Electronic Supplementary Information (ESI)

Highly sensitive detection of DNA methylation level by using quantum dots-based FRET method

Yunfei Ma [a,b], Honglian Zhang [b], Fangming Liu [b], Zhenhua Wu [b], Shaohua Lu[c], Qinhui Jin [b], Jianlong Zhao [b], Xinhua Zhong [a*], Hongju Mao [b*]

Synthesis of CdSe/CdS/ZnS core/shell/shell QDs

Chemicals. oleylamine (OAm,70%), 1-hexadecylamine (98%), 1-dodecylamine (>99%), oleic acid (99%), 1-octadecene(ODE, >95%) were obtained from Aldrich and used without further purification. All organic solvents such as hexane, dichloromethane, ethanol, methanol were of analytical grade and obtained from commercial sources and used as received. Deionized water was used throughout.

Stock solutions preparation. Se precursor solution (2.4 mL) was prepared by dissolving selenium (79.0 mg) in TOP (4.0 mL) and ODE (6.0 mL) via supersonication. The Cd precursor solution (0.1 M) was made by dissolving CdO (128.4 mg, 1 mmol) in oleic acid (2.0 mL) and ODE (8.0 mL) at 160 ^{o}C. The sulfur precursor solution (0.1 M) was prepared by dissolving sulfur in ODE at 120 ^{o}C. The Zn precursor solution (0.1 M) was obtained by dissolving $Zn(OAc)_2 \cdot 2H_2O$ (219.5 mg, 1 mmol) in ODE (10.0 mL) at 160 ^{o}C.

Synthesis of CdSe Core QDs. CdSe core was synthesized according to the method reported.[1] Typically, CdO (25.6 mg, 0.2 mmol), TOPO (1.3 g), oleic acid (1.0 mL), and ODE (4.0 mL) were placed in a 50 mL three-neck flask. The mixture was heated to 320 ^{o}C under N_2 protection. After cooling to 310 ^{o}C, Se precursor solution (2.4 mL) was injected into the above mixure. The reaction system was then set at ~270 ^{o}C in temperature for 10s for the growth of the CdSe core and finally cooled to ~60 ^{o}C to obtain the CdSe core. The as-prepared CdSe core with emission wavelengths of 550nm was further extracted by using 10.0 mL of hexane/CH_3OH (v/v, 1:1) as the extraction solvent and then purified by centrifugation with acetone. The precipitate was re-dissolved in hexane for further using.

Synthesis of CdSe/CdS/ZnS Core/Shell/Shell QDs. Oleic acid/OAm-capped oil-soluble CdSe/CdS/ZnS core/shell/shell QDs were prepared following the successive ion layer adsorption and reaction (SILAR) technique.[2,3] In a typical procedure, the purified CdSe core in hexane solution above, ODE (4.0 mL) and oleylamine (1.0 mL) were placed in a 50 mL three-neck flask. The reaction system was heated to 230 ^{o}C under N_2 protection, and then the Cd precursor stock solution was injected into it. After reacting for 10 min, an equimolar amount of S precursor stock solution was added into the reaction mixture for the growth of the CdS shell as the first monolayer deposited around the CdSe cores. Another CdS shell was then formed by adding Cd/S precursor solution alternately at approximately 10 min intervals. The volume of the precursor stock solution added in each cycle was the amount needed for a whole monolayer of CdS shell which was calculated from the respective volumes of concentric spherical shells with 0.35 nm thickness for one monolayer of CdS (e.g. 0.7, 1.0, 1.3 mL for the 1st, 2nd, and 3rd monolayer, respectively). After the formation of CdS layers, the reaction temperature was set at 200 ^{o}C for the overgrowth of ZnS shell by adding the Zn/S precursor stock solution into the reaction system at intervals of 20 min. Aliquots of the sample were taken to record their corresponding UV-vis and PL spectra. Finally, the reaction was terminated by allowing the reaction mixture to cool down to room temperature to obtain the desired CdSe/CdS/ZnS Core/Shell/Shell QDs. The prepared CdSe/CdS/ZnS QDs with emission wavelengths of 600nm were purified by the similar procedure to that for CdSe core QDs.

1 L. Qu and X.Peng, *J. Am. Chem. Soc*., 2002, **124**, 2049.

2 J. J. Li, Y. A. Wang, W. Guo, J. C. Keay,T. D. Mishima, M. B. Johnson and X. Peng, *J. Am. Chem. Soc*., 2003, **125**, 12567.

