Supporting Information

Platinum ions mediate the interaction between DNA and carbon quantum dots: diagnosis of MRSA infections

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Experimental section

Materials. Calcium chloride, potassium chloride, magnesium chloride, sodium chloride, hydrochloric acid, sodium phosphate monobasic, sodium phosphate dibasic anhydrous, sodium phosphate tribasic, and tris(hydroxymethyl)aminomethane were purchased from Mallinckrodt Baker (New Jersey, USA). Spermidine trihydrochloride, citric acid, sodium hydroxide, potassium hexachloroplatinate(IV), agarose LE and other metal salts used in this study were purchased from Sigma-Aldrich (St Louis, USA). Diammonium citrate was purchased from Showa Chemical Industry (Okayama, Honshu, Japan). Primers were purchased from Mission Biotech (Taipei, Taiwan) and LAMP reagent kits were purchased from New England BioLabs (Massachusetts, USA). DNA extraction kits and PD Minitrap™ G-25 column was purchased from GE Healthcare (Pittsburgh, USA). Milli-Q ultrapure water (18.2 MΩ·cm; EMD Millipore, Billerica, MA, USA) was used in all experiments.

Synthesis of CQDs. CQDs were prepared by a simple dry heating procedure according to our previous work.[1] Briefly, citric acid (50 mg), diammonium citrate (50 mg) or spermidine trihydrochloride (50 mg) was heated in a muffle furnace at 180–270 ℃ for 3 h. The residues were cooled to room temperature and dissolved in 5.0 mL of DI water. The samples were then sonicated for 1 h and subsequently centrifuged at a relative centrifugal force (RCF) of 35,000 *g* for 1 h to remove larger particles. The supernatant containing CQDs (10 mL) was then dialyzed against ultrapure water (~2 L) through a dialysis membrane (MWCO = 0.5–1.0 kD, Float-A-Lyzer G2, Spectrum Laboratories, Rancho Dominguez, CA, USA) for 5 h with the water replaced every 1 h. After 5 h, the ultrapure water was replaced every 12 h for 2 days. The solution of purified CQDs was quantified by the freeze-drying method and stored at 4 °C when not in use.

Characterization of CQDs. Transmission electron microscopy (TEM) images of the CQDs were obtained using a Tecnai 20 G2 S-Twin TEM (Philips/FEI, Hillsboro, OR, USA). Fluorescence and

UV-Vis absorption spectra of the as-prepared CQDs were recorded using a monochromatic microplate spectrophotometer (Synergy 4 Multi-Mode; Biotek Instruments, Winooski, VT, USA). The hydrodynamic size and zeta potentials (ζ) of the CQDs were assessed using a Zetasizer (Nano ZS, Malvern Instruments, Worcestershire, UK). An Agilent Cary 640 FT-IR spectrometer (Santa Clara, CA, USA) was used to identify the functional groups present in the CQDs. Elemental analysis of the CQDs was performed after collection of 5 batches of as-prepared CQDs by using a vario EL cube analyzer (Elementar, Hanau, Germany) for N, C, H, and O. For inductively coupled plasma mass spectrometry (ICP-MS; Agilent 7700 Series ICP-MS, Agilent Technologies, California, USA) measurements, the CQDs samples were prepared in 2% HNO_3. The fluorescence lifetimes of the CQDs were recorded using a photo-counting PicoHarp 300 system (PicoQuant, Berlin, Germany) and a diode laser emitting at 375 nm (FluoTime 300) as the light source.

Preparation of CQD_{SPDs}/Pt^{4+} probe. To prepare a CQD_{SPDs}/Pt^{4+} stock solution, a mixture of the as-prepared CQDs (100 μg mL^{-1}) synthesized from spermidine·3HCl (i.e., CQD_{SPDs}) and Pt^{4+} ions solution (0.1 mM) was kept in Tris-acetate buffer (5 mM, pH 7.0) at room temperature for 1 h. We used Amicon® Ultra Centrifugal Filters to purify unbounded metal ions and quantified the adsorbed Pt^{4+} ions. Around 25% of Pt^{4+} ions readily capped to the CQD_{SPDs} through complexation, which was confirmed by inductively coupled plasma mass spectrometry (ICP-MS; Agilent 7700 Series ICP-MS, Agilent Technologies, California, USA) measurement. The CQD_{SPDs}/Pt^{4+} probe solution was stable for at least three months when stored at 4 ℃ in the dark.

