Electronic Supplementary Information

An efficient phosphorescence energy transfer between quantum dots and carbon nanotubes for ultrasensitive turn-on detection of DNA

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EXPERIMENTAL SECTION

Apparatus. Phosphorescence spectra were recorded on an LS-55 fluorescence spectrophotometer (PerkinElmer company, USA) equipped with a quartz cell (1cm×1cm) in the phosphorescence mode. Ultraviolet-visible absorption spectra were measured on UV-3010 spectrophotometer (Hitachi, Japan). X-ray powder diffraction (XRD) patterns were obtained from a Japan Rigaku DMax-γA rotation anode X-ray diffractometer equipped with graphite monochromatized Cu-K radiation (λ=1.54178 Å). High-resolution transmission electron microscope (HRTEM) observations for the morphological measurements of Mn-ZnS QDs were performed on JEOL-2010F with an acceleration voltage of 200 KV. Energy dispersive spectrometer (EDS) was carried out on Inca Oxford equipped on JEOL-2010F. Decay curves measurements were performed with the time correlated single photo counting technique on the combined steady state and lifetime spectrometer (Edinburgh Analytical Instruments, FLS920). pHS-3C pH meter was used for pH measurement (Shanghai, China). Electrochemical measurements were performed on CHI 760D electrochemical workstation (CHI, Shanghai) with a conventional three-electrode, single compartment electrochemical cell which consists of a glass carbon electrode or composite-modified electrode as working electrode, an Ag/AgCl (in 3 M KCl) as reference electrode, and a platinum wire as the counter electrode.

Materials. All chemicals were of analytical grade or better and were used as received without further purification. $ZnSO_4 \cdot 7H_2O$, $Na_2S \cdot 9H_2O$, $MnCl_2 \cdot 4H_2O$, 1-ethyl-3-(3-dimethyllaminopropyl) carbodiimide hydrochloride (EDC·HCl), 3-mercaptopropionic acid (MPA), N-hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich. CNTs (single-walled carbon nanotubes, diameter≤5 nm, lengths 5-15 μm) were purchased from Sangon (Shanghai, China) and oxidized before use. DNA oligonucleotides with a concentration of 100 μM were purchased from Sangon (Shanghai, China), and the sequences are listed as follows:

Capture ssDNA: 5′-NH_2-TGC ATT ACT AAT CAG TGA GGC CTT-3′

Target ssDNA(perfect match with capture ssDNA): 5′-AAG GCC TCA CTG ATT AGT AAT GCA-3′

Single-base mismatch ssDNA: 5′-AAG GCC TCA C**A**G ATT AGT AAT GCA-3′

Triplex distilled water (Mill-Q, Millipore, 18.2 MΩ) resistivity was used throughout the experiments.

Synthesis of Mercaptopropionic Acid-Capped Mn-Doped ZnS QDs (MPA-QDs). In order to label Mn-Doped ZnS QDs with DNA, MPA capped Mn-doped ZnS QDs was synthesized in aqueous solution according to previous report with minor modification.[1,2] Briefly, 0.01 mol $ZnSO_4$, 0.4 mmol $MnCl_2$, and 2 mmol MPA were co-dissolved in 50 mL ultrapure water and then transferred into a 100 mL three-necked flask. The mixed solution was adjusted to pH 11 with 1M NaOH and stirred at room temperature, following by N_2 bubbling for 30 min to purge air. In the deaerated solution, 5 mL of 0.1M Na_2S was quickly injected into the solution and stirred for 20 min, and then aged at 50℃ under air for 2h. The resultant MPA capped Mn-ZnS QDs were precipitated with absolute ethanol, separated by centrifuging, washed with absolute ethanol, and finally dried in vacuum.

Procedures for the Oxidation of Carbon Nanotubes (oxCNTs). In order to obtain homogeneous solution, the pristine SWNTs were oxidized according to the literatures.[3-5] Briefly, 0.5g CNTs were dispersed in 200 mL HCl solution (2 $molmL^{-1}$) and refluxed for 24 h, the CNTs were collected by centrifugation (12000 rpm, 30min) and washed with Milli-Q water, then the purified CNTs were oxidized by 16 mL HNO_3/H_2SO_4 (1:3, v/v) in an ultrasonic bath for 2h, after washed to neutral pH, the resulted oxCNTs were dried at 105℃ for 24 h and stored in a desiccator. 0.1g oxCNTs were suspended by 100 mL ultrapure water and the concentration of oxCNTs was 1 mg mL^{-1}. This procedure provided a black aqueous suspension of oxCNTs.

