Supporting Information

Spontaneous, One-Pot Assembly of pH-Responsive Hydrogen-Bonded Polymer Capsules

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Materials

Hydroxyethylmethacrylate (HEMA), ethyl α-bromoisobutyrate (EBiB), succinic anhydride (SA) or maleic anhydride (MA), anhydride pyridine, sodium hydride (NaH), N, N, N', N'', N''-pentamethyldiethylenetriamine (PMDETA), copper(I) bromide (CuBr), 2-hydroxy-2-methylpropiophenone (HMPPh) photoinitiator, adipic acid dihydrazide (AADH), N-hydroxysulfosuccinimide sodium salt (sulfo-NHS), Methanol-d_4 (d_4-MeOH) and Nile Red were purchased from Sigma Aldrich. Dimethylformamide (DMF) and pyridine were acquired from Alfa Aesar. Poly(N-vinylpyrrolidone) (PVP) (M_w= 10 kDa, 29 kDa, 55 kDa and 1300 kDa) was supplied by Polysciences, Inc. 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC) was purchased from Thermo Scientific, and Alexa Fluor 488 dihydrazide (Alexa-488) – from Invitrogen Co., CA. Ultrapure Milli-Q water (Millipore) with a resistivity of ~18 MΩ/cm was used in all experiments. The substrates for Fourier transform infrared spectroscopy (FTIR) were 525±25 μm thick, single-side-polished, N-type (110) silicon wafers purchased from Virginia Semiconductor, VA. Silica nanoparticles (140 nm non-functionalized silica nanoparticles) were purchased from Nanocomposix Inc., USA. Deuterium oxide (D_2O, D, 99.96%) was purchased from Cambridge Isotope Laboratories.

Synthesis and Assembly Procedures

Synthesis of polyacids. Poly(2-succinyloxyethyl methacrylate) (PSEMA) and poly(2-maleyloxyethyl methacrylate) (PMEMA) were prepared by modification of a parent poly(hydroxyethylmethacrylate) (PHEMA, 6 kDa) which was synthesized using ATRP polymerization as described elsewhere.[1] For ATRP polymerization of HEMA, EBiB (0.07 mmol, 10.70 μl), HEMA (4.52 mmol, 550.00 μl), PMDETA (0.02 mmol, 5.00 μl) and 3 mL DMF were mixed into a 25 mL Schlenk flask. The solution was processed by three freeze-pump-thaw cycles, and then CuBr (0.01 mmol, 1.40 mg) was added into the reaction solution under a protection of argon flow. The polymerization was allowed to proceed under continuous stirring at room temperature and under argon atmosphere overnight. The reaction was terminated by exposure to air. The catalyst was removed by passing through silica gel. The obtained polymers were precipitated by diethyl ether and dried under vacuum overnight.

Synthesis of PSEMA followed the procedure described elsewhere.[1a,c] Briefly, PHEMA (3.85 mmol, 0.50 g) was modified with SA (7.70 mmol, 0.58 g) in anhydrous pyridine (2.00 mL)

solution for 24 h at 25 °C. For synthesis of PMEMA, PHEMA (3.85 mmol, 0.50 g) was treated with sodium hydride (5.78 mmol, 0.14 g) in 3 ml of anhydrous DMF, followed by a reaction with MA (7.70 mmol, 0.58 g) for 3 hours at 25 °C. PSEMA and PMEMA were purified by repeated precipitation in diethyl ether, and dried in vacuum. The molecular weights of PSEMA or PMEMA were ~ 10 kDa.

Fluorescently modified PSEMA (PSEMA).* Labeling of PSEMA with Alexa-488 to obtain PSEMA* was performed using a procedure similar to that previously reported for other polycarboxylic acids.[2] Briefly, solutions of 5 μL of 10 mg/mL PSEMA (M_w 10 kDa, PDI<1.20) (6.34×10^{-7} mol), 20 μL of 50 mg/mL EDC (5.22×10^{-6} mol) and 20 μL of 60 mg/mL of Sulfo-NHS (5.53×10^{-6} mol) were prepared in 0.1 M phosphate buffer at pH 5 , mixed and continuously stirred for 1 hour. Then, 25 μL of 10 mg/mL Alexa-488 in 0.1 M phosphate buffer at pH 5 buffer was added to the mixture. The reaction was stirred overnight and then diluted to 2 mL with 0.1 M phosphate buffer at pH 7. The solution was dialyzed against 0.01 M phosphate buffer at pH 7 also containing 0.1 M NaCl for 2 days, followed by dialysis against Milli-Q water for another 2 days. The molecular weight cutoff for the dialysis membrane was 2 KDa.

