Supplementary information for:

Polymersomes functionalized via "click" chemistry with the fibronectin mimetic peptides PR_b and GRGDSP for targeted delivery to cells with different levels of $\alpha_5\beta_1$ expression

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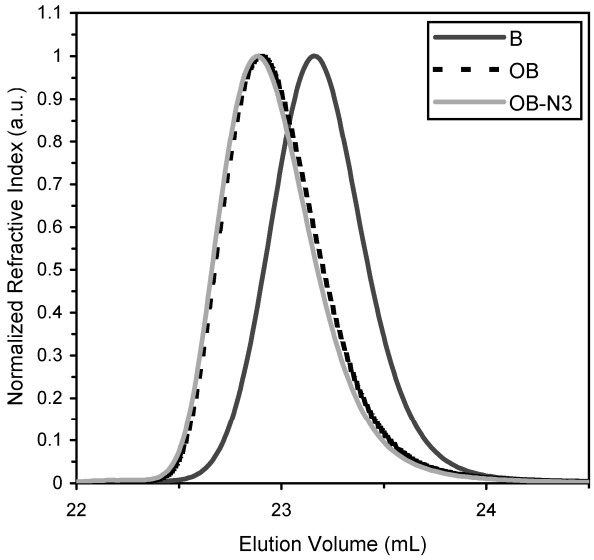

Figure S1. SEC traces of the precursor PB homopolymer, B, the hydroxyl end-capped PEO-PB diblock copolymer, OB, and the azide end-capped diblock copolymer, OB-N_3.

