Reversible fluorescence modulation through energy transfer with ABC triblock copolymer micelles as scaffolds†

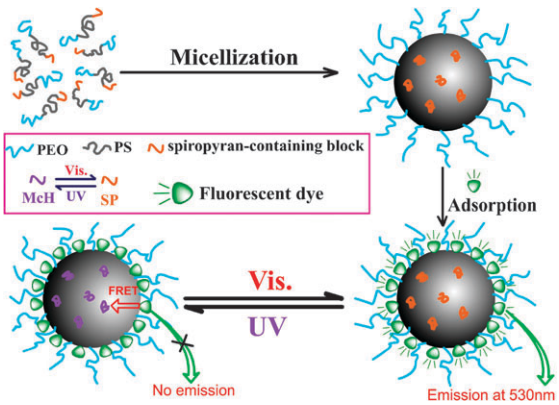
Jian Chen, Fang Zeng,* Shuizhu Wu,* Jianqing Zhao, Qiming Chen and Zhen Tong

Received (in Cambridge, UK) 25th June 2008, Accepted 19th August 2008
First published as an Advance Article on the web 25th September 2008
DOI: 10.1039/b810677k

The micelle system formed by an amphiphilic triblock copolymer in water serves as a novel scaffold for fluorescence resonance energy transfer as well as light-induced reversible fluorescence modulation for a hydrophobic fluorescent dye.

Fluorescent labels and sensors have been used for a wide range of applications in chemical analysis, biological research, and clinical diagnosis, owing to the valuable advantages of fluorescence technology such as high sensitivity and low-cost instrumentation;[1] in these fluorescence-based applications the fluorescence resonance energy transfer (FRET), a well-characterized photophysical tool, has been extensively adopted.[1,2] Recently, the development of particle-based labels and sensors, such as ligand-capped quantum dots,[3] dye-doped silica[4] and polymer particles[5] as well as the fluorophore-containing supramolecular aggregates,[6] represents a new trend in fluorescence-involved cellular biological studies due to their higher brightness and improved photostability. Moreover, the particle-based devices hold the most promise as 3-dimensional matrices for the development of new tunable and versatile sensing and detection devices since the selectivity and the operating range can be monitored, without any synthetic efforts, by changing the donor and/or the acceptor, thus they can be utilized as novel FRET-based devices. [3–6]

Owing to the sustained interest of chemo-sensors and labels in biology, the design and development of new particle-based fluorescent devices, which are non-toxic, biocompatible and usable in aqueous media, is a topic of great interest. The advancements in controlled radical polymerizations are conducive to microstructure designing and synthesis of block copolymers, leading to various fascinating architectures such as micellar structures.[7] Micellar structures formed from amphiphilic block copolymers in aqueous media are among the widely explored systems. The hydrophilic polymer poly(ethylene oxide) (PEO), which is used for a wide range of biomedical applications because of their non-toxic, non-antigenic and non-immunogenic properties,[8] can be readily linked to hydrophobic polymer chain(s) to constitute amphiphilic block copolymers and then form micelles in water.[9] Herein, we designed an amphiphilic triblock copolymer, in which PEO serves as the hydrophilic block, with polystyrene and a spiropyran-containing block as the hydrophobic blocks, and we have demonstrated the potential of the nano-sized micelles formed by this copolymer as a novel FRET-based scaffold. As illustrated in Scheme 1, in aqueous media, the amphiphilic micelles can incorporate a hydrophobic fluorescent dye and then form an energy transfer system with the fluorescent dye as the donor and spiropyran moieties as the acceptor, the fluorescence modulation for the dye can be achieved through an intra-micelle FRET process. For this system, the fluorescence intensity of the incorporated dye can be reversibly modulated upon alternating visible light/UV irradiation.



Scheme 1 Formation of a FRET system by a triblock copolymer micelle and reversible fluorescence modulation for a hydrophobic fluorescent dye with the micelle as the scaffold.

For the synthesis of the triblock copolymer, first a spiropyran-containing methacrylate, 2-{3-[3′,3′-dimethyl-6-nitrospiro (indoline-′,2-[2*H*-1] benzopyran)-1′-yl] propanoyloxy}-ethyl-methacrylate (SPMA) was synthesized, then a PEO_{110}-*b*-PS_{85}-*b*-$PSPMA_3$ triblock copolymer was obtained by atom transfer radical polymerization (ATRP) (with experiment details and characterizations on pp. 2–22 in the ESI†). The amphiphilic triblock copolymer can form micelles in water, with a critical

College of Materials Science & Engineering, South China University of Technology, Guangzhou, 510640, China.
E-mail: mcfzeng@scut.edu.cn; shzhwu@scut.edu.cn;
Fax: 86 20 87110273; Tel: 86 20 22236262

