

# Release of hydrophobic molecules from polymer micelles into cell membranes revealed by Förster resonance energy transfer imaging

Hongtao Chen<sup>\*†</sup>, Sungwon Kim<sup>‡</sup>, Li Li<sup>\*†</sup>, Shuyi Wang<sup>\*§</sup>, Kinam Park<sup>\*¶</sup>, and Ji-Xin Cheng<sup>\*†¶</sup>

<sup>\*</sup>Weldon School of Biomedical Engineering, <sup>†</sup>Department of Chemistry, and <sup>‡</sup>Department of Pharmaceutics and Biomedical Engineering, Purdue University, West Lafayette, IN 47907

Edited by Milan Mrksich, University of Chicago, Chicago, IL, and accepted by the Editorial Board February 21, 2008 (received for review July 27, 2007)

It is generally assumed that polymeric micelles, upon administration into the blood stream, carry drug molecules until they are taken up into cells followed by intracellular release. The current work revisits this conventional wisdom. The study using dual-labeled micelles containing fluorescently labeled copolymers and hydrophobic fluorescent probes entrapped in the polymeric micelle core showed that cellular uptake of hydrophobic probes was much faster than that of labeled copolymers. This result implies that the hydrophobic probes in the core are released from micelles in the extracellular space. Förster resonance energy transfer (FRET) imaging and spectroscopy were used to monitor this process in real time. A FRET pair, DiIC<sub>18(3)</sub> and DiOC<sub>18(3)</sub>, was loaded into monomethoxy poly(ethylene glycol)-block-poly(D,L-lactic acid) micelles. By monitoring the FRET efficiency, release of the core-loaded probes to model membranes was demonstrated. During administration of polymeric micelles to tumor cells, a decrease of FRET was observed both on the cell membrane and inside of cells, indicating the release of core-loaded probes to the cell membrane before internalization. The decrease of FRET on the plasma membrane was also observed during administration of paclitaxel-loaded micelles. Taken together, our results suggest a membrane-mediated pathway for cellular uptake of hydrophobic molecules preloaded in polymeric micelles. The plasma membrane provides a temporal residence for micelle-released hydrophobic molecules before their delivery to target intracellular destinations. A putative role of the PEG shell in the molecular transport from micelle to membrane is discussed.

**B**lock copolymer micelles (1–4) have attracted growing interest as carriers to deliver drugs including antitumor reagents to the target site via the enhanced permeability and retention effect (5). Hydrophobic molecules, such as paclitaxel (PTX), can be incorporated into the core of polymeric micelles by physical entrapment. The core composed of hydrophobic chains is expected to regulate the retention and the release of loaded drug molecules on an appropriate time scale (6–8). With micelle-based drug formulations reaching clinical trials (9, 10), the impetus for understanding the pathways involved in micellar drug delivery starts to emerge. It is thought that intact polymeric micelles are taken into cells, followed by intracellular release of drug molecules (8). Kabanov's group (11) reported that pluronic copolymer micelles could be internalized by an endocytic pathway and increase drug absorption by inhibition of P-glycoprotein drug efflux system in cancer cells. Allen *et al.* (12) suggested that poly(ethylene oxide)-poly( $\epsilon$ -caprolactone) (PEO-PCL) micelles enter PC12 cells via endocytosis. To directly monitor the micelles, Luo *et al.* (13) and Savic *et al.* (14) chemically conjugated a rhodamine molecule to the end of the PCL block and investigated the internalization of PEO-PCL micelles into P19 and PC12 cells. In their study, micelles were reported to enter the cell by endocytosis after nonspecific association with the cell membrane (8). It was argued later that these observations were not indicative of the intracellular distribution of the nonionic micelles because the modification with a cationic marker could

change the property of copolymer (15). At present, the pathway for cellular internalization of polymeric micelles and incorporated drug molecules remains unclear.

