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Electronic Supplementary Information

Materials and Methods

Poly(ethylene glycol)-b-poly(butadiene) (PBD_{22}-PEG_{14}; average molecular weight for the PBD and PEG block: 1200 and 600, respectively) was purchased from Polymer Source Inc. (Quebec, Canada). 1,2-dioleoyl-3-trimethylammonium propane (DOTAP), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) and 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (Rhodamine-PE) were purchased from Avanti Polar Lipids (Alabaster, USA). N-Dodecanoyl-NBD-sulfatide (NBD-SF) was from Matreya LLC (Pleasant Gap, USA). Alexa Fluor 633-labeled transferrin was from Invitrogen (Carlsbad, USA) and APC-labeled anti-LAMP1 antibody from Biolegend (San Diego, USA).

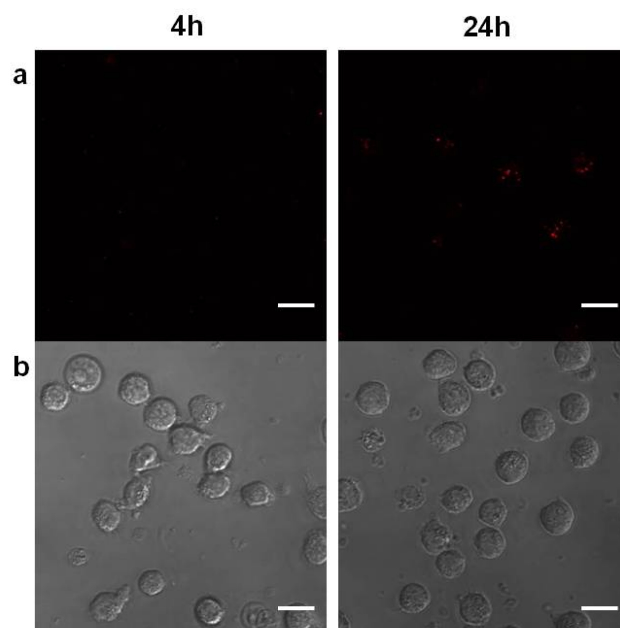
Pure and hybrid polymersomes were formed by film rehydration. Briefly, 5 mg/mL stock solution of PEG-PBD was mixed with 25 mol% DOTAP or POPC and 1 mol% Rhodamine-PE in chloroform. For loading of sulfatide, NBD-SF was also added to the mixture at 1 mol%. The lipid/polymer solution was evaporated under a gentle stream of nitrogen and desiccated for 4h to obtain a dry, thin film. Vesicles were formed by adding 1 mL of *phosphate-buffered saline* (PBS, 0.1 mM phosphate, pH 7.4) to the dried film and stirring at room temperature overnight at 700 rpm. The vesicles suspension was then *extruded* 8 times each through a 0.45 µm followed by a 0.22 µm membrane to yield *vesicles of ~200 nm.* The suspension was purified by dialysis against PBS (Spectrum Laboratories, MWCO 50 000) to remove any non-incorporated molecules.

The resulting polymersomes and their stability were characterized by dynamic light scattering (DLS) using a ZetaPALS dynamic light scattering detector (Brookhaven Instruments). Vesicle samples (in triplicate) were diluted 100 × in 2.5 mL PBS, pH 7.4 and measurements were performed 5 times per sample at each time point. The diameter, count rate and polydispersity index were reported as the average of the measurements with standard deviations.

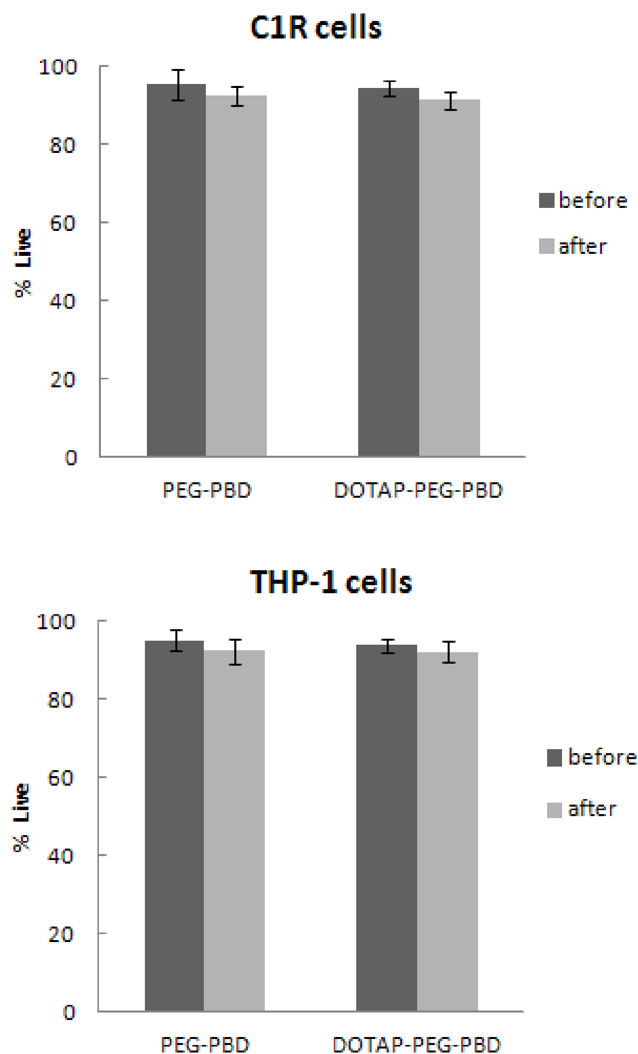
The association of Rhodamine-PE and NBD-SF with the polymersomes were confirmed by filtering the dialyzed suspension through Vivaspin centrifugal filters (1 MDa MWCO PES, GE Healthcare) at 3000 rpm where polymersomes were retained on the filter while any free molecule passes through. The amount of non-incorporated molecules in the filtrate was quantified using a fluorescence microplate reader (Tecan). The filtration of free Rhodamine-PE and NBD-SF showed a recovery of ~90% while fluorescence dropped to background levels in the case of polymersomes, showing that all dye and cargo was associated with vesicles and no free molecules remained following dialysis. The loading efficiency of NBD-SF in polymersomes was determined by comparing fluorescent intensities of the initial and final dialyzed suspensions.

