

# Supporting Information for: Self-Assembly/Disassembly of Giant Double-Hydrophilic Polymersomes at Biologically-Relevant pHs

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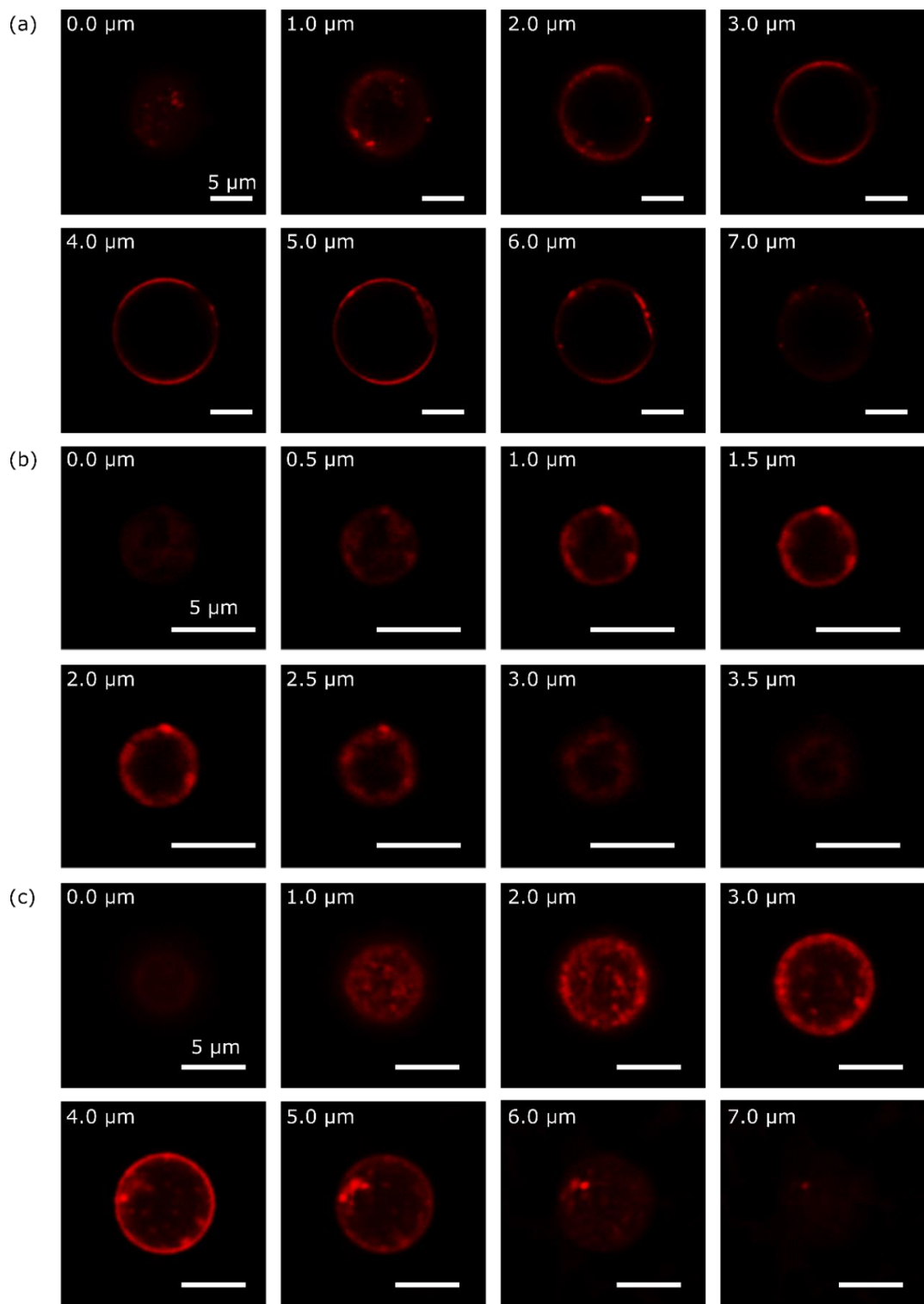
## 1. Experimental details

