Electronic Supplementary Information

Cellular uptake and trafficking of polydiacetylene micelles

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A. Chemical Synthesis

1. General

Unless otherwise specified, chemicals were purchased from Sigma-Aldrich and used without further purification. Tetrahydrofuran (THF) and diethyl ether were distilled from sodium/benzophenone before use. Flash chromatography was carried out on Kieselgel 60 (230–240 mesh, Merck). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance DPX 400 spectrometer at 400 and 100 MHz respectively. Chemical shifts (δ) are given in ppm relative to the NMR solvent residual peak and coupling constants (*J*) in hertz.

2. Synthesis of diacetylene polyethylene glycol (DA–PEG) ligands

*2.1. Synthesis of pentacosa-10,12-diyn-1-ol (**5**).*

Under nitrogen, a solution of pentacosa-10,12-diynoic acid (**4**, 1 g – 2.7 mmol – 1 equiv.) in diethyl ether (50 mL) was cooled at 4 °C before lithium aluminium hydride (205 mg – 2 equiv.) was added. After stirring for 1.5 h at room temperature, the reaction was cooled to 4 °C and 15% sodium hydroxide (200 µL) was added, followed by water (600 µL). The resulting pink precipitate was filtered off on Celite. The organic phase was washed with hydrochloric acid (2 × 20 mL), dried on magnesium sulfate, filtered, and concentrated under vacuum. Product **5** was obtained as a white solid (938 mg – 2.6 mmol – 96% yield).

^{1}H NMR (400 MHz, $CDCl_3$, δ): 3.63 (t, *J* = 7 Hz, 2H; CH_2−OH), 2.23 (t, *J* = 7 Hz, 4H; CH_2−C≡), 1.60-1.45 (M, 6H; CH_2), 1.44-1.24 (M, 28H; CH_2), 0.86 ppm (t, *J* = 7 Hz, 3H; CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 77.5 (−C≡), 77.4 (−C≡), 65.2 (−C≡), 65.2 (−C≡), 63.1 (CH_2−OH), 32.7 (CH_2), 31.9 (CH_2), 29.6 (3 CH_2), 29.4 (CH_2), 29.3 (3 CH_2), 29.1 (CH_2), 29.0 (CH_2), 28.8 (CH_2), 28.7 (CH_2), 28.3 (CH_2), 28.2 (2 CH_2), 25.7 (CH_2), 22.6 (CH_2), 19.2 (CH_2), 14.1 ppm (CH_3).

*2.2. Synthesis of 1-bromopentacosa-10,12-diyne (**6**).*

Under nitrogen, triphenylphosphine (550 mg – 1.5 equiv.) and compound **5** (500 mg – 1.4 mmol – 1 equiv.) were solubilized in dichloromethane (3 mL). Tetrabromomethane (700 mg – 21.5 equiv.) was added in portions and the reaction was stirred at room temperature for 15 min. After addition of cold water (2 mL) the organic phase was separated, dried over magnesium sulfate, filtered, and concentrated under vacuum. Purification on a silica plug (elution with dichloromethane) afforded the desired product **6** as a yellowish varnish (585 mg – 1.38 mmol – quantitative yield).

^{1}H NMR (400 MHz, $CDCl_3$, δ): 3.40 (t, *J* = 7 Hz, 2H; CH_2−Br), 2.24 (t, *J* = 7 Hz, 4H; CH_2−C≡), 1.85 (td, *J* = 7 Hz, 2H; CH_2), 1.60-1.45 (M, 6H; CH_2), 1.45-1.25 (M, 26H; CH_2), 0.88 ppm (t, *J* = 7 Hz, 3H; CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 77.6 (−C≡), 77.4 (−C≡), 65.3 (−C≡), 65.1 (−C≡), 34.0 (CH_2−Br), 32.8 (CH_2), 31.9 (CH_2), 29.6 (3 CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 29.1 (2 CH_2), 28.9 (CH_2), 28.8 (CH_2), 28.7 (CH_2), 28.6 (CH_2), 28.3 (CH_2), 28.2 (CH_2), 28.1 (CH_2), 22.7 (CH_2), 19.2 (CH_2), 14.1 ppm (CH_3).