Conjugation of Mn-ZnS QDs with Oligonucleotides (ssDNA). The protocols for conjugation of QDs with oligonucleotide (ssDNA) were based on previous literature with a small modification.[6] A quantity of 2 mg QDs was dispersed in 3.0 mL of 0.1 mol L^{-1} phosphate buffer (pH 7.0) by ultrasonication and a homogenous dispersoid was obtained. An amount of 20 mg of succinic anhydride was added to the solution, and then the mixture solution was allowed to react for 2h under stirring. After reaction, the as-prepared mixture solution was centriguged and washed with phosphate butter (pH 7.0), and the re-dispersed in 3.0 mL of 0.05 M Tris-HCl buffer containing 0.02M NaCl (pH 7.2) 1.2 mg of EDC and 1.8mg of NHS were added to the solution and allowed the reaction to react for 30 min under stirring, and 50 μL of DNA(ssDNA) was the added, and the reaction was incubated for 12h. The resulting solution was centrifuged and washed with 0.05M Tris-HCl, the particles were resuspended in 3.0mL of 0.05M Tris-HCl buffer containing 0.02M NaCl (pH 7.2), and therefore QDs conjugated with oligonucleotide(ssDNA) (QDs-ssDNA) were obtained.

Gel Electrophoreses. In order to further verify the specificity of cDNA-QDs-SWNTs system, the un-captured tDNA or mDNA are analyzed by 10% polyacrylamide gel electrophoresis (PAGE). To accomplish this, 40 nM tDNA (or mDNA) was introduced into cDNA-QDs-SWNTs system. After hybridization for 30 min, the mixture solution was centrifuged and washed with buffer, and then the supernatant was collected for electrophoretic indentification. The control solutions (i.e., hybridization for 0 min) was obtained with the same procedures. The samples were put on a polyacrylamide gel (15% acrylamide, 29:1, acrylamide/bisacrylamide) for electrophoretic indentification.

Phosphorescence Quenching and Hybridization Assay. The steady-state phosphorescence spectra were measured at 581 nm by an LS-55 fluorescence spectrophotometer equipped with a plotter unit and a quartz cell (1cm×1cm) in the phosphorescence mode at an excitation of 316 nm.

References:

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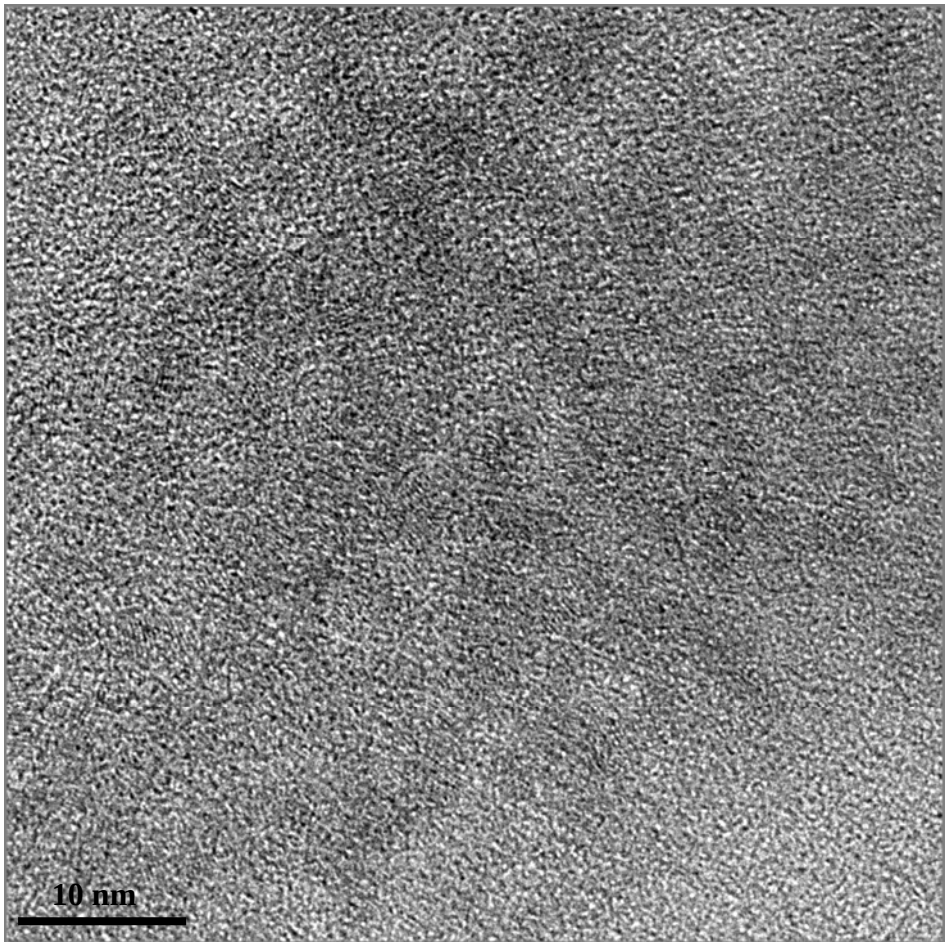
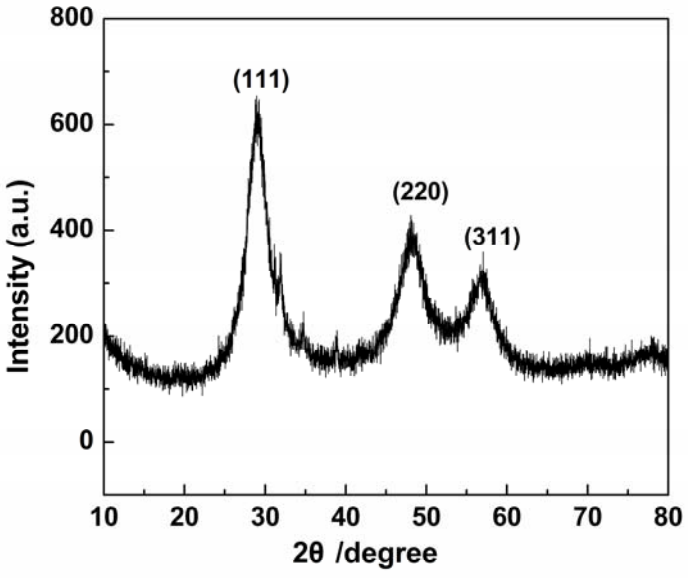
FIGURES

