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Quantum-dot-coated encoded silica colloidal crystals beads for multiplex coding

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Experiment

Materials: Poly (allylamine hydrochloride) (PAH), M_w 70,000 g mol^{-1}, poly (sodium 4-styrensulfonate) (PSS), M_w 70,000 g mol^{-1}, poly (acrylic acid) (PAA; M_w 45, 000 g mol^{-1}, 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and N-Hydroxysulfosuccinimide sodium salt (Sulfo-NHS) were purchased from Sigma Chemicals. 10×TE buffer (100 mM Tris-HCl, 10 mM EDTA, pH 8.0) and wash buffer (PBS, 10 mM phosphate sodium buffer solution, pH 7.4, 100 mM NaCl) from Shanghai Your Sun Biological Technology Co., Ltd. were all used as-received. Hybridization buffer (750 mM NaCl, 150 mM sodium citrate, pH 7.4) was self-prepared. Oligonucleotides were acquired from TaKaRa Biotechnology (Dalian) Co., Ltd.

Synthesis of water-soluble CdTe QDs: Mercaptopropionic acid stabilized CdTe QDs were synthesized according to published procedures.[S1] Using a cathodic stripping Te electrode, the CdTe precursor could be easily formed in the presence of 3-mercaptopropionic acid (MPA) at pH 9. Subsequently, the solution of CdTe precursor was heated in a water bath at 80°C under stirring, and CdTe QDs were gradually

crystallized. The heating time of precursor controlled the desired size of QDs. All CdTe QDs emitted PL at room temperature between 520 nm and 680 nm.

Synthesis of SCCBs: Various sizes of silica particles (200-300 nm in diameter) were prepared by hydrolysis-condensation of tetraethoxysilane in $EtOH/H_2O/NH_3$ mixture following the well-known Stöber process.[S2] The monodisperse and size-controlled SCCBs were fabricated by a microfluidic device,[S3] and their average size was about 200 μm. Then the SCCBs were treated with piranha solution (30% hydrogen peroxide and 70% sulfuric acid) for 6 hours in order to get the negatively charged beads.

Synthesis of QD-Coated SCCBs by Layer-by-Layer Assembly: PAH, PSS and PAA stock solutions were prepared in 0.5 M NaCl (1 mg mL^{-1}). The three-layer primer film was formed by the alternate adsorption of PAH and PSS from 1 mg mL^{-1} solutions onto negatively charged SCCBs by adding 0.5 mL of the PE solution to SCCBs (~100 spheres), allowing 20 min for adsorption, and then removing excess PE by four repeated water wash. Then the PE_3-coated SCCBs were dispersed into 0.2 mL of an aqueous dispersion of CdTe nanocrystals capped by mercaptopropionic acid (0.8 mmol L^{-1} with reference to Te) for 60 min. Subsequent PE and QD layers were deposited in identical fashion under the same experimental conditions.

Immobilization of probe DNA on to QD-Coated SCCBs: Probe sequences were covalently attached to the carboxyl groups at the surface of the QD-coated SCCBs by the two-step carbodiimide method.[S4,S5] The prepared QD-coated SCCBs (~100 spheres) with carboxyl groups were first added to 10 μL of amine-functionalized probe DNA (100 μM). Then 50 μL of freshly prepared 4.2 mg mL^{-1} EDC stock solution was added to the mixture. At the same time, the active intermediate was stabilized with 40 μL of sulfo-NHS (5 mg mL^{-1}). The samples were incubated for 2 h at room temperature under shaking in the dark and kept overnight at 4°C.

The melting temperatures of dsDNA: For multiplexed assays, the oligo lengths and sequences were optimized so that all probes had similar melting temperatures. The single stranded configuration of DNA shows a stronger absorbance of ultraviolet light at 260 nm than does the double stranded form. UV absorption experiments were performed using the ultraviolet-visible spectrophotometer equipped with

thermoelectrically controlled cell holder. The plots of the absorbance (measure at 260 nm) versus temperature were as follows:

