

Supporting Information

Backbone-Degradable Polymers Prepared by Chemical Vapor Deposition

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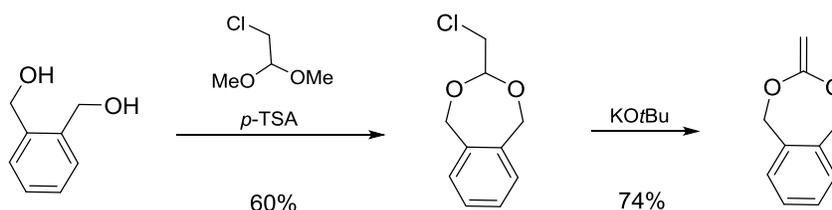
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Experimental Section

CVD precursors

The non-functionalized [2.2]paracyclophane (PCP-N) was purchased from *Parylene Coatings Services Inc.*. 4-Hydroxy-methyl[2.2]paracyclophane (PCP-CH₂OH) and 4-ethynyl[2.2]paracyclophane (PCP-alkyne) were synthesized as reported previously.^[1-2] 5,6-Benzo-2-methylene-1,3-dioxepane (BMDO) was synthesized according to literature-known procedures with slight modifications.^[3-5]

Synthesis of BMDO



Scheme S1. Synthesis of 5,6-benzo-2-methylene-1,3-dioxepane (BMDO). The product was synthesized via a slightly modified literature-known procedure.

Synthesis of 2-(chloromethyl)-5,6-benzo-1,3-dioxepane

1,2-Benzendimethanol (16.3 g, 118 mmol), chloroacetaldehyde dimethyl acetal (14.0 mL, 122 mmol) and *p*-toluenesulfonic acid (105 mg, 0.60 mmol) were dissolved in diglyme (33 mL) and the flask was equipped with a 3-way 75° bend distillation head adapter and a flask to collect the generated methanol. The mixture was heated to 150 °C and stirred for 24 hours until no more methanol was collected. After cooling to room temperature the reaction solution was poured into hexane (200 mL). The precipitated product was isolated. After washing with hexane (3 x 80 mL) the product was obtained as a white solid (14.2 g, 60%). – ¹H-NMR (500 MHz, CDCl₃): 7.29–7.20 (m, 4H, aromatic), 5.12 (t, *J* = 5.1 Hz, 1H, OCH), 4.98 (d, *J* = 14.5 Hz, 2H, OCH₂), 4.95 (d, *J* = 14.5 Hz, 2H, OCH₂), 3.63 (d, *J* = 5.1 Hz, 2H, CH₂Cl) ppm. – ¹³C-NMR (125 MHz, CDCl₃): 138.5, 127.8, 127.5, 106.2, 72.0, 43.9 ppm. Spectroscopic data is in agreement with the literature.^[3-5]

Synthesis of 5,6-benzo-2-methylene-1, 3-dioxepane

Under argon, 2-(Chloromethyl)-5,6-benzo-1,3-dioxepane (11.2 g, 56.1 mmol) and potassium *t*-butoxide (7.74 g, 69.0 mmol) were dissolved in tetrahydrofuran (125 mL) and the mixture was heated to 50 °C. After 72 hours the suspension was cooled to room temperature and the solvent was removed under reduced pressure. Next, diethyl ether (350 mL) was added and the solid was filtered. After removal of the solvent, the crude product was purified by vacuum distillation (0.001 bar, 62 °C) to yield the product as a colorless liquid that crystallized upon standing at room temperature (6.76 g, 74%). – **¹H-NMR** (500 MHz, CDCl₃): 7.29–7.26 (m, 2H, aromatic), 7.13–7.10 (m, 2H, aromatic), 5.09 (s, 4H, OCH₂), 3.75 (s, 2H, CH₂) ppm. – **¹³C-NMR** (125 MHz, CDCl₃): 164.2, 135.8, 127.4, 126.2, 72.1, 69.5 ppm. The spectroscopic data are in agreement with the literature.^[3-5]

