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Self-Assembly of Quantum Dots and Carbon Nanotubes for Ultrasensitive DNA and Antigen Detection

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A highly selective, ultrasensitive, fluorescence detection method for DNA and antigen based on self-assembly of multiwalled carbon nanotubes (CNTs) and CdSe quantum dots (QDs) via oligonucleotide hybridization is reported. Mercaptoalkyloligonucleotide molecules bind to the quantum dots, while amineoalkyloligonucleotides bind to CNTs with −COCl surface groups. QDs and CNTs further assemble into nanohybrids through DNA hybridization in the presence of target complementary oligonucleotides. The method is achieved with good repeatability with the detection limit of 0.2 pM DNA molecules and 0.01 nM antigen molecules. This novel detection system can also be used for multicomponent detection and antigen−antibody immunoreaction. The novel system has great potential in applications such as ultrasensitive pathogen DNA or antigen or antibody detection, molecular imaging, and photoelectrical biosensors.

Carbon nanotubes, because of unique mechanical, physical and chemical properties, have great potential applications in various fields, including molecular electronics, medical chemistry, and biomedical engineering.[1−3] Carbon nanotubes (CNTs) can be functionalized to achieve improved properties and functions, such as good biocompatibility and biomolecular recognition capabilities.[4−7] Semiconductor nanomaterials have generated great interest in the past two decades. The properties and applications of fluorescent semiconductor nanocrystals stand among the most exciting research fields in chemistry, physics, and biology.[8−10] Semiconductor nanocrystals or quantum dots (QDs) exhibit interesting size-tunable optical properties due to the confinement of their electronic wave functions.[11−14] A QD−biomolecule assembly constructed using bionanotechnology may facilitate development of a novel class of hybrid sensing materials.[15,16] Thus far, a metal nanoparticle system, which exclusively involves oligonucleotide-functionalized nanoparticles as the building blocks, has been extensively studied.[17,18] The system has already increased our understanding of particle−particle interactions on the nanometer scale and has led to the development of a new and highly selective colorimetric detection technology for oligonucleotides.[19,20] Carbon nanotubes can bind with QDs[21] and cause a change in the photoluminescence properties of quantum dots,[22] and QD-activated luminescent carbon nanotubes have been explored for in vivo imaging.[23]

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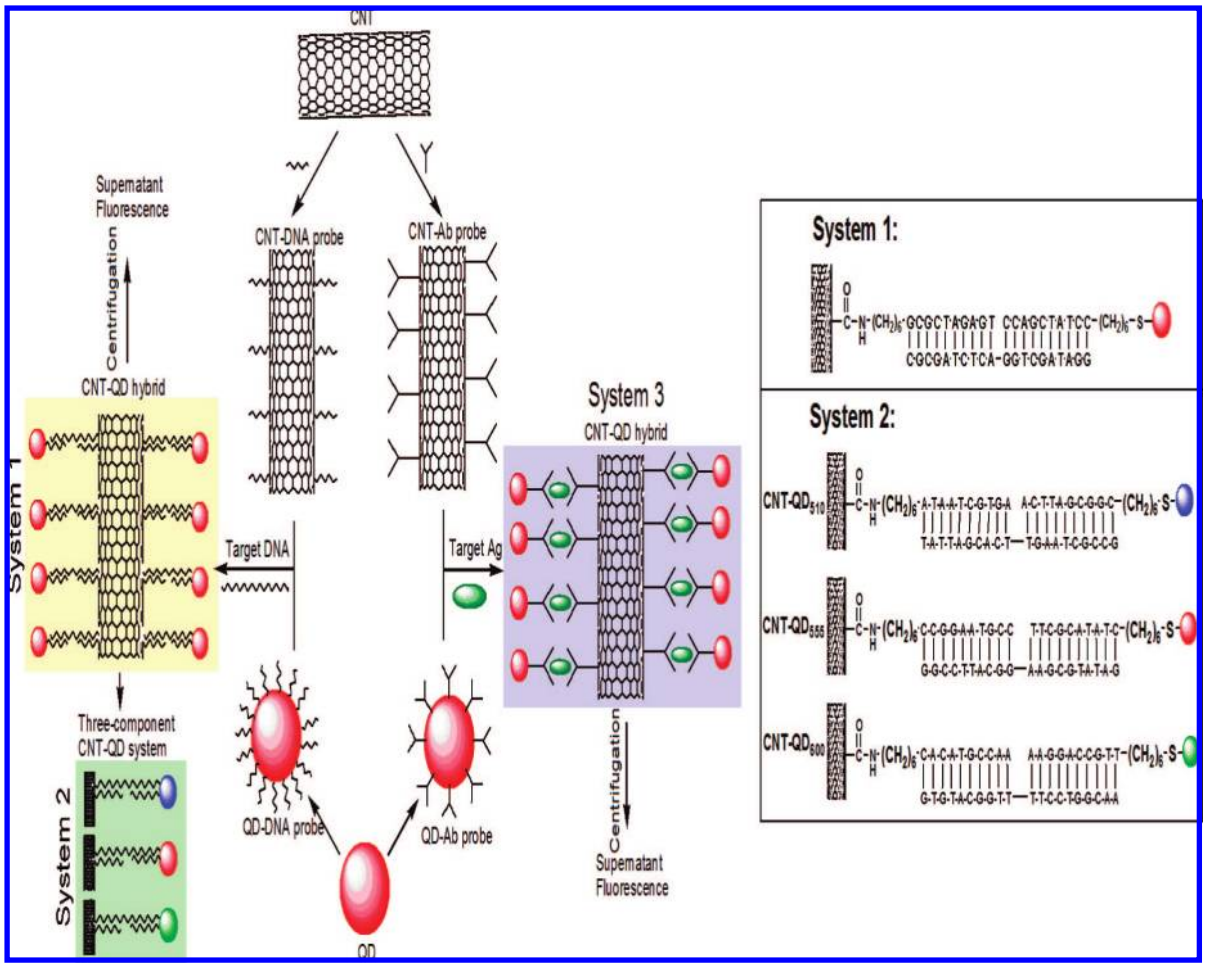
Scheme 1. Surface Functionalization of CNT (or QD) with Oligonucleotide/Antibody (Ab), Forming aCNT−DNA (or −Ab) Probe and QD−DNA (or −Ab) Probe, and Subsequent Addition of Target Oligonucleotide (or Antigen) to Form a CNT−QD Assembly[a]