**Materials.** Poly(ethylene oxide)-*b*-poly(acrylic acid) with different molecular weights (P6348, P6351, and P18436) and poly(ethylene oxide)-*b*-polybutadiene (P10191) were purchased from Polymer Source Inc. Sucrose and agarose were obtained from Sigma Aldrich; polydimethylsiloxane (PDMS) prepolymer and curing agent (Sylgard 184 elastomer kit) from Dow Corning; Texas Red 1,2- dihexadecanoyl-sn-glycero-3-phosphoethanolamine, triethylammonium salt (TR) and Alexa Fluor 488 (AF) from Molecular Probes; methanol from EMD Chemicals; chloroform from Acros Organics; tetrahydrofuran (THF), hydrochloric acid (HCl), and sodium hydroxide (NaOH) from Fisher Chemical. All reagents were used without further purification.

**Vesicle preparation by gel-assisted rehydration.** Agarose gel film was prepared with 285 mM sucrose and 1 % w/v agarose on glass coverslips. Polymer solutions were prepared by dissolving the polymer in preferred organic solvent with 0.5 mol% TR for confocal and epifluorescence imaging purpose. The concentration of polymer solution was 5.96 mg/mL in THF:ethanol 6:10 (v/v) for **EA1**, 3.75 mg/mL in THF for **EA2**, 10 mg/mL in THF for **EA3**, and 3.05 mg/mL in THF for PEO-PBD. 40  $\mu$ L of polymer solution was uniformly deposited on the agarose gel film using circular motions with a needle. Once organic solvent was evaporated, a PDMS well was adhered to the gel/polymer film surface. The coverslip was placed into a humidity chamber that was preheated to 55 °C. 400  $\mu$ L of 285 mM sucrose solution at pH 2.3 (335 mOsm/kg) was added to the PDMS well and then the coverslip was incubated in humidity chamber at 55 °C for 90 min. To raise the pH, 12  $\mu$ L of 0.2 M NaOH (333 mOsm/kg) was added to the vesicle solution. For the dye encapsulation experiment, 400  $\mu$ L of 100  $\mu$ M AF in sucrose solution at pH 2.3 (336 mOsm/kg) was added to the gel/polymer film surface and then the coverslip was incubated in humidity chamber at 55 °C for 90 min. After dye encapsulation, the vesicle solution was rinsed with pH 2.3 sucrose solution 3 times to remove unencapsulated dye.

**Characterization.** Confocal micrographs were captured on an inverted microscope (Olympus FV1000) equipped with a 40 $\times$  objective, a multiline Ar laser and a HeNe laser. Quantitative analysis of intensity profile data was performed using ImageJ software. Epifluorescence micrographs were captured on an inverted microscope (Olympus IX81) in epifluorescence with a 100 $\times$  objective equipped with Orca-Flash 4.0 cMOS camera (Hamamatsu Photonics) for image collection. Images were processed using Fiji software. Bulk pH was measured using pH meter (Orion Star A211, Thermo Scientific) with pH electrode (Ohaus STMICRO5). Dynamic light scattering (DLS) and  $\zeta$  potential measurements were performed using a Malvern Zetasizer Nano ZS (ZEN3600, Malvern Instruments) equipped with a 4 mW HeNe gas laser operating at a wavelength of 633 nm and a scattering angle of 175°. <sup>1</sup>H NMR experiments were performed using a Bruker Advance III 500 MHz spectrometer. Cryo-electron microscopy (cryo-EM) image was captured on a high-resolution electron microscope (JEOL 2010) operating at 200 kV accelerating voltage equipped with a CCD camera (Orion, Gatan) for image collection. Osmolarity was measured using osmometer (model 3320, Advanced Instruments, Inc.).

