ELECTRONIC SUPPORTING INFORMATION (ESI) FOR:

Synergy between quantum dots and 1,10-phenanthroline-copper(II) complex towards cleaving DNA

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Materials and Methods.

Materials. All reagents and chemicals were obtained from commercial sources and used without further purification.

Copper (II) chloride, 1,10-phenanthroline, cadmium oxide (CdO, 99.5%), tri-*n*-octylphosphine oxide (TOPO, 99%), tri-*n*-butylphosphine (TBP, 97%), hexadecylamine (HDA, 98%), diethylzinc ($ZnEt_2$,1M in hexane), hexametyldisilathiane [$(TMS)_2S$ 98%], nickel (II) chloride, *N*-hydroxysuccinimide (NHS, 98%) and *N*-*N*-bis(carboxymethyl)-L-lysine hydrate were purchased from Sigma Aldrich. 1-Ethyl-3-[3'-(dimethylamino)propyl]carbodiimide was purchased from Ademtech. Stearic acid (≥98.5) was purchased from Fluka. Selenium powder (Se, 99.999%) was obtained from Alfa Aesar. 1,2-Dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (PEG(2000)PE) and 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000] (DSPE-PEG(2000)Amine) were supplied from Avanti Polar Lipids, Inc. Plasmid pUC18 (0.5 μg/μL, 1500 μM in nucleotides) in 10 mM Tris-HCl (pH 7.6) and 1 mM EDTA was purchased from Fermentas. A High Pure PCR Product Purification Kit was supplied from Roche Diagnostics, Germany.

The 1:2 Cu(II) complex of 1,10-phenanthroline (phen) was prepared by adopting a literature procedure;[1] the complex was found to conform to the general formula $Cu(phen)_2Cl_2 \cdot H_2O$, on the basis of elemental analysis.

Hydrophobic core-shell CdSe-ZnS QDs were synthesized and purified as we described previously.[2] The results reported in this study are for nanocrystals which had the first absorption band at 600 nm and a maximum emission peak at 645 nm

with excitation at 325 nm. The size and concentration of the QDs solutions were estimated by the method of Peng *et al*.[3], and confirmed by TEM and ICP-OES.

Synthesis of QD micelles.

Hydrophobic QDs were dissolved in 150 μL of chloroform ([QD] = 6.7 μM). 1,2-Dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (1 mg, 0.4 μmol) and 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000] (1 mg, 0.4 μmol) were dissolved in 50 μL of chloroform. The hydrophobic QDs and phospholipid solutions were then mixed together in a 5 mL round-bottomed flask. The flask was left open at room temperature overnight in a fume hood to slowly evaporate the chloroform solvent. Any remaining chloroform was removed under vacuum using a rotary evaporator for 2 h to form a thin film. The flask containing the QDs was then heated in water bath set to 80 °C for 30 s, after which 600 μL of doubly deionized water was quickly added. Addition of water forms a suspension which contains unreacted hydrophobic QDs and QDs encapsulated into the phospholipids micelles. This suspension was transferred into Ependorff tubes and centrifuged at 12470 g for 3 min. The unreacted QDs formed a pellet which was discarded while the micelles containing QDs remained in solution. This process was repeated several times to remove all suspended nanoparticles from the supernatant solutions.

QD-filled micelles were characterised by HRTEM, EDS, UV-vis, fluorescence spectroscopy and ICP-OES.

The CdSe-ZnS QDs had an average diameter of 5.2 nm (4.0 nm CdSe core diameter and 0.6 nm ZnS shell thickness). The number of Cd, Se, and Zn and S atoms per QD was calculated using the bulk densities and formula weights of CdSe and ZnS, and was confirmed experimentally by ICP-OES: ca. 600 Cd and Se atoms, and ca. 1000 Zn and S atoms.

