Supporting Information for

Tailoring Polymersome Bilayer Permeability Improves Enhanced Permeability and Retention Effect for Bioimaging

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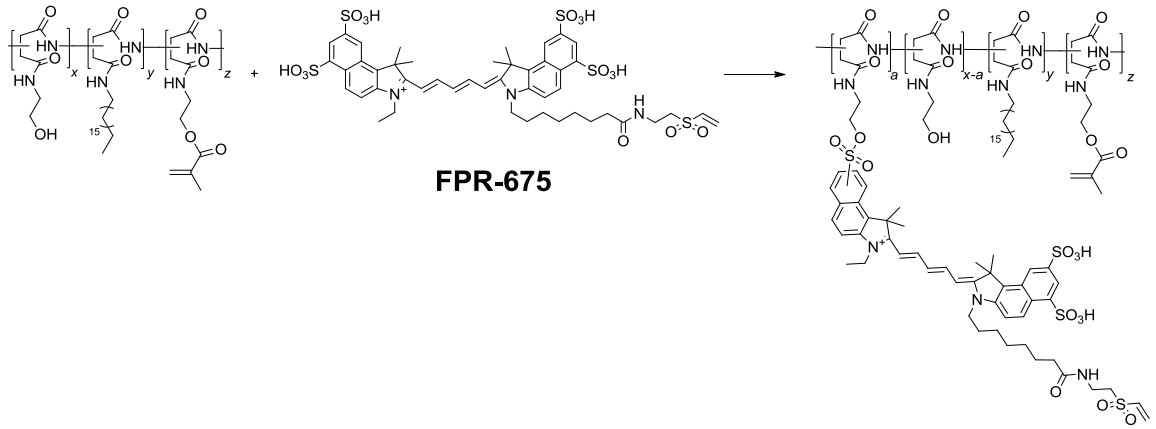
METHODS

Characterization of PHEA-C_{18} and PHEA-C_{18}-MA polymers: ^{1}H NMR (Varian Unity 500 MHz) was applied to analyze the polymer dissolved in DMSO-d_6 (Cambridge Isotope Laboratories, Inc.). The integrals of characteristic peaks were used to quantify the degree of substitution of octadecyl chains (DS_{C18}) and the degree of substitution of methacrylate groups (DS_{MA}) in each sample by Equation (1) and (2).

$$DS_{C18}\ (\text{mol}\%) = \frac{\text{The integral of the peak in } 0.85-0.95 \text{ ppm}/3}{\text{The integral of the peak in } 4.3-4.7 \text{ ppm}} \times 100\ \% \quad \text{..... (1)}$$

$$DS_{MA}\ (\text{mol}\%) = \frac{\text{The sum of the integrals of the peak at } 5.7 \text{ ppm and at } 6.1 \text{ ppm}/2}{\text{The integral of the peak in } 4.3-4.7 \text{ ppm}} \times 100\ \% \quad \text{..... (2)}$$

Cytotoxicity evaluation of PHEA-C_{18}-MA polymersomes: C166 mouse endothelial cells (ATCC) were seeded on a 96-well plate at 10^4 cells per well. Cells were incubated for 24 hours with MA-2.7 polymersomes or with MA-4.8 polymersomes. MTT reagent ((3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, ATCC) was then added to the cell culture media. Metabolically active cells convert the MTT reagent to formazan dye, which can be quantified by measuring its absorbance at 570 nm (Tecan Infinite 200 PRO, Tecan AG). Cellular metabolic activity is presented as a percent of a control, i.e. cells cultured without the presence of polymersome. Error bars are shown as the standard deviation of three replicates.



Scheme S1. PHEA-C_{18}-MA functionalized with a near-infrared (NIR) fluorescent probe, FPR-675.

