Supporting Information for Chemical Communications

Orthogonal Thiol-ene 'Click' Reactions: A Powerful Combination for Fabrication and Functionalization of Patterned Hydrogels

N. Cengiz, T. N. Gevrek, R. Sanyal, and A. Sanyal

Department of Chemistry, Bogazici University, Istanbul, Turkey

e-mail: amitav.sanyal@boun.edu.tr

Materials. The furan protected maleimide-containing methacrylate (FuMaMA) monomer was synthesized according to a previous report.[1] Poly(ethylene glycol) methyl ether methacrylate (PEGMEMA, M_w = 300 $gmol^{-1}$) was purchased from Sigma Aldrich and purified by passing through an aluminium oxide column prior to use. Poly (ethylene glycol) (PEG, M_w = 8000 $gmol^{-1}$), was purchased from Fluka, 3-(trimethoxysilyl)propyl methacrylate (TMSMA), triethylamine (TEA), Copper(I)bromide, 2,2′-bipyridyl and 2,2′-(ethylenedioxy)diethanethiol, furfuryl mercaptan, 2-Hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone and 2,2-dimethoxy-2-phenylacetophenone (DMPA) were obtained from Sigma Aldrich, 2-bromo-2-methylpropionyl bromide was obtained from Acros and used as received. Biotin-SH was obtained from Nanoscience Instruments. Qdot® 605 Streptavidin conjugate was obtained from Sigma. 4,4-Difluoro-1,3,5,7-tetramethyl-8-[(10-mercapto)]-4-bora-3a,4a-diaza-*s*-indacene (BODIPY-SH) was synthesized according to the literature procedure.[2] Methanol was purchased from Merck. Anhydrous solvents such as dichloromethane (DCM), tetrahydrofuran (THF) and toluene were obtained from a SciMatCo purification system and other solvents were dried over molecular sieves. Column chromatography was performed using silica gel 60 (43-60 nm, Merck). Thin layer chromatography was performed using silica gel plates (Kieselgel 60 F254, 0.2 mm, Merck).

The plates were viewed under 254 nm UV lamp and/or developed by $KMnO_4$ stain. Blak-Ray® B-100 AP/R High Intensity 100 Watt/365 nm UV lamp is used for both crosslinking process and radical mediated thiol-ene reaction.

Characterization. ^{1}H-NMR spectra were recorded with a Varian Mercury VX 400 MHz spectrometer, and referenced to $CDCl_3$ or CD_2Cl_2. The weight and number average molecular weights (Mw and Mn) as well as the polydispersity index (PDI) were determined by gel permeation chromatography (GPC) using a Shimadzu GPC system furnished with a PSS-SDV (length/ID 8×300 mm, 10μm particle size) linear M column. Polystyrene standards (1-150 kDa) from Viscotek were used for calibration. Tetrahydrofuran (THF) was used as eluent at a flow rate of 1 $mLmin^{-1}$ at 30 ^oC. FT-IR spectra were recorded with a Thermo Fischer Scientific Inc. Nicolet 380 instrument (spectral range between 4000 and 450 cm^{-1}). Scanning electron microscopy (SEM) micrographs were taken using Philips XL-30 instrument with an acceleration voltage of 10 kV. X-ray photoelectron spectra were obtained using Surface Science Instruments. The instrument was operated in a fixed analyzer transmission mode using a monochromatic Al *Kα* X-ray source. The pass energy of 150 eV with a 400 μm spot size was used for survey spectra. Immobilization experiments to confirm the attachment of BODIPY-SH and Streptavidin conjugated quantum dot adsorption were determined using LD-A-Plan 20x/0.30 objective in Zeiss Axio Observer inverted microscope (ZEISS Fluorescence Microscopy, Carl Zeiss Canada Ltd, Canada). Zeiss Filter set 38 (Excitation BP 470/40, Emission BP 525/50) was used for imaging of BODIPY-SH attached hydrogel surface. For visualization of streptavidin conjugated quantum dot, filter set 43 (Excitation BP 545/25, Emission BP 605/70) was used. Images were processed using Zeiss AxioVision software. Hydrogel rheological behaviors were evaluated by using an Anton PAAR MCR 302 rheometer.

