**Supporting Information for**

# PHEA-*g*-PEO Well-Defined Graft Copolymer Exhibiting Synchronous Encapsulation of Both Hydrophobic Pyrene and Hydrophilic Rhodamine 6G

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## Experimental section

### Materials

2,2'-Azobis(isobutyronitrile) (AIBN, Aldrich, 98%) was recrystallized from anhydrous ethanol twice. Copper (I) chloride (CuCl, Aldrich, 98%) was purified by stirring overnight over $CH_3CO_2H$ at room temperature, followed by washing the solid with ethanol, diethyl ether, and acetone prior to drying at 40°C *in vacuo* for one day. *N*-Phenyl-1-naphthylamine (PNA, Alfa Aesar, 97%) was purified by recrystallization in ethanol for three times. Tetrahydrofuran (THF, Aldrich, 99%) and toluene (Aldrich, 99%) were dried over $CaH_2$ and distilled from sodium and benzophenone under $N_2$ prior to use. Dimethyl formamide (DMF, Aldrich, 99.8%) and triethylamine (TEA, Aldrich, 99.5%) were dried over KOH and distilled from $CaH_2$ under $N_2$ prior to use. Chloroform (Aldrich, 99%) was distilled from $CaH_2$ under $N_2$ prior to use.

Poly(ethylene oxide) monomethyl ether (PEO-OH, $M_n$ = 2,000 g/mol, Aldrich), 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (HTEMPO, Aldrich, 97%), succinic anhydride (Aldrich, 99%), 4-di(methylamino)pyridine (DMAP, Aldrich, 99%), thionyl chloride ($SOCl_2$, Aldrich, 99%), pyrene (Aldrich, 99%), rhodamine 6G (R6G, Aldrich, 99%), and *N*,*N*,*N*',*N*',*N*''-pentamethyldiethylenetriamine (PMDETA, Aldrich, 99%) were used as received. 2-Hydroxyethyl 2-((2-chloropropanoyloxy)methyl)acrylate (HECPMA)[60] and cumyl dithiobenzoate (CDB)[61] were synthesized according to previous literatures.

**Characterizations**

FT-IR spectra were recorded on a Nicolet AVATAR-360 FT-IR spectrophotometer with a resolution of 4 $cm^{-1}$. All $^1H$ NMR (400 MHz) and $^{13}C$ NMR (100 MHz) analyses were performed in $CDCl_3$, $CD_3OD$, DMSO-$d_6$, and $D_2O$ on a JEOL JNM-ECZ400 spectrometer, in particular $^1H$ NMR and $^{13}C$ NMR spectra of PEO-TEMPO were measured in $CD_3OD$ in the presence of stoichiometric $HCOONH_4$ and Pd/C.[34] Relative molecular weights and molecular weight distributions were measured by conventional gel permeation chromatography (GPC) system equipped with a Waters 1515 Isocratic HPLC pump, a Waters 2414 refractive index detector, and a set of Waters Styragel columns (HR3 (500-30,000), HR4 (5,000-600,000) and HR5 (50,000-4,000,000), 7.8×300 mm, particle size: 5 μm). GPC measurements were carried out at 35°C using DMF as eluent with a flow rate of 1.0 mL/min. The system was calibrated with linear polystyrene standards. Absolute molecular weights were

determined by GPC equipped with a multi-angle light scattering detector (GPC/MALS). DMF was used as solvent with a flow rate of 1.0 mL/min, detectors: Wyatt Optilab rEX refractive index detector and Wyatt DAWN HELEOS 18-angle light scattering detector with a 50 mW solid-state laser operating at 658 nm. UV/vis spectra were measured by a Hitachi U-2910 spectrophotometer with a rate of 200 nm/min. Steady-state fluorescence spectra were measured at 20°C on a Hitachi F-2700 spectrophotometer with the band width of 5 nm for excitation and emission, the emission intensity at 418 nm ($\lambda_{ex}$ = 340 nm) was recorded to determine the critical micelle concentration (*cmc*) and the emission intensity of R6G at 551 nm ($\lambda_{ex}$ = 500 nm) was recorded. Hydrodynamic diameter ($D_h$) was measured by dynamic light scattering (DLS) with a Malvern Nano-ZS90 Zetasizer. TEM images were obtained by a JEOL JEM-2100 instrument operated at 80 kV.

