Electronic Supplementary Material (ESI) for ChemComm.

Evolving Polymersomes Autonomously Generated in and Regulated by a Semibatch pH Oscillator

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Key words:
pH oscillatory chemistry; PISA; giant vesicles; protocell; self-assembly

Experimental Section

Materials

Macromolecular poly(ethylene glycol) chain transfer agent (PEG-CTA), poly(ethylene glycol) 4-cyano-4-(phenylcarbonothioylthio)pentanoate, was synthesized by the esterification reaction between methoxy poly(ethylene glycol) (mPEG, 1900 Da, Alfa Aesar) and 4-cyano-4-(thiobenzoylthio) pentanoic acid (Strem), with the help of N,N'-dicylohexylcarbodiimide (DCC, Sigma-Aldrich) and *N,N'*-dimethylaminopyridine (DMAP, Alfa Aesar), according to literature. [1] Methanol-d_4 for NMR tests, was purchased from Cambridge Isotope Laboratories, Inc.. Butyl acrylate (BA, Sigma-Aldrich), 2-methoxyethyl methacrylate (MEMA, Sigma-Aldrich), sodium bromate ($NaBrO_3$, Sigma-Aldrich), sodium sulfite (Na_2SO_3, anhydrous, Sigma-Aldrich), sulfuric acid (H_2SO_4, 10 Normal, Ricca Chemical Company), anhydrous dichloromethane (DCM, Sigma-Aldrich), N,N-dimethylformamide (DMF, HPLC grade, VWR), and lithium bromide (LiBr, anhydrous, 99.99%, VWR) were used without further purification. Sodium sulfite (Na_2SO_3) solution was freshly prepared and used on the same day.

General measurements

The pH change of the aqueous solution in semi-batch experiments was monitored by a Benchtop pH/mV Meter (Sper Scientific Direct) equipped with a pH Electrode with BNC + PIN connector (Hanna Instruments), pH values were collected once per second. ^{1}H-NMR spectra of polymers were recorded on a 500 MHz Varian Unity/Inova 500B spectrometer in methanol-d_4. Gel permeation chromatography (GPC) analysis was conducted on an Agilent 1260 system equipped with a refractive index detector, using DMF with 0.05 mol/L LiBr as an eluent, with a flow rate of 1.0 mL/min at 50 ℃. Standard curve was obtained using monodispersed polystyrene (PS) standards (Agilent Technologies) with molecular weights ranging from 580 to 3.2×10^6 Da. Dynamic light scattering (DLS) analysis was conducted on a Delsa Nano C particle size and zeta potential analyzer (Beckman Coulter, Inc.). Morphology of the self-assembled polymersomes was observed under field scanning electron microscope (FESEM, Supra 55VP) and/or transmission electron microscope (TEM, JEOL JEM-2100), and/or Scanning electron cryomicroscopy (Cryo-SEM) (Zeiss NVision 40 focus ion beam scanning electron microscope (FIB-SEM)).

Polymerization induced self-assembly driven and controlled by bromate-sulfite (B-S) semi-batch pH oscillator (pH-O-PISA) of poly(ethylene glycol)-block-poly(butyl acrylate) (PEG-b-PBA)

PEG-CTA and hydrophobic monomer were added into the semibatch B-S pH oscillator, the radicals generated by pH oscillator were used to initiate RAFT (reversible addition-fragmentation chain transfer) polymerization, which lead to the formation of amphiphilic block copolymers and the following self-assembled polymersomes. The reaction process is given here using butyl acrylate (BA) as monomer. Briefly, 20 mL 0.1 M sodium bromate ($NaBrO_3$) solution was added in a 50 mL tall beaker/reactor, and a freshly calibrated pH meter was inserted in at 40 °C. 100 μL of 0.1 M H^+ (H_2SO_4) was added into the sodium bromate solution to bring the pH value down to ~3.1. Then a mixture of 17.3 mg PEG-CTA (8 μmol) and 114.8 μL BA (800 μmol, DP_{target} = feeding ratio of monomer/CTA = 100) were added into the solution before a mixed solution of 2.0 M sodium sulfite (Na_2SO_3) and 0.185 M H^+ (H_2SO_4) was continuously pumped into the reaction mixture using a peristaltic pump (LongerPump i150) with a constant flow rate of 0.30 mL/h, and the system was stirred at 400 rpm at 40 °C. The pH value change of the reaction mixture was monitored during the whole reaction process by collecting pH value data once per second. At the minimal and maximal pH values of the first five pH oscillation cycles and pre-set time points, 60 μL reaction mixture was sampled out and quickly froze in ice that pre-frozen at -20 °C to stop polymerization, then water was removed by freeze-drying before 600 μL methanol-*d₄* was added to dissolve the polymer for ^{1}H-NMR tests. The average hydrodynamic diameters (Ave. D.) and polydispersity indexes (PDI) of self-assembled polymersomes at 30, 60, 120, 150 and 180 min were determined by DLS. The morphology of polymersomes in samples collected at 60, 120, 150 and 180 min was observed by SEM, TEM, and/or Cryo-SEM. The final PEG-b-PBA product at 180 min was collected and dialyzed against deionized water to remove salt, and freeze-dried before being used for

NMR and GPC studies.

