Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information

Cell-Activatable CdSe Fluorescence Probe for Dual-Targeted Imaging and Drug Application

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1. Materials and Methods

1.1. Materials and Apparatus

selenium (99.9%, powder), sodium borohydride, $CdCl_2$, 2-aminoethanethiol (AET), 3, 3'-dithiodipropionic acid, dimethyl sulfoxide (DMSO), Folic acid, Doxorubicin (Doxo), N-hydroxysuccinimide (NHS), ethanediamine, glutathione (GSH), and 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) were purchased from Sigma-Aldrich and used without further purification. 0.1 M PBS buffer (pH 7.4) were prepared according to the standard methods. All other reagents were of analytical grade. Double distilled water was used for all experiments.

Cells: Ramos cells (CRL-1596, B-cell, human Burkitt's lymphoma) and MCF-7 cells were obtained from Chinese Academy of Medical Sciences. The cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 100 IU/mL penicillin-Streptomycin. The cell density was determined using a hemocytometer, and this was performed prior to any experiments. After which, ~1 million cells dispersed in RPMI 1640 cell media buffer were centrifuged at 3000 rpm for 5 min and redispersed in cell media three times and were then redispersed in 1 mL cell media buffer. During all experiments, the cells were kept in an ice bath at 4 ºC.

Transmission electron microscopy (TEM) images were recorded using a JEOL JSM-6700F instrument (Hitachi). Photoluminescence (PL) spectra were obtained on an RF-540 spectrophotometer (Shimadzu).Scanning Laser Confocal Microscopy was performed on a Leica TCS SP5 II apparatus. The specimen were excited using 488 nm laser beam (Argon ion laser), observed using an HCX PL APO 40/0.85 objective, and the fluorescence images in the range 585-660 nm were recorded along with the transmitted-light image.

1.2. Synthesis of CdSe -NH_2 nanocrystals

Briefly, 0.05 g of selenium powder and 0.037 g of sodium borohydride were added to a small flask, then 4 mL of ultrapure water was added. The solution was degassed, refilled with nitrogen and heated to 80 ℃. After the selenium powder disappeared completely, the resulting clear NaHSe of 0.1 M was obtained.

Colloidal CdSe-NH_2 nanocrystals were prepared as described with a slight modification [s1]. Briefly, freshly prepared 0.1 M NaHSe solution was added to 1.25 mM N_2-saturated $CdCl_2$

solution, then 2-aminoethanethiol (AET) as the stabilizing agent was added, and the pH was adjusted to 5.6-5.9. The molar ratio of Cd^{2+}/AET/HSe- was fixed at 1:2.4:0.5. After the mixture was vigorously stirred for 10 min, it was refluxed for 3 h to control the growth of the CdSe nanocrystals.

1.3. Conjugation of 3, 3'-dithiodipropionic to CdSe-NH_2 nanocrystals

The solution of EDC (0.1 mol/L, 100 μL) and NHS (0.025 mol/L, 100 μL) were added to 3 mL of 3, 3'-dithiodipropionic acid dissolved in dimethyl sulfoxide (DMSO), and incubated at 25 ºC for 30 min. Then 1 mL of the QDs solution was added to the mixed solution and incubated at 4 ºC for 12 h. After isolation of impurity through ultrafiltration, the resulting QDs were redispersed in 1 mL of water.

1.4. Amino-functionalization of folic acid

Folic acid (0.0812 g) was dissolved in 4 mL of ultrapure water, then EDC (0.1 mol/L, 100 μL) and NHS (0.025 mol/L, 100 μL) were added to the solution and incubated for 30 min. After 1 mL of ethanediamine was added and shaked gently for 12 h at room temperature, the solution was distilled to remove superfluous ethanediamine and water, and dissolved in 10 mL of PBS (pH 7.4).

1.5. Activation of doxorubicin hydrochloride

The doxorubicin hydrochloride (1.1 mg) was dissolved in 1 mL of PBS (pH 8.4). Then the resulting solution was centrifuged, and the solid pallet was dissolved in 1 mL of DMSO.

1.6. Synthesis of the doxorubicin-QDs-folate nanoconjugate

EDC (100 μL, 0.1 mol/L) and NHS (100 μL, 0.025 mol/L) were added to the solution of CdSe -dithiodipropionic acid conjugate, and incubated for 30 min. Then the solution was added to the mixture of amino-functionalized folic acid (1 mL) and doxorubicin hydrochloride (1 mL), and incubated at 4 ºC for 12 h. After ultrafiltration two times, the resulting doxorubicin-QDs-folate nanoconjugate were redispersed in 1 mL of PBS (10 mM).

1.7. The doxorubicin-QDs-folate nanoconjugate for targeted imaging and therapy of cancer cells

500 μL of Ramos cells were centrifuged at 2000 rpm for 3 min and redispersed in 200 μL of PBS (10 mM). Then 200 μL of the above QDs probe was added to the cells buffer and incubated at 37 °C for 30 min with gentle shaking, then the solution was centrifuged, washed, and resuspended in buffer.

1.8. Microscopy Sample Preparation imaging

For confocal microscopy imaging, 10 μL sample solution was deposited onto a microscope slide and covered with a standard microscope slide. The fluorescent QDs and labeled cancer cells were observed with HCX PL APO 40/0.85 objective. The apoptosis of cancer cell were observed by optical microscopy (OM) and fluorescent microscopy (FM). 380-420 nm excitation light source were employed for this experiment. The Nikon E800 inverted microscope with a Nikon Digital sight DS-U1 camera was employed for OM and FM imaging.

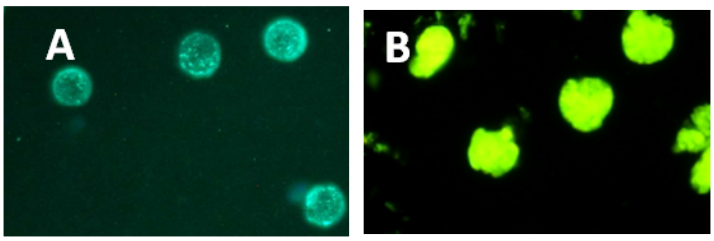

Figure S1: (A) Fluorescence microscopy images of FR(+) cells stained by green fluorescein and preincubated with excess free folicacid, before incubation with the activatable QDs probe; (B) FR(-) cells were treated with doxorubicin-QDs-folate nanoconjugate

References

[s1] S. Santra, C. Kaittanis, J. M. Perez, Mol. Pharmaceutics, 2010, 7, 1209.