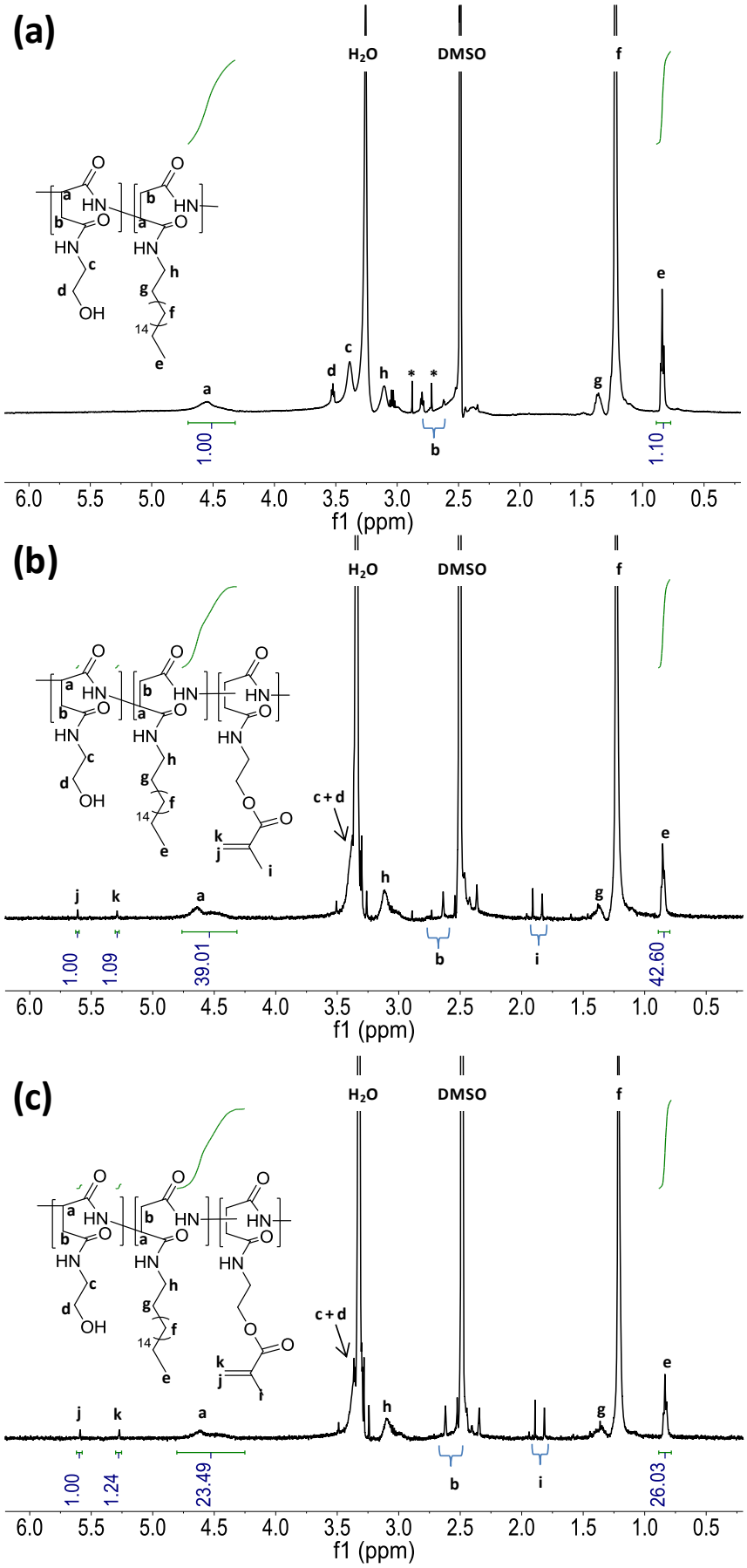

(a)
H2O
DMSO
f
e
d
c
h
a
g
b
1.00
1.10
6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)
(b)
H2O
DMSO
f
c+d
e
h
j
k
a
g
b
i
1.00
1.09
39.01
42.60
6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)
(c)
H2O
DMSO
f
c+d
e
h
j
k
a
g
b
i
1.00
1.24
23.49
26.03
6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)

Figure S1. ^{1}H NMR (DMSO-d_6, 500 MHz) spectra of **(a)** PHEA-C_{18} without methacrylate groups (MA-0.0), **(b)** PHEA-C_{18}-MA at DS_{MA} of 2.7 mol% (MA-2.7), and **(c)** PHEA-C_{18}-MA at DS_{MA} of 4.8 mol% (MA-4.8).