[a] The unbound QD probe was obtained by simple centrifugation separation, and the supernatant fluorescence intensity of QDs was monitored by spectrofluorometer. (System 1) Formation of CNT−QD hybrid in the presence of complementary DNA target. (System 2) Three-component CNT−QD system with the purpose to detect three different DNA targets simultaneously. (System 3) CNT−QD protein detection system based on antigen−antibody immunoreaction.

Here, we reporte a novel ultrasensitive DNA or antigen detection strategy based on the CNT−QD assembly shown in Scheme 1. Multiwalled CNTs and QDs, their surfaces functionalized with oligonucleotide DNA or antibody (Ab), can be assembled into nanohybrid structure upon the addition of a target complementary oligonucleotide or antigen (Ag), as shown in Scheme 1. Nanomaterial building blocks that vary in chemical composition, size, or shape are arranged in space on the basis of their interactions with complementary linking oligonucleotides for potential application in biosensors.[24–27] We show how this oligonucleotide-directed assembly strategy could be used to prepare binary (two-component) assembly materials comprising two differently shaped oligonucleotide-functionalized nanomaterials. Importantly, the proof-of-concept demonstrations reported herein suggest that this strategy could be extended easily to a wide variety of multicomponent systems.

EXPERIMENTAL SECTION

Materials. Multiwalled carbon nanotubes were obtained from Carbon Nanotechnologies, Inc. (CAS no. 7782-42-5, purity 99.9%), and their lengths are controlled within 100 nm by a filtration method. The CdSe QDs were synthesized as previously described.[8,9] Thionyl chloride ($SOCl_2$) and alkyloligonucleotides were obtained from Sigma Company. Anti-BRCAA1 polyclonal antibodies were purchased from American Alpha Genix Company. BRCAA1 (breast cancer associated antigen 1, AF208045) peptides were prepared and kept in our laboratory.[28,29] The preparation of CNT−DNA, QD−DNA probes, CNTs coated with anti-BRCAA1 polyclonal antibodies (CNTs−Ab), and QDs coated with anti-BRCAA1 polyclonal antibody (QDs−Ab) are described in detail in the Supporting Information.