Primer design of *mecA* and *femA* for LAMP assay. The primer was designed following the method published by Koide and co-workers.[2] Briefly, LAMP primer candidates were obtained from the nucleotide sequence of *mecA* and *femA* genes by Primer Explorer version 2 (Fujitsu) at the Net Laboratory website and the sequence of MRSA from the GenBank at the National Center for Biotechnology Information (NCBI) website. The candidates were then aligned by Genetyx ver.

8 (Genetyx, Tokyo, Japan). A set of LAMP primers (**Table S4**) targeting six distinct regions, including forward inner primer (FIP) with the complementary sequence of F1 (F1c), linker and F2, backward inner primer (BIP) with the complementary sequence of B1 (B1c), linker and B2, the outer primers F3 and B3 located outside of the F2 and B2 regions.

Bacterial culture. *S. aureus* (BCRC10781) and MRSA [methicillin-resistant *Staphylococcus aureus*, (ATCC 43300)] were grown separately in Luria–Bertani medium (LB broth). Individual colonies were isolated and inoculated in LB broth. The bacterial cultures were then incubated at 37 °C until the optical density at 600 nm ($OD_{600\ nm}$) reached 1 (optical path length = 1 cm). Each bacterial culture (1 mL) was centrifuged at a RCF of 5000 *g* at 25 °C for 15 min and washed with phosphate-buffered saline [PBS; containing 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 2.0 mM KH_2PO_4; adjusted to pH 7.4 using HCl (pH 7.4)] twice prior to further use.

LAMP-mediated amplification of target gene and gel electrophoresis. A total of 24 specimens were collected as per the procedure in compliance with relevant laws and institutional guidelines from the approval of the Research Ethics Board of MacKay Memorial Hospital in Taiwan using sterile swabs. The swabs were rubbed in a rotating manner to cover up to a 5 × 5 cm area. Each swab was suspended in 1 mL of 7% sodium chloride nutrient broth (Becton Dickinson, San Jose, CA, USA) in a 2-mL freezer tube and vortexed. Each container with a sponge was filled with 10 mL of the same broth to soak the sponge and vortexed. The tubes and containers were incubated at 37 ℃ with loose caps overnight for enrichment. After 24 h of enrichment, 1 mL broth was taken from each specimen and extracted using Genomic DNA extraction kit (GeneDirex NA023-100). The LAMP reaction utilized the Loopamp DNA amplification kit from Eiken. Typically, 1 μL *Bst* polymerase (8 units), 2.5 μL premixed primer (1.6 μM each of inner primers and 0.2 μM of each outer primers), and 5.0 μL template (extracted genomic DNA from MRSA in water, urine or blood background) were added together to yield a final volume of 25 μL reaction solution. The reaction

was allowed to proceed under the isothermal condition at 65 ℃ by dry bath for 60 min. After the incubation, the reaction was terminated by heating at 80 ℃ for 2 min. The samples were analyzed by gel electrophoresis in 1.0% agarose gel and 40 mM Tris-acetate buffer/1 mM EDTA (TAE buffer, pH 8.0) at 100 V for 30 min. The gel was then stained with a 1:100,000 dilution of HealthView nucleic acid stain (Genomics BioSci & Tech Corp., New Taipei City, Taiwan), photographed and visualized using a Gel Dox XR (Bio-Rad, Madison, CA, USA) photograph system with ultraviolet light.

Detection of MRSA genes using CQD_{SPDs}/Pt^{4+} probe. The *mecA*-LAMP or *femA*-LAMP products obtained from the amplification of the cultured bacterial samples or clinical samples were both diluted 100-folds and mixed with CQD_{SPDs}/Pt^{4+} ($[CQD_{SPDs}]$ = 10 μg mL^{-1}; $[Pt^{4+}]$ = 10 μM) in 5.0 mM Tris-acetate buffer solution (pH 7.0) for 2 h. After incubation, the fluorescence intensity was measured by a monochromatic microplate spectrophotometer with excitation at 365 nm and emission from 385 to 700 nm.

References

(1) Y.-J. Li, S. G. Harroun, Y.-C. Su, C.-F. Huang, B. Unnikrishnan, H.-J. Lin, C.-H. Lin and C.-C. Huang, *Adv. Healthcare Mater.*, 2016, **5**, 2545–2554.

