Semiconductor quantum dots photosensitizing release of anticancer drug†

Zhenzhen Liu, Qiuning Lin, Qi Huang, Hui Liu, Chunyan Bao,* Wenjin Zhang, Xinhua Zhong and Linyong Zhu*

Received 29th October 2010, Accepted 18th November 2010
DOI: 10.1039/c0cc04676k

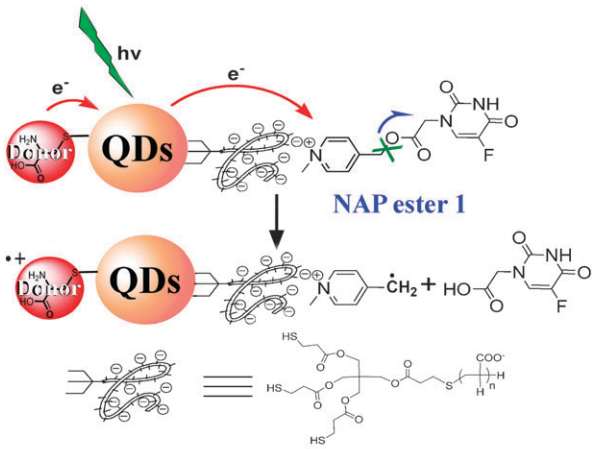
A new photo-controlled anticancer drug release system is reported based on the photo-induced electron transfer (PET) between semiconductor quantum dots (QDs) and *N*-methyl-4-picolinium (NAP) ester 1 under the excitation of visible light.

Recently, photo-responsive controllable releases have attracted more and more attention and interest for their amazing applications in the area of drug delivery and cancer therapy, where light offers a highly orthogonal external stimulus and provides a control at a specific time and location for phototherapy.[1–3] Generally, changes in physical and chemical properties induced by light controls the photo-responsive release. For example, the use of photothermal effects of gold nanomaterials to achieve photo-controlled release is a typical physical method.[4] For chemical methods, there are two strategies for facilitating photo-responsive controllable release. The first strategy is often referred as "caging" and involves temporarily deactivating a biologically active drug molecule by employing a phototrigger. Commonly used phototriggers include nitrobenzyl alcohol,[5] phenacyl ester,[6] and coumarinyl[7] derivatives. The second strategy is to utilize bistable photoswitches, such as the use of azobenzene derivatives to control release by the photoisomerization of azobenzene.[8] One significant limitation of the aforementioned strategies is the use of UV light, the intensity of which is attenuated quickly in tissue.[9] Among many attempts to avoid using cytotoxic UV light, one effort is to design new photo-triggers that can absorb at higher wavelengths.[10] However this approach encounters difficulty of synthesizing and extending the conjugation of the phototriggers without adversely affecting the bond cleavage rates and selectivity. Another approach is to utilize two-photon excitation,[11] however it is expensive due to the need of a fs-mode laser and less sensitive due to the low two-photon absorption cross section.

In this paper we demonstrate a new anticancer drug release system based on sensitized photo-induced electron transfer (PET) upon the irradiation with visible light. In this system, an excited sensitizer initiates an electron transfer to a photolabile group, which induces the release of the attached drug molecule (as shown in Scheme 1). This promising method decouples the light absorption step from photo-responsive release, and makes it possible to optimize each of the two steps independently. The *N*-alkyl-4-picolinium (NAP) has been reported as an excellent PET-based photolabile group by the Falvey group, allowing activation by a wide variety of photo-reductants.[12,13] In addition, semiconductor quantum dots (QDs) are often used as excellent photosensitizers due to their appropriate reduction potentials at *ca* −1.1 V *vs* Fc/Fc^{+}[14] and outstanding photosensitive property that can generate 4 or more electron-hole pairs upon photoexcitation.[15] Based on these properties, we prepared a new NAP ester **1** linked with an anticancer drug 5-fluorouracil acid (5-FUA) as an electron acceptor to generate photo-controlled release. In this system, water soluble QDs capped by pentaerythritol tetra-(3-mercaptopropionate)-polyacrylic acid (PTMP-PAA) and L-cysteine were used as the photosensitizer and electron donor, respectively. Herein, the QDs are expected to act as net electron shuttles to transfer an electron between the electron donor and the NAP ester **1** (as shown in Scheme 1). To the best of our knowledge, this is the first example of the application of PET method to photo-responsive drug release system.