*2.3. Synthesis of pentacosa-10,12-diyn-1-oxypentatetracontaethyleneglycol (**7**).*

Under nitrogen, polyethylene glycol (MW = 2 000, 1.5 g – 0.75 mmol – 1 equiv.) in anhydrous acetonitrile (15 mL) was added to a suspension of sodium hydride (36 mg – 2 equiv.) in anhydrous acetonitrile (7 mL). The mixture was refluxed for 30 min and allowed to cool down to room temperature. Compound **6** (317 mg – 1 equiv.) dissolved in tetrahydrofuran (3 mL) was slowly added and the reaction was stirred at room temperature for 96 h. After concentration under vacuum, purification by column chromatography (silica gel, dichloromethane/methanol 95:5) afforded the desired product as a yellow solid (600 mg – 0.3 mmol – 40% yield).

^{1}H NMR (400 MHz, $CDCl_3$, δ): 3.65 (M, 180H; CH_2−O), 3.42 (t, *J* = 7 Hz, 2H; CH_2−O), 2.22 (t, *J* = 7 Hz, 4H; CH_2−C≡), 1.60-1.45 (M, 6H; CH_2), 1.35-1.20 (M, 28H; CH_2), 0.88 ppm (t, *J* = 7 Hz, 3H; CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 77.4 (−C≡), 77.3 (−C≡), 72.4 (CH_2−O), 71.4 (CH_2−O), 70.5 (86 CH_2−O), 70.2 (CH_2−O), 69.9 (CH_2−O), 65.3 (−C≡), 65.2 (−C≡), 61.5 (CH_2−OH), 31.8 (CH_2), 29.5 (4

CH_2), 29.4 (CH_2), 29.3 (3 CH_2), 29.2 (2 CH_2), 29.0 (CH_2), 28.9 (CH_2), 28.7 (CH_2), 28.2 (2 CH_2), 25.9 (CH_2), 22.6 (CH_2), 19.1 (CH_2), 14.0 ppm (CH_3).

2.4. *Synthesis of carboxylic derivative **1**.*

Under nitrogen, pentacosa-10,12-diyn-1-oxypolyethyleneglycol (468 mg – 0.2 mmol – 1 equiv.) in tetrahydrofuran (5 mL) was added to a suspension of sodium hydride (12 mg – 2.5 equiv.) in tetrahydrofuran (5 mL). The mixture was refluxed for 30 min and allowed to cool down to room temperature. 2-Bromoacetic acid (195 mg – 7 equiv.) in tetrahydrofuran (2 mL) was slowly added and the reaction was stirred at room temperature for 24 h. After concentration under vacuum, purification by column chromatography (silica gel, dichloromethane/methanol 95:5) afforded the desired product as a yellow solid (384 mg – 0.16 mmol – 80% yield).

^{1}H NMR (400 MHz, $CDCl_3$, δ): 4.20 (s, 2H, O−C$\underline{H_2}$−CO_2H), 3.80-3.60 (M, 180H; CH_2−O), 3.51 (t, *J* = 7 Hz, 2H; CH_2−O), 2.24 (t, *J* = 7 Hz, 4H; CH_2−C≡), 1.65-1.47 (M, 6H; CH_2), 1.40-1.25 (M, 28H; CH_2), 0.88 ppm (t, *J* = 7 Hz, 3H; CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 169.1 (CO_2H), 77.5 (−C≡), 77.3 (−C≡), 72.4 (CH_2−O), 71.6 (CH_2−O), 70.6 (86 CH_2−O), 70.1 (CH_2−O), 70.0 (CH_2−O), 68.6 (O−$\underline{C}H_2$− CO_2H), 65.2 (−C≡), 65.1 (−C≡), 61.6 (CH_2−O), 31.6 (CH_2), 29.6 (3 CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (2 CH_2), 29.2 (CH_2), 29.1 (CH_2), 29.0 (CH_2), 28.8 (2 CH_2), 28.7 (CH_2), 28.2 (2 CH_2), 26.2 (CH_2), 22.6 (CH_2), 19.2 (CH_2), 14.0 ppm (CH_3).