CVD polymerization

The degradable polymers were synthesized by feeding a selected [2.2]paracyclophane derivative together with BMDO into the CVD system. The feed ratio of the precursors is variable. For the synthesis of co-polymer 1, the molar ratio of the two precursors PCP-CH₂OH and BMDO was 1:15. For co-polymer 2, the molar ratio of PCP and BMDO was 3:5. For co-polymer 3, the molar ratio of PCP-alkyne and BMDO was 1:5. The precursors sublimated or evaporated under 0.07 Torr at around 100 °C and were then transferred to the pyrolysis zone (530 °C) using a stream of argon carrier gas (20 sccm). Radicals generated from the pyrolysis of [2.2]paracyclophanes were further transferred into the deposition chamber together with the vaporized BMDO. The radicals and BMDO adsorbed and polymerized on the substrates placed on a metal stage set at 15 °C. The CVD deposition rate was kept at 0.1 to 0.2 Å/s and was constantly monitored by a quartz crystal microbalance (QCM).

Polymer characterization

Co-polymer 1 was characterized by FTIR, XPS and TGA. All FTIR data reported in this study were generated using a Nicolet 6700 spectrometer with a MCT-A detector and a smart specular apertured grazing angle (Smart SAGA) accessory with an 80° fixed angle

of incidence. XPS was performed on a Kratos Axis Ultra XPS equipped with a monochromatic Al K α X-ray source. Passing energy applied was 160 eV for survey scans and 20 eV for high-resolution analysis. All spectra were calibrated in reference to the non-functionalized aliphatic carbon with peak position fixed at 285.0 eV. TGA experiments were performed on a TA Instruments Discovery TGA with a heating rate of 10 °C/min in the range from 20 °C to 750 °C in nitrogen atmosphere with a purge rate of 25 mL/min.

Polymer degradation

Gold (100 nm by e-beam evaporation, with 10 nm Ti as adhesion layer) coated polished silicon surfaces were used as substrates to facilitate Fourier transform infrared spectroscopy (FTIR) and ellipsometry measurements, which were used to monitor the film degradation process. Samples coated with co-polymer 1 were incubated in an aqueous solution with 0.1 M sodium bicarbonate and 0.1 M sodium carbonate (v/v=9:1, Na₂CO₃/NaHCO₃) at 37 °C. The pH value of this degradation solution was 10.6 at 37 °C. At different time intervals, the samples were taken out of the degradation solution, washed thoroughly with deionized-water and air-dried. Then the samples were measured by FTIR and ellipsometry (Accurion, Nanofilm EP³-SE, Germany) to monitor their change in chemical composition and thickness. Ellipsometric parameters were fitted using a *Cauchy* model. After full degradation of co-polymer 1 coatings, the degradation solution was extracted with chloroform and analyzed by Electrospray Ionization–Mass Spectrometry (ESI-MS) to analyze the final degradation products.

Cell viability tests

Tissue culture polystyrene (TCPS) coverslips (Thermo Scientific Nunc Thermanox Coverslips) were coated with PPX-CH₂OH or co-polymer 1. Some of the samples coated with co-polymer 1 were degraded for 5 days before the cell viability test. TCPS itself served as a positive control and organ-tin (dibutyltin maleate, 2 wt%) stabilized PVC (Ot-PVC) with high cytotoxicity was used as a negative control. NIH/3T3 cells were cultured at 37°C, 5% CO₂ in DMEM with 10% FBS. Cells were then passaged after reaching 80% confluence and seeded onto the non-coated TCPS, the CVD coated TCPS and the Ot-

PVC at a density of 1×10^4 cells/cm². The cells were then cultured at 37°C under 5% CO₂ for 3 days. After 3 days in culture, the medium was removed and replaced with fresh medium. Cytotoxicity was then determined using an XTT cell proliferation assay kit (ATCC, Manessa VA). Activated XTT solution was added to the medium and incubated at 37°C under 5% CO₂ for 2 hours. Next, the absorbance of the medium was measured using a microplate reader (BioTek Synergy 2, Winooski VT) at two wavelengths, 475 nm and 660 nm. Cell viability was determined by normalizing the measured mitochondrial activity of the NIH/3T3 cells expanded on CVD coated TCPS with cells expanded on non-coated TCPS.

