
# Supporting Information

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# Controlled Microstructuring of Janus Particles Based on a Multifunctional Poly(ethylene glycol)

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## Controlled Microstructuring of Janus Particles Based on a Multifunctional Poly(ethylene glycol)

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## Experimental Section

### Materials

Allyl glycidyl ether (AGE), benzyl glycidyl ether (BnGE), naphthalene, potassium, 2,2′-azobis(2-methylpropionitrile) (AIBN), poly(DL-lactide-co-glycolide) (PLGA, 85:15, $M_w$ = 50 – 75,000 g mol$^{-1}$), 2α-mannobiose, sodium periodate, N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide (EDC), calcium chloride, manganese chloride, poly[(m-phenylenevinylene)-*alt*-(2,5-dibutoxy-p-phenylenevinylene)] (MEHPV, blue emission dye), poly[tris(2,5-bis(hexyloxy)-1,4-henylenevinylene)-*alt*-(1,3-phenylenevinylene)] (PTDPV, green emission dye), Tween 20, chloroform, N,N′-dimethylformamide (DMF) were purchased from Sigma-Aldrich. 2-Methoxyethanol, methyl mercaptoacetate were purchased from Alfa Aesar. Hydrazine hydrate (80% in water), calcium hydride, sodium were purchased from Merck KGAA. Tetrahydrofuran (THF), chloroform, methanol were purchased from BDH Prolabo. TRITC-labeled Concanavalin A (TRITC-ConA) was purchased from Vector Laboratories. N-hydroxysulfosuccinimide (sulfo-NHS) was purchased from Thermo Scientific, rhodamine polyethylene glycol acid (RB-PEG-COOH, $M_w$ = 3,000 g mol$^{-1}$) was purchased from Nanocs, Inc. N-2-Hydroxyethylpiperazine-N-2-ethane sulfonic acid (HEPES, 1M buffer solution, Gibco) was purchased from Life Technologies. Tissue-Tek O.C.T. compound was purchased from Andwin Scientific. THF was distilled from sodium and used immediately thereafter. Naphthalene was recrystallized in methanol. Potassium naphthalenide

was prepared as described elsewhere.[1] 2-Methoxyethanol, AGE and BnGE were freshly distilled from calcium hydride. Dialysis membrane Spectra/Por 6 (MWCO = 1,000 g $mol^{-1}$) was purchased from Spectrum Laboratories.

**Characterization**

$^{1}H$ NMR and $^{13}C$ NMR spectra were recorded using a Bruker Avance III 500 spectrometer with $CDCl_3$ and DMSO-$d_6$ as solvents. Working frequencies were 500 MHz for $^{1}H$ and 125 MHz for $^{13}C$ NMR. GPC was performed on a Toson EcoSEC GPC system with an RI-detector, using THF as mobile phase at 30 °C with an elution rate of 1.0 mL $min^{-1}$. Three PSS SDV columns (100 Å, 5 µ, 8.0 x 300 mm 1000 Å, 5 µ, 8.0 x 300 mm and 100000 Å, 5 µ, 8.0 x 300 mm) were calibrated by polystyrene standards (PSS). MALDI-TOF-MS spectra were recorded on an AB SCIEX 4800 Proteomics Analyzer. α-Cyano-4-hydroxycinnamic acid and dithranol were used as the matrixes and sodium iodide as an ionizing agent. The particles were visualized using a Confocal Laser Scanning Microscopy (CLSM) (Olympus, FluoView 500). 405 nm laser, 488 nm Argon laser, and 533 nm Helium-Neon green (HeNeG) laser were used to excite MEHPV, PTDPV and TRITC (labeling dyes for ConA), respectively. The barrier filters were set to 430–460 nm for MEHPV, 505–525 nm for PTDPV, and > 560 nm for TRITC. The confocal Raman microscopy was performed on WITec alpha300R utilizing 532 nm laser. Microfibers were dried under vacuum overnight prior to measurements to remove any remaining solvents. Spectra were acquired using an integration time of 0.5 sec per pixel with image scan area of 100 pixels x 100 pixels.

