Electronic Supplementary Information for:

Poly(3-hydroxyalkanoate)-derived Amphiphilic Graft Copolymer for the Design of Polymersomes

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Experimental Part

Materials

Acetone ≥99.5%, Anhydrous MeOH 99.8%, poly(ethylene glycol) methyl ether average M_n ~550 (MeO-PEG_{550}) potassium thioacetate 98%, and *α,α′*-Azoisobutyronitrile ≥ 98% (AIBN) were purchased from Sigma Aldrich. p-toluenesulfonyl chloride 99+% (TsCl) and 4-dimethylaminopyridine 99% (DMAP) were purchased from Acros Organics. Hydrochloric acid 37% and pyridine synthesis grade were obtained from Carlo Erba. CH_2Cl_2 was obtained from Carlo Erba and was distilled over CaH_2 before use. THF and toluene were obtained from Carlo Erba and were distilled over sodium/benzophenone before use. All other materials were used without further purification.

PHOU was obtained from EMPA, Swiss Fed Labs Mat Testing & Res, Lab Biomat, CH-9014 St Gallen, Switzerland, synthesized following a previously reported procedure.[1] Its molar composition was determined by ^1H NMR in $CDCl_3$ by comparing the integration of the ethylenic proton **g** to the main chain protons **b, b'** (Figure S1 a). $PHOU_{14\%}$: 14% of unsaturated undecenoate units and 86% of 3-hydroxyoctanoate. $PHOU_{31\%}$: 31% of undecenoate units and 69% of 3-hydroxyoctanoate. It has to be noted that PHOU are mainly composed of 3-hydroxyoctanoate and 3-hydroxy-10-undecenoate units; however, they possess a wider chain length distribution (3-hydroxyhexanoate; 3-hydroxy-6-heptenoate; 3-hydroxy-8-nonenoate), as a consequence of biosynthesis. Their molar masses were determined by SEC

(THF, polystyrene standards): $PHOU_{14\%}$: $\overline{Mn}$ = 98600 g.mol^{-1}, PDI = 1.8, and $PHOU_{31\%}$: $\overline{Mn}$ = 95600 g.mol^{-1}, PDI = 1.8.

Synthesis of PEG-SH

Thiolated PEG was synthesized using a modified procedure.[2]

Tosylation of PEG_{550}: MeO-PEG_{550} (30 g, 54.5 mmol) and DMAP (333 mg, 2.7 mmol) were dissolved in 160 mL of CH_2Cl_2/ Pyridine (1:1) solution at 0°C under argon atmosphere. p-toluenesulfonyl chloride (18.7 g, 98.1 mmol) dissolved in 50 mL of CH_2Cl_2 was added dropwise and the solution was stirred overnight (from 0°C to RT). 200 mL of cold water were added and the aqueous phase was extracted three times with 150 mL of CH_2Cl_2. The combined organic phases were successively washed with 3x 250 mL HCl 1M solution and 3x 250 mL of saturated NaCl solution. After drying over $MgSO_4$, the organic phase was concentrated under reduced pressure and the resulting product (PEG_{550}-OTs) dried under vacuum (m = 36.4 g, yield 95%). ^{1}H NMR ($CDCl_3$) δ 7.76 (d, J = 8.3 Hz, 2H), 7.30 (d, J = 8 Hz, 2H), 4.12 (m, 2H), 3.49-3.66 (m, 41H), 3.34 (s, 3H), 2.41 (s, 3H).

Synthesis of PEG_{550}-thioacetate

PEG_{550}-OTs (36 g, 51 mmol) was dissolved in 200 mL of THF under Ar. Potassium thioacetate (11.66 g, 102 mmol) was added and the solution was refluxed for 20h. A white fluffy precipitate formed. The solution was cooled to room temperature and 400 mL of water were added. The flask contents were extracted with 3x 150 mL CH_2Cl_2 and the brown colored organic extract was washed with 3x 200 mL of water, treated with activated charcoal, filtered and rotary evaporated to afford 23.3 g (38.3 mmol) of light yellow oil (yield 75%). ^{1}H NMR ($CDCl_3$) δ 3.49-3.62 (m, 43H), 3.34 (s, 3H), 3.05 (t, J = 6.5 Hz, 2H), 2.29 (s, 3H).

