Electronic Supporting Information

Functional Polymeric Molecules for Performing Autonomous Synthesis of Particles with Core-Shell Structures and Customizable Shapes

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1. Materials and Methods

Poly(L-lactide) (PLA) (a high number average molecular weight, M_n, of 59,000 and ester terminated or a low number average molecular weight, M_n, of 5,000 and ester terminated), poly(D, L-lactide-*co*-glycolide) (PLGA) (weight average molecular weight, M_w, of 76,000 to 115,000 and ester terminated), polystyrene (PS) (carboxylic acid-terminated with weight average molecular weight, M_w, of 200,000 or CH_3 terminated with weight average molecular weight, M_w, of 192,000), poly(acrylic acid) (PAA) (viscosity average molecular weight, M_v, of 450,000), brilliant blue G (BBG), paracetamol, chloroform, dimethyl sulfoxide (DMSO), oil red O, deuterated methanol, potassium dihydrogen phosphate, trimethylamine, phosphoric acid, acetonitrile, and iron oxide powder (max. particle size 50 μm) were purchased from Sigma Aldrich. Sodium hydroxide (NaOH) was purchased from VWR. UV-curable adhesive (Norland, NOA 64) was purchased from Norland Products. 184 Silicon Elastomer was purchased from Dow Corning for preparing the polydimethylsiloxane (PDMS).

Preparing the template with different shapes. For fabricating the templates with cavities of different shapes, objects of the complementary shapes were first prepared by either of the two methods: 3D printing or photolithography. For 3D printing, objects of different geometries were first drawn using a computer program (i.e., AutoCAD) and were then printed by a 3D printer (i.e., Form 2, FormLab) using a photopolymer resin. For photolithography, a mold was first assembled. The mold consisted of two slabs of PDMS (0.5 mm in height, 20 mm in length, and 3 mm in width) that were both placed flat on a surface (i.e., PDMS with the dimension of 22 mm in width, 50 mm in length, and 8 mm in thickness). These two slabs of PDMS were placed at a distance of 15 mm horizontally apart and arranged in parallel. They served as spacers for creating the space in the mold. A piece of glass was placed over the top of the two spacers. Hence, a space was created in the middle of the assembly by the bottom

surface, the two spacers placed horizontally apart, and the piece of glass on top. A UV-curable liquid monomer (Norland, NOA 64) was filled into the space. A photomask designed with the desired patterns was placed on top of the piece of glass. Finally, the whole assembly was secured by clamping the two ends of the long side of the structure. The liquid monomer was cured by applying UV light (Omnicure S2000) with a wavelength of 365 nm and an intensity of 10 mW cm^{-2} through the photomask and the piece of glass for about 30 s. After polymerization, the assembly was taken apart and the unpolymerized regions were washed away by ethanol and acetone. The polymerized objects of the desired shapes were thus obtained.

These objects were attached to the bottom of a Petri dish. The PDMS pre-polymer liquid and curing agent were first mixed in a weight ratio of 20:1, and then poured into the Petri dish until the liquid covered the objects. It was cured by placing it in an oven operated at 80 °C for 3 h. The cured piece of PDMS was separated from the Petri dish and the objects. This piece of PDMS was the template with the cavities of the desired shapes.

Preparing the core-shell particles of different shapes. For fabricating the core-shell particles of different shapes, it first required the preparation of a mixture of an organic phase and an aqueous phase. In one type of experiments, the organic phase used was a 7.5 wt% of PLA (number average molecular weight of 59,000) dissolved in chloroform. The aqueous phase consisted of BBG dye (1 mM) dissolved in deionized water. 0.2 mL of the organic phase was mixed vigorously with 0.1 mL of the aqueous phase by vortexing the mixture for 2 – 5 min. The PDMS template with cavities of the desired shapes was prepared as described in the previous section. The temperature of the PDMS template was lowered by placing it on top of a piece of ice. The liquid mixture was then injected into the cavities of the PDMS template;

the lowered temperature was needed for reducing the rate of evaporation of chloroform. Excessive liquid on the top of the template was removed with a blade. The liquid in the cavity was then simply left undisturbed overnight (or at least for 4 h). The core-shell particles formed spontaneously with time within the cavities. After formation, the particles were obtained by extracting them from the PDMS template.