**Polymer Synthesis**

*Polymerization*

Polymerization was carried out in a flame-dried Schlenk-flask under argon atmosphere at room temperature. Potassium naphthalenide was added to the solution of 2-methoxyethanol in

dry THF till the green color remained. Next, the monomer (25 w $v^{-1}$%) was introduced into the reaction vessel and reaction mixture was stirred for 72 h. The reaction was terminated by the addition of acidified methanol, and the solvent was evaporated in vacuo. Monomer conversion was calculated based on $^1$H NMR spectra of crude polymer. The obtained polymer was further purified chromatographically on a silica gel column with gradient elution (chloroform to chloroform/methanol (10:0.7) mixture as an eluent).

*Poly(Ally Glycidyl Ether) (PAGE, **1a**)*. The initial ratio 2-methoxyethanol:AGE was 1:60. Conversion: 99%; yield after chromatographic purification: 97%. GPC: $M_n$ 6900 g mol$^{-1}$, $M_w$ 7700 g mol$^{-1}$, PDI 1.11. $^1$H NMR (500 MHz, $CDCl_3$, δ): 5.93–5.82 (m, 1H, $CH_2$=C<u>H</u>), 5.20 (dd, 2H, C<u>H$_2$</u>=CH), 3.98 (d, 2H, $CH_2$=CHC<u>H$_2$</u>O), 3.68–3.43 (m, 5H, $CH_{2\text{-PEG}}$ + $CH_{PEG}$ + $CH_{PEG}$C<u>H$_2$</u>O), 3.37 (s, 3H, $CH_3O$); $^{13}$C NMR (125 MHz, $CDCl_3$, δ): 135.1 ($CH_2$=<u>C</u>H), 116.9 (<u>C</u>$H_2$=CH), 79.0–78.8 ($CH_{PEG}$), 72.4 ($CH_2$=CH<u>C</u>$H_2$O), 70.4–69.9 (2C, $CH_{2\text{-PEG}}$ + $CH_{PEG}$<u>C</u>$H_2$O), 59.2 ($CH_3O$).

*Poly(Ally Glycidyl Ether-co-Benzyl Glycidyl Ether) (P(AGE-co-BnGE), **1b**)*. The ratio 2-methoxyethanol:AGE:BnGE was 1:20:40. Conversion: 99% (AGE), 97% (BnGE); yield after chromatographic purification: 98%. GPC: $M_n$ 7700 g mol$^{-1}$, $M_w$ 8200 g mol$^{-1}$, PDI 1.07. $^1$H NMR (500 MHz, $CDCl_3$, δ): 7.34–7.18 (5H, $H_{Ar}$), 5.83 (1H, $CH_2$=C<u>H</u>), 5.16 (2H, C<u>H$_2$</u>=CH), 4.46 (2H, OC<u>H$_2$</u>Ph), 3.92 (2H, $CH_2$=CHC<u>H$_2$</u>O), 3.75–3.37 (5H and 5H, $CH_{2\text{-PEG}}$ + $CH_{PEG}$ + $CH_{PEG}$C<u>H$_2$</u>O), 3.33 (3H, $CH_3O$); $^{13}$C NMR (125 MHz, $CDCl_3$, δ): 138.6 ($C_{Ar}$), 135.1 ($CH_2$=<u>C</u>H), 128.4 (2C, $CH_{Ar}$), 127.7 (2C, $CH_{Ar}$), 127.6 ($CH_{Ar}$), 116.8 (<u>C</u>$H_2$=CH), 79.1–78.8 ($CH_{PEG}$), 73.4 (O<u>C</u>$H_2$Ph), 72.3 ($CH_2$=CH<u>C</u>$H_2$O), 70.5–69.8 (2C, $CH_{2\text{-PEG}}$ + $CH_{PEG}$C<u>H$_2$</u>O), 59.1 ($CH_3O$).

*Thiol-Ene Reaction*

Methyl mercaptoacetate (5 eq) and AIBN (0.15 eq) were added to the solution of purified polymer **1** (1 eq C=C) in dry THF under an argon atmosphere. The mixture was refluxed for

5 h. The solvent was removed in vacuo and the residue was purified on silica gel column with gradient elution (chloroform to chloroform/methanol (10:0.7) mixture as an eluent).

