

Supporting Information for

Modular Design of Ultrahigh-Intensity Nanoparticle Probe for Cancer Cell Imaging and Rapid Visual Detection of Nucleic Acid

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Experimental Section

Table S1. DNA sequences used in this work.

Name	Sequences
S1	5'- AAA CGA GTC AGT T GTA TCG GAT AGT CTA TCC AGC ATG CAT ACT CCC AGT TTC ATC A-NH ₂ -3'
S2	5'- ACT ATC CGA TAC TGA ACT GGA TCC AGT TCT ACT GAC TCG TTT -3'
S3	5'- CTA GCA AGA TCC TAC TTT AGA CGG TTG ACT AAA CGA GTC AGT -3'
S4	5'- GTA TCG GAT AGT TAC TTT AGA CGG TTG ACT TAC AGG AGC TTA -3'
S5	5'- GGA TCT TGC TAG TTC CTT GAC GTC AAG GAT TAA GCT CCT GTA -3'
S6	5'- GTC AAC CGT CTA AAG TTT TTT T -SH-3'
S7	5'- ACT ATC CGA TAC ACT GAC TCG TTT T AAC ACC GGG AGG ATA GTT CGG TGG CTG TTC AGG GTC TCC TCC CGG TGT TT -3'
S8	5'- ACT ATC CGA TAC ACT GAC TCG TTT T GCT ACC GAG TGC AGC - 3'
S9	5'- CGG ACG TGG TGG CAC GCT GCA CTC GGT AGC - 3'
S10	5'- GTG CCA CCA CGT CCG TTTT -NH ₂ - 3'
S11	5'- CGG TCG TGG TGG CAC GCT GCA CTC GGA AGC - 3'
S12	5'- ACT TCC AGA CTC AAT ACG TAT GGG ACA CAA - 3'
S13	5'- SH-TTT TTT AAC ACC GGG AGG ATA GTT CGG TGG CTG TTC AGG GTC TCC TCC CGG TG - 3'

Reagents: All oligonucleotides were purchased from SBS Genetech. Co. Ltd. (China). And the DNA sequences were presented in Table S1. N-hydroxysulfosuccinimide (sulfo-NHS), 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), mercaptopropionic acid (MPA), tris(2-carboxyethyl)phosphine hydrochloride (TCEP), glutaraldehyde (GA), and (3-aminopropyl) trimethoxysilane (APS) were purchased from Sigma-Aldrich. Cadmium chloride hemipentahydrate (CdCl₂·2.5H₂O), tellurium powder, sodium borohydride (NaBH₄), and sodium dodecyl sulfate (SDS) were obtained from Sinopharm Chemical Reagent Beijing Co. Ltd. (China). Carboxyl-modified polystyrene (PS) microbeads (size: 500 nm) were from Baseline

chromatography technology development center (Tianjin, china).

All reagents were analytical grade and used without further purification. Double distilled, deionized water (DDW) was used throughout. The buffers involved in this work are as follows. DNA hybridization buffer: 10 mM PBS, 1.0 mM EDTA, and 0.1 M NaCl (pH 7.4).

SSC buffer, pH 7.0, is 0.15 M sodium chloride/ 0.015 M trisodium citrate.

Apparatus: The Nikon E800 inverted microscope with a Nikon Digital sight DS-U1 camera (Nikon, Japan) was employed for fluorescence microscopy (FM) imaging. Confocal fluorescence microscope (CFM) images were captured with a Leica TCS SP5 II confocal laser scanning microscope equipped with an Andor EMCCD camera (Germany). Transmission electron microscopy (TEM) imaging was taken with JEM-2000EX/ASID2 instrument (Hitachi, Japan). Scanning electron microscopy (SEM) imaging was performed by a JEOL JSM-6700F instrument (Tokoy, Japan).

Synthesis of Water-Soluble CdTe QDs: Synthesis of water-soluble CdTe QDs was performed following reported methods¹ with small modifications. The Te precursor solution was prepared by dissolving 80 mg Te powder and 50 mg NaBH₄ in 2 mL of water under the protection of highly pure nitrogen until the solution attained a clear, dark purple color. The Cd precursor solution was prepared by adding 33 μ L of MPA stabilizer and 36 mg CdCl₂·2.5H₂O into 63 mL of water and adjusting the pH to 8.0 with 0.2 M NaOH solution. The resulting clear solution was bubbled with highly pure nitrogen for 30 min. Then 250 μ L of Te precursor solution was quickly added into the Cd precursor solution. The mixture solution was refluxed at 100 °C for 8 h under the protection of nitrogen. The reaction was stopped when the solution became orange.