Assembly and Characterization of CNTs and QDs through DNA Hybridization (Scheme 1, System 1). The methodology of DNA detection by fluorescence measurement is shown in System 1. Two microliters of target oligonucleotide with different concentrations were added into the mixture of CNT−DNA (50 μL, [CNT] = 1 mg/mL, [DNA] = 0.1 nM) and QD−DNA probes (50 μL, [DNA] = 0.1 nM). This was mixed and incubated at 75

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°C for 5 min and then at 25 °C for 20 min. After hybridization with target DNA, CNT−QD assemblies were formed and then removed by centrifugation at 2000 rpm for 5 min. The unbound QD−DNA probes in supernatant PBS buffer were immediately used to carry out fluorescence measurement with a Perkin-Elmer LS-55 spectrofluorometer. The wavelength $\lambda = 480$ nm of the laser source was used for the excitation of the CNT−QD detection system. The fluorescence signal was recorded over a range from $\lambda = 450$ nm to $\lambda = 700$ nm. NoncDNA target (CGC GAT CTC AGG TCG ATA GG) was used as the control experiment. TEM images, AFM images, UV−vis spectroscopy, and Rahman spectroscopy are given in the Supporting Information.

Three-Component CNT−QD System with the Purpose of Detecting Three Different DNA Targets Simultaneously (Scheme 1, System 2). Three QDs with different emission wavelengths at 510, 555, and 600 nm were used to simultaneously detect three target DNA molecules, as shown in system 2, called QD_{510}, QD_{555}, and QD_{600} probes, respectively. There are six probes in this system: three CNT−DNA probes (50 μL of each, [CNT] = 1 mg/mL and [DNA] = 0.1 nM) and 3 QD−DNA probes (50 μL of each, [DNA] = 0.1 nM) (as shown in Scheme1, System 2). A mix of the six probes formed a uniform solution. Three cDNA targets were used in this system. Two microliters of each DNA target with different concentration was added into the six-probe mixture, individually. NoncDNA target (CGC GAT CTC AGG TCG ATA GG) was used as the control experiment. In addition, we mixed the three DNA targets (2 μL of each) to form a 6 μL DNA solution containing three different DNA targets, and then incubated the three DNA targets with the six-probe mixture simultaneously. The mixture solution containing probes and targets was incubated at 75 °C for 5 min and then at 25 °C for 20 min. After hybridization with target DNA molecules, CNT−QD assemblies were formed and then removed by centrifugation at 2000 rpm for 5 min. The unbound QD−DNA probes in supernatant PBS buffer were used immediately to carry out fluorescence measurement with a Perkin-Elmer LS-55 spectrofluorometer.

CNT−QD System for Antigen Detection via Antigen−antibody Immunoreaction (Scheme 1, System 3). To confirm that the CNT−QD system can be used for antigen detection, a novel CNT−QD immunoassay system based on antigen−antibody immunoreaction was established. BRCAA1 protein was chosen as a typical example.[26,29] CNT−Ab and QD−Ab probes were prepared as shown in the Supporting Information. The immunoreaction procedure is described as follows: 50 μL of CNT−Ab probe ([CNT] = 1.0 mg/mL, [Ab] = 1.0 nM) was reacted with 10 μL of BRCAA1 antigen (concentration from 0 to 1.0 nM) for 30 min, then 50 μL of QD−Ab probe (1.0 nM) was added, and the mixture was incubated for 2 h at room temperature. After the immunoreaction, the sandwich-type immunocomplex was formed on the surface of CNT probes. The unbound QDs−pAb was obtained by simple centrifugation separation and the supernatant fluorescence intensity of QDs was monitored by spectrofluorometer.

RESULTS AND DISCUSSION

CNT−QD Assembly System for DNA Target Detection (System 1). The supernatant fluorescence spectra in Figure 1 show that the QD−DNA probe has strong fluorescence, and the maximum fluorescence wavelength for CdSe was at ~570 nm. The

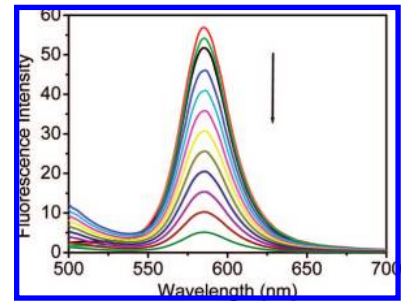

Figure 1. Fluorescence intensity of supernatant after adding target 1 with different concentrations (from top to bottom): 0, 10, 16, 25, 40, 50, 60, 75, 90, 120, 150, and 200 pM.

cDNA target (sequence shown in Scheme 1, system 1) with concentration ranges of 0−200 pM was added to the mixture of CNT−DNA and QD−DNA probes to form CNT−QD nanohybrids. After centrifugation at 2000 rpm for 5 min, the CNT−QD nanohybrids were removed; therefore, only unbounded QD−DNA existed in the supernatant buffer. The decrease in the fluorescence intensity was the most marked change in the fluorescence spectrum observed upon addition of the cDNA target.