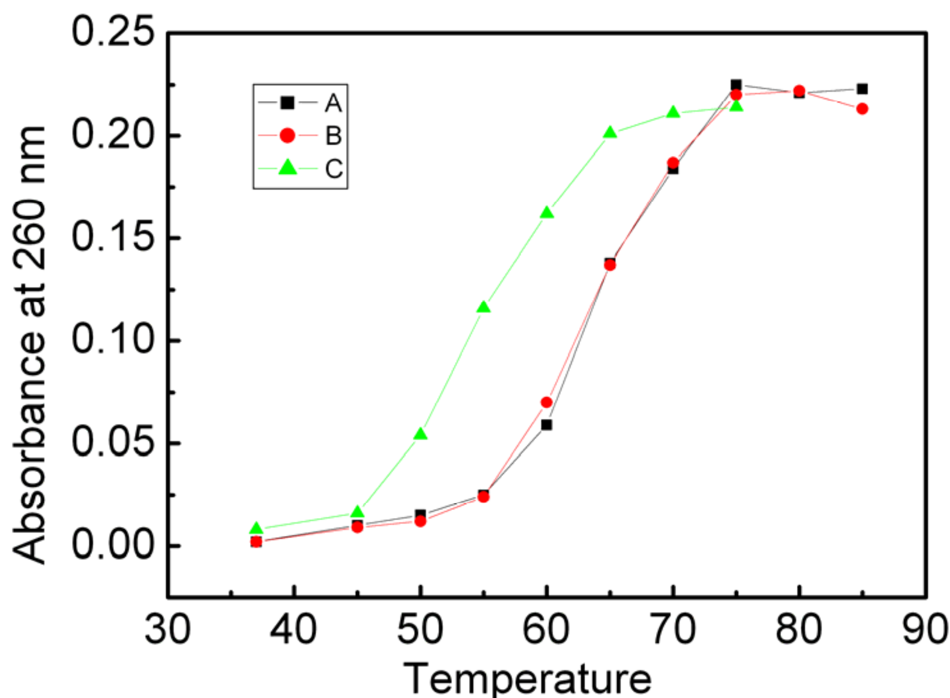

Fig. S1 The melting temperatures curve of DNA A (A), DNA B (B) and DNA C (C).

As seen from Figure 2, the melting temperatures of dsDNA involved in this work are 63, 63 and 54°C respectively. And the melting temperatures of dsDNA provided from TaKaRa Biotechnology Co., Ltd were 65, 65 and 56°C respectively. So the bioactivity of DNA is not impaired when being immobilized to the support surface. The melting temperatures of dsDNA involved in this work are between 54 and 63°C. So the reaction solution was incubated at 37°C for hybridization.

Selectivity of the DNA assay: After immobilization of probe DNA A on to the QD-coated encoded SCCBs, the carrier-probe conjugates were added to target DNA A (1 µM, with AMC label) and mismatched target (1 µM, with AMC label) for hybridization respectively. Two kinds of mixture were incubated with continuous shaking at 37°C for 30 min. Before fluorescence measurement, the beads were cleaned three times with wash buffer. As shown in Figure S1, the fluorescence intensity for one-base mismatched target was significantly weaker than that of the fully complementary target.

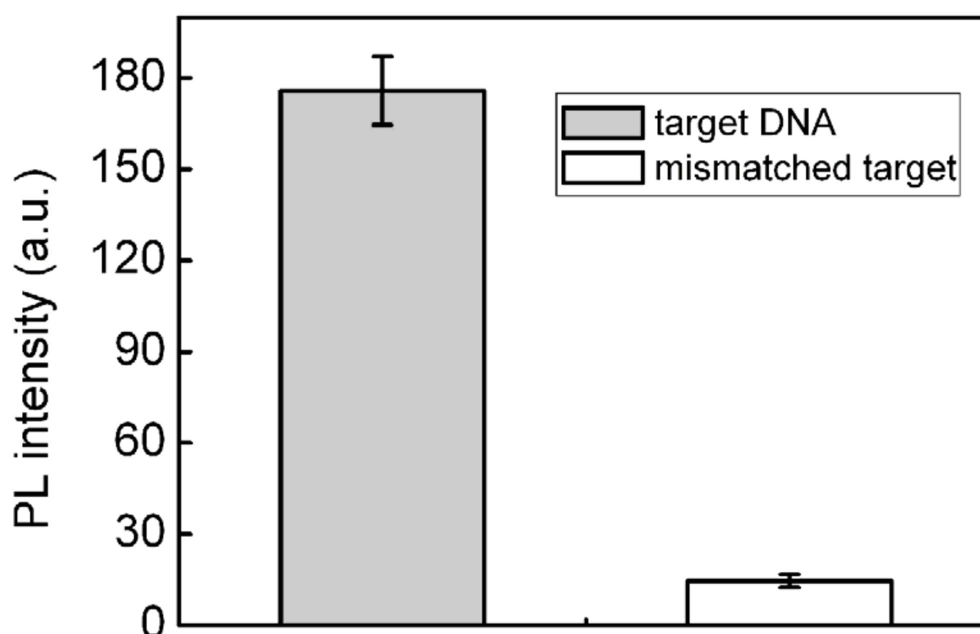

Fig. S2 Fluorescence signals of QD-coated encoded SCCBs after hybridization with target DNA and mismatched target respectively.