† Electronic supplementary information (ESI) available: Experimental details; ^{1}H NMR and ^{13}C NMR spectra; GPC curves; determination of CMC; AFM image and particle size distribution; absorption spectra for monomer and triblock copolymer in solutions; surface tension *vs.* concentration curve for triblock copolymer in water; illustration for light-induced structural variation; fluorescence emission spectra; photographs and emission spectra of micelles upon UV or visible light irradiation; fluorescence intensity changes of NBD/micelle dispersion upon alternate irradiation with UV and visible light; calculation of Förster critical radius and determination of experimental energy transfer efficiency; and illustration for effective and noneffective FRET volume in a micelle core. See DOI: 10.1039/b810677k

micelle concentration (CMC) of 0.18 mg ml^{-1} at 25 °C (Fig. S10, ESI†), which consist of a hydrophobic core resulting from the aggregation of PS blocks and P(SPMA) blocks surrounded by a hydrophilic corona formed by PEO (Scheme 1). The hydrodynamic diameter for the micelles determined by dynamic light scattering is 59 nm (Fig. S11, ESI†). In the dried state, the micelles are spherical in shape with an averaged diameter of 51 nm, as determined by atomic force microscopy (AFM) (Fig. S11, ESI†).

The absorption spectra for the triblock copolymer micelles in water upon UV or visible light irradiation are shown in Fig. 1A. It is well known that spiropyran can assume one of two stable states: the open-ring state, known as the protonated merocyanine (McH) form, and the closed-ring state, known as the spiro (SP) form (Fig. S12, ESI†).[10–11] Upon visible light irradiation, spiropyran moieties in the micelles assumed the SP form and exhibited no absorption from 450 to 700 nm; while with UV irradiation, a new absorption band at *ca.* 570 nm appeared due to the formation of the McH form. Fig. 1A clearly indicates that with spiropyran moieties incorporated, the micelle dispersion exhibits photochromic properties.

To form the FRET-based donor–acceptor pairs within a micelle particle, we made use of the amphiphilic nature of the micelles to incorporate (absorb) a hydrophobic fluorescent dye, a nitrobenzoxadiazolyl (NBD) derivative with an 8-carbon alkyl tail, into the micelles. The NBD dye exhibits very low absorbance and emission in pure water (Fig. S13 and S14, ESI†) owing to its very low solubility in water; however, as the dye was incorporated into the micelles, the dispersion exhibited a prominent absorption at *ca.* 470 nm and a strong fluorescent emission at 530 nm. In addition, the absorption maximum displays a blue-shift for the dye in the micelle system compared with that in pure water, indicating that the NBD dye molecules reside in a more hydrophobic environment (compared with that of water) in micelles,[12] likely in the core–corona interface (Fig. S15, ESI†). Although both the fluorescent dye and spiropyran are included into one micelle, the absorption and fluorescence spectra for the micelle dispersion (Fig. 1 and Fig. S13, ESI†) show no sign of any π–π interaction between the dye and the spiropyran, indicating that in the micelles the dye is electronically insulated from the π system of the spiropyran. The purpose for introducing polystyrene block into the copolymer is two-fold: first, it has been found by Matyjaszewski and coworkers[11] that the micelles of *diblock* copolymer with a PEO block and a spiropyran-containing block will be disrupted by UV irradiation, and the existence of PS block can prevent the dissociation of micelles upon UV irradiation; second, PS block can provide spiropyran with a preferable environment, thus not only separating the donor and the acceptor, but also alleviating the solvation effect on spiropyran moieties by water molecules and ensuring the relatively rapid photochromic interconversion rate.[13] On the other hand, in this study we covalently linked the spiropyrans to the polymer chains, this may restrict the migration of spiropyran molecules within the micelles. Upon UV irradiation, the low polar SP form of spiropyran will turn into the high polar and zwitterionic McH form; if spiropyran molecules are not covalently bound to the polymer chains, chronically the migration of the McH may lead to phase separation, and the zwitterionic McH may be stabilized by water molecules (in this case, the conversion of McH back to SP will become much slower[13]) if the free McH molecules migrate into the loose corona layer of the micelle particle.

With the fluorescent dye incorporated, the micelles containing both the donor (NBD dye) and acceptor (spiropyran) may serve as the scaffolds for FRET process. Fig. 1B shows the modulation of fluorescence intensity of NBD by spiropyran moieties in the micelle systems. As a NBD/micelle dispersion was irradiated by UV light (300 nm), the characteristic fluorescence emission for NBD at 530 nm was efficiently quenched, a new emission band at 640 nm appeared, which is the emission of the open-ring state (McH) of spiropyran moieties, suggesting the FRET may occur from NBD to the McH form of spiropyran; while after irradiation by visible light (525 nm), the fluorescence intensity at 530 nm recovered. Moreover, we found that the fluorescence of the NBD/micelle system can be reversibly quenched and "activated" by alternating the visible and UV light irradiation, and as a result, the appearance of the dispersion also reversibly changed markedly, as shown in Fig. 1B.