*Ester PEG (**2a**)*. Conversion: 99%; yield after chromatographic purification: 91%. GPC: $M_n$ 9400 g mol$^{-1}$, $M_w$ 10400 g mol$^{-1}$, PDI 1.11.$^1$H NMR (500 MHz, $CDCl_3$, δ): 3.73 (s, 3H, $COOCH_3$), 3.64–3.40 (m, 7H, $CH_{2\text{-PEG}}$ + $CH_{PEG}$ + $CH_{PEG}C\underline{H}_2O$ + $OC\underline{H}_2CH_2$), 3.37 (s, 3H, $CH_3O$), 3.23 (s, 2H, $SC\underline{H}_2COOMe$), 2.70 (t, 2H, $SC\underline{H}_2CH_2$), 1.85 (m, 2H, $SCH_2C\underline{H}_2CH_2O$); $^{13}$C NMR (125 MHz, $CDCl_3$, δ): 170.9 (C=O), 79.0–78.8 ($CH_{PEG}$), 71.1–70.0 (2C, $CH_{2\text{-PEG}}$ + $CH_{PEG}\underline{C}H_2O$), 69.6 ($O\underline{C}H_2CH_2$), 52.4 ($COO\underline{C}H_3$), 33.4 ($S\underline{C}H_2COOMe$), 29.5 ($S\underline{C}H_2CH_2$), 29.2 ($SCH_2\underline{C}H_2CH_2O$).

*Copolymer of ester PEG (**2b**)*. Conversion: 99%; yield after chromatographic purification: 81%. GPC: $M_n$ 8300 g mol$^{-1}$, $M_w$ 9100 g mol$^{-1}$, PDI 1.10. $^1$H NMR (500 MHz, $CDCl_3$, δ): 7.35–7.18 (5H, $H_{Ar}$), 4.46 (2H, $OC\underline{H}_2Ph$), 3.69 (3H, $CH_3$), 3.66–3.36 (7H and 5H, $CH_{2\text{-PEG}}$ + $CH_{PEG}$ + $CH_{PEG}C\underline{H}_2O$ + $OC\underline{H}_2CH_2$), 3.37 (3H, $CH_3O$), 3.17 (2H, $SC\underline{H}_2COOMe$), 2.64 (2H, $SC\underline{H}_2CH_2$), 1.80 (2H, $SCH_2C\underline{H}_2CH_2O$); $^{13}$C NMR (125 MHz, $CDCl_3$, δ): 171.0 (C=O), 138.6 ($C_{Ar}$), 128.4 (2C, $CH_{Ar}$), 127.6 (3C, $CH_{Ar}$), 79.1–78.7 ($CH_{PEG}$), 73.4 ($O\underline{C}H_2Ph$), 71.1–68.8 (2C, $CH_{2\text{-PEG}}$ + $CH_{PEG}\underline{C}H_2O$), 69.7 ($O\underline{C}H_2CH_2$), 52.5 ($COO\underline{C}H_3$), 33.5 ($S\underline{C}H_2COOMe$), 29.5 ($S\underline{C}H_2CH_2$), 29.2 ($SCH_2\underline{C}H_2CH_2O$).

*Conversion to Hydrazide-functionalized PEG derivatives*

Polymer **2** (1 eq COOMe) was refluxed with 80% water solution of hydrazine hydrate (100 eq) in THF for 5 h. The solvent was evaporated and the product was purified as specified for each polymer.

*Hydrazide PEG (PHZ, **3a**)*. The raw product was dialyzed against water for 48 h and dried in vacuo. Yield 87%. $^1$H NMR (500 MHz, DMSO-$d_6$, δ): 9.11 (s, 1H, NH), 4.29 (s, 2H, $NH_2$), 3.65–3.20 (m, 7H, $CH_{2\text{-PEG}}$ + $CH_{PEG}$ + $CH_{PEG}C\underline{H}_2O$ + $OC\underline{H}_2CH_2$), 3.25 (s, 3H, $CH_3O$), 3.04 (s, 2H, $SC\underline{H}_2CONHNH_2$), 2.60 (t, 2H, $SC\underline{H}_2CH_2$), 1.76 (m, 2H, $SCH_2C\underline{H}_2CH_2O$); $^{13}$C NMR

(125 MHz, DMSO-$d_6$, δ): 168.5(C=O), 78.3–78.0 ($CH_{PEG}$), 70.4–69.3 ($CH_{2\text{-}PEG}$ + $CH_{PEG}\underline{C}H_2O$), 69.1 ($O\underline{C}H_2CH_2$), 32.6 ($S\underline{C}H_2CONHNH_2$), 28.9 ($S\underline{C}H_2CH_2$), 28.6 ($SCH_2\underline{C}H_2CH_2O$).