This procedure was repeated for different types of core-shell particles that had different compositions. One of the experiments involved using an aqueous phase that was a basic aqueous solution at pH 11 (i.e., without the BBG dye). The organic phase remained the same. The two phases were then mixed and added into the cavities for preparing the core-shell particles.

In other sets of experiments, the PLA was replaced with either PLGA or PS (carboxylic acid-terminated with weight average molecular weight of 200,000). Two types of experiments were performed for each type of polymer. One of them involved an aqueous phase that consisted of 1 mM of BBG solution, whereas the other involved a basic aqueous phase at pH 11. Both of them involved an organic phase that consisted of 7.5 wt% of the polymer dissolved in chloroform. Besides the difference in chemical compositions of the two types of liquid mixtures, the rest of the procedure for preparing the core-shell particles of different shapes was the same for both of these types of polymers used.

Investigating the formation of core-shell particles using polymers with different properties. For investigating the effect of the molecular weight of the polymer on the formation of the core-shell particle, PLA with a number average molecular weight of either 5,000 or 59,000 was used. The organic phase was 7.5 wt% of PLA (number average molecular weight of either 5,000 or 59,000) dissolved in chloroform. The aqueous phase

consisted of a basic solution of NaOH at pH 11. 0.2 mL of the organic phase was mixed vigorously with 0.1 mL of the aqueous phase by a vortex mixer for 2 – 5 min. The liquid mixture was then placed either into a cubic PDMS template or the flat surface of PDMS.

For investigating the hydrophobicity or hydrophilicity of the polymeric molecules on the formation of the core-shell particle, either PLA (number average molecular weight of 59,000) or PAA (viscosity average molecular weight, M_v, of 450,000) was used. The experiment involved adding 24 mg of either PLA or PAA (i.e., 7.5 wt%), 0.2 mL of chloroform, and 0.1 mL of the aqueous pH 11 solution into a vial. The mixture was mixed vigorously by a vortex mixer for 2 – 5 min. The liquid mixture was then placed either into a PDMS template with cubic cavities or the surface of PDMS.

Investigating the stability of the emulsion under different conditions. One experiment involved examining the stability of the emulsion by varying the pH of the aqueous solutions. For this experiment, the organic phase was 7.5 wt% of PLA (number average molecular weight of 59,000) dissolved in chloroform. The aqueous phase consisted of a solution of NaOH that had pH of either 10, 11, 12, or 13. 0.2 mL of the organic phase was mixed vigorously with 0.1 mL of the aqueous phase by a vortex mixer for 2 – 5 min. After mixing, the liquid mixture was left undisturbed. Images of the states of the emulsion were taken at times 10 s, 120 s, and 600 s.

Another experiment involved examining the stability of the emulsion by using polymeric molecules with different terminal groups. The organic phase was 7.5 wt% of PS (either CH_3 terminated with weight average molecular weight of 192,000 or carboxylic acid-terminated with weight average molecular weight of 200,000) dissolved in chloroform. The aqueous phase consisted of a solution of NaOH at pH 11. 0.2 mL of the organic phase was

mixed vigorously with 0.1 mL of the aqueous phase by a vortex mixer for 2 – 5 min. The stability of the emulsion was observed with time.

Quantifying the proportion of solid versus liquid of the particles. For this analysis, a relatively large cubic core-shell particle (~2 mm in length) was first fabricated. After preparing the particle, it was placed on a high-resolution balance for ~4 h. The liquid in the core gradually evaporated into the surrounding atmosphere with time. The loss in weight of the particle was monitored by the balance (Fig. S3).