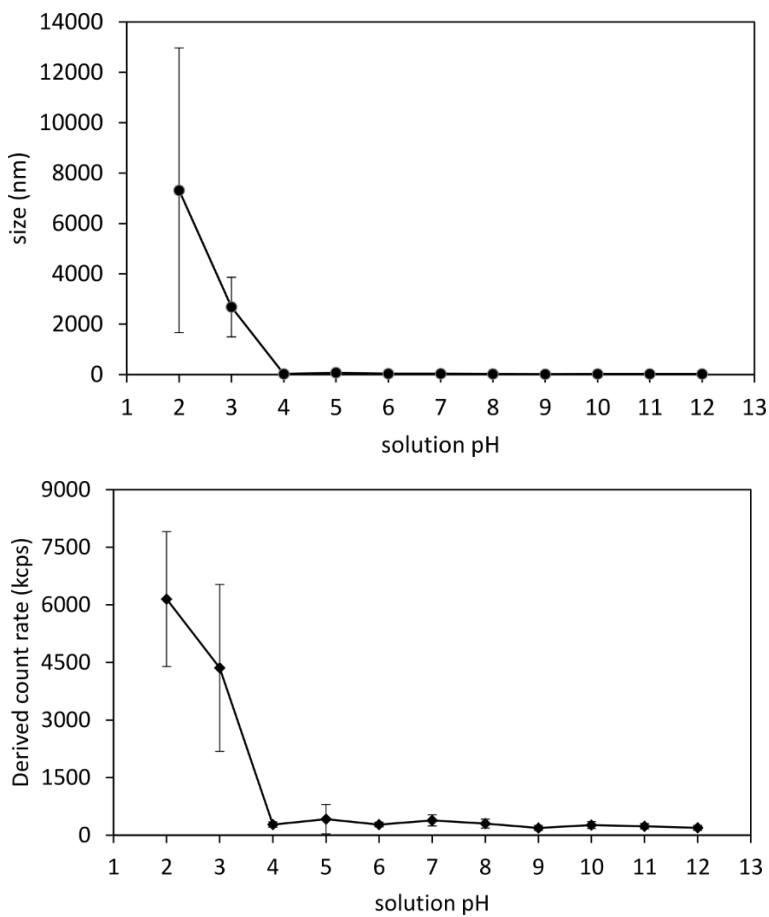
## 2. Z-stack of confocal micrographs of PEO-PAA vesicles at pH 2.3



**Figure S1.** Z-stack of confocal micrographs of polymer vesicles (a) EA1, (b) EA2, and (c) EA3 prepared in pH 2.3 aqueous sucrose solutions.

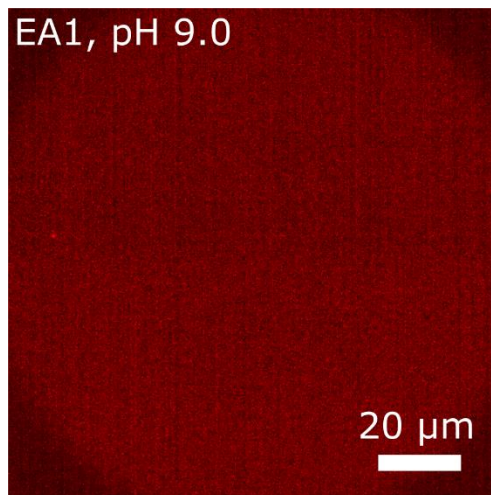
### 3. Solubility of PEO-PAA at different pH

Hydrodynamic diameter of aggregates formed by PEO-PAA polymer in aqueous solution was measured at various pH by using DLS to check solubility of the polymer. The PEO-PAA (**EA1**) polymer was completely dissolved in deionized water adjusted to pH 12 (5 mg/mL) and the pH of solution was incrementally decreased to 2 by adding HCl and stirring for 15 min between each pH measurement. After the pH of solution reached to 2, the pH of solution was incrementally increased back to 12 by adding NaOH and stirring for 15 min between each pH measurement. All samples were measured at room temperature.



