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Supporting Information

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Chemically Orthogonal Three-Patch Microparticles**

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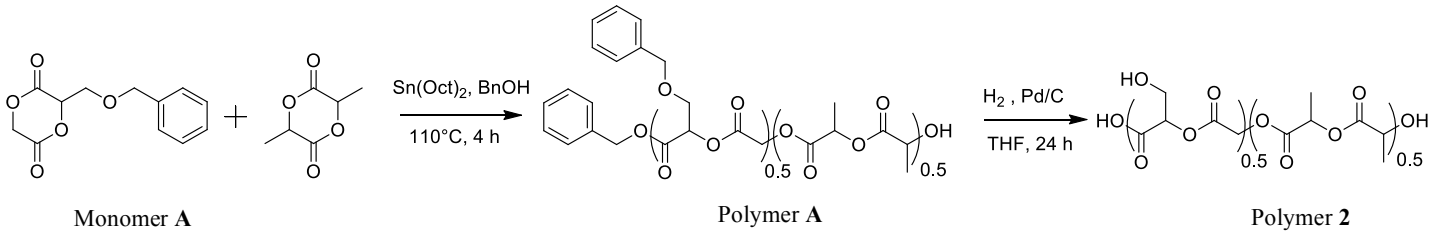
Supplemental Section 1: Materials

O-benzyl-L-serine was purchased from Alfa Aesar and diethyl ether was purchased from Acros Organics. Triethyl amine, bromoacetyl bromide, dimethyl amino pyridine, benzyl alcohol, l-lactide, tin (II) ethyl hexanoate, anhydrous toluene, palladium 10% wt. on activated carbon, anhydrous tetrahydrofuran (THF), 4-methylbenzophenone, carbon tetrachloride, N-Bromosuccinimide, benzoyl peroxide, sodium cyanide, hydrochloric acid, tetraethylammonium chloride, palladium acetate, diphenylphosphine, 1-methyl-2-aminoterephthalate, potassium iodide sodium sulfite, sodium sulfate, sodium chloride, sodium methoxide, dimethylsulfoxide, ethyl acetate, magnesium sulfate, silver perchlorate, methyl glycolate, potassium *tert* butoxide, bromoform, hexane, cycloheptene, poly(lactide-co-glycolide) (PLGA) 85:15, chloroform, dimethylformamide (DMF), MEHPV (polymeric dye), methanol, Tween 20, phosphate buffered saline (PBS), and *N*-(3-Dimethylaminopropyl)-*N*'-ehtylcarbodiimide hydrochloride (EDC) were purchased from Sigma-Aldrich, USA. Sulfuric acid, acetonitrile, sodium nitrite, dichloromethane, sodium carbonate, acetone, Optimal Cutting Temperature (OCT) compound, and *N*-(hydroxysulfosuccinimide) (Sulfo-NHS) were purchased from Fisher Scientific, USA. Hydrogen was provided by Cryogenic Gases. Amine-PEG-FITC molecular weight 5 kDa, azide-PEG-FITC molecular weight 3.4 kDa, amine-PEG-Rhodamine molecular weight 3.4 kDa, and carboxyl-PEG-rhodamine molecular weight 3.4 kDa were purchased from Nanocs, USA. Albumin from Bovine Serum (BSA), Tetramethylrhodamine conjugate, TRITC-Streptavidin™, and Alexa Fluor ® 647 Streptavidin™ were purchased from Life Technologies-Invitrogen, USA. Amine-dPEG23-biotin molecular weight 1.3 kDa and azide-dPEG47-biotin molecular weight 2.4 kDa were purchased from Quanta Biodesign, Ltd., USA.

Supplemental Section 2.1: Polymerization, deprotection, and characterization of polymer **2**



Monomer **A** Polymer **A** Polymer **2**

Scheme 1: Synthesis of the hydroxylated poly-lactide copolymer, polymer **2**.