Control experiments were carried out by adding cDNA target to the QD−DNA solution in the absence of the CNT−DNA probe (Figure 2A). In addition, buffer solutions with pH ranging from 4.0 to 11.0 were used to investigate the pH effect on the fluorescence properties of the QD−DNA probe (Figure 2B). As shown in Figure 2A and B, little PL change can be observed for QD−DNA probes after adding complementary target DNA solutions without CNT−DNA probe at pH's from 4.0 to 11.0, which highly suggests that the QD fluorescence intensity was stable in the presence of oligonucleotides at different pH's. From the plot of fluorescence intensity versus DNA concentration (Figure 2D), their increasing concentrations caused a linear reduction in the fluorescence intensity of QDs at the target 1 concentration range of 0−200 pM. Furthermore, noncDNA target was used as a control, and after addition of noncDNA target, little fluorescence change can be observed (Figure 2C), indicating no CNT−QD assembly can be formed in the presence of noncDNA (sequence shown in the Experimental Section). In this experiment, the available DNA detection range is from 0 to 200 pM, and according to the measurement results on the samples of gradually diluted DNA targets, the available good repeatability limit of detection is as low as ~0.2 pM (Figure 2D).

Simultaneous Three DNA Target Detection Based on CNT−QD Assembly System (System 2). Three QDs with different emission wavelengths at 510, 555, and 600 nm were used as probes to detect three target DNA molecules simultaneously (called QD_{510}, QD_{555}, and QD_{600} probes). Their spectrally resolved fluorescence signal is displayed in Figure 3A. As can be seen, the fluorescence signal can be split into three bands with emission wavelengths of 510 (QD_{510}), 555 (QD_{555}), and 600 nm (QD_{600}). Plotting the fluorescence intensities for the three wavelengths, that is, $\lambda = 510$, $\lambda = 555$, and $\lambda = 600$ nm, respectively, against the concentration of DNA targets results in the linear decrease of fluorescence intensity given in Figure 3B−F. In Figure 3B, we used a noncDNA target (sequence shown in the Experimental Section) as control experiment, no fluorescence changes of the supernatant after incubation of noncDNA can be observed,

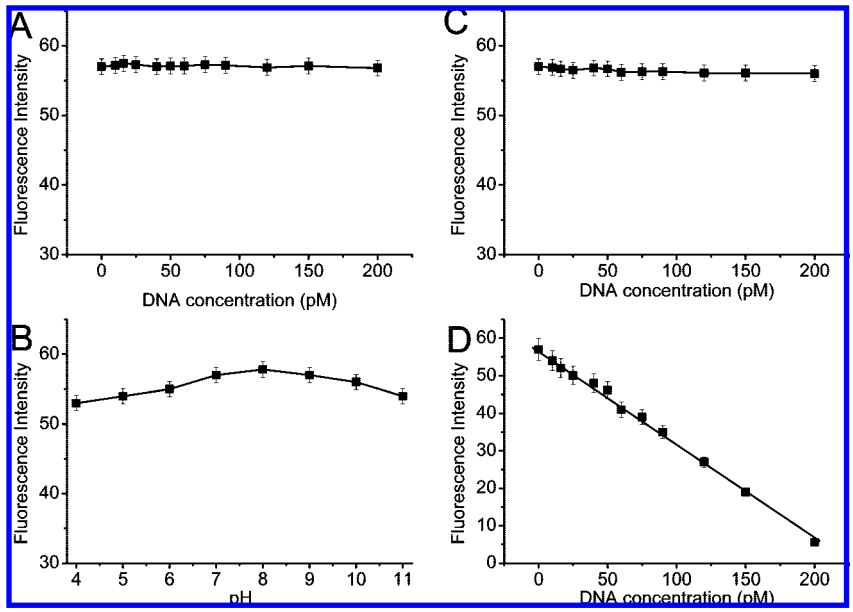

Figure 2. Plot of supernatant fluorescence intensity versus DNA concentration: (A) effect of cDNA target concentration on fluorescence intensity of QD−DNA probe; (B) fluorescence of QD−DNA probe at different pH's from 4.0 to 11.0; (C) noncDNA target with concentration from 0 to 200 pM incubated with CNT−DNA and QD−DNA probes (no fluorescence changes in supernatant); and (D) marked fluorescence decrease found for CNT−DNA/QD−DNA mixture solution after the incubation with cDNA target.