In this study, we investigated the cellular uptake of hydrophobic molecules preloaded in PEG-polyester micelles. Monomethoxy poly(ethylene glycol)-*b*-poly(D,L-lactic acid) (PEG-PDLLA) micelles were studied because they have been widely used as carriers of PTX (10). PEG-PDLLA was labeled with fluorescein isothiocyanate (FITC) to monitor the micelle itself, and DiIC<sub>18(3)</sub>, a hydrophobic fluorescence probe, was used to represent a hydrophobic drug loaded in the micelle cores. It was found that cellular uptake of DiIC<sub>18(3)</sub> was much faster than that of FITC-labeled copolymers, indicating two separate cell entry pathways for DiIC<sub>18(3)</sub> and the copolymers. To clarify this process, we used Förster resonance energy transfer (FRET) to monitor the release of core-loaded molecules from micelles in real time. A FRET pair of hydrophobic dyes, DiIC<sub>18(3)</sub> as acceptor and DiOC<sub>18(3)</sub> as donor (referred to as DiI and DiO, respectively), was physically loaded into the micelle cores. FRET between DiO and DiI was recently used to detect plasma membrane heterogeneity (16). In our case, FRET occurred in intact micelles because of intimate DiI/DiO contacts in the cores, whereas the decomposition of micelles by acetone or the release of DiI and DiO from micelles caused a loss of the FRET effect. The results of this study show that during micelle–membrane interactions, the core-loaded molecules are effectively transferred to the plasma membrane, from which they can either be endocytosed or diffuse to the targets; i.e., specific intracellular structures.

## Results

Dual-labeled PEG-PDLLA micelles (Fig. 1*A Inset*; containing 10% FITC-PEG-PDLLA and 0.15% DiI) with an average diameter of 52 nm were prepared by dialysis at the 2 mg/ml administration dose and used for the study of cellular uptake of copolymers and core-loaded hydrophobic molecules. KB cells were imaged after a 24-h incubation with 0.2 mg/ml dual-labeled micelles at 37°C. Almost no FITC-labeled copolymers (green in Fig. 1*A*) were found inside of cells, whereas the DiI (red in Fig. 1*B*) initially loaded in the micelle core was significantly internalized into cells. Moreover, the DiI delivered by micelles (Fig.

Author contributions: K.P. and J.-X.C. designed research; H.C., S.K., L.L., and S.W. performed research; and H.C. wrote the paper.

The authors declare no conflict of interest.

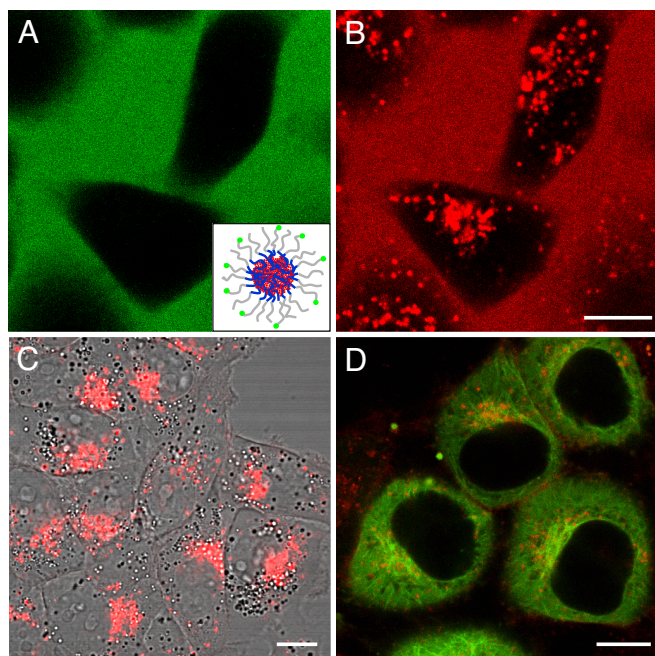
This article is a PNAS Direct Submission. M.M. is a guest editor invited by the Editorial Board.

<sup>§</sup>Permanent address: College of Medical Device Engineering, University of Shanghai for Science and Technology, Shanghai 200093, China.

<sup>¶</sup>To whom correspondence may be addressed. E-mail: kpark@purdue.edu or jcheng@purdue.edu.