(2) Y. Koide, H. Maeda, K. Yamabe, K. Naruishi, T. Yamamoto, S. Kokeguchi and S. Takashiba, *Lett. Appl. Microbiol.*, 2010, **50**, 386–392.

Table S1. Elemental analyses and zeta potentials of the as-prepared CQD_{CAs}, CQD_{ACs}, and CQD_{SPDs}.

	Elemental compositions (wt%)					Zeta potential (mV; n = 5)
	C[a]	O[a]	N[a]	H[a]	Cl[b]	
CQD_{CAs}	41.67	52.81	<0.01	5.34	<0.01	−34.9 ± 6.3
CQD_{ACs}	42.67	39.17	12.23	4.90	<0.01	−27.9 ± 6.9
CQD_{SPDs}	39.34	8.83	12.63	11.06	28.09	+35.4 ± 3.9

[a] Determined by the elemental analyzer
[b] Determined by the inductively coupled plasma mass spectrometry

Table S2. The sequences of random DNA and its complementary DNA.

DNA	Sequence
random DNA	5’-CGG CTG ATT ACT CTT GTT GGT GTG GTA TCG CTA AAC TGC GTC GCG GAG CCT TAT GGC ATA GTC GTC CGC GGA GCA CTC TG-3’
complementary DNA	5’-CAG AGT GCT CCG CGG ACG ACT ATG CCA TAA GGC TCC GCG ACG CAG TTT AGC GAT ACC ACA CCA ACA AGA GTA ATC AGC CG-3’

Table S3. LAMP assays of *mecA* and *femA* genes for the identification of different bacterial strains.

Bacteria strain	*mecA*[a,b]	*femA*[a,b]
MRSA	P	P
S. aureus	N	P
MR-CoNS	P	N
E. coli	N	N
A. bumannii	N	N
P. aeruginosa	N	N

[a] Positive

[b] Negative

Table S4. Primers used in the LAMP reaction for *mecA* and *femA* genes.

Target gene	Primer	Sequence
mecA	F3	5'-AAG ATG GCA AAG ATA TTC AAC-3'
	B3	5'-AGG TTC TTT TTT ATC TTC GGT TA-3'
	FIP	5'-ACC TGT TTG AGG GTG GAT AGC ATG ATG CTA AAG TTC AAA AGA GT-3'
	BIP	5'-GCA CTT GTA ACA CCT TCA CTT CGT TAC TCA TGC CAT AC-3'
femA	F3	5'-GTC CTG AAA ATA AAA AAG CAC AT-3'
	B3	5'-ACT TCC GGC AAA ATG ACG-3'
	FIP	5'-TGT TCT TCT TGT AGA CGT TTA CCT TGA GAT AAC TTA CAA CAA CAA CTT G-3'
	BIP	5'-ACC TAT CTC TGC TGG TTT CTT CTA ATG CAT TTG ATG TAC CAC C-3'

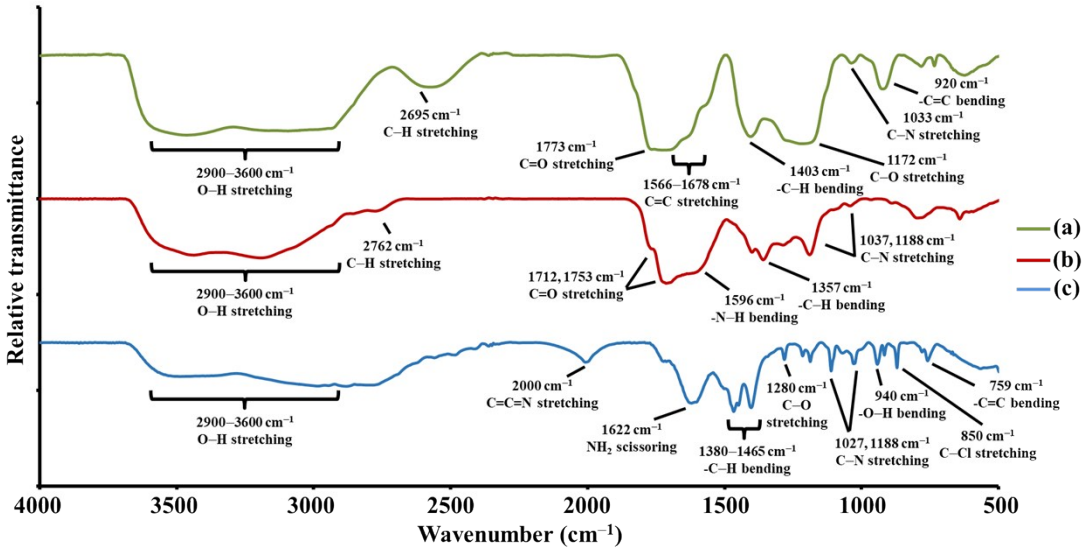

Fig. S1. FTIR spectra of (a) CQD_{CAs}, (b) CQD_{ACs}, and (c) CQD_{SPDs}.