Self-assembly of polymer capsules. Polymer capsules were prepared by mixing PSEMA (or PMEMA) with PVP aqueous solutions at a neutral pH, followed by lowering the solution pH. For instance, to prepare capsules with a diameter of ~110 nm, PSEMA (or PMEMA) with M_w of 10 kDa was mixed with polyvinylpyrrolidone with M_w of 55 kDa (PVP55) solution at pH 6 to achieve final concentrations of PSEMA (or PMEMA) and PVP of 0.2 mg/mL and 0.4 mg/mL, respectively. The pH of the mixture solution was then gradually lowered to pH 3 by adding 0.1M HCl. To obtain different sizes of submicron- and micron-size capsules, different molecular weights of PVP were mixed with PSEMA (or PMEMA) solutions at a constant molar ratio of PVP-to-PSEMA (PMEMA) units of 4:1. In all mixture solutions, the concentration of PSEMA (or PMEMA) was maintained at 0.2 mg/mL.

Crosslinking of polymer capsules. In order to achieve stability of the capsules in a wide range of pH, the polymer capsules shells were crosslinked either via chemical or UV crosslinking. To that end, PMEMA capsules with a 100 nm size were first prepared by lowering pH of PMEMA (10 kDa) and PVP (55 kDa) solutions to 3 for 1h. Then, 0.01 v/v% of HMPPh was added to the capsule solution. The capsule solution was then loaded into a 6 mm diameter and 4 cm tall glass

vail, and the solution was exposed to Allanson 501B ultraviolet (UV) mercury grid lamp (120 V, 450 W) for 10 minutes to crosslink PMEMA capsule walls. The distance between sample and the source was ~ 10 cm. As the solution was stored in a glass vail, the wavelength to activate the photoinitiator from the mercury grid lamp was around 350 nm.

Nanoparticle encapsulation. The silica nanoparticles encapsulation by PVP55/PSEMA or PVP55/PMEMA was achieved by pre-mixing silica nanoparticles with PVP55/PSEMA or PVP55/PMEMA mixture solution at pH 7 solution first. The concentration of silica nanoparticles was 0.05 mg/mL, and the concentration of PSEMA or PMEMA was 0.2 mg/mL. Then, the solution pH was lowered to pH=3 using 0.1 M HCl. The pH-triggered release of silica nanoparticles from PVP55/PSEMA was achieved by increasing solution pH to 7. Stabilization of a pH-responsive coating of PVP55/PMEMA on silica nanoparticles was performed via UV-assisted crosslinking using the same procedure as described above for nanoparticle-free PVP55/PMEMA capsules. Prior to TEM characterization, nanoparticles coated with crosslinked films were exposed to solutions of pH 3 or 7 for 20 minutes.

Characterization Techniques

^{1}H NMR spectra. ^{1}H NMR spectra were recorded using a Varian Inova 400-MHz NMR spectrometer in D_2O or d_4-MeOH at room temperature.

Optical density measurements. Turbidity of polymer solutions was measured using UV−Vis spectrophotometer Lambda 25 (Perkin Elmer, USA) at a wavelength 400 nm after one hour of solution mixing.

Fourier transform infrared spectroscopy (FTIR). Bruker Tensor-27 spectrometer equipped with DTGS detector and OPUS 6.5 software was used for FTIR analysis. The total number of scans was 64 with 4 cm^{-1} resolution. The IR-transparent silicon wafers were cut into 1.50 ×1.50 cm^2 size with a Fletcher steel wheel glass cutter, exposed to UV for 2 hours and treated with concentrated sulfuric acid to clean the wafer surface. About 60 µl of capsule solutions were deposited on the wafer surface and dried prior to FTIR measurements. The ionization degree of PSEMA was determined by FTIR from the absorbances of the nonionized and ionized forms of the polyacids at 1730 cm^{-1} and 1570 cm^{-1}, which are associated with the stretch vibrations of the uncharged form of carboxylic groups (ν, C=O) and the asymmetric stretching vibrations of the carboxylate groups (ν_a, COO-), respectively. Assuming equal values for the extinction

coefficients of the 1730 and 1570 cm^{-1} PSEMA absorption bands (based on the data reported for poly(methacrylic acid) [3]), the ionization degree has been calculated as the ratio of absorbance of the 1570 cm^{-1} band to the sum of absorbances of the 1730 and 1570 cm^{-1} bands.