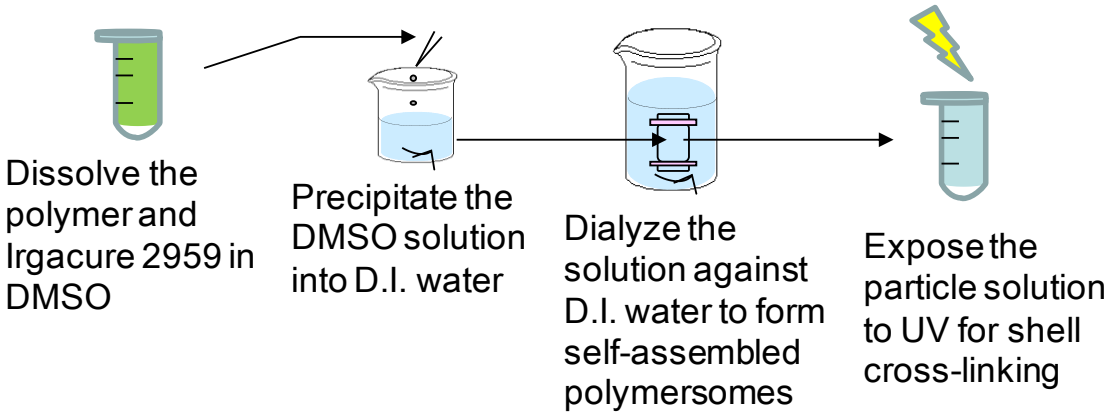


Figure S2. Schematic description of the solvent exchange process to prepare PHEA-C_{18}-MA polymersomes with cross-linked bilayers.

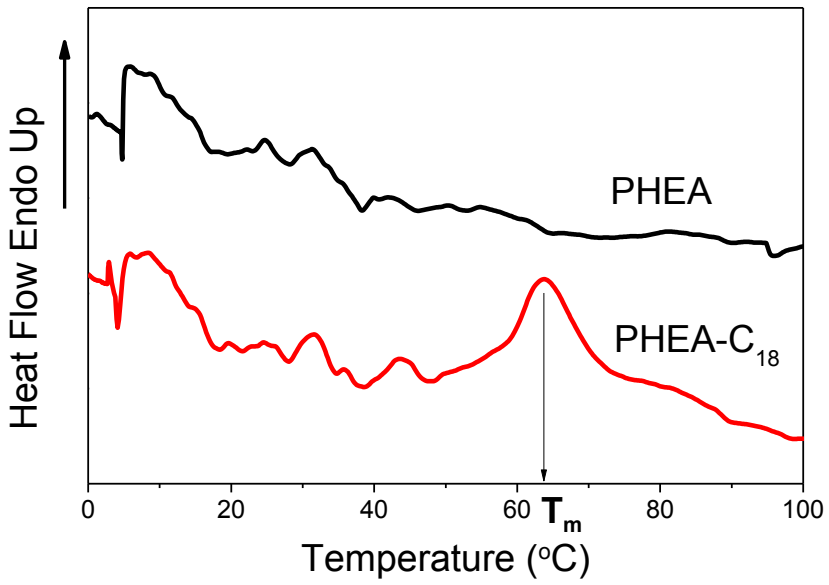


Figure S3. Differential scanning calorimetry (DSC) thermogram of PHEA and PHEA-C_{18}. DSC measurements were performed under a nitrogen atmosphere at a heating rate of 10 °C/min using Perkin Elmer Diamond DSC, and the heat flow of the polymer during the heating process was measured.

