Supporting Information

Ratiometric and Time-Resolved Fluorimetry from Quantum Dots Featuring Drug Carriers for Real-Time Monitoring of Drug Release in Situ

Rijun Gui,[a,b] Ajun Wan,[b,c,*] Yalei Zhang,[c] Huili Li,[a,*] and Tingting Zhao[b]

[a] School of Pharmacy, Shanghai Jiao Tong University, Shanghai 200240, P.R. China
[b] Department of Chemistry, School of Chemistry and Chemical Engineering, Shanghai Jiao Tong University, Shanghai 200240, PR China
[c] State Key Laboratory of Pollution Control and Resources Reuse, National Engineering Research Center of Facilities Agriculture, Tongji University, Shanghai 200092, P.R. China

* Fax: +86 21 5474 5706. Tel.: +86 21 3420 1245. E-mail address: lihl@sjtu.edu.cn (H. Li); wanajun@sjtu.edu.cn (A. Wan).

Part S1. Synthesis of MPA-capped CdSe/ZnS QDs (MPA-QDs)

Lipophilic hexadecylamine (HDA)-capped CdSe/ZnS QDs (HDA-QDs) were first prepared by using the reported method,[1] and then modified by MPA to achieve water solubility (MPA-QDs) based on a surface-ligand exchange.[2,3] In detail, 1 mL of HDA-QDs dissolved in toluene was selected to react (12 h) with 1 mL of MPA in the dark. After surface-ligand exchange between HDA and MPA, these QDs were transferred from toluene to an aqueous phase by adding NaOH solution (1 M) and shaking. The aqueous phase was separated, and the excess of MPA was removed from water-soluble QDs by the precipitation of QDs with acetone, centrifugation (12000 rpm, 15 min), followed by re-dispersion of MPA-QDs in Milli-Q water for subsequent experiments.

Reference

(1) Čapek, R. K.; Lambert, K.; Dorfs, D.; Smet, P. F.; Poelman, D.; Eychmüller, A.; Hens, Z. *Chem. Mater.* **2009**, *21*, 1743.
(2) Ruedas-Rama, M. J.; Hall, E. A. H. *Analyst* **2008**, *133*, 1556.
(3) Patolsky, F.; Gill, R.; Weizmann, Y.; Mokari, T.; Banin, U.; Willner, I. *J. Am. Chem. Soc.* **2003**, *125*, 13918.

Part S2. Preparation of PEG-NH_2 conjugated MPA-QDs (QDs-PEG)

Under the action of ultrasonic, 1.0 mg/mL of MPA-QDs dispersed in water was treated with 10 mg/mL of 6-arm poly(ethylene glycol)-amine (PEG-NH_2) for 10 min. After that, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) hydrochloride was added (10 mM), and the resulting mixed solution was sonicated for another 1 h, followed by adding EDC (40 mM) and N-hydroxysuccinimide (NHS, 20 mM), stirring for 24 h. The reaction was terminated by adding mercaptoethanol. The final reaction solution was further purified by centrifugation (12000 rpm) for 1 h, and the supernatant was collected to obtain products (QDs-PEG), which were properly diluted with PBS to prepare QDs-PEG aqueous suspension (with different pHs) for further uses in following experiments.

Supporting Information

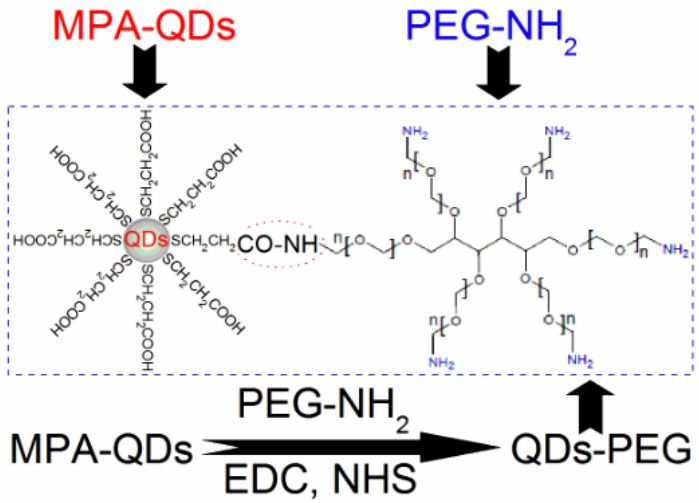

Figure S1 Schematic illustration of the chemical structure and preparation procedure of QDs-PEG based on a carboxy-amine coupling reaction.