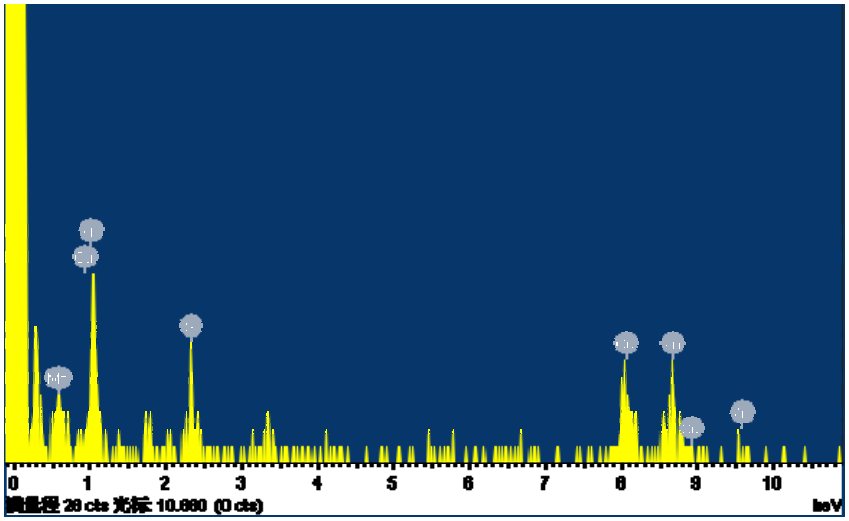
Fig. S1 Typical HRTEM image (A), XRD patterns (B), EDX patterns(C), and UV-vis absorbtion spectrum (D) of Mn-doped ZnS QDs.