DNA Cleavage. Solutions of the copper(II) complex for strand scission experiments were freshly prepared prior to each experiment. In a typical experiment, stock copper(II) complex and buffer solutions were degassed with argon for 15 min prior to initiation. Reaction mixtures were immediately prepared in a nitrogen-filled glovebag by addition of the appropriate volumes of 200 μM copper (II) complex stock solution,

0.5 μL of pUC18 DNA solution (0.5 μg/μl, 1500 μM in base pairs), and appropriate amounts of 0.1 M cacodylate buffer (pH = 6.0) solution to complete 18 μL. After 5 minutes, QDs micelles were added to achieve the desired final metal complex and QD concentrations (20 μL total volume). Then, the samples were incubated at 20 °C under dark conditions in the same nitrogen-filled glovebag. To stop the reaction a quench buffer solution (3 μL) consisting of bromophenole blue (0.25%), xylene cyanole (0.25%), and glycerol (30%), and 100 mM EDTA was added. The solution was then subjected to electrophoresis on 0.8% agarose gel in 0.5X TBE buffer (0.045 M Tris, 0.045 M boric acid, and 1 mM EDTA) containing 2 μL/100 mL of a solution of ethidium bromide (10 mg/mL) at 80 V for 2 h. The bands were photographed on an UVIdoc HD2 capturing system (UVItec Cambridge).

For photocleavage studies, the reactions were carried out in the same way but in this case the solutions were not degassed and the reactions were carried out under illuminated conditions with samples irradiated in a carousel inside a Luzchem photoreactor (CCP-4V) equipped with 10 LZC-vis lamps with red filters which cut off light below 600 nm (LZC-red) from Luzchem Research.

The relative amounts of different plasmid structures were quantified with the aid of ImageJ 1.34s (Wayne Rasband, ImageJ 1.34s. National Institutes of Health, Bethesda, MD, U.S.A.). A correction factor of 1.42 was used for the assessment of supercoiled DNA (Form I) because the intercalation between ethidium bromide and Form I DNA is relatively weak compared to that of nicked (Form II) and linear (Form III) DNA.[4]

Kinetic studies were carried out in the same way quantifying DNA cleavage at different times. DNA recovery assays were performed using a High Pure PCR Product Purification Kit following the manufacture's instructions (Roche Diagnostics, Germany).

Transmission electron microscopy. High-resolution transmission electron microscopy (HRTEM) and energy-dispersive X-ray spectroscopy (EDS) studies were conducted on a JEOL JEM-2011 electron microscope operating at 200 kV. The samples were prepared by depositing a drop of a solution of nanocrystals onto a copper specimen grid coated with a holey carbon film and allowing it to dry.

References:

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2. (a) A. Touceda-Varela, E. I. Stevenson, J. A. Galve-Gasión, D. T. F. Dryden and J. C. Mareque-Rivas, *Chem. Commun.*, 2008, 1998; (b) N. G. Blanco, C. R. Maldonado and J. C. Mareque-Rivas *Chem. Commun.,* 2009, 5257; (c) T. A. Barr, M. Krembuszewski, M. Gupta, D. Gray and J. C. Mareque-Rivas, *Mol. BioSyst.*, 2010, **6**, 1572.

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4. C. W. Haidle, R. S. Lloyd and D. L. Robberson, *In Bleomycin: Chemical, Biochemical, and Biological Aspects*; Hecht, S. M., Ed.; Springer-Verlag: New York, 1979; pp 222-243.