*Copolymer of Hydrazide PEG (P(HZ-co-BnGE, **3b**)*. The raw product was dissolved in chloroform, washed with water, brine and dried over $Na_2SO_4$. The solvent was removed in vacuo. Yield 71%. $^1$H NMR (500 MHz, $CDCl_3$, δ): 7.27 (5H, $H_{Ar}$), 4.47 (2H, $OC\underline{H}_2Ph$), 3.80–3.30 (7H and 5H, $CH_{2\text{-}PEG}$ + $CH_{PEG}$ + $CH_{PEG}C\underline{H}_2O$ + $OC\underline{H}_2CH_2$), 3.34 (3H, $CH_3O$), 3.16 (broad s, 2H, $SC\underline{H}_2CONHNH_2$), 2.60 (2H, $SC\underline{H}_2CH_2$), 1.78 (2H, $SCH_2\underline{C}H_2CH_2O$); $^{13}$C NMR (125 MHz, $CDCl_3$, δ): 169.6 (C=O), 138.5 ($C_{Ar}$), 128.5 (2C, $CH_{Ar}$), 127.7 (3C, $CH_{Ar}$), 79.1–78.7 ($CH_{PEG}$), 73.4 ($O\underline{C}H_2Ph$), 70.5–69.5 (2C, $CH_{2\text{-}PEG}$ + $CH_{PEG}\underline{C}H_2O$), 62.6 ($O\underline{C}H_2CH_2$), 34.4 ($S\underline{C}H_2CONHNH_2$), 29.8 ($S\underline{C}H_2CH_2$), 29.2 ($SCH_2\underline{C}H_2CH_2O$).

**Fabrication of Microparticles and Their Surface Modification**

*Fabrication of Bicompartmental PLGA/Polymer **3b** Microspheres*

Two different polymer solutions were prepared in separate vials. One solution contained 9 w $v^{-1}$% PLGA dissolved in a solvent mixture of chloroform and DMF (95:5, v $v^{-1}$). Another solution was made by mixing 9 w $v^{-1}$% of PLGA with 0.9 w $v^{-1}$% of polymer **3b** (10% by weight of PLGA) in the same ratio of solvents. The experimental setup of EHD co-jetting includes a syringe pump (Fisher Scientific, Inc., USA), a power supply (DC voltage source, Gamma High Voltage Research, USA), and a grounded collector. Each one of the two polymer solutions was delivered at a constant flow rate of 0.4 ml $h^{-1}$ via vertically positioned side-by-side syringes equipped with 26 G needles (Hamilton Company). When a driving voltage of 4.5 kV was applied to the polymer solutions, a stable Taylor Cone was formed and microspheres were collected at a distance of 40 cm.

*Fabrication of Bicompartmental PLGA/Polymer **3b** Microfibers*

In the case of microfibers, the experimental setup was similar to that of the microspheres except the concentration of PLGA was 30 w $v^{-1}$% in both solutions, and 15 w $v^{-1}$% of polymer **3b** (50% by weight of PLGA) was introduced in one of the solutions. Each one of the two polymer solutions was delivered at a constant flow rate of 0.05 ml $h^{-1}$ with a driving voltage of 12 kV. The microfibers were collected at a distance of 7 cm using a rotary collector.

*Fabrication of Bicompartmental PLGA/Polymer **3b**Microcylinders*

Once the bicompartmental PLGA/polymer **3b** microfibers were fabricated, the cylinders were produced by cryosectioning as previously reported.[2] Briefly, the microfibers were embedded in a freezing medium (OCT) and sectioned at –20 °C by a cryostat microtome (HM550 OMC, Microme) with a desired length of 70 μm. After the samples were collected, remaining OCT was removed by extensive washing with 0.01 v $v^{-1}$% Tween 20/DI-water at room temperature.

