Electronic Supplementary Material (ESI) for Polymer Chemistry

Supplemental Information for

Intracellular pH-Activatiable PEG-*b*-PDPA Wormlike Micelles for Hydrophobic Drug Delivery

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Experimental Section

Matarials and Methods. Methoxy polyethylene glycol 5000 ($mPEG_{114}$-OH), copper bromide (CuBr, 99.99%), DMF, isopropyl alcohol (*i*-PA), and N,N,N',N',N'-Pentamethyldiethylenetriamine (PMDETA) were ordered from Sigma-Aldrich and used as recived. 2-(Diisopropylamino) ethyl methacrylate (DPA) was purchased from Scientific Polymer Products Inc. (Ontario, NY, USA) and purified by vacuum distillation over calcium hydride. 2-Hydroxyethyl methacrylate (HEMA) was ordered from Sigma-Aldrich and purified by vacuum distillation. Dialysis tubing (MWCO 3500 Da) was ordered from Fisher Scientific. Inc. (IL, USA). Nile red fluorescence probe was ordered from MP Biomedicals, LLC. (Ohio, USA). Tetramethylrhodamine-5-carbonyl-azide (TMR-azide), LysoTracker® green (DND-26), and Hoechst 33342 was obtained from Life Technologies.

Syntheses of mPEG-*b*-PDPA diblock copolymers via ATRP. Macroinitiator $mPEG_{114}$-Br was prepared according to the same procedure as described elsewhere.[1] $mPEG_{114}$-Br (0.50 g, 0.010 mmol), CuBr (14.3 mg, 0.250 mmol), PMDETA (43.3 mg, 0.250 mmol) and DPA (2.13 g 10.0 mmol) were charged into a schlenk flask. One milliliter of DMF/i-propanol mixture (v/v 1:1) was added as the solvent. The reactant mixture was degassed by three freezing-thawing cycles under vacuum condition. The polymerization was carried out at 40 °C for 24 h. The polymer solution was diluted by THF and passed ove a neutral alumina column to remoce copper catalist. The product was purified by dialyzing against Milli-Q water and dried by lyophilizing (see Fig. SI 1A for chemical structure). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.8~1.1 (broad, 15 H x 42, -$CH(CH_3)_2$, -CCH_3), 1.6~2.1 (broad, 2 H x 42, -CCH_2-), 2.62 (broad, 2 H x 42, -$CH(CH_3)_2$), 2.98 (broad, 2 H x 42, -CH_2CH_2N-), 3.64 (broad, 4 H x 113, -$O(CH_2)_2$-), 3.75~4.0 (broad, 2 H x 42, -NCH_2CH_2O-). Their molecular weights (*Mn*,*Mw*) were determined by gel permation chromatography (GPC) measureemnts.

Synthesis of mPEG-*b*-P(DPA-co-HEMA) diblock copolymer and TMR labeling. The $mPEG_{114}$-*b*-P(DPA_{25}-co-$HEMA_3$) diblock copolymer was synthesized by ATRP method following the procedure described above. ^{1}H NMR (500 MHz, Bruke, $CDCl_3$): δ 0.8~1.1 (broad, 15 H x 42, -$CH(CH_3)_2$, -CCH_3), 1.6~2.1 (broad, 2 H x 42, -CCH_2-), 2.62 (broad, 2 H x 42, -$CH(CH_3)_2$), 2.98 (broad, 2 H x 42, -CH_2CH_2N-), 3.64 (broad, 4 H x 113, -$O(CH_2)_2$-), 3.75~4.0 (broad, 2 H x 42, -NCH_2CH_2O-).

The $mPEG_{114}$-*b*-P(DPA_{25}-co-$HEMA_3$) diblock copolymer was labeled with TMR-azide dye according to manufacture's procedure. The reaction product was purified by dialyzing against ethanol and water, respectively. Any trace TMR dye was removed by multiple ultracentrifugation (Milli-pore, molecular weight cut-off 100 kDa). The TMR conjugation percentage was about 90% as determined by ^{1}H NMR spectra.