Determining that the emulsion was water-in-oil. The liquid mixture used for preparing the emulsion consisted of 0.2 mL of PLA (number average molecular weight of 59,000) dissolved in chloroform (7.5 wt%) as the organic phase and 0.1 mL of BBG dissolved in deionized water as the aqueous phase (1 mM). The mixture was mixed vigorously by a vortex mixer for 5 min. 10 μL of this liquid mixture was extracted from the emulsion and added into a vial filled with 0.5 mL of deionized water. Observations showed that the entire volume of the emulsion added was found to be immiscible with the deionized water and sank to the bottom of the tube. This result showed that the mixture was composed of water-in-oil emulsion: the continuous organic phase did not mix with the water. If the emulsion were oil-in-water, the continuous water phase would have mixed with the water in the tube and resulted in a much smaller volume of the immiscible phase that sank to the bottom.

Tensile stress-strain curve of the polymeric shell. First, a relatively large core-shell block (48 mm × 8 mm × 2 mm) was prepared as described in a previous section. The organic phase

was 7.5 wt% of PLA (number average molecular weight of 59,000) dissolved in chloroform. The aqueous phase was BBG dye (1 mM) dissolved in deionized water. The mixture was added into the PDMS template with the cavities of the corresponding sizes. After formation of the core-shell block, it was cut open. By cutting out one of the flat surfaces of the block, a flat sheet of the polymer was obtained. It was then trimmed (i.e., the sides) to remove any uneven surface, rinsed with deionized water, and dried. The final dimension of the sheet of polymer was 15 mm × 6 mm × 0.15 mm. The tensile stress measurements were done at a strain rate of 5 mm/min using a mechanical tester (Instron Tensile Tester, Instron 5542).

Demonstrating that the organic phase formed a layer on top of the aqueous phase. A 2 mL glass bottle was first half filled with distilled water. 10 μL of chloroform stained with a red organic dye (i.e., oil red O) for visualizing the effect clearly was placed on the surface of the distilled water. The layer of chloroform was observed to stay on top of the distilled water. The experiment was repeated with different combinations of the substances used. In the first case, the experiment was repeated by placing the pure 10 μL chloroform without staining it with the oil red O on the surface of the distilled water. In the second case, the experiment was repeated by placing the pure 10 μL chloroform without staining it with the oil red O on the surface of a 1 mM of BBG aqueous solution (i.e., instead of distilled water). In all these experiments, the chloroform was observed to stay on top of the aqueous phase.

Studying the controlled release of the core-shell particles. Cylindrical (2 mm in diameter and height) core-shell particles were used for studying the controlled release. The core-shell particles loaded with the dye, BBG, were prepared as described in a previous section. For monitoring the release of BBG in real time, one particle was placed in a cuvette. The cuvette

was then filled with water. The top of the cuvette was covered to reduce the evaporation of water. The release of BBG from the particle into the water with time was monitored by a UV-vis spectrophotometer (Shimadzu UV-3600). The experiment was performed in triplicate. In addition, this experiment for studying the controlled release of BBG from the core-shell particle was repeated by using phosphate buffer solution (0.01 M, pH 7.4) instead of water as the liquid medium.

For studying the release of paracetamol, the core-shell particles loaded with paracetamol was first prepared. In this case, the organic phase was 7.5 wt% PLA (number average molecular weight of 59,000) in chloroform. The organic phase was mixed with an aqueous basic solution (pH 11) containing 1 mM of paracetamol. This liquid mixture was then added into the cavities of a PDMS template of the desired shapes for preparing the core-shell particles as described previously. Cylindrical (2 mm in diameter and height) core-shell particles were used for studying the controlled release. The particles loaded with paracetamol were immersed in 40 mL of deionized water. Samples were collected at regular time intervals and analyzed by High Performance Liquid Chromatography (HPLC, Shimadzu UV-3600). HPLC separation was performed in an Agilent Eclipse Plus C18 column (4.6 mm × 150 mm, 3.5 μm). The flow rate was 1.0 mL/min and the injection volume was 10 μL. The elution time of paracetamol was at 5.5 min. Sharp peaks were detected at the wavelength of 515 nm. Two mobile phases were used. The first mobile phase was a pH 4.0 buffer composed of 0.013 M potassium dihydrogen phosphate, 0.01 M trimethylamine, and phosphoric acid. The second mobile phase was acetonitrile. The experiment was performed in triplicate.