Surface reactivity test

In order to test the surface reactivity of co-polymer 3 (co-polymer of PCP-alkyne and BMDO with a molar feed ratio of 1:5), copper catalyzed alkyne-azide click chemistry^[2, 6] was applied on the surface using micro-contact printing. The fabrication of the mold and polydimethylsiloxane (PDMS) stamps for micro-contact printing is described elsewhere.^[7-8] PDMS stamps were first oxidized by UV ozone treatment for 30 minutes in order to facilitate the wetting process. One type of “ink” applied was an aqueous solution consisting of 5 µg/ml Alexa Fluor 488 azide (Life Technologies), 20 mg/ml sodium ascorbate and 1 mM copper sulfate pentahydrate. The other type of “ink” applied was an aqueous solution consisting of 0.2 mg/ml biotin-PEG₃-azide (Sigma-Aldrich), 20 mg/ml sodium ascorbate and 1 mM copper sulfate pentahydrate. The printing time was 2 hours for both inks and the printed samples were thoroughly washed with deionized water afterwards. Since the second ink did not contain a fluorescent dye, samples with biotin immobilized on the surface were incubated in 5 µg/ml Streptavidin-Cy3 (Sigma-Aldrich) PBS solution with 1 mg/ml bovine serum albumin (BSA) for half an hour in order to visualize the pattern. Biotin and streptavidin are biomolecules which are often used as a model in biomedical research due to their high binding affinity to each other.^[9] BSA was added to stabilize the protein in the solution and prevent nonspecific binding of the protein on the coating surface. The surface was thoroughly rinsed with BSA/PBS solution, PBS solution and deionized water before fluorescence imaging.