Preparation of mPEG-*b*-PDPA wormlike micelles: Typically, 15 mg of mPEG-*b*-PDPA diblock copolymer was dissolved in organic solvent (THF, methanol, DCM or Acetonitrile) overnight to ensure the complete releaxation of polymer chain. Add the solution into 8 mL glass vial and dried the solvent by N_2 flow. Adding 3 mL of DI water into the glass vial, and disperse the polymer film into wormlike micelles via ultrasonic irradiation. The nile red-loaded wormlike micelles (0.25 wt% loading density) were prepared by following the same procedure except adding nile red ethanol solution into the copolymer THF solution before film preparation.

DSC measurement: the crytallization of PEG-*b*-PDPA solid film was examined by DSC measurement (Perkin-Elmer-7). Temperature range: 0~100 ^{o}C, temperature increase/decrease rate: 10 ^{o}C·min^{-1}.

Fluorescent spectra and images: the fluorescnce spectra of TMR-conjugated or nile red-loaded wormlike micelles were determined by fluoresecence spectrameter (Jasco, FP-6500, Japan). The TMR fluorescence spectra was recorded with 519 nm excitation wavelength. The nile red fluorescence spectra was recorded with 490 nm excitation wavelength. The fluoresecent images were obtained by exciting the micelle samples with a UV lamp at 365 nm and imaging with a digital camera.

Cell culture: A549 non-small lung cancer cells were obtained from Dr. John Minna's labrotary (UTSouthwestern Medical Center at Dallas, TX) and used for all cell culture study. The cells were cultured in DMEM cell culture medium with 10% FBS supplementary, and 37 ^{o}C incubator with 5% CO_2 supply. Micelle concentration of 0.5 mg·mL^{-1} was applied for all cell culture study. The micelles were purified with 450 nm syringe filter for cell culture study.

Live cell imaging. All cell images were taken with invert Laser Scanning Microscope (Axio Observer Z.1, Carl Zeiss, Germany). The cell nucleus was stained with Hoechst 33342 (0.25 μg·mL^{-1}) 30 min before image taken (DAPI filter set). The acidic endocytosis organelles (i.e. late endosomes/lysosomes) were stained with Lysotacker-green (DND-26, 75 nmol) 1 h before image taking and imaged with GFP filter set (Ex 470/40, Em 525/50). TMR or NR fluoresence was imaged with Cy-3 filter set (Ex 550/25, Em 605/70).

Photodynamic treatment of cancer cells. A549 lung cancer cells were seeded into 96-well tissue culture plate at a density of 1.0×10^4 cells/well 24 h before micelle incubation. And then the A549 cells were pre-incubated with 2.0 wt% Ce6-loaded wormlike micelles at deasired micelle concentration for 24 h. The cells were then washed with fresh cell culture medium and irradiated with 655 nm laser (output 1.0 W/cm^2) for 3 min. The cellular viability were determined by MTT assay 24 h later after light irradiation, whice was expressed as relative cellular viability as normalized with that of untreated cell control. The cells were stained with Hoechst 33342 (2.5 μg/mL) and Calcein AM (2 μM/mL) for live-dead assay.

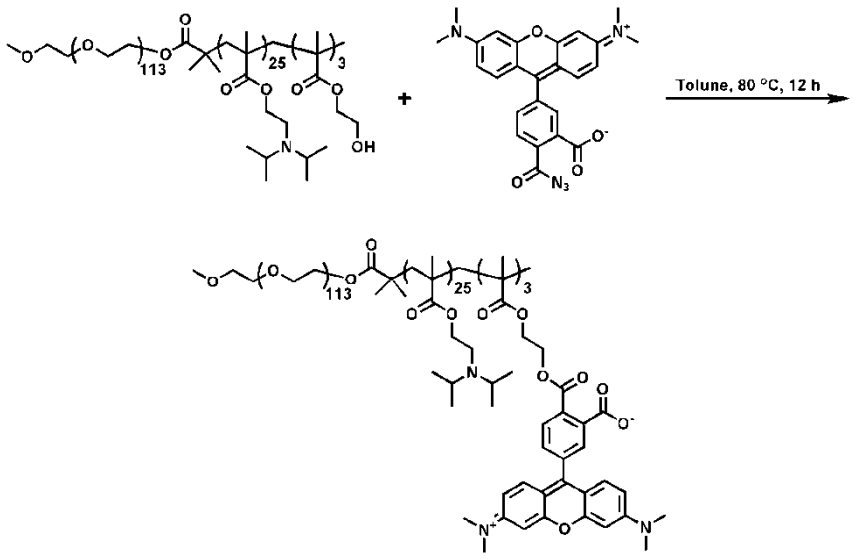
References

1.H. Yu, Y. Zou, Y. Wang, X. Huang, G. Huang, B. D. Sumer, D. A. Boothman and J. Gao, *ACS Nano*, **5**, 9246-9255.