This article contains supporting information online at [www.pnas.org/cgi/content/full/0707046105/DCSupplemental](http://www.pnas.org/cgi/content/full/0707046105/DCSupplemental).

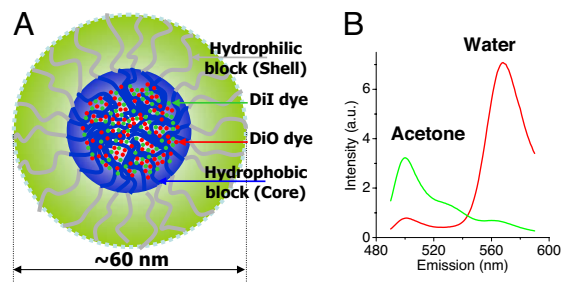
© 2008 by The National Academy of Sciences of the USA



**Fig. 1.** Cellular internalization of fluorescently labeled micelles and core-loaded hydrophobic probes inspected by confocal fluorescence microscopy. (A and B) KB cells treated with 0.2 mg/ml dual-labeled micelles for 24 h. The green color represents the FITC signal from labeled micelles, and the red color represents the DiI signal. (Inset) Diagram of a micelle labeled with FITC and loaded with DiI. (C) KB cells incubated with free DiI in DMSO at the final concentration of 0.1  $\mu\text{g/ml}$  for 24 h. (D) HeLa cells incubated with 0.2 mg/ml micelles (physically loaded with 0.15% DiI) for 2 h. DiI was accumulated in the microtubule organizing center area. (Scale bars: 10  $\mu\text{m}$ .)

1B) and the free DiI delivered by DMSO (Fig. 1C) exhibited the same pattern of perinuclear accumulation. These observations imply that DiI escaped from micelles and entered the cells separately. The intracellular distribution of DiI was further examined by using HeLa cells stably transfected with GFP-tubulin. After a 2-h incubation at 37°C with 0.2 mg/ml micelles loaded with 0.15% DiI, vesicles containing DiI were found to be accumulated in the microtubule organizing center area (Fig. 1D), indicative of endosomal localization of DiI. The same pattern was observed in HeLa cells incubated with free DiI (data not shown). Taken together, these results suggest that DiI could escape from the micelles and internalize into cells via endocytosis.

To monitor the release of the core-loaded molecules in real time, we physically loaded a FRET pair into micelles (referred to as FRET micelles), as shown in Fig. 2A. The FRET micelles containing 0.75% DiI and 0.75% DiO were prepared by dialysis at an initial concentration of 2 mg/ml copolymer. The micelles were stable for >3 months with an average diameter of 60 nm measured by dynamic light scattering (DLS) and a zeta potential of  $-6.19 (\pm 0.06)$  mV. To verify the occurrence of FRET, fluorescence spectra of 0.2 mg/ml micelles in deionized water or acetone were measured on a spectrometer with 484-nm excitation. For micelles in deionized water, a strong DiI signal was observed (red curve in Fig. 2B) due to the close proximity of DiI and DiO in the micelle core, with a FRET ratio  $I_R/(I_G + I_R)$  of 0.90, where  $I_R$  and  $I_G$  are fluorescence intensities at 565 nm and 501 nm, respectively. After micelle decomposition by acetone, FRET disappeared (green curve in Fig. 2B) because DiI and DiO could not be closely retained anymore, resulting in a FRET ratio  $I_R/(I_G + I_R)$  of 0.17. Fluorescence spectra of micelles loaded with 0.75% DiI alone or 0.75% DiO alone [supporting



**Fig. 2.** PEG-PDLLA FRET micelles. (A) Diagram of a FRET micelle prepared with 0.75% DiO and 0.75% DiI at 2 mg/ml polymer concentration. (B) Spectra of micelles diluted by 10 $\times$  water (red curve) and 10 $\times$  acetone (green curve), respectively.

information (SI Fig. S1) excited at 484 nm show that cross-talk and direct acceptor excitation are minor effects in comparison with the FRET inside of the micelle core. We have also determined the homo-FRET by measuring the fluorescence anisotropy from micelles loaded with different amounts of DiI or DiO. The result shows self-quenching of DiO and DiI fluorescence increased with the loading concentration (Fig. S2). With these characterizations, it is possible to monitor the liberation of DiI and DiO from micelles or decomposition of micelles by measuring the FRET effects, as described below.