indicating no CNT−QD hybrid formation in this system. By adding three cDNA targets all together to the CNT−DNA and QD−DNA probe solution (six-probe solution), we found a fluorescence decrease at the three wavelengths (510, 555, and 600 nm; Figure 3C), indicating CNT−QD hybrids formed in all the probes corresponding to three different wavelengths. In Figure 3D, only one cDNA target (sequence shown in Scheme 1, system 2, CNT−QD_{510}) was incubated with the six-probe solution, and we found fluorescence decreased only at 510 nm, and no fluorescence changes were found at 555 and 600 nm, indicating there is only CNT−QD_{510} hybrid formed, but no CNT−QD_{555} and CNT−QD_{600} hybrids. Similar results can be seen from Figure 3E and F. Only CNT−QD_{555} and CNT−QD_{600} hybrids were formed upon the addition of only corresponding cDNA target, respectively. Briefly, we demonstrated the CNT−QD system can be used as simultaneous multicomponent detection system with detection limits of ~0.2 pM for each DNA target.

From Figure 3C−F and Figure 2D, we noticed that fluorescence intensity is 60 at [DNA] = 0, but on the other hand, fluorescence intensity was 0 at [DNA] = 225 pM. Although the fluorescence intensity = 0, the amount of target DNA is 2 μL × 225 pM = 4.5×10^{-16} mol. In the CNT−DNA probe and QD−DNA solution, [DNA] = 1.0 nM and volume = 50 μL, so the DNA amount in the CNT−DNA probe or QD−DNA probe solution is 50 μL × 0.1 nM = 5×10^{-15} mol. From Figure 2D, if we assumed all QD particles were binding to the CNT surface at the point of fluorescence intensity = 0, we concluded that the QD number should be equal to the DNA amount (i.e., 4.5×10^{-16}), so the number of DNA molecules on each QD particle is: 5×10^{-15} mol/4.5×10^{-16} = 11. That is to say, there are 11 DNA molecules on each QD, and only 1 DNA molecule performed DNA hybridization to produce the CNT−QD hybrid. But we could not calculate the number of DNA probes on each CNT, since the lengths of the CNT particles were not known.

According to Figure 2D and Figure 3C−F, the QD fluorescence intensity (F) scales with the DNA concentration [DNA] (pM) through the following:

$$F = -26.7[\mathrm{DNA}] + 60 \quad (1)$$

CNT−QD Assembly System for Antigen Detection via Antigen−antibody (Ag−Ab) Immunoreaction (System 3). The purpose of the CNT−QD assembly system is to ultrasensitively detect target biomolecules, trying to confirm that the CNT−QD ultrasensitive detection system can be used not only for a cDNA target, but also for an antigen−antibody system. The formation of the CNT−QD hybrid via immunoreaction of an antigen with CNT−Ab and QD−Ab probes can be demonstrated from the fluorescence decrease of unbound QD fluorescence, as shown in Figure 4. Similar to system 1, we used the QD particles with fluorescence emission wavelength at 570 nm. Upon incubating with target BRCAA1 antigen, CNT−QD hybrid was removed by a simple centrifugation step, and supernatant QD fluorescence intensity decreased linearly at an antigen concentration ranging from 0 to 1.0 nM (Figure 4a). By measuring the gradually diluted samples, we found that we can obtain good repeatability results within the concentration scope of more than ~0.01 nM, which is also located within the scope of linearity between the QDs fluorescence and BRCAA1 concentration. For the control experiment, in the absence of the target antigen, no fluorescence decrease was observed from Figure 4b.

From Figure 4, we noticed that the fluorescence intensity is 0 at the point of the target [Ag] = 1.1 nM; that is,the amount of antigen is 1.1 nM × 10 μL = 1.1×10^{-14} mol. In CNT−Ab and QD−Ab probes, the antibody amount is 1.0 nM × 50 μL = 5×10^{-14} mol. The antibody number on each QD particle is (5×10^{-14} mol)/(1.1×10^{-14} mol) = 4.5, so we concluded that 4.5 antibody molecules are on each QD particle, and only 1 antibody on QD bound to the antigen to form the CNT−QD hybrid.