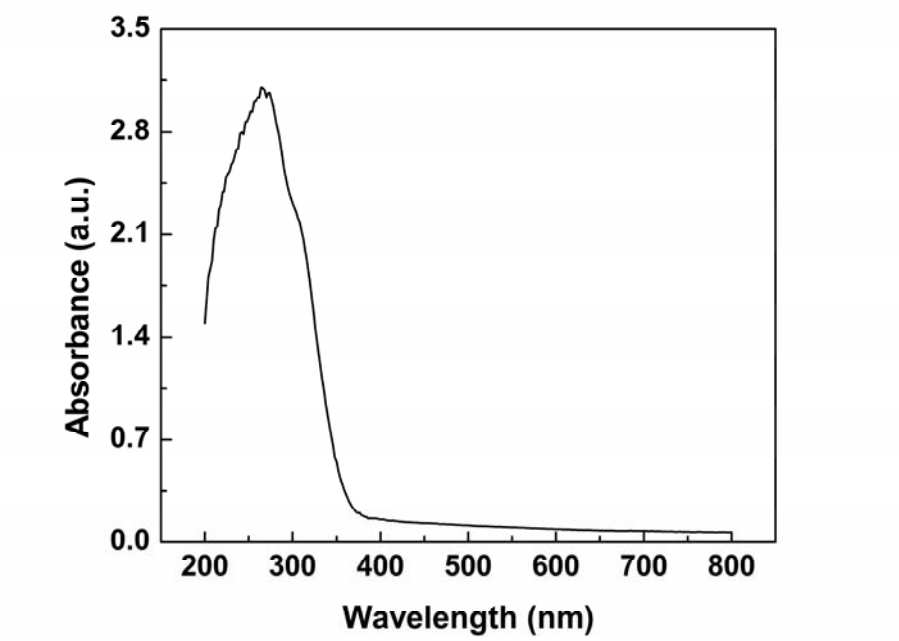
(A)



(B)


0
1
2
3
4
5
6
7
8
9
10
keV

(C)


3.5
2.8
2.1
1.4
0.7
0.0
Absorbance (a.u.)
200
300
400
500
600
700
800
Wavelength (nm)

(D)

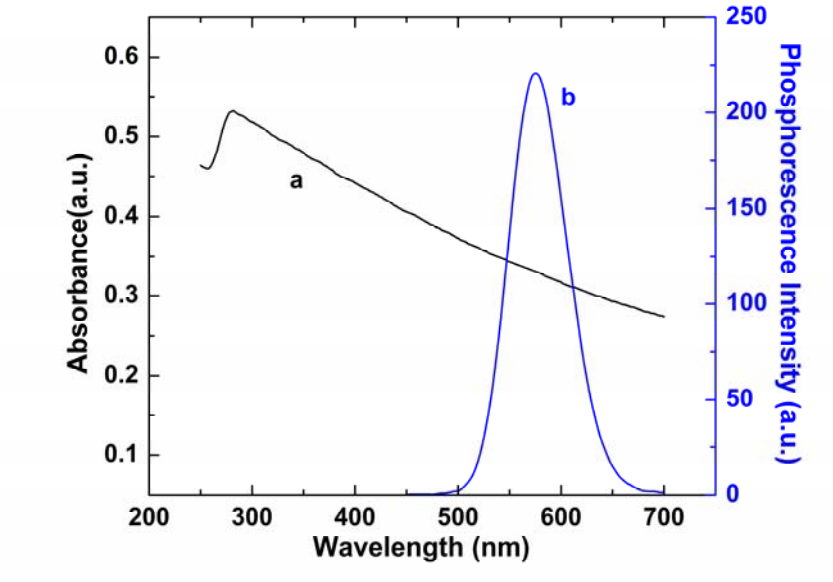


Fig. S2 The spectral overlay of adsorption spectrum (curve a) of carbon nanotubes (0.12 μg mL^{-1}) and phosphorescence spectrum (curve b) of cDNA-QDs (0.03 μg mL^{-1}).

