Thiol-Isocyanate "Click" Reactions: Rapid Development of Functional Polymeric Surfaces

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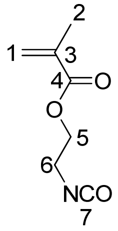
Supporting Information.

Materials and methods. All reagents and solvents were obtained at the highest purity available from Aldrich Chemical Company or Fisher Scientific and used without further purification unless otherwise specified. 2-Hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone (Irgacure 2959) was purchased from Ciba Specialty Chemicals. Mercaptopropylisobutyl Polyhedral Oligomeric Silsesquioxane (POSS)® was generously donated by Hybrid Plastics. 2-Isocyanatoethyl methacrylate was purchased from TCI America and passed through neutral alumina before use to remove the BHT inhibitor.

Characterization. A Varian Mercury Plus 300MHz NMR spectrometer operating at a frequency of 300 MHz with VNMR 6.1C software was used for proton and carbon analysis. Contact angle goniometry was performed using a Ramé-hart 200-00 Std.-Tilting B. goniometer. Static (θ_{sw}) contact angles were measured using 10 μL water droplets. Ellipsometric measurements were carried out using a Gaertner Scientific Corporation LSE ellipsometer with a 632.8 nm laser at 70° from the normal. Refractive indices of 3.89 for silicon, 1.46 for silicon oxide, 1.43 for initiator, and 1.5 for the polymer were used. ATR-FT-IR spectra of surface polymers were carried out using a Nicolet 8700 with a gradient-angle ATR attachment using Omnic software. Spectra were taken with a resolution of 4 cm^{-1} by accumulating a minimum of 64 scans per run. Nitrogen was constantly purged through the attachment to reduce interference of carbon dioxide and water. Optical images of the micropatterns were taken using a Keyence VHX digital microscope with a multi-illumination lighting system. Fluorescent images were taken using a Nikon eclipse 80i with a fict filter, Plan-Fluor 20x/0.50 scope, and a photometrics coolsnap cf camera using NIS-Elements F software. AFM imaging was performed in tapping mode on a Multimode Nanoscope IIIa (Digital Instruments/Veeco Metrology Group) using silicon AFM probes with a nominal spring constant of 40 N/m and a resonant frequency of 300 kHz. The scan rate used was 0.374 Hz.

^{1}H and ^{13}C NMR of 2-Isocyanatoethyl Methacrylate. NMR analysis reflects the pure monomer solution after removing the BHT inhibitor.



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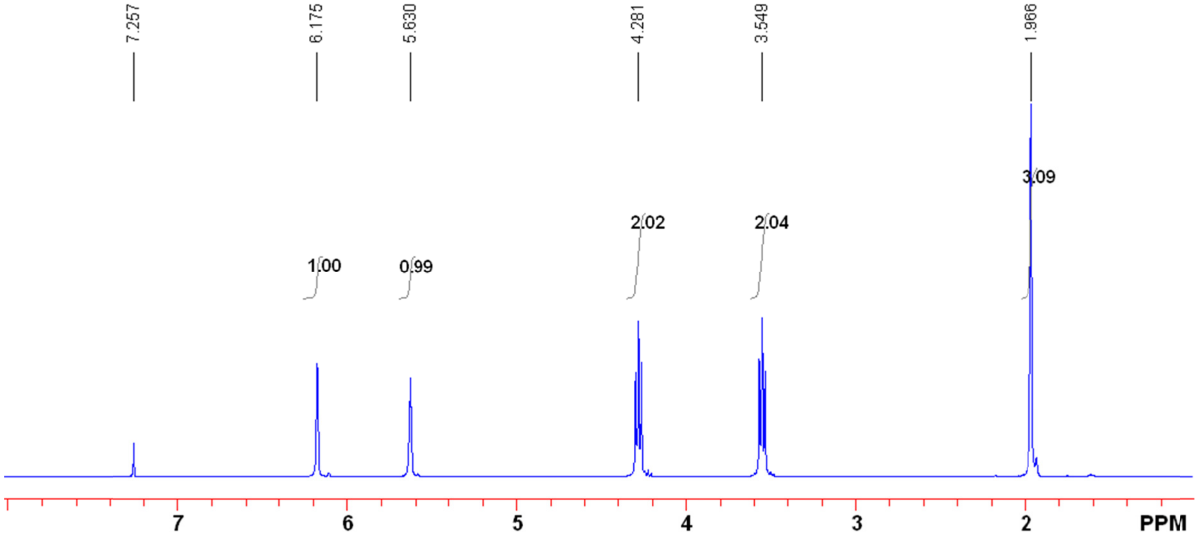
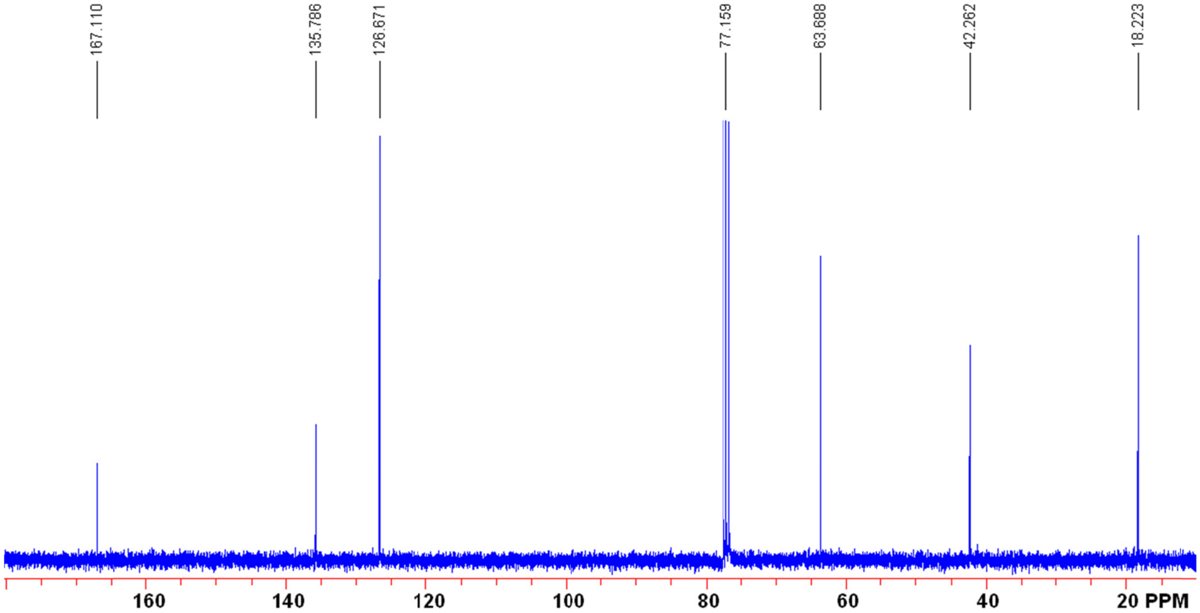