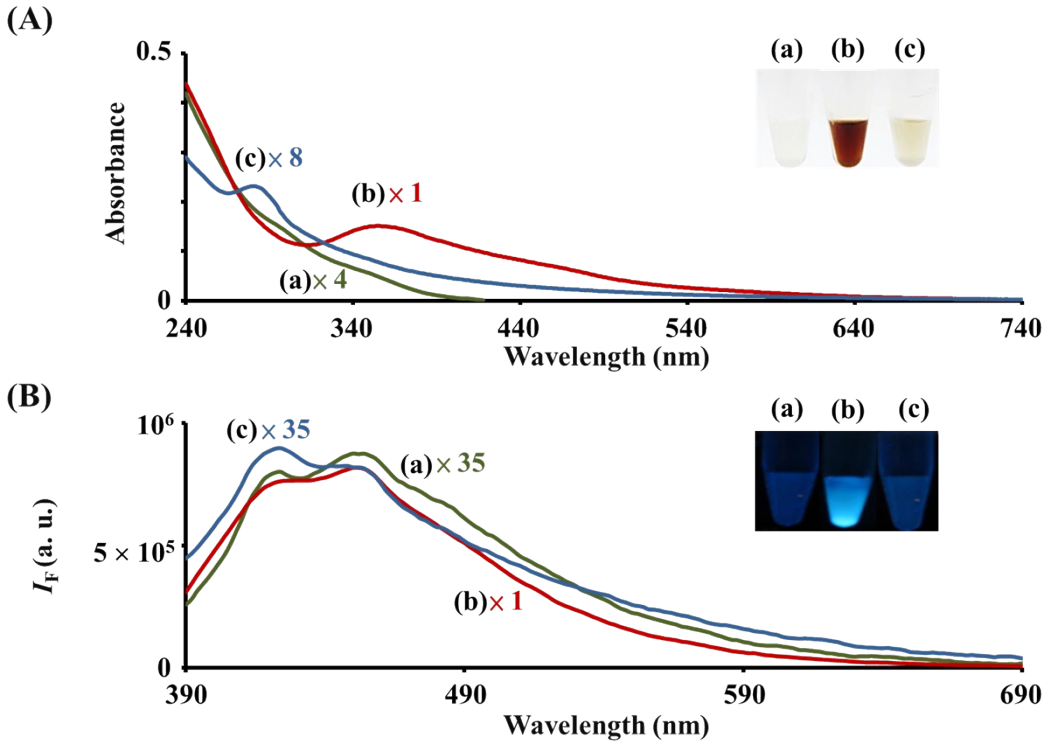

Fig. S2. (A) UV-Vis absorption and (B) fluorescence spectra of (a) CQD_{CAs}, (b) CQD_{ACs}, and (c) CQD_{SPDs} in 5.0 mM Tris-acetate buffer solution (pH 7.0). The concentration of all CQDs is 10 μg mL^{-1} for the UV-Vis absorption and fluorescence measurements. Insets in panels (A) and (B): photographs of the corresponding solutions (A) before and (B) during excitation with a UV lamp (365 nm). Fluorescence spectra of CQDs were recorded at an excitation wavelength of 365 nm. The fluorescence intensities (I_F) are plotted in arbitrary units (a. u.).