3 R. Xie, U. Kolb, J. Li, T. Basche and A. Mews, *J. Am. Chem. Soc.,* 2005, **127**, 7480.

gene	forward primer (5'–3')	reverse primer (5'–3')	product size (bp)	Sequencing primer
PCDHGB6	AATTTGAGGGGGATGTATATTT	biotin-AAAATCCCAAACCAAAAACT	171	GAATTTAAAATGAAAAAT
HOXA9	GAGTTGTGGTTGTTTTTTTTTG	biotin-ACCTTTCAAAACTCCTTCCTC	118	TTTTGGGTTTTGTATTTTTT
RASSF1A	AGTTTTYGTAGTGGAGAGTAGAG	biotin-CCRTATTTCTAAAAACCAACTT	204	AGTTTTYGTAGTGGAGAGTAGA

Table S1 Sequences of primers for amplifying the promoter regions in bisulfate-modified DNA.

gene	sample	QDs-based FRET method (%)	Pyrosequencing (%)
PCDHGB6	1# NT sample	11.11	7.19
	1# cancer sample	21.70	23.34
	2# NT sample	23.49	18.61
	2# cancer sample	56.77	49.27
	3# NT sample	25.81	16.33
	3# cancer sample	76.11	61.77
HOXA9	1# NT sample	15.69	14.86
	1# cancer sample	21.88	21.53
	2# NT sample	27.39	14.35
	2# cancer sample	80.39	63.61
	3# NT sample	25.22	19.23
	3# cancer sample	78.61	67.95
RASSF1A	1# NT sample	21.19	17.73
	1# cancer sample	12.04	8.59
	2# NT sample	19.38	15.12
	2# cancer sample	62.58	52.0
	3# NT sample	18.59	12.84
	3# cancer sample	55.10	37.25
	4# NT sample	25.44	24.67
	4# cancer sample	67.14	68.34

Table S2 Comparisons of detected methylation levels of parts of NT and cancer samples in different gene promoters between QDs-based FRET method and bisulfite pyrosequencing.

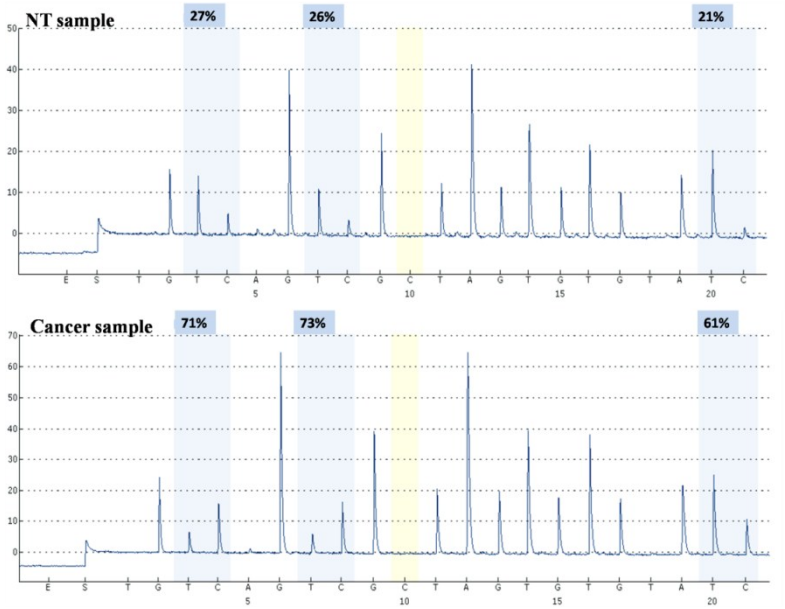

Fig. S1 Methylation level of the RASSF1A gene in one pair of NT and cancer samples as indicated by pyrosequencing.

Data. S1

Theoretical calculation of the Förster distance R_0

In this study, we estimate the Förster distance R_0 (the distance showing 50% FRET efficiency) by using the formula $R_0 = \left(\frac{9000(\ln 10)k_p^2 Q_D}{N_A 128\pi^5 n_D^4} I \right)^{1/6}$ according to the FRET model.

In the formula above, k_p^2 is the orientation factor (2/3 for randomly oriented dipoles); Q_D is the quantum yield of the amino-QDs donor (34% in our study); N_A is the Avogadro's number; n_D is the refractive index of the surrounding medium (~1.4 for biomolecules in aqueous solution). I, defined by equation $I = \int_0^\infty PL_{D-corr} \varepsilon_A(\lambda) \lambda^4 \, \mathrm{d}\lambda$, is the spectral overlap integral; it is a function of the normalized donor emission spectrum $PL_{D\text{-}corr}$, and the acceptor absorption spectrum (expressed as an extinction coefficient $\varepsilon_A(\lambda)$, $\varepsilon_A(647) = 250{,}000$ $cm^{-1}M^{-1}$ for A647).

Thus, by using the normalized UV–vis absorption and fluorescence emission spectra of A647 and amino-QDs (Fig. 2 in the manuscript), the quantum yield of QDs and the values of other parameters above, the Förster distance R_0 of ~6.91 nm is estimated according to the Förster formula. The donor–acceptor separation drived from electrostatic interactions in our sudy was considered much shorter than Förster distance R_0 and the FRET efficiency exceeding 50% was expected.