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Liposome–Quantum Dot Complexes Enable Multiplexed Detection of Attomolar DNAs without Target Amplification

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Supporting Information

ABSTRACT: Sensitive detection of DNA usually relies on target amplification approaches such as polymerase chain reaction and rolling circle amplification. Here we describe a new approach for sensitive detection of low-abundance DNA using liposome–quantum dot (QD) complexes and single-particle detection techniques. This assay allows for detection of single-stranded DNA at attomolar concentrations without the involvement of target amplification. Importantly, this strategy can be employed for simultaneous detection of multiple DNA targets.

Sensitive detection of DNA is of great importance in biomedical research, clinical diagnosis, and gene expression studies.[1] Target amplification is usually employed to achieve high sensitivity.[2−5] Among the techniques, polymerase chain reaction (PCR) is the most widely used technique for the amplified detection of DNA,[2] but PCR involves both multiple primers and special DNA polymerases and requires high-precision thermal cycling to separate the two DNA strands, which limits its practical applications.[2c] Alternatively, several isothermal amplification techniques, such as rolling circle amplification,[3] strand displacement amplification,[4] and loop-mediated isothermal amplification,[5] have been developed. These isothermal amplification methods proceed at a constant temperature and provide high amplification efficiency; however, some special requirements such as the ligation of a padlock probe,[3] an initial heating denaturation step, and the use of multiple primers[5] and special DNA polymerases[3−5] increase the experimental complexity and cost. Therefore, the development of new approaches for sensitive detection of DNA without the involvement of target amplification is highly desirable.

Due to its remarkable advantages of high signal-to-noise ratio, low sample consumption, and improved sensitivity,[6] single-molecule detection has become a promising approach in the research of chemical analysis,[7] molecular assembly,[8] medical diagnosis,[9] and dynamic study of biological processes.[10] Organic fluorescent dyes are usually employed in single-molecule detection.[6,7] Recently, owing to their unique optical properties of broad excitation, narrow emission, high quantum yield, and photochemical stability,[11] semiconductor quantum dots (QDs) have been widely used in place of organic fluorescent dyes in biomedical research, biological labeling, and in vitro/in vivo imaging.[11,12] Single-particle detection which combines single-molecule detection with the QDs[13−16] has shown distinct advantages of a high signal-to-noise ratio, improved sensitivity, low sample consumption, and near-zero background signal in comparison with the conventional ensemble fluorescence measurements. Single-particle detection enables the detection of biomolecules at single-particle level,[13−16] and its sensitivity can reach femtomolar.[13a] With the involvement of target amplification, its sensitivity can be further improved to attomolar.[14] However, the detection of biomolecules with attomolar sensitivity without the involvement of target amplification has never been reported.

In the conventional QD-based nanosensors, signal enhancement is usually achieved by the assembly of multiple target molecules on the surface of a single QD,[13−16] thus the sensitivity is mainly limited by the availability of the amount of both target molecules and the QDs. To break through the bottleneck of signal enhancement, we have developed a new method for sensitive detection of DNA using liposome–QD (L/QD) complexes and single-particle detection techniques. As shown in Scheme 1, a L/QD complex is composed of hundreds of QDs. The presence of target DNA leads to the generation of a sandwich hybrid which consists of a L/QD complex-tagged reporter probe, a magnetic bead-modified capture probe, and a target DNA. The separation of sandwich hybrids from the free reporter probes by the magnetic beads and the subsequent disruption of L/QD complexes result in the release of QDs, which can be sensitively counted by single-particle detection.

In this research, liposomes are used to encapsulate the QDs to form the L/QD complexes on the basis of two facts: (i) liposomes are prominent cargo carriers which can engulf many hydrophobic nanoparticles due to their unique amphiphilic structures;[17] (ii) the unique nature of liposomes such as accessible functionality and desirable biocompatibility endows them with promising applications in the biomedical researches.[17] In addition, simultaneous detection of multiple DNAs can be easily achieved by using two types of L/QD complexes with different colors.

The high-quality green and red QDs were synthesized according to a reported procedure.[18] The as-obtained QDs were evaluated by transmission electron microscope (TEM), UV–vis absorption, and steady-state fluorescence spectroscopy (see Supporting Information [SI], Figure S1). TEM images show that both the green and red QDs are highly