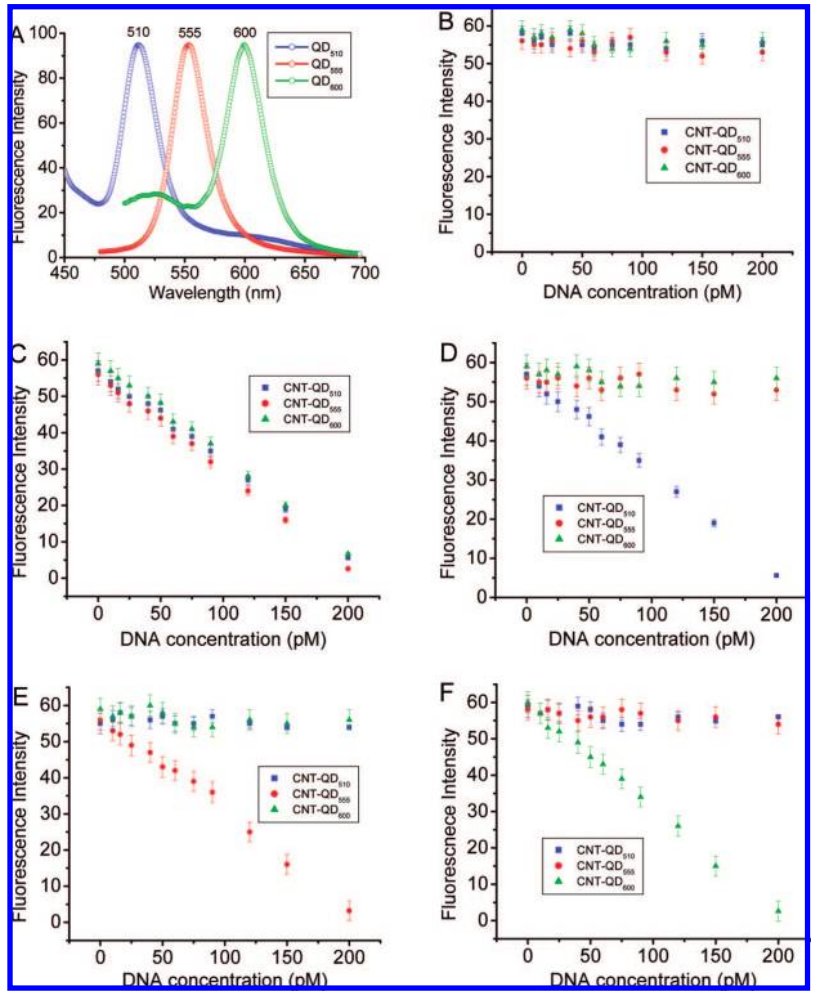

Figure 3. Sensitivity and linearity analysis of the three-component CNT−QD DNA detection system by plotting fluorescence intensity against target DNA concentration. (A) QD probes with three different fluorescence wavelengths at 510, 555, and 600 nm; (B) plotting of supernatant fluorescence intensity against DNA concentration upon incubation of noncDNA target with CNT−DNA and QD−DNA probes; (C) fluorescence decreased at the three peaks 510, 555, and 600 nm upon the adding three cDNA targets to the six-probe solution; (D) only the 510 nm band decreased by adding the only DNA target corresponding to CNT−QD_{510} system; and (E, F) fluorescence decreases at 555 and 600 when cDNA corresponding to CNT−QD_{555} and CNT−QD_{600} system, respectively, is added.

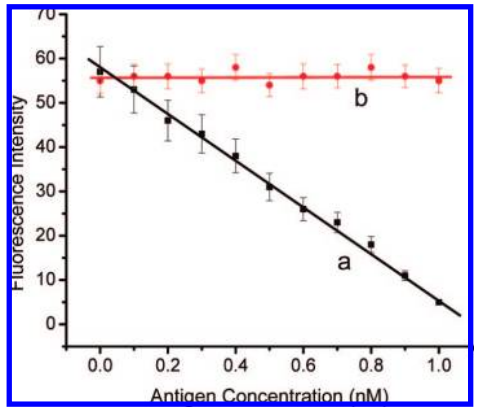

Figure 4. CNT−QD assembly system for antigen detection. (a) Linear plot of fluorescence intensity in supernatant against antigen concentration and (b) control experiment were conducted in the absence target antigen.