**RAFT Homopolymerization of HECPMA**

AIBN (9.3 mg, 0.057 mmol) and CDB (46.3 mg, 0.17 mmol) were first added into a 10 mL Schlenk flask (flame-dried under vacuum prior to use) sealed with a rubber septum for degassing and kept under $N_2$. Next, HECPMA **1** (1.00 g, 4.25 mmol) and 0.22 mL of anhydrous DMF were added via a gastight syringe. The flask was degassed by three cycles of freezing-pumping-thawing followed by immersing the flask into an oil bath set at 80°C. The polymerization was terminated by immersing the flask into liquid $N_2$ after 8 h. THF was added to dilute the solution and the solution was precipitated into cold diethyl ether. The crude product was purified by

repeated dissolution and precipitation followed by drying *in vacuo* overnight to give 0.832 g of pink powder.

To remove the dithiobenzoate end moiety, AIBN (0.46 g, 2.8 mmol) and 0.7 g of pink powder (0.085 mmol of dithiobenzoate group determined from $M_{n,GPC/MALS}$ = 8,223 g/mol) were first added to a 100 mL Schlenk flask (flame-dried under vacuum prior to use) sealed with a rubber septum for degassing and kept under $N_2$. Next, 30 mL of anhydrous THF was added via a gastight syringe. The flask was immersed into an oil bath set at 60°C and the reaction was quenched by liquid $N_2$ after 48 h. The solution turned colorless and was precipitated into cold diethyl ether after concentration. After repeated purification via dissolution and precipitation, 0.50 g of white powder, poly(2-hydroxyethyl 2-((2-chloropropanoyloxy)methyl)acrylate) (PHECPMA) **2**, was obtained by drying *in vacuo* overnight. GPC: $M_{n,GPC}$ = 5,600 g/mol, $M_w/M_n$ = 1.10. GPC/MALS: $M_{n,GPC/MALS}$ = 8,223 g/mol, $M_w/M_n$ = 1.08. FT-IR: $\nu$ ($cm^{-1}$): 3433 ($\nu_{O-H}$), 2951 ($\nu_{C-H}$), 2878 ($\nu_{C-H}$), 1735 ($\nu_{C=O}$), 1450, 1380, 1253, 1174, 1076, 1008, 970, 912, 844, 748. $^1$H NMR (DMSO-$d_6$): $\delta$ (ppm): 1.18 (12H, terminal $C(CH_3)_2$), 1.66 (3H, $CHClCH_3$), 1.98 (2H, $CH_2CCO_2$), 3.56 (2H, $CO_2CH_2CH_2OH$), 3.82 (2H, $CO_2CH_2CH_2OH$), 4.07 (2H, $CO_2CCH_2O$), 4.63 (1H, $CHClCH_3$), 4.88 (1H, $CH_2OH$), 7.11-7.30 (5H, terminal $C_6H_5$). $^{13}$C NMR (DMSO-$d_6$): $\delta$ (ppm): 21.8 ($CHClCH_3$), 44.3 ($CH_2CCO_2$), 47.8 ($CHClCH_3$), 53.3 ($CO_2CH_2CH_2OH$), 58.5 ($CO_2CCH_2O$), 64.6 ($CH_2CCO_2$), 67.4 ($CO_2CH_2CH_2OH$), 126.0, 128.1 ($C_6H_5$), 169.2 ($CH_2CCO_2$), 172.3 ($CO_2CHCl$).

**Preparation of PEO-TEMPO**

PEO-OH (4.00 g, $M_n$ = 2,000 g/mol, 2 mmol), succinic anhydride (0.5 g, 5 mmol), DMAP (0.0244 g, 0.2 mmol), and dry chloroform (20 mL) were added to a 250 mL three-neck flask and the mixture was stirred at 80°C for 24 h. Chloroform was removed by rotary evaporation and the crude product was dissolved in water. Unreacted succinic anhydride was eliminated by washing with a mixture of ethyl acetate and *n*-hexane (v:v = 1:1) several times. The aqueous phase was extracted by $CH_2Cl_2$ (50 mL×3) and all organic layers were merged followed by drying over $MgSO_4$ overnight. The filtrate was concentrated and dried *in vacuo* overnight to give 3.45 g of white solid, carboxyl-functionalized PEO (PEO-COOH, 86.3% yield). FT-IR: υ ($cm^{-1}$): 3507, 2870, 1734, 1454, 1108, 853. $^1$H NMR: $\delta$ (ppm): 2.60 (4H, C$H_2$C$H_2$CO$_2$H), 3.36 (3H, OC$H_3$), 3.55 (2H, CO$_2$CH$_2$C$H_2$), 3.62 (4H, OC$H_2$C$H_2$), 4.21 (2H, CO$_2$C$H_2$CH$_2$). $^{13}$C NMR: $\delta$ (ppm): 29.0 (*C*H$_2$*C*H$_2$CO$_2$H), 58.6 (O*C*H$_3$), 63.5 (CO$_2$*C*H$_2$CH$_2$), 70.1 (O*C*H$_2$*C*H$_2$ and CO$_2$CH$_2$*C*H$_2$), 172.0 (*C*O$_2$CH$_2$CH$_2$), 173.9 (CH$_2$CH$_2$*C*O$_2$H).