Table SI 1. Molecular parameters of PEG-*b*-PDPA copolymers investigated in this study

Entry	Compositions[a]	PEG (wt)	*Mn*[a] (*10^3)	*Mw*[b] (*10^3)	*Mn*[b] (*10^3)	*PDI*[b](*Mw/Mn*)	Morphology[c]
1	$mPEG_{23}$-*b*-$PDPA_{65}$	0.67	14.9	22.9	21.1	1.09	N
2	$mPEG_{45}$-*b*-$PDPA_{35}$	0.21	9.47	14.5	18.9	1.30	S
3	$mPEG_{45}$-*b*-$PDPA_{42}$	0.18	10.9	19.6	23.1	1.18	N
4	$mPEG_{114}$-*b*-$PDPA_{11}$	0.68	7.35	11.9	12.8	1.07	S + W
5	$mPEG_{114}$-*b*-$PDPA_{25}$	0.48	10.5	11.8	13.4	1.13	W
6	$mPEG_{114}$-*b*-$PDPA_{30}$	0.44	11.4	15.9	18.5	1.17	W
7	$mPEG_{114}$-*b*-$PDPA_{40}$	0.36	14.0	20.7	17.1	1.21	W
8	$mPEG_{114}$-*b*-$PDPA_{80}$	0.23	22.1	36.2	44.8	1.24	N
9	$mPEG_{227}$-*b*-$PDPA_{20}$	0.70	14.3	22.0	24.7	1.12	S
10	$mPEG_{227}$-*b*-$PDPA_{40}$	0.54	18.5	26.1	29.4	1.23	S

a, ^{1}H-NMR spectra determined number average molecular weight (M_n); b, GPC measurement determined weight average molecular weight (M_w), number average molecular weight (M_n), and polydispersity index (PDI); c, (N) non-dispersible solid, (S) spherical micelles, and (W) wormlike micelles.



Scheme SI 1. Synthesis of TMR-conjugated PEG-*b*-PDPA copolymers.