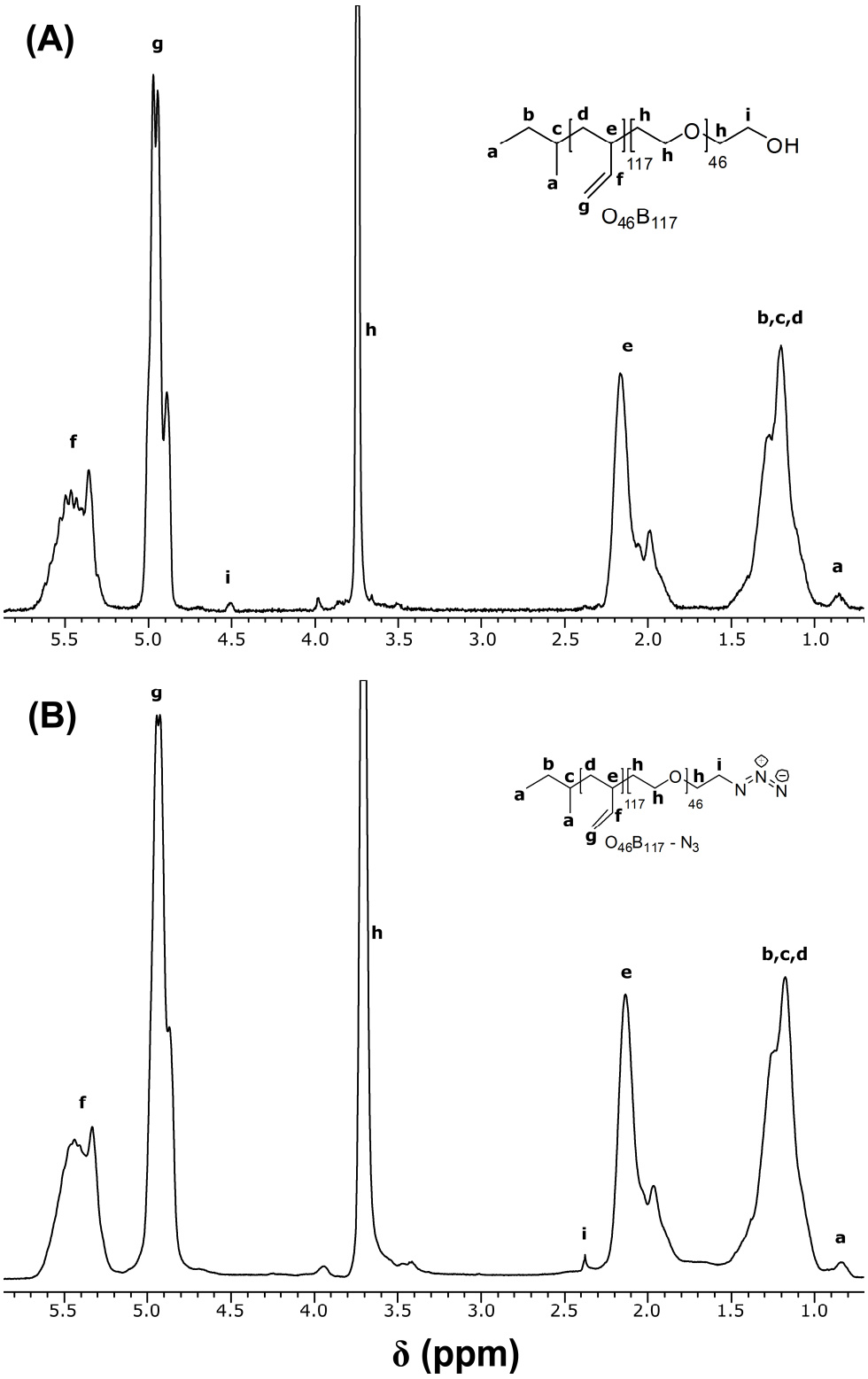

Figure S2. ^{1}H NMR spectra of OB (A), and OB-N_3 (B) diblock copolymers in $CDCl_3$ + 1% (v/v) trifluoroacetic anhydride.

Table S1. Peptide conjugation yield of click chemistry reaction.

mol% Conjugation [a]	**% OB-N_3** [b]	**% Yield** [c]
0%	0.0%	n/a
0%	0.0%	n/a
4.9% PR_b	5.0%	98%
5.6% PR_b	5.5%	102%
10.3% PR_b	12.3%	83%
17.3% PR_b	28.8%	60%
22.7% PR_b	39.8%	57%
29.8% PR_b	54.7%	54%
5.2% GRGDSP	5.3%	98%
5.2% GRGDSP	5.3%	97%
29.5% GRGDSP	30.1%	98%
33.5% GRGDSP	32.1%	104%

[a] mol% peptide with respect to OB diblock. [b] Weight percent of OB-N_3 polymer loaded in the polymer vesicles. [c] Percent of OB-N_3 on the surface of the polymer vesicle that was conjugated with peptide.

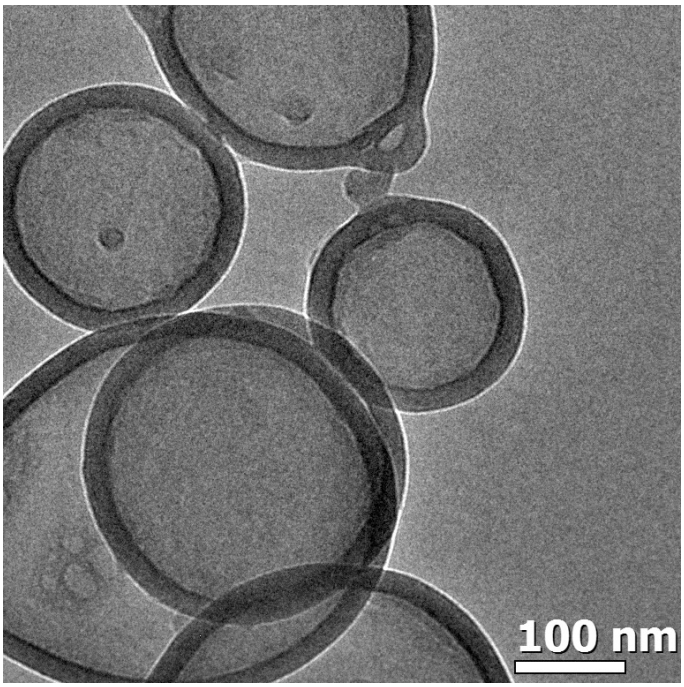

Figure S3. Cryogenic transmission electron microscopy (cryo-TEM) image of non-functionalized OB polymer vesicles.