Monomer A Synthesis:

Monomer **A** has been synthesized using the literature reported procedure.[1]

Random copolymer synthesis:

Ring-opening co-polymerizations of monomer **A** and l-lactide were performed in the melt using benzyl alcohol (BnOH) and $Sn(Oct)_2$ as the initiator and catalyst, respectively. Briefly, monomer **A** (4.56 g, 19.3 mmol) and recrystallized l-lactide (2.78 g, 19.3 mmol) were placed in an oven dried Schlenk flask equipped with a small stirring bar under a dry argon atmosphere. BnOH (10.5 mg, 0.1 mmol) and catalyst $Sn(Oct)_2$ (39.6 mg, 0.1 mmol) were added by an argon purged dry syringe, and the flask was kept under vacuum for 2 hours. The flask was then closed and immersed in an oil bath thermostated at 110 °C for 4 hours. The resulting polymer **A** was dissolved in dichloromethane, precipitated in cold methanol, and dried in vacuum. Polymerizations proceeded in good yield (90%).

^{1}H NMR: ($CDCl_3$) δ: 1.5-1.7 (m, 6H), 3.8-4.0 (m, 2H), 4.5-4.6 (m, 2H), 4.6-5.0 (m, 2H), 5.1-5.3 (m, 2H), 5.4-5.5 (m, 1H), 7.2-7.4 (m, 5H).

Deprotection of Polymer A to yield Polymer 2 has been done using literature reported procedure.[1] The yield of the final polymer **2** was around 80%. Found Mn: 46333; Mw: 52883; PDI: 1.14; (Molecular weight has been determined by GPC using polystyrene as calibration standard).

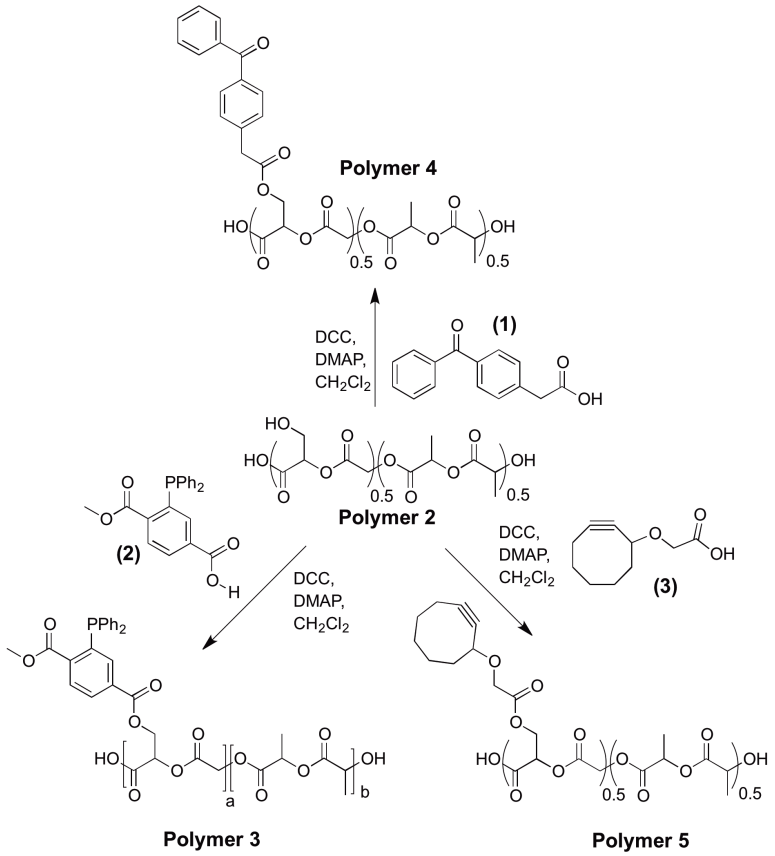
Polymer 2 characterizations:

^{1}H NMR: ($CDCl_3$) δ: 1.5-1.7 (m, 6H), 3.8-4.0 (m, 2H), 4.6-5.0 (m, 2H), 5.1-5.3 (m, 2H), 5.4-5.5 (m, 1H)

FT-IR: ν = [1100, 1300, 1190, 1200, 1260, 1350, 1380, 1400, 1450, 1770, 2920, and 2980 cm^{-1}].

Raman: ν = [1440, 1750, 2876, 2900, 2943, 3000 cm^{-1}].