Electron energy loss spectroscopy (EELS). EELS data were collected using a FEI CM-20 FEG TEM/STEM (FEI Company) equipped with a Gatan Enfina EELS spectrometer. The microscope operated at 200 keV with an 0.8 eV energy resolution. Samples were cooled to -167 °C using the cryo-holder. Energy loss data were collected from spectral images with a 10 nm pixel size and a 2.5 s pixel dwell time with a 0.75 nm diameter probe.

Dynamic light scattering (DLS). The hydrodynamic sizes of crosslinked PVP55/PNEMA capsules were determined using Zetasizer Nano-ZS (Malvern Instruments, Ltd).

Thermogravimetric analysis (TGA). TGA was performed in a nitrogen gas flow using a Polymer Laboratories TG1000 instrument. The silica particles with the average diameter of 140 nm were encapsulated using the PVP55/PSEMA system at pH 3, and centrifuged after the addition of 1/3 volume of acetone at 13,000 rpm for 30 min. The supernatant solution was discarded, the precipitate rinsed with acetone and collected for TGA measurements. The samples were equilibrated at 110 °C for 20 min prior to measurements to remove remaining solvents. The TGA measurements were then taken between 50 °C and 500 °C at a heating rate of 15 °C min^{-1}.

Imaging techniques. SEM (Zeiss Auriga FIB-SEM with Leica VCT-100 cryo-system), TEM (FEI CM20 FEG S/TEM), CLSM (5 PASCAL laser scanning microscope, Zeiss, Germany), and AFM (NSCRIPTOR™ dip pen nanolithography system, Nanoink) were used to characterize capsules. For TEM imaging, capsules were deposited on 300 mesh Cu TEM grids covered with carbon support films. Cryo-SEM was used to study samples in the frozen hydrated state in order to analyze the native state of the capsules. Cryo-samples were prepared with a high pressure freezer (Leica EM HPM 100) to prevent structural damages during freezing. For cryo-imaging, samples were sublimated for 10 min at -105 °C and were coated with gold for 20 seconds, prior to imaging at -135 °C. TEM was operated at 200 kV and images were collected at both 8,800 X and 20,000 X magnifications, at the defocus of -2 μm. CLSM fluorescence images of the capsule in solution were obtained using a C-Apochromat 60X/1.4 oil immersion objective. For imaging of PVP10/PSEMA* capsules, the fluorophores were excited by a laser at λ=488 nm, and emission was collected after filtering with a BP 500-560 pass filter. To image Nile-Red-stained

PVP10/PSEMA capsules, the fluorophores were excited by a laser at λ=543 nm, and emission was collected after filtering with a LP560 pass filter. For AFM characterization, samples were deposited on Si wafers and on 300 mesh Cu TEM grids covered with carbon support films in the case of uncrosslinked and crosslinked capsules, respectively. The instrument operated in the AC (tapping) mode using Aspire CT130 conical soft tapping mode AFM probes with a nominal force constant of 6 N/m.