Preparation of DNA-CdTe QD Conjugates: Mercapto-modified DNA strands (HS-DNA S6) were conjugated to CdTe QDs via the cadmium-thiolate bonds. 100 μ L of 10 mM TCEP was added to 1.2 mL of 50 μ M HS-DNA S6 in acetate buffer (10 mM, pH 5.6) to activate mercapto groups for 1 h. Then 1.2 mL of freshly prepared CdTe QDs solution was added into the mixture solution and shook gently for 12 h. The DNA-CdTe QD conjugates solution was aged in salts (0.3 M NaCl, 10 mM acetate buffer) for 24 h. Excess reagents were removed by centrifuging at 20,000 rpm for 1 h. The precipitate was washed, and centrifuged again. The resulting DNA-CdTe QD conjugates were dispersed into 1.2 mL of 10 mM PBS (pH 7.4) and stored at 4 °C.

Preparation of the Modular NP Probe: Firetly, 10 μ L of carboxyl-modified PS microbeads were washed three times with 500 μ L of 0.1 M imidazole buffer (pH 6.8). For the immobilization of DNA, the carboxy-modified PS microbeads were activated with 10 mM EDC/sulfo-NHS in imidazole buffer for 2 h and subsequently incubated with a 10 μ M solution of DNA S1 in 1 mL of 10 mM PBS (pH 7.4) for 24 h at 37 °C under gentle shaking. S1-modified PS microbeads were centrifuged at 3000 rpm for 3 min to remove unbound DNA. The precipitate was washed twice with 1 mL of 10 mM PBS (pH 7.4) and then resuspended in 1 mL of PBS.

Secondly, 1 mL of 1 μ M DNA S2, S3, S4 and S5 were added to 1 mL S1-modified PS microbeads solution. DNA nanowires grew on the surface of the PS microbeads after annealing

the mixture solution in a water bath by cooling from 90 °C to 18 °C over 12 h. Then 1 mL of 10 μM DNA S7 (for the construction of cellular probe) or S8 (for DNA detection) was added into the mixture solution and incubated at 18 °C for 2 h to bind to the end of DNA nanowires.

Thirdly, 1.2 mL DNA-CdTe QD conjugates was added into the mixture solution and incubated at 18 °C for 24 h with gentle shaking. The as-prepared probe solution were centrifuged at 3000 rpm for 3 min. The precipitate was washed twice with 1 mL of 10 mM PBS (pH 7.4) and then resuspended in 200 μL PBS before use.

Cell Studies: Ramos cells (CRL-1596, B-cell, human Burkitt's lymphoma) and CCRF-CEM cells (CCL-119, T lymphoblast, human acute lymphoblastic leukemia) were obtained from the American Type Culture Collection. The cells were cultured in RPMI-1640 medium containing 10% fetal bovine serum and 100 units/mL penicillin-streptomycin in cell culture flasks at 37 °C. The amount of cells was determined by using a hemocytometer prior to each experiment. After removing the cell culture medium, cells were washed twice with Dulbecco's phosphate-buffered saline, supplemented with 5 mM glucose, and then resuspended in 1 mL Dulbecco's phosphate-buffered saline (approximately 5×10^5 cells mL⁻¹).

For cellular imaging, 20 μL of probe solution was added into Ramos sample and incubated at room temperature for 20 min. 10 μL of incubated Ramos cells solution was deposited onto a microscope slide and covered with a standard microscope slide. The cells were observed by a Nikon E800 upright microscope.