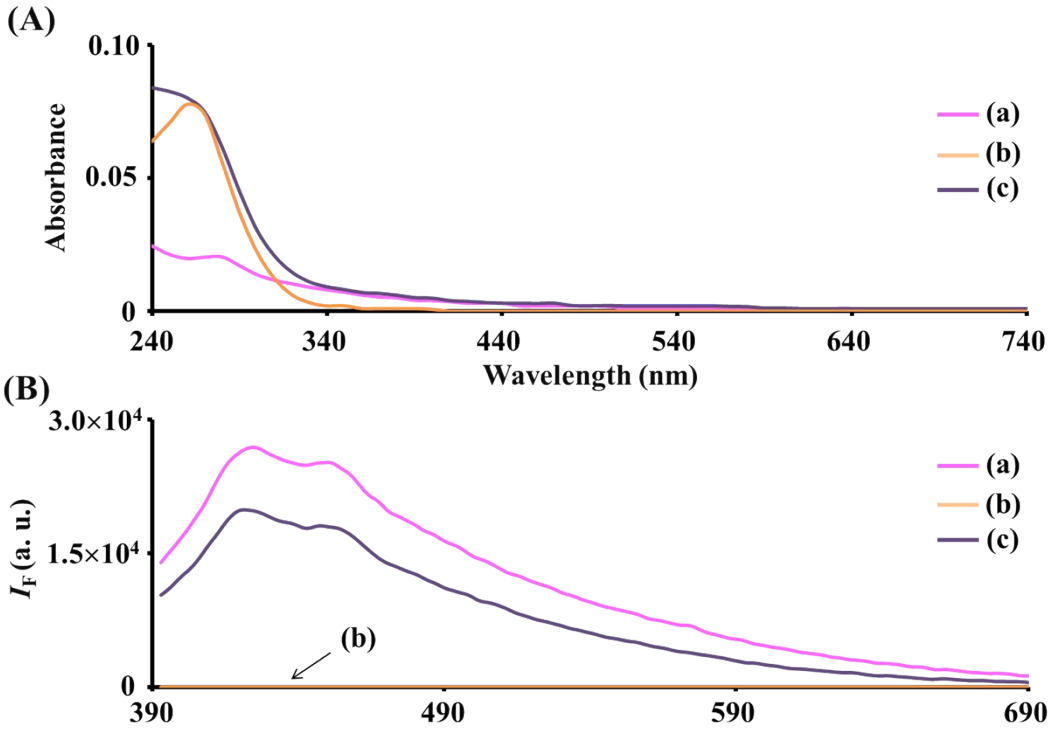

Fig. S3. (A) UV-Vis absorption and (B) fluorescence spectra of (a) CQD_{SPDs}, (b) Pt^{4+}, and (c) the mixture of CQD_{SPDs} and Pt^{4+} in 5.0 mM Tris-acetate buffer solution (pH 7.0). The concentration of CQDs and Pt^{4+} is 10 μg mL^{-1} and 10 μM, respectively. Fluorescence spectra of CQDs were recorded at an excitation wavelength of 365 nm. The fluorescence intensities (I_F) are plotted in arbitrary units (a. u.).