The FRET micelles were first applied to model membranes to examine the release of the core-loaded hydrophobic probes to lipid bilayers. Homogeneous supported bilayer and small unilamellar vesicle (SUV) samples were prepared as described in ref. 17. A supported bilayer composed of dioleoyl phosphatidylcholine (DOPC) was incubated with 0.2 mg/ml FRET micelles at room temperature for 2 h. The bilayer was then washed with deionized water. Two-color fluorescence imaging was performed with 488-nm excitation on a confocal microscope. Both DiO fluorescence (Fig. 3A) and DiI fluorescence (Fig. 3B) from the bilayer were observed, with a ratio  $I_{DiI}/(I_{DiI} + I_{DiO})$  of 0.37. As a control, 0.2 mg/ml FRET micelles in water were also imaged (Fig. 3C and D), and a higher ratio  $I_{DiI}/(I_{DiI} + I_{DiO})$  of 0.89 was obtained. These results indicate that DiI and DiO were effectively relocated to the lipid bilayer during the micelle–bilayer interaction.

To monitor the dynamics of such transfer, 5  $\mu\text{l}$  of 2 mg/ml FRET micelles were mixed with 2 ml of DOPC SUV solution (4 mM), resulting in a final micelle concentration of 5  $\mu\text{g/ml}$ . The time-resolved spectra measured every 15 min over a 2-h period are shown in Fig. 3E. A burst decrease of DiI signal was found in the first 15 min, followed by very small changes. On the contrary, the DiO signal increased gradually during the 2-h period. The increase of DiO intensity and decrease of DiI intensity indicate a reduction of FRET due to release of the probes from micelles. To explain the different patterns of intensity change between DiI and DiO, a time-resolved fluorescence study of micelles loaded with DiI or DiO alone was carried out. The results are shown in Fig. 3F and G. For DiO-loaded micelles at 484-nm excitation, mixing with SUVs caused a decrease of DiO fluorescence in the first 15 min, followed by a substantial increase. The initial stage (i.e., within 15 min) can be attributed to the burst release of probes located at the hydrophobic-hydrophilic interface inside of the micelle (18, 19), in which the intensity drop shows the effect of local environment (micelle versus lipid membrane) on the probe fluorescence. The later stage can be attributed to the release of high-density probes from the micelle core. Because the release also reduced the probe density inside of the core, the self-quench was effectively attenuated, resulting in an increase of total DiO intensity as shown in Fig. 3G. The DiI-loaded micelles displayed the similar





shown in Fig. 6B confirms a strong FRET outside of the cell (red curve) and a weak FRET inside of the cell (green curve), consistent with the case of PEG-PDLLA FRET micelles shown in Fig. 4A and B. These results suggest that release of hydrophobic molecules from self-assembled PEG-PCL micelles to cells occurred in a way similar to that for PEG-PDLLA micelles. We also used DOPC SUV solution to monitor the dynamics of DiI and DiO release from micelles to lipid bilayers. The time-resolved emission spectra measured for a 2-h period with 5  $\mu\text{g}/\text{ml}$  micelles are shown in Fig. 6C. A gradual increase of the DiO fluorescence and simultaneous diminution of the DiI fluorescence were observed. Accordingly, the FRET ratio decreased from 0.68 at 0 h to 0.59 at 2 h. It is notable that no burst release was observed, implying an enhanced retention of hydrophobic molecules in the PCL core.

## Discussion

Understanding the mechanisms underlying the drug delivery by micelles to cellular and subcellular targets is critical for the design of micelles as effective drug carriers (30, 31). Although internalization of micelles was reported previously (14, 32, 33), the current work showed that internalization of micelles was much slower than that of core-loaded hydrophobic molecules (Fig. 1A and B). By FRET experiments in model membranes (Fig. 3) and in cells (Figs. 4–6), we found that core-loaded DiI and DiO were released to plasma membranes and internalized by cells afterward. These results show that micelles exhibit membrane-mediated transportation of hydrophobic agents to cells.