Synthesis of PEG ATRP Macro-initiator. A procedure reported by Leroux and coworkers was adapted.[3] A solution of HO-PEG-OH (M_n=8000 $gmol^{-1}$, 4.0 g, 0.502 mmol) was dissolved in toluene and dried azeotropically using rotary evaporation, followed by drying under vacuum. The polymer HO-PEG-OH and triethylamine (0.15 mL, 1.06 mmol) was dissolved in anhydrous methylene chloride (30 mL) under a nitrogen atmosphere and the flask was immersed in an ice-water bath. Then, 2-bromoisobutyryl bromide (0.62 mL, 5.02 mmol) was slowly added to the reaction mixture. The solution was warmed to room temperature and stirred for 24 hours. The crude product was dissolved in methylene chloride (250 mL) and washed with saturated $NaHCO_3$ solution (100 mL) three times. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated in vacuum. The crude product was dissolved in a minimum amount of methylene chloride and then precipitated in cold diethyl ether. The precipitate was recovered by simple filtration to yield the desired polymer as a white powder (yield: 85 %). ^{1}H NMR (δ, ppm, $CDCl_3$) 4.32-4.29 (4H, m, -COO-*CH_2*-), 3.81-3.78 (4H, m, -COO-CH_2-*CH_2*-), 3.73-3.71 (4H, m, -COO-CH_2CH_2O-*CH_2*), 3.66-3.59(712H, br s, -CH_2CH_2O-), 3.45-3.43 (4H, m, -CH_2CH_2O-), 1.92 (12H, s, -CH_3-C-COO-).

General Polymerization Procedure for the synthesis of A–B–A triblock copolymers. In a 10 mL flask, the PEG macroinitiator (Br−PEG−Br) [M_n=8300 Da] (0.100 g, 0.01 mmol), FuMaMA monomer (0.075 g, 0.26 mmol) and PEGMEMA [M_n=300 Da] (0.231 g, 0.77 mmol) were dissolved in a mixture of MeOH (1.8 mL) and H_2O (0.2 mL). The flask was sealed with a rubber septum and thoroughly degassed under nitrogen purge. Polymerization was initiated by addition of a solution of 2, 2'-bipyridine (0.049 g, 0.31 mmol) and Cu(I)Br (0.022 g, 0.11 mmol) dissolved in MeOH (0.6 mL) and the polymerization was carried out at room temperature for 2 hours. After the polymerization, the reaction mixture was precipitated into cold diethyl ether (100 mL). The viscous liquid obtained after discarding

the diethyl ether layer was diluted with DCM and passed through a short column of basic alumina to remove the catalyst. After evaporating the organic solvent, the residue was dried under vacuum at room temperature (yield 50%). M_n=23 kDa, PDI=1.2, ^{1}H NMR (δ, ppm, $CDCl_3$) 6.51 (s, 2H, *CH=CH* protected), 5.23 (s, 2H, *CH* bridgehead protons), 4.32-4.01 (m, 6H, -COO-*CH_2*-), 3.91-3.78 (m, 6H, -COO-CH_2-*CH_2*-), 3.66-3.54, (712+14H, br s, -*CH_2CH_2*O-), 3.45-3.43 (4H, m, -CH_2C*H_2*O-), 3.35 (s, 3H, O-*CH_3*), 2.86 (s, 2H,*CH-CH*, bridge protons), 1.91–0.84 (m, 7H,NCH_2*CH_2*CH_2O, *CH_2* and *CH_3* along polymer backbone.

