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Specific Detection of DNA Using Quantum Dots and Magnetic Beads for Large Volume Samples

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Abstract Here we present a sensitive DNA detection protocol using quantum dots (QDs) and magnetic beads (MBs) for large volume samples. In this study, QDs, conjugated with streptavidin, were used to produce fluorescent signals while magnetic beads (MBs) were used to isolate and concentrate the signals. The presence of target DNAs leads to the sandwich hybridization between the functionalized QDs, the target DNAs and the MBs. In fact, the QDs-MBs complex, which is bound using the target DNA, can be isolated and then concentrated. The binding of the QDs to the surface of the MBs was confirmed by confocal microscopy and Cd elemental analysis. It was found that the fluorescent intensity was proportional to concentration of the target DNA, while the presence of non-complementary DNA produced no significant fluorescent signal. In addition, the presence of low copies of target DNAs such as 0.5 pM in large volume samples up to 40 mL was successfully detected by using a magnet-assisted concentration protocol which consequently results in the enhancement of the sensitivity more than 100-fold.

Keywords: DNA detection, quantum dots, magnetic beads, magnet-assisted concentration

INTRODUCTION

Detection of specific oligonucleotides has become more important in medical diagnoses, especially in the areas of genetic diseases, the detection of infectious agents and forensic sciences. Hence, demand for the development of more rapid, cheaper, simpler, and highly sensitive DNA detection methods has also increased [1-3]. In general, DNA sequence detection systems are based on the specific hybridization of oligonucleotide probes to complementary DNA targets. Many methods have been reported in recent years, including those based upon optical assays (fluorescence tagging or nanoparticle-based), gravimetric assays (such as surface plasmon resonance and quartz crystal microbalance) and electrochemical or colorimetric assays [4-11]. Although the optical assay is one of more sensitive detection methods, its use is limited in studies, primarily due to its higher cost of operation.

The main focus of this study is the fluorescent detection of oligonucleotides using DNA functionalized QDs alongside a magnetic concentration. Quantum dots (QDs) are a new type of fluorescent marker offering several advantages compared to conventional fluorescent dyes [12,13]. First, conventional dyes generally have a narrow excitation and broad emission spectra with a relatively small Stokes shift, which means that the optimal excitation wavelength is close to the emission peak. In contrast, QDs are very efficient in that their absorption is broad and the emission is both narrow and independent of the excitation wavelength. Second, QDs have a negligible photobleaching and higher quantum yield compared with conventional dyes. Besides, the emission color is adjustable by varying the size of the QDs. Therefore multiplexed detection can be possible using multicolor QDs [14-17]. Owing to these special properties, many researchers are currently using QDs in various applications, such as biological imaging and analysis [18-20]. A recent modifying process has allowed researchers to functionalization the QDs with biomolecules, such as DNA, proteins and antibodies [6].

Magnetic separation is a rapid, simple and efficient method for the isolation of biological targets, such as DNA, protein, small biomolecules and cells, without the need for centrifugation, filtration or any other steps. Magnetic beads and nanoparticles are popularly used for the rapid detection of biomolecules [2,21-25]. The strategy employing magnetic beads in the detection of biomolecules is normally based on a "sandwich" binding between the probe conjugated magnetic beads, the target compounds and another probe that can produce an electrochemical or optical signal.

In this study, DNA functionalized quantum dots and magnetic beads were used to produce a fluorescence signal and isolate the QDs-MB complex, which is linked via a target DNA. By this method, the QDs-MB complex can be separated and concentrated very simply and rapidly

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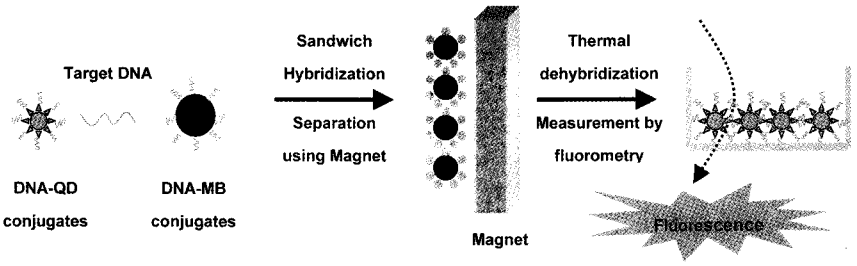

Fig. 1. Schematic showing the fluorescent DNA detection method using DNA functionalized Qdots and magnetic beads.

from solution containing unbound QDs. It was demonstrated that a magnet-assisted concentration protocol enhances the sensitivity of DNA detection for large volume samples.