*Selective Fluorescence-Labeling of PLGA/Polymer **3b** Microspheres*

PLGA/polymer **3b** microspheres were first collected and suspended in an aqueous solution containing 0.01% Tween 20. To visually confirm the presence of hydrazide groups on the particle surface, rhodamine-labeled carboxyl groups were conjugated using EDC/NHS chemistry. Briefly, 10 mM RB-PEG-COOH/PBS containing 0.01% Tween 20 in a total volume of 1 ml was activated with 40 mM EDC for 10 min, and then with 10 mM sulfo-NHS for another 10 min. Next, approximately 300 μg of the particles were reacted with the solution for 2 h. Finally, the particles were washed 20 times by centrifugation to remove any unreacted chemicals, and the selective binding of rhodamine on the particles was confirmed through CLSM.

*Selective Sugar-Lectin Binding of PLGA/Polymer **3b** Microcylinders*

Prior to the sugar-lectin reaction, 2α-mannobiose was first oxidized with sodium periodate to obtain free aldehyde groups. The aldehyde groups were then selectively conjugated with hydrazide groups on the cylinder surface. The reaction condition was as follows. Approximately 50,000 bicompartmental PLGA/polymer **3b** microcylinders were kept rotating with 0.85 mg of 2α-mannobiose and 2.14 mg of sodium periodate in Eppendorf tube containing 1 mL of 0.01 v $v^{-1}$%Tween 20/DI-water for 5 h at room temperature. The unreacted molecules were removed by repeated washing with 0.01 v $v^{-1}$% Tween 20/DI-water. In order to confirm selective immobilization of 2α-mannobiose on the microcylinders, a sugar-lectin reaction was performed by incubating the mannose-binding lectin ConA. The 2α-mannobiose-bound microcylinders were reacted with 100 μg of TRITC-ConA and gently rotated in the buffer solution (10 mM HEPES, 1 mM $CaCl_2$, 1 mM $MnCl_2$, 0.01% Tween 20) for 3 h at room temperature. The unreacted molecules were removed by washing with the same buffer solution.

[1] J. Zhang, Y.-J. Zhao, Zh.-G. Su, G.-H. Ma, *J. Appl. Polym. Sci.* **2007**, *105*, 3780.

[2] S. Bhaskar, J. Hitt, S.-W. L. Chang, J. Lahann, *Angew. Chem. Int. Ed.* **2009**, *48*, 4589.

