Supplemental figures



Supplemental Figure 1. FT-IR spectra of PMPC-COOH, deprotected PMPC, and PMPC-Mal.

The functional chemical species presenting in PMPC polymer were characterized by FT-IR analysis using a Bruker Alpha ATR-FTIR spectrometer. The ATR accessory was used for signal enhancement of chemical species by internal total reflectance. The number of scans was set to 32 with a resolution on four for all samples analyzed.



Supplemental Figure 2. The ¹H NMR spectra of a) PMPC100-COOH, b) deprotected PMPC100-COOH, and c) PMPC100-Mal.

The number of repeat units in the PMPC backbone was characterized by proton nuclear magnetic resonance spectroscopy (¹H NMR) analysis using a Bruker 400 MHz NMR spectrometer. The PMPCs were prepared in D_2O at a concentration of 1 mg/mL and analyzed at room temperature with 64 scans for determination of degree of polymerization.

Additionally, the percent conversion from PMPC-COOH to PMPC-Mal was calculated by ¹H-NMR analysis, and the PMPC-Mal samples were prepared for ¹H-NMR and with 256 number of scans to resolve the end group proton signals appearing at δ = 6.8 ppm.

PMPC100-COOH: ¹H NMR (400 MHz, D₂O, δ) from one side: 0.7-1.1 (a, 3H, -C*CH3*), 1.6-2.2 (b, 2H, -*CH2*C-; c, 3H, -C*CH3*), 2.4-2.8 (d and e, 4H, HOOC*CH2CH2*-), 3.2 (j, 9H, -N*(CH3)3*), 3.6 (i, 2H, -*CH2*N-), 3.8-4.2 (f, g, and h, 6H, -COO*CH2CH2*OPO*CH2*-), 7.4-8.0 (k, l, and m, 5H, -C*CHCHCHCHCH*).

Deprotected PMPC100-COOH: ¹H NMR (400 MHz, D₂O, δ) from one side: 0.7-1.1 (a, 3H, -CCH3), 1.6-2.2 (b, 2H, -CH2C-; c, 3H, -CCH3), 2.4-2.8 (d and e, 4H, HOOCCH2CH2-), 3.2 (j, 9H, -N(CH3)3), 3.6 (i, 2H, -CH2N-), 3.8-4.2 (f, g, and h, 6H, -COOCH2CH2OPOCH2-).

PMPC100-Mal: ¹H NMR (400 MHz, D₂O, δ) from one side: 0.7-1.1 (a, 3H, -C*CH3*), 1.6-2.2 (b, 2H, -*CH*2C-; c, 3H, -C*CH3*), 2.4-2.8 (d and e, 4H, HOOC*CH*2*CH*2-), 3.2 (j, 9H, -N(*CH3*)3), 3.6 (i, 2H, -*CH*2N-), 3.8-4.2 (f, g, and h, 6H, -COO*CH*2*CH*2OPO*CH*2-), 6.8 (k, 2H, -C*CHCH*C-).



Supplemental Figure 3. Whole body imaging of NSG mice treated with AF647 or AFTmab.

Mice (n=3) were retro-orbitally injected with 100 μ L AFTmab (10 mg/kg) or AF647 in PBS. AF647 was used as equivalent to the total fluorescent intensity of AFTmab. The mouse on the far left was treated with PBS as a negative control (n=1). The images were taken on an IVIS imaging system 12 hours post-injection.



Supplemental Figure 4. ELISA standard curves of Tmab and AFTmab.

A 96 ELISA well-plate was coated with anti-human IgG antibody (1 μ g/mL in carbonate buffer, pH 9.5) at 4 °C overnight, followed by three washes with PBST (0.1% Tween in PBS). Non-specific binding was blocked with blocking buffer (2% BSA in PBST) at room temperature for two hours, followed by three washes with PBST. Tmab and AFTmab were diluted with blocking buffer (concentration ranging from 0 to 10 ng/mL) and incubated in the well at room temperature for two hours, followed by five washes with PBST. HRP-coupled anti-human IgG antibody (10 μ g/mL) was added to each well and incubated at room temperature for one hour, followed by five washes with PBST. Finally, 3,3',5,5'-Tetramethylbenzidine and H₂O₂ solutions were added and incubated at room temperature for 15 minutes. The UV adsorption at 450 nm was measured with a Varioskan LUX plate reader.



Supplemental Figure 5. CLSM images of SKOV-3 cells treated with PBS, AF647, AFIgG, or AFTmab.

SKOV-3 cells (2 x 10⁵) were seeded on glass coverslips in 12-well plate. After 24 hours, cells were incubated with AFTmab (2 μ g/mL), AF647, AFIgG (equivalent to the fluorescent intensity of AFTmab), and PBS (10 μ L) at 37 °C for two hours, followed by 4% paraformaldehyde for 15 minutes at room temperature for fixation after three washes with PBS. Nuclei were stained by 10 μ g/mL Hoechst 33342 at room temperature for 20 minutes. Fluorescent images were taken with a Nikon A1R/SIM confocal microscope.



Supplemental Figure 6. Tmab-dependent binding of AFTmab to SKOV-3 cells.

SKOV-3 cells (1 x 10⁵) were incubated with AFTmab (2 μ g/mL) and AF647 (equivalent to the fluorescent intensity of AFTmab) for two hours at 37 °C. The cells were then washed by PBS, harvested, and fixed. The mean-fluorescent intensity of the cell surface was measured by flow cytometry.



Supplemental Figure 7. Assay for Tmab-saturation binding.

SKOV-3 cells (2 x 10⁵) in FACS buffer were included with AFTmab and Tmab (concentration ranging from 0.001 to 300 nM) for two hours at 4 °C, followed by three washes with PBS. The mean-fluorescent intensity of the cell surface was measured by flow cytometry.

Entry	MPC: CTA: VA-044	Conversion ratio (%)	modification	Yield (%)
	(molar ratio)		ratio (%)	
PMPC50-COOH	50: 1: 0.2	74		62
PMPC100-COOH	100: 1: 0.2	56		48
PMPC200-COOH	200: 1: 0.2	78		74
PMPC50-Mal			60.9	51
PMPC100-Mal			51.9	62
PMPC200-Mal			11.3	79

Supplemental Table 1. The summary of DP, yield, and modification ratio of PMPC-COOHs, and PMPC-Mals with different designated feed ratios.