Partial Activation of A–B–A triblock copolymer. Triblock copolymer P1 (25 mg, $1x10^{-3}$ mmol) was dissolved in toluene (10 mL) and heated at 100 °C for 50 min to give the partially activated copolymer P2. Copolymer P2 was precipitated in cold diethyl ether (yield = 92%). ^{1}H NMR (δ, ppm, CD_2Cl_2) 6.76 (s, 2H, *CH=CH* deprotected), 6.51 (s, 2H, *CH=CH* protected), 5.23 (s, 2H, CH bridgehead protons), 4.32-4.01 (m, 8H, -COO-*CH_2*-), 3.91-3.78 (m, 8H, -COO-CH_2-*CH_2*-), 3.66-3.54, (br s, 712+14H, -CH_2CH_2O-), 3.45-3.43 (m, 4H, -CH_2C*H_2*O-), 3.35 (s, 3H, O-CH_3), 2.86 (s, 2H, CH-CH, bridge protons), 1.91–0.84 (m, 7H,$NCH_2CH_2CH_2O$, CH_2 and CH_3 along polymer backbone.

Functionalization of Deprotected Maleimide Side Chains (P3). Benzyl mercaptan (14.6 mg, 0.118 mmol), triethylamine (1.21 mg, $1.2x10^{-2}$ mmol) and copolymer P2 (70 mg, $3x10^{-3}$ mmol) were dissolved in anhydrous THF (0.5 mL) and stirred for 3 hours at room temperature. Copolymer P3 was precipitated in cold diethyl ether ^{1}H NMR (δ, ppm, CD_2Cl_2) 7.32-7.14 (m, 5H, Ar-) 6.51 (s, 2H, CH=CH protected), 5.23 (s, 2H, CH bridgehead protons), 4.32-4.01 (8H, m, -COO-*CH_2*-), 3.91-3.78 (m, 8H, -COO-CH_2-*CH_2*-), 3.66-3.54, (br s, 712+14H, -CH_2CH_2O-), 3.45-3.43 (m, 4H, -CH_2C*H_2*O-), 3.35 (s, 3H, O-CH3), 3.00-2.89 (br s, 2H, Ar-*CH2*-S-) 2.86 (s, 2H,CH-CH, bridge protons), 1.91–0.84 (m, 7H,$NCH_2CH_2CH_2O$, CH_2 and CH_3 along polymer backbone.

Functionalization of Furan-protected Maleimide Side Chains (P4). Copolymer P3 (60 mg, 28.8 x 10^{-4} mmol) and DMPA (5.78 mg, 2.26 x10^{-2} mmol) was dissolved in 30 μL methanol. Thioglycerol (12.22 mg, 0.1128 mmol) was added to the reaction vial and the solution was degassed under a slow stream of nitrogen for 20 minutes. Finally, the solution was subjected to 365 nm UV exposure for 45 min. The resulting polymer was precipitated in cold diethyl ether. ^{1}H NMR (δ, ppm, CD_2Cl_2) 7.32-7.14 (m, 5H, Ar-), 4.76 (br s, 1H, -CH bridgehead), 4.70 (br s, 1H, -CH bridgehead) 4.32-4.01 (8H, m, -COO-*CH_2*-), 3.91-3.78 (m, 8H, -COO-CH_2-*CH_2*-), 3.66-3.54, (br s, 712+14H, -CH_2CH_2O-), 3.45-3.43 (4H, m, -CH_2C*H_2*O-), 3.35 (s, 3H, O-CH_3), 3.00-2.89 (br s, 2H, Ar-*CH_2*-S-), 2.89-2.81 (br s, 2H, *CH_2*-S-), 2.81-2.65 (br s, 2H,CH-CH-C(O)N), 1.91–0.84 (m, 7H,$NCH_2CH_2CH_2O$, CH_2 and CH_3 along polymer backbone.

Reverse Sequence of Functionalization:

Partial Functionalization of Copolymer P1′ via Thiol-Ene Reaction; P2′. For these studies, a copolymer P1′ with similar PEGMEMA/FuMaMA ratio as copolymer P1 but Mn=15 kDa was used. Copolymer P1′ (114 mg, 7.6x10^{-3} mmol) and DMPA (4.82 mg, 1.8x10^{-2} mmol) was dissolved in 50 μL degassed methanol. Benzyl mercaptan (11.7 mg, 9.4x10^{-2} mmol) was added to the reaction vial. The solution was then subjected to 365 nm UV exposure for 15 minutes. The resulting partially modified polymer, P2′ was precipitated in cold diethyl ether (Figure S1).