Construction of the DNA biosensor: The process for modifying capture DNA to glass slides was performed as follows. Glass slides (18 mm × 18 mm × 0.17 mm) were dipped in freshly prepared piranha solution (H₂SO₄ : H₂O₂ = 3 : 1) for 1 h and then rinsed with DDW, and dried at 100 °C for 30 min. The cleaned glass slides were immersed in a solution of 10 mM sodium acetate/acetic acid buffer (pH 5.0) containing 5% (v/v) APS at 80 °C for 2 h and then rinsed with DDW, and dried at 100 °C for 1 h. The amino-modified glass slides were immersed in a 2.5% (v/v) GA solution at room temperature for 1 h and rinsed with DDW. Subsequently, The GA-activated glass slides were immersed in a 10 mM PBS buffer (pH 7.4, 100 mM NaCl) containing 10 nM capture DNA S10 at room temperature for 1 h, washed twice with 2 × SSC buffer (pH 7.0) containing 0.01% (v/v) SDS. The capture DNA-modified glass slides were then dipped in a 100 mM glycine solution at room temperature for 1 h to block the active binding sites.

DNA analysis was performed by hybridizing target DNA S9 and the NP probe to the capture DNA-modified glass slides. Briefly, capture DNA-modified glass slides were dipped in 2 mL target DNA solution at 37 °C for 1 h. The unbound target DNA strands were washed with 2 × SSC buffer (pH 7.0) containing 0.01% (v/v) SDS. The glass slides were then incubated with 2 mL 100-fold-diluted probe solution at 37 °C for 1 h. The unbound probes were also washed away. Finally, the glass slides were observed by a Nikon E800 upright microscope.

Calculation of the Mean QD Coverage: The mean QD coverage of the NP probe was calculated as follows. Because the PS microbeads have a density of 1.03 g cm⁻³ and an average diameter of

505 nm, the average mass of one PS microbead is estimated to be 6.94×10^{-14} g. Based on a density of 0.025 g mL^{-1} (w/v) for PS microbeads in aqueous solution, the concentration of PS microbeads store solution was estimated to be $3.60 \times 10^{11} \text{ particles mL}^{-1}$.

The average diameter of PS microbeads: $d_{\text{PS}} = 505 \text{ nm}$

The volume of one PS microbead: $V_{\text{PS}} = 4/3\pi r^3 = 6.74 \times 10^{-14} \text{ cm}^3$

The average mass of one PS microbead: $m_{\text{PS}} = \rho_{\text{PS}} \times V_{\text{PS}} = 1.03 \times 6.74 \times 10^{-14} = 6.94 \times 10^{-14} \text{ g}$

The concentration of PS microbeads solution: $C_{\text{PS}} = 0.025 \text{ g mL}^{-1} / (6.94 \times 10^{-14} \text{ g})$
 $= 3.60 \times 10^{11} \text{ particles mL}^{-1}$

The total amount of CdTe QDs loaded on PS microbeads was detected by dissolving the probe in a strongly acidic solution and then detecting cadmium ions levels by differential pulse anodic stripping voltammetry (DPASV) method. A calibration curve for different concentrations of CdTe QDs solution was obtained using DPASV method as follows. Briefly, the concentration of the as-prepared CdTe QDs solution was obtained to be $8.99 \times 10^{-6} \text{ mol L}^{-1}$ according the adsorption peak in Figure 1C and Peng's empirical equations.² 10 μL of different concentrations of CdTe QDs solution (or the probe solution) were dissolved in 20 μL of 0.5 M HNO_3 solution for 10 min. Then the acidic solution containing Cd^{2+} was transferred into an analytical cell containing $1.8 \times 10^{-4} \text{ M}$ Hg^{2+} and the final volume was adjusted to 2 mL with 0.01 M acetate buffer (pH 5.3). DPASV detection was performed on a CHI 660C electrochemical working station (Texas, USA) using a three-electrode system consisted of a platinum wire auxiliary electrode, an Ag/AgCl reference electrode and a glass carbon working electrode (GCE). Mercury film was in situ prepared on the surface of a GCE with a deposition time of 300 s and a deposition potential of -1.2 V . After a rest time of 20 s, a anodic stripping peak of Cd^{2+} located at -0.56 V were obtained.