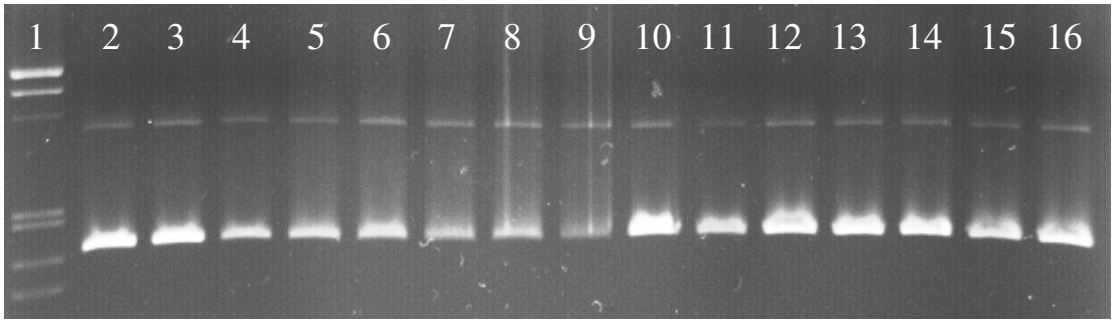


Fig S1. QDs-pUC18 interaction: gel electrophoresis of supercoiled pUC18 DNA at 20 ˚C in 0.1 M cacodylate buffer (pH 6.0) under dark conditions. Lane 1, Marker; Lane 2, control pUC18 DNA; Lanes 3-9, DNA + QDs (25, 50, 75, 100, 125, 187.5 and 250 nM); Lanes 10-16 DNA + QDs (25, 50, 75, 100, 125, 187.5 and 250 nM) in 50 mM NaCl.

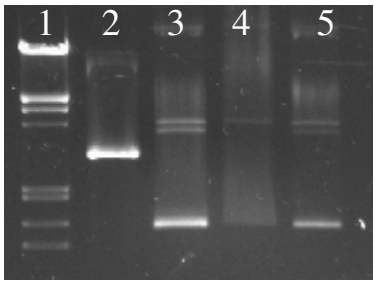
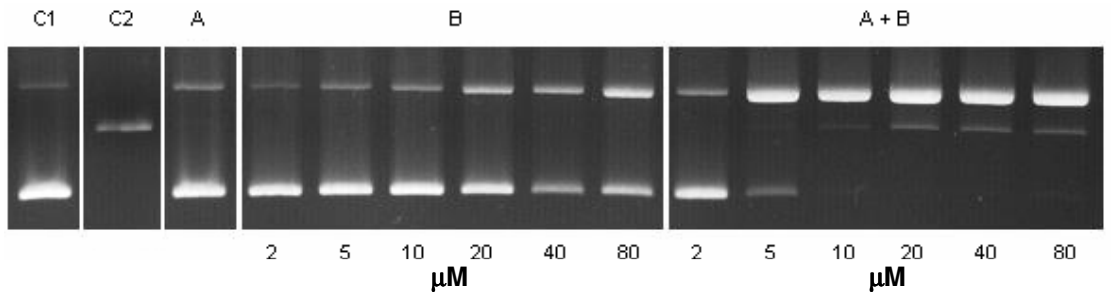


Fig. S2. Recovery of DNA after QDs-pUC18 DNA interaction: gel electrophoresis of supercoiled pUC18 DNA at 20 ˚C in 0.1 M cacodylate buffer (pH 6.0) under dark conditions. Lane 1, Marker; Lane 2, lineal DNA control; Lane 3, pUC18 DNA control; Lane 4, pUC18 DNA + 200 nM QDs; lane 5, pUC18 DNA + 200 nM QDs after passing through the DNA purification kit.



7	0	12	5	8	12	23	37	42	15	83	96	91	91	94	Form II (%)
0	100	0	0	0	0	0	0	0	0	0	4	9	9	6	Form III (%)
93	0	88	95	92	88	77	63	58	85	17	0	0	0	0	Form I (%)

Fig. S3. Representative plasmid cleavage assay of supercoiled pUC18 DNA with QD-filled micelles containing only PEG(2000)PE at 20 ˚C in 0.1 M cacodylate buffer (pH 6.0) under dark conditions. In the presence of A (100 nM QDs), B (2-80 μM) Cu(II) complex), and A + B after incubation period of 5 h. C1 corresponds to DNA control and C2 to DNA + EcoRI.