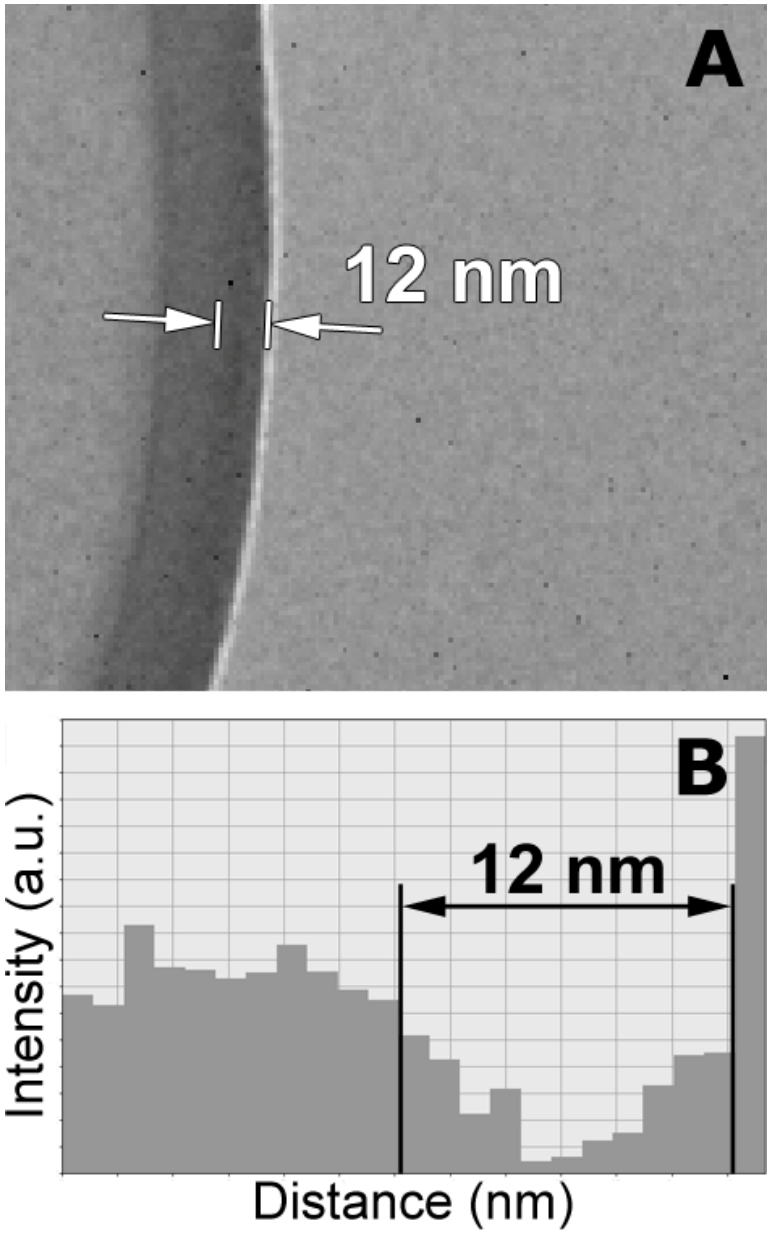

Figure S4. Cryo-TEM image (A) of a non-functionalized OB membrane, demonstrating the measurement of the PB core thickness. The core thickness is measured from an optimally focused image, and is less than the total thickness of the dark band. Because TEM is a 2D projection of a 3D object the actual PB core thickness is most accurately measured as the darkest region of the dark band. A representative intensity profile (B) shows the pixel intensity moving radially from the interior of the dark band to the outer edge. The final estimate of the core thickness is the average of fifty different analyzed intensity profiles.

Table S2. Polymersome size distributions and molar loadings of encapsulates.

mol% Conjugation [a]	**D_h (nm)** [b]	**Molar Loading ($\times 10^2$)** [c]
0%	258.2 ± 68.3	141 ± 2 [d]
0%	237.4 ± 52.6	4.6 ± 0.1 [e]
4.9% PR_b	260.8 ± 38.4	211 ± 3 [d]
5.6% PR_b	273.9 ± 84.7	4.7 ± 0.2 [e]
10.3% PR_b	271.6 ± 33.8	4.6 ± 0.2 [e]
17.3% PR_b	201.9 ± 115.7	4.7 ± 0.1 [e]
22.7% PR_b	264.4 ± 57.3	4.5 ± 0.1 [e]
29.8% PR_b	222.2 ± 125.2	120 ± 3 [d]
5.2% GRGDSP	251.9 ± 60.3	4.7 ± 0.1 [e]
5.2% GRGDSP	232.6 ± 117.3	267 ± 4 [d]
29.5% GRGDSP	245.7 ± 101.8	184 ± 5 [d]
33.5% GRGDSP	263.7 ± 97.4	4.6 ± 0.1 [e]

[a] mol% peptide with respect to OB diblock. [b] Z-average hydrodynamic diameter of polymersome formulation as measured by dynamic light scattering (DLS). [c] The mole ratio of encapsulate (carboxyfluorescein or doxorubicin) to OB block copolymer in each polymersome formulation. [d] Doxorubicin encapsulated. [e] Carboxyfluorescein encapsulated.