Additional information on co-polymer 1

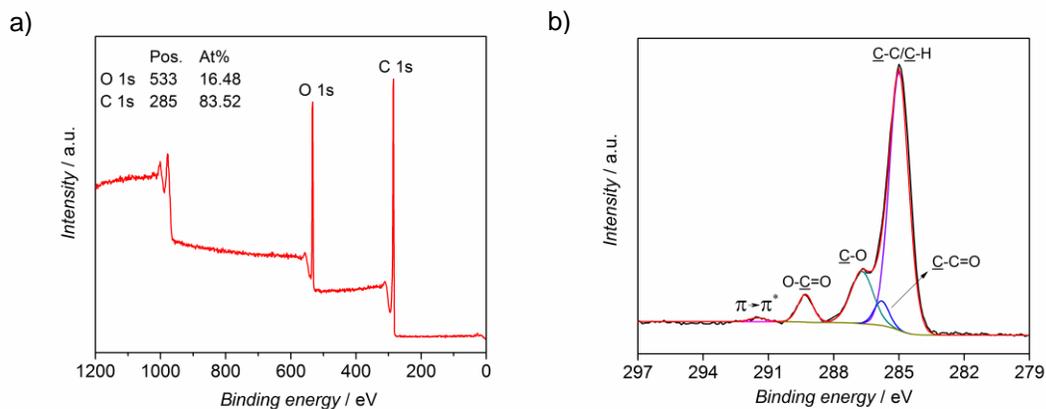
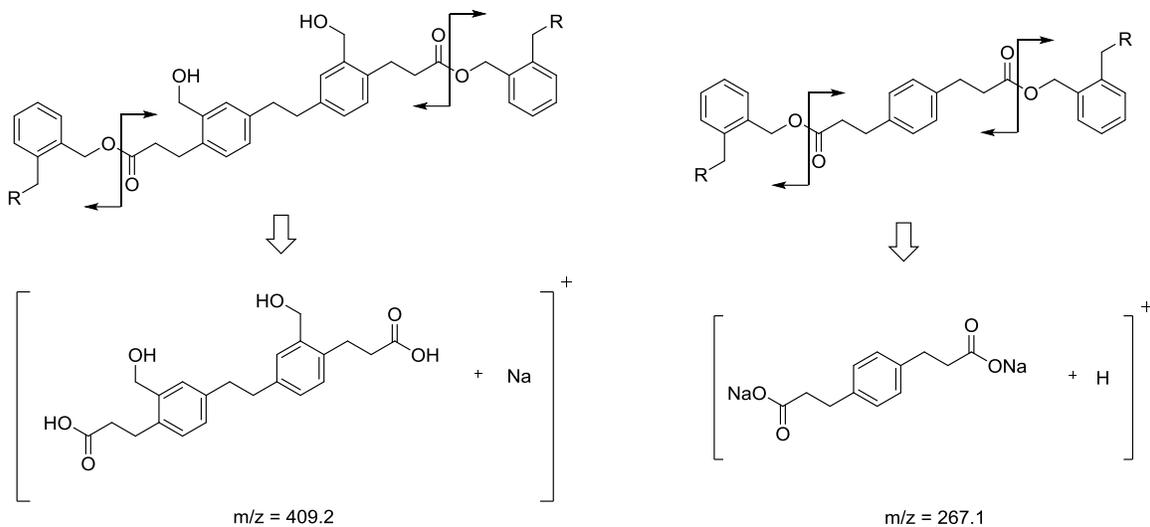


Figure S1. (a) XPS survey spectrum of co-polymer 1; (b) high resolution XPS C 1s spectrum of co-polymer 1 (co-polymer of 4-hydroxymethyl-[2.2]paracyclophane and BMDO).



Scheme S2. Possible fragmentation patterns of co-polymer 1 after degradation. The carboxyl terminated products could be detected by ESI-MS analysis.

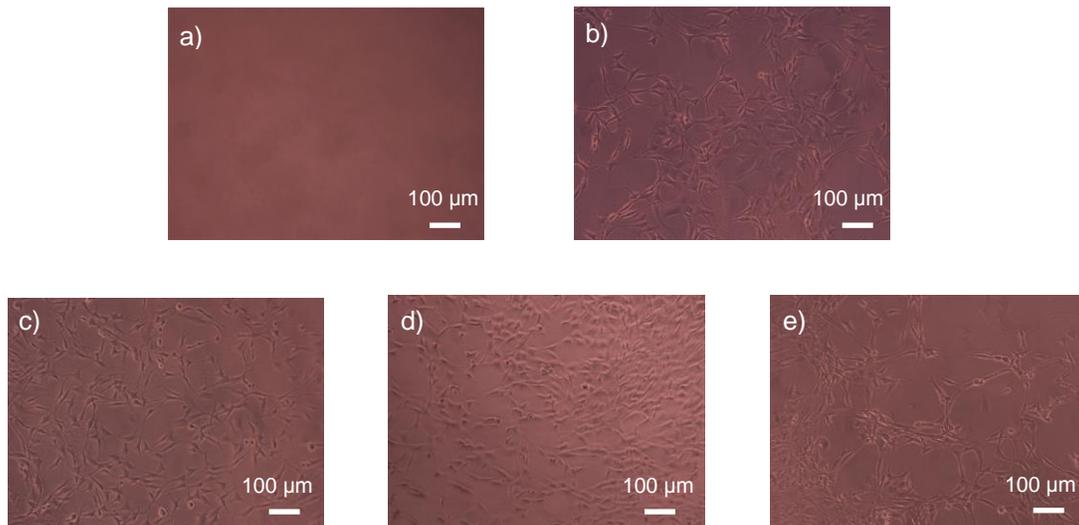
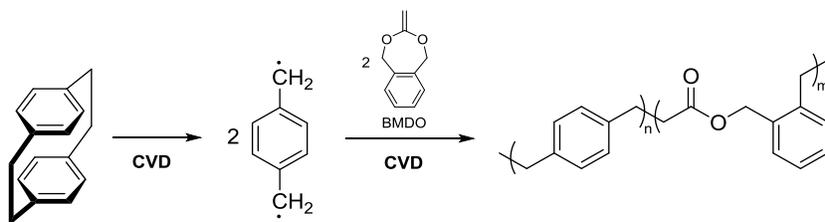