The pH-O-PISA experiments of PEG-b-PBA with DP_{target} = 75 and 150 were also conducted. Except the amount of added monomer, all other compositions were kept the same as the compositions of DP_{target} =100, and the pH of bromate solution was also adjusted to ~3.5 by adding 100 μL of 0.1 M H^+ (H_2SO_4). The pH oscillation curves and corresponding compositions are shown in **Figure S1**.

To investigate the effect of polymerization to pH oscillation, a pH-O-PISA experiment was designed by adding BA monomer with a DP_{target} =75 first, and adding the same amount of BA after the pH oscillations stopped, the total DP_{target} = 150. The pH oscillation curve is shown in Figure S3.

pH oscillations of pure B-S pH oscillator at different H_2SO_4 concentrations

The pH oscillation of pure B-S pH oscillator was also conducted. Much lower concentration of H^+ is necessary to restore pH oscillations with other compositions (without using PEG-CTA and monomer) being kept the same as used in pH-O-PISA of PEG-b-PBA with DP_{target} = 100 (Figure 1a), such as a H_2SO_4 solution of 0.06 M H^+. The corresponding pH oscillation curve of pure B-S pH oscillator is shown in Figure S4a. At the same H_2SO_4 concentration used in pure B-S pH oscillator (0.06 M H^+), the addition of PEG-CTA and monomer brought the system out of oscillatory regime (Figure S4b).

While, at the same H_2SO_4 concentration used in pH-O-PISA of PEG-b-PBA with DP_{target} = 100, but without adding PEG-CTA and monomer, pure B-S pH oscillator was also out of oscillatory domain too. The corresponding pH change curve is shown in

Figure S5.

pH-O-PISA of different monomer

To demonstrate the wide adaptability of pH-O-PISA to different vinyl monomers, a pH-O-PISA experiment of PEG-b-PMEMA, using 2-methoxyethyl methacrylate (MEMA) as monomer, was also conducted. The pH oscillation curve and the composition used are shown in Figure S6. The concentration of H^+ (H_2SO_4) was increased to 0.25 M to maintain large pH oscillation amplitude and make the pH oscillations last longer (Figure S6).

Table S1. Molecular weights and polydispersity index (PDI) of PEG, PEG-CTA, and PEG-b-PBA (DP_{target} = 100, polymerized for 3 hours) determined by ^{1}H-NMR and GPC.

Polymer	M_n-NMR (Da)	M_n-GPC (Da)	M_w-GPC (Da)	PDI	DP of BA
mPEG[a]	1953[b]	2320	2656	1.15	0
mPEG-CTA	2230[c]	2481	3421	1.38	0
PEG-b-PBA	9049[d]	8983	19403	2.16	53.2[d]/50.7[e]

[a]The mPEG with a molecular weight of 1900Da (provided by the company) was purchased from Fluke; [b]Calculated by the integration ratio between -CH_2**CH_2**O- (3.59-3.68 ppm) and terminal –**CH_3** (~3.35ppm) group; [c]The CTA modification efficiency, determined by the integration ratio between **CH_2** of the terminal of PEG that near the newly formed ester group (mPEG-O-CH_2-**CH_2**-OCO-CTA, ~4.15 ppm) and the terminal –**CH_3** of mPEG (~3.35ppm), was 99.3%, so the M_n of PEG-CTA determined by NMR is 2230 Da; [d]Determined by NMR of the final reaction mixture, calculated by area ratios A(-**CH_2**OCO-)/A(PEG); [e]Determined by GPC.

Table S2. The composite reactions and their categories in B-S pH oscillator (reproduced from reference 32 in main text).