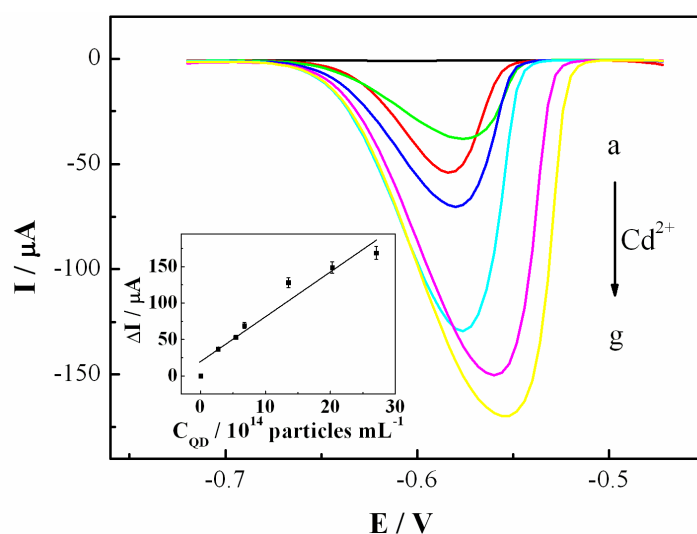


Figure S1 DPASV signals of Cd^{2+} corresponding to different concentrations of CdTe QD solution. Inset: calibration curve for the CdTe QD solution.

Figure S1 showed the DPASV signals of Cd^{2+} corresponding to different concentrations of CdTe QDs solution. The electrochemical signals of Cd^{2+} increased with increasing concentrations of CdTe QDs solution. CdTe QDs solution could be quantified over a concentration range of 2.7×10^{14} to 27.1×10^{14} particles mL^{-1} . The calibration curve was calculated to be $\Delta I = 6.16 C_{\text{QD}} + 19.79$ (ΔI is the relative anodic stripping peak currents of Cd^{2+} , μA ; C_{QD} is the concentration of CdTe QDs solution, 10^{14} particles mL^{-1} ; $n = 7$; $R = 0.9699$; the inset in Figure S2). Based on the calibration curve, the concentration of CdTe QDs loaded on PS microbeads could be calculated to be 2.36×10^{15} particles mL^{-1} . Furthermore, the probe was prepared by incubating 10 μL of PS microbeads store solution with CdTe QDs solution and the final volume was adjusted to 200 μL . With a view to the decrease in PS microbeads concentration in multiple centrifugal separation process, the mean QD coverage of the NP probe was estimated to be more than 1.31×10^5 particles per microbead.

The relative anodic stripping peak current of Cd^{2+} dissolved from the probe: $\Delta I = -165.26 \mu\text{A}$

The concentration of CdTe QDs loaded on PS microbeads: $C_{\text{QD}} = 2.36 \times 10^{15}$ particles mL^{-1}

The number of CdTe QDs loaded on PS microbeads: $n_{\text{QD}} = C_{\text{QD}} \times V_{\text{QD}} = 2.36 \times 10^{15}$ particles $\text{mL}^{-1} \times 10 \mu\text{L} = 2.36 \times 10^{13}$ particles

The number of PS microbeads: $n_{\text{PS}} = C_{\text{PS}} \times V_{\text{PS}} = 3.60 \times 10^{11}$ particles $\text{mL}^{-1} \times 10 \mu\text{L} \times 10 \mu\text{L} / 200 \mu\text{L} = 1.80 \times 10^8$ particles

The mean QD coverage: $n_{\text{QD}} / n_{\text{PS}} = 2.36 \times 10^{13}$ particles / $(1.8 \times 10^8 \text{ particles}) = 1.31 \times 10^5$ particles per microbead

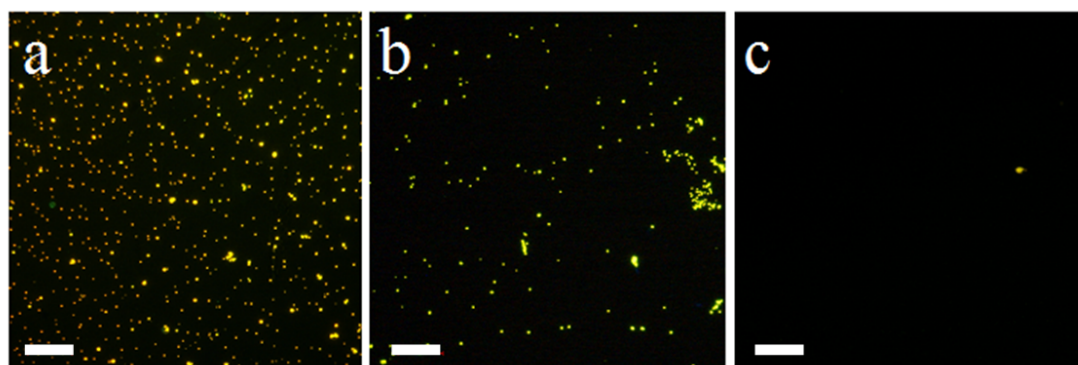


Figure S2 FM images of the DNA biosensor for the detection of 5 pM target DNA S9, two-base mismatched DNA S11 and noncomplementary DNA S12. Exposure time: 0.25 s; scale bar: 10 μm .