Figure S2. Microscopy images of NIH3T3 fibroblasts grown on different surfaces for the XTT cell viability assay: (a) poly(vinyl chloride) (PVC) surface (negative control); (b) TCPS (positive control); (c) PPX-CH₂OH; (d) co-polymer 1; (e) co-polymer 1 partially degraded. NIH3T3s show different spreading responses with respect to the different surfaces.

Additional information on co-polymer 2



Scheme S3. Synthesis of co-polymer 2 *via* co-polymerization of [2.2]paracyclophane and BMDO (feeding molar ratio 3:5).

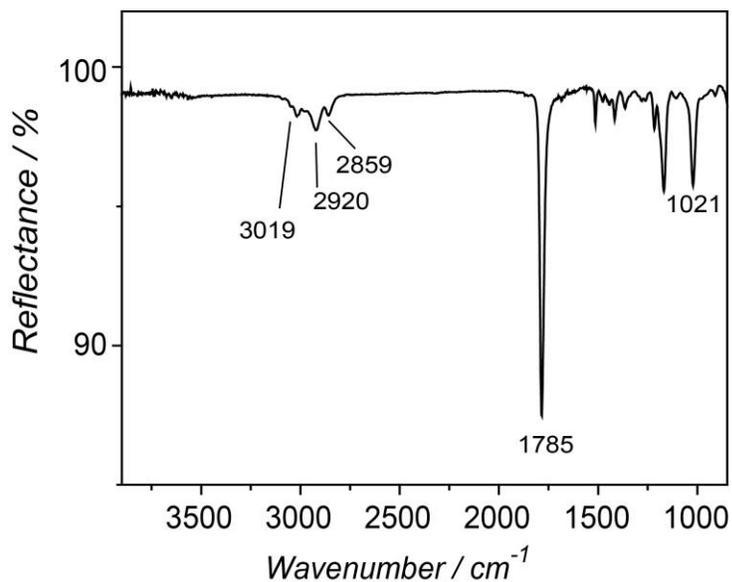


Figure S3. FT-IR spectrum of co-polymer 2 film. The film has been stable in carbonate buffer for at least two weeks and no reduction of its ellipsometric thickness has been observed during that period.