Targeting by shape specificity. Disk-shaped (diameter: 1.6 mm and height: 0.8 mm) and rod-shaped (diameter: 0.7 mm and height: 4 mm) core-shell particles were first prepared. In

this case, the core-shell particles loaded with the dye, BBG, were fabricated in the same way as previously described except that the aqueous phase also contained 10 wt% iron oxide particles. Two types of PDMS substrates (diameter: 36.7 mm and height: 2 mm) were made as illustrated in Fig. 5 of the main text. One of them contained only disk-shaped cavities (diameter: 1.65 mm and depth: 0.8 mm). The other contained both disk-shaped (diameter: 1.65 mm and depth: 0.8 mm) and rectangular cavities (width: 0.7 mm, length: 4 mm, and depth: 1 mm). These cavities were designed to resemble a tree pattern. The PDMS substrate was treated by air plasma and fixed to the bottom of a 50 mL beaker containing 25 mL water with the cavities facing up. The air plasma made the PDMS hydrophilic and allowed it to be immersed in water without air bubbles attached to it easily. For the PDMS substrate that contained only the disk-shaped cavities, one disk-shaped particle was added to the beaker. For the PDMS substrate that contained both the disk-shaped and rectangular cavities, five disk-shaped particles and five rod-shaped particles were added into the beaker. The beaker was then agitated. Specifically, the axial axis of the cylinder was first tilted to around 30° to 45° to the vertical. After tilting, the agitation involved repeated cycles of linear agitation and rotation. The linear agitation was performed along the axial axis of the cylindrical beaker at a frequency of 6 Hz and an amplitude of ~1 cm for 10 seconds. The beaker was then rotated slowly clockwise for 45° in 3 – 5 s. This repeated cycles of linear agitation and rotation were continued until the particle(s) moved into the cavity(ies) of the PDMS substrate.

Magnetic resonance imaging (MRI). Two types of cubic PLA core-shell particles (dimensions: 3.0 × 3.0 × 3.0 mm) loaded with BBG were first prepared as previously described. One type contained ~10 wt% of iron oxide particles, whereas the other type did not contain any iron oxide particles. These particles were embedded in agarose gel for imaging by MRI. The agarose gel is a medium widely used in MRI for mimicking the tissue

in a human body; in addition, it allowed the core-shell particles to be fixed in place during imaging.

The procedure involved first adhering two cubic core-shell particles that contained the iron oxide particles onto the inner bottom surface of a Petri dish (diameter: 30 mm). In a separate step, a 1.0 wt% agarose gel was prepared by mixing 4 g of the agarose gel with deionized water into a solution with a total mass of 400 g. After mixing the solution vigorously, it was placed in a microwave oven until the solution boiled. Within 1 – 2 min after the solution boiled, the Petri dish that contained the two cubic core-shell particles was immersed carefully into the liquid solution until it was placed at the bottom of the beaker using a pair of tweezers. The 1 L beaker was then placed in a fridge at 4 °C for solidifying the agarose gel. After the agarose gel solidified, the core-shell particles were thus embedded in the gel and fixed in place. For the control experiment, the procedure was repeated except that three core-shell particles that did not contain any iron oxide particles were used instead of the particles that contained iron oxide. The cubic core-shell particles were analysed by MRI using an isotropic proton density spin-echo sequence (TR, 2000 ms; TE, 14 ms; flip angle, 150°; slice thickness, 0.4 mm; FOV, 140 mm; matrix, 384 × 384) on a 3.0 T MRI system (Siemens 3T Prisma, Erlanger, Germany).