2.5. *Synthesis of dimethylamine derivative **2**.*

Under nitrogen and at 4 °C, compound **2** (150 mg – 60 µmol – 1 equiv.) and diisopropylethylamine (13 mg – 1.7 equiv.) were dissolved in dry tetrahydrofuran (1 mL) before addition of isobutyl chloroformate (10 mg – 1.2 equiv.). After 1 h at 4 °C, *N*,*N*-dimethylaminopropylamine (100 mg – 16 equiv.) was added and the mixture was stirred overnight at room temperature. The mixture was then concentrated under vacuum and the residue was purified by column chromatography (silica

gel, dichloromethane/methanol 98:2→85:15) to afford the desired product as a yellow solid (100 mg – 40 µmol – 67% yield).

^{1}H NMR (400 MHz, $CDCl_3$, δ): 3.99 (s, 2H, O−C$\underline{H_2}$−CO−NH), 3.80-3.60 (M, 180H; CH_2−O), 3.41 (m, 2H; CH_2−O), 3.06 (m, 2H, Me_2N−C$\underline{H_2}$−CH_2), 2.79 (s, 6H, $(CH_3)_2N$) 2.22 (t, *J* = 7 Hz, 4H; CH_2−C≡), 2.06 (m, 2H, Me_2N−CH_2−C$\underline{H_2}$), 1.62-1.45 (M, 6H; CH_2), 1.40-1.20 (M, 28H; CH_2), 0.86 ppm (t, *J* = 7 Hz, 3H; CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 170.9 (CO−NH), 77.5 (−C≡), 77.2 (−C≡), 72.6 (CH_2−O), 71.5 (CH_2−O), 71.0 (O−$\underline{C}H_2$−CO−NH) 70.6-70.4 (86 CH_2−O), 70.2 (CH_2−O), 70.0 (CH_2−O), 65.3 (−C≡), 65.2 (−C≡), 61.6 (CH_2−O), 55.6 (Me_2N−$\underline{C}H_2$−CH_2), 53.4 (CO−NH−$\underline{C}H_2$), 42.9 (($\underline{C}H_3)_2N$), 35.7 (Me_2N−CH_2−$\underline{C}H_2$), 31.9 (CH_2), 29.7 (3 CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.4 (2 CH_2), 29.3 (CH_2), 29.1 (CH_2), 29.0 (CH_2), 28.8 (2 CH_2), 28.7 (CH_2), 28.3 (2 CH_2), 26.0 (CH_2), 24.9 (CH_2), 22.6 (CH_2), 19.2 (CH_2), 14.1 ppm (CH_3).

*2.6. Synthesis of methyl ether derivative **3**.*

Under nitrogen, polyethylene glycol monomethyl ether (MW = 2 000, 250 mg – 0.13 mmol – 1 equiv.) in anhydrous acetonitrile (10 mL) was added to a suspension of sodium hydride (6 mg – 2 equiv.) in anhydrous acetonitrile (2 mL). The mixture was refluxed for 30 min and allowed to cool down to room temperature. Compound **6** (211 mg – 4 equiv.) in tetrahydrofurane (2 mL) was slowly added and the reaction was stirred at room temperature for 96 h. After concentration under vacuum, purification by column chromatography (silica gel, dichloromethane/methanol 95:5) afforded the desired product as a yellow solid (265 mg – 0.11 mmol – 90% yield).