MATERIALS AND METHODS

DNA Synthesis

All nucleotides were obtained from GenoTech Co. (Deajeon, Korea). The following DNA strands were synthesized for this experiment: target DNA, 5′-TTAGAGATGC-TTGGTATAAC TGCTCTTACT-3′; non-complementary DNA, 5′-ATGTTAGTTGCCATACTCTTAAGCGTAAAT-3′; QDs-probe, 5′-ACCAAGCATCTCTAA-A_5-biotin-3′; MB-probe, 5′-biotin-A_5-AGTA AGAGCAGTTAT-3′. The QD-probe and MB-probe are both complementary to the target DNA but on different portions. The synthesized DNA strands were dissolved in distilled water (18 mΩ, Millipore) to a final concentration of 100 μM.

Materials

CdSe-ZnS quantum dot streptavidin conjugates (30 nm in size) having a maximum emission wavelength of 525 nm (QD525 Streptavidin conjugates) were purchased from Quantum Dots Co. (Hayward, CA, USA). The stock concentration of the QD525 Streptavidin conjugates is 1 μM and they were dissolved in 50 mM borate buffer (pH 8.3). Superparamagnetic beads, modified with streptavidin on the surface (Dynabeads MyOne Streptavidin C_1, diameter 1 μm), were obtained from Dynal Biotech Inc. (Lake Success, NY, USA). Streptavidin modified magnetic beads (having an iron oxide core) were suspended in pH 7.4 phosphate buffered saline (PBS). They are hydrophilic, negatively-charged beads.

Functionalization of Quantum Dots and Magnetic Beads with DNA Probes

Immobilization of the biotinylated probes on the surface of the streptavidin coated QDs and MBs is achieved using the avidin-biotin interaction. Functionalization of the oligonucleotide (QDs-probes) to quantum dots conjugated with streptavidin was prepared according to manufacturer's protocol. At first, 20 μL of QD525 streptavidin (SA) conjugates (1 μM) were incubated with 10 μL of the biotinylated QDs-probes (10 μM) in 200 μL of borate buffer (50 mM, pH 8.3) for 1 h at room temperature. The molar ratio of the DNA probes/QDs was a 5:1. To confirm that the QDs-probes were immobilized on the surface of the QD525 SA conjugates, 5 μL of the reaction solution, along with the QD525 SA conjugates solution (100 nM) alone, were loaded onto a 0.5% agarose gel and electrophoresed for 20 min at 100 V.

The magnetic beads (MB) coated with streptavidin were functionalized with biotinylated ssDNA (MB-probes) using the protocol suggested by manufacturer. Before immobilization, 50 μL of the streptavidin modified magnetic beads (10 mg/mL, approx. 7~12 × 10^9 beads) were washed two times with 100 μL of 2 × Binding & Washing (B&W) buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 2.0 M NaCl) to remove any preservatives and then resuspended in 100 μL of 2 × B&W buffer. Next, 100 μL of the MB-probes (10 μM), in D.W., were mixed with the washed MBs suspension and incubated for 10 min at room temperature with gentle agitation. The magnetic beads, now coated with MB-probes, were separated from the weakly bound MB-probes and washed three times with 1 × B&W buffer using a magnet (Dynal MPC-S, Dynal Biotech Inc.). Finally, the DNA-probe functionalized magnetic beads were resuspended in hybridization buffer (1 × SSC (150 mM sodium chloride, 15 mM sodium citrate), 1% (w/v) SDS). To confirm that the MB-probes were immobilized on the surface of the magnetic beads, the amount of unbound MB-probe was measured using a NanoDrop ND1000 (NanoDrop Co., USA) after the isolation of the MB-probe functionalized MBs.