Supplemental Section 2.2: Functionalization and characterization of polymers **3**, **4**, and **5**



Scheme 2: Synthesis of poly-lactide derivatives.

Functionalized poly-lactide derivative 3: Compound **2** (3-(diphenylphosphino)-4-(methoxycarbonyl) benzoic acid) was synthesized according to the literature reported protocol.[2] 56 mg of compound **2** was dissolved in 1 ml of dry dichloromethane under dry argon atmosphere. Then 42 mg of DCC (dicyclohexyl carbodiimide) and 12.5 mg of DMAP (N,N dimethyl amino pyridine) was added to this solution. The solution was stirred for 10 min, then 15 mg of polymer **2** was added under argon and stirred for overnight at RT. Next, all the solvents were evaporated to dryness. Then the resulting polymer **3** was dissolved in minimal amount of dichloromethane, precipitated in cold methanol, and dried in vacuum. Functionalization proceeded in good yield at 90% conversion. This polymer is air sensitive and after work up we found that around 20% of triphenyl phosphine functionality of polymer **3** was converted to its phosphine oxide analogues. Hence, we stored the polymer in oxygen free atmosphere (vacuum dessicator) for future use. We have used this mixture of polymers (70% polymer **3**, 20% phosphine oxide analogue of polymer **3** and 10% polymer **2**) for subsequent study.

Polymer 3 characterization:

^{1}H NMR: ($CDCl_3$) δ 1.42-1.67 (bs, 6H), 3.62-3.82 (s, 3H), 4.42-4.97 (m, 4H), 5.02-5.3 (m, 2H), 5.38-5.58 (bs, 1H), 7.18-7.78 (m, 13H).

^{31}P NMR: ($CDCl_3$) δ: -4.3 ppm

FT-IR: ν = [1100, 1200, 1180, 1260, 1280, 1350, 1380, 1430, 1580, 1730, 1760, 2940, and 2980 cm^{-1}].

Raman: ν = [1430, 1596, 1732, 2896, 2900, 2943, 2977, 3057 cm^{-1}].

Functionalized poly-lactide derivative 4: Compound **1** (2-(4-benzoylphenyl) acetic acid) was synthesized according to the literature reported protocol.[3] 35 mg of compound **1** was dissolved in 1 ml of dry dichloromethane under argon atmosphere. Then 42 mg of DCC and 12.5 mg of DMAP was added to this solution. The solution was stirred for 10 min, then 15 mg of polymer **2** was added under argon and stirred for overnight at RT. All solvents were evaporated to dryness, then the resulting polymer **4** was dissolved in minimal amount of dichloromethane, precipitated in cold methanol, and dried in vacuum. Functionalization proceeded in good yield at 100% conversion.

Polymer 4 characterization:

^{1}H NMR: ($CDCl_3$) δ 1.42-1.71 (bs, 6H), 4.58-4.97 (m, 4H), 5.05-5.25 (m, 2H), 5.5-5.7 (bs, 1H), 7.39-7.55 (bs, 2H), 7.55-7.65 (bs, 1H), 7.70-7.9 (bs, 4H), 8.05-8.25 (bs, 2H).

FT-IR: ν = [1090, 1120, 1180, 1270, 1300, 1350, 1380, 1450, 1580, 1660, 1760, 2930, and 2980 cm^{-1}].

Raman: ν = [1440, 1610, 1660, 1730, 2865, 2943, 2983, 3067 cm^{-1}].

Functionalized poly-lactide derivative 5: Compound **3** (2-(cyclooct-2-yn-l-yloxy) acetic acid) has been synthesized according to the literature reported protocol.[4] 28 mg of compound **3** was dissolved in 1 ml of dry dichloromethane under dry argon atmosphere. Then 42 mg of DCC and 12.5 mg of DMAP was added to this solution. The solution was stirred for 10 min, then 15 mg of polymer **2** was added under argon and stirred for overnight at RT. All solvents were evaporated to dryness, then the resulting polymer **5** was dissolved in minimal amount of dichloromethane, precipitated in cold methanol, and dried in vacuum. Functionalization proceeded in good yield at around 90% conversion.