NAP ester **1** was synthesized using general ester synthesis techniques, and water soluble PTMP-PAA-capped QDs were prepared as usual (see supporting information†). Generally, NAP ester **1** was mixed with QDs and electron donor in phosphate-buffered saline (PBS solution) (pH = 7.4, 0.01M). The obtained solutions were then purged with N_2 for ten minutes and irradiated with visible light (UV-cut, $\lambda > 400$ nm, 62 mW cm^{-2}) for prescribed periods of time, and then analyzed by HPLC spectroscopy.

The feasibility of a photorelease mechanism for this system was first supported by two characterizations as follows.



Scheme 1 Schematic presentation of QDs photosensitized drug release process by photo-induced electron transfer mechanism.

Key Laboratory for Advanced Materials, Institute of Fine Chemicals, East China University of Science and Technology, Shanghai, 200237, China. E-mail: baochunyan@ecust.edu.cn, linyongzhu@ecust.edu.cn; Fax: +86-21-64253742; Tel: +86-21-64253742

† Electronic supplementary information (ESI) available: Full experimental details and the Stern–Volmer analysis. See DOI: 10.1039/c0cc04676k

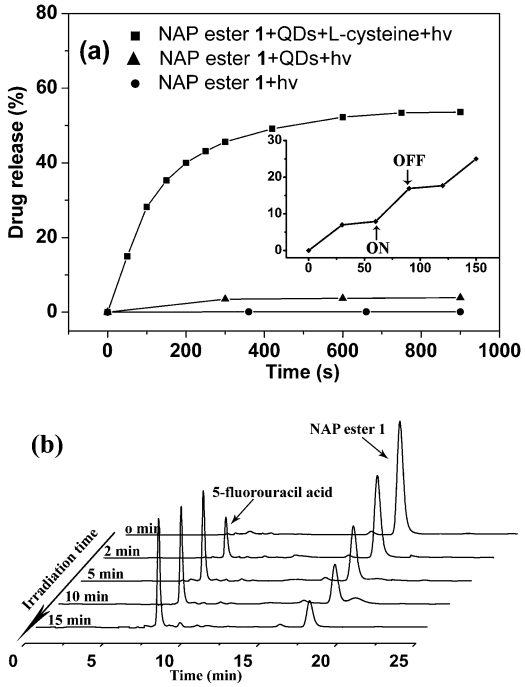


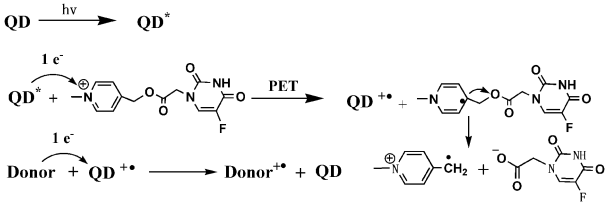
Fig. 1 (a) Time course of photolysis process for drug release of 5-fluorouracil acid in PBS solution (pH = 7.4) under different conditions. Inset is the partial process for drug release of the PET-based drug release system (NAP ester **1** + QDs + L-cysteine) under bright and dark conditions. "ON" indicates the beginning of light irradiation; "OFF" indicates the ending of light irradiation. (b) HPLC charts detected at 254 nm for the system of NAP ester **1** + QDs + L-cysteine subjected to visible light irradiation for different lengths of time. The concentrations are 0.6 mM for NAP ester **1**, 0.1 μM for QDs (λ_{em} = 602 nm), and 0.03 M for L-cysteine, respectively. The data in Fig. 1a were determined by HPLC analysis, relative to NAP ester **1** in dark conditions, and the used light was visible light (UV-cut, λ > 400 nm, 62 mW cm^{-2}).