PEO-COOH (3.00 g, 1.5 mmol) and thionyl chloride (11 mL, 150 mmol) were added to a 100 mL flask and the mixture was stirred at room temperature for 4 h. Unreacted thionyl chloride was removed under vacuum at 50°C to give acyl-chloride-functionalized PEO (PEO-COCl). Next, HTEMPO 1.03 g, 6 mmol), DMAP (0.022 g, 0.18 mmol), TEA (0.2 mL, 1.44 mmol), and dry THF (40 mL) were added to a 250 mL three-neck flask and the solution was cooled to 0°C for adding PEO-COCl in 80 mL of dry THF. The system was stirred at 0°C for 1 hour and was raised to room

temperature with further stirring for 1 day. Water was added to quench the reaction. The solvent was removed by rotary evaporation and the crude product was dissolved in water. The aqueous system was washed with a mixture of ethyl acetate and *n*-hexane (v:v = 1:1) several times and then extracted by $CH_2Cl_2$. All organic layers were merged followed by drying over $MgSO_4$ overnight. The filtrate was concentrated and dried *in vacuo* overnight to give a red solid of TEMPO- functionalized PEO (PEO-TEMPO) **3** (2.54 g, 84.7% yield). FT-IR: υ ($cm^{-1}$): 2883, 1735, 1467, 1114, 842. $^1$H NMR ($CD_3OD$ in the presence of Pd/C and $HCOONH_4$): $\delta$ (ppm): 1.15 (12H, C*H*$_3$ of TEMPO), 1.53, 1.85 (4H, C*H*$_2$ of TEMPO), 2.60 (4H, C*H*$_2$C*H*$_2$CO$_2$), 3.35 (3H, OC*H*$_3$), 3.56 (2H, $CO_2CH_2CH_2$), 3.64 (4H, OC*H*$_2$C*H*$_2$), 4.22 (2H, $CO_2CH_2CH_2$), 5.01 (C*H* of TEMPO). $^{13}$C NMR ($CD_3OD$ in the presence of Pd/C and $HCOONH_4$): $\delta$ (ppm): 19.9 (*C*H$_3$ of TEMPO), 31.2 (*C*H$_2$ of TEMPO and *C*H$_2$*C*H$_2$CO$_2$), 58.8 (O*C*H$_3$), 60.9 (*C*(CH$_3$)$_2$ of TEMPO), 63.4 ($CO_2CH_2CH_2$), 69.7 (O*C*H$_2$*C*H$_2$, $CO_2CH_2CH_2$ and *C*H of TEMPO), 169.0 ($CH_2CH_2CO_2$).

**ATNRC between PHECPMA and PEO-TEMPO**

CuCl (0.005 g, 0.05 mmol) and PHECPMA **2** (0.012 g, $M_{n,GPC/MALS}$ = 8,223 g/mol, $M_w/M_n$ = 1.08, $N_{HECPMA}$ = 34.0, 0.05 mmol (-OCOCH(CH$_3$)Cl group) were added to a 10 mL Schlenk flask (flame-dried under vacuum prior to use) sealed with a rubber septum under $N_2$. After three cycles of evacuating and purging with $N_2$, PEO-TEMPO **3** (0.1353 g, 0.06 mmol) in 3 mL of dry toluene, DMF (3 mL), and PMDETA (10 μL, 0.05 mmol) were charged via a gastight syringe. The flask was degassed by three

cycles of freezing-pumping-thawing. Next, the flask was immersed into an oil bath set at 90°C. The coupling reaction lasted 3 h and it was terminated by putting the flask into liquid $N_2$. The reaction mixture was diluted with THF and passed through a neutral alumina column to remove the residual copper complex. The crude product was purified by dialysis (MW cutoff = 25 KDa) against $CH_3OH$ for three days to remove unbound PEG-TEMPO. The target product, PHEA-*g*-PEO **4** graft copolymer, was obtained by drying *in vacuo* overnight. GPC: $M_n$ = 41,900 g/mol, $M_w/M_n$ = 1.16. FT-IR: $\upsilon$ ($cm^{-1}$): 3430, 2886, 1735, 1433, 1359, 1343, 1168, 823. $^1H$ NMR ($CDCl_3$): $\delta$ (ppm): 0.89, 1.21 (12H, C*H*$_3$ of TEMPO), 1.25, 1.38 (4H, C*H*$_2$ of TEMPO), 1.73 (3H, C*H*$_3$CHCl and 3H, C*H*$_3$CHON), 2.06 (2H, C*H*$_2$CCO$_2$), 2.62 (4H, O$_2$CC*H*$_2$C*H*$_2$CO$_2$), 3.38 (3H, OC*H*$_3$), 3.56 (2H, CO$_2$CH$_2$C*H*$_2$O and 2H, CO$_2$CH$_2$C*H*$_2$OH), 3.64 (4H, OC*H*$_2$C*H*$_2$), 3.88 (2H, CO$_2$C*H*$_2$CH$_2$OH), 4.25 (2H, CO$_2$C*H*$_2$CH$_2$O and 2H, CO$_2$CC*H*$_2$O), 4.35 (1H, CH$_3$C*H*Cl and 1H, CH$_3$C*H*ON), 5.00 (1H, C*H* of TEMPO).