Polymer 5 characterization:

^{1}H NMR ($CDCl_3$) δ 0.9-1.4 (m, 8H), 1.45-1.95 (m, 4H), 1.95-2.1 (m, 1H), 2.1-2.2 (m, 2H), 2.2-2.3 (m, 1H), 4.05-4.25 (m, 2H), 4.33 – 4.42 (m, 1H), 4.5-4.95 (m, 4H), 5.11-5.25 (m, 2H), 5.4-5.6 (m, 1H).

FT-IR: ν = [1100, 1130, 1190, 1200, 1240, 1350, 1380, 1400, 1450, 1770, 2920, and 2980 cm^{-1}].

Raman: ν = [1440, 1750, 2200, 2852, 2876, 2943, 2973 cm^{-1}].

Supplemental Section 2.3: Fabrication of multicompartmental microparticles through EHD co-jetting, the microparticles' surface modifications & characterization

Microparticles fabrication and characterization

Microparticles were fabricated as described previously by Bhaskar, *et al.*[5] The jetting solutions were composed of PLGA 85:15 at 30% w/v in a 97:03 ratio of chloroform to DMF. For microparticles containing polymers **1**-**5**, the functional polymers were added as an additive at 25% weight of the PLGA 85:15, with an exception to the polymer **5** used in **Figures 4**. In this case, the polymer **5** used had a molecular weight of approximately 70 kDa and was used as 25% w/w of the PLGA 85:15 and not as an additive. To fabricate the fibers, the polymer solutions were flown through syringes tipped with 26 gauge metal needles at 0.03-0.05 ml/hr. The EHD conditions were a distance of 5-10 cm, RT, and a voltage of 10-12 kV. A rotating cylinder wrapped with aluminum foil and coated with OCT was used as the collector. Once jetted, the collector was placed in vacuumed to dry the fibers and then embedded in a mold filled with OCT for cryosectioning.[6] A HISTO-550 Cryostat at the Microscopy & Imaging Laboratory facilities at the University of Michigan was used to section the embedded fibers at 5 μm. Once sectioned, the microparticles were washed several times with deionized (DI) water and 1% Tween 20 to remove the excess OCT. The microparticles were filtered with 40 and 10 μm filters to remove larger aggregates before use. The microparticle's characterizations were done with CLSM (Confocal Laser Scanning Microscopy) using an Olympus Confocal Microscope and an Amray SEM (Scanning Electron Microscopy) at Microscopy & Imaging Laboratory facilities at the University of Michigan.

Surface modification of microparticles:

For EDC/Sulfo-NHS chemistry with polymer **1**, microparticles were incubated with 0.33 mg of EDC in 0.33 ml of buffer for 10 min, followed with 10 minutes incubation with 0.03 mg of sulfo-NHS in 0.33 ml of buffer to activate the carboxyl groups. Once activated, 1 mg of amine-PEG-Rhodamine, 0.22 mg of amine-PEG-biotin, or 1 mg of amine-PEG-FITC in 0.33 ml of buffer were added to the solution and incubated for 2 hours. The microparticles were then washed numerous times to remove unreacted material. Microparticles reacted with amine-PEG-biotin were further incubated with 100 μg of Alexa Fluor ® 647 Streptavidin™ in 1 ml of buffer for 3 hours to label the biotin before being washed and imaged with CLSM. The buffer used was PBS with 1% v/v Tween 20 and all reactions were done at RT on a rotator.

For EDC/Sulfo-NHS chemistry with polymer **2**, 0.33 mg of EDC in 0.33 ml of buffer was added to 1-1.5 mg of carboxyl-PEG-Rhodamine for 10 min, followed with 10 minutes incubation with 0.033 mg of sulfo-NHS in 0.33 ml of buffer to activate the carboxyl groups. Once activated, the microparticles containing polymer **2** in 0.33 ml of buffer were added to the mixture and incubated for 2-3 hours. The microparticles were washed numerous times before imaging with CLSM. The buffer used was PBS with 1% v/v Tween 20 and all reactions were done at RT on a rotator.