^{1}H-NMR ($CDCl_3$; ppm): 1.97 (3H, C2), 3.55 (2H, C5), 4.28 (2H, C6), 5.63 (1H, C1), 6.18 (1H, C1)



^{13}C-NMR ($CDCl_3$; ppm): 18.22 (C2), 42.26(C6), 63.69(C5), 126.67 (C1, C7), 135.79 (C3), 167.11 (C3)

Thiol-Isocyanate "Click" Reactions. All thiol-isocyanate reactions were catalyzed using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) under ambient laboratory conditions (i.e. r.t. and normal atmosphere) for 12 minutes unless otherwise specified. No catalyst was required for amine-isocyanate reactions. Reaction mixtures were *not* degassed prior to use. After X-isocyanate (X = thiol or amine) reactions, the samples were washed extensively with THF, and toluene. Details of the various X-isocyanate reactions are given below. In all cases, a significant change in wettability could be observed within *seconds* of initiation. However, reaction times were chosen to ensure complete conversion of the isocyanate units on the surface.

3-mercaptoproprionic acid. A solution of 3-mercaptoproprionic acid (1.2 mL, 13.8 mmol) and THF (3.6 mL), 1:3 (v/v) ratio, was prepared. A 500:1 (mol/mol) ratio of thiol:DBU (4.12 µL DBU) was used to catalyze the reaction. The thiol solution was placed into the reaction vessel containing the isocyanate-functionalized polymer brush followed by the addition of the catalyst, and subsequently allowed to react for 12 minutes.

1-dodecanethiol. A solution of 1-dodecanethiol (1.5 mL, 6.26 mmol) and THF (4.5 mL), 1:3 (v/v) ratio, was prepared. A 500:1 (mol/mol) ratio of thiol:DBU (1.91 µL DBU) was used to catalyze the reaction. The thiol solution was placed into the reaction vessel containing the isocyanate-functionalized polymer brush followed by the addition of the catalyst, and subsequently allowed to react for 12 minutes.

1-thioglycerol. A solution of 1-thioglycerol (1.2 mL, 13.84 mmol) and THF (3.6 mL), 1:3 (v/v) ratio, was prepared. A 500:1 (mol/mol) ratio of thiol:DBU (4.13 µL DBU) was used to catalyze the reaction. The thiol solution was placed into the reaction vessel containing the isocyanate-functionalized polymer brush followed by the addition of the catalyst, and subsequently allowed to react for 12 minutes.

N-acetyl-L-cysteine. A solution of N-acetyl-L-cysteine (0.25g, 1.5 mmol) and a 2:1 (v/v) THF:DMF (3.2 mL, 1.6mL) was prepared. A 300:1 (mol/mol) ratio of thiol:DBU (0.75 µL DBU) was used to catalyze the reaction. The thiol solution was placed into the reaction vessel containing the isocyanate-functionalized polymer brush followed by the addition of the catalyst, and subsequently allowed to react for 12 minutes.