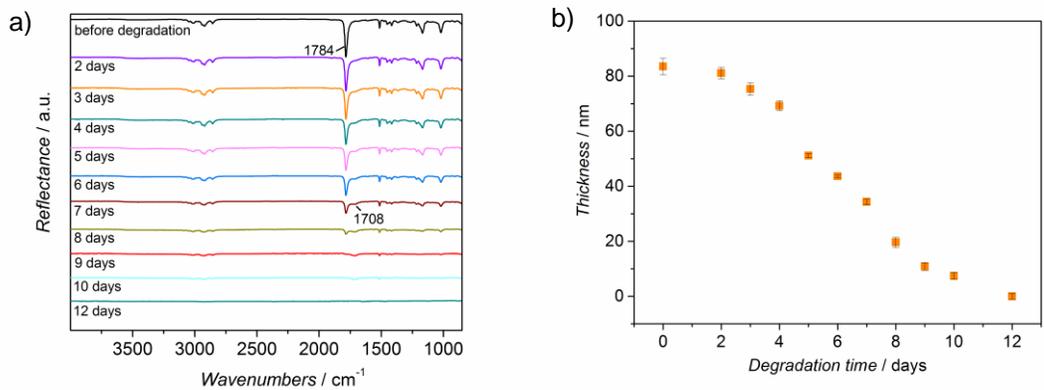


Figure S4. (a) FT-IR spectra of co-polymer 2 degrading in 5 mM KOH/isopropanol solution; (b) changes in the layer thickness of co-polymer 2 degrading in 5 mM KOH/isopropanol solution measured by ellipsometry.

Additional information on PPX-CH₂OH

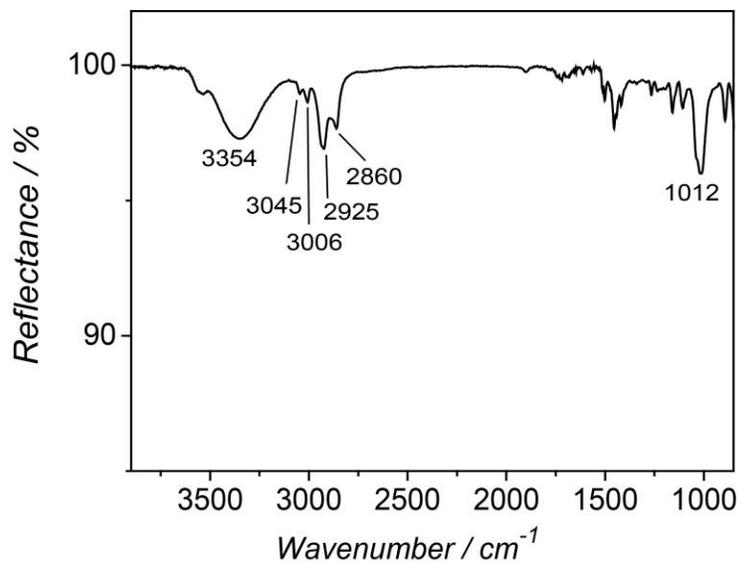


Figure S5. FT-IR spectrum of PPX-CH₂OH film. The thickness of the polymer film is 88 nm, as measured by ellipsometry.

Additional information on co-polymer 3

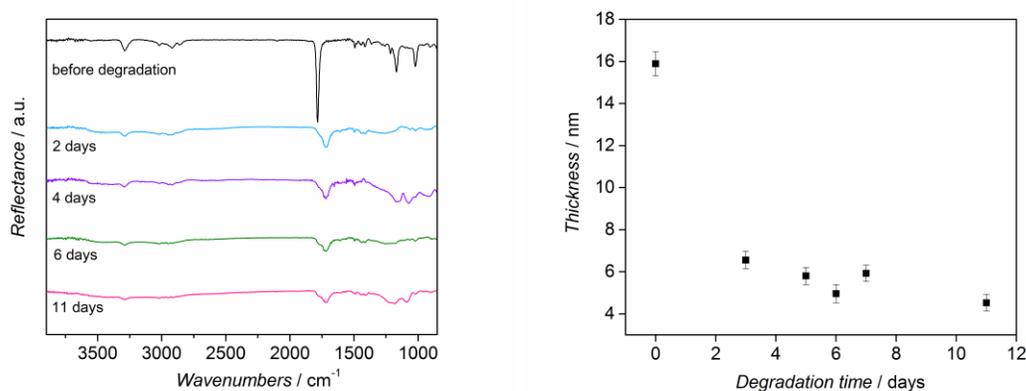


Figure S6. (a) FTIR spectra of co-polymer 3 degrading over time; (b) changes in the layer thickness of co-polymer 3 degrading in 5 mM KOH/isopropanol solution measured by ellipsometry.