For Staudinger ligation with polymer **3**, microparticles were incubated overnight with 20 mg of azide-PEG-biotin in 1 ml of buffer. The microparticles were then washed numerous times to remove unreacted material. Once washed, the microparticles were incubated with 100 μg of TRITC-Streptavidin™ to label the biotin before washing again to remove unreacted material and imaging with CLSM. The buffer used was DI water with 1% v/v Tween 20 and all reactions were done at RT on a rotator.

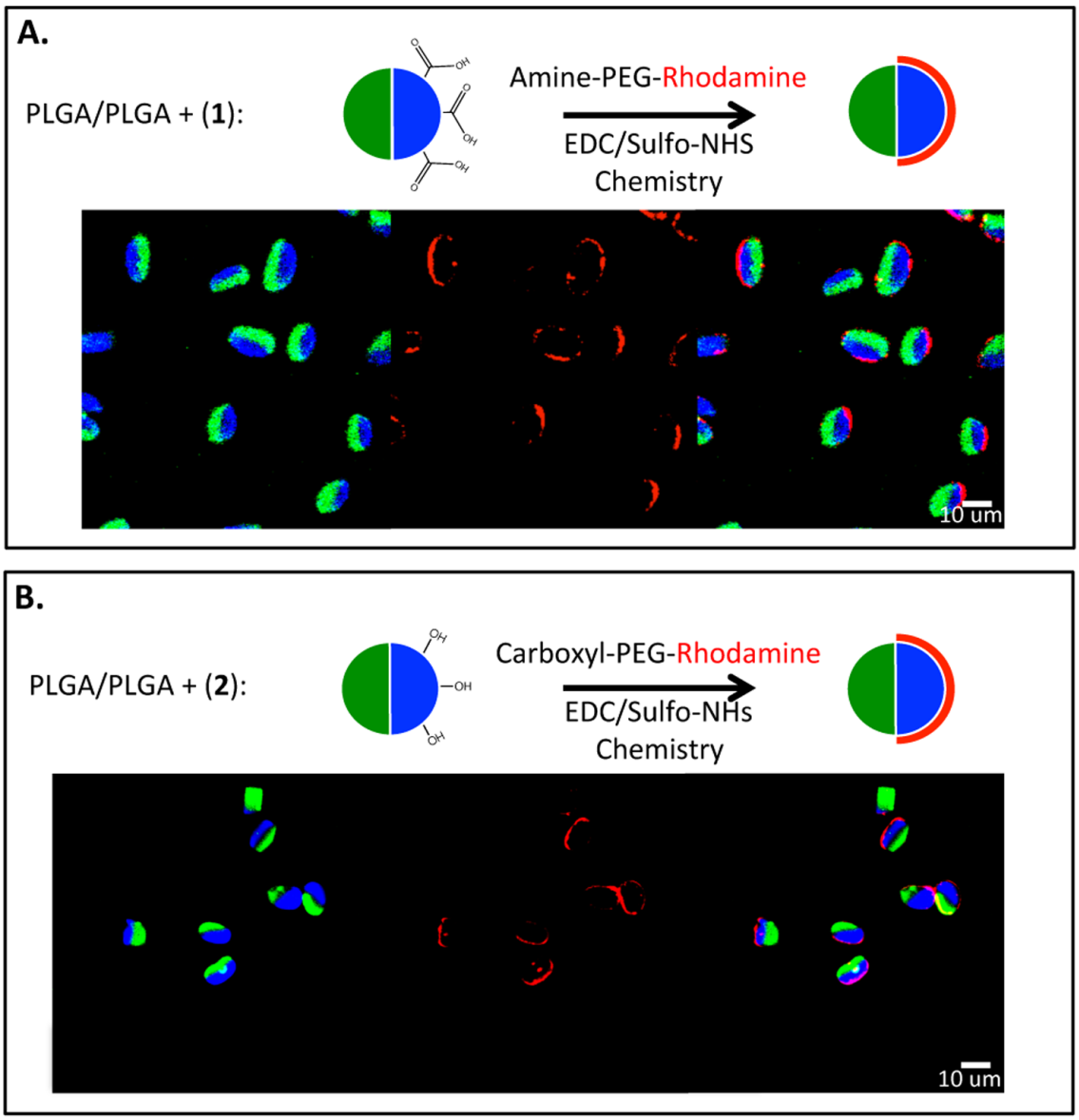
For photoreactive chemistry with polymer **4**, the microparticles were incubated with 0.4-1 mg of BSA-tetramethylrhodamine conjugate in 1 ml of buffer for 1.5-2.5 hours before UV'ing at 365 nm for 30 minutes with a handheld UV lamp from Ultra Violet Products,