No.	Reactions	Rate laws	Rate constants (45°C)
1	$SO_3^{2-} + H^+ \rightleftharpoons HSO_3^-$	$r_1 = k_1[SO_3^{2-}][H^+]$;	k_1: 2.0 ×10^{10} $M^{-1}s^{-1}$;
		$r_{-1} = k_{-1}[HSO_3^-]$	k_{-1}: 2.0 ×10^3 s^{-1}
2	$HSO_3^- + H^+ \rightleftharpoons H_2SO_3$	$r_2 = k_2[HSO_3^-][H^+]$;	k_2: 1.2 ×10^{10} $M^{-1}s^{-1}$;
		$r_{-2} = k_{-2}[H_2SO_3]$	k_{-2}: 2.0 ×10^8 s^{-1}
3	$3HSO_3^- + BrO_3^- \rightarrow 3SO_4^{2-} + Br^- + 3H^+$	$r_3 = k_3[HSO_3^-][BrO_3^-]$	k_3: 0.13 $M^{-1}s^{-1}$
4	$3H_2SO_3 + BrO_3^- \rightarrow 3SO_4^{2-} + Br^- + 6H^+$	$r_4 = k_4[H_2SO_3][BrO_3^-]$	k_4: 30 $M^{-1}s^{-1}$
5	$6H_2SO_3 + BrO_3^- \rightarrow 3H_2S_2O_6 + Br^- + 3H_2O$	$r_5 = k_5[H_2SO_3][BrO_3^-]$	k_5: 2.0 $M^{-1}s^{-1}$

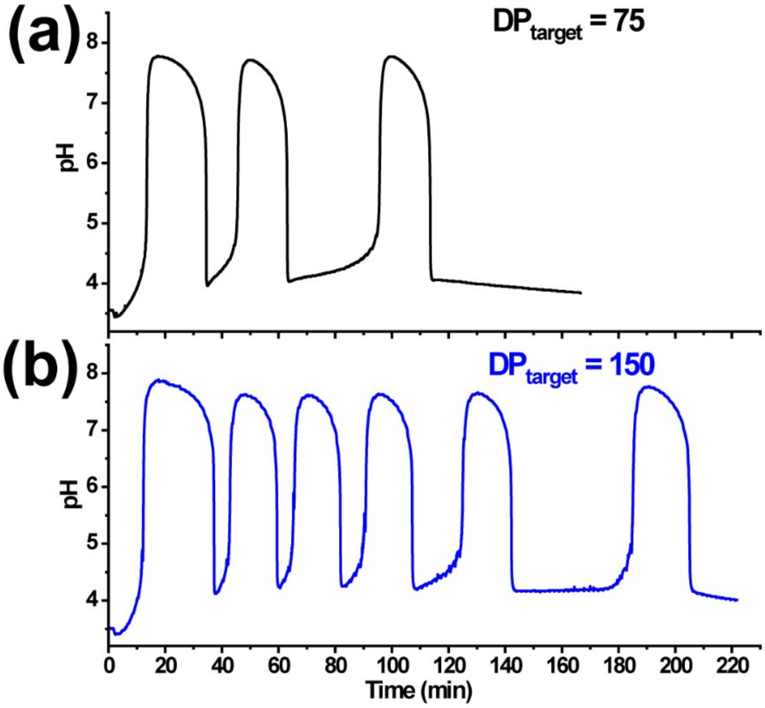

Figure S1. pH oscillation curves for pH-O-PISA of PEG-b-PBA with a DP$_{target}$ = 75 (**a**), and 150 (**b**).

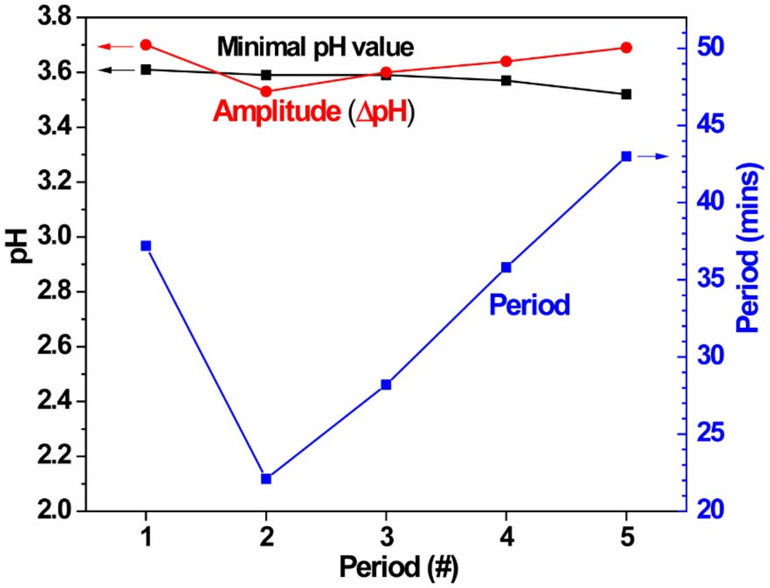

Figure S2. Changes in the period length (blue squares), amplitudes (red circles), and minimal pH values (black squares) of different periods in pH-O-PISA of PEG-b-PBA.