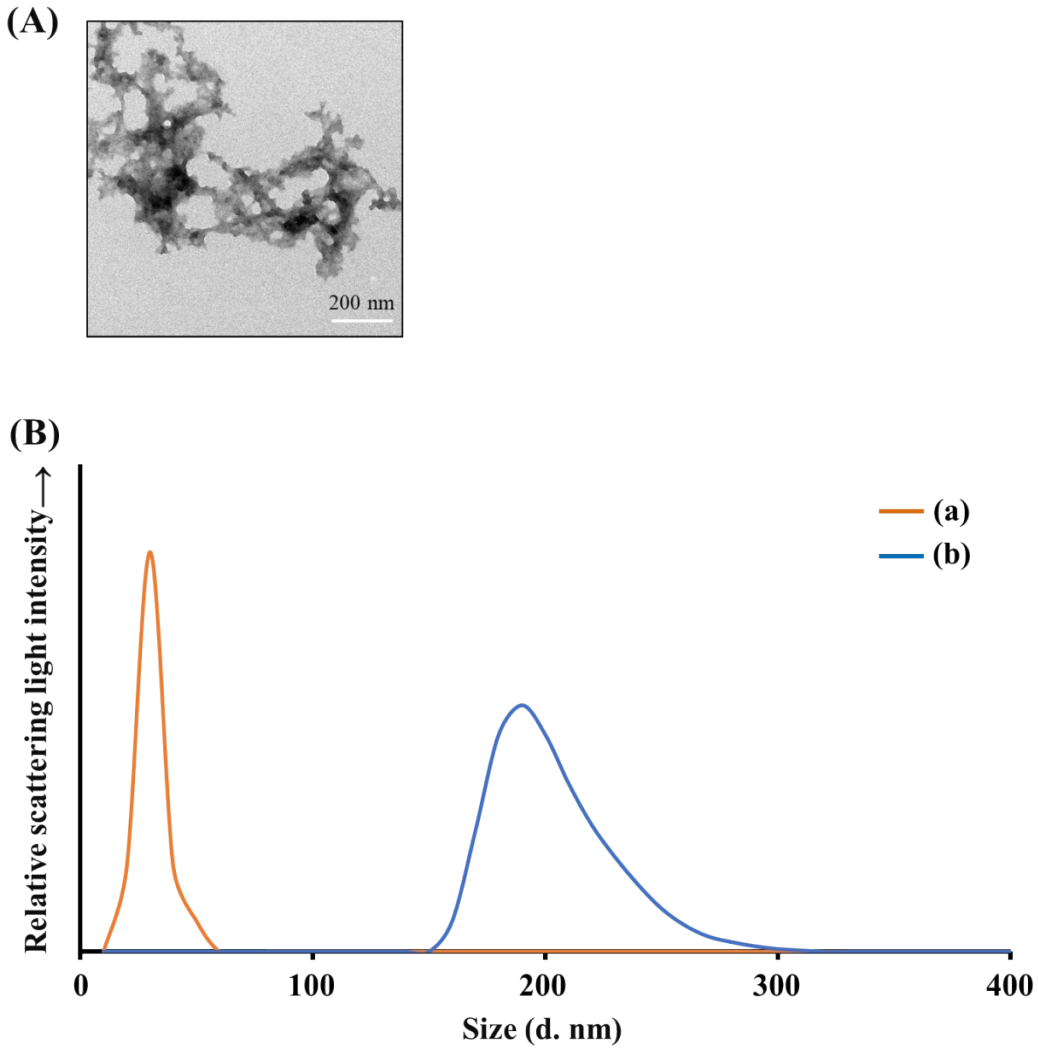

Fig. S4. (A) TEM image of the mixture of CQD_{SPDs}/Pt^{4+} ([CQD_{SPDs}] = 10 μg mL^{-1}; [Pt^{4+}] = 10 μM) in the presence of dsDNA (100 nM). (B) Hydrodynamic radius of CQD_{SPDs}/Pt^{4+} in the (a) absence and (b) presence of dsDNA (100 nM) in 5.0 mM Tris-acetate buffer solution (pH 7.0).