Ltd. The microparticles were then washed to remove unreacted material before imaging via CLSM. The buffer used was PBS with 1% v/v Tween 20 and all reactions were done at RT.

For copper free click chemistry with polymer **5**, microparticles were incubated overnight with 20 mg of azide-PEG-biotin or 10 mg of azide-PEG-FITC in 1 ml of buffer. The microparticles were then washed numerous times to remove unreacted material. Microparticles reacted with azide-PEG-biotin were incubated with 100 μg of Alexa Fluor ® 647 Streptavidin^{TM} to label the biotin. The microparticles were washed again to remove unreacted material before imaging with CLSM. The buffer used was DI water with 1% v/v Tween 20 and all reactions were done at RT on a rotator.

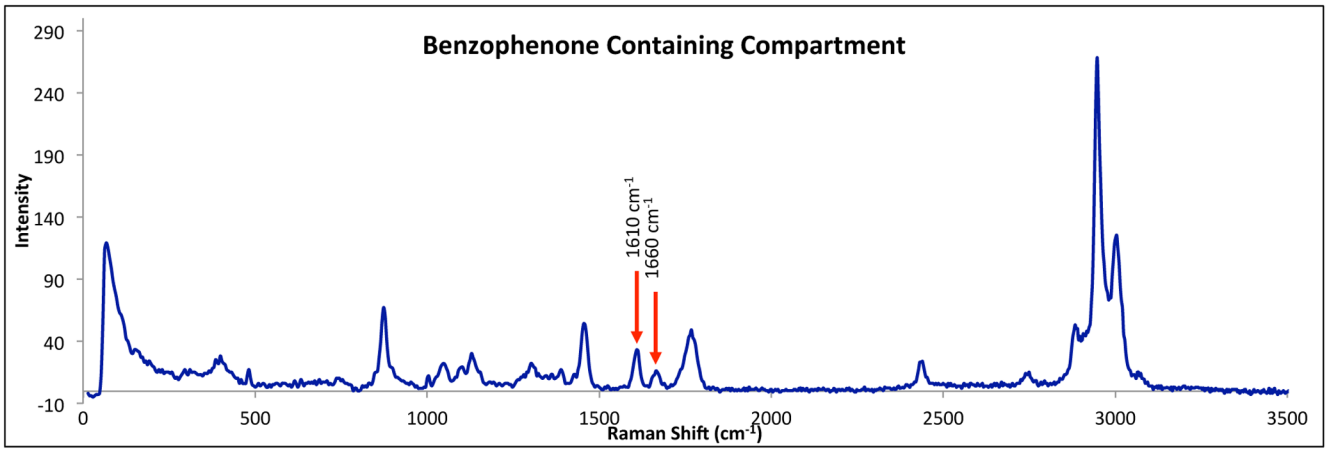
For Raman-Confocal imaging, the microparticles were lyophilized to remove excess water, deposited on glass slides, and characterized via the Raman-Confocal microscope using a 532 nm laser and an integration time of 0.5 seconds.