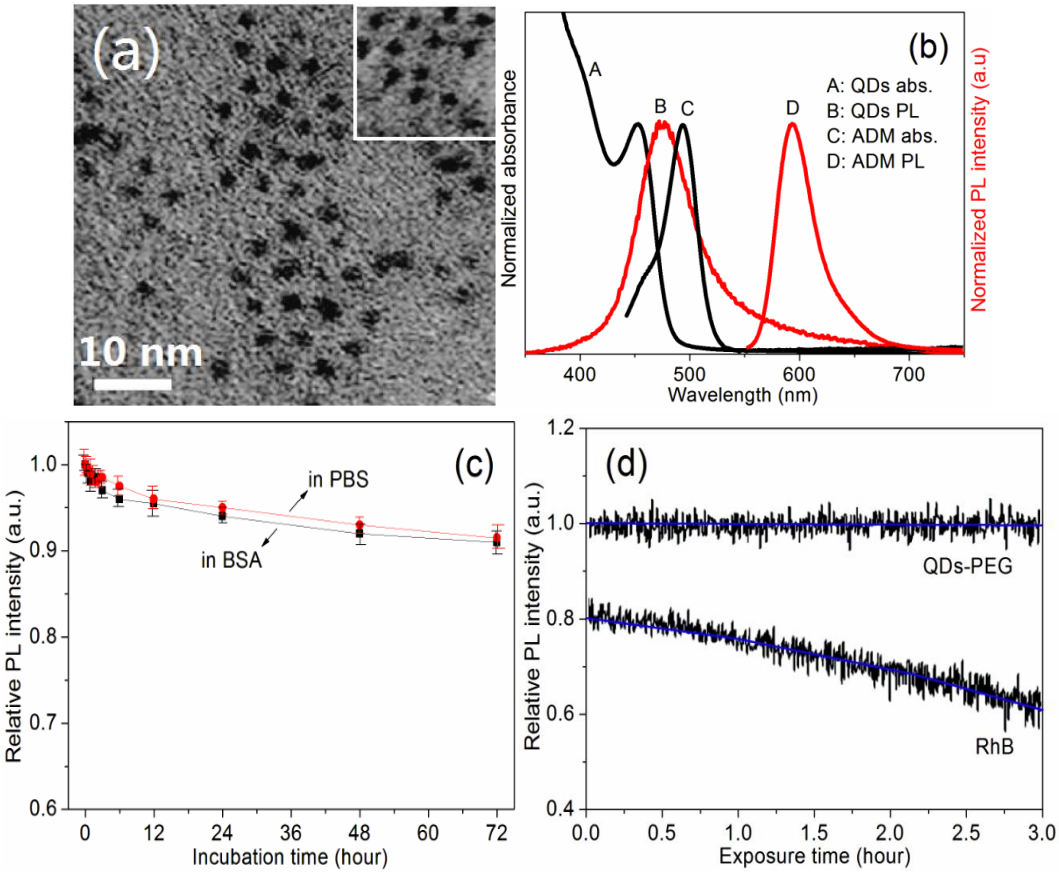

Figure S2 (a) Wide-filed transmission electron microscope (TEM) images of MPA-QDs (inserted) and QDs-PEG. (b) Normal UV-vis absorption and PL emission spectra of QDs (from QDs-PEG) and ADM. (c) Colloidal stability of QDs-PEG in 1 mM of PBS (pH 7.4) and 10 mM of BSA at 37 ^{o}C. (d) Photostability of QDs-PEG and Rhodamine B (RhB) (a commercial fluorescent dye) in PBS (1 mM, pH 7.0) at 25 ^{o}C, continuously excited with a 50 mW of 450 nm (475 nm) laser for QDs-PEG (RhB). PL intensities of QDs-PEG (RhB) were measured at different incubation or exposure (excitation) times.