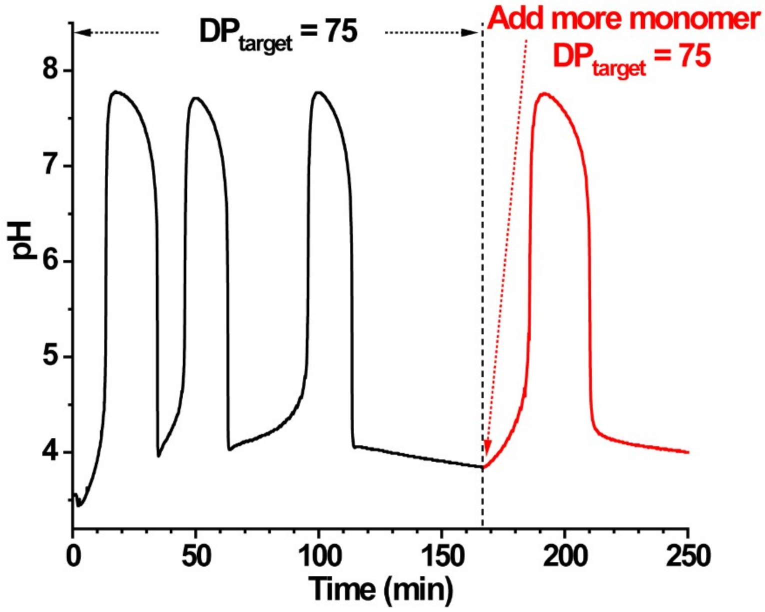

Figure S3. pH oscillation curves for pH-O-PISA of PEG-b-PBA with a DP$_{target}$ = 75 and additional monomer (DP$_{target}$ = 75) was added after pH oscillations stopped.

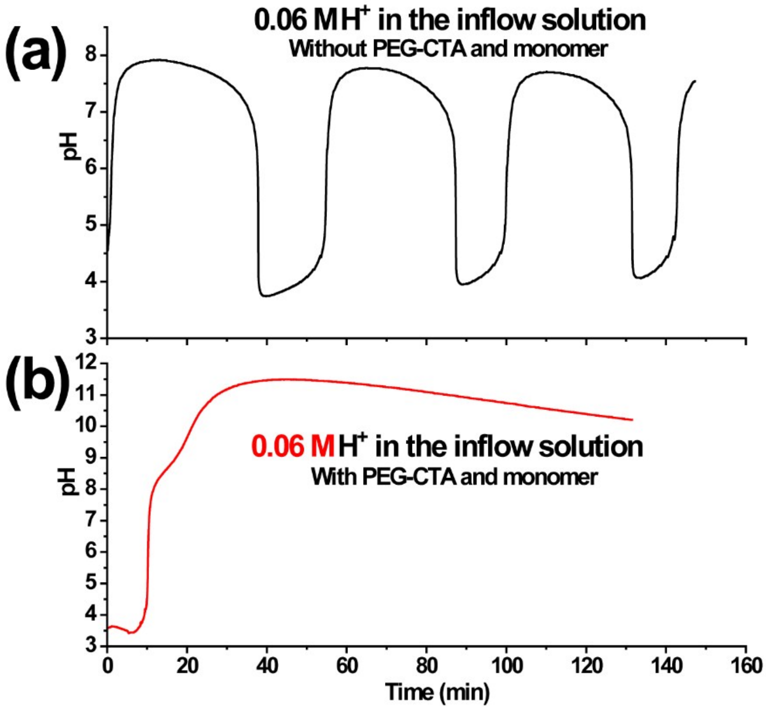

Figure S4. pH change curves of pure B-S pH oscillator at oscillatory state (**a**) and the same system with PEG-CTA and monomer (**b**). The recipe and experimental process is presented here: (**a**) to a solution of 20 mL 0.1 M $NaBrO_3$, a mixed solution [2.0 M Na_2SO_3+ 0.06 M H^+ (H_2SO_4)] was inflowed with a flow rate of 0.30 mL/h at 40 ^oC; (**b**) To a 20 mL mixed solution of $NaBrO_3$ (0.1 M), PEG-CTA (17.3 mg), and BA (114.8 μL) (100 μL 0.1 M H^+ (H_2SO_4) was added in to bring the pH value down to ~3.5 before the experiment), a mixed solution [2.0 M Na_2SO_3 + 0.06 M H^+ (H_2SO_4)] was inflowed with a flow rate of 0.30 mL/h at 40 ^oC.