Supplementary Figures

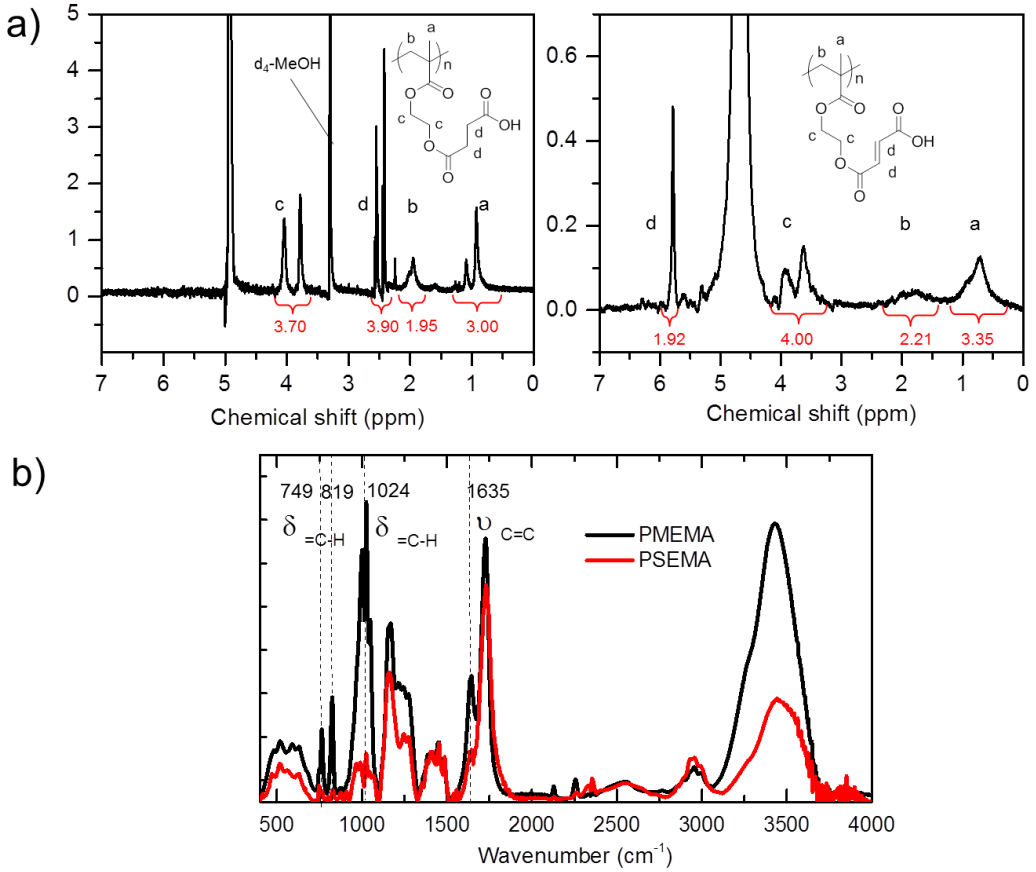

Figure S1. Polymer characterization: (a) ^{1}H-NMR of 5 mg/mL solutions of PSEMA and PMEMA (left and right, respectively). PSEMA was dissolved in d_4-MeOH, and PMEMA ─ in D_2O at pH 8. (b) FTIR spectra of PSEMA and PMEMA was collected using the KBr pellet method.

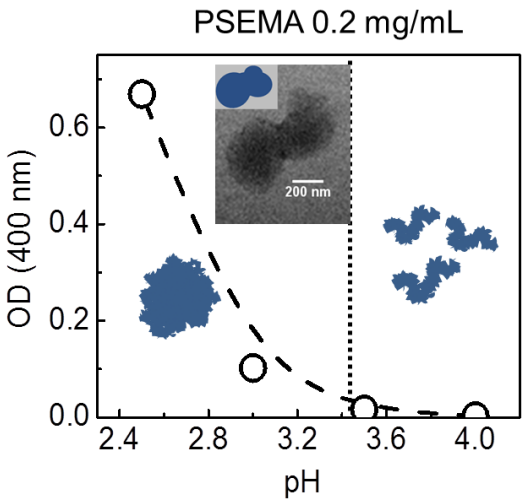

Figure S2. Turbidity developed in 0.2 mg/mL PSEMA solutions after a one-hour exposure to acidic media. Inset shows a representative TEM image of phase-separating polymer-rich droplets, and the dotted line separates phase separation and molecular solubility regions.