Activation of P2′ via Retro Diels-Alder reaction; P3′. Partially modified P2′, (36 mg, 2.4x10^{-3} mmol) was dissolved in toluene (15 mL) and heated at 100 °C for 8 hours to give the activated copolymer P3′. It was precipitated in cold diethyl ether to purify (Figure S1).

Functionalization of P3′ via Michael Addition; P4′. Furfuryl mercaptan (1.84 mg, 1.6x10^{-2} mmol), triethylamine (0.16 mg, 0.0016 mmol) and copolymer P3′ (18 mg, 1.2x10^{-3} mmol) were dissolved in anhydrous THF (125 μL) and stirred for 3 hours at room temperature.

Resulting polymer was precipitated in cold diethyl ether to obtain copolymer P4′ (Figure S1).

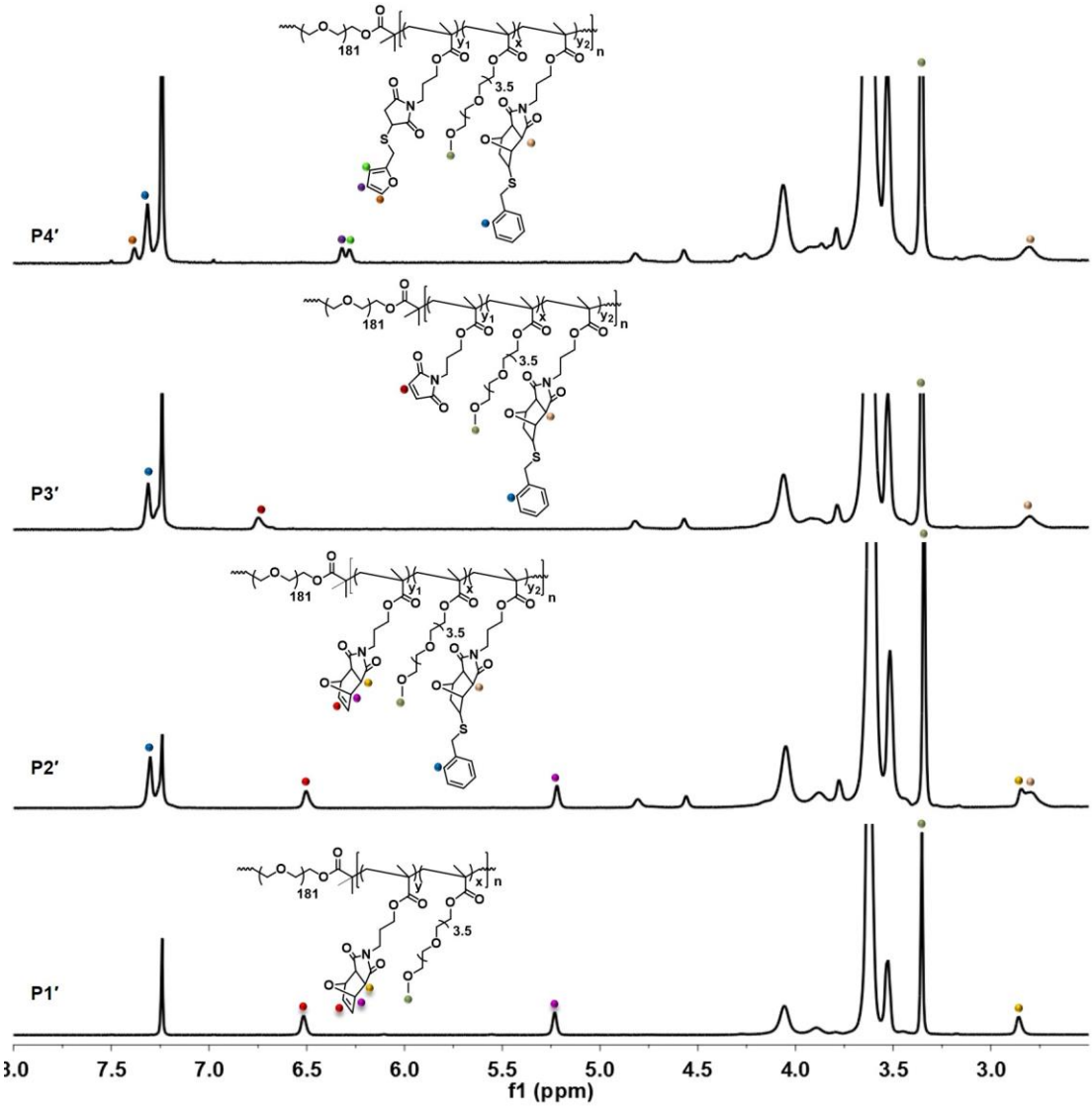

Figure S1. 1H NMR spectra for orthogonal functionalization of copolymer P1′ using partial thiol-ene addition of benzyl mercaptan, followed by activation and subsequent Michael addition of furfuyl mercaptan.

Fabrication of Bulk Hydrogel via Michael addition. Partially activated copolymer P1′ (18 mg) and triethylamine (0.026 mg, 2.5×10^{-4} mmol) was dissolved in H_2O/EtOH (10:1) mixture. After adding 2,2′-(ethylenedioxy)diethanethiol (0.24 mg, 1.2×10^{-3} mmol) the mixture was left overnight to obtain hydrogel.

