Supplementary Material (ESI) for Chemical Communications

Supporting Information:

Multivalent Polymer Vesicles via Surface Functionalization

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General Procedures and Materials. Poly(butadiene-b-ethylene oxide) (PBD-PEO) (PDI 1.10) with a composition of 6500 g/mol PBD (> 80 % 1,2 addition) and 3900 g/mol was purchased from Polymer Source (Dorval, Canada). Dry dichloromethane was obtained from a solvent purification system. Pyridine was distilled over CaH_2. NMR chemical shifts are reported in ppm and are calibrated against residual solvent signals of $CDCl_3$ (δ 7.26, 77.2) or CD_3OD (δ 3.31, 48.9). All coupling constants (J) are reported in Hz.

Synthesis of PBD-PEO-N_3 (3)

PBD-PEO (**1**) (50 mg, 4.8 μmol, 1.0 equiv.) was dissolved in dry CH_2Cl_2 (0.5 mL). Azido acetic acid[1] (9.7 mg, 96 μmol, 20 equiv), dicyclohexylcarbodiimide (DCC) (20 mg, 96 μmol, 20 equiv), 4,4-dimethylaminopyridine (DMAP) (2.9 mg, 24 μmol, 5.0 equiv) and 4,4-dimethylaminopyridinium p-toluenesulfonate (DPTS) (7.1 mg, 24 μmol, 5.0 equiv) were added, then the reaction mixture was stirred overnight under nitrogen at room temperature. The reaction mixture was diluted with CH_2Cl_2, then the dicyclohexylurea (DCU) byproduct was filtered off using a cotton plug and washed with a small amount of CH_2Cl_2. The solvent was evaporated and the resulting residue was taken up in EtOAc. Residual DCU was removed by filtering the resulting suspension. The filtrate was dialysed against 200 mL of DMF for 24 h using a 25K MWCO membrane (Spectra/Por, regenerated cellulose). The DMF was evaporated to provide polymer **3** (46 mg, 91 %). ^{1}H NMR (400MHz, $CDCl_3$): δ 5.60-5.21 (m, 152H), 5.00-4.84 (m, 196H), 4.35 (t, 2H, J=4.7Hz), 3.91 (s, 2H), 3.82-3.45 (m, 355H), 2.17-1.80 (m,

174H), 1.38-0.95 (m, 264H). IR (thin film from $CHCl_3$) ν_{max}/cm^{-1}: 2108, 1830, 1733, 1640.

Synthesis of anhydride 5

A solution of BOC-protected β-alanine,[2] (0.30 g, 1.6 mmol, 1.0 equiv.) in 3 mL of anhydrous CH_2Cl_2 was cooled to 0˚C. To this was added a solution of DCC (0.16 g, 0.79 mmol, 0.50 equiv.) in 2 mL of anhydrous CH_2Cl_2 and the resulting mixture was stirred at 0˚C for 15 minutes. After warming to room temperature and stirring an additional 2 h, the reaction mixture was filtered and the solvent was removed under reduced pressure. The product was redissolved in EtOAc and filtered over cotton to remove any residual DCU byproduct. The solvent was removed under reduced pressure providing **13** (0.54 g, 95%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 5.11 (br s, 2H), 3.42 (t, 4H, J=5.9Hz), 2.68 (t, 4H, J=6.0Hz), 1.43 (s, 18H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 177.3, 155.9, 79.6, 35.8, 34.3, 28.2. IR (thin film from CH_2Cl_2) ν_{max}/cm^{-1}: 3351, 1820, 1701, 1522. MS: calcd $[M+H]^+$ ($C_{16}H_{29}N_2O_7$): 361.2. Found: (ES+) 361.1.

Synthesis of dendron 6

To a solution of dendron **4**[3] (0.30 g, 0.35 mmol, 1.0 equiv.), DMAP (0.15 g, 1.3 mmol, 3.7 equiv.) and distilled pyridine (83 μL, 1.0 mmol, 2.9 equiv.) in 10 mL of anhydrous CH_2Cl_2, was added a solution of anhydride **5** (2.5g, 7.0 mmol, 20 equiv.) in 10 mL of anhydrous CH_2Cl_2. The mixture was stirred overnight at room temperature under a nitrogen atmosphere. The reaction was quenched with 10 mL of water and stirred for three h, until no anhydride could be detected by ^{1}H NMR. The solution was then diluted with 50 mL of CH_2Cl_2 and washed with 1M HCl (3x 20 mL), 10% Na_2CO_3 (3x 20 mL),