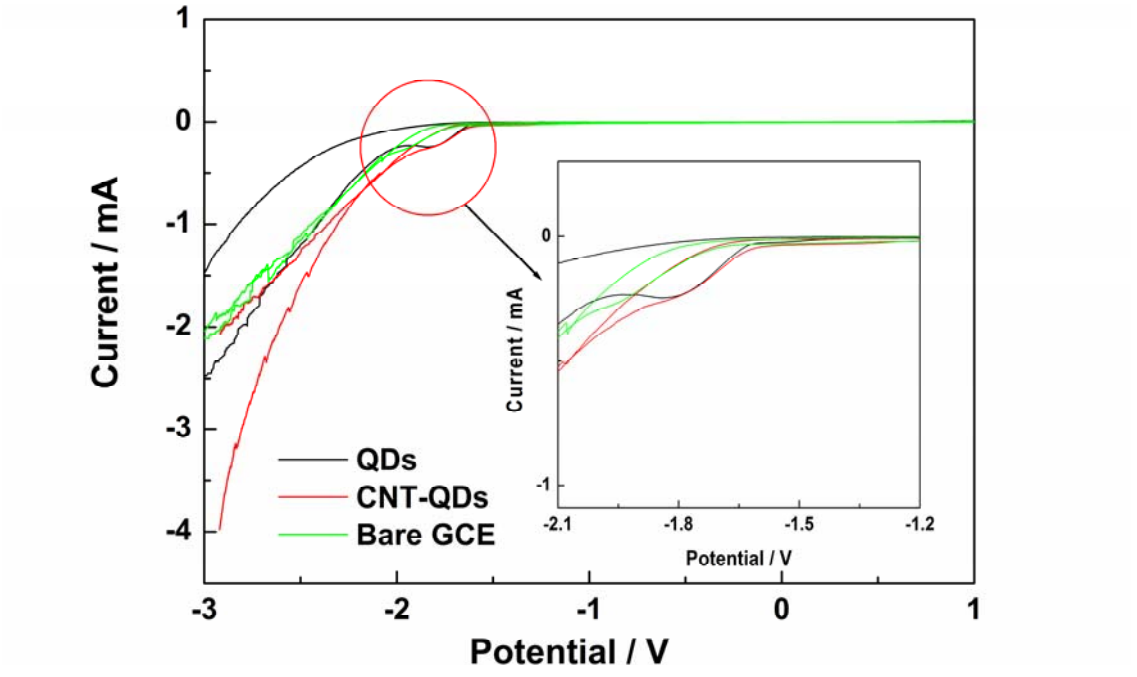


Fig. S3 The cyclic voltammograms of bare glassy carbon electrode (GCE) (green line), GCE modified with Mn-ZnS QDs (black line), and GCE modified CNT-QDs mixture(red line) in buffer solution with a scan rate of 100 mV s^{-1}.

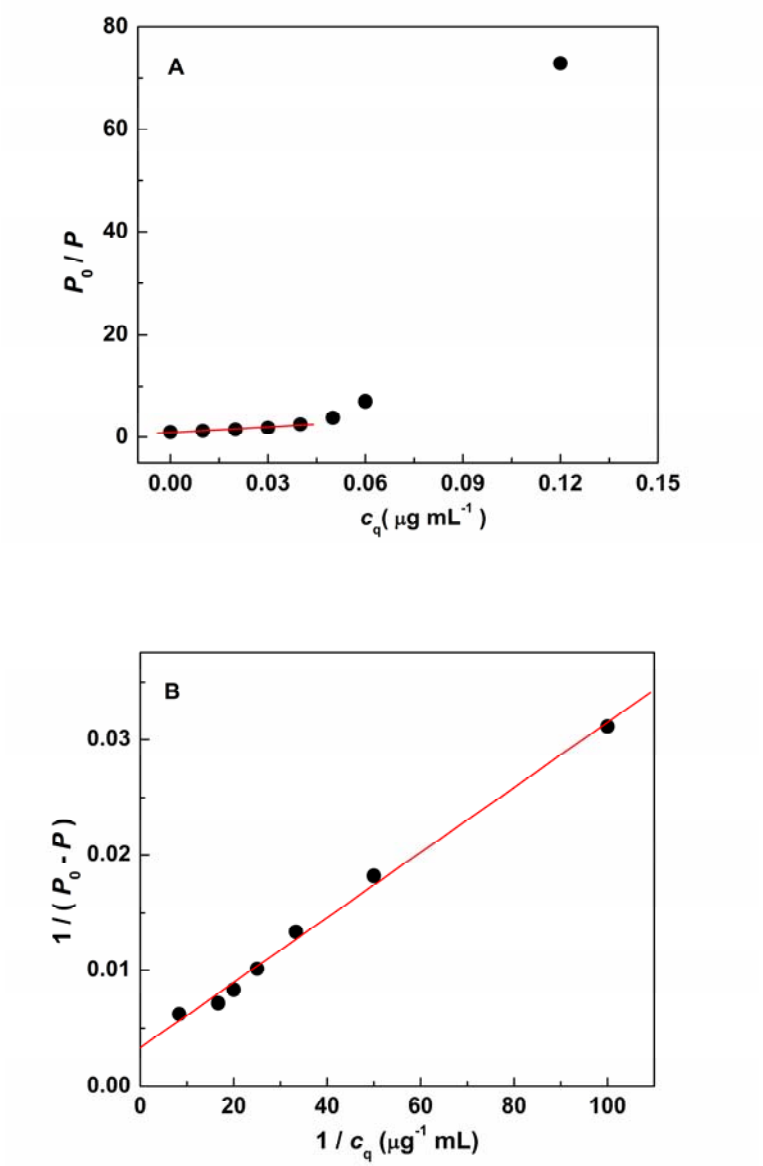


Fig. S4 The plot of Stern-Volmer (A) and Lineweaver-Burk (B). P_0 and P represent the relative phosphorescence intensity of cDNA-QDs in the absence and presence of SWNTs, respectively. The data are obtained from Fig. 1A.