The dynamic instability of polymeric micelles provides a possible mechanism for the release of hydrophobic molecules from micelle core to lipid bilayers. However, it was shown that dynamic exchange between micelles requires days (34, 35), much more time than the release observed in our experiments. We propose that the fast release is facilitated by the PEG shell in micelles. PEG-induced fusion of model membranes has been well studied (36, 37). It is hypothesized that PEG can force very close contact between vesicle membranes by lowering the activity of water adjacent to the membrane (38). In addition, PEG can dehydrate the lipid bilayer, leading to the formation of nonlamellar structures, raising the gel-to-fluid phase transition temperature (39, 40), and enhancing membrane permeability (41). It was also reported that lipid-soluble probes on the membrane of labeled cells could diffuse to the membrane of unlabeled cells at a high PEG concentration, indicating a PEG bridge between the phospholipid membranes of adjacent cells (42). In our study, although the overall PEG concentration in the solution is low with 0.1 mg/ml, the PEG density at the micelle surface is locally high because of the self-assembly of copolymers—e.g., assuming 1,000 copolymer molecules in a micelle of 60 nm diameter, the average PEG density would be 73 mg/ml. The high-density PEG could bridge the transfer of hydrophobic molecules from micelle core to lipid membrane.

Our observation of the fast release of drug molecules from micelles to lipid sinks explains the previous study by Burt *et al.* (43), who observed that PTX rapidly dissociated from the micelle in the blood. Such dissociation is likely due to the transfer of PTX to the abundant lipid components and carriers in the blood. This uncontrolled drug release limits the applications of micelles as a drug delivery system. Although the targeting efficiency can be enhanced by functionalizing micelles with ligands that have selective affinity for recognizing and interacting with a specific cell, tissue, or organ in the body (44), it cannot avoid the drug release during *in vivo* transportation. Shell-cross-linked biodegradable micelles would be a practical method for overcoming the uncontrolled release of drug to lipid environment. Physically incorporated drug molecules can be shielded tightly by the cross-linked corona, and biodegradable cross-

linking can allow the release of the drug from micelles in a controlled manner.

## Materials and Methods

**Materials.** Unless stated otherwise, all of the reagents and solvents were used as originally procured.  $\alpha$ -Amino-methoxy PEG (MPEG-NH<sub>2</sub>; MW 5,000) was purchased from Nektar. D,L-lactide (LA), 10-hydroxydecanoic acid (HDA), dicyclohexyl carbodiimide (DCC), *N*-hydroxysuccinimide (NHS), triethylamine (TEA), stannous 2-ethyl-hexanoate [Sn(Oct)<sub>2</sub>], dibutyltin dilaurate (DBDL), bis-amino-PEG (H<sub>2</sub>N-PEG-NH<sub>2</sub>), cytochalasin D, and sodium azide were purchased from Sigma–Aldrich. PTX was supplied by Samyang Genex. DiI, DiO, FITC, and the MTT assay kit were purchased from Invitrogen. DOPC was purchased from Avanti Polar Lipids.

**Polymer Synthesis and Micelle Preparation.** Details on synthesis of copolymers and their fluorescent conjugates can be found in *SI Methods*. Dual-labeled PEG-PDLLA micelles were prepared by the precipitation and membrane dialysis method. Block copolymers (90 mg of PEG-PDLLA, 10 mg of FITC-PEG-PDLLA) were dissolved in 5 ml of acetone with 0.15 mg of DiI. After stirring for 30 min, the solution was dropped to 50 ml of deionized water at 2 ml/min speed and followed by 3 h of stirring to vaporize acetone. Block copolymer aqueous solution was then dialyzed against 2 liters of deionized water (Spectra/Por MWCO 3500) for 2 days. Water was changed after 1 day. Finally, the solution was filtered through a 0.45- $\mu\text{m}$  filter to remove undesirable aggregates, and stored at 4°C. The administration concentration of 2 mg/ml was calculated according to the copolymer and deionized water used.