Synthesis of PEG_{550}-SH

PEG_{550}-thioacetate (23 g, 37.7 mmol) was dissolved in 200 mL of anhydrous MeOH under argon. 15 mL of HCl 37% were added and the solution was refluxed for 2h. 200 mL of water

were added and the solution was extracted with 3x 150 mL CH_2Cl_2. The combined organic phases were washed with 3x 200 mL of water, 3x 250 mL of saturated NaCl solution, dried over $MgSO_4$ and filtered. The resulting solution was rotary evaporated to give 17.52 g (30.9 mmol) of colorless oil, thiolated PEG (yield 82%). ^{1}H NMR ($CDCl_3$) δ 3.50-3.63 (m, 43H), 3.34 (s, 3H), 2.66 (dt, J = 8.2, 6.4 Hz, 2H), 1.56 (t, J = 8.2 Hz, 1H).

Synthesis of PHOU oligomers[3]

PHOU (300 mg) was dissolved in 12 mL of CH_2Cl_2. 12 mL of anhydrous MeOH were introduced to the solution and 0.9 mL of H_2SO_4 was added. The solution was then stirred at 100°C (the vessel was hermetically closed) for 7 minutes to obtain the desired molar mass. After cooling in an ice bath, 8 mL of distilled water were added; after decantation, the organic layer was washed again with distilled water, dried over $MgSO_4$ and filtered. The solvent was evaporated and the resulting product was dried under vacuum at 40°C to afford 279 mg of PHOU oligomers (yield 93%). See Figure S2 for complete ^{1}H NMR attribution and Table 1 for molar mass determination.

Typical synthesis of PHOU-*g*-PEG

$PHOU_{14\%}$ oligomers (278 mg, 1.88 mmol, 0.263 mmol of double bonds), PEG_{550}-SH (747 mg, 1.31 mmol) and AIBN (21 mg, 0.131 mmol) were dissolved in 10 mL of anhydrous toluene under argon, and the solution was heated at 80°C for 20h. Toluene was rotary evaporated and the crude was dissolved in 30 mL of acetone. The solution was poured in 30 mL of water, and the resulting colloidal suspension was then transferred into a dialysis tube (SpectraPor, molecular weight cut off, 6-8000 Da) and dialyzed against water for 3 days. In order to remove all unreacted PEG, acetone was added to break the vesicles till complete clearance of the solution, and put again to dialyze for 3 days. Water was removed under reduced pressure and the resulting product dried under vacuum (394 mg, yield 92%). See Figure S1 for complete ^{1}H NMR attribution and Table 1 for molar mass determination.

Sample Preparation. Self-assembled vesicles were prepared using two different methods. In a first manner, nanoprecipitation was used. 20 mg of the copolymer were dissolved in 2 mL of acetone and precipitated in 4 mL of water. Acetone was removed from the dispersion by extensive dialysis against water for 48h.

Dialysis was also used to elaborate polymeric vesicles. 50 mg of the copolymer were dissolved in 10 mL of acetone and extensively dialyzed against water for 4 days (Spectra/Por dialysis tubing, molecular weight cut off 6-8000 Da). The volume of water was two liters, and was renewed 4 times per day.

Dynamic Light Scattering (DLS). Samples for DLS experiments were adjusted at the concentration of 1 $g.L^{-1}$ and filtrated through a 0.45 µm filter prior to measurement in the case of nanoprecipitated polymersomes (measurement without filtration has been made to check the possible presence of aggregates or large vesicles; none of them has been observed). Polymersomes made by dialysis were analyzed without filtration.