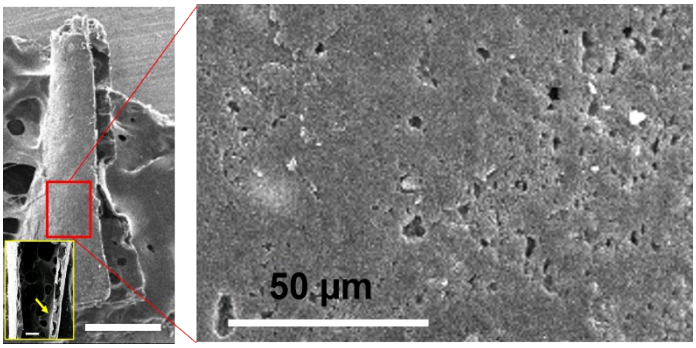

Fig. S1 SEM images of the cone-shaped core-shell particle after it was cut open. The scale bar for the image on the left is 500 µm. The inset shows that the thickness of the shell was ~50 µm. The scale bar in the inset is 50 µm. The image on the right shows that the polymeric shell was continuously solid.

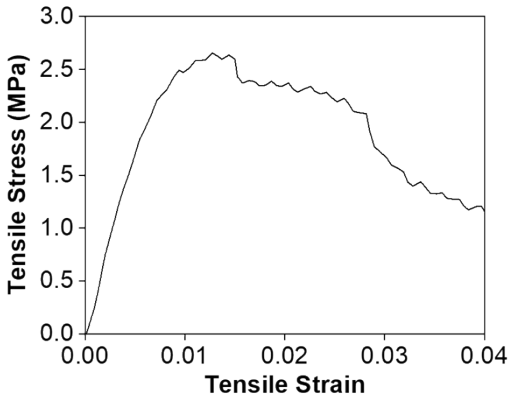

Fig. S2 Tensile stress-strain curve of the polymeric (i.e., polylactic acid, PLA) shell of the core-shell particle. The analysis showed that the Young's modulus of the polymeric shell was ~400 MPa.

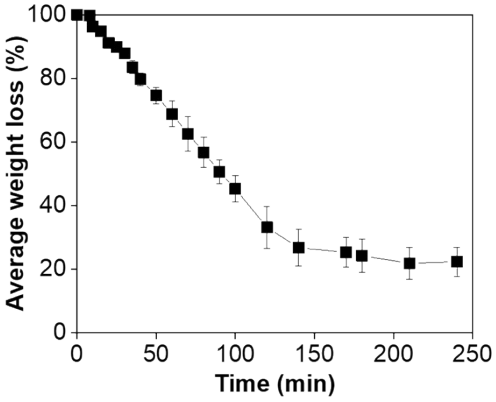

Fig. S3 Determining the proportion of liquid in the core-shell particle by evaporation and weighing the particle. After complete evaporation of the liquid with time, we determined that the polymeric shell was ~20 wt% and the liquid was ~80 wt%.

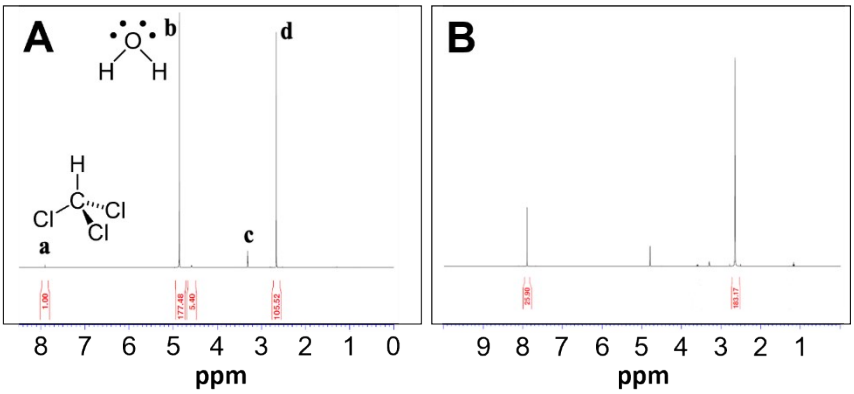

Fig. S4 ^{1}H-NMR analysis of the aqueous solution loaded in the core of the particle. The analysis of (A) the solution loaded in the core of the particle and (B) a control sample that consisted of an equal molar ratio of chloroform and DMSO in deuterated methanol. The peaks at 7.906 and 2.660 ppm were due to chloroform and DMSO (i.e., the reference) respectively. The peaks at 4.804 ppm and in the range of 3.3 to 3.6 ppm were due to water and deuterated methanol (i.e., the solvent) respectively.