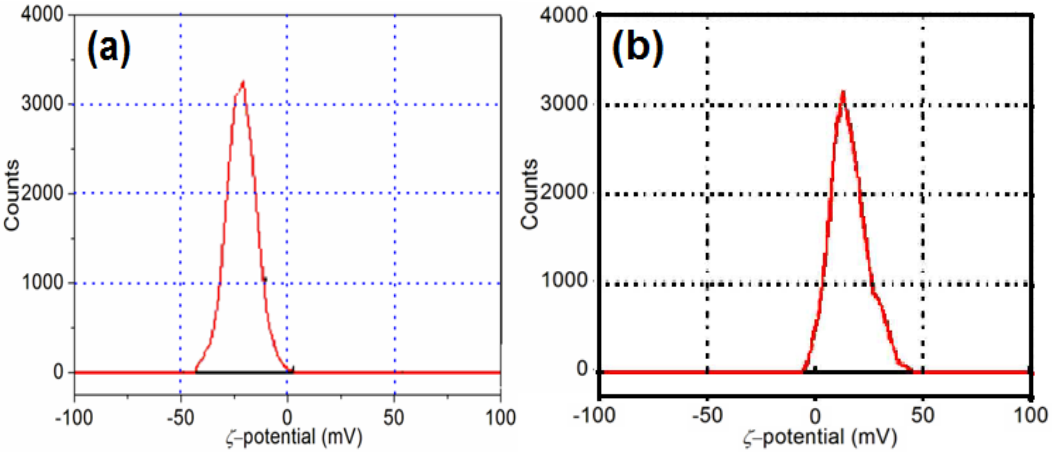

Figure S3 Zeta (ζ) potential of the as-prepared QDs-PEG conjugates (a) and QDs-PEG-ADM drug carriers (b). The two peaks are centerted at -20.5 mV and +14.3 mV, respectively.

Part S3. The calculation of LC and LE of ADM in QDs-PEG-ADM

Loading content (LC) and efficiency (LE) of ADM in QDs-PEG were provided as below. In a typical experiment, 1.0 mg/mL of QDs-PEG was mixed with 2.0 mg/mL of ADM in 1 mM of PBS (pH 7.4), and then stirred at room temperature for 12 h in the dark. The reaction products were separated by dialysis (MWCO of 3500) frequently against water for 48 h, together with the bath solution changed with water every 4 h. The as-obtained products were further purified by lyophilizing. The LC and LE were measured by dispersing final products (QDs-PEG-ADM) into PBS (1 mM, pH 7.4), and determining the absorbance at 475 nm. According to the following equations, LC and LE of ADM loaded into QDs-PEG-ADM were calculated to be 17.4% and 34.1%, respectively.

ADM-LC (%) = 100 × (weight of ADM loaded into products) / weight of products (1)

ADM-LE (%) = 100 × (weight of ADM loaded into products) / weight of total ADM (2)

Part S4. The concentration of ADM released from QDs-PEG-ADM

The release of ADM from QDs-PEG-ADM was studied at 37 ^{o}C in 1 mM of PBS with pH of 5.5, 6.0, 6.5 and 7.4, respectively. Briefly, 50 mg of QDs-PEG-ADM was dispersed in 100 mL water. An aliquot of 10 mL of the solution was transferred into a dialysis membrane (MWCO of 3500), which was immersed in 1 mM of PBS (40 mL) with different pH values at 37 ^{o}C, together with constant shaking (150 rpm). After incubation for desired time intervals (0-24 h), 1 mL of the solution after ADM release was taken for the concentration analysis of [ADM].

To calculate the concentration of ADM released from QDs-PEG-ADM, aqueous suspension of QDs-PEG-ADM was centrifuged, and washed with water twice to remove releasing ADM. The mass of ADM loaded in QDs-PEG-ADM (after ADM release, M_1) was calculated by measuring the absorbance at 475 nm (UV-vis spectrophotometer based on the Lambert-Beer law). The mass of ADM in the supernatant (*i.e.*, released ADM, M) was calculated by subtracting M_1 from the initial (*i.e.*, total) mass of ADM (M_0) in the aqueous suspension, as below;

Released ADM (%), $M = 100 \times (M_0 — M_1) / M_0$ (3)