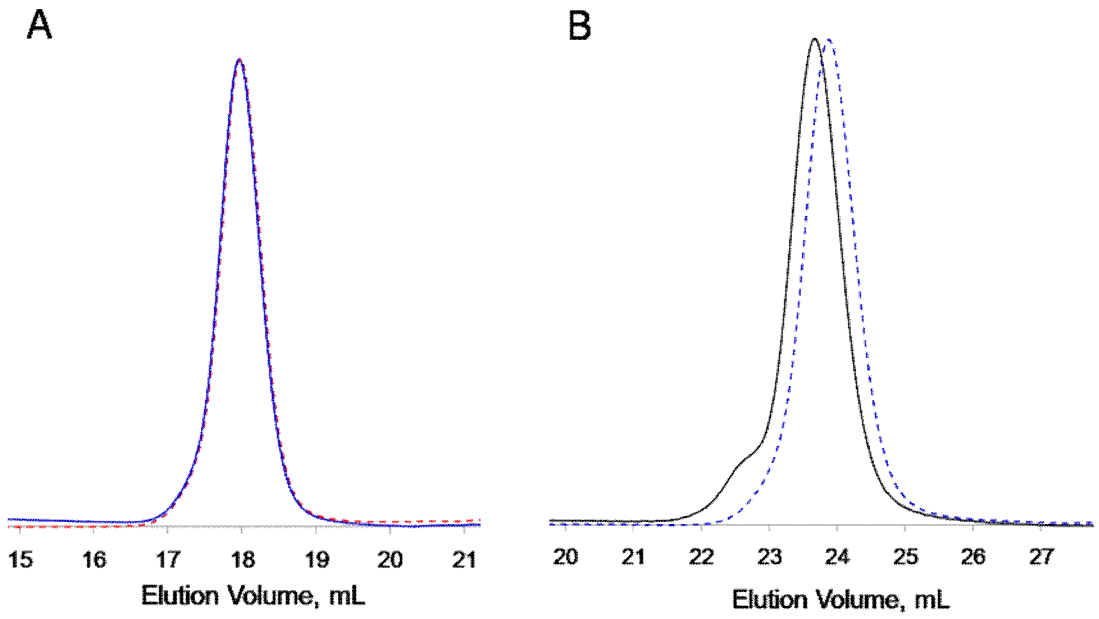


*Figure S1.* Gel permeation chromatograms of polymer **1b** before (red dashed line) and after (blue solid line) chromatographic purification on silica gel (A). Gel permeation chromatograms of polymer **1b** (blue dashed line) and **2b** (black solid line). Measurements were performed in THF relative to polystyrene calibration standards.

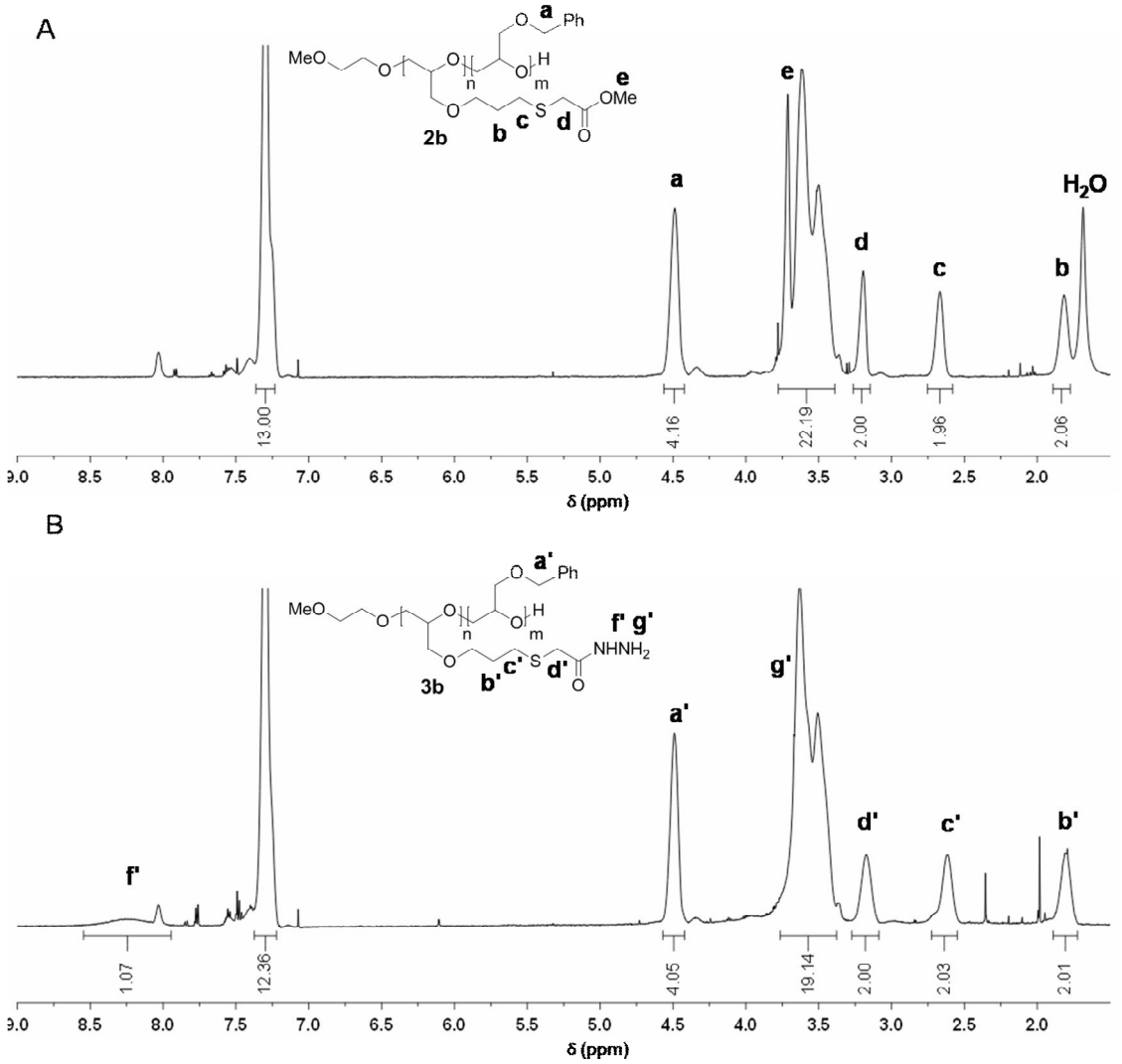


*Figure S2.* Comparative $^{1}$H NMR spectra of the polymers **2b** (A) and **3b** (B). Solvent $CDCl_3$.