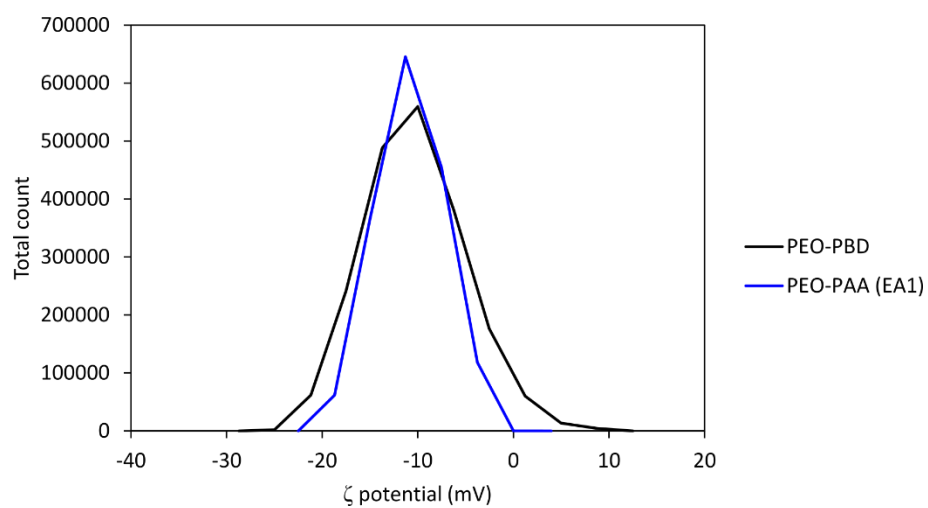
**Figure S2.** (a) Hydrodynamic diameter of aggregates formed by PEO-PAA polymer in aqueous solution at various pH and (b) count rate of same solution.

#### 4. Epifluorescence micrograph of PEO-PAA at pH 9.0



**Figure S3.** Epifluorescence micrograph of a sample of PEO-PAA (EA1) after gel-assisted rehydration at pH 9.0.

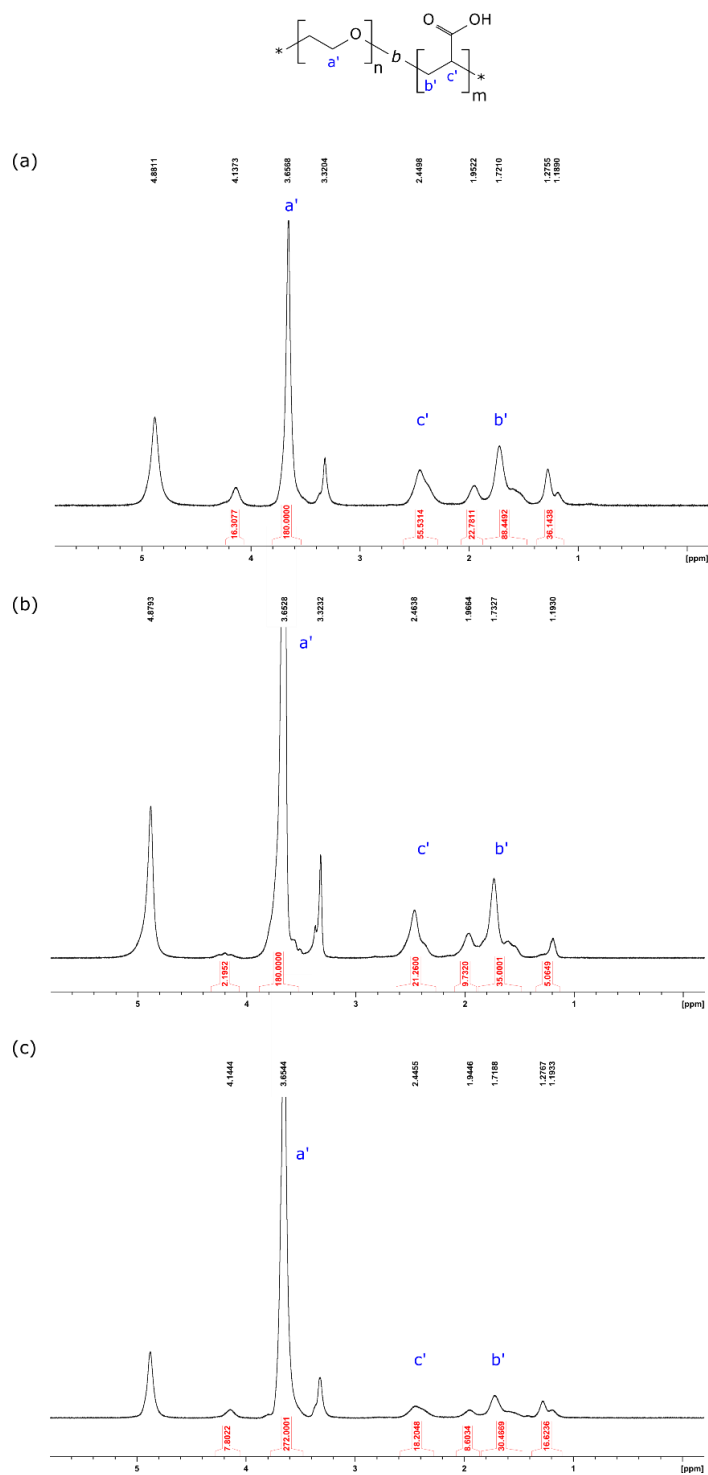
#### 5. $\zeta$ potential distributions of polymer vesicles