Fluorescence Detection

The procedure used to measure the fluorescence is shown in Fig. 1. The sample was prepared by mixing the DNA functionalized MBs (2 μL) with the ssDNA coated QD525 SA conjugates (20 μL) and the complementary target DNA (10 pM to 500 nM) in 400 μL of hybridization buffer. The hybridization was performed by gently mixing this solution at 30°C in the dark for 4 h. The sample was then washed three times with 400 μL of hybridization buffer to remove excess and weakly bound QD525 SA conjugates. Finally, after the solution was concentrated to a final volume of 100 μL, the sample was heated at 94°C for 5 min to detach QD525 SA conjugates from the magnetic beads. The fluorescence inten-

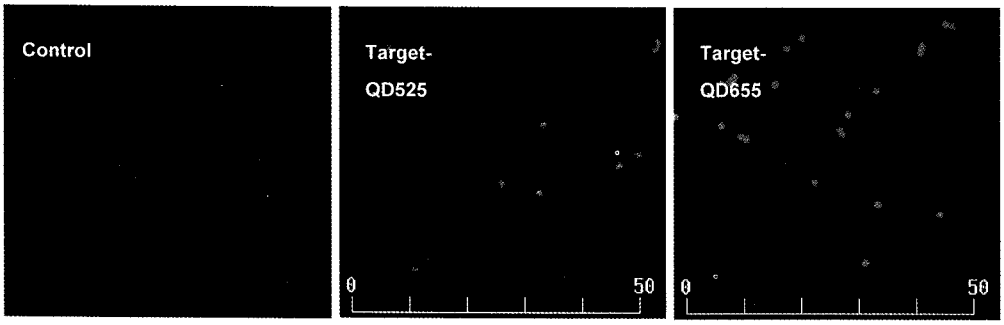

Fig. 2. Confocal microscopy images. (A) In absence of the target DNA, no fluorescent signal is seen since the QDs and MBs cannot bind. (B) Magnetic beads incubated with the target DNA and QD525 SA conjugates fluoresced green. (C) QD655 SA conjugates were tested according to same procedure. The scale bar is a 50 μm.

sity of the isolated QDs solution (100 μL) was measured with a fluorometer (VICTOR3, PerkinElmer Co., USA) using an excitation wavelength of 355 nm, emission filter S535 (bandwidth 25 nm), CW-lamp energy 10000 and measurement time of 3 sec. To test the effective concentration of QDs with a magnet in large volume sample, the same amount of functionalized QDs, MBs, and target DNA were incubated in 400 μL, 4 and 40 mL buffer solution, respectively, for overnight (16 h). The fluorescence was measured as explained in the above.

Confocal Microscopy and Elemental Analysis

To confirm that sandwich binding between QD525 SA conjugates and streptavidin modified MBs occurs via the target DNA, confocal microscopy was carried out. Samples were prepared according to the procedure described above. For confocal microscopy, 500 nM of the target oligonucleotides were incubated with the DNA functionalized QD525 SA conjugates and MBs. To compare the fluorescence color, samples using QD655 SA conjugates instead of QD525 SA conjugates were also prepared using the same procedure. After hybridization for 4 h, the QDs-coated MBs were separated from the solution and concentrated to 10 μL with a magnet. This solution was dropped on a slide glass and analyzed by confocal microcopy (MRC-1024, BioRad Lab.), which was performed using a 1,000 × magnification, 50% laser power and a 519 nm/680 nm emission filter set. In addition, conjugation of QDs and MBs via hybridization was further confirmed by an elemental analysis technique. The elemental analysis for cadmium (Cd), which is a major component of QDs, was performed by inductively coupled plasma – mass spectrometry (ICP-MS, Agilent 7100, USA) after QDs were detached from MBs and isolated.