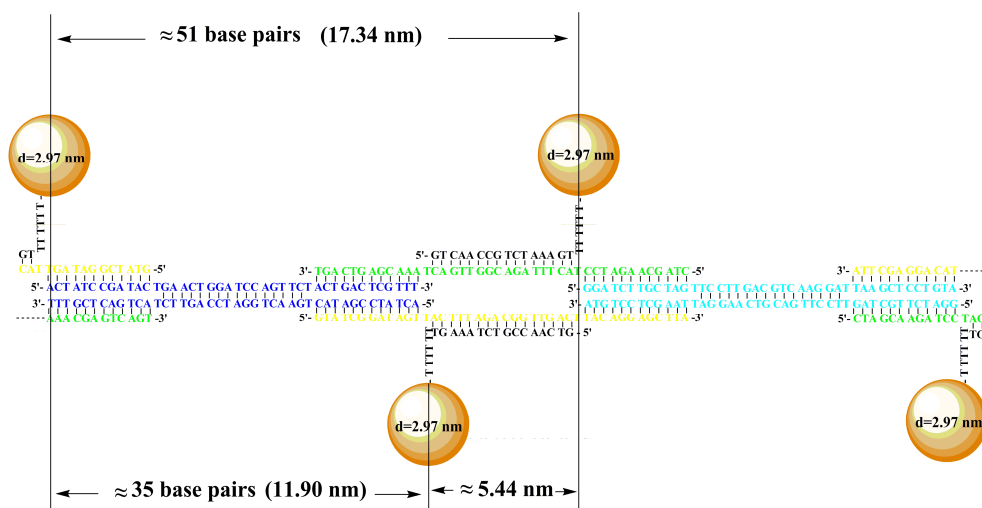


Figure S3 The relative position of quantum dots in the DNA nanowire, calculate the distance between two adjacent quantum dots according to the spacing between the DNA bases.

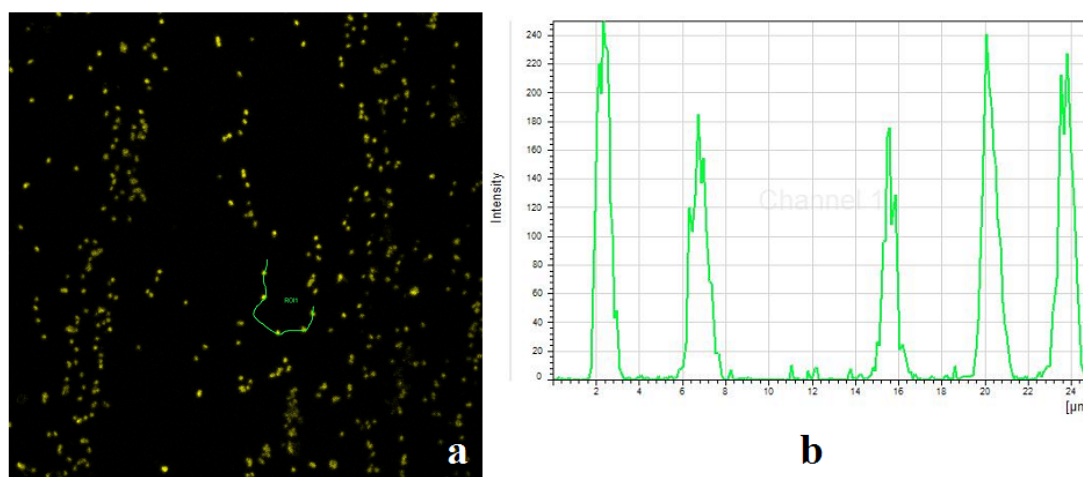


Figure S4 (a)Confocal laser scanning microscope photograph of the NP probe (DNA nanowire-CdTe quantum dot@ polystyrene microsphere probe); (b) Relative intensity of five light spot in green curve. The standard deviation of the fluorescence intensity between the NP probe particles is 15.59%. The length of the DNA nanowire (n, repeat unit number) can not be accurately calculated. QD coverage of the NP probe was calculated to be 1.31×10^5 particles per microbead (see Supporting Information for details).