**Figure S4.**  $\zeta$  potential distributions for PEO-PAA and PEO-PBD vesicles in pH = 2.4 water

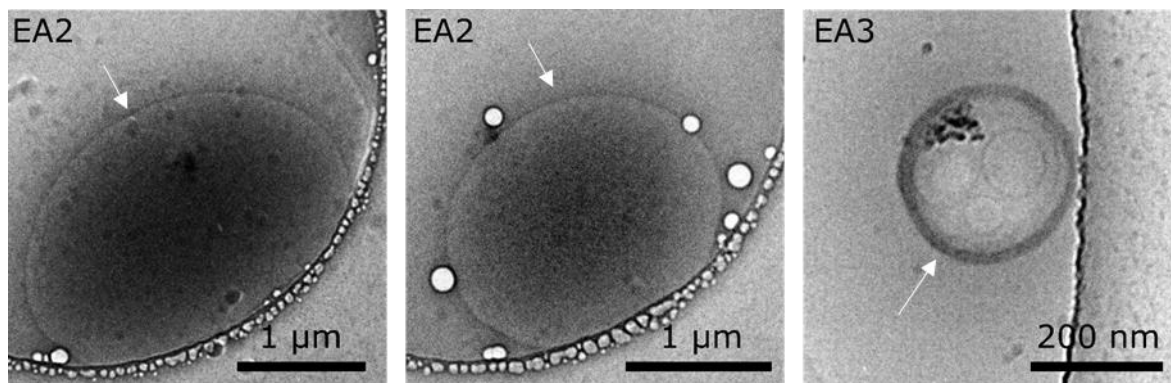


## 6. $^1\text{H}$ NMR spectra of as received PEO-PAA



**Figure S5.**  $^1\text{H}$  NMR spectra of (a) EA1, (b) EA2, and (c) EA3 in methanol- $d_4$ . Polymer composition was determined for each polymer based on the integration of the protons in the poly(acrylic acid) block ( $b'$  and  $c'$ ) compared to the integration of the protons in the poly(ethylene oxide) block ( $a'$ ), using the molecular weight information provided by the supplier (Polymer Source) to calibrate the number of protons in the poly(ethylene oxide) block for each polymer (2000 D for EA1, EA2; 3000 D for EA3).

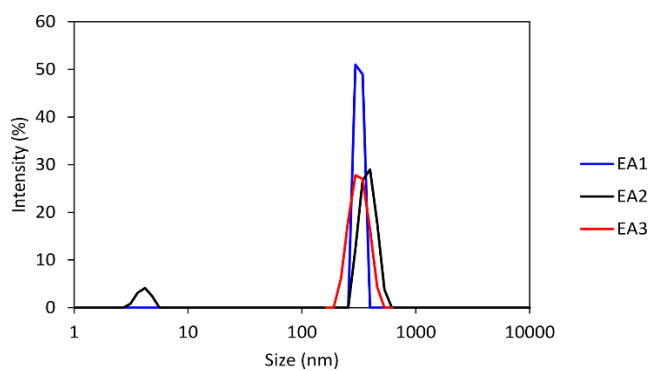
## 7. Cryo-EM images of PEO-PAA vesicles



**Figure S6.** Cryo-EM images of **EA2** and **EA3** vesicles, designated by white arrows, formed at pH 2.3. Vesicles were prepared by gel-assisted rehydration and transferred to a cryo-EM sample grid.