**Determination of Critical Micelle Concentration**

PNA was used as fluorescence probe to measure the *cmc* of PHEA-*g*-PEO **4** graft copolymer in aqueous media, NaCl (0.2 M and 0.8 M), and $Na_2SO_4$ (0.3 M) aqueous solutions. Acetone solution of PNA ([PNA] = 2 mM) was added to a large amount of water (or NaCl, $Na_2SO_4$ aqueous solution) until [PNA] reached 0.002 mM. The solutions for fluorescence measurement were obtained by adding different amounts of THF solutions of copolymer **4** (1, 0.1, 0.01, 0.001, or 0.0001 mg/mL) to water (or NaCl, $Na_2SO_4$ aqueous solution) containing PNA ([PNA] = 0.002 mM).

**Micellar Morphology**

PHEA-*g*-PEO **4** graft copolymer was added dropwise to deionized water (or NaCl (0.2 M and 0.8 M), and $Na_2SO_4$ (0.3 M) aqueous solutions) under vigorous stirring until the concentration of copolymer reached 0.1 mg/mL. THF was evaporated by stirring moderately overnight at room temperature. For TEM studies, 10 μL of micellar solution was deposited on an electron microscopy copper grid coated with carbon film and the water was evaporated at room temperature.

**Encapsulation of Hydrophobic Pyrene in Micelles**

THF solution containing PHEA-*g*-PEO **4** graft copolymer and pyrene was added dropwise to deionized water under vigorous stirring until the concentrations of copolymer and pyrene reached 0.08 mg/mL and 0.01 mmol/L, respectively. THF was evaporated by stirring moderately overnight at room temperature. For the control experiment, only pyrene was added to deionized water. Both solutions were filtered through a 0.45 μm syringe filter and the obtained solutions were employed for the measurement of UV absorption spectroscopy.

**Encapsulation of Hydrophilic Rhodamine 6G in Micelles**

THF solution of PHEA-*g*-PEO **4** graft copolymer was added dropwise to deionized water under vigorous stirring until the concentration of copolymer reached 0.08 mg/mL. Next, aqueous solution of R6G was added to the above solution until the concentration of R6G reached 0.02 mmol/L. THF was evaporated by stirring

moderately overnight at room temperature. The resulting solution was dialyzed against water using dialysis membrane ($MW_{cut\text{-}off}$ = 3.5 kDa) until the dialysate did not show any detectable UV signal. The obtained solution was used for the measurements of UV absorption and fluorescence spectroscopy. Similar procedure was used for $PEG_{113}$-*b*-$PS_{100}$ diblock copolymer.

**Coencapsulation of Rhodamine 6G and Pyrene in Micelles**

THF solution containing PHEA-*g*-PEO **4** graft copolymer and pyrene was added dropwise to deionized water under vigorous stirring until the concentration of copolymer and pyrene reached 0.08 mg/mL and 0.01 mmol/L, respectively. R6G aqueous solution was then added to the above solution until the concentration of R6G reached 0.02 mmol/L. THF was evaporated by stirring moderately overnight at room temperature. The resulting solution was dialyzed against water using dialysis membrane ($MW_{cut\text{-}off}$ = 3.5 kDa) until the dialysate did not show any detectable UV signal. The obtained solution was used for the measurement of UV absorption spectroscopy.

**References and Notes**


1. Jiang, X. Y.; Lu, G. L.; Feng, C.; Huang, X. Y. *Polym. Chem.* **2014**, *5*, 4915-4925.
2. Moad, G.; Chiefari, J.; Chong, Y. K.; Krstina, J.; Mayadunne, R. T. A.; Postma, A.; Rizzardo, E.; Thang, S. H. *Polym. Int.* **2000**, *49*, 993-1001.