Notes: The dynamic quenching can be described by Stern-Volmer equation (equation 1), while the static quenching can be described by the Lineweaver-Burk equation (equation 2) as following.

$$P_0 / P = 1 + K_{SV} \times c_q \qquad (1)$$

$$1 / (P_0 - P) = 1 / P_0 + K_{LB} / (P_0 c_q) \qquad (2)$$

Herein, P_0 and P represent the relative phosphorescence intensity of cDNA-QDs in the absence and presence of SWNTs, respectively, and c_q is the concentration of the quencher (SWNTs). K_{SV} is the dynamic quenching constant, and K_{LB} is the static quenching constant.

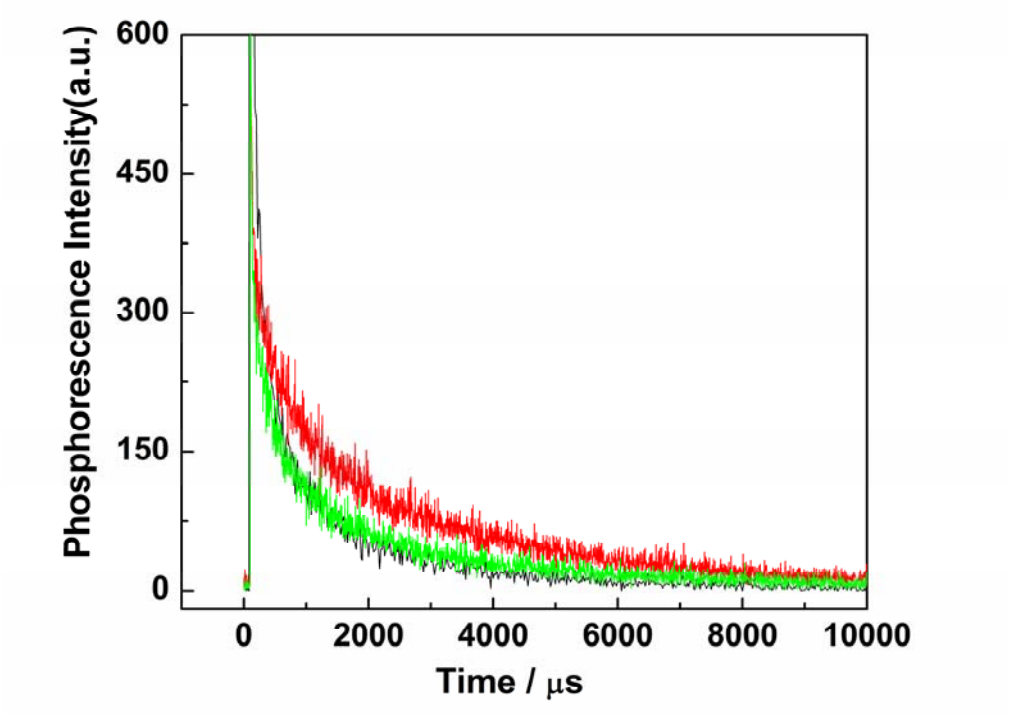


Fig. S5 Decay curves of the phosphorescence emission of cDNA-QDs (red line), cDNA-QDs +0.04 μg mL^{-1} CNTs (green line), and cDNA-QDs +0.12 μg mL^{-1} CNTs (black line).

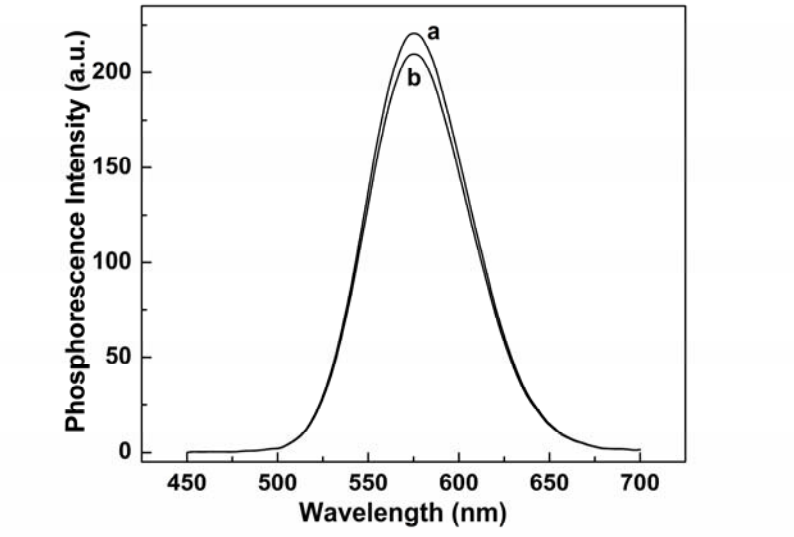


Fig. S6 Phosphorescence spectra of QDs in the absence (curve a) and presence (curve b) of SWNTs. QDs: 0.03 μg mL^{-1}, SWNTs: 0.12 μg mL^{-1}.