The specificity of hybridization assay was also examined by detecting the fluorescently signals of one kind of carrier-probe conjugate hybridization with the mixture of perfectly complementary target and one-base mismatched strand. Mismatched strand is labeled with AMC and no fluorescent dye was labeled to complementary target. In this experiment, fluorescence signals were weak and can be neglected. These assays showed a high degree of sequence specificity and a low level of nonspecific adsorption.

Multicolor imaging: Epi-illumination and fluorescence images were obtained with an inverted fluorescence microscope (Olympus IX71) and a CCD camera (MediaCybernetics Evolution MP 5.0). Broad-band excitation in the near-UV range (330-385 nm) was provided by a 100 W mercury lamp. A longpass dichroic filter (DM 400, Chroma Technologies, Brattleboro, VT) was used to reject the scattered light and to pass the Stokes-shifted fluorescence signals. Reflection and fluorescence spectra of SCCBs were recorded by an optical microscope equipped with a fiber-optic spectrometer (Ocean Optics, USB2000-FLG).

PL and reflection spectra of deposition of different-layer and different-sized CdTe on encoded SCCBs

The QD deposition process has very little effect on the resulting spectra of SCCBs.

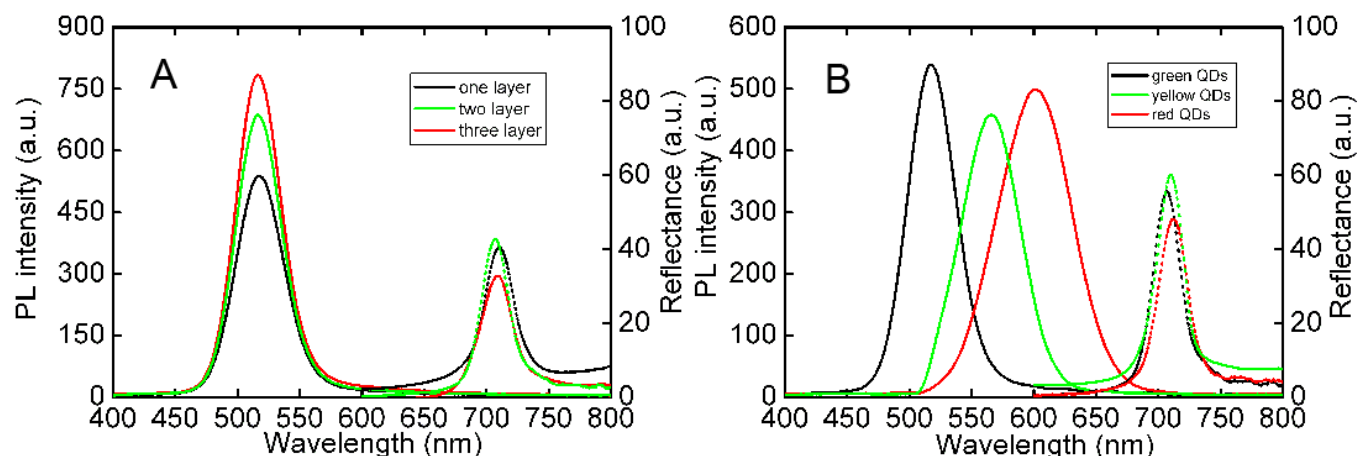

Fig. S3 The spectra of deposition of different-layer CdTe (A) and different-sized CdTe (B) on encoded SCCBs. The solid lines are the PL spectra of the QD-coated SCCBs, and the dashed lines are the reflection spectra of the QD-coated SCCBs.

SEM micrographs of SCCBs before and after coated with QDs

The formation of the multilayer films on the beads was visualized by scanning electron microscopy (SEM). There is no damage to the original structure of the SCCBs after coated with $(PE_3/CdTe\ QD)_3/PE_3$ and QDs on the beads surface are rather uniform (**Figure S4**).