RESULTS AND DISCUSSION

Functionalization of Quantum Dots and Magnetic Beads with ssDNA Probe

To confirm that the biotinylated DNA probes were attached to the surface of the QD525 SA conjugates, the QD-probes (10 μM) and QD525 SA conjugates (1 μM) were incubated together at a QDs/DNA probe molar ratio of 1:5 and 1:10. The fluorescence bands generated from each sample were then compared after electrophoresis in a 0.5% agarose gel. The DNA (20 mer) functionalized QD525 SA conjugates ran faster than the QD525 SA conjugates due to the negative charge of the immobilized DNA (QD-probes) (data not shown). Additionally, the fluorescence bands of the two samples (1:5 and 1:10 molar ratio of QDs/DNA probe) ran about the same distance, indicating that a similar number of DNA probes were immobilized on the QDs (data not shown). Therefore, a molar ratio of 1:5 was sufficient to saturate the QDs.

Likewise, the amount of MB-probe bound to the magnetic beads was measured after magnetic separation. About 80% of biotinylated DNA that was added was bound to the streptavidin modified MBs (data not shown). It was calculated that about 1.2×10^6 molecules of the MB-probe were immobilized on the surface of each streptavidin modified MB, based on the number of MBs added and DNA probes immobilized on MBs.

Binding of the Quantum Dots to the Magnetic Beads

Confocal microscopy was performed to confirm that the QDs were bound to the surfaces of the MBs in the presence of target DNA. The images are shown in Fig. 2. The 1 μm magnetic beads fluoresced green when coated with QD525 SA conjugates in the presence of target DNA (Fig. 2, second panel). However, when the magnetic beads were incubated with the same amount of DNA functionalized QD525 SA conjugates but without the target DNA, they didn't fluoresce (Fig. 2, first panel). These results clearly show that the QD525 SA conjugates require the target DNA in order for them to couple with the MBs. A similar test with the target DNA was performed using QD655 SA conjugates, which fluoresce red, and the results are shown in Panel 3 of Fig. 2.

In addition to confocal microscopy analysis, the binding of QDs to MBs was also confirmed by an elemental analysis. The content of Cd, 1,652.33 ± 78.15 ng/mL, was measured in the sample incubated with 500 nM of target DNA, while the amount of Cd in control sample

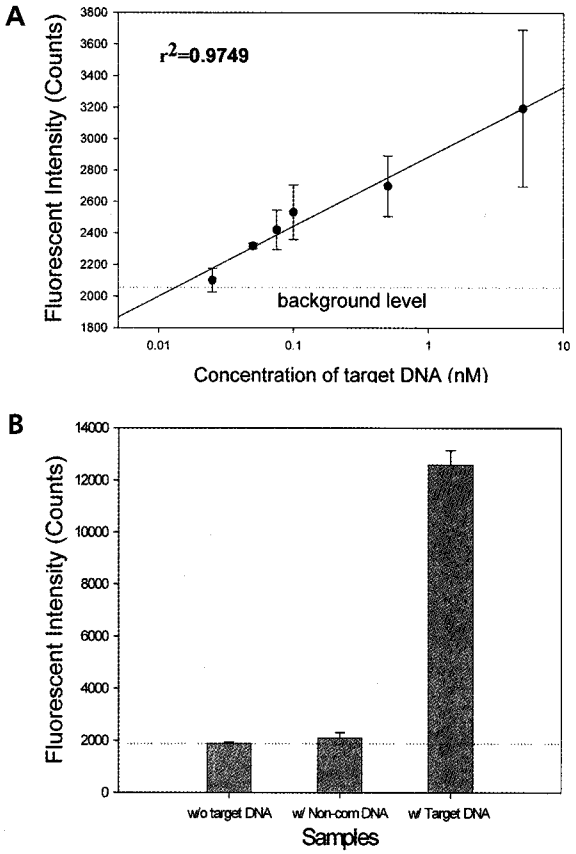

Fig. 3. (A) Fluorescence detection of the target DNA at 535 nm. The fluorescence intensity increased proportional to the concentration of the target DNA (r^2 = 0.9749). (B) Specificity test using non-complementary DNA. Clear fluorescent signals were seen from samples incubated with the target DNA, but not the non-complementary DNA.