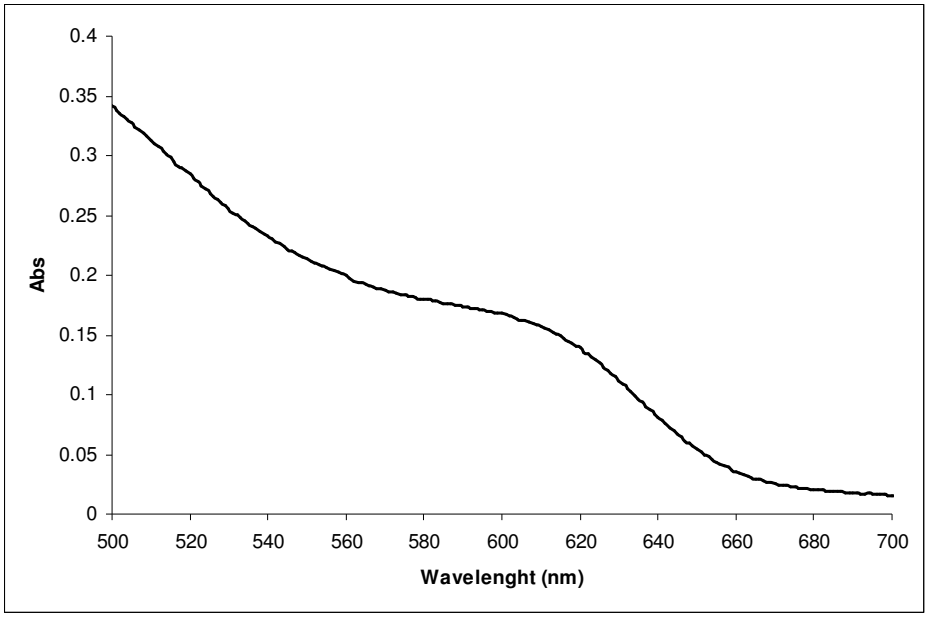


Fig. S4. Visible spectra of QDs-filled micelles.