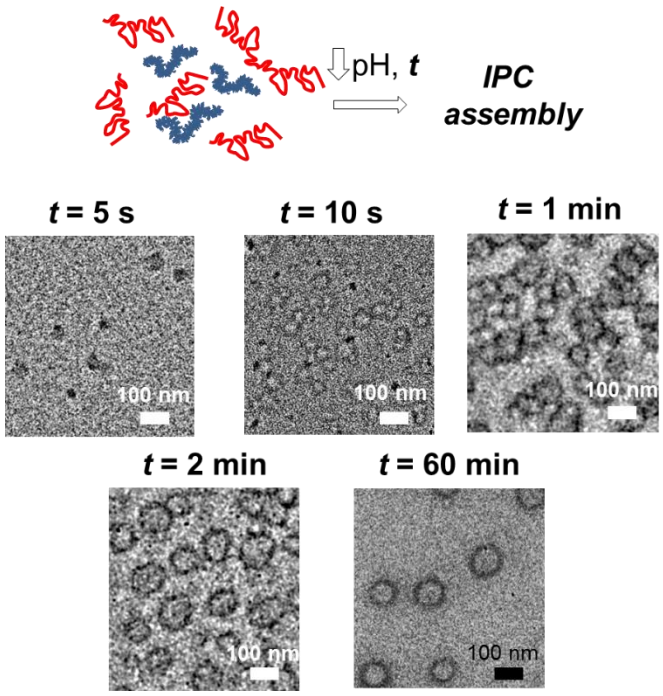

Figure S3. Schematics and representative TEM images of the capsule size evolution in PVP55/PSEMA solutions, where *t* denotes the capsule assembly time after lowering the solution pH from 7 to 3.

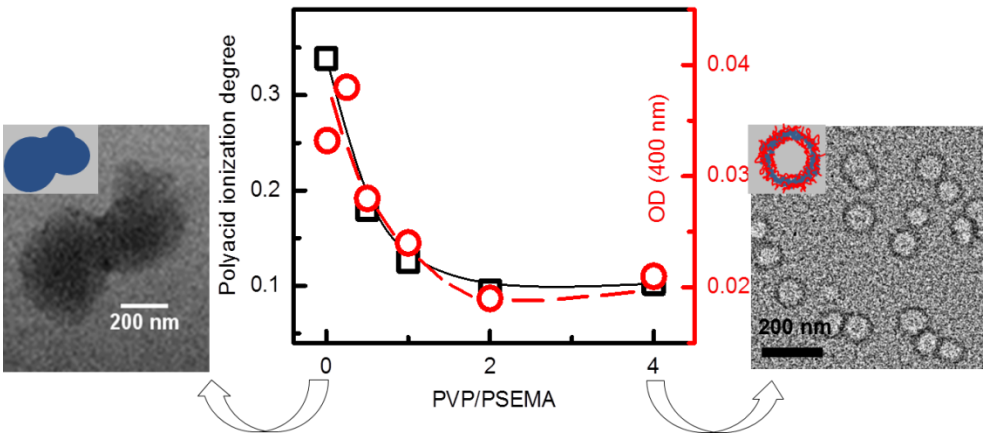

Figure S4. Center: Ionization degree of PSEMA (black squares) and optical density (red circles) measured in PVP55/PSEMA solutions with different molar ratios of polymer units one hour after adjusting the solution acidity to pH 3. TEM images illustrate morphology of PVP55/PSEMA mixtures with molar unit ratios 0:1 and 4:1 (left and right, respectively).

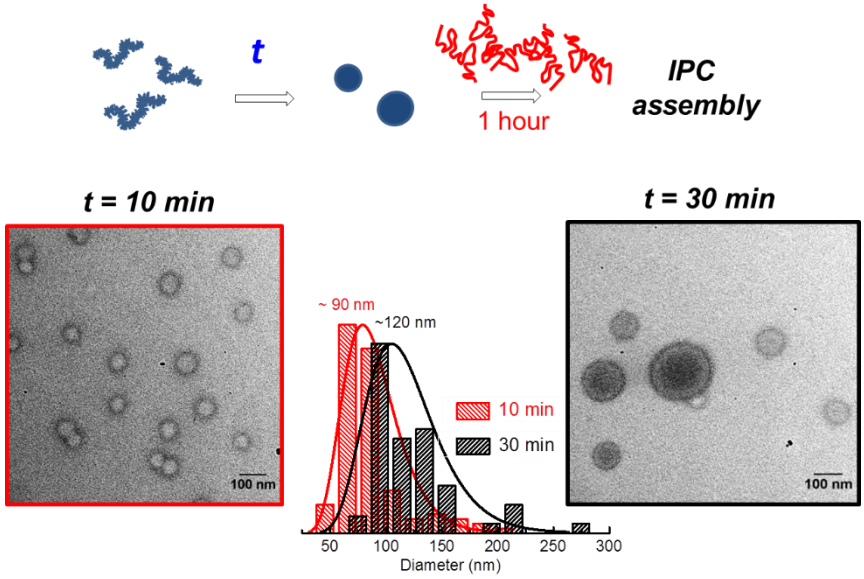

Figure S5. Schematics of control experiments and representative TEM images and size histograms of assembled structures emerging in PVP55/PSEMA solutions upon delayed addition of PVP when PSEMA was allowed to phase separate in the absence of PVP for 10 and 30 min at pH 3.