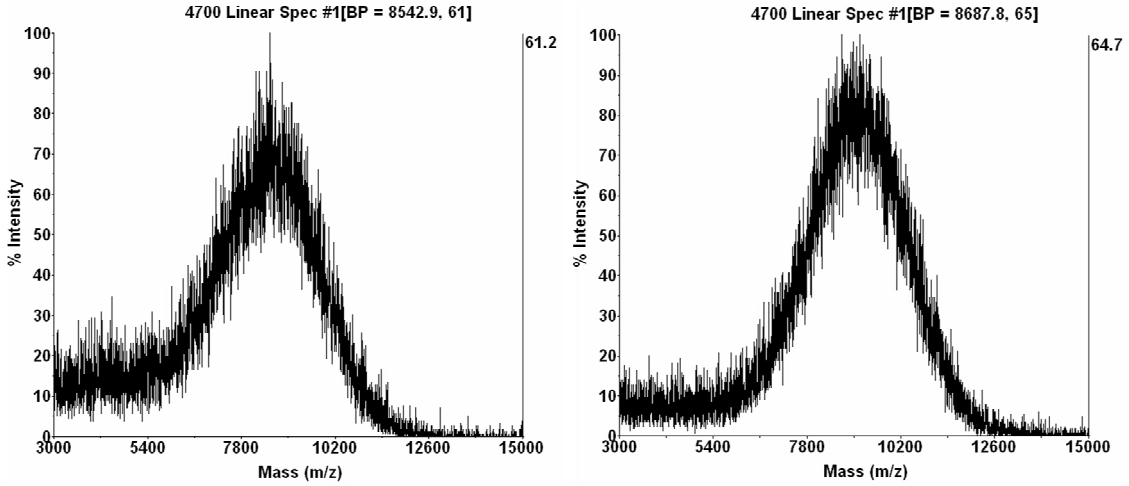


*Figure S3.* MALDI-TOF spectra of the polymers **2b** (A) and **3b** (B).

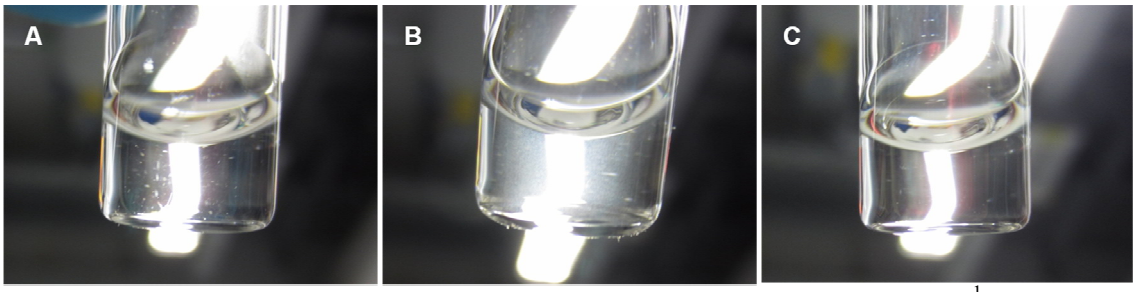


*Figure S4.* Comparison of the solubility in water of the polymers **3a** (5 μg $ml^{-1}$) (A) and **3b** (0.5 μg $ml^{-1}$) (B). The concentration of the polymer **3b** in the solution shown in image B is one hundred times lower than that of the polymer **3a** shown in image A; however solution of the polymer **3b** is cloudy, while solution of the polymer **3a** is completely clear. This indicates a considerably higher solubility of the polymer **3b** as compared to the polymer **3a** which is achieved due to the incorporation of hydrophobic phenyl moieties into the polymer structure. Image of pure water is shown for comparison (C).