THP-1, a human monocytic leukemia cell line and C1R, a human B lymphoblastic cell line were grown in RPMI-1640 medium supplemented with 10% FBS, kanamycin, sodium pyruvate, MEM non-essential amino acids and glutamax at 37 °C in a 5% CO_2 incubator. Cells were seeded at 2 x 10^5 cells/well in 96 well tissue culture-treated plates and polymersomes (125 µg/mL) with and without loaded NBD-SF (0.625 µg/mL) were added to the cells for 4 or 24 h at 37 °C. After washing with PBS to remove unbound vesicles, the cells were transferred to µ-slide VI 0.4 (ibiTreat, tissue culture treated, Ibidi) and imaged live under a confocal microscope (Olympus FV1000). Cell viability before and after incubation of cells with vesicles was determined by trypan blue exclusion. For determining the intracellular location of delivered NBD-SF, THP-1 cells were stained live with 5 µg/mL Alexa Fluor 633-labeled transferrin for 4h to identify early/recycling endosomes or fixed (4% paraformaldehyde), permeabilized (2% saponin) and stained with 1 µg/mL APC-labeled anti-LAMP1 for late endosomes/ lysosomes. Colocalization of NBD-SF and the endolysosomal markers was then visualized via confocal microscopy.

Supplementary Figures



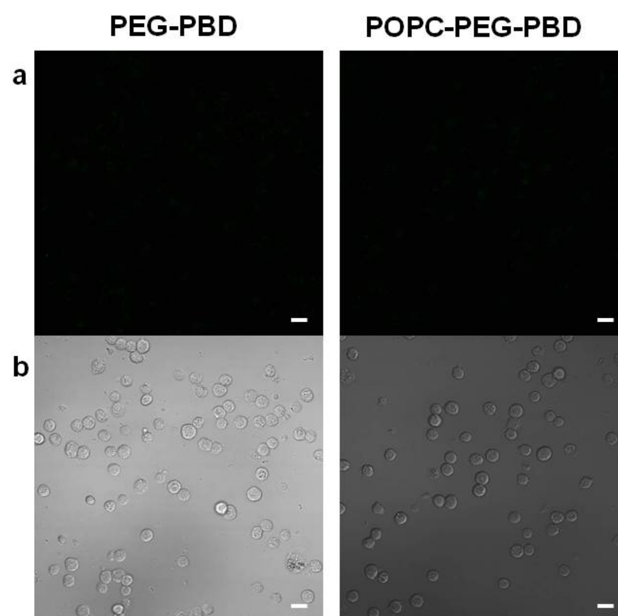
Supplementary Fig. 1 Representative confocal images of THP-1 cells with hybrid POPC-PEG-PBD polymersomes (a, rhodamine fluorescence; b, bright field) for 4 or 24 h at 37 °C after washing to remove unbound vesicles. Scale bars 20 µm.



Supplementary Fig. 2 The viability of C1R and THP-1 cells before and after treatment with unmodified PEG-PBD and hybrid DOTAP-PEG-PBD polymersomes, as determined by trypan blue exclusion.

Compositions	Sulfatide Loading Efficiency (%)
PEG-PBD	86.5 ± 7.4
DOTAP-PEG-PBD	88.2 ± 6.6
POPC-PEG-PBD	84.6 ± 10.1

Supplementary Fig. 3 Loading efficiencies of sulfatide in PEG-PBD, DOTAP- or POPC-PEG-PBD hybrid polymersomes, quantified by fluorescence measurements on post-dialysis suspensions.



Supplementary Fig. 4 Representative confocal images of THP-1 cells incubated with NBD-SF-loaded PEG-PBD or POPC-PEG-PBD vesicles at 37 °C for 24 h, washed and imaged by confocal microscopy (a, NBD fluorescence; b, bright field). Scale bars 20 µm.