Firstly, from the thermodynamics calculations, the driving force for the electron transfer step was estimated by the reduction potential, E_{red}, of NAP ester **1** and QDs. The reduction potential of NAP ester **1** was −0.91 V *vs* Fc/Fc$^+$, determined by cyclic voltammetry (CV) experiments at a scan rate 100 mV s^{-1}, and the reduction potential of PTMP-PAA capped water-soluble CdSe/ZnS QDs was around −1.1 V *vs* Fc/Fc$^+$, which was sufficiently negative to reduce our NAP ester **1** in a mediated PET mechanism (see supporting information†).Secondly, the Stern–Volmer analysis (see supporting information†) also indicated that the NAP ester reacts rapidly with the singlet states of the photosensitizer QDs with the rate constants *ca* ~10^{11} M^{-1} S^{-1}, which is significantly larger than the diffusion controlled kinetics (near 1 × 10^{10} M^{-1} S^{-1}).[16] This analysis also suggested some type of binding interaction, and NAP ester **1** should be absorbed on the surface of the QDs by electrostatic action in the drug release system, which promotes the process of PET-based release mechanism.

To further confirm the PET-based release, control photolysis experiments that lack either photosensitizer QDs or the combination of QDs and L-cysteine were carried out as shown in Fig. 1a. The lack of any components resulted in an insignificant amount of 5-FUA, which suggested that the release occurred effectively upon the irradiation of light only when photosensitizer QDs, NAP ester **1**, and electron donor L-cysteine coexisted in the system as a supramolecular complex. Here, the photosensitizer QDs and not the prodrug adsorbed the light, and then the sensitized QDs transferred an electron to NAP ester **1** which induced the cleavage of the 5-FUA. The donor in the system then supplied an electron to QDs which promoted the progress of cleavage (as shown in Scheme 2).[12] The allowance of the light absorption step decoupling from the drug release provided the opportunity to tune the properties of QDs to optimize the release, thus permitting more control over the wavelengths of light used in the release process.

The precise control of the photolytic release was demonstrated by monitoring the progress of 5-FUA release after periods of exposure to light and dark conditions, as shown in inset of Fig. 1a. The distinctive "stepped" profile revealed that the drug release proceeded under light conditions and realized the "light-controlled precise release". HPLC analysis profiles in Fig. 1b further confirmed that the release of 5-FUA was increased in line with the consumption of prodrug under visible light irradiation. In the HPLC detection process, the radical fragment of *N*-methyl-4-picolinium (NAP) salt generated from NAP ester **1** was not found. It may be attributed to the radical decays through a variety of pathways, since the fate of radical fragment under these conditions was not known with certainty.[12]

To optimize the release conditions, NAP ester **1** was subjected to filtered (λ > 400 nm) irradiation with two kinds of CdSe/ZnS QDs with different emission wavelength, as shown in Table 1. In both cases, 5-FUA was released efficiently under identical photolysis conditions. The drug release for QDs with the emission at 625 nm (QDs_{625}) was lower than that of QDs_{602}; we attribute this to the relatively larger reduction potentials which promoted the photolysis process. The higher electron transfer rate constant for QDs_{602} also agreed with the higher release efficiency (see supporting information†). Recently, transition metal ion-doped quantum dots not containing heavy metal ions have attracted much attention because of their low toxicity compared to CdSe QDs.[17] In this work, Mn doped ZnSe (Mn:ZnSe) QDs (λ_{em} = 585 nm) was used as a photosensitizer for release of 5-FUA. As shown in Table 1, 5-FUA was released successfully with 64% drug release achieved after 30 min irradiation. These studies indicate that we have the chance to optimize our drug release system by using suitable QDs with longer emission wavelength and less toxic components.