A similar procedure was used to prepare PEG-PDLLA micelles with 0.15% DiI, PEG-PDLLA FRET micelles, PTX-loaded FRET micelles, and PEG-PCL (5,000:2,000) FRET micelles. In the FRET micelle preparation, DiI and DiO were mixed with 100 mg of copolymer at a 0.75% weight ratio in 5 ml of acetone solution. In the PTX-loaded FRET micelle preparation, 10 mg of PTX was supplied to this 5-ml acetone solution.

The average size of block copolymer micelles were measured by DLS at 23°C. DLS measurement was carried out using a DynaPro99 molecular-sizing instrument equipped with a microsampler (Protein Solutions). The intensity autocorrelation was measured at a scattering angle of 90°. The program CORAN was used for data analysis to obtain the size distribution of micelles. The zeta potential was measured by using the ZetaPALS (Brookhaven Instruments). The anisotropy was measured by using the SpectraMax M5 (Molecular Devices).

**Cell Culture.** KB cells, a tumor cell line, were obtained from American Type Culture Collection (ATCC CCL-17). A GFP-tubulin stably transfected HeLa was a kind gift from Xiaoqi Liu (Purdue University). Cells were cultured at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub> and grown continuously in RPMI medium 1640 supplemented with 10% FBS, 100 unit/ml penicillin, and 100  $\mu\text{g}/\text{ml}$  streptomycin. Coverslip-bottomed Petri dishes (MatTek) were used for high-resolution imaging. Before each experiment,  $6 \times 10^4$  cells in 1 ml of growth medium were deposited into a Petri dish and incubated for 3–4 days to encourage adherence and cell confluence. The cells in 0.9 ml of culture medium containing 10% FBS were supplemented with 100  $\mu\text{l}$  of micelles and incubated at 37°C or 4°C for the desired lengths of time before imaging.

**FRET Imaging, Microspectroscopy, and Spectroscopy.** Imaging was performed by using a FV1000 confocal system (Olympus). A 543-nm HeNe laser was used to excite DiI. An Ar<sup>+</sup> laser was used to excite DiO, FITC, and GFP. A 405/488 nm excitation dichroic mirror was used to reflect the 488-nm beam to the sample. FRET images were acquired with 488-nm excitation, spectral filter of 555–655 nm for DiI detection, and spectral filter of 500–530 nm for DiO detection. All of the images were obtained and processed with Fluoview software (Olympus). Raw images in OIB format were converted to 8-bit tiff files with pseudocolors for display. Only brightness and contrast were optimized, and no other image processing was used. Microspectroscopy at pixels of interest was carried out by using a spectral detector, with emission scan from 490 to 590 nm. Fluorescence spectra of FRET micelles were measured by using the AMINCO-Bowman series 2 luminescence spectrometer (SLM Aminco Bowman) with 484-nm excitation and emission scan from 490 to 590 nm.

**ACKNOWLEDGMENTS.** This work was supported by a seed grant from the Purdue University Oncological Sciences Center, National Science Foundation Grant 0416785-MCB (to J.-X.C.), and National Institutes of Health Grants GM065284 and HL078715 (to K.P.).