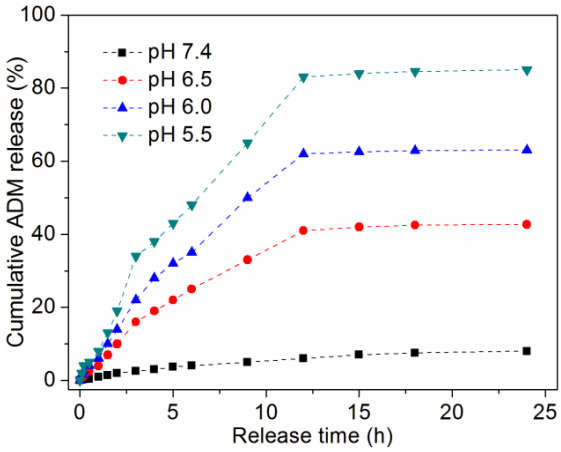

Figure S4 Time-dependent ADM release profiles from QDs-PEG-ADM (0.1 mg/mL) dispersed in 1 mM of PBS with different pH values: 5.5, 6.0, 6.5 and 7.4. Results of [ADM] were calculated by measuring the absorbance at 475 nm, using the Lambert-Beer law.

Table S1 Examples of PL lifetimes ($\tau_{1\text{-}4}$) and normalized pre-exponential factors (fractional weights, $a_{1\text{-}4}$) of ADM, QDs-PEG and QDs-PEG-ADM at different ADM release times.

[a] Sample	τ_1/ns	a_1/%	τ_2/ns	a_2/%	τ_3/ns	a_3/%	τ_4/ns	a_4/%	[b] τ_{ave}/ns	χ^2
QDs-PEG	0.53	10	5.83	16	9.97	32	25.14	42	20.54	1.129
ADM	4.69	100							4.69	1.012
QDs-PEG-ADM, 10 min	0.31	38	2.53	28	8.04	24	19.78	10	11.93	1.193
QDs-PEG-ADM, 1 h	0.35	37	2.93	26	8.52	25	20.13	12	12.69	1.181
QDs-PEG-ADM, 2 h	0.32	37	3.31	27	8.53	23	21.15	13	13.61	1.212
QDs-PEG-ADM, 3 h	0.31	36	3.01	24	8.97	25	21.54	15	14.57	1.158
QDs-PEG-ADM, 6 h	0.36	35	2.19	23	9.28	24	21.67	18	15.74	1.205

[a] PL lifetimes of QDs from QDs-PEG and QDs-PEG-ADM are measured under 475 nm of λ_{em} (λ_{ex} = 450 nm). For the case of ADM, the λ_{em} is 595 nm (λ_{ex} = 495 nm).

[b] The τ_{ave} is calculated by the following equation,[4,5] as below;

$$\tau_{ave}\,(\text{ns}) = \sum a_i \cdot (\tau_i)^2 / \sum a_i \cdot \tau_i \; (i = 1, 2, 3, 4) \qquad (4)$$

Reference

(4) Ruedas-Rama, M. J.; Orte, A.; Hall, E. A. H.; Alvarez-Pez, J. M.; Talavera, E. M. *Chem. Commun.* **2011**, *47*, 2898.

(5) Ruedas-Rama, M. J.; Orte, A.; Hall, E. A. H.; Alvarez-Pez, J. M.; Talavera, E. M. *Analyst* **2012**, *137*, 1500.

Part S5. Cell apoptosis (fluorescence activated cell sorter, FACS) assay

HeLa cells were loaded into a 6-well plate (1×10^4 cells/well). After incubation for 24 h, these cells were treated with QDs-PEG at a concentration of 0.1 mg/mL for 24 h at 37 ^{o}C. Afterward, these cells were collected, repeatedly washed with PBS (1 mM, pH 7.4), followed by incubation with anti-annexin V-fluorescein isothiocyanate (FITC) and propidium iodide (PI). Single-cell suspensions were analyzed by the FACS. Here, acinomycin D (0.1 μM) was used for the apoptosis positive control groups.

For Figure 3a-d in the text (manuscript), the cells appearing in the upper left quadrant (Q_1) stood for the necrosis

cells, while the cells appearing in the lower left quadrant (Q_3) denote the normal cells. In addition, those appearing in the upper right quadrant (Q_2), and in the lower right quadrant (Q_4) represent the cells in the late and early stages, respectively.

Part S6. In vitro cytotoxicity of QDs-PEG and QDs-PEG-ADM

In detail, HeLa cells were cultured as subconfluent monolayers on 25 cm^2 cell culture plates with vent caps in 1 × minimum essential α medium supplemented with fetal bovine serum (10%) in a humidified incubator at 37 ^oC containing CO_2 (5%). After grown to subconfluence, these cells were dissociated from the surface with a solution of trypsin (0.25%), and aliquots (100 μL) were seeded (1×10^4 cells) into a 96-well plate. After 24 h incubation at 37 ^oC, the medium was replaced with 10 μL of serum-free Dulbecco modified Eagle medium (DMEM) containing QDs-PEG (0-1 mg/mL). These cells were incubated for 12, 24, 48 and 72 h at 37 ^oC in the dark, while those cells treated with alone medium were used for low cell death controls. Finally, cell viabilities were quantitated by using a standard (3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) MTT assay.