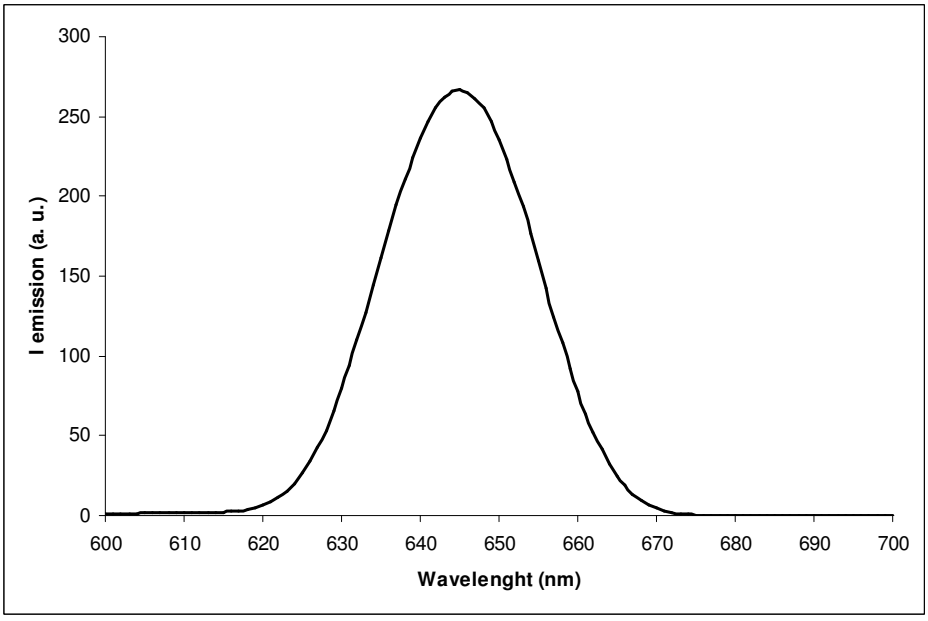
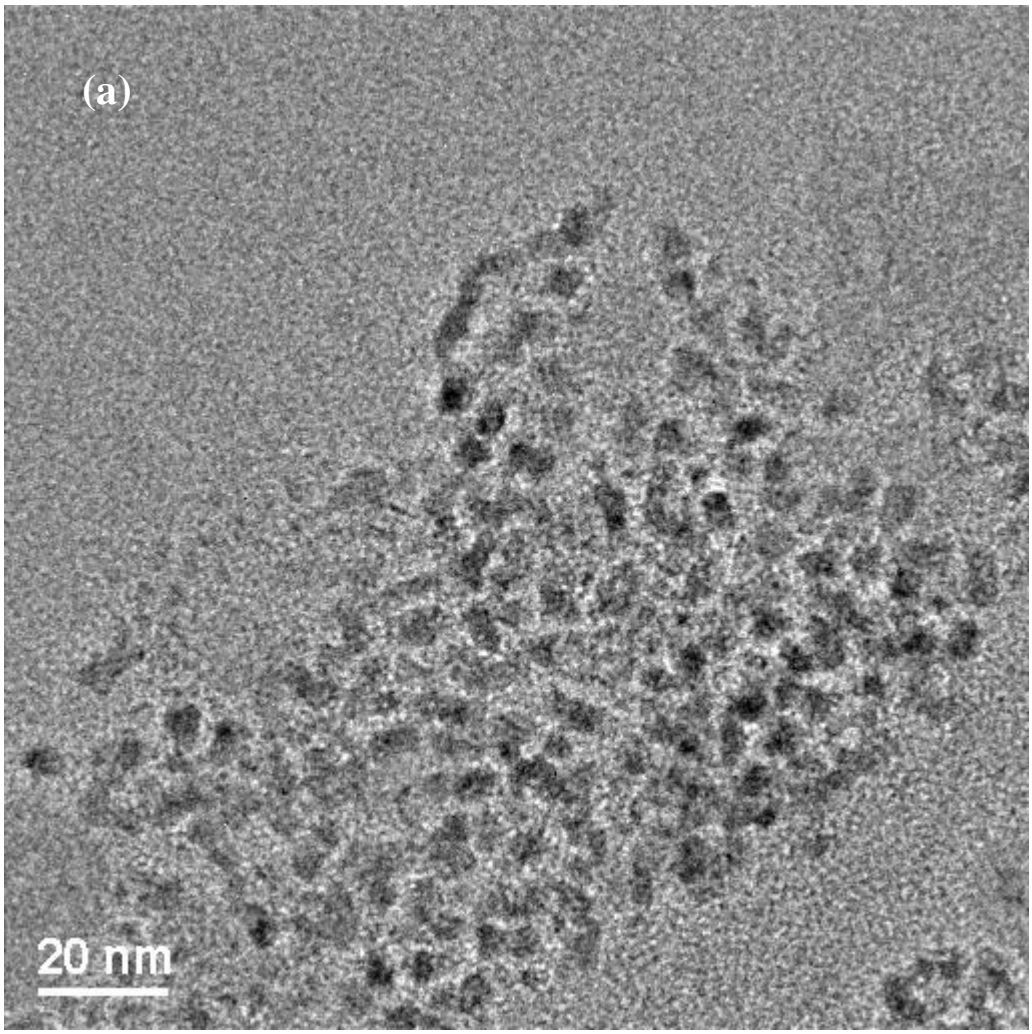


Fig. S5. Emission spectra of QD-filled micelles with excitation at 325 nm.