Table S1. XPS analysis results of co-polymer 2 and 3.

	B.E. (eV) ^[a]	Co-polymer 2 experimental (%) ^[b]	Co-polymer 3 experimental (%) ^[b]
C-C/H	285±0.1	68.1	69.1
C-C=O	285.7±0.1	6.8	8.3
C-O	286.8±0.1	7.6	8.7
O-C=O	289.3±0.1	4.2	4.9
π→π*	291.5±0.1	1.3	1.0
O	533±0.1	12.0	8.0

[a] Binding Energy; [b] Atomic percent.

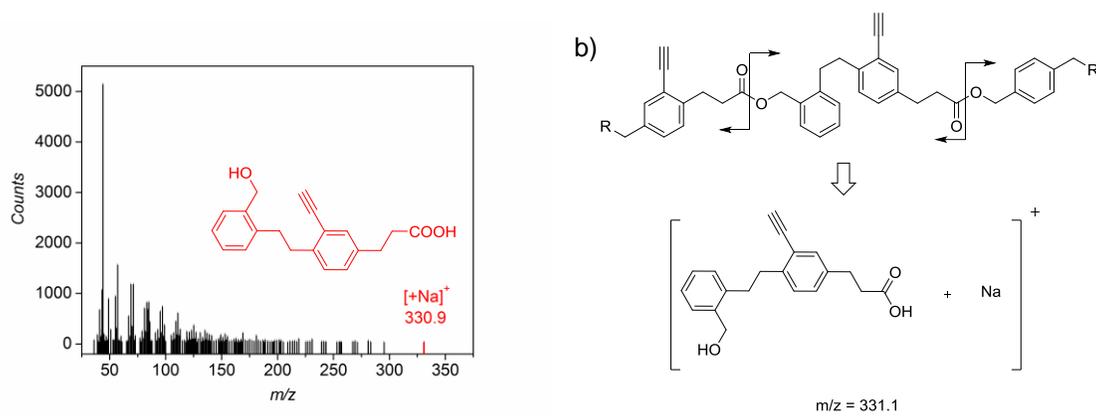


Figure S7. (a) Expanded ESI-mass spectrum of degradation products of co-polymer 3; (b) possible fragmentation pattern of co-polymer 3 after degradation.