Critical Aggregation Concentration (CAC). Samples for CAC determination were prepared in a two-steps procedure: first, 10µL of a stock solution of pyrene ($1.4x10^{-4}$ M) in dichloromethane was deposited on the bottom of a flask and left to evaporate, and then 2 mL of a copolymer solution with the appropriate concentration was added to the flask and shaken for two days before measurements (the final concentration of pyrene in the samples was $7x10^{-7}$ M).

Cryo-Transmission Electron Microscopy (cryo-TEM). The morphology and size of the polymer nanoparticles (NPs) were determined from cryo-TEM images. A drop of NPs dispersion (0.1 wt% solid content) was deposited on "quantifoil"® (Quantifoil Micro Tools GmbH, Germany) carbon membrane. The excess of liquid on the membrane was blotted out with a filter paper and, before evaporation, the membrane was quench-frozen in liquid ethane to form a thin vitreous ice film in which NPs were captive. Once mounted in a Gatan 626

cryo-holder cooled with liquid nitrogen, the samples were transferred in the microscope and observed at low temperature (-180 °C).

Instrumentation. ^{1}H NMR spectra were recorded in $CDCl_3$ using a Bruker 400 MHz NMR spectrometer. Size exclusion chromatography (SEC) experiments were performed in THF (1 $mL.min^{-1}$) at room temperature using a Shimadzu LC10 AD pump, and two PLgel Polymer Laboratories columns (5 μm Mixte-C). A Wyatt Technology Optilab Rex interferometric refractometer was used as detector. The molar masses were calibrated with polystyrene standards. DLS experiments were made using a Malvern Instruments Zetasizer Nano ZS operating with a He-Ne laser source at the wavelength of 633 nm. All measurements were performed at 25°C and at an angle of 173°. The autocorrelation functions were analyzed using the cumulant method to obtain mean diameter and distribution. The pyrene fluorescence was recorded at 23°C with a SLM Aminco 8100 by excitation at 335 nm with slits fixed at 16 and 0.5 nm, respectively, at the excitation and emission. Cryo-TEM images were recorded on ultrascan 2k ccd camera (Gatan, USA), using a LaB_6 JEOL 2100 (JEOL, Japan) cryo microscope operating at 200kV with a JEOL low dose system (Minimum Dose System, MDS) to protect the thin ice film from any irradiation before imaging and reduce the irradiation during the image capture . The size measurements were carried out using ImageJ software.