## 8. Size distributions of PEO-PAA vesicles by DLS measurements

Vesicle	$Z_{avg}$ (nm)	PDI
<b>EA1</b>	$888.3 \pm 94.9$	$0.78 \pm 0.1$
<b>EA2</b>	$577.3 \pm 70.4$	$0.53 \pm 0.1$
<b>EA3</b>	$729.7 \pm 142.8$	$0.61 \pm 0.1$

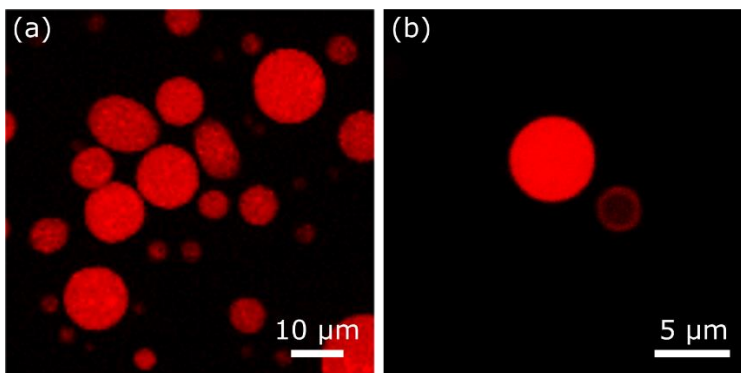


**Figure S7.** Z-average diameters and size distributions of PEO-PAA vesicles in pH 2.4 water by DLS measurements.



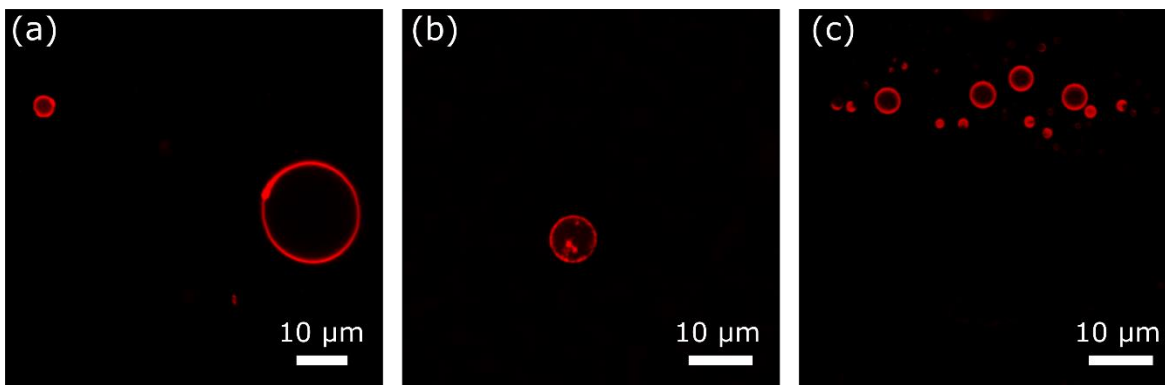
## 9. Swollen polymer droplets

We note that vesicle formation depends on the preparation method that is employed. When the polymer solution was slowly exchanged from THF to pH 2.3 water, only swollen polymer droplets that appear to have a uniform fluorescence intensity throughout their volume were observed (Fig. S8a). Such swollen polymer droplets were also observed from vesicle solution prepared by gel-assisted rehydration method. However, vesicles with hollow interiors were distinguishable from the polymer droplets by confocal microscopy (Fig. S8b).