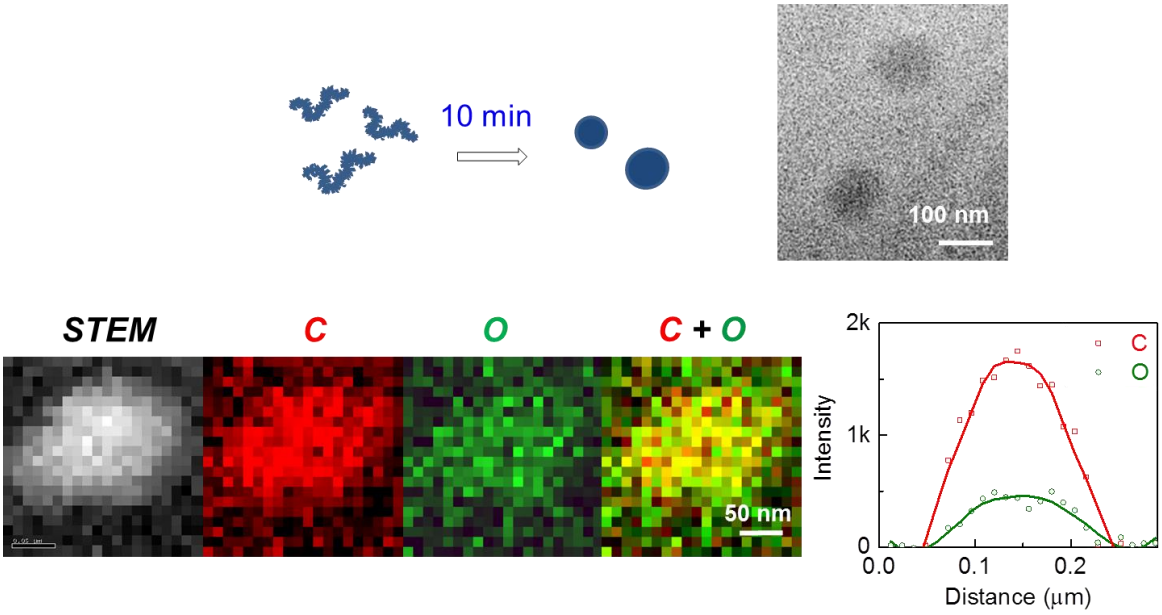

Figure S6. Top: Schematics of control experiments and representative TEM images of structures emerging in 0.2 mg/mL PSEMA solutions after a 10-min exposure to pH 3. Bottom: EELS mapping of C, O in phase-separating PSEMA particles, analyzed by the rotational averaging with respect with the particle center (bottom right).

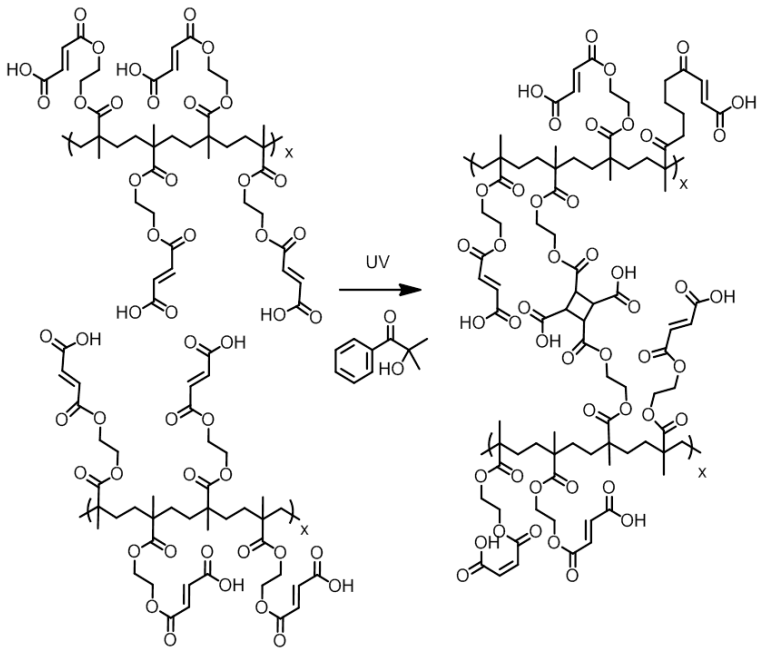

Figure S7. Schematic illustration of UV-induced crosslinking between PMEMA units.