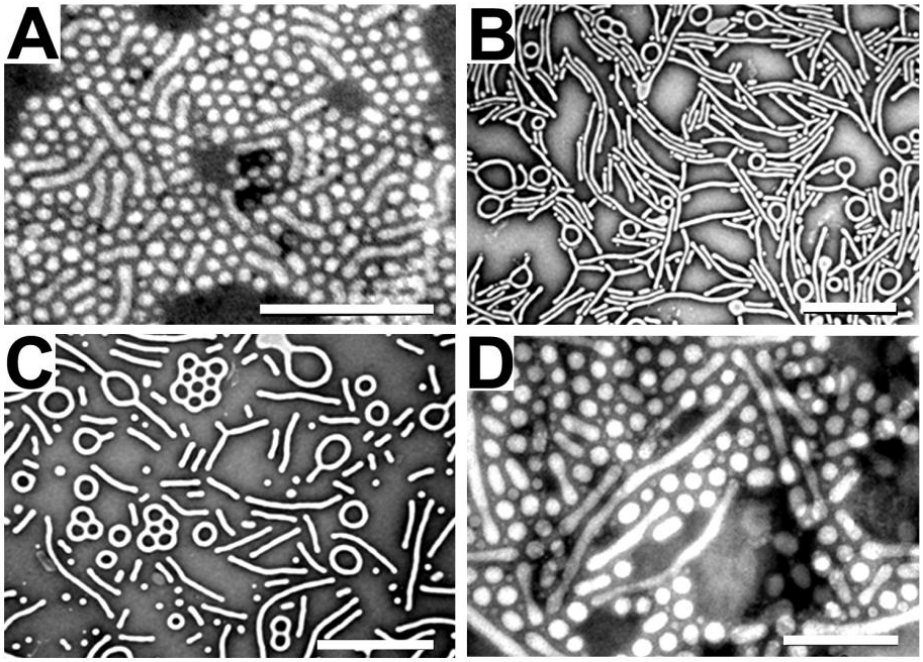


Figure SI 1. TEM images of ultrasonic irradiation prepared micelles with different copolymer compositions: (A) PEG_{113}-*b*-$PDPA_{11}$, (B) PEG_{113}-*b*-$PDPA_{25}$, (C) PEG_{113}-*b*-$PDPA_{40}$, (D) PEG_{225}-*b*-$PDPA_{40}$ (all samples were purified with 450 nm sysnringe filter, scale bar 200 nm applied for all images).

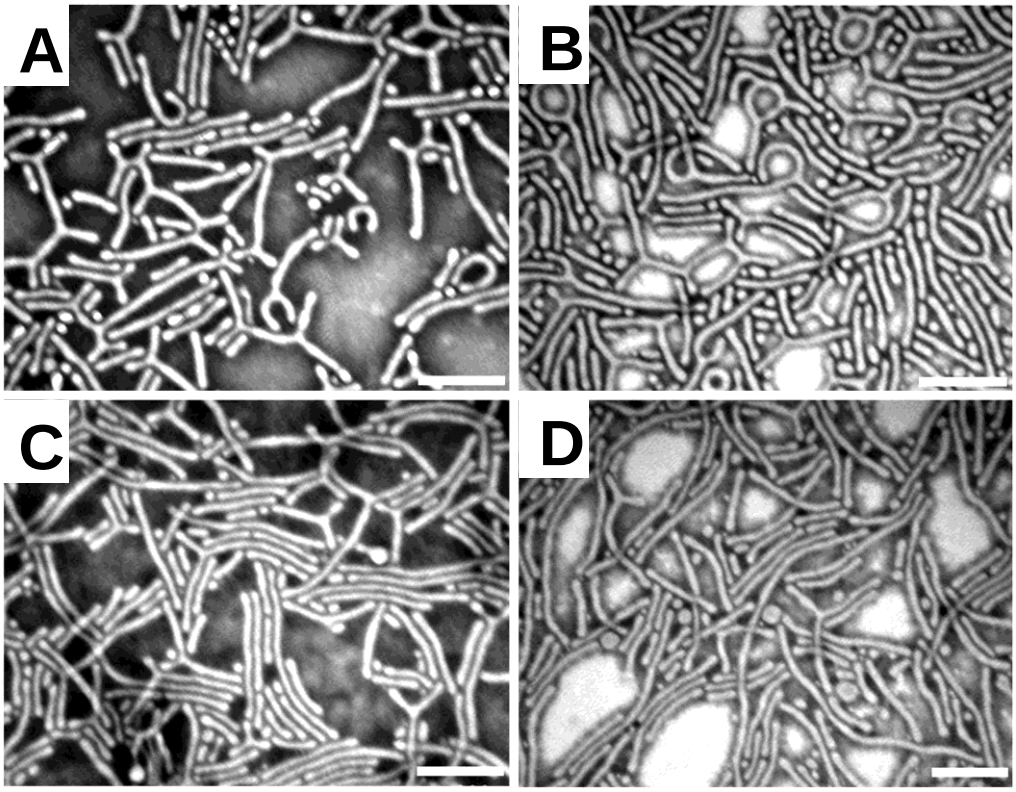


Figure SI 2. TEM images of wormlike micelles prepared from PEG_{114}-*b*-$PDPA_{25}$ in various organic solvents: (A) Acetonnitrile; (B) Methanol; (C) Tetrahydrofunan; (D) Chloroform (all samples were purified with 450 nm sysnringe filter, scale bar 200 nm applied for all images).

