and a colorimetric droplet platform.[10] These methods based on $Ru(bpy)_2(dppx)^{2+}$ and QDs can be used to differentiate between dsDNA and single-stranded DNA (ssDNA), while it cannot be used to detect specific sequence ssDNA.

In order to solve the problem discussed above, herein, we developed a highly sensitive and selective DNA detection biosensor that combines HCR with QDs and a ruthenium complex for the detection of specific ssDNA sequences. In this work, a human papillomavirus 18 L1 gene oligonucleotide sequence was used as a model. As shown in Fig. 1, the carboxylated magnetic microparticles (MMPs) were firstly functionalized with amino-modified DNA (A DNA) which can hybridize with the 3′ end of target DNA. The modified MMPs were prepared using a modified protocol suggested by the manufacturer (see ESI†). The 3′ end of capture DNA can hybridize with the 5′ end of the target DNA and the 5′ end of capture DNA can be hybridized with the 3′ end of H1. Then the 5′ exposed end of H1 is complementary with the 3′ end of H2 and the exposed 5′ end of H2 can hybridize with another H1 and start a new cycle. The target DNA hybridizes with the A DNA and the capture DNA,

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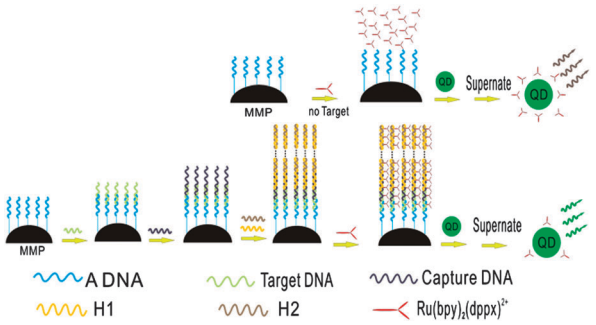

Fig. 1 Schematic representation of label-free and ultrasensitive detection of human papillomavirus 18 L1 gene using hybridization chain reaction with CdTe quantum dots and a ruthenium complex.

thus, a cascade of hybridization events between H1 and H2 can lead to long-range self-assemblies and form HCR products (shown in Fig. S1, ESI†) that link to the MMPs, and even a single target DNA can trigger the connection of a long concatamer to a capture probe on the MMPs. Then, a large amount of $Ru(bpy)_2(dppx)^{2+}$ can intercalate into the HCR products and restore the fluorescence of the QDs. In the absence of the target DNA, the HCR products cannot be conjugated to the MMPs so that the fluorescence of the QDs is quenched by $Ru(bpy)_2(dppx)^{2+}$. By monitoring the change in the fluorescence signal of the QDs, the concentration of the target DNA can be determined.

We first explored the quenching between $Ru(bpy)_2(dppx)^{2+}$ and QDs. As illustrated in Fig. 2, the QDs exhibit green fluorescence with an emissive peak at 525 nm. We discovered that the fluorescence intensity of QDs was reduced with the increase in the concentration of $Ru(bpy)_2(dppx)^{2+}$. The fluorescence intensity of QDs decreased with a proportional relationship when the concentration of $Ru(bpy)_2(dppx)^{2+}$ ranged from 1 μM to 3 μM, and the fluorescence intensity no longer decreased with the increase in $Ru(bpy)_2(dppx)^{2+}$ when the concentration was higher than 6 μM, therefore 6 μM was used in this research.

The feasibility of our design is presented in Fig. 3. Only a weak fluorescence peak was observed (Fig. 3a) for the reason that without the target DNA, although H1 and H2 can self-assemble, the HCR products were washed by the washing

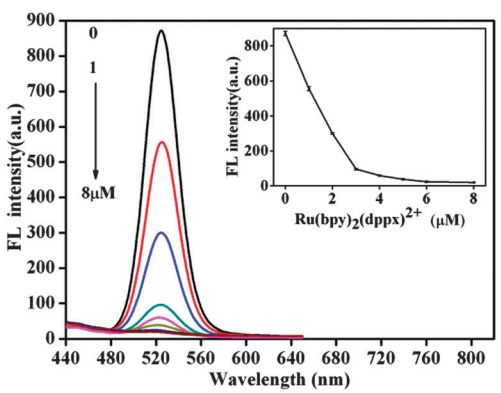

Fig. 2 Changes in the fluorescence spectra of CdTe QDs (50 nM), with increasing concentration of $Ru(bpy)_2(dppx)^{2+}$. Inset: the curve of quenched fluorescence intensity of 525 nm QDs upon increasing the $Ru(bpy)_2(dppx)^{2+}$ concentration.