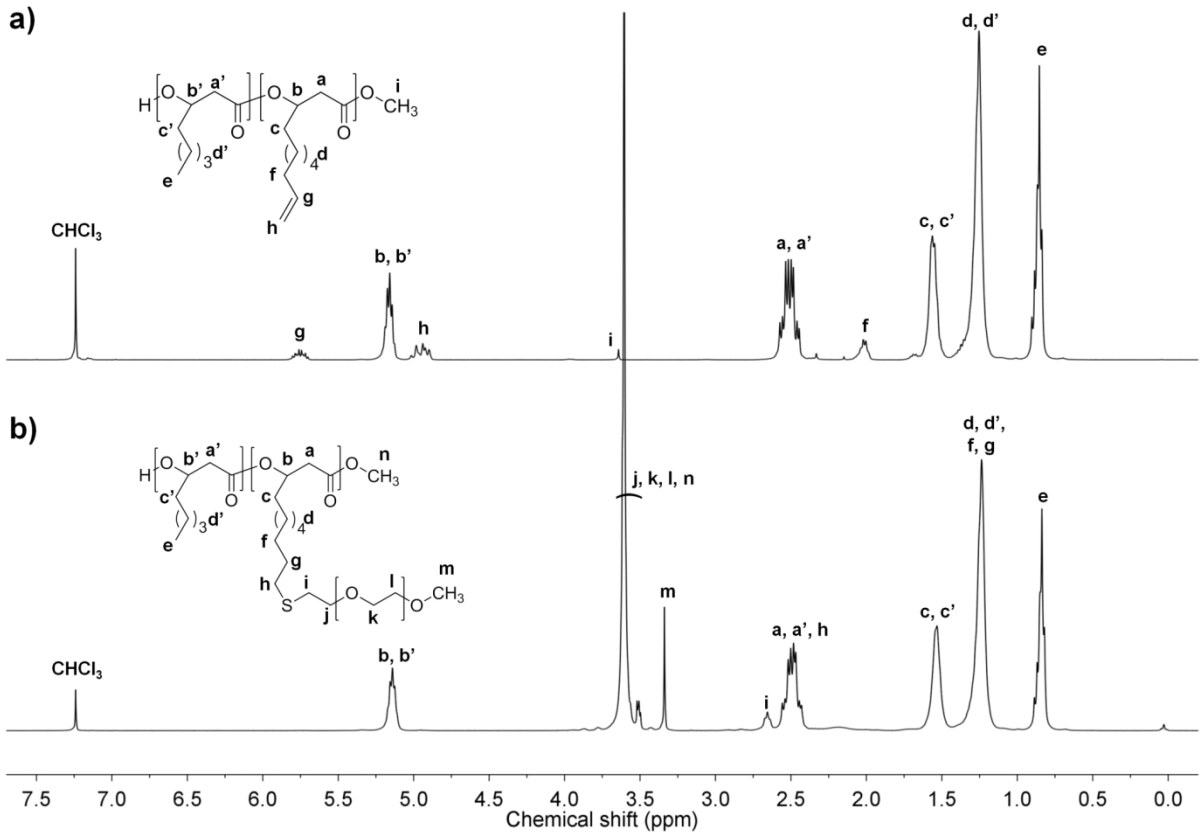

Figure S1. ^{1}H NMR of a) $PHOU_{14\%}$ oligomers and b) $PHOU_{14\%}$-*g*-PEG.

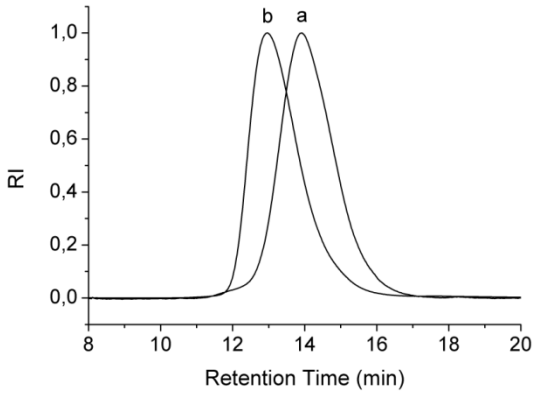

Figure S2. SEC chromatograms of a) $PHOU_{14\%}$ oligomer and b) $PHOU_{14\%}$-*g*-PEG.