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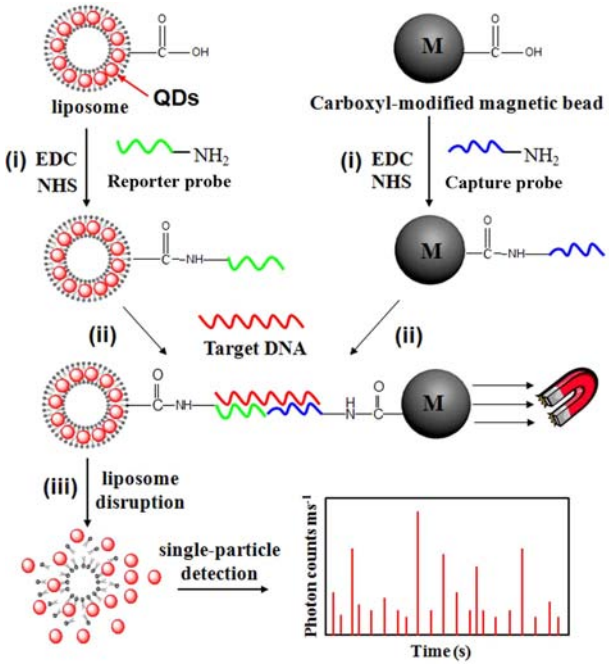
Scheme 1. Schematic Illustration for Sensitive Detection of Attomolar DNA Using Liposome−QD Complexes and Single-Particle Detection Techniques[a]



[a]This method involves three steps: (i) preparation of L/QD complexes, L/QD complex-tagged reporter probes and magnetic bead-modified capture probes; (ii) formation of sandwich hybrids in the presence of target DNA and further purification through a magnet separation; (iii) release of QDs from L/QD complex and subsequent measurement by single-particle detection.

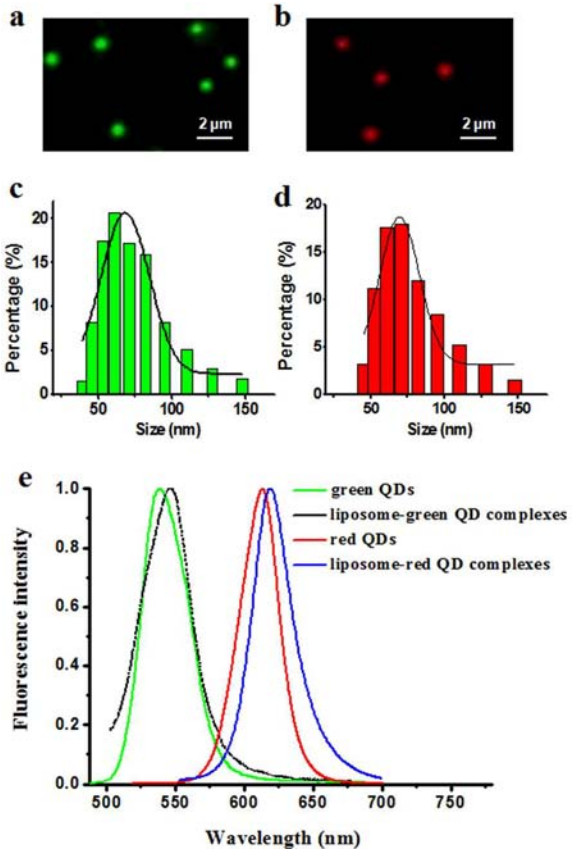


Figure 1. Characterization of two types of L/QD complexes with different colors. Fluorescence imaging of (a) L/QD green complexes and (b) L/QD red complexes. Size distribution histogram of (c) L/QD green complexes and (d) L/QD red complexes. (e) Normalized fluorescence emission spectra of pristine green QDs (green line), L/QD green complexes (black line), pristine red QDs (red line) and L/QD red complexes (blue line).

monodispersed and uniform in size. Measurement of fluorescence spectra indicates that the emission peak is 537 nm for the green QDs and 612 nm for the red QDs. The average size is estimated to be 2.8 ± 0.25 and 4.6 ± 0.34 nm for the green and red QDs, respectively.[19]

Two types of L/QD complexes with different colors were prepared based on a reported procedure with some midfications.[20] Fluorescence images show that both L/QD green complexes and L/QD red complexes are spherical in shape, remarkably bright, and uniform in size (a and b of Figure 1). Size, polydispersity index, and surface charge of L/QD complexes were measured by a Zetasizer Nano-ZS. The L/QD complexes are uniform with a polydispersity index of 0.274 ± 0.036 for L/QD green complexes and 0.236 ± 0.016 for L/QD red complexes (see SI, Figure S2 and Table S1). Analysis of size distributions reveals the average size of 82 ± 3.8 nm for the L/QD green complexes (Figure 1c) and 90 ± 3.5 nm for the L/QD red complexes (Figure 1d), much larger than that of 2.8 ± 0.25 nm for pristine green QDs and 4.6 ± 0.34 nm for pristine red QDs (see SI, Figure S1), suggesting the successful encapsulation of QDs inside the liposomes. Zeta potential is measured to be −32.7 mV for L/QD green complexes and −37.2 mV for L/QD red complexes, indicating that the L/QD complexes are highly dispersible in aqueous solution. The narrow and symmetrical fluorescence spectra (Figure 1e) further confirm the excellent optical behavior of two types of L/QD complexes with different colors, with a red-shift of 5−7 nm in the emission peak as compared with the pristine QDs due to the interaction of QDs with the lipid layer.[21] In addition, L/QD complexes were characterized by TEM. The TEM images show that the L/QD complexes are nearly spherical with the encapsulation of hundreds of QDs (see SI, Figure S3). On the basis of the three-dimensional model with the encapsulation of QDs in the lipid interior of a liposome bilayer and the calculation using the data obtained experimentally, it was found that each liposome can encapsulate either ∼1063 green QDs or ∼648 red QDs (see SI, Figure S4).