and saturated brine (1x 20 mL). The organic phase was dried with $MgSO_4$, filtered and evaporated to provide the BOC-protected derivative of **6** (0.68 g, 86%). ^{1}H NMR (400MHz, $CDCl_3$): δ 5.23 (br s, 8H), 4.75 (d, 2H, J=2.6Hz), 4.33-4.17 (m, 28H), 3.40-3.37 (m, 16H), 2.59 (t, 1H, J=2.3Hz), 2.55 (t, 16H, J=6.0Hz), 1.44 (s, 72H), 1.33 (s, 3H), 1.27 (s, 6H), 1.25 (s, 12H). ^{13}C NMR (150MHz, $CDCl_3$): δ 172.0, 171.5, 171.3, 171.0, 155.8, 79.5, 76.3, 75.8, 65.7, 65.5, 65.3, 53.4, 46.7, 46.4, 46.3, 36.1, 34.3, 28.7, 17.8, 17.5, 17.4. IR (thin film from CH_2Cl_2) ν_{max}/cm^{-1}: 3398, 2119, 1740, 1714. MS: calcd $[M + Na]^+$ ($C_{102}H_{164}N_8NaO_{46}$): 2260.0, Found: (ES+) 2259.8.

The BOC-protected derivative of **6** (0.68 g, 0.30 mmol) was dissolved in 2 mL of 1:1 trifluoroacetic acid/CH_2Cl_2 and the solution was stirred at room temperature for 2 h. The solvent was removed in vacuo to provide **6** (0.70 g, 98 %). ^{1}H NMR (400MHz, CD_3OD): δ 4.81 (d, 2H, J=2.3Hz), 4.31 (m, 28H), 3.23 (t, 16H, J=6.7Hz), 3.02 (t, 1H, J=2.5Hz), 2.81 (t, 16H, J=6.6Hz), 1.36 (s, 3H), 1.32 (s, 6H), 1.29 (s, 12H). ^{13}C NMR (150MHz, CD_3OD): δ 173.6, 173.4, 173.3, 171.9, 77.2, 75.6, 67.2, 67.0, 66.8, 54.0, 48.2, 48.1, 47.8, 36.4, 32.3, 18.4, 18.3, 18.1. IR (thin film from THF/MeOH) ν_{max}/cm^{-1}: 2119, 1740, 1683. MS: calcd $[M+H]^+$ ($C_{62}H_{101}N_8O_{30}$): 1437.6, Found: (MALDI-TOF) 1437.7.

Fluorescent labeling of dendron 6 to provide 8

Dendron **6** (25 mg, 11 μmol, 1.0 equiv.) and activated rhodamine dye **7**[4] (8.0 mg, 11 μmol, 1.0 equiv.) were dissolved in 1.0 mL of pyridine. N,N-Diisopropylethylamine (DIPEA) (27 mg, 0.22 mmol, 20 equiv.) was added and the reaction mixture was stirred at room temperature overnight. To remove byproducts and excess dye, the product was dialysed against 200 mL of DMF for 24 h using a 12-14K MWCO membrane

(Spectra/Por, regenerated cellulose). The solvent was evaporated to give the dye-labeled dendron **8** (15 mg, ~ 65 %). The extinction coefficient ε was measured in $CHCl_3$/MeOH (60/40). ε varied somewhat from batch to batch due to different conjugation yields, but typically ranged from 35000 to 60000 L $mol^{-1}cm^{-1}$ at 560 nm.

Vesicle formation with PBD-PEO-N_3 (3)

In a 10 mL round bottom flask, 5.0 mg of polymer was mixed with 50 μg of the hydrophobic dye Nile Red in 0.5 mL of chloroform and then the solvent was evaporated under a stream of nitrogen gas. The resulting thin film was further dried in vacuo to remove traces of solvent. Vesicles were formed upon hydration of the thin film in 0.5 mL of H_2O at 35 °C overnight, with rapid stirring. The vesicles were then sonicated for 30 min using a Fisher Scientific FS20H ultrasonic bath, and then imaged by confocal laser scanning microscopy (see Fig. 1a, supp. info).