Scheme 2 Proposed mechanism for QDs-based photolysis release.

Table 1 Data for NAP ester **1** photolysis release experiments with different QDs and different donors

	[NAP ester **1**] (mM)	[QD] (0.1 μM) λ_{em}	E_{red}[a]	Donor (0.03 M)	Drug release (%)	NAP ester **1** consumed (%)
1	0.6	CdSe/ZnS QD_{602}	−1.14	L-cysteine	53.6	75.3
2	0.6	CdSe/ZnS QD_{625}	−1.06	L-cysteine	36.0	38.0
3	0.6	CdSe/ZnS QD_{602}	−1.14	DTT	56.3	87.4
4	0.6	CdSe/ZnS QD_{602}	−1.14	TEA hydrochloride	12.0	14.0
5	0.6	CdSe/ZnS QD_{602}	−1.14	EDTA	7.9	11.3
6	0.6	Mn:ZnSe QD_{585}	−1.16	L-cysteine	64.0[b]	75.0[b]

Light source: UV-cut, λ > 400 nm, 62 mW cm^{-2}; irradiation time is 15 min.[a] Reduction potential using ferrocene as internal standard. [b] Irradiation time is 30 min.

The effect of electron donors for this PET-induced photorelease was also detected by using different donors. As shown in Table 1, several donors, like DL-dithiothreitol (DTT), triethanolamine (TEA) hydrochloride, and ethylenediaminetetraacetic acid (EDTA), were used for comparing with L-cysteine. In these cases, the efficient release of the anticancer drug 5-FUA were observed only when DTT and L-cysteine were used as donors. This can be explained from the electron donating ability and the molecular structures of the donors. As known, DTT and L-cysteine were reported as strong reductors and always used as efficient electron donors. On the other hand, electron transfer between the donor and acceptor is ultrafast and plays a role when the distance is short. For DTT and L-cysteine, the existence of –SH groups make them easy to adsorb on the surface of QDs to form a supramolecular system which promotes the process of the PET-induced photolysis efficiently. In addition, the stronger coordinating ability of DTT with two –SH groups might determine the higher efficiency of drug release compared with that of L-cysteine with only one –SH group. All the results indicate that our PET-based photo-responsive release could control drug release by external manipulation of irradiation time, properties of photosensitizer and donors.

In conclusion, these experiments demonstrate a successful controllable anticancer drug release system under the irradiation of visible light based on photo-induced electron transfer and utilizing QDs nanoparticles as sensitizers. To the best of our knowledge, this is the first account of the application of easily prepared QDs as photosensitizers for photochemical release strategies. All the control experiments indicated that this PET-based photo-responsive release could be controlled by external manipulation including irradiation time and properties of photosensitizer and donors. We envision that this novel method has the potential to fulfil an increasing need for versatile and controllable drug delivery systems.

We thank the Innovation Program of Shanghai Municipal Education Commission (092259), NSFC (20903039, 21073062), "Chen Guang" project (09CG25), and Shanghai Sci. Tech. Comm. (09ZR1408600) for financial support.