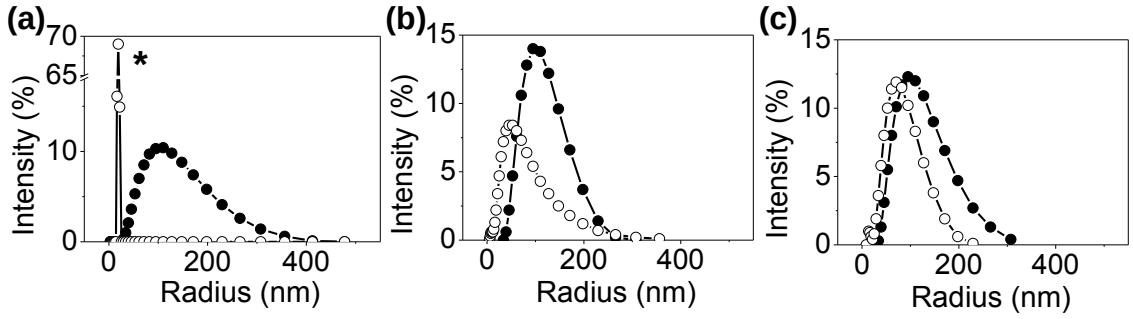


Figure S4. Effects of DS_{MA} on the size distribution of the polymersomes of **(a)** MA-0.0, **(b)** MA-2.7, and **(c)** MA-4.8. Bilayers of MA-2.7 and MA-4.8 polymersomes were cross-linked. The filled and open circles represent the polymersomes suspended in PBS before and after incubation at 90 °C for 24 h, respectively. *The majority of MA-0.0 polymersomes disappeared after incubation at 90 °C.

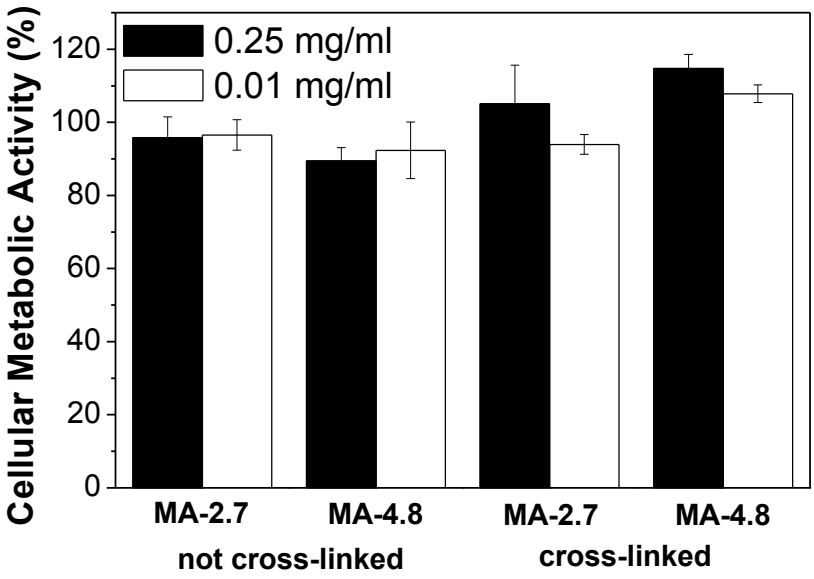


Figure S5. Cellular metabolic activity of C166 mouse endothelial cells analyzed with an MTT reagent.