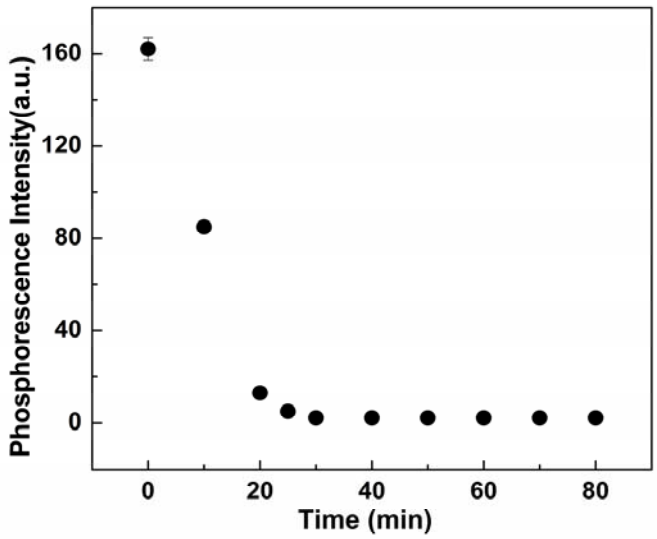


Fig.S7 Time dependence of phosphorescence quenching of 0.03 μg mL^{-1} cDNA-QDs by 0.12μg mL^{-1} SWNTs. All experiments were performed in phosphate buffer (0.01 M, 0.15 M NaCl, pH 7.4) under excitation at 316 nm.

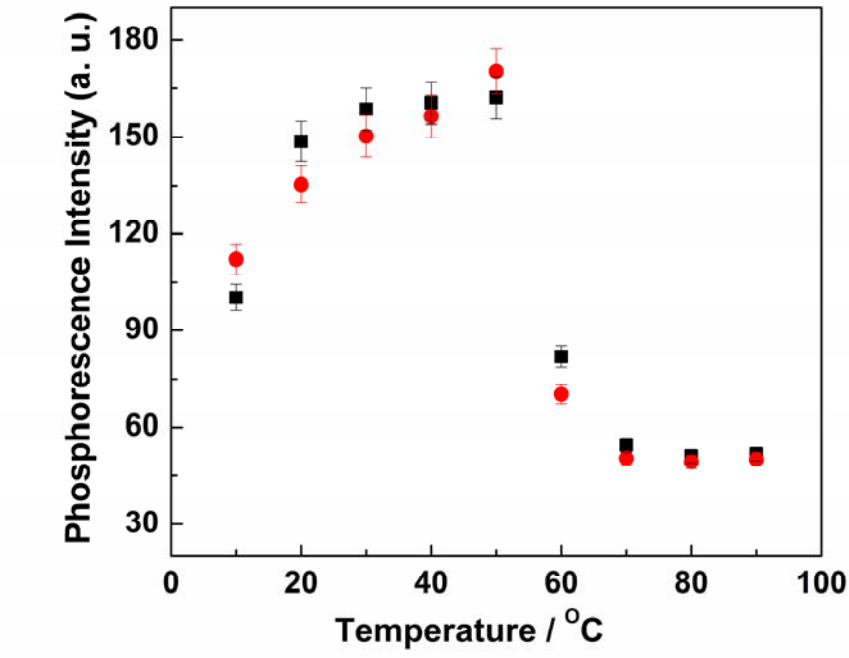


Fig. S8 Effects of temperature on the phosphorescence intensity of the Mn-doped ZnS QDs (black dots) and cDNA-QDs-CNTs (red dots).