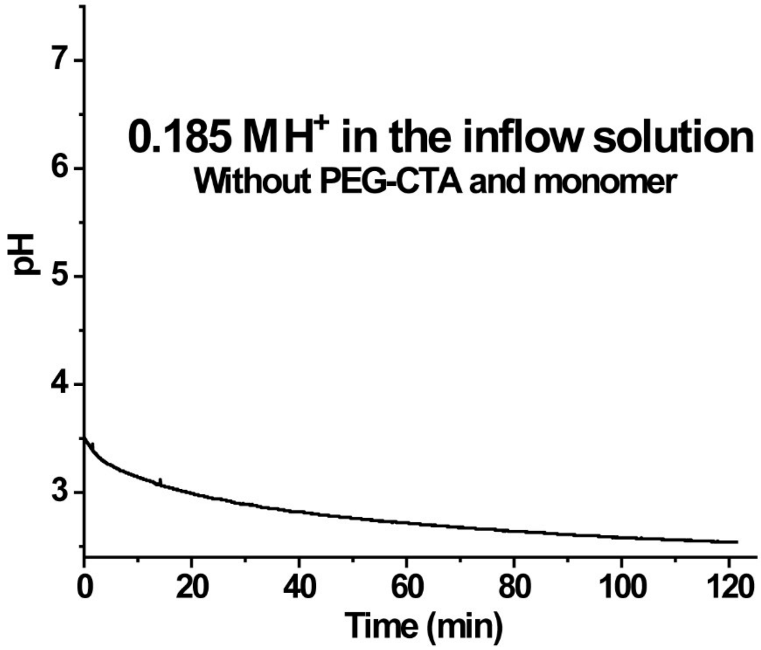

Figure S5. pH change curve of pure B-S pH oscillator at non-oscillatory state. The recipe is the same as pH-O-PISA of PEG-b-PBA with a DP_{target} =100, except no PEG-CTA or monomer was used: to a solution of 20 mL 0.1 M $NaBrO_3$, a mixed solution [2.0 M Na_2SO_3 + 0.185 M H^+ (H_2SO_4)] was inflowed with a flow rate of 0.30 mL/h at 40 °C. 100 μL 0.1 M H^+ (H_2SO_4) was added into the $NaBrO_3$ solution to bring the pH value down to ~3.5 before the experiment.

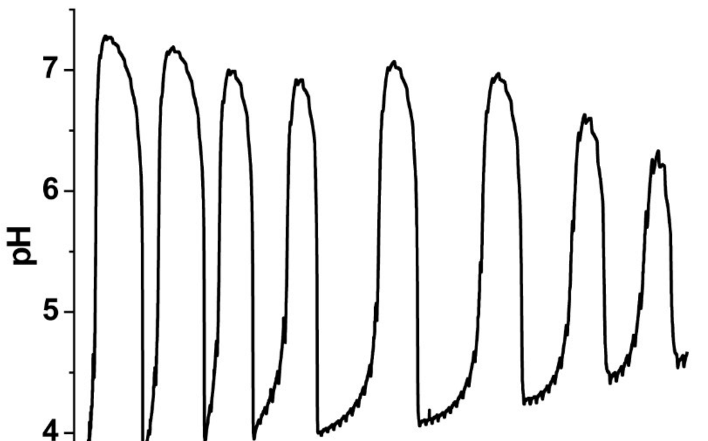

Figure S6. pH oscillation curve of pH-O-PISA of PEG-b-PMEMA (using 2-methoxyethyl methacrylate (MEMA) as monomer, $DP_{target} = 200$) with the following formulation: PEG-CTA 17.3 mg (8 μmol), MEMA (232.3 μL), 20 mL $NaBrO_3$ (0.1 M), in-flow solution [2.0 M Na_2SO_3 + 0.25 M H^+ (H_2SO_4)], 0.30 mL/h, 40 °C. 100 μL 0.1 M H^+ (H_2SO_4) was added into the $NaBrO_3$ solution to bring the pH value down to ~3.5 before the experiment.