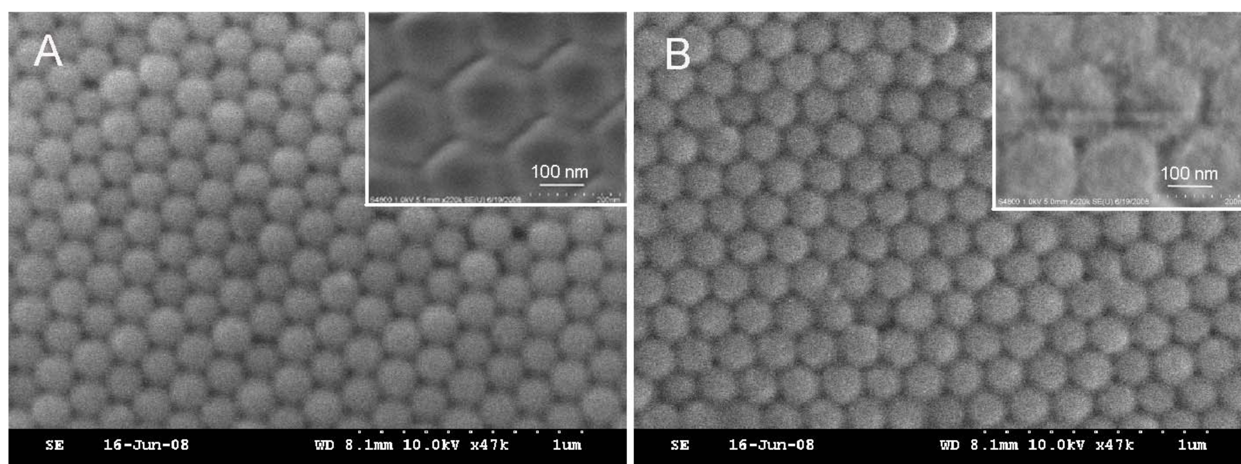

Fig. S4 SEM micrographs of SCCBs coated with $(PE_3/CdTe\ QD)_3/PE_4$, before(A) and after(B), illustrating the uniformity of the coating and the presence of the CdTe nanocrystals(Inset).

Sequences of oligonucleotides

The sequences of oligonucleotides were shown in Table S1. Probe DNA sequences were amine-functionalized with a $(CH_2)_6$ spacer at the 5' end. And target DNA sequences were labeled by AMC at the 5' end. For multiplexed assays, the oligo lengths and sequences were optimized so that all probes had similar annealing temperatures (Tm = 56–65°C) and hybridization kinetics (30 min).

Table S1. Sequences of oligonucleotides used in this work

base sequence	name
5'-NH_2-C_6-GCG GCC TTC ATC ATT TCG CTT TCA GAA CTG-3'	probe DNA A
5'-NH_2-C_6-CGG GTC AGG CGA TTG CTA ACC GTT TTA CTT-3'	probe DNA B
5'-NH_2-C_6-TGA TCG CGG TGT CAG TTC TTT-3'	probe DNA C
5'-CAG TTC TGA AAG CGA AAT GAT GAA GGC CGC-3'	target DNA A
5'-AMC-AAG TAA AAC GGT TAG CAA TCG CCT GAC CCG-3'	target DNA B
5'-AMC-AAA GAA CTG ACA CCG CGA TCA-3'	target DNA C
5'- AMC-CAG TTC TGA AAG CGG AAT GAT GAA GGC CGC-3'	mismatched target

References

S1 C. W. Ge, M. Xu, J. Liu, J. P. Lei, H. X. Ju, *Chem. Commun.* **2008**, *4*, 450.

S2 W. Stober, A. Fink, E. Bohn, *J. Colloid Interface Sci.* **1968**, *26*, 62.

S3 Y. J. Zhao, X. W. Zhao, C. Sun, J. Li, R. Zhu, Z. Z. Gu. *Anal. Chem.* **2008**, *80*, 1598.

S4 J. V. Staros, R. W. Wright, D. M. Swingle, *Anal. Biochem.* **1986**, *156*, 220.

S5 S. Derveaux, B. G. De Geest, C. Roelant, K. Braeckmans, J. Demeester, S. C. De Smedt, *Langmuir* **2007**, *23*, 10272.