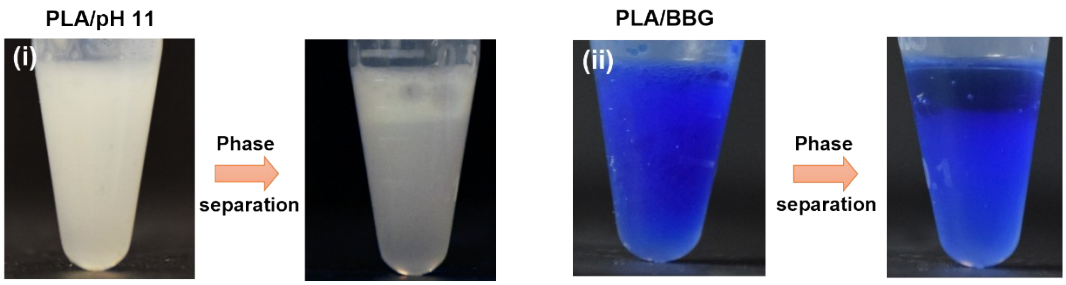

Fig. S5 Molecular self-assembly for aggregating the molecules via the temporarily stable emulsion. (i) The first experiment involved mixing the solutions of PLA in chloroform and aqueous basic (pH 11) vigorously. (ii) The second experiment involved mixing the solutions of PLA in chloroform and aqueous BBG solution. For both these experiments, the solutions were initially observed to be stable. After leaving the mixture undisturbed for some time (i.e., minutes to hours), the liquid separated into two phases. These results indicated that the emulsions were temporarily stable.

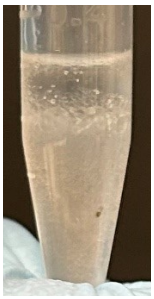

Fig. S6 Emulsion prepared using 0.1 M NaCl aqueous solution and 7.5 wt% PLA in chloroform. Phase separation occurred instantaneously after mixing.

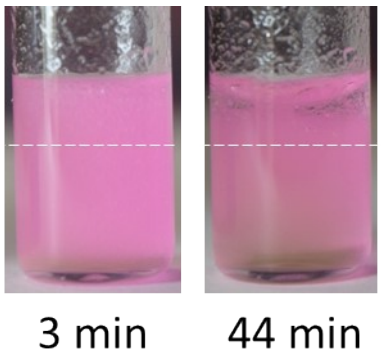

Fig. S7 Emulsion prepared using 1 mM rhodamine B in water and 7.5 wt% PLA in chloroform. Temporarily stable emulsion was achieved. Phase separation occurred only after 40 min of mixing.