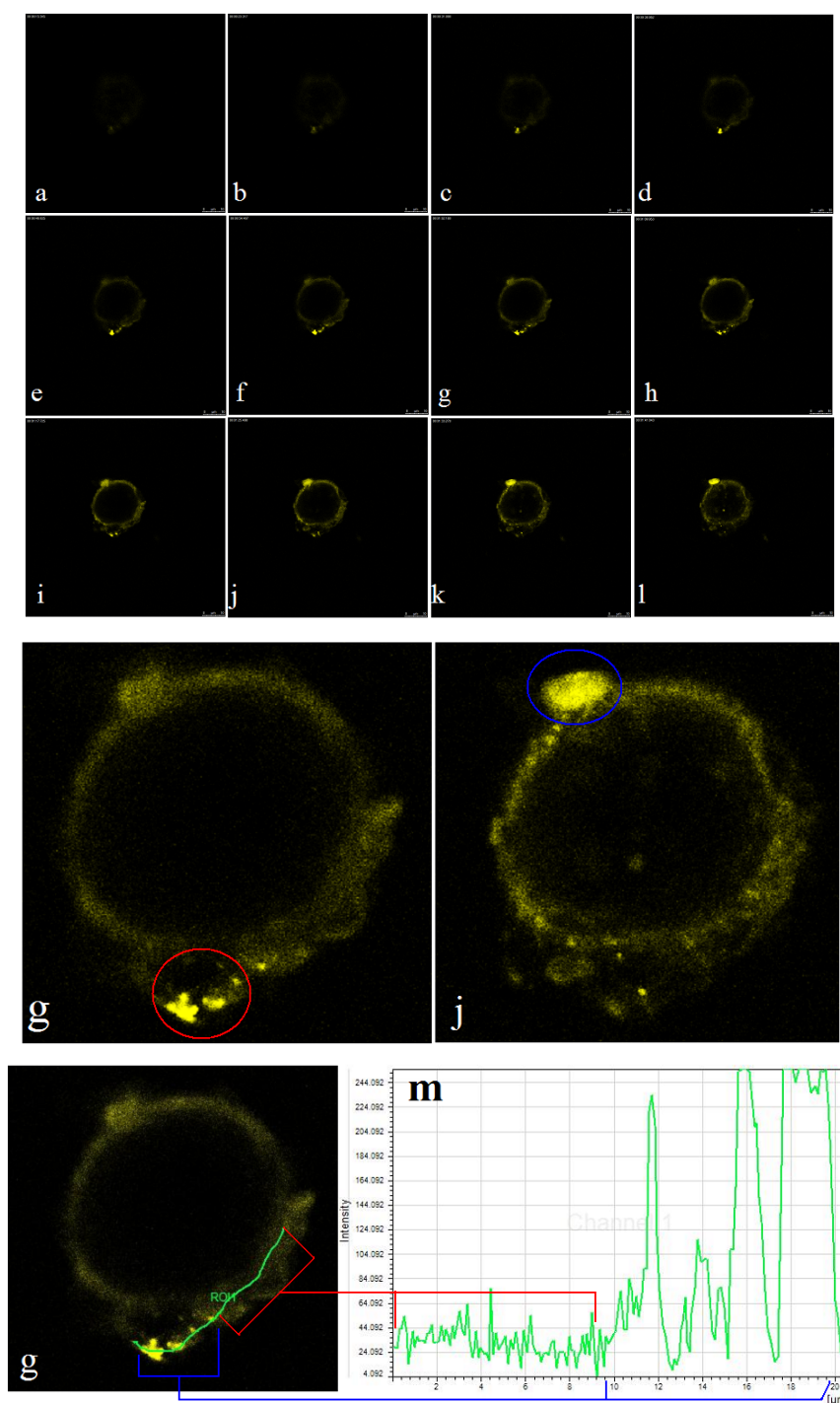


Figure S5 Z-axis section images of single Ramos cell (a-l); the amplificatory images of (g) and (j); (m) the relative fluorescence intensity between NP probe (DNA nanowire-CdTe quantum dot@ polystyrene microsphere) and standard aptamer/CdTe QD probe in g.

References

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