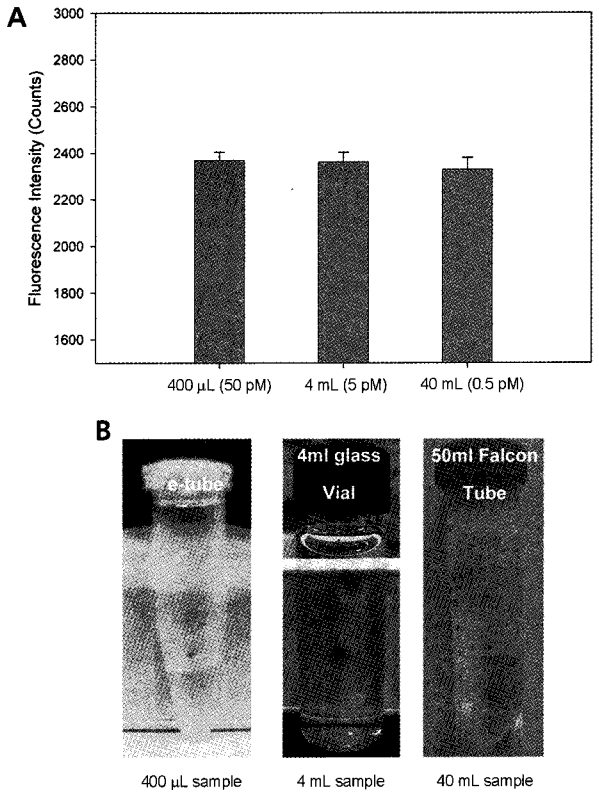

Fig. 4. Adaptation of a magnet-assisted concentration protocol to enhance the sensitivity. (A) The fluorescence values from the 0.4, 4, and 40 mL samples containing same amount of target DNA were nearly identical in error range. (B) Photographs showing how the magnet-assisted concentration protocol implemented for three different samples. The spot sizes showing QDs-MBs complex in three different cases seen to be different, but the total amount of QDs-MBs complex are almost same. The spot in 4 mL glass vial looks like larger than one in e-tube due to the refraction of buffer solution, when taken a photograph.

without target DNA was negligible as 23.78 ± 3.41 ng/mL. This data supports the result of confocal microscopy which QD525 SA conjugates (CdSe-ZnS core) is bound to MBs via target DNA.

Fluorescence Detection of Specific Sequenced Oligonucleotides

Previous studies found that an excess of MBs result in a high background and, thus, decease the signal-to-noise ratio [22,26]. Therefore, in this study, to avoid any influence the MBs may have on the fluorescence, the QD525 SA conjugates were detached from the QDs/target/MB complex before measuring the fluorescence. Detachment of the QDs was achieved by thermal dehybridization, *i.e.* 94°C for 5 min. Therefore, the fluorescent intensities of the QD525 SA conjugates were measured before and after heating to be certain that the fluorescence is stable. We found that there was no change when the QD525 SA conjugates were tested at low concentrations, while higher concentrations (more than 10 nM) showed a slight decrease of about 10~20%. Since sandwich hybridization requires only 5 nM of probe coated QDs, thermal dehybridization could be used in this study.

Next, the fluorescence intensity of the samples hybridized with varying amounts of target DNA was measured. As shown in Fig. 3A, the fluorescence intensity (counts) at a wavelength of 535 nm increased proportional to the concentration of the target DNA. Single each QD525 SA conjugates has, on average, five DNA probes (QD-probe) on its surface, the hypothetical binding ratio of the QD525 SA conjugates to the target DNA would be 1:5.