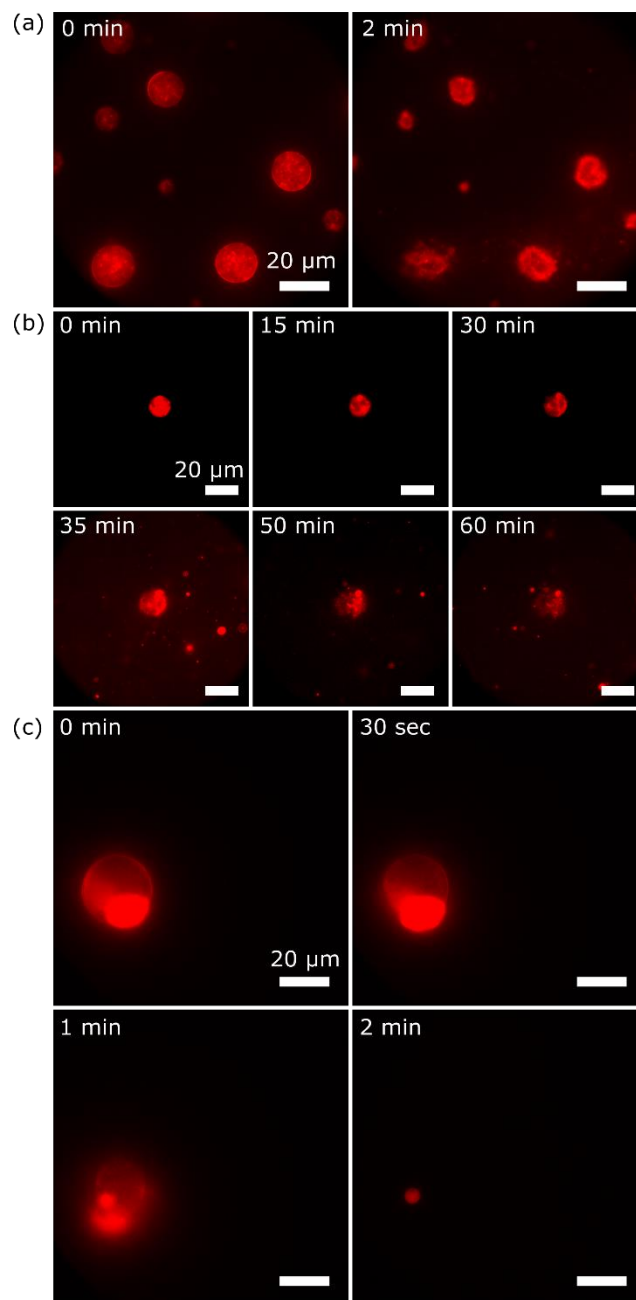
**Figure S8.** Confocal micrograph of (a) swollen PEO-PAA droplets prepared by solvent exchange method from THF to pH 2.3 water and (b) swollen PEO-PAA droplet and vesicle rehydrated on agarose layer at pH 2.3.

## 10. Yield of PEO-PAA vesicles



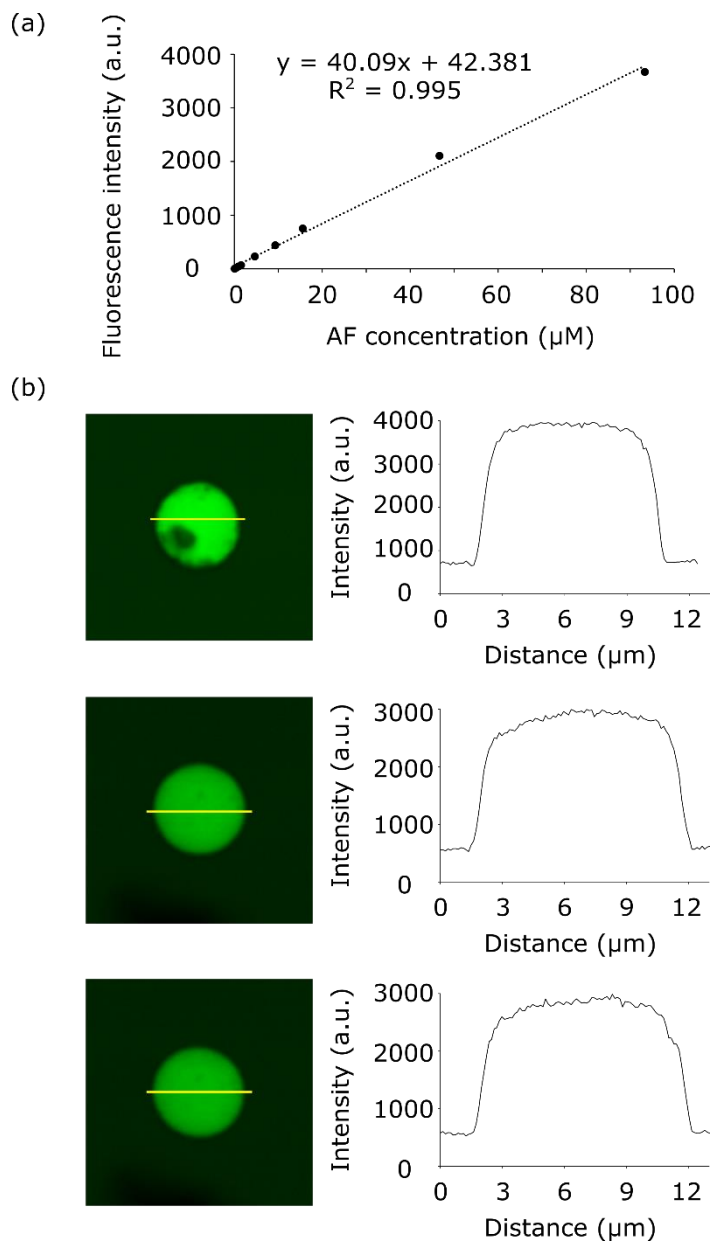
**Figure S9.** Confocal micrographs of (a) PEO-PBD vesicles at pH 7.4, (b) a fully detached **EA3** vesicle at pH 2.3, and (c) hemispheres of **EA3** vesicles still attached to the agarose gel at pH 2.3