Supplemental Section 2.4: Selective surface functionalization of microparticles containing either polymers **1** or **2**



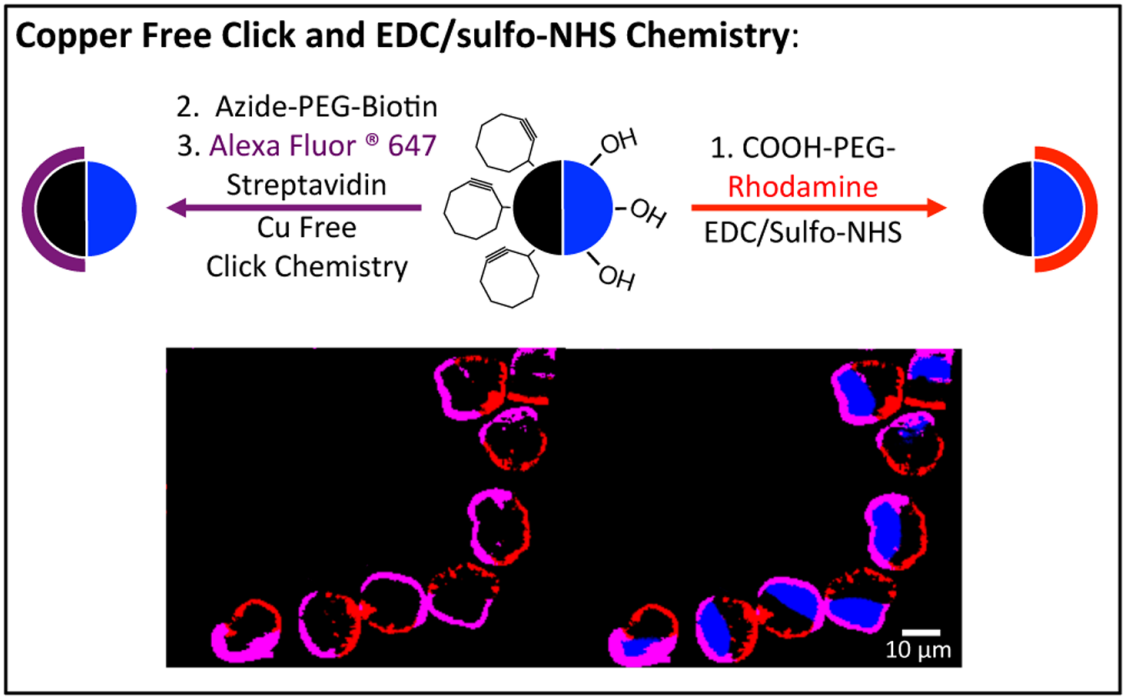
Supplemental Figure 1: *The selective surface modification of bicompartmental microparticles containing polymer **1** or **2** in one hemisphere. In **S.1A**, the microparticles contain polymer **1** in the blue patch and PLGA in the green patch. The carboxyl groups in polymer **2** are used to conjugate amine-PEG-Rhodamine (red) to the surface of one hemisphere using EDC/Sulfo-NHS chemistry. In **S.1B**, the microparticles contained polymer **2** in the blue hemisphere and PLGA in the green hemisphere. The hydroxyl groups in polymer **2** were used to attach carboxyl-PEG-Rhodamine using EDC/Sulfo-NHS chemistry.*

Supplemental Section 2.5: Full Raman spectra of polymer **4** containing compartment.