According to Figure 4, the QD fluorescence intensity (F) scales with the antigen concentration [Ag] (nM) through the following:

$$F = -51.8[\mathrm{Ag}] + 57 \qquad (2)$$

As a comparison, the ELISA detection method for BRCAA1 antigen was performed according to the testing procedure for antigen−antibody interaction. The novel CNT−QD hybrids method reported herein was compared with ELISA.[28−30] BRCAA1 antigen samples were especially selected to be used for the immunoassay using the ELISA methods, and the result was ~0.5 nM. In the case of protein detection, BRCAA1 antigen with a concentration <0.5 nM cannot be detected by using the ELISA methods. In the CNT−QD method, BRCAA1 antigen can be detected with good repeatability at ~0.1 nM below the detection limit. The results suggest the excellent CNT−QD method has a higher sensitivity than ELISA. The protein detection limit is ~0.1 ppm by using a Dot−Blot fluorescent staining method.[31] In the case of oligonucleotide detection, the detection limit of the colorimetric

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polynucleotide detection method[32] is the same as that of our method. Bio-Barcodes assays have been studied and work comparably well over the 20−700 nM target concentration range.[33] Therefore, quantification and detection of DNA/protein can be performed with higher accuracy and sensitivity.

Regarding the potential mechanism, the CNT probe and QD probe can form the nanocomposites under the existence of the complementary target oligonucleotides; the distance between CNT and QD highly depends on the length of the oligonucleotides, as observed in experiments; CNTs can quench the fluorescence signal of QDs, as we reported in previous paper;[22] and the dynamic quenching and photoluminescence resonance energy transfer between CNTs and QDs should be responsible for the phenomena. When the CNT−QD method is used to detect the DNA or antigen molecules, the ratio of the CNT and QD probes is very important. According to our experience, 1:1 is suitable for almost all detected samples. When the sample concentration is lower than the concentration of the CNT and QD probes, part CNT probe and QD probe will be redundant because the distance between uncomplementary CNT probe and QD probe is far more than 10 nm. Therefore, the quenching degree of the QD probe caused by the CNT probe is much less, and the quenching degree can be detected as a control group. By measuring these gradually diluted samples with known concentration, the dose−effects standard curve can be set up before the samples with unknown concentration are detected. Therefore, the redundant CNT probes do not affect the final result of the detected samples.

Regarding the specificity and efficiency of the CNT−QD method, as is known, oligonucleotide hybridization has been broadly used for genetic diagnosis, chip detection, and so on. Its specificity and hybridization efficiency have been confirmed. Therefore, in this work, we did not focus on investigating the specificity and efficiency of the CNT−QD method. Our further work will evaluate its specificity and dynamic efficiency based on one-, two-, and three-base-pair mismatch probes and various temperature conditions as well as large quantities of background genomic and oligonucleotide existence as nonselective matrix effects.

Regarding the stability of the CNT and QD probes, asODN-modified CNTs can enhance markedly the water solubility and dispersability of CNTs as reported.[34] We have also observed that the CNT probe is very stable at room temperature in PBS buffer for several months. The asODN-modified CdTe QDs are also water-soluble and very stable. Their photoluminescence intensities do not change at room temperature and in a dark environment for almost 2 years, and are almost not affected by different pH values, as reported.[22] Conversely, the photoluminescence intensity of unmodified QDs can be affected seriously by different pH values.

In conclusion, we have developed a novel, efficient DNA/protein detection method for biomolecules, such as oligonucleotide and antigen, that is based on DNA hybridization and antibody−antigen immunoreaction by using asODN-labeled CNT and QD as the molecular probes. The CNT and QD probes functionalized with alkyloligonucleotide can be assembled into a nanohybrid structure upon the addition of a target oligonucleotide. This strategy based on oligonucleotide hybridization assembly can be used to prepare multicomponent assembly materials comprising differently shaped oligonucleotide-functionalized nanomaterials. We have also developed a promising nanoscale CNT probe and QD probe for the direct, rapid, inexpensive, and sensitive detection and quantification of DNA/protein. Our probe combines the DNA hybridization and antigen−antibody interaction and is versatile and capable of simultaneous processing of multiple samples. The established method has great potential in applications such as ultrasensitive pathogen DNA or antigen or antibody detection, molecular imaging, and photoelectrical biosensors.

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SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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