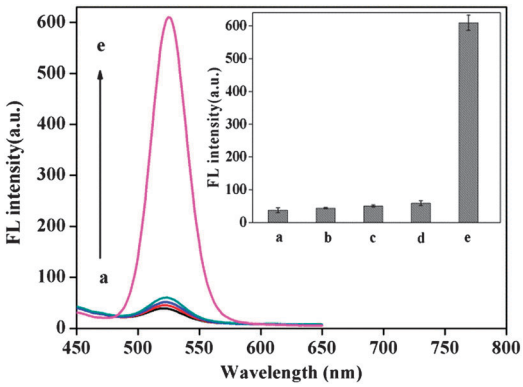

Fig. 3 Fluorescence intensities for the specificity analysis with (a) H1 + H2; (b) target only; (c) target and H2; (d) target and H1; (e) target and H1 + H2. Experimental conditions: MMPs, 70 μg; capture DNA, 25 nM; target DNA, 1 nM; H1, 1 μM; H2, 1 μM.

buffer because they were not hybridized to the surface of MMPs, so that a large amount of $Ru(bpy)_2(dppx)^{2+}$ was suspended in the supernatant. Afterwards QDs were added, and the fluorescence of QDs was quenched by $Ru(bpy)_2(dppx)^{2+}$. In the presence of the target DNA, some $Ru(bpy)_2(dppx)^{2+}$ were bound to MMPs and a slightly larger peak was obtained (Fig. 3b). If there was only H2 or H1 in the solution, the HCR products were not formed (Fig. 3c and d) and the fluorescence signal of QDs was still quenched largely. In the presence of the target DNA, the solution containing 1 μM H1 and 1 μM H2 (Fig. 3e), the HCR products were firmly immobilized onto the MMPs *via* the target DNA and the capture DNA. Thus, most of $Ru(bpy)_2(dppx)^{2+}$ were intercalated to the surface of MMPs and the fluorescence signal of QDs was restored.

To further achieve optimal experimental results, we investigated the effect of various experimental conditions including hybridization time and the concentrations of H1 and H2. The effect of hybridization time and the concentrations of H1 and H2 are shown in Fig. S2 and S3 (ESI†). The optimal hybridization time is 100 min and the optimal concentration of H1 and H2 is 1.0 μM. Fig. 4 shows representative fluorescence responses with

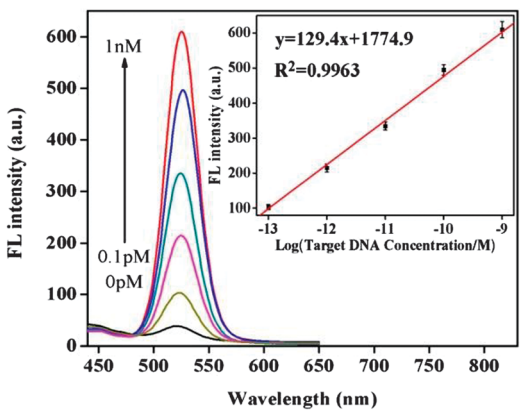

Fig. 4 The fluorescence response of different concentrations of the target DNA: 10^{-13}, 10^{-12}, 10^{-11}, 10^{-10}, 10^{-9} M (from bottom to top). Inset: the linear relationship between the fluorescence and the logarithm of the target DNA concentration.

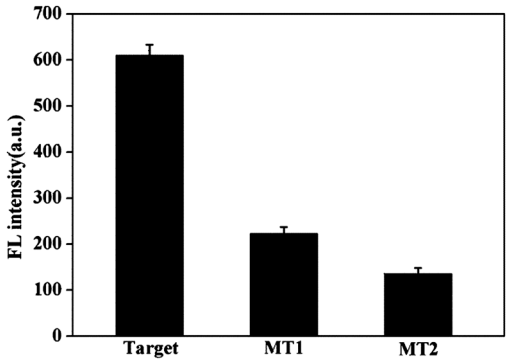

Fig. 5 Bar chart of the fluorescent responses of various DNA sequences. The target DNA and other mismatched strands were all 1 nM.

the different concentrations of the target DNA under optimal experimental conditions. A linear dependence between the fluorescence intensity and the logarithm of the target DNA concentration was obtained in the range from 0.1 pM to 1 nM with a limit of detection (LOD) as low as 6.75×10^{-14} M (3σ). This detection sensitivity is comparable to that of the existing signal amplification fluorescence detection methods.[4,5c,11] A series of five parallel measurements of 10 pM target DNA were used for estimating the precision, and the relative standard deviation (RSD) was 2.65%, and the blank standard deviation was 2.78%.

To embody the merit of the selectivity, we challenged the assay with the single-base mismatched target (MT1) and the two-base mismatched target (MT2), and the results are shown in Fig. 5. The fluorescence intensity of MT1 was about 37% of that of the perfect target DNA and about 22% of MT2 at the same concentration. All these observations indicate that the present biosensor can easily differentiate between perfect matched and mismatched targets, and therefore, it provides a great opportunity for single-nucleotide polymorphism analysis.

For comparison, we also investigated the performance of the assay in complex matrices (Fig. S4, ESI†). The serum sample was spiked with 1 nM, 0.1 nM and 10 pM target DNA to test the performance of the assay in complex matrices. There was no obvious difference between the fluorescence intensities obtained from the serum sample and those in the buffer solution, indicating the potentiality of the proposed assay for DNA detection in real biological samples.

In conclusion, we have developed a highly sensitive and selective DNA detection assay using HCR signal amplification, CdTe QDs and a ruthenium complex. This DNA biosensor does not require any kind of enzymes or sophisticated equipment. And we can detect special sequence DNA using CdTe QDs and a ruthenium complex. This developed strategy also shows high selectivity against single- and two-base mismatched sequences, which makes our new HCR-based method a useful addition to the amplified DNA detection arena. In addition, this method can be expanded to detect a wide range of DNAs in general with a rational design of the corresponding DNA sequences. In view of these advantages, this ultrasensitive fluorescent DNA biosensor has great potential in the area of DNA diagnostics and clinical analysis.

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