1. Kataoka K, Kwon GS, Yokoyama M, Okano T, Sakurai Y (1993) Block copolymer micelles as vehicles for drug delivery. *J Controlled Release* 24:119–132.
2. Kwon GS, Kataoka K (1995) Block copolymer micelles as long-circulating drug vehicles. *Adv Drug Delivery Rev* 16:295–309.
3. Moghimi SM, Hunter AC, Murray JC (2001) Long-circulating and target-specific nanoparticles: Theory to practice. *Pharmacol Rev* 53:283–318.
4. Gaucher G, et al. (2005) Block copolymer micelles: Preparation, characterization and application in drug delivery. *J Controlled Release* 109:169–188.
5. Maeda H, Wu J, Sawa T, Matsumura Y, Hori K (2000) Tumor vascular permeability and the EPR effect in macromolecular therapeutics: A review. *J Controlled Release* 65:271–284.
6. Washington C (1990) Drug release from microdisperse systems: A critical review. *Int J Pharm* 58:1–12.
7. Lim Soo P, Luo L, Maysinger D, Eisenberg A (2002) Incorporation and release of hydrophobic probes in biocompatible polycaprolactone-block-poly(ethylene oxide) micelles: Implications for drug delivery. *Langmuir* 18:9996–10004.
8. Hubbell JA (2003) Materials science: Enhancing drug function. *Science* 300:595–596.
9. Kim SC, et al. (2001) In vivo evaluation of polymeric micellar paclitaxel formulation: Toxicity and efficacy. *J Controlled Release* 72:191–202.
10. Kim T-Y, et al. (2004) Phase I and pharmacokinetic study of Genexol-PM, a cremophor-free, polymeric micelle-formulated paclitaxel, in patients with advanced malignancies. *Clin Cancer Res* 10:3708–3716.
11. Venne A, Li S, Mandeville R, Kabanov A, Alakhov V (1996) Hypersensitizing effect of Pluronic L61 on cytotoxic activity, transport, and subcellular distribution of Doxorubicin in multiple drug-resistant cells. *Cancer Res* 56:3626–3629.
12. Allen C, Yu Y, Eisenberg A, Maysinger D (1999) Cellular internalization of PCL<sub>20</sub>-b-PEO<sub>44</sub> block copolymer micelles. *Biochim Biophys Acta* 1421:32–38.
13. Luo L, Tam J, Maysinger D, Eisenberg A (2002) Cellular internalization of poly(ethylene oxide)-b-poly( $\epsilon$ -caprolactone) diblock copolymer micelles. *Bioconjugate Chem* 13:1259–1265.
14. Savic R, Luo L, Eisenberg A, Maysinger D (2003) Micellar nanocontainers distribute to defined cytoplasmic organelles. *Science* 300:615–618.
15. Moghimi SM, et al. (2004) Cellular distribution of nonionic micelles. *Science* 303:626–628.
16. Sengupta P, Holowka D, Baird B (2007) Fluorescence resonance energy transfer between lipid probes detects nanoscopic heterogeneity in the plasma membrane of live cells. *Biophys J* 92:3564–3574.
17. Li L, Wang H, Cheng J-X (2005) Quantitative coherent anti-Stokes Raman scattering imaging of lipid distribution in coexisting domains. *Biophys J* 89:3480–3490.
18. Matsumoto J, Nakada Y, Sakurai K, Nakamura T, Takahashi Y (1999) Preparation of nanoparticles consisted of poly(L-lactide)-poly(ethylene glycol)-poly(L-lactide) and their evaluation in vitro. *Int J Pharm* 185:93–101.
19. Ahmed F, Discher DE (2004) Self-orienting polymersomes of PEG-PLA and PEG-PCL: Hydrolysis-triggered controlled release vesicles. *J Controlled Release* 96:37–53.
20. Steendam R, van Steenbergen MJ, Hennink WE, Frijlink HW, Lerk CF (2001) Effect of molecular weight and glass transition on relaxation and release behaviour of poly(D,L-lactic acid) tablets. *J Controlled Release* 70:71–82.
21. Yamamoto Y, Yasugi K, Harada A, Nagasaki Y, Kataoka K (2002) Temperature-related change in the properties relevant to drug delivery of poly(ethylene glycol)-poly(D,L-lactide) block copolymer micelles in aqueous milieu. *J Controlled Release* 82:359–371.
22. McGuire WP, Rowinsky EK (1995) *Paclitaxel in Cancer Treatment* (Dekker, New York).