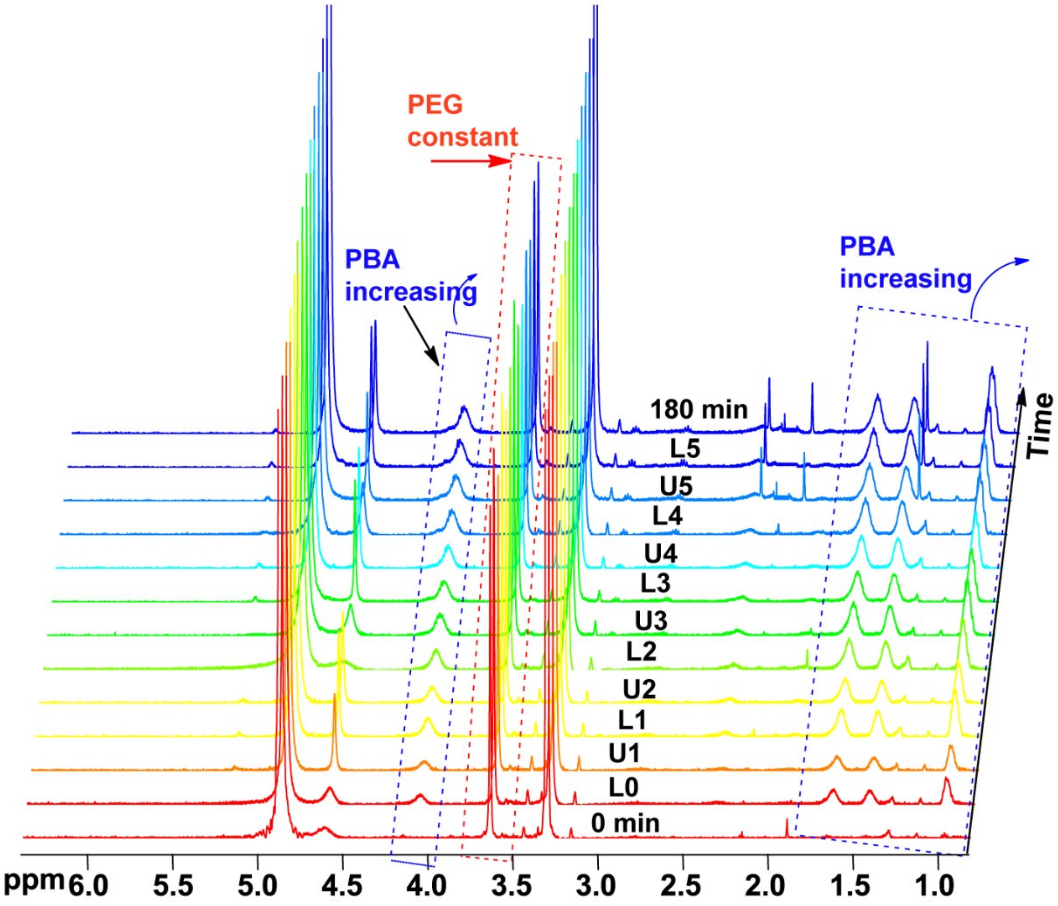

Figure S7. The ^{1}H-NMR spectra of the freeze-dried reaction mixtures sampled out at different time points labelled out in Figure 1a.

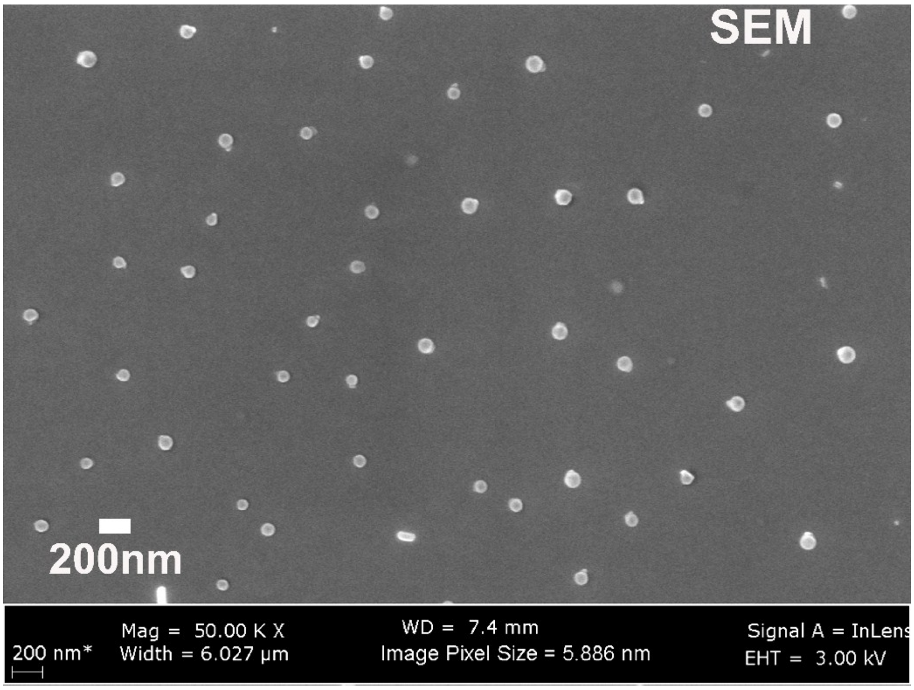

Figure S8. SEM image of the 30 min sample for pH-O-PISA of PEG-b-PBA (DP_{target} = 100).