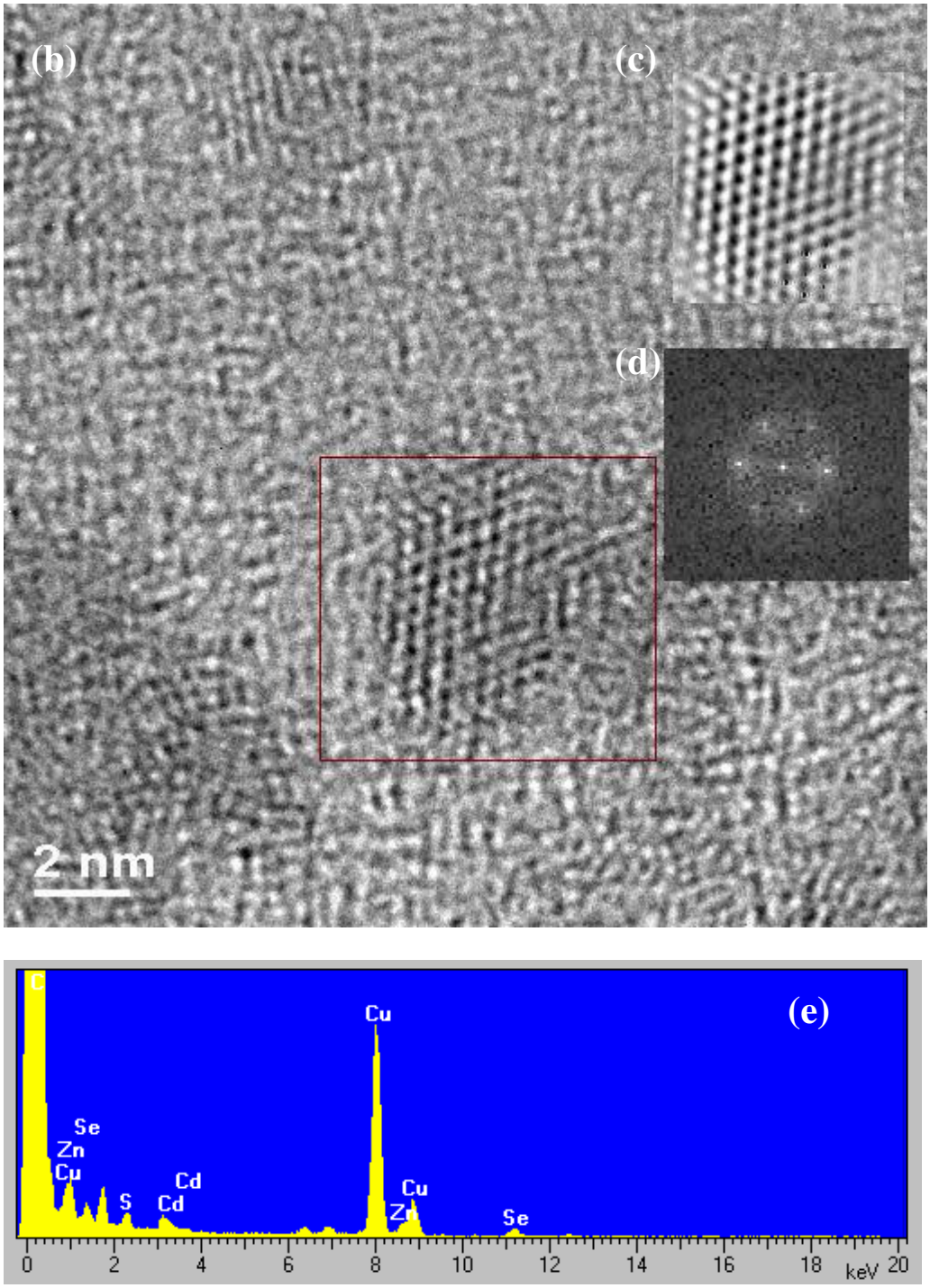


Fig. S6. High-resolution transmission electron microscopy (HRTEM) images of QD-filled micelles (a) and (b), fast Fourier transform (FFT) of the QD inside the box (c) and corresponding inverse FFT (IFFT) image (d), EDX spectra of these QDs (e).