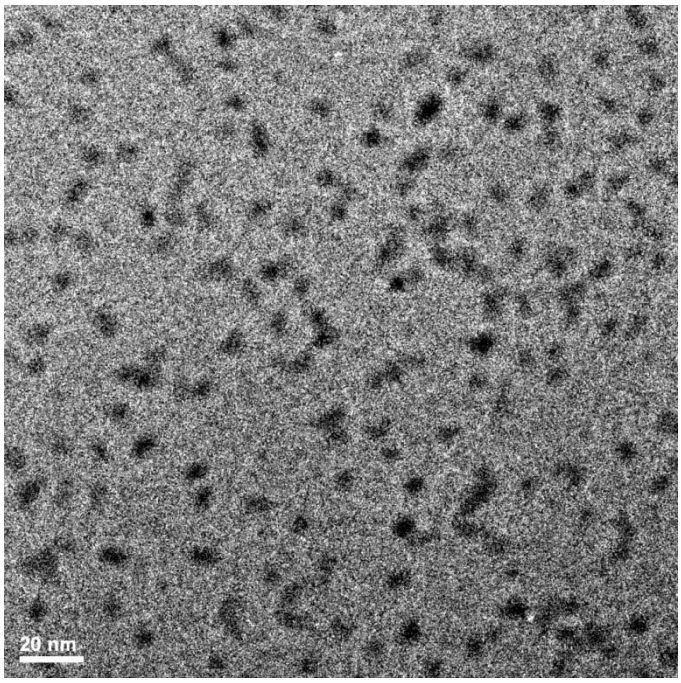

Figure S3. Cryo-TEM picture of $PHOU_{31\%}$-*g*-PEG in water at $1g.L^{-1}$

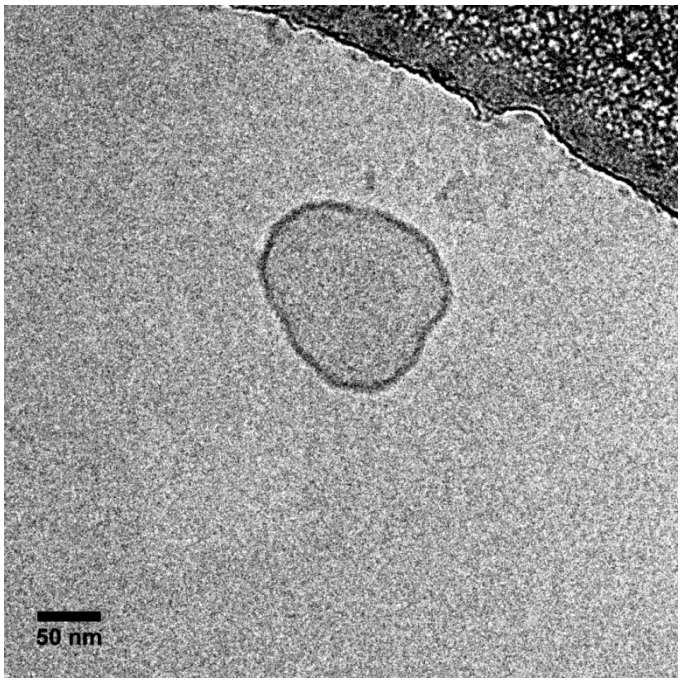

Figure S4. Cryo-TEM picture of $PHOU_{14\%}$-*g*-PEG prepared by dialysis. The darker area corresponds to the carbon membrane of the grid.

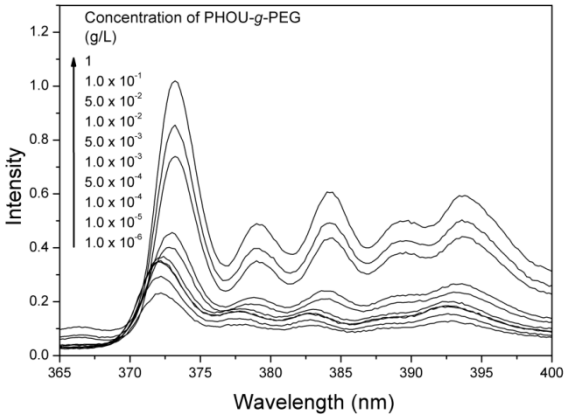

Figure S5. Fluorescence emission spectra of the pyrene probe for $PHOU_{14\%}$-*g*-PEG copolymer at various concentrations ([pyrene] = 7 x 10^{-7} M).

1. R. Hany, C. Bohlen, T. Geiger, R. Hartmann, J. Kawada, M. Schmid, M. Zinn and R. H. Marchessault, *Macromolecules*, 2004, **37**, 385-389.
2. D. Lee, R. L. Donkers, J. M. DeSimone and R. W. Murray, *J. Am. Chem. Soc.*, 2003, **125**, 1182-1183.
3. J. Babinot, E. Renard and V. Langlois, *Macromol. Rapid Commun.*, 2010, **31**, 619-624.