Radical Thiol-ene Functionalization of Bulk Hydrogel. 1 mg BODIPY thiol and 0.117 mg DMPA was dissolved in 1 mL of MeOH. 20 µL of the solution was added into a vial with 5 mg of swollen hydrogel and placed under UV for 15 minutes. As a control experiment, hydrogel was incubated in same amount of BODIPY thiol solution and placed in a dark. After washing with MeOH, they were visualized via fluorescence microscope (Figure S2).

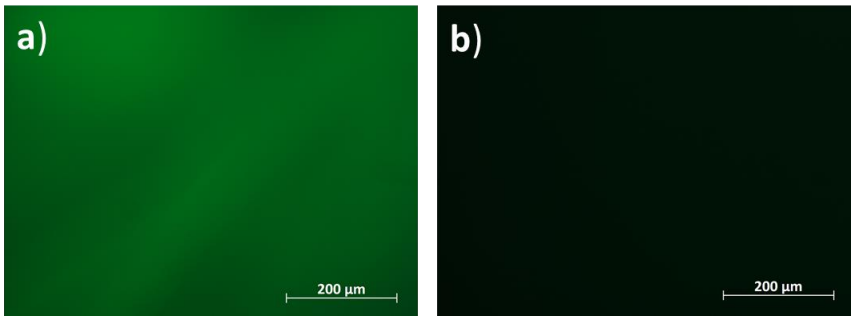

Figure S2. a) Functionalization of hydrogel with BODIPY-SH using thiol-ene reaction with UV. b) Control experiment of thiol-ene click without UV-irradiation.

Fabrication of Bulk Hydrogels via radical thiol-ene in 10:1 EtOH:H_2O

Copolymer P1′ (46.7 mg, $3.13x10^{-3}$ mmol), DMPA (0.19 mg, $7.6x10^{-4}$ mmol) and 2,2′-(ethylenedioxy)diethanethiol (0.69 mg, $3.8x10^{-3}$ mmol) were first dissolved in 103 µL of EtOH/H_2O (10:1) mixture in a glass vial, and irradiated with UV (365 nm). Hydrogels were swollen in water. Frequency and strain sweep tests were applied at 1% strain and 10 rad s^{-1} angular frequency, respectively, to obtain rheological data (Figure S3).