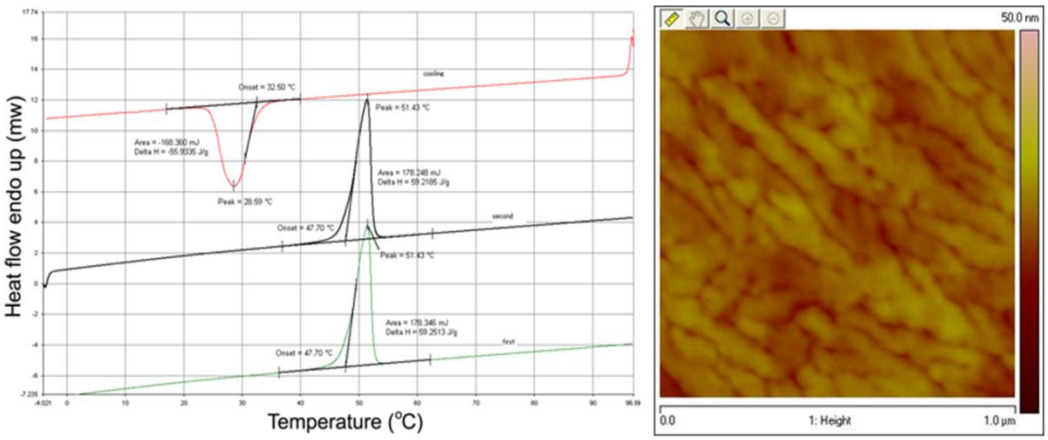


Figure SI 3. DSC curve (left) and AFM image (right) of PEG_{114}-*b*-$PDPA_{25}$ solid film prepared by N_2 flow drying.

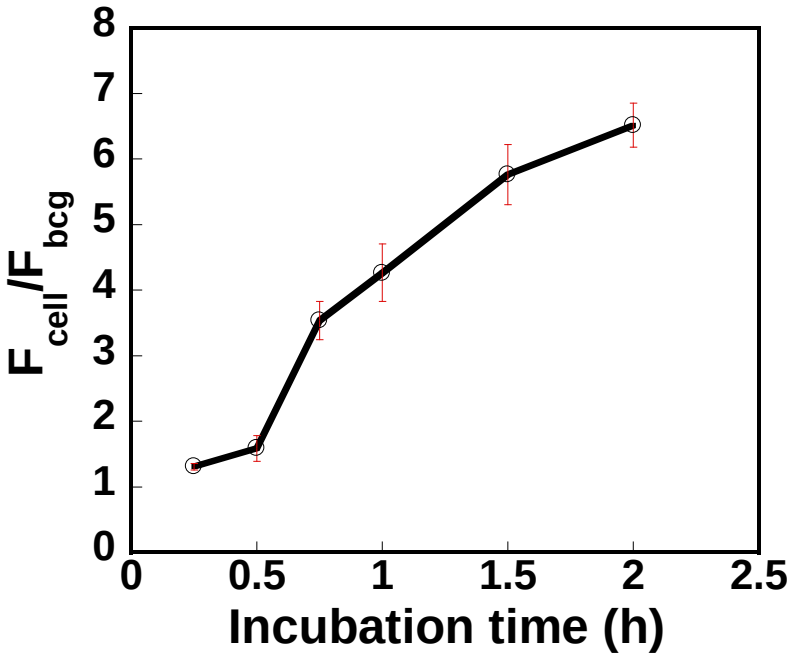


Figure SI 4. Signal to noise ratio as calculated by comparing the cellular fluorescence intensity with background noise at a function of incubation time.