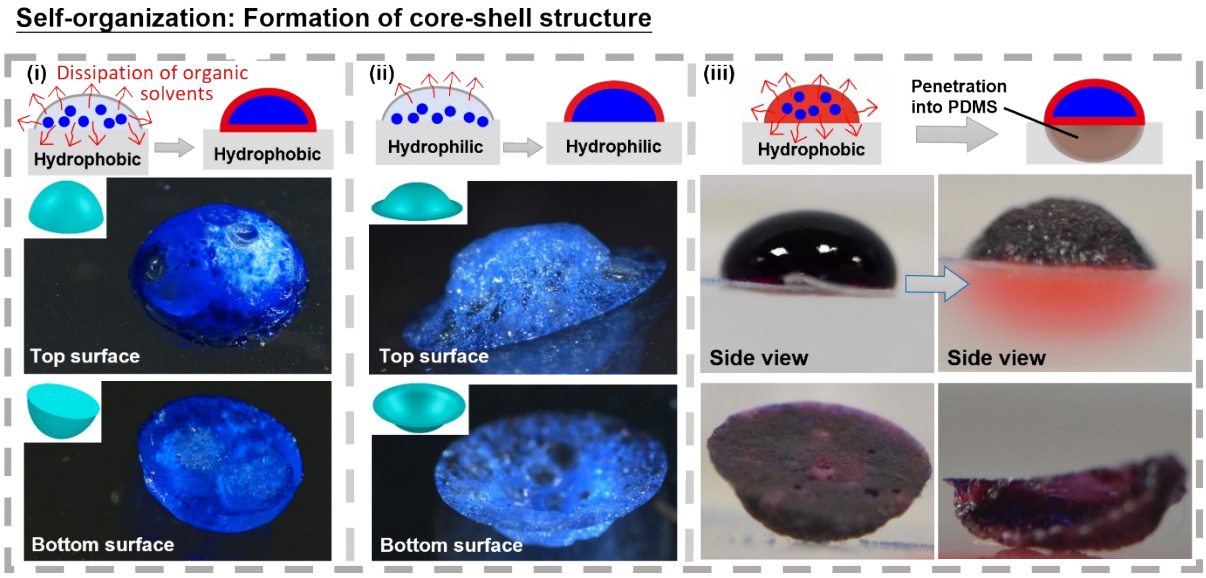

Fig. S8 Molecular self-organization for forming the spatially distinct core and shell structures. This set of experiments involved placing a droplet of the solution onto a flat surface for analyzing the formation of the core-shell structure clearly. (i) The first experiment involved placing a drop of the liquid that consisted of PLA in chloroform and BBG in water on the flat surface of PDMS. The liquid transformed into the full core-shell structure, including the top

of the liquid exposed to air (image labeled as “Top surface”) and bottom in contact with PDMS (image labeled as “Bottom surface”). (ii) The second experiment involved placing the same drop of liquid as (i) on a flat hydrophilic glass slide instead of the hydrophobic PDMS. In this case, the polymeric layer formed at the top (image labeled as “Top surface”) but did not form at the bottom in contact with the glass slide (image labeled as “Bottom surface”). Hydrophobicity of the template was thus needed. (iii) The third experiment involved placing a drop of the same liquid with an additional hydrophobic red dye in the organic phase on the flat surface of PDMS. Penetration of the red organic phase into the PDMS polymer was observed (image on the top right labeled as “Side view”). Image on the bottom left showed the polymeric shell after detaching from the surface. After cutting the structure into half, liquid flowed out from the core; the remaining material clearly showed a full core-shell structure (image on the bottom right).

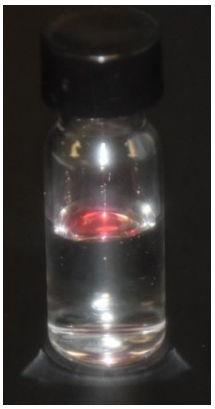

Fig. S9 Chloroform (stained with a red dye) sat on top of the surface of distilled water due to surface tension effects. Chloroform has a much higher density (~1.5 g cm^{-3} at room temperature) than water (~1.0 g cm^{-3} at room temperature) and sinks in water quickly. In this experiment, we gently placed a small droplet of 10 μL of chloroform (stained with a red dye for visualization) on the surface of distilled water in a vial. In this case, the chloroform was found to stay on top of the surface of water at equilibrium as shown in this experimental image. We repeated the experiment using chloroform without staining it with dye and placing it on top of distilled water. We also repeated the experiment using chloroform without staining it with dye and placing it on top of a 1 mM BBG aqueous solution. For all these cases, we observed that the chloroform stayed on top of the aqueous phase instead of sinking to the bottom of the vial due to its high density. These results showed clearly that due to the high surface tension of the aqueous phase, it is favorable for the chloroform to be sandwiched between the air and the aqueous phase for achieving a more energetically favorable state. This effect naturally allowed the self-organization of the organic phase onto the top of the liquid mixture in the cavity for forming the top polymeric shell of the core-shell particle.