The specificity in our DNA detection system depends on the sequence of the target oligonucleotides, which should be complementary to both the DNA probes immobilized with QD525 SA conjugates and those on the magnetic beads. Therefore, non-complementary oligonucleotides were tested with QD525 SA conjugates to confirm that this detection system is specific for DNA sequence. As shown in Fig. 3B, the sample containing 500 nM of non-complementary oligonucleotides produced a fluorescent signal that was the same as the background level, while the fluorescence from the sample containing

500 nM target DNA was significantly higher. These results demonstrate that a significant fluorescent signal is seen only in the presence of the specific sequenced oligonucleotides.

To test if it is possible to enhance the sensitivity through the use of a magnet-assisted concentration protocol, three samples having different reaction volumes were tested. The same amount of target DNA and DNA functionalized QDs and MBs were incubated at room temperature overnight in different volumes of hybridization buffer (400 μL, 4 and 40 mL). Therefore, the target DNA concentration of each sample, respectively, is 50, 5, and 0.5 pM. In Fig. 4, the fluorescent values from the 4 and 40 mL samples were indistinguishable from those of the 400 μL sample, *i.e.*, the normal test condition. Therefore, it is clear that 0.5 pM target DNA could be detected when magnetic concentration is used. This could be further improved upon through an optimization of the hybridization conditions and the use of a more powerful magnet. Therefore, this study shows that even if a small amount of target DNA exists in a large sample volume, it can be detected through the use of this coupled quantum dot and magnet-assisted concentration protocol. In addition, even if not tested in this study yet, it is evident that this method can be expanded for the simultaneous detection of various DNA sequences when multi-colored QD SA conjugates are used.

CONCLUSION

This study presents an implementation concept for the fluorescent detection of specifically sequenced oligonucleotides using DNA functionalized QDs and MBs. This QD-MB based fluorescence detection method was specific for only select DNA sequences and nonresponsive to non-complementary sequences. The sensitivity of this method was increased to 0.5 pM by adopting a magnet-assisted concentration protocol, which would be extremely helpful in situations where a large sample volume is being tested. The sensitivity of this method might be further enhanced by using a stronger magnet and/or optimized hybridization time and conditions.

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REFERENCES

[1] Taton, T. A., C. A. Mirkin, and R. L. Letsinger (2000) Scanometric DNA array detection with nanoparticle probes. *Science* 289: 1757-1760.

[2] Wang, J., G. D. Liu, and A. Merkoci (2003) Electrochemical coding technology for simultaneous detection of multiple DNA targets. *J. Am. Chem. Soc.* 125: 3214-3215.

[3] Yoo, S. M., K. C. Keum, S. Y. Yoo, J. Y. Choi, K. H. Chang, N. C. Yoo, W. M. Yoo, J. M. Kim, D. Lee, and S. Y. Lee (2004) Development of DNA microarray for pathogen detection. *Biotechnol. Bioprocess Eng.* 9: 93-99.

[4] Li, H. X. and L. Rothberg (2004) Colorimetric detection of DNA sequences based on electrostatic interactions with unmodified gold nanoparticles. *Proc. Natl. Acad. Sci. USA* 101: 14036-14039.

[5] Guedon, P., T. Livache, F. Martin, F. Lesbre, A. Roget, G. Bidan, and Y. Levy (2000) Characterization and optimization of a real-time, parallel, label-free, polypyrrole-based DNA sensor by surface plasmon resonance imaging. *Anal. Chem.* 72: 6003-6009.

[6] Gerion, D., F. Q. Chen, B. Kannan, A. H. Fu, W. J. Parak, D. J. Chen, A. Majumdar, and A. P. Alivisatos (2003) Room-temperature single-nucleotide polymorphism and multiallele DNA detection using fluorescent nanocrystals and microarrays. *Anal. Chem.* 75: 4766-4772.

[7] Drummond, T. G., M. G. Hill, and J. K. Barton (2003) Electrochemical DNA sensors. *Nat. Biotechnol.* 21: 1192-1199.

[8] Wang, J., D. K. Xu, A. N. Kawde, and R. Polsky (2001) Metal nanoparticle-based electrochemical stripping potentiometric detection of DNA hybridization. *Anal. Chem.* 73: 5576-5581.