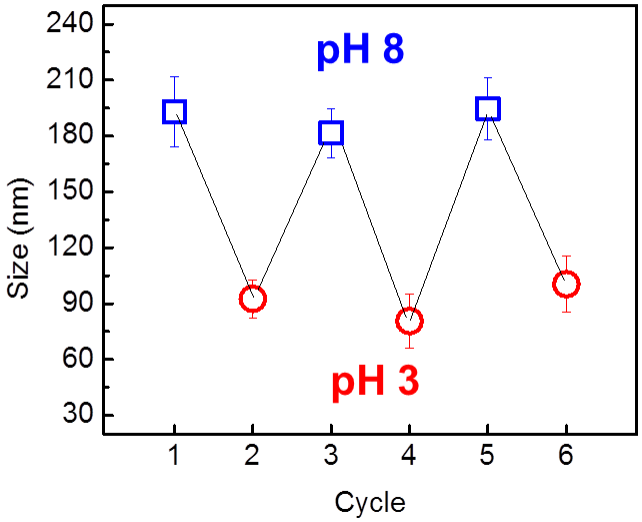

Figure S8. Reversible pH-triggered changes in the hydrodynamic diameter of photocrosslinked PVP55/PMEMA capsules as measured by DLS.

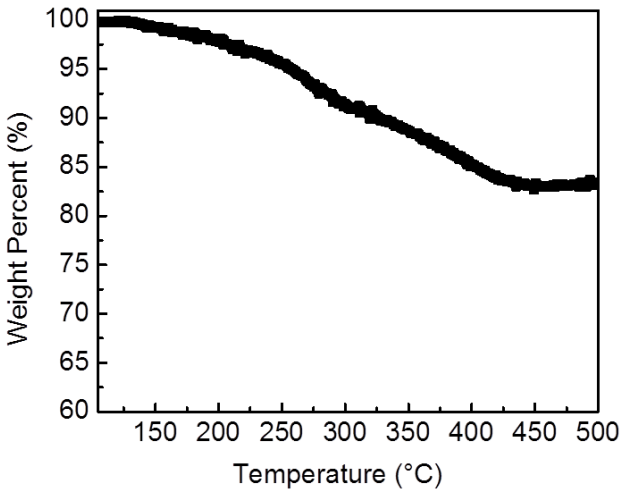

Figure S9. TGA of PVP55/PSEMA-coated silica particles. The particles with a diameter of 140 nm were encapsulated at pH 3.

References

1. (a) Shi, Y.; Selin, V.; Wang, Y.; Sukhishvili, S. A., Multiresponsive Block Copolymer-Modified "Hairy" Gold Nanoparticles for Remote Control of Interfaces. *Particle & Particle Systems Characterization* **2013,** *30* (11), 950-957; (b) Beers, K. L.; Boo, S.; Gaynor, S. G.; Matyjaszewski, K., Atom Transfer Radical Polymerization of 2-Hydroxyethyl Methacrylate. *Macromolecules* **1999,** *32* (18), 5772-5776; (c) Bories-Azeau, X.; Mérian, T.; Weaver, J. V. M.; Armes, S. P.; van den Haak, H. J. W., Synthesis of Near-Monodisperse Acidic Homopolymers and Block Copolymers from Hydroxylated Methacrylic Copolymers Using Succinic Anhydride under Mild Conditions. *Macromolecules* **2004**, *37* (24), 8903-8910.

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3. (a) Kharlampieva, E.; Sukhishvili, S. A., Ionization and pH Stability of Multilayers Formed by Self-Assembly of Weak Polyelectrolytes. *Langmuir* **2003,** *19* (4), 1235-1243; (b) Sukhishvili, S. A.; Granick, S., Layered, Erasable Polymer Multilayers Formed by Hydrogen-Bonded Sequential Self-Assembly. *Macromolecules* **2002,** *35* (1), 301-310; (c) Xie, A. F.; Granick, S., Local Electrostatics within a Polyelectrolyte Multilayer with Embedded Weak Polyelectrolyte. *Macromolecules* **2002,** *35* (5), 1805-1813.