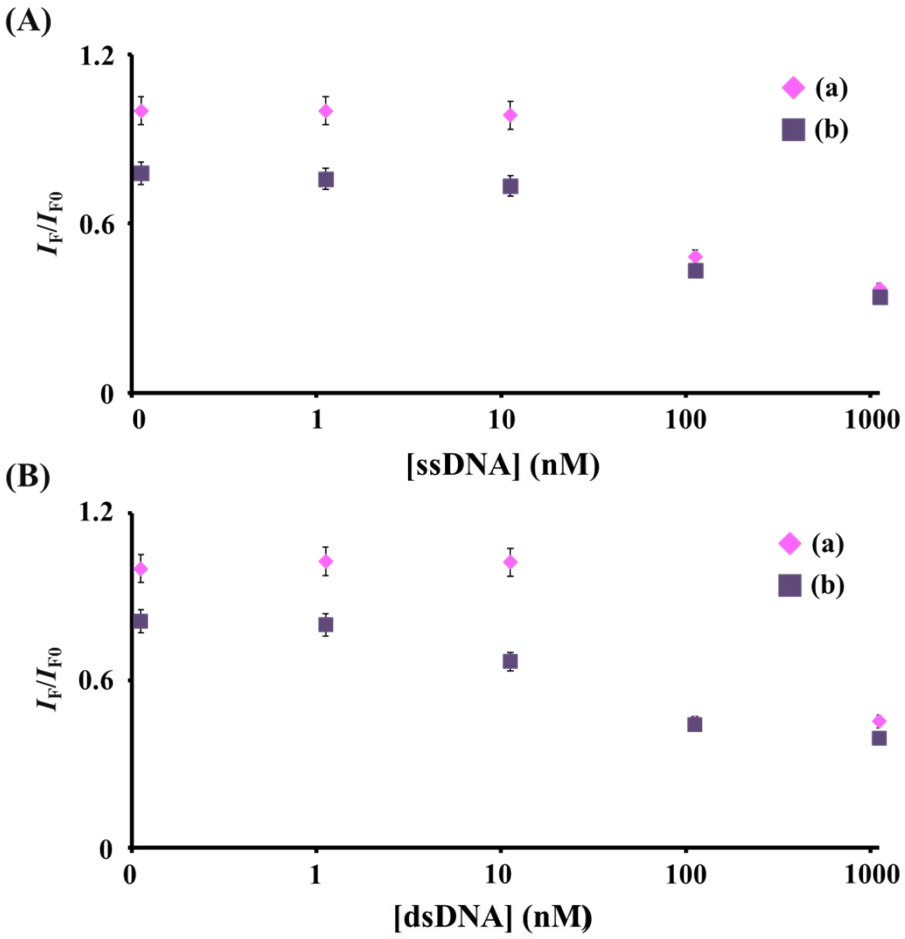

Fig. S5. The relative changes in the fluorescence intensity (I_F/I_{F0}) of (a) CQD_{SPDs} and (b) CQD_{SPDs}/Pt^{4+} in the presence of (A) random ssDNA (80 mer) and (B) random dsDNA (80 bp) in 5.0 mM Tris-acetate buffer solution (pH 7.0). I_{F0} and I_F represent the fluorescence intensities of the CQD_{SPDs} (10 μg mL^{-1}) solutions at 450 nm in the absence and presence of Pt^{4+} (10 μM) and DNA (0–1000 nM), respectively. Error bars represent the standard deviations of experiments in triplicate.

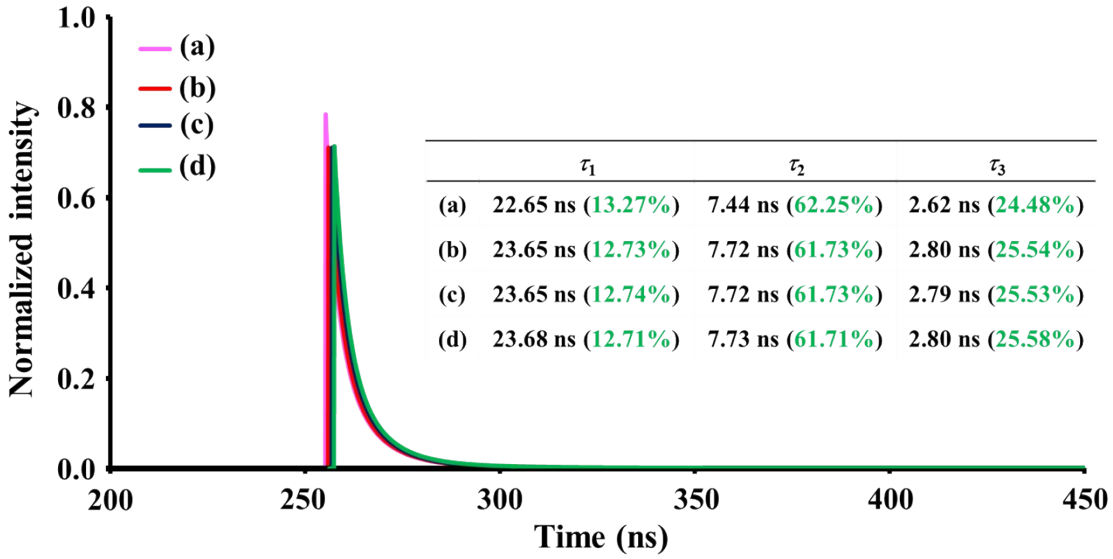

Fig. S6. Fluorescence decay profile of the (a) CQD_{SPDs}, (b) a mixture of CQD_{SPDs} and dsDNA, (c) a mixture of CQD_{SPDs} and Pt^{4+}, and (d) a mixture of CQD_{SPDs}, Pt^{4+}, and dsDNA. The concentration of CQD_{SPDs}, Pt^{4+}, and dsDNA (80 bp) are 100 μg mL^{-1}, 100 μM, and 1.0 μM, respectively. The fluorescence decay was fitted to a triexponential decay: $I(t) = a_1 \exp(-t/\tau_1) + a_2 \exp(-t/\tau_2) + a_3 \exp(-t/\tau_3)$. The values for the lifetimes and parameters (a_1, a_2, and a_3 components) are listed in the inset.