Subsequently, the QDs-PEG, QDs-PEG-ADM and ADM were added into HeLa cell culture medium at selected concentrations (0-1 mg/mL). Typically, HeLa cells (1×10^4 cells/well) were incubated in the DMEM containing calf serum (wt. 10 %) and 100 units mL^{-1} penicillin in a fully humidified incubator with CO_2 (vol. 5%) at 37 ^oC. When the cells reached 80% of confluence with a normal morphology, QDs-PEG, QDs-PEG-ADM and free ADM were added into cell dishes, respectively. Then, these cell dishes containing additives were put into incubators for 72 h at 37 ^oC. After 72 h incubation, the culture medium was replaced by 20 μL of MTT reagent (diluted in the culture medium, 0.5 $mg\cdot mL^{-1}$), followed by incubation for an additional 2 h. Finally, MTT medium was carefully removed and 150 μL of dimethyl sulfoxide (DMSO) was added into each well for dissolving crystals, and the absorbance (A) of colored solutions (individual well) was recorded at 570 nm with a Multiskan MK3 enzyme-labeled Instrument. All experiments were performed in triplicate, and each result was averaged. The cell viability (survival) rates were determined according to the following equation as below;

Cell viability rate (%) = $100 \times (A_{\text{test cells}} / A_{\text{control cells}})$ (5)

Part S7. The detection of [ADM] in HeLa cells treated with QDs-PEG-ADM

The prepared QDs-PEG-ADM (0.2 mg/mL) was ultrasonically dispersed in 10 mL of PBS (1 mM, pH 5.5, 6.0, and 6.5, respectively). Under continuous stirring, 10 mL aqueous suspension of HeLa cells (1×10^4 cells/well) was added to form 20 mL of mixed solution at room temperature. At different time intervals (release times of 1, 2, 3 and 6 h), the resulting mixed solution was centrifuged, and washed with water twice to remove the free ADM released from QDs-PEG-ADM. Both the initial (before release) and the residual (after release) mass of ADM loaded in the QDs-PEG-ADM were calculated by UV-vis spectrophotometer (absorbance) at 475 nm using the Lambert-Beer law. The real-time concentration of released ADM (%) was calculated by the following equation, as below;

Released ADM (%) = $100 \times (M_{\text{initial-ADM}} - M_{\text{residual-ADM}}) / M_{\text{initial-ADM}}$ (6)

Table S2 Results of [ADM] in HeLa Cells Incubated with 0.1 mg·mL^{-1} of QDs-PEG-ADM (in 1 mM of PBS) for Different Times of ADM Release, Measured by the Methods of I_{QDs}/I_{ADM} and τ_{ave} of QDs.

Sample	[a] [ADM]	[b] I_{QDs}/I_{ADM}	[c] RSD	[d] τ_{ave}	RSD
pH 5.5					
1 h	8.1	8.0	2.9	7.9	2.5
2 h	19.2	19.0	2.6	19.3	2.4
3 h	34.5	34.5	1.8	34.3	3.1
6 h	48.6	48.8	2.0	48.7	0.9
pH 6.0					
1 h	6.0	5.8	2.8	5.9	1.7
2 h	13.8	13.9	3.2	13.8	0.7
3 h	21.4	21.1	1.1	21.5	2.1
6 h	34.3	34.5	2.3	34.0	2.5
pH 6.5					
1 h	3.9	4.1	2.4	4.0	3.0
2 h	9.7	10.0	1.5	9.9	1.9
3 h	15.6	15.5	2.2	15.4	3.3
6 h	24.2	24.2	1.6	24.0	2.7

Note: All measured results of [ADM]: [a, b, d] from the methods of absorbance (Part S4, SI), I_{QDs}/I_{ADM} (Figure 2d) and τ_{ave} of QDs (Figure 2f). All results are expressed as the mean of six repeated measurements. [c] The relative standard deviation (RSD) is calculated as (standard deviation / mean) × 100%.