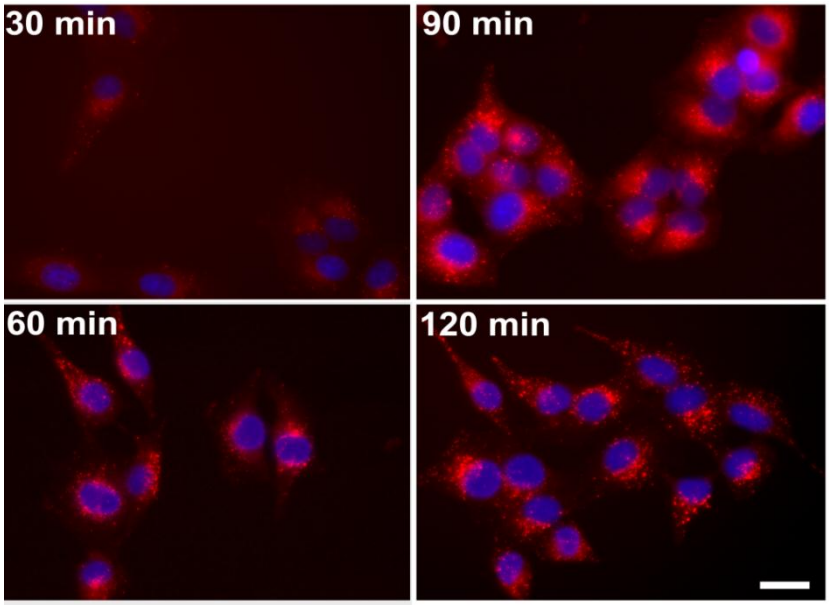


Figure SI 5. Fluoresecence images of A549 lung cancer celles incubated with TMR-conjugated wormlike micelles after different incubation time (scale bar 10 μm).

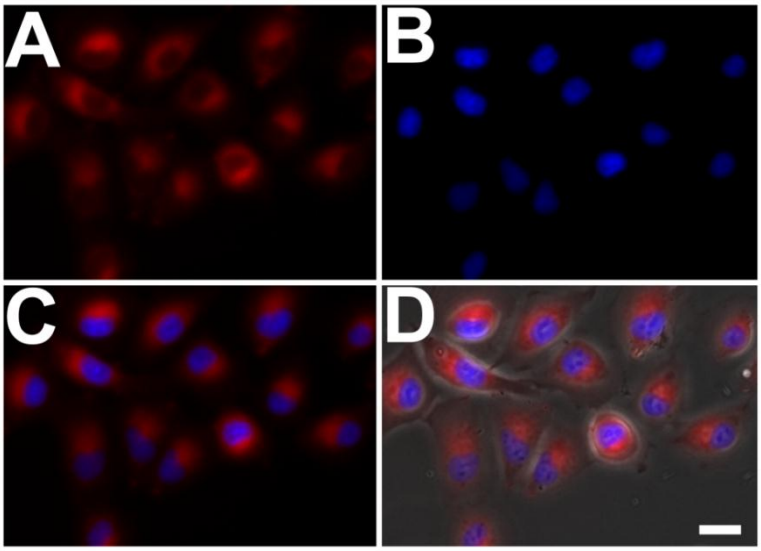


Figure SI 6. Fluorescence images of A549 celles incubated with free nile red dye for 4 h (scale bar 10 μm) (1.0 mg·mL^{-1} NR in DMSO, diluted into 250 ng·mL^{-1} for cell culture study. Hoechst 33342 0.25 ug·mL^{-1} for nucleus staining).

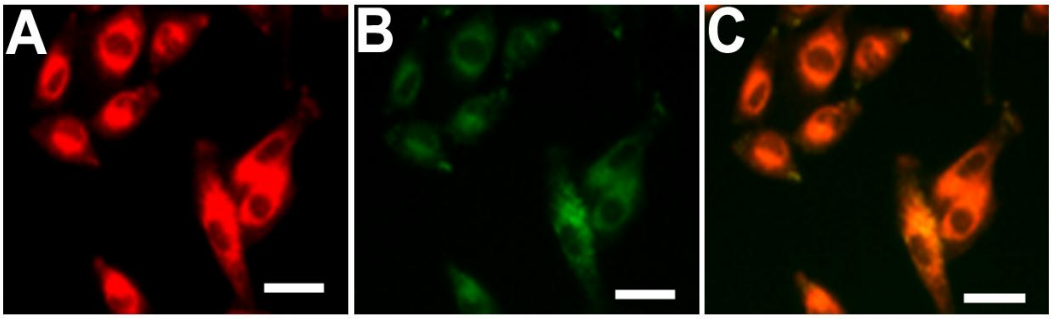


Figure SI 7. Fluorescence images of A549 cells treated for NR-loaded PEG_{114}-*b*-$PDPA_{25}$ wormlike micelles for 4 h. (A) NR, (B) Lysotracker green, and (C) overlay of red and green chaneels (scale bar 15 μm, applied for all three images).