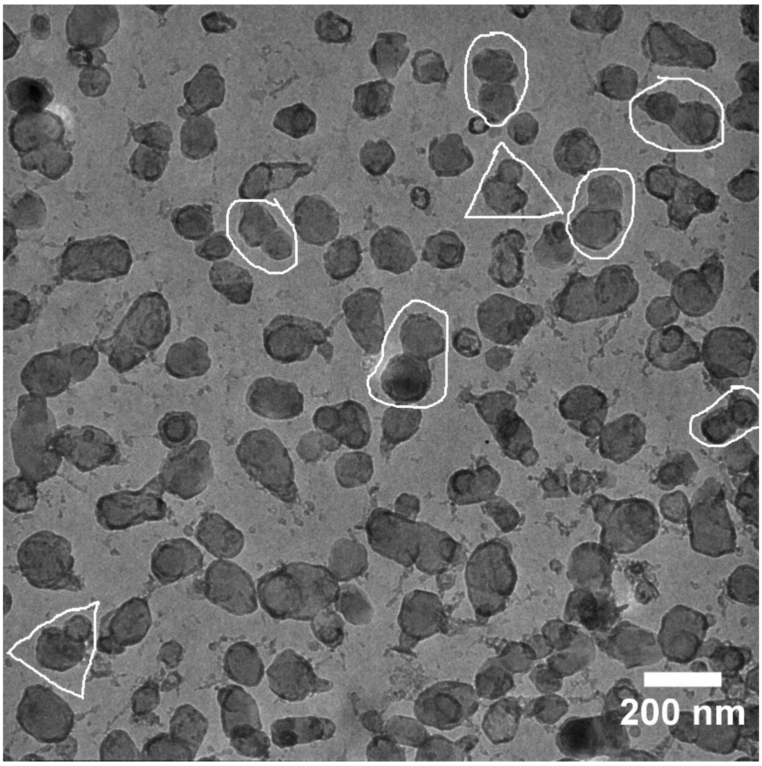

Figure S9. TEM image of small PEG-b-PBA vesicles obtained by pH-O-PISA (DP_{target} = 100) at 60 min. Representative vesicle division (in white cycle) and vesicle budding (in white triangle) are labeled in the image.

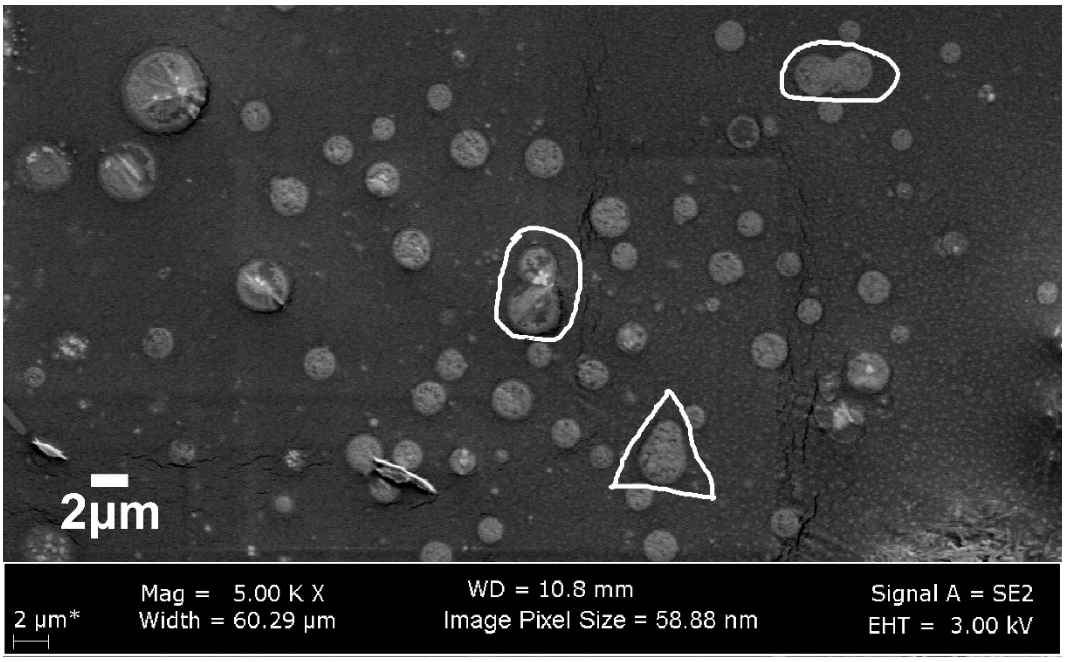

Figure S10. Low magnification SEM image of PEG-b-PBA large vesicles obtained by pH-O-PISA (DP_{target} = 100) at 180 min (non-washed). Representative vesicle division (in white cycle) and vesicle budding (in white triangle) are labeled in the image.