Notes and references

1 N. K. Mal, M. Fujiwara and Y. Tanaka, *Nature*, 2003, **421**, 350–353; S. K. Choi, T. Thomas, M. Li, A. Kotlyar, A. Desai, J. James and R. Baker, *Chem. Commun*., 2010, **46**, 2632–2634.
2 M. Noguchi, M. Skwarczynski, H. Prakash, S. Hirota, T. Kimura, Y. Hayashi and Y. Kiso, *Bioorg. Med. Chem*., 2008, **16**, 5389–5397; C. P. McCoy, C. Rooney, C. R. Edwards, D. S. Jones and S. P. Gorman, *J. Am. Chem. Soc*., 2007, **129**, 9572–9573.
3 M. Y. Jiang and D. Dolphin, *J. Am. Chem. Soc*., 2008, **130**, 4236–4237; S. S. Agasti, A. Chompoosor, C. -C. You, P. Ghosh, C. K. Kim and V. M. Rotello, *J. Am. Chem. Soc*., 2009, **131**, 5728–5729.
4 M. S. Yavuz, Y. Cheng, J. Chen, C. M. Cobley, Q. Zhang, M. Rycenga, J. Xie, C. Kim, K. H. Song, A. G. Scheartz, L. V. Wang and Y. Xia, *Nat. Mater*., 2009, **8**, 935–939; T. Kuo, V. A. Hovhannisyan, Y. Chao, S. Chao, S. Chiang, S. Lin, C. Dong and C. Chen, *J. Am. Chem. Soc*., 2010, **132**, 14163–14171.
5 H. J. Schuster, B. Krewer, J. M. von Hof, K. Schmuck, I. Schuberth, F. Alvesb and L. F. Tietze, *Org. Biomol. Chem*., 2010, **8**, 1833–1842.
6 R. Orth and S. A. Sieber, *J. Org. Chem*., 2009, **74**, 8476–8479.
7 V. Hagen, S. Frings, J. Bendig, D. Lorenz, B. Wiesner and U. B. Kaupp, *Angew. Chem., Int. Ed*., 2002, **41**, 3625–3628.
8 J. Lu, E. Choi, F. Tamanoi and J. I. Zink, *Small*, 2008, **4**, 421–426; Y. Zhu and M. Fujiwara, *Angew. Chem., Int. Ed*., 2007, **46**, 2241–2244.
9 A. Schwarz, S. Ständer, M. Berneburg, M. Böhm, D. Kulms, H. Steeg, K. Grosse-Heitmeyer, J. Krutmann and T. Schwarz, *Nat. Cell Biol*., 2002, **4**, 26–31.
10 Y. Chen and M. G. Steinmetz, *Org. Lett*., 2005, **7**, 3729–3732; I. Aujard, C. Benbrahim, M. Gouget, O. Ruel, J.-B. Baudin, P. Neveu and L. Jullien, *Chem.–Eur. J*., 2006, **12**, 6865–6879.
11 Q. Lin, Q. Huang, C. Li, C. Bao, Z. Liu, F. Li and L. Zhu, *J. Am. Chem. Soc*., 2010, **132**, 10645–10647.
12 C. Sundararajan and D. E. Falvey, *J. Org. Chem*., 2004, **69**, 5547–5554; J. B. Borak, S. Lpez-Sola and D. E. Falvey, *Org. Lett*., 2008, **10**, 457–460.
13 C. Sundararajan and D. E. Falvey, *Org. Lett*., 2005, **7**, 2631–2634; J. B. Borak and D. E. Falvey, *J. Org. Chem*., 2009, **74**, 3894–3899.
14 F. M. Paymo and I. Yildiz, *Phys. Chem. Chem. Phys*., 2007, **9**, 2036–2043.
15 V. V. Matylisky, L. Dworak, V. V. Breus, T. Baché and J. Wachtveitl, *J. Am. Chem. Soc*., 2009, **131**, 2424–2425; R. Bakalova, H. Ohba, Z. Zhelev, M. Ishikawa and Y. Baba, *Nat. Biotechnol*., 2004, **22**, 1360–1361; J. Liu, C. Bao, X. Zhong, C. Zhao and L. Zhu, *Chem. Commun*., 2010, **46**, 2971–2973.
16 J. R. Lakowicz, *in Principle of Fluorescence Spectroscopy*, Springer Science & Business Media, LLC, New York, 2006, ch. 8, pp. 278–285.
17 N. Pradhan, D. Goorskey, J. Thessing and X. Peng, *J. Am. Chem. Soc*., 2005, **127**, 17586–17587; D. Zhu, X. Jiang, C. Zhao, X. Sun, J. Zhang and J. Zhu, *Chem. Commun*., 2010, **46**, 5226–5228.