23. Zhang X, et al. (1997) An investigation of the antitumor activity and biodistribution of polymeric micellar paclitaxel. *Cancer Chemother Pharmacol* 40:81–86.
24. Huh KM, et al. (2005) Hydrotropic polymer micelle system for delivery of paclitaxel. *J Controlled Release* 101:59–68.
25. Balasubramanian SV, Straubinger RM (1994) Taxol-lipid interactions: Taxol-dependent effects on the physical properties of model membranes. *Biochemistry* 33:8941–8947.
26. Ali S, Minchey S, Janoff A, Mayhew E (2000) A differential scanning calorimetry study of phosphocholines mixed with paclitaxel and its bromoacylated taxanes. *Biophys J* 78:246–256.
27. Hamm-Alvarez SF, Sonee M, Loran-Goss K, Shen W-C (1996) Paclitaxel and nocodazole differentially alter endocytosis in cultured cells. *Pharm Res* 13:1647–1656.
28. Lin W-J, Juang L-W, Lin C-C (2003) Stability and release performance of a series of pegylated copolymeric micelles. *Pharm Res* 20:668–673.
29. Park EK, Lee SB, Lee YM (2005) Preparation and characterization of methoxy poly(ethylene glycol)/poly( $\epsilon$ -caprolactone) amphiphilic block copolymeric nanospheres for tumor-specific folate-mediated targeting of anticancer drugs. *Biomaterials* 26:1053–1061.
30. Maysinger D, Berezovska O, Savic R, Lim Soo P, Eisenberg A (2001) Block copolymers modify the internalization of micelle-incorporated probes into neural cells. *Biochim Biophys Acta* 1539:205–217.
31. Allen TM, Cullis PR (2004) Drug delivery systems: Entering the mainstream. *Science* 303:1818–1822.
32. Liaw J, Aoyagi T, Kataoka K, Sakurai Y, Okano T (1999) Permeation of PEO-PBLA-FITC polymeric micelles in aortic endothelial cells. *Pharm Res* 16:213–220.
33. Mahmud A, Lavasanifar A (2005) The effect of block copolymer structure on the internalization of polymeric micelles by human breast cancer cells. *Colloids Surf B Biointerfaces* 45:82–89.
34. Wang Y, Kausch CM, Chun M, Quirk RP, Mattice WL (1995) Exchange of chains between micelles of labeled polystyrene-block-poly(oxyethylene) as monitored by nonradiative singlet energy transfer. *Macromolecules* 28:904–911.
35. Haliloglu T, Bahar I, Erman B, Mattice WL (1996) Mechanisms of the exchange of diblock copolymers between micelles at dynamic equilibrium. *Macromolecules* 29:4764–4771.
36. Lentz BR (1994) Polymer-induced membrane fusion: Potential mechanism and relation to cell fusion events. *Chem Phys Lipids* 73:91–106.
37. Lee J, Lentz BR (1997) Evolution of lipidic structures during model membrane fusion and the relation of this process to cell membrane fusion. *Biochemistry* 36:6251–6259.
38. Arnold K, Zschoernig O, Barthel D, Herold W (1990) Exclusion of poly(ethylene glycol) from liposome surfaces. *Biochim Biophys Acta* 1022:303–310.
39. Boni LT, Stewart TP, Alderfer JL, Hui SW (1981) Lipid-polyethylene glycol interactions: II. Formation of defects in bilayers. *J Membr Biol* 62:71–77.
40. Tilcock CPS, Fisher D (1979) Interaction of phospholipid membranes with poly(ethylene glycol)s. *Biochim Biophys Acta* 557:53–61.
41. Aldwinckle TJ, Ahkong QF, Bangham AD, Fisher D, Lucy JA (1982) Effects of poly(ethylene glycol) on liposomes and erythrocytes: Permeability changes and membrane fusion. *Biochim Biophys Acta* 689:548–560.
42. Ahkong QF, Desmazes JP, Georgescauld D, Lucy JA (1987) Movements of fluorescent probes in the mechanism of cell fusion induced by poly(ethylene glycol). *J Cell Sci* 88:389–398.
43. Burt HM, Zhang X, Toleikis P, Embree L, Hunter WL (1999) Development of copolymers of poly(D,L-lactide) and methoxypolyethylene glycol as micellar carriers of paclitaxel. *Colloids Surf B Biointerfaces* 16:161–171.
44. Vasir JK, Reddy MK, Labhasetwar VD (2005) Nanosystems in drug targeting: Opportunities and challenges. *Curr Nanosci* 1:47–64.