General Procedure for Surface Conjugation by Click Chemistry

Vesicles were prepared as described above for PBD-PEO-N_3 (**3**) except that mixtures of copolymers **1** and **3** with specified percentages of **3** ranging from 0–100 % were used and no Nile Red was used. To the sonicated vesicles were added aqueous solutions of copper(II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$), sodium ascorbate, bathophenanthrolinedisulfonic acid disodium salt hydrate, and dendron **8** in sequence such that the reaction mixture contained 1 mM $CuSO_4$, 25 mM sodium ascorbate, 2.3 mM bathophenanthrolinedisulfonic acid and 4 equiv. of **8** with respect to **3**. The reaction

mixture was stirred at room temperature overnight and then dialyzed against water overnight using a 25K MWCO dialysis membrane (Spectra/Por, regenerated cellulose).

Confocal Laser Scanning Microscopy (CLSM)

The aqueous solution from the dialysis was directly used to prepare microscope slides (glass). In some cases the slides were allowed to sit for 2 hours prior to imaging in order to minimize vesicle movement and thus facilitate imaging. Images were obtained using a confocal laser scanning microscope (LSM 510, Carl Zeiss Inc.) using a 63X (N.A. = 1.4) oil immersion objective and an excitation wavelength of 543 nm (He-Ne laser).

Quantification of surface dendritic groups

Following dialysis, the water was evaporated and the resulting material was weighed, and then taken up in 1 mL of $CHCl_3$/MeOH (3/2). The solution was centrifuged at 1000 g for 1 h to remove any insoluble material, then the absorbance was measured at 560 nm using a Cary 50 UV-visible spectrometer. The degree of surface functionalization was calculated using the measured ε for the dye labeled dendron **8** in the same solvent, and the mass of polymer recovered (generally > 90 %).

Reconstitution of functionalized vesicles

The $CHCl_3$/MeOH solution used for the surface group quantification was evaporated to provide a thin film of polymer. The film was dried in vacuo to remove trace solvent. Water (0.5 mL) was added then vesicles were formed by sonication for 20 min using a

Fisher Scientific FS20H ultrasonic bath, followed by heating at 35 °C overnight with rapid stirring. The resulting vesicles were imaged by CLSM (Fig. 1b, supp info).

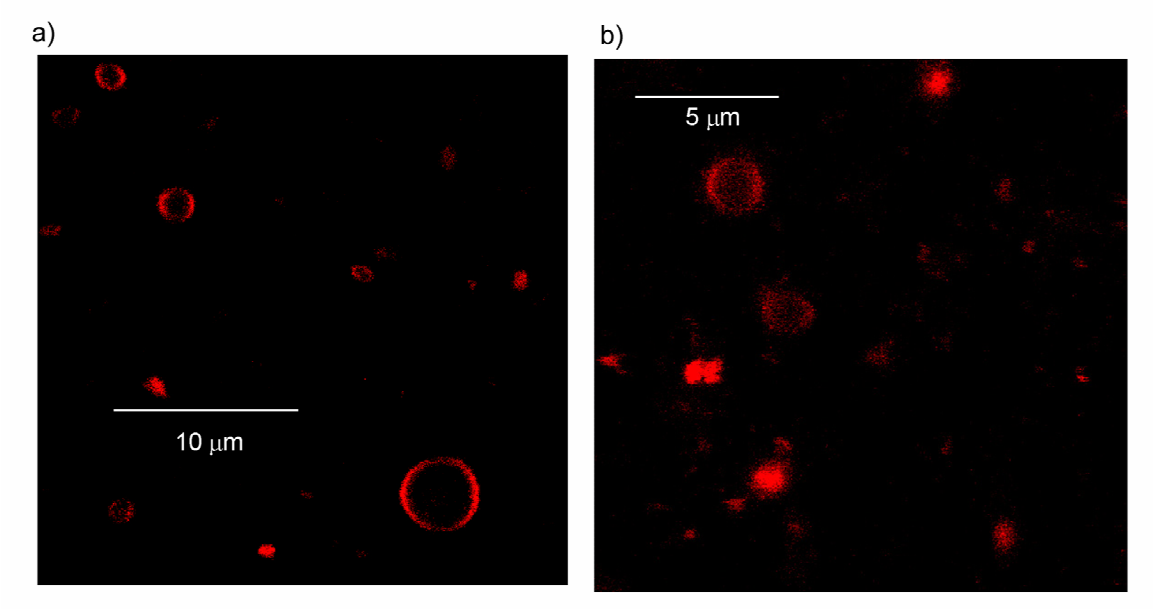

Fig. 1 CLSM images of a) vesicles formed from pure **3** in the presence of 1 wt% Nile Red and b) Reconstituted surface functionalized vesicles **9f**.

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