The typical photoresponsive behavior as well as the reversible nature of the fluorescence modulation of NBD/micelle complex in water upon exposure to alternating cycles of UV (300 nm) and visible light (525 nm) illumination is illustrated in Fig. 2. Upon irradiation with UV, the fluorescence intensity gradually decreased in 5 min, while it increased upon irradiation with 525 nm light, and it took about 50 min for the complex to recover its fluorescence intensity. Moreover, the visible light or UV can be applied to reversibly "turn on" and

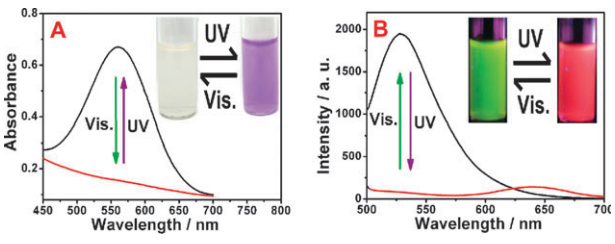


Fig. 1 Absorption spectra of copolymer micelle dispersion (with no NBD) (A) and fluorescence spectra (excited at 490 nm) of NBD/micelle dispersion (B) upon UV-vis light irradiation. The inset in (A) shows the appearance change of the dispersion upon UV-vis; inset in (B) is the fluorescence change upon UV or Vis. (Fig. S16, ESI†).

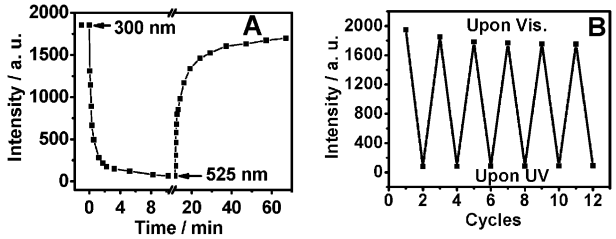


Fig. 2 Fluorescence response (excited at 490 nm and emitted at 530 nm) of the NBD/micelle dispersion on irradiation with 300 nm UV and then 525 nm visible light (A); and fluorescence intensity change for the dispersion after UV illumination and visible light irradiation cycles (B). The concentration of NBD in dispersion is 1.27×10^{-5} mol l^{-1}.

"turn off" the fluorescence of NBD and the switching of fluorescence intensity of NBD is due to the photochemical conversion between the two states of the spiropyran moieties. Fig. 2 indicates that the NBD/micelle system exhibits fairly good light-induced reversible fluorescence modulation properties.

Generally, the FRET process requires that the emission band of the fluorophore (donor) overlaps the absorption band of the acceptor, and the distance between them be within the Förster radius.[1,2] For the present case, the emission band (500–650 nm) of the fluorophore NBD well overlaps the absorption band (500–650 nm) of the McH state of spiropyran in micelles; however, the SP form does not exhibit any absorption band within the emission band of NBD (500 nm to 650 nm), as shown in Fig. 1. Thus, the energy transfer from the excited state of NBD to the SP form of spiropyran is impossible, while energy transfer to the McH form is possible. Therefore, the light-induced interconversion of the spiropyran structure can be employed to modulate the fluorescence of NBD through intra-micelle energy transfer.

As for the distance requirement on this donor–acceptor pair in the micelle system, according to Förster energy transfer theory,[1,2] the effective distance between donor and acceptor is governed by Förster's critical distances R_0, and the energy transfer is effective over distances in the $R_0 \pm 50\%$ R_0 range.[1,14,15] For this micelle system, R_0 has been calculated to be 2.8 nm (see the ESI for details), thus the effective distance between the NBD dye to the McH form of spiropyran is from 1.4 nm to 4.2 nm. In the core–corona micelles, the NBD molecules (donor) reside in the core–corona interface (as indicated in Fig. S15, ESI†). Since the upper limit of the effective energy transfer distance for the sample is 4.2 nm, an outer sphere about 4.2 nm thick, which represents about 47% of the volume of the hydrophobic core (with the estimated core diameter of 44.6 nm) of the micelle system, can serve as the effective area[15] in which the McH form of spiropyran can quench the fluorescence emission of NBD (see Scheme S2 in the ESI†). The above results and analysis demonstrate that the micelle system can provide a suitable scaffold for the FRET process, by allowing the donors to locate within the Förster radius of a sufficient number of the acceptors, and effectively preventing the interaction between the two π systems of the donor and acceptor.

In conclusion, the well-defined and water-dispersable micelles formed by the triblock copolymer can act as novel and preferable scaffolds for the FRET process. The micelle system can realize reversible fluorescence modulation and be used to selectively highlight the cells;[16] moreover, this strategy can be used in other FRET-based applications like biological sensing and detection by incorporating a suitable ligand (receptor) into the core–corona interface and linking a fluorophore in the hydrophobic core of the micelle particles. This strategy could open new perspectives to a wider application of block copolymer micelles. However, for this approach, further work needs to be done to reduce the CMC of the triblock copolymer at ambient temperature, since the current CMC (0.18 mg ml^{-1} at 25 °C) is relatively high and would lead to a large amount of soluble polymer chains in water and may interfere with the detections.

Acknowledgment is made to the NSFC (No. 50573023, 50773022) and GDNSF (No. 07006497) for support of this research.

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