[9] Wang, J. (2003) Nanoparticle-based electrochemical DNA detection. *Anal. Chim. Acta* 500: 247-257.

[10] Park, J. W., H. S. Jung, H. Y. Lee, and T. Kawai (2005) Electrical recognition of label-free oligonucleotides upon streptavidin-modified electrode surfaces. *Biotechnol. Bioprocess Eng.* 10: 505-509.

[11] Yoon, H. C. and H. S. Kim (2004) Bioelectrocatalyzed signal amplification for affinity interactions at chemically modified electrodes. *Biotechnol. Bioprocess Eng.* 9: 107-111.

[12] Bruchez, M., M. Moronne, P. Gin, S. Weiss, and A. P. Alivisatos (1998) Semiconductor nanocrystals as fluorescent biological labels. *Science* 281: 2013-2016.

[13] Alivisatos, A. P. (1996) Semiconductor clusters, nanocrystals, and quantum dots. *Science* 271: 933-937.

[14] Bailey, R. E. and S. M. Nie (2003) Alloyed semiconductor quantum dots: Tuning the optical properties without changing the particle size. *J. Am. Chem. Soc.* 125: 7100-7106.

[15] Li, Y. G., Y. T. H. Cu, and D. Luo (2005) Multiplexed detection of pathogen DNA with DNA-based fluorescence nanobarcodes. *Nat. Biotechnol.* 23: 885-889.

[16] Gao, X. H., W. C. W. Chan, and S. M. Nie (2002) Quantum-dot nanocrystals for ultrasensitive biological labeling and multicolor optical encoding. *J. Biomed. Opt.* 7: 532-537.

[17] Ho, Y. P., M. C. Kung, S. Yang, and T. H. Wang (2005) Multiplexed hybridization detection with multicolor colocalization of quantum dot nanoprobes. *Nano Lett.* 5: 1693-1697.

[18] Chan, W. C. W. and S. M. Nie (1998) Quantum dot bioconjugates for ultrasensitive nonisotopic detection. *Science* 281: 2016-2018.

[19] Alivisatos, P. (2004) The use of nanocrystals in biological detection. *Nat. Biotechnol.* 22: 47-52.

[20] Michalet, X., F. F. Pinaud, L. A. Bentolila, J. M. Tsay, S. Doose, J. J. Li, G. Sundaresan, A. M. Wu, S. S. Gambhir, and S. Weiss (2005) Quantum dots for live cells, *in vivo*

imaging, and diagnostics. *Science* 307: 538-544.
[21] Pumera, M., M. T. Castaneda, M. I. Pividori, R. Eritja, A. Merkoci, and S. Alegret (2005) Magnetically trigged direct electrochemical detection of DNA hybridization using Au-67 quantum dot as electrical tracer. *Langmuir* 21: 9625-9629.
[22] Su, X. L. and Y. B. Li (2004) Quantum dot biolabeling coupled with immunomagnetic separation for detection of *Escherichia coli* O157:H7. *Anal. Chem.* 76: 4806-4810.
[23] Sun, X. L., W. X. Cui, C. Haller, and E. L. Chaikof (2004) Site-specific multivalent carbohydrate labeling of quantum dots and magnetic beads. *Chembiochem* 5: 1593-1596.
[24] Nam, J. M., C. S. Thaxton, and C. A. Mirkin (2003) Nanoparticle-based bio-bar codes for the ultrasensitive detection of proteins. *Science* 301: 1884-1886.
[25] Patolsky, F., Y. Weizmann, E. Katz, and I. Willner (2003) Magnetically amplified DNA assays (MADA): Sensing of viral DNA and single-base mismatches by using nucleic acid modified magnetic particles. *Angew. Chem. Int. Ed. Engl.* 42: 2372-2376.
[26] Yang, L. J. and Y. B. Li (2005) Quantum dots as fluorescent labels for quantitative detection of *Salmonella* typhimurium in chicken carcass wash water. *J. Food Protect.* 68: 1241-1245.

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