Fabrication of Bulk Hydrogels via radical thiol-ene in 10:1 H_2O:EtOH

Copolymer P1′ (34 mg, $2.27x10^{-3}$ mmol) was dissolved in 68 µL water and 2-Hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone (0.12 mg, $2.72x10^{-3}$ mmol) in 6.8 µL EtOH was added on it. After adding 2,2′-(ethylenedioxy)diethanethiol (0.12 mg, $5.44x10^{-4}$ mmol), the mixture was irradiated with UV (365 nm). Hydrogels were swollen in water. Frequency and strain sweep tests were applied at 1% strain and 10 rad s^{-1} angular frequency, respectively, to obtain rheological data (Figure S3).

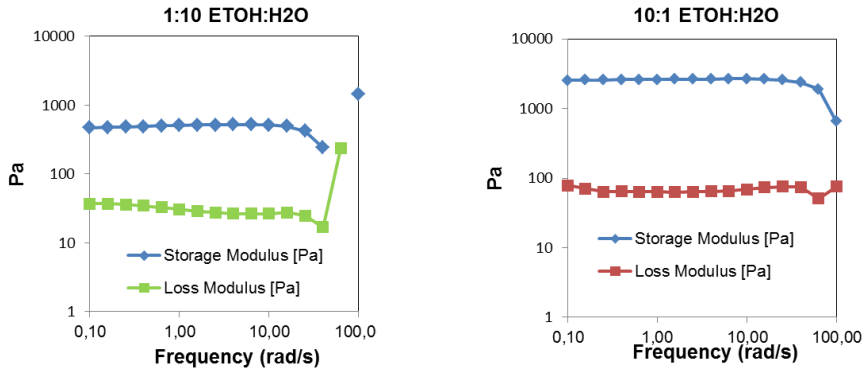

Figure S3. Frequency tests for hydrogels

Swelling Study of Photo-crosslinked Bulk hydrogels.

Dry hydrogels which synthesized in H_2O/EtOH (10:1) or EtOH/ H_2O (10:1) mixtures as solvent were cut into small pieces and weighed. Each hydrogel was placed into a beaker with 10 mL deionized water at room temperature. At regular time intervals, the hydrogels were taken out of the water, surface moisture was removed by tissue paper, and they were weighed again. Then the hydrogels were returned to the beaker and the water uptake was measured until the maximum mass was obtained. The percentage amount of water uptake (Wup) was calculated using the following equation: $Wup(\%) = (Wmax - Wdry)/Wdry \times 100$ where Wmax = maximum weight of the swollen hydrogel and Wdry = weight of the dry hydrogel. The equilibrium water uptakes were calculated as 285 % and 480 % for EtOH/ H_2O (10:1) and H_2O/EtOH (10:1) hydrogels respectively.

Swelling Study of Patterned Hydrogel H2. The height of dry hydrogel patterns H2 synthesized in H_2O/EtOH (10:1) was measured using profilometer. After swelling in 10 mL water for 30 minutes, surface moisture was removed by tissue paper, and then the height was measured again. After incubation in water, height of hydrogel patterns increased from 400.3 to 643.7 nm.

Modification of silicon wafer for promoting hydrogel adhesion. TMSMA was used for modification of silicon wafer to promote the covalent adhesion between the surface and the hydrogel. Silicon wafers were first treated with acidic NOCHROMIX solution (0.5 g /20 mL NOCHROMIX/H_2SO_4) for 1 hour to remove the native oxides, organic impurities and metallic contaminants, rinsed with deionized water, and then sonicated in acetone and isopropyl alcohol respectively and blown dry using stream of nitrogen. TMSMA was dissolved in anhydrous toluene (1 wt % solution) and clean surfaces were incubated in this solution under nitrogen atmosphere overnight. The modified wafer was washed several times in toluene and methanol and then dried under vacuum.

Fabrication of Hydrogel Pattern (H1). Copolymer P1 (11 mg, $4.9x10^{-4}$ mmol) was mixed with 2,2′-(ethylenedioxy)diethanethiol (0.278 mg) and DMPA (0.846 mg, $3.3x10^{-3}$ mmol) to prepare a 10 wt% polymer solution in EtOH/H_2O (10:1). Selective photo patterning was performed through a photomask. For this aim, dried glass substrate was coated with polymer solution by spin coating at 500 rpm for 10 s and 2000 rpm for 30 s. The prepared polymer films were lithographically patterned by selective 365 nm UV exposure through a photomask for 10 minutes, then washed with THF and dried under nitrogen (Figure 3).