For sensitive detection of target DNA, a typical sandwich format was constructed. The carboxyl-functionalized L/QD complexes and carboxyl-modified magnetic beads were covalently conjugated with the amino-terminated oligonucleotides,[22] producing the reporter probes and the capture probes, respectively (see details in SI). As a proof of concept, one target oligonucleotide of HIV-1 was sandwich hybridized with a L/QD green complex-tagged reporter probe 1 and a magnetic bead-modified capture probe 1 on the basis of Watson−Crick base pairing. Another target oligonucleotide of HIV-2 was sandwich hybridized with a L/QD red complex-tagged reporter probe 2 and magnetic bead-modified capture probe 2. The use of two types of L/QD complexes with different colors made it possible for simultaneous detection of HIV-1 and HIV-2. Finally, the separation and purification of sandwich hybrids from the free reporter probes was realized using the magnetic beads and an external magnetic field.

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In contrast to single QD-based nanosensors,[13−16] the disruption of liposomes and the subsequent counting of released single QDs are the keys to the improvement of detection sensitivity in this research. A variety of organic solvents including methanol, ethanol, 1-propanol, 1-butanol, and surfactant of triton X-100 were examined for their capability to disrupt the liposomes.[23] Although the liposomes could be disrupted by these solvents, the fluorescence of QDs decreased greatly. Chloroform proved to be the most efficient solvent with the capability of disrupting the liposome and, at the same time, maintaining the strong fluorescence of single QDs. The selection of chloroform might be attributed to two facts: (1) DSPC, DSPE−PEG−COOH, and cholesterol which form the liposome are easy to dissolve in chloroform;[17b,20] consequently, the vesicle structure of liposome cannot be preserved in chloroform; (2) the encapsulated QDs are prone to escape upon the disruption of liposome and redisperse in chloroform due to the presence of hydrophobic ligands on the surface of QDs. To characterize the released QDs from the liposomes, particle size analysis, steady-state fluorescence and UV−Vis spectra were investigated. As shown in Figure 2a, the

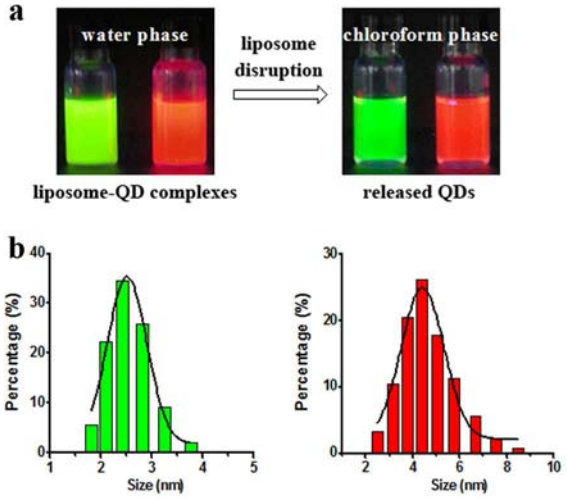

Figure 2. Release of green and red QDs from the L/QD green complexes and L/QD red complexes, respectively. (a) Fluorescence imaging of L/QD complexes in PBS solution and the released QDs in chloroform under a UV lamp with the excitation wavelength of 365 nm. (b) Size distribution histograms of the released green QDs (left) and the released red QDs (right).

released single QDs display bright fluorescence under UV excitation, indicating that the optical performance of QDs are well preserved during the incorporation and disruption processes. This is also supported by no obvious change in either the fluorescence emission spectra or the maxima absorption position of UV−vis spectra between pristine QDs and the released single QDs (see SI, Figure S5). In addition, analysis of size distributions reveals the average size of 2.5 ± 0.28 nm for the released green QDs and 4.3 ± 0.45 nm for the released red QDs (Figure 2b), consistent with that of 2.8 ± 0.25 nm for pristine green QDs and 4.6 ± 0.34 nm for pristine red QDs, suggesting the successful release of single QDs from the liposomes without the existence of QD aggregation.