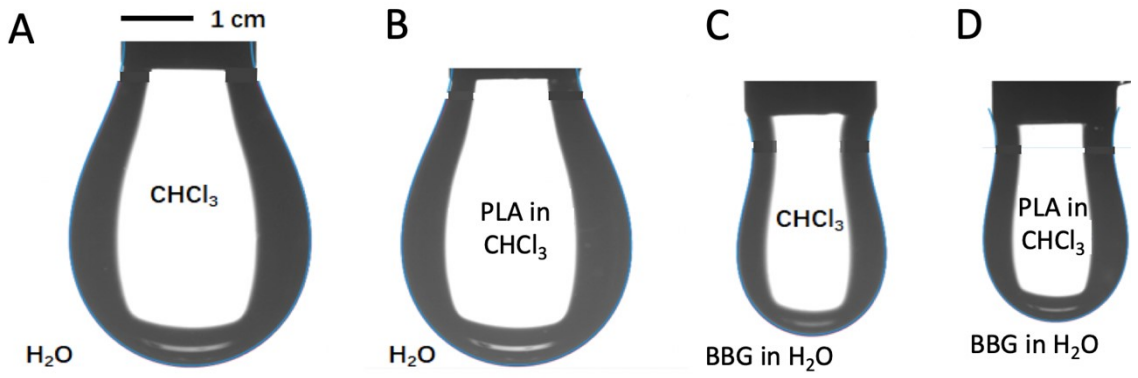

Fig. S10 Pendant drop method for measuring interfacial tension using a goniometer (DSA100). An organic droplet was injected slowly via a syringe into an immiscible aqueous phase. The injection was controlled by a computer connected to the goniometer. The organic droplet was injected vertically downward into the aqueous phase. Because the density of the organic phase was higher than that of the aqueous phase, the organic droplet detached from the tip of the needle and fell downward when it reached a critical size. The equipment then automatically provided the measurement of the interfacial tension based on the shape of the pendant droplet. Each experiment was performed 7 times. Four combinations of liquids were tested. (A) The first combination consisted of chloroform as the organic phase and deionized water as the aqueous phase. (B) The second combination consisted of 5 wt% of PLA in chloroform as the organic phase and deionized water as the aqueous phase. (C) The third combination consisted of chloroform as the organic phase and 0.2 mM of BBG in deionized water as the aqueous phase. (D) The fourth combination consisted of 5 wt% of PLA in chloroform as the organic phase and 0.2 mM of BBG in deionized water as the aqueous phase. The sizes of the droplets were significantly smaller when BBG was included in the aqueous phase; hence, the interfacial tension between the organic and aqueous phases was reduced when BBG was present.

Table S1 Interfacial tension between the different types of mixtures of organic and aqueous phases.

Mixture	**Interfacial Tension [mN m^{-1}]**
Chloroform – Water	28.96 ± 0.20
PLA in Chloroform – Water	27.27 ± 0.37
Chloroform – BBG solution	8.36 ± 0.46
PLA in Chloroform – BBG solution	7.80 ± 0.18

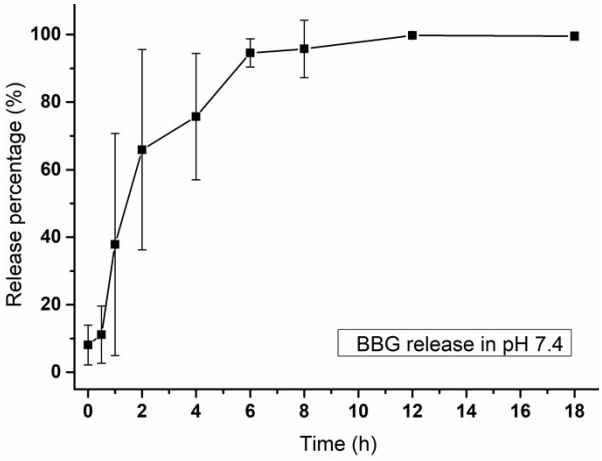

Fig. S11 Sustained release of BBG from core-shell particle in a phosphate buffer solution (0.01 M, pH 7.4).