Fabrication of Hydrogel Pattern (H2). Copolymer P1 (3 mg, $1.4x10^{-4}$ mmol) was mixed with 2,2′-(ethylenedioxy)diethanethiol (0.046 mg, $2.5x10^{-4}$ mmol) and DMPA (0.013 mg, $5x10^{-5}$ mmol) to prepare a 10 wt% polymer solution in EtOH/H_2O (10:1). Selective photo patterning was performed through a photomask. For this aim, dried glass substrates were coated with polymer solution by spin coating at 500 rpm for 10 s and 2000 rpm for 30 s. The prepared polymer films were lithographically patterned by selective 365 nm UV exposure through a photomask for 5 minutes, then washed with THF and dried under nitrogen (Figures 4a, b).

Fabrication of Hydrogel Pattern (H3). Copolymer P1 (3 mg, $1.4x10^{-4}$ mmol) was mixed with 2,2′-(ethylenedioxy)diethanethiol (0.069 mg, $3.8x\ 10^{-4}$ mmol) and DMPA (0.019 mg, $7.4x\ 10^{-5}$ mmol) to prepare a 10 wt% polymer solution in EtOH/H_2O (10:1). Selective photo patterning was performed through a photomask. For this aim, dried silicon wafer substrates were coated with polymer solution by spin coating at 500 rpm for 10 s and 2000 rpm for 30 s. The prepared polymer films were lithographically patterned by selective 365 nm UV exposure through a photo mask for 5 minutes, then washed with THF and dried under nitrogen (Figures 4c, d).

Activation of Hydrogel Patterns. To activate hydrogel pattern through retro Diels-Alder reaction, patterned surfaces were placed in a vacuum oven at 100 ^{o}C for 30 min. This furnished surfaces with micro-patterned hydrogels containing thiol reactive maleimide groups.

Dye Immobilization on Hydrogel Patterns. A solution of BODIPY-SH in THF (1 mg/mL) was prepared. Then, maleimide group containing hydrogel surface H2 were incubated with the BODIPY-SH solution. After 18 hours, patterned surface was washed with copious amounts of THF to remove unbound dye molecules. Feature size of square patterns (100 μm) are coherent with photomask (Figure 4 and Figure S4).

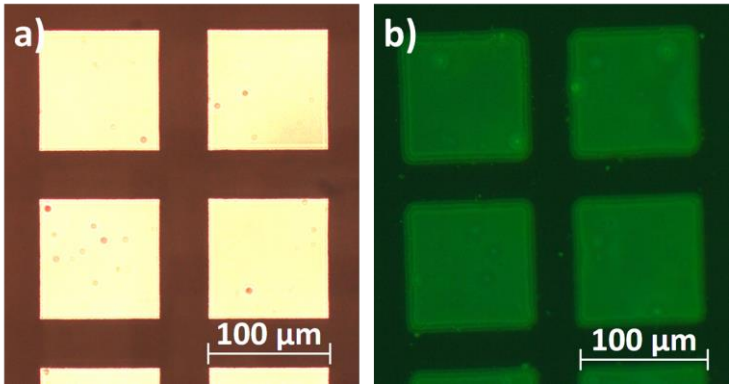

Figure S4. a) Optical microscope image of photomask and b) fluorescence microscope image of dye immobilized patterns.

Bioimmobilization on Hydrogel Patterns. A solution of Biotin-SH in MeOH (1 mg/mL) was prepared and dropped onto a dried hydrogel pattern H3. After 18 hours, the sample was washed with MeOH several times and dried. A solution of Qdot® 605 Streptavidin Conjugate (20 μL, 0.5 mM in H_2O) was dropped on the biotinylated patterned surface. The pattern was placed a dark place for 45 min, and then gently rinsed with copious amounts of water (Figure 4c).