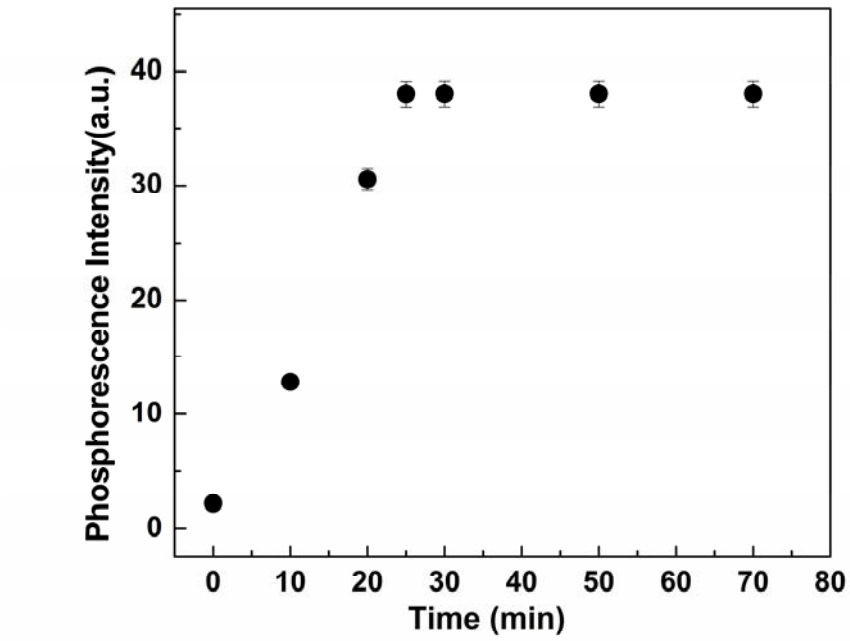


Fig. S9 Phosphorescence recovery of cDNA-QDs -SWNTs system by 10 nM tDNA as a function of incubation time. cDNA-QDs: 0.03 μg mL^{-1}; SWNTs: 0.12 μg mL^{-1}.

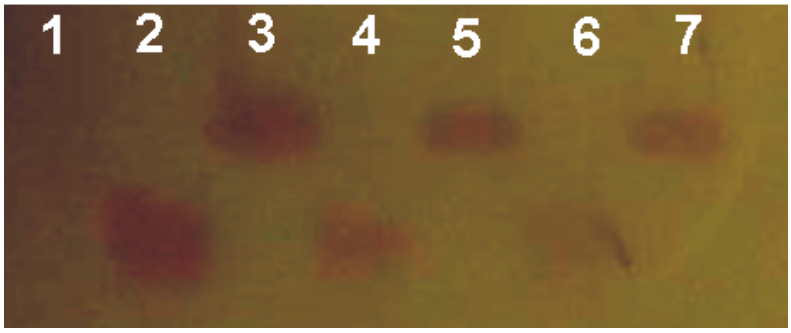


Fig. S10 Polyacrylamide gel electrophoresis(PAGE) of QDs-SWNTs (lane 1), target DNA(lane 2), mismatch DNA(lane 3), residual target DNA after cDNA-QDs-SWNTs and target DNA complex were incubated for 0 min (lane 4), residual mismatch DNA after cDNA-QDs-SWNTs and mismatch DNA complex were incubated for 0 min(lane 5), residual target DNA after cDNA-QDs-SWNTs and target DNA complex were incubated for 30 min(lane 6), residual mismatch DNA after cDNA-QDs-SWNTs and mismatch DNA complex were incubated for 30 min(lane 7).

Table S1 Comparison of biosensor performances for DNA detection with different optical schemes.

Method	Signal reporter	Linear range (nM)	Detection limit (nM)	Ref
FRET	PEI-coated $NaYF_4$:Yb, Er upconversion phosphors	0.1-6.0	0.036	1
FRET	SYBR green I	0.5-100	0.31	2
FRET	fluorescein isothiocyanate (FITC)-doped fluorescent silica nanoparticles	0-35.0	0.003	3
LSPR light scattering	DNA–silver nanoparticle conjugates	0.3-2.0	0.195	4
FRET	CdTe QDs	50-1500	12	5
Fluorimetry	Gold nanoparticles	0.1-1	0.1	6
Phosphorescence-based molecular beacon	Eu^{3+} complex	0-120	0.5	7
FRET	CdTe QDs	10-20000	9.39	8
Phosphorescence	Mn-ZnS/octa(3-aminopropyl)octasilsequioxane octahydrochloride nanohybrids	125-10000	54.9	9
PRET	cDNA/ Mn-ZnS QDs/SWNTs system	0-45	0.027	This work

FRET, fluorescence resonance energy transfer; PEI, Poly(ethylenimine);
LSPR, localized surface plasmon resonance.

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