^{1}H NMR (400 MHz, $CDCl_3$, δ): 3.80-3.45 (M, 180H; CH_2−O), 3.33 (s, 3H, O–CH_3), 2.19 (t, *J* = 7 Hz, 4H; CH_2−C≡), 1.57-1.45 (M, 6H; CH_2), 1.37-1.17 (M, 28H; CH_2), 0.83 ppm (t, *J* = 7 Hz, 3H; CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 77.4 (−C≡), 77.3 (−C≡), 72.5 (CH_2−O), 71.9 (CH_2−O), 70.7-70.2 (86 CH_2−O), 70.3 (CH_2−O), 70.0 (CH_2−O), 65.3 (−C≡), 65.2 (−C≡), 61.6 (CH_2−OH), 58.9 (O–CH_3), 31.8 (CH_2), 29.6 (CH_2), 29.5 (3 CH_2), 29.4 (CH_2), 29.3 (3 CH_2), 29.2 (CH_2), 29.1 (CH_2), 29.0 (CH_2), 28.8 (CH_2), 28.7 (CH_2), 28.3 (2 CH_2), 26.0 (CH_2), 22.6 (CH_2), 19.1 (CH_2), 14.0 ppm (CH_3).

B. Supplementary figures

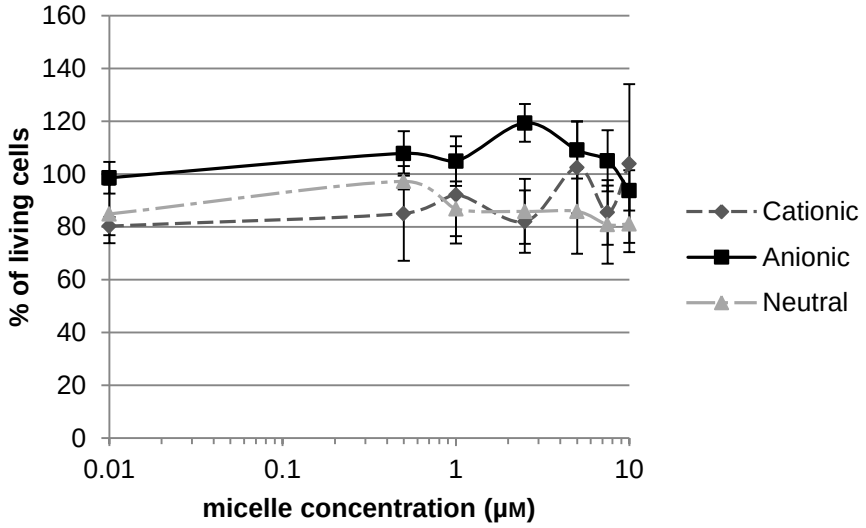

Figure S1. MTT test results.

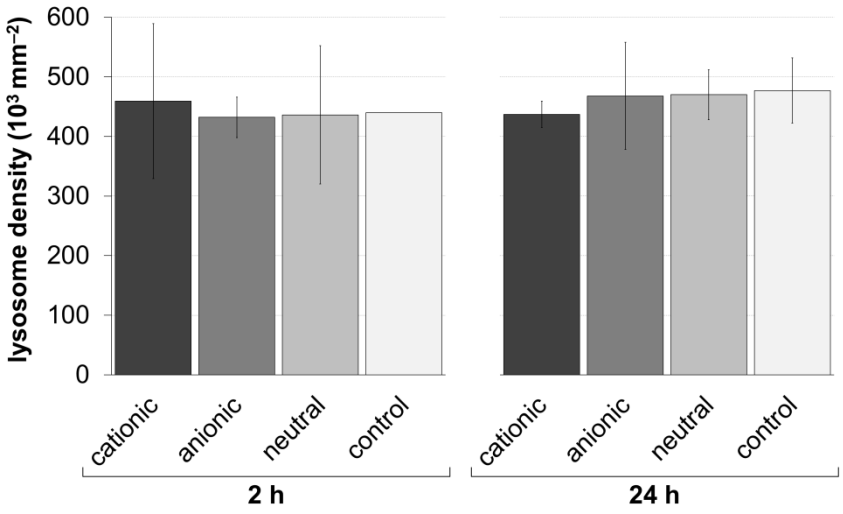

Figure S2. Density of lysosomes in mCF-7 cells after incubation with micelles.