## 11. pH-sensitive PEO-PAA vesicles



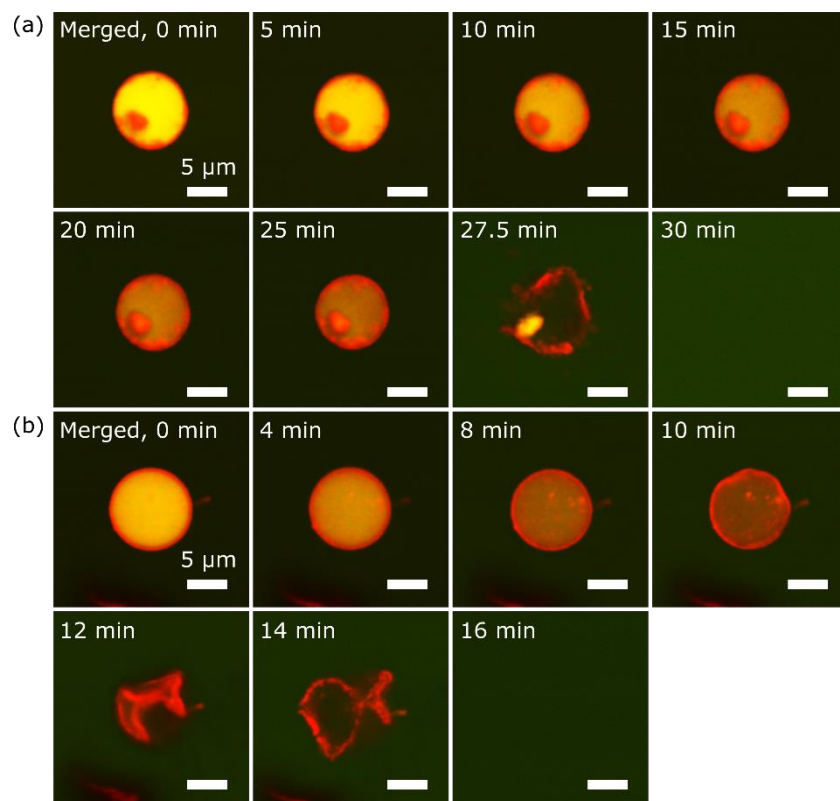
**Figure S10.** Epifluorescence micrographs of (a) EA1, (b) EA2, and (c) EA3 vesicles after addition of iso-osmolar NaOH solution showing degradation of PEO-PAA vesicles at pH 10.2. The timescale for vesicle degradation varied widely in all cases (from minutes to hours), which we attribute to diffusive mass transfer limitations and incomplete mixing with the addition of the basic aqueous solution.

## 12. Dye encapsulation efficiency



**Figure S11.** (a) A calibration curve for Alexa Fluor 488 (AF) in pH 2.3 sucrose solution. (b) Confocal micrographs of AF dye encapsulated in PEO-PAA vesicles (**EA3**) and intensity profiles of AF dye.

### 13. Dye release from PEO-PAA vesicles



**Figure S12.** Confocal micrographs of PEO-PAA vesicles (**EA3**) with merged channel (a) at pH 5.9 and (b) at pH 8.1 showing complete degradation of vesicle and release of encapsulated AF dye.