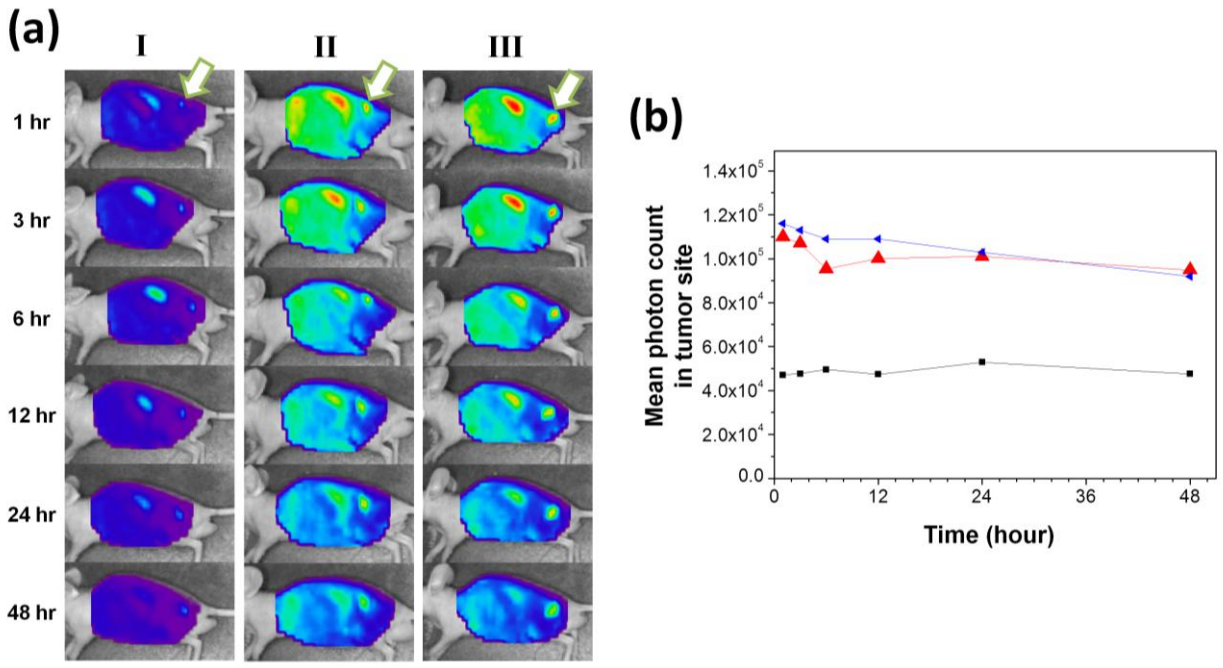


Figure S6. In vivo evaluation of PHEA-C_{18}-MA polymersomes in targeting and imaging tumors. **(a)** NIR fluorescence images of tumor-bearing mice after the intravenous injection of (I) free FPR-675 probes, (II) FPR-675-labeled MA-2.7 polymersomes with cross-linked bilayers, and (III) FPR-675-labeled MA-4.8 polymersomes with cross-linked bilayers. Arrows mark the tumor site. **(b)** NIR fluorescence intensity changes over 48 h in the tumor tissues of the corresponding mice shown in **(a)**. (—■—) represents free FPR-675 dye, (—●—) represents FPR-675-labeled MA-2.7 polymersomes with cross-linked bilayers, and (—▲—) represents FPR-675-labeled MA-4.8 polymersomes with cross-linked bilayers.

Table S1. Molecular analysis of PHEA-C_{18} and PHEA-C_{18}-MA with controlled DS_{MA}.

Sample	DS_{C18} [mol%][a)]	DS_{MA} [mol%][a)]	mass percentage of the hydrophilic grafting to the total macromoleculue[b)]	Morphology[c)]
MA-0.0	36.7	0.0	44.1	polymersome
MA-2.7	36.4	2.7	42.2	polymersome
MA-4.8	36.9	4.8	40.0	polymersome

[a)] Determined with the ^{1}H NMR spectra of the polymers; [b)] Calculated by $\frac{(100 - DS_{C18} - DS_{MA}) \times \text{mass of PHEA unit substituted with a hydroxyl group}}{\text{averaged unit molecular weight}} \times 100\%$, where the average unit molecular weight is calculated by averaging the mass of the PHEA unit substituted with a hydroxyl group (174 g mol^{-1}), substituted with a octadecyl group (381 g mol^{-1}), and substituted with a methacrylate group (242 g mol^{-1}) by the degree of substitution; [c)] The amphiphilic PHEA-C_{18} substituted with a mass percentage of the hydrophilic grafting to the total macromolecule in the range of 25-45 % have been shown to favor the polymersome formation in aqueous solution.[1-3]

REFERENCES

1. Lee, H. J.; Yang, S. R.; An, E. J.; Kim, J.-D. Biodegradable Polymersomes from Poly(2-hydroxyethyl aspartamide) Grafted with Lactic Acid Oligomers in Aqueous Solution. *Macromolecules* **2006**, *39*, 4938-4940.

2. Lai, M.-H.; Jeong, J. H.; DeVolder, R. J.; Brockman, C.; Schroeder, C.; Kong, H. Ellipsoidal Polyaspartamide Polymersomes with Enhanced Cell-Targeting Ability. *Adv. Funct. Mater.* **2012**, *22*, 3239–3246.

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