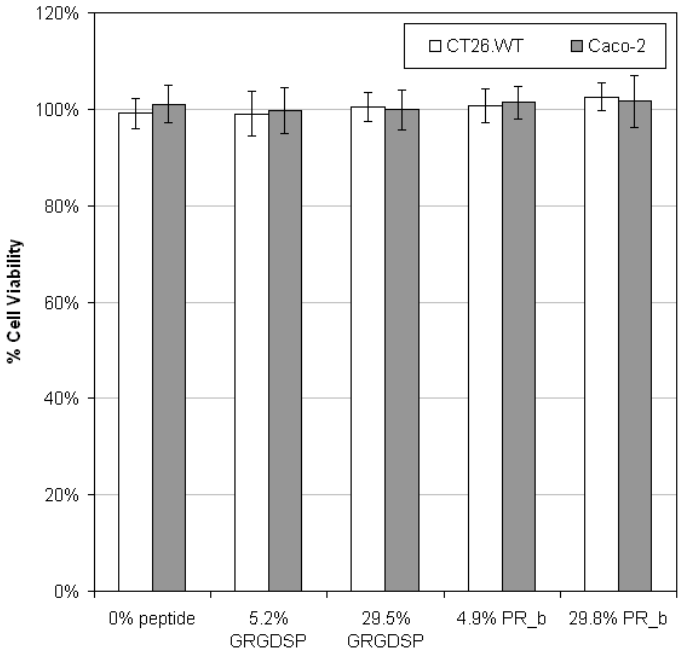

Figure S5. Percent cell viability resulting from delivery of "empty" polymer vesicles (vesicles not loaded with doxorubicin) to CT26.WT and Caco-2 cells *in vitro*. Cells were incubated with vesicle formulations with the indicated mol% of peptide functionalization for 24 h at 37 °C, after which cell viability was measured using the MTT assay. One-hundred percent cell viability is representative of untreated cells. Data is the mean ± standard deviation of two separate experiments (n=2), and each experiment was performed in quadruplicate.