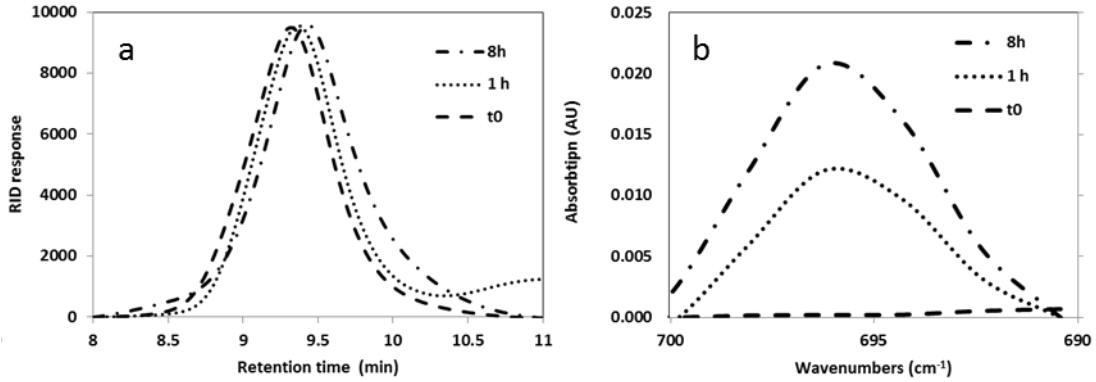

Figure S5. a) GPC Spectrum of polymers t_o:before retro, after 1 h of r-DA, and after 8 h of r-DA. b) C-H (Double bond) stretching of polymers in IR spectra, t_o: before retro, after 1 h of r-DA, and after 8 h of r-DA.

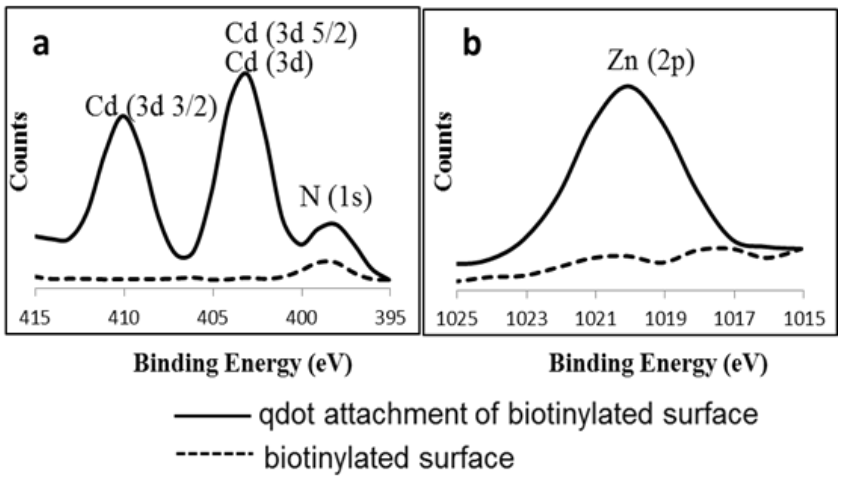

Figure S6. XPS Spectra of patterned hydrogel H3 (a) Binding energy of Cd and N on biotinylated surface and after attachment of streptavidin conjugated QDs. (b) Binding

energy of Zn of biotinylated surface and after attachment of streptavidin conjugated QDs.

References

1. T. Dispinar, R. Sanyal and A. Sanyal, *J. Polym. Sci., Part A: Polym. Chem.*, 2007, **45**, 4545-4551.
2. J. L. Shepherd, A. Kell, E. Chung, C. W. Sinclar, M. S. Workentin, D. Bizzotto, *J. Am. Chem. Soc., 2004,* **126**, 8329-8335.
3. M. Ranger, M. C. Jones, M. A. Yessine and J. C. Leroux, *J. Polym. Sci., Part A: Polym. Chem.*, 2001, **39,** 3861-3874.