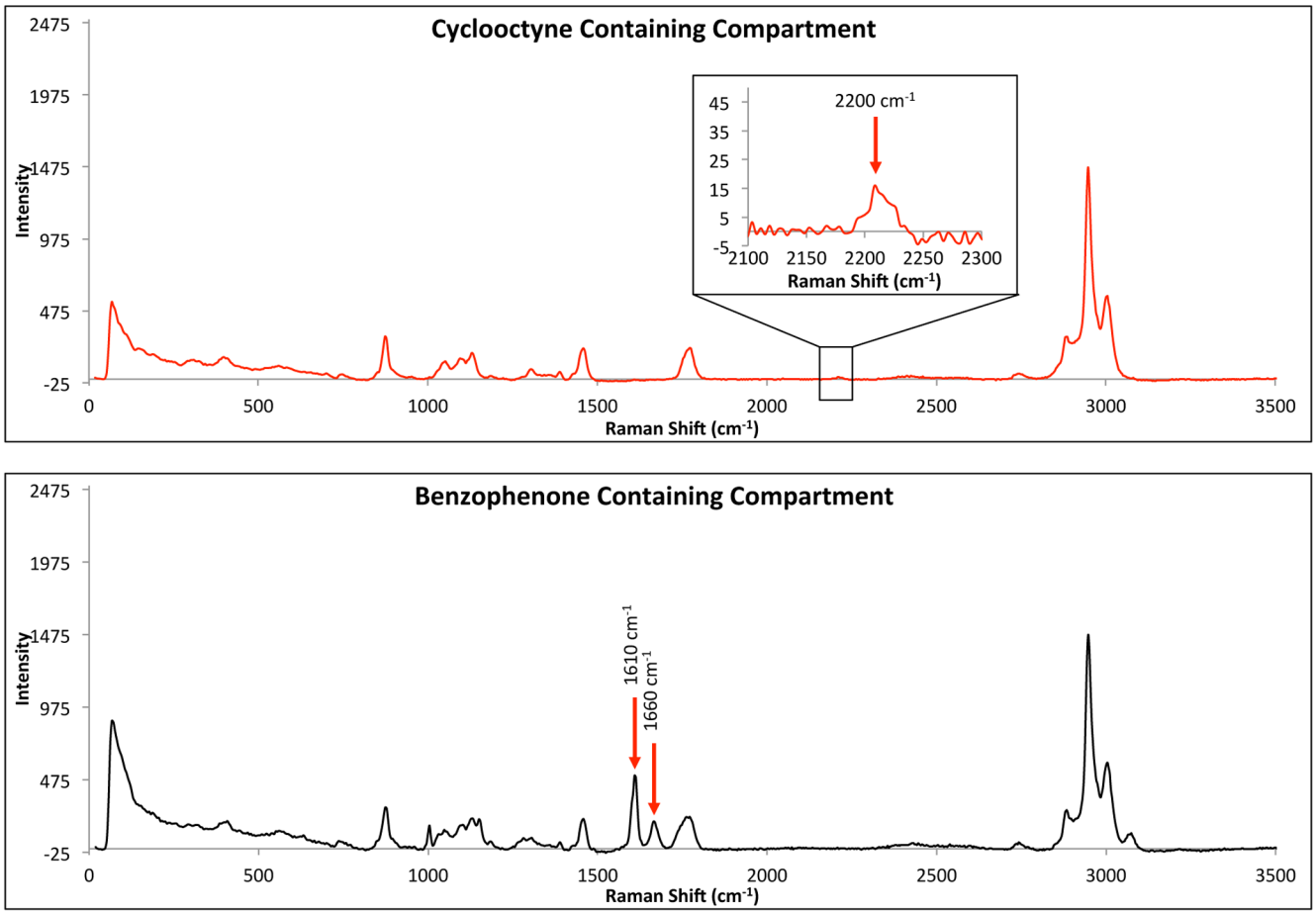
Supplemental Figure 2: *The full Raman spectra of the compartment containing polymer **4** and PLGA. Here, the 1610 and 1660 nm signals (shown with red arrows) signify the benzophenone functional groups present in this polymer.*

Supplemental Section 2.6: Selective surface modification of microparticles containing polymer **2** and **5.**



Supplemental Figure 3: *The selective surface modification of bicompartmental microparticles containing polymer* ***2*** *and* ***5*** *in separate hemispheres. Here, the blue compartment contains polymer* ***2*** *and the black compartment (no dyes) contains polymer* ***5****. The surface of the blue patch was first reacted with cooh-PEG-Rhodamine through EDC/Sulfo-NHS chemistry, followed by the click reaction on the other hemisphere using the azide-PEG-biotin and the cyclooctynes present on the surface. The biotins were then labeled with Alexa Fluor ® 647 Streptavidin for imaging purposes.*

Supplemental Section 2.7: Full Raman spectra of polymer **4** and **5** containing compartments



Supplemental Figure 4: *The full Raman spectra of the compartments containing polymer* ***4*** *or* ***5*** *in tricompartmental microparticles in* ***Figure 4.*** *Here, the top spectra is of the compartment containing polymer* ***5*** *and PLGA, with the appropriate signals at 2200 cm^{-1} for cyclooctyne, and the bottom spectra is of the compartment containing polymer* ***4*** *and PLGA, with the peaks at 1610 and 1660 cm^{-1} for benzophenone.*

References:

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