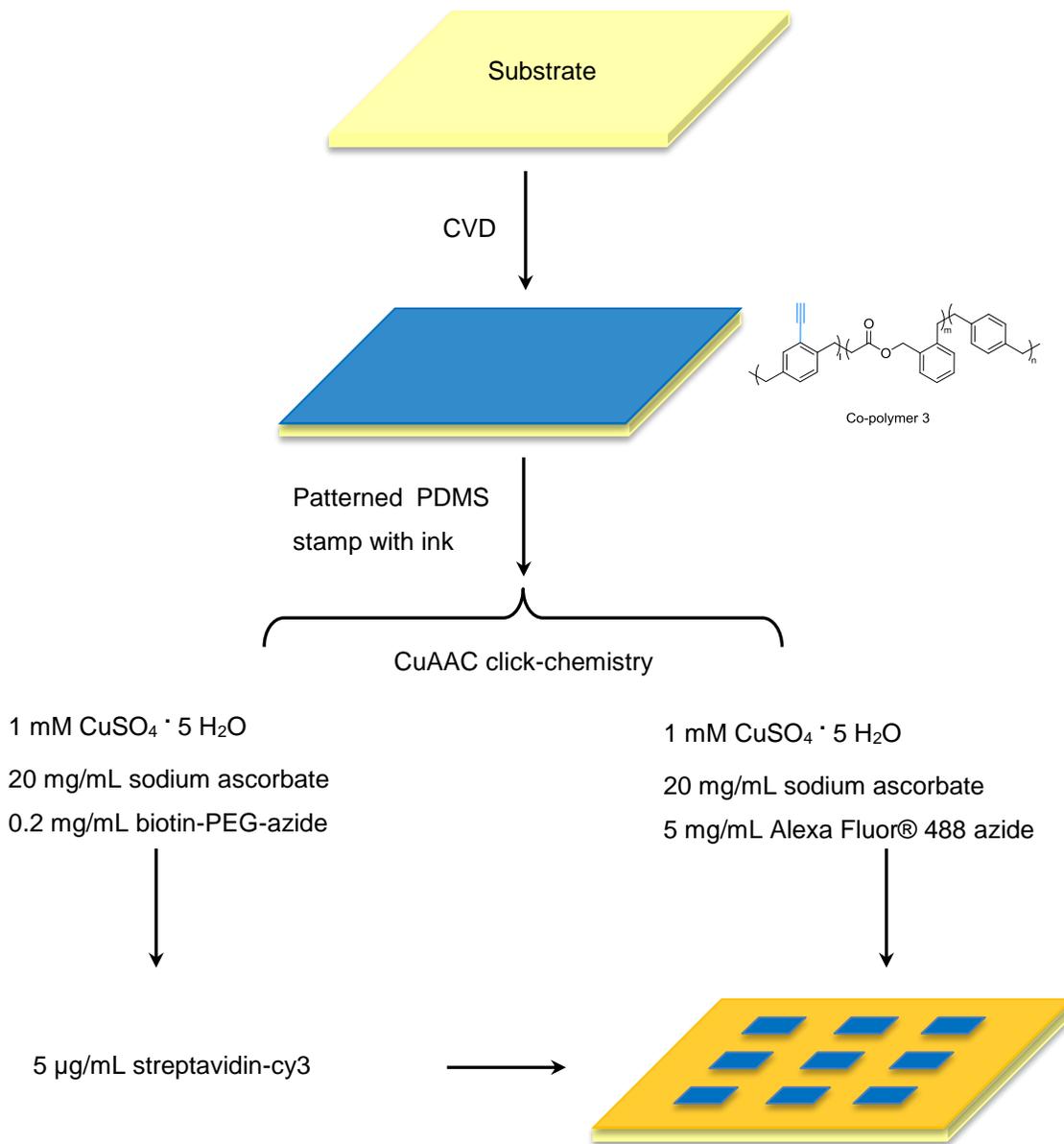


Figure S8. Schematic illustration showing the click-chemistry procedure used to demonstrate the chemical activity of co-polymer 3.

References

- [1] X. Jiang, H. Y. Chen, G. Galvan, M. Yoshida, J. Lahann, *Adv. Funct. Mater.* **2008**, *18*, 27-35.
- [2] H. Nandivada, H.-Y. Chen, L. Bondarenko, J. Lahann, *Angew. Chem. Int. Ed.* **2006**, *45*, 3360-3363.
- [3] W. J. Bailey, Z. Ni, S. R. Wu, *Macromolecules* **1982**, *15*, 711-714.
- [4] H. Wickel, S. Agarwal, *Macromolecules* **2003**, *36*, 6152-6159.
- [5] G. G. d'Ayala, M. Malinconico, P. Laurienzo, A. Tardy, Y. Guillaneuf, M. Lansalot, F. D'Agosto, B. Charleux, *J. Polym. Sci., Part A: Polym. Chem.* **2014**, *52*, 104-111.
- [6] X. Deng, C. Friedmann, J. Lahann, *Angew. Chem. Int. Ed.* **2011**, *50*, 6522-6526.
- [7] J. L. Wilbur, A. Kumar, E. Kim, G. M. Whitesides, *Adv. Mater.* **1994**, *6*, 600-604.
- [8] H. Y. Chen, J. Lahann, *Adv. Mater.* **2007**, *19*, 3801-3808.
- [9] P. Weber, D. Ohlendorf, J. Wendoloski, F. Salemme, *Science* **1989**, *243*, 85-88.