The released QDs were further quantified via single-particle detection. Panels a−c of Figure 3 show the representative trace of fluorescence bursts from the released green QDs and the

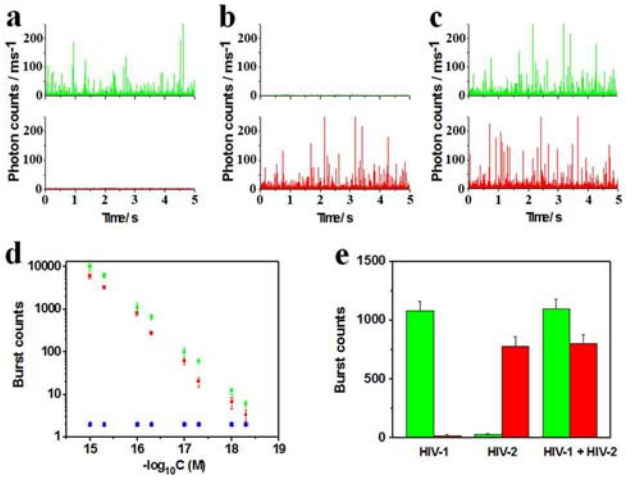

Figure 3. Simultaneous detection of multiple DNAs. (a−c) Representative trace of fluorescence bursts from the released QDs in the presence of (a) HIV-1, (b) HIV-2, and (c) both HIV-1 and HIV-2. The concentration of HIV-1 and HIV-2 is 5 fM. (d) Variance of burst counts from the released QDs as a function of the concentrations of HIV-1 (green) and HIV-2 (red). No change in the burst counts is observed in the control groups with noncomplementary DNA (black and blue). (e) Simultaneous detection of HIV-1 (green) and HIV-2 (red). The concentrations of HIV-1 and HIV-2 are each 0.1 fM. The concentration of L/QD complex-tagged reporter probes is 1.2 μM, and the concentration of magnetic bead-tagged capture probes is 1.2 μM. Error bars show the standard deviation of three experiments.

released red QDs, respectively. In the presence of HIV-1, only the fluorescence bursts from the green QDs are observed, but no fluorescence burst from the red QDs is detected (Figure 3a). While in the presence of HIV-2, only the fluorescence bursts from the red QDs are observed, but no fluorescence burst from the green QDs is detected (Figure 3b). These results indicate the excellent specificity of the proposed method. In contrast, the fluorescence bursts from both the green QDs and the red QDs are observed simultaneously in the presence of both HIV-1 and HIV-2 (Figure 3c). Thus, this method can be used for simultaneous detection of both HIV-1 and HIV-2. Moreover, the near-zero background noise warrants the ultrasensitive detection of target DNA. As shown in Figure 3d, the burst counts of green QDs increase with the target concentration for HIV-1 detection, and the burst counts of red QDs increase with the target concentration for HIV-2 detection. In contrast, in the control group with noncomplementary DNA, no obvious change is observed in the burst counts of either green QDs or red QDs. The detection limit can reach 1 aM for HIV-1 and 2.5 aM for HIV-2. Notably, the detection sensitivity of the proposed method has improved by as much as 5 orders of magnitude as compared with that of fluorescence-tagged microbead-based nanosensors,[24] and 3 orders of magnitude as compared with that of single-QD-based nanosensors.[13a] It should be noted that one bead/QD might correspond to multiple target DNAs in the fluorescence-tagged microbead-based biosensors[24] and single QD-based nanosensors.[13−16] In contrast, the current approach makes one target DNA correspond to hundreds of QDs upon the release of single QDs from the L/QD complexes, thus significantly improving the detection sensitivity even without the involvement of target amplification.

To demonstrate the capability of multiplex detection, the released green QDs and red QDs in the presence of HIV-1 and

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HIV-2 were detected simultaneously by single-particle detection (see SI, Figure S6). As shown in Figure 3e, only green-QD signals can be detected in the presence of HIV-1, and only red-QDs signals can be observed in the presence of HIV-2. While in the presence of both HIV-1 and HIV-2, both green-QD and red-QD signals can be detected simultaneously. These results clearly demonstrate that this method can be used for multiple DNA detection.

In conclusion, we have developed a new approach for sensitive detection of DNA using L/QD complexes and single-particle detection techniques. The release of hundreds of single QDs from the L/QD complexes and the subsequent counting at single-particle level are crucial to the improvement of detection sensitivity. Without the involvement of target amplification, the detection limit of the proposed method can reach attomolar, which has improved by as much as 5 orders of magnitude as compared with that of fluorescence-tagged microbead-based nanosensors,[24] and 3 orders of magnitude as compared with that of single QD-based nanosensors.[13a] Moreover, taking advantage of two types of L/QD complexes with different colors, simultaneous detection of two DNA targets can be achieved. This method has significant advantages of high sensitivity, multiplex analysis, and low sample consumption and might be further extended to sensitive detection of miRNAs and proteins in high-throughput screening and early clinical diagnosis.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental details and supplementary figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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