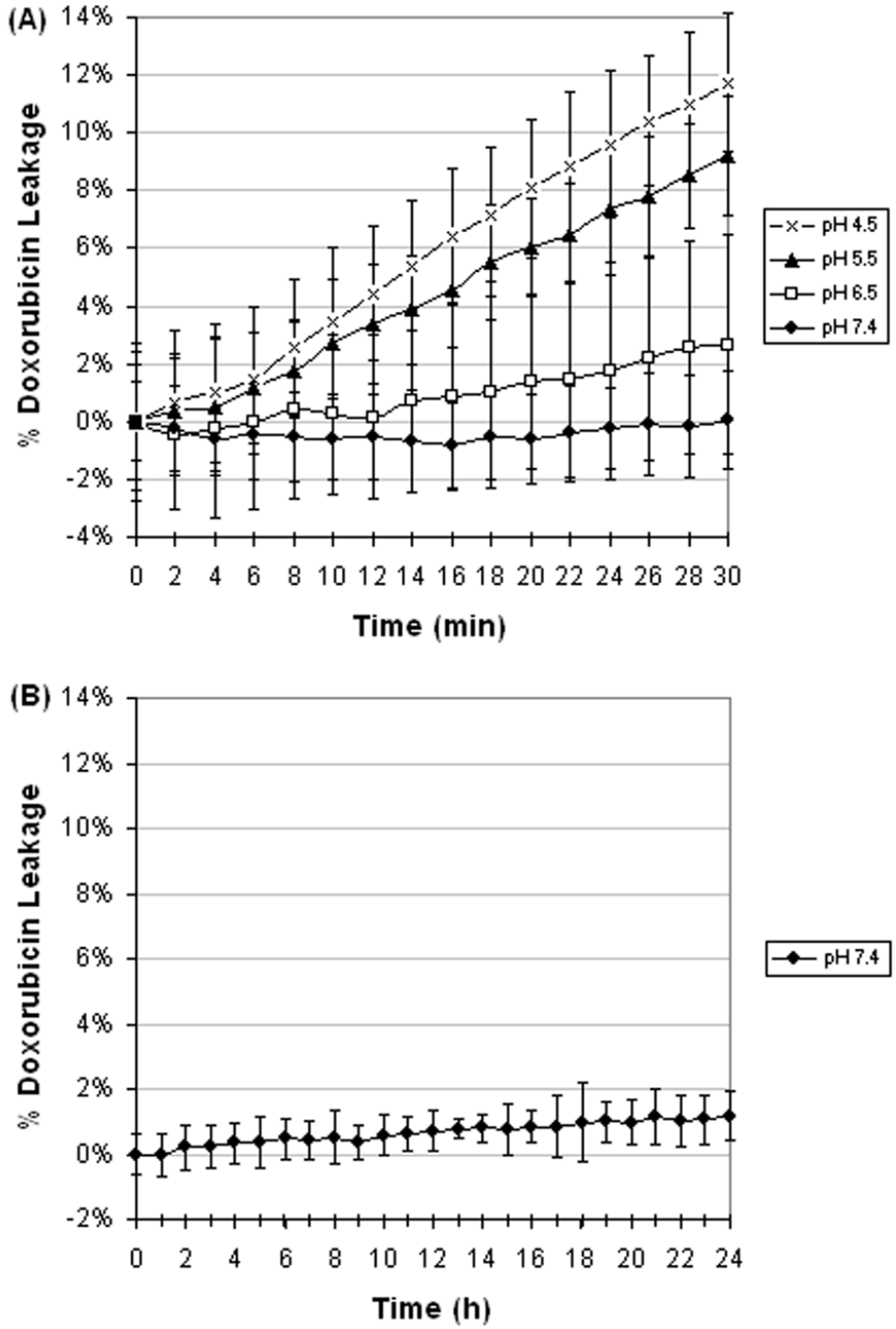

Figure S6. Release profiles of doxorubicin from within polymersomes in buffers of the indicated pH at 37 °C over the course of (A) 30 minutes and (B) 24 hours. Doxorubicin release is expressed as a percentage of complete release of encapsulated doxorubicin. Data is the mean ± standard deviation of two separate experiments (n=2), and each experiment was performed in triplicate. These release profiles were found to be representative of both non-functionalized and peptide functionalized polymersomes. Under storage conditions (4 °C, pH 7.4) no detectable leakage was observed over the time span of vesicle storage.

Movie S1. A movie showing a 3D representation of the "z-slice" confocal images for CT26.WT cells after 24 h at 37 °C incubation with non-functionalized polymer vesicles. Polymer vesicles were fluorescently tagged green, cell nuclei were labeled blue, and cell membranes were labeled red. The movie shows sequential "z-slice" images moving up from the coverslip surface and a rotating 3D representation of the stacked images.

Movie S2. A movie showing a 3D representation of the "z-slice" confocal images for CT26.WT cells after 24 h at 37 °C incubation with polymer vesicles functionalized with 5.2 mol% of the GRGDSP peptide. Polymer vesicles were fluorescently tagged green, cell nuclei were labeled blue, and cell membranes were labeled red. The movie shows sequential "z-slice" images moving up from the coverslip surface and a rotating 3D representation of the stacked images.

Movie S3. A movie showing a 3D representation of the "z-slice" confocal images for CT26.WT cells after 24 h at 37 °C incubation with polymer vesicles functionalized with 5.6 mol% of the PR_b peptide. Polymer vesicles were fluorescently tagged green, cell nuclei were labeled blue, and cell membranes were labeled red. The movie shows sequential "z-slice" images moving up from the coverslip surface and a rotating 3D representation of the stacked images.

Movie S4. A movie showing a 3D representation of the "z-slice" confocal images for CT26.WT cells after 24 h at 37 °C incubation with polymer vesicles functionalized with 33.5 mol% of the GRGDSP peptide. Polymer vesicles were fluorescently tagged green, cell nuclei were labeled blue, and cell membranes were labeled red. The movie shows sequential "z-slice" images moving up from the coverslip surface and a rotating 3D representation of the stacked images.

Movie S5. A movie showing a 3D representation of the "z-slice" confocal images for CT26.WT cells after 24 h at 37 °C incubation with polymer vesicles functionalized with 22.7 mol% of the PR_b peptide. Polymer vesicles were fluorescently tagged green, cell nuclei were labeled blue, and cell membranes were labeled red. The movie shows sequential "z-slice" images moving up from the coverslip surface and a rotating 3D representation of the stacked images.

Movie S6. A movie showing a 3D representation of the "z-slice" confocal images for CT26.WT cells after 24 h at 37 °C incubation with polymer vesicles functionalized with 17.3 mol% of the PR_b peptide. Polymer vesicles were fluorescently tagged green, cell nuclei were labeled blue, and cell membranes were labeled red. The movie shows sequential "z-slice" images moving up from the coverslip surface and a rotating 3D representation of the stacked images.

Movie S7. A movie showing a 3D representation of the "z-slice" confocal images for Caco-2 cells after 24 h at 37 °C incubation with polymer vesicles functionalized with 17.3 mol% of the PR_b peptide. Polymer vesicles were fluorescently tagged green, cell nuclei were labeled blue, and cell membranes were labeled red. The movie shows sequential "z-slice" images moving up from the coverslip surface and a rotating 3D representation of the stacked images.