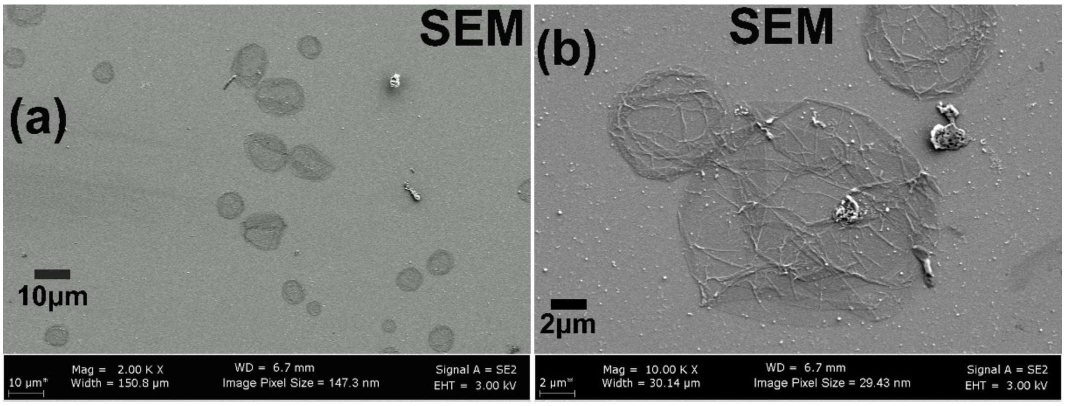

Figure S11. Low magnification SEM image (**a**) and representative vesicle budding SEM images (**b**) of PEG-b-PBA vesicles obtained by pH-O-PISA (DP_{target} = 100) at 180 min (washed).

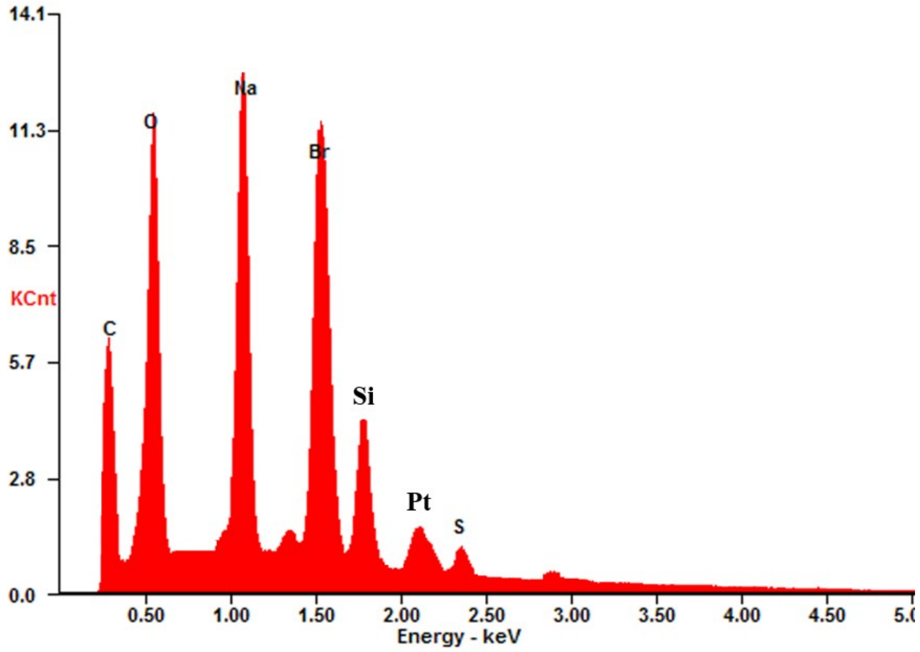

Figure S12. EDS spectrum of vesicles of PEG-b-PBA block copolymers imbedded in salt layer in the unwashed sample of 180 min (see SEM image in **Figure 3e1**). The coexistence of sodium (Na), bromate (Br), and sulfur (S) with carbon (C) and oxygen (O) proved the existence of salt encapsulated in and distributed surrounding PEG-b-PBA vesicles.