Benzyl mercaptan. A solution of benzyl mercaptan (1.2 mL, 9.28 mmol) and THF (3.6 mL), 1:3 (v/v) ratio, was prepared. A 500:1 (mol/mol) ratio of thiol:DBU (2.77 µL DBU) was used to catalyze the reaction. The thiol solution was placed into the reaction vessel containing the isocyanate-functionalized polymer brush followed by the addition of the catalyst, and subsequently allowed to react for 12 minutes.

1-adamantanethiol. A solution of 1-adamantanethiol (0.25g, 1.49 mmol) and THF (4.8 mL) was prepared. A 300:1 (mol/mol) ratio of thiol:DBU (0.71 µL DBU) was used to catalyze the reaction. The thiol solution was placed into the reaction vessel containing the isocyanate-functionalized polymer brush followed by the addition of the catalyst, and subsequently allowed to react for 12 minutes.

Thiocholesterol. A solution of thiocholesterol (0.15g, 0.37 mmol) and THF (4.8 mL) was prepared. A 100:1 (mol/mol) ratio of thiol:DBU (0.56 µL DBU) was used to catalyze the reaction. The thiol solution was placed into the reaction vessel containing the isocyanate-functionalized polymer brush followed by the addition of the catalyst, and subsequently allowed to react for 12 minutes.

Mercaptopropylisobutyl Polyhedral Oligomeric Silsesquioxane (POSS)®. A solution of POSS (1.0g, 0.0011 mmol) and THF (4.8 mL) was prepared. A 300:1 (mol/mol) ratio of thiol:DBU (0.56 µL DBU) was used to catalyze the reaction. The thiol solution was placed into the reaction vessel containing the isocyanate-functionalized polymer brush followed by the addition of the catalyst, and subsequently allowed to react for 12 minutes.

Furfuryl mercaptan. A solution of furfuryl mercaptan (1.2 mL, 11.82 mmol) and THF (3.6 mL), 1:3 (v/v) ratio, was prepared. A 500:1 (mol/mol) ratio of thiol:DBU (3.53 µL DBU) was used to catalyze the reaction. The thiol solution was placed into the reaction vessel containing the isocyanate-functionalized polymer brush followed by the addition of the catalyst, and subsequently allowed to react for 12 minutes.

Hexyl amine. A solution of hexyl amine (0.4 mL, 3.02 mmol) and THF (1.2 mL), 1:3 (v/v) ratio, was prepared. The amine solution was placed into the reaction vessel containing the isocyanate-functionalized polymer brush and allowed to react for 12 minutes.

Benzyl amine. A solution of benzyl amine (0.4 mL, 3.67 mmol) and THF (1.2 mL), 1:3 (v/v) ratio, was prepared. The amine solution was placed into the reaction vessel containing the isocyanate-functionalized polymer brush and allowed to react for 12 minutes.

Table SI.1: IR Absorption Bands of "Functional" Polymer Brushes.[1] Peak assignments provided:

Figure	Description	Absorption (cm^{-1})	Peak Assignment
a)	p(isocyanatoethyl methacrylate) Brush	2275	N=C=O
		1729	C=O; ester
b)	3-mercaptopropionic acid	3430 – 3174	NH-CO
		3430 – 3174	O-H (associated w/ COOH)
		2930	C-H
		1725	C=O; ester
		1650	NH-CO-S
		1528	NH-CO (amide band II)
c)	1-dodecanethiol	3442 - 3215	NH-CO
		2954, 2924, 2852	C-H
		1731	C=O; ester
		1653	NH-CO-S
		1516	NH-CO (amide band II)
		1463	$(CH_2)_n$
d)	1-thioglycerol	3573 – 3125	O-H
		3573 – 3125	NH-CO
		2930, 2882	C-H
		1725	C=O; ester
		1650	NH-CO-S
		1528	NH-CO (amide band II)
e)	N-acetyl-L-cysteine	3450 - 3162	NH-CO
		3450 - 3162	NH-CO-CH_3
		2989, 2942	C-H
		1725	C=O; ester
		1654	NH-CO-S
		1531	NH-CO (amide band II)
f)	Benzyl mercaptan	3326	NH-CO
		3084, 3058, 3025	=C-H
		2942, 2859	C-H
		1725	C=O; ester
		1657	NH-CO-S
		1517, 1493, 1451	C=C
g)	1-admantanethiol	3350	NH-CO
		2906, 2850	C-H
		1731	C=O; ester
		1669	NH-CO-S
h)	Thiocholesterol	3350	NH-CO
		2936, 2903, 2865, 2850	C-H
		1728	C=O; ester
		1671	NH-CO-S
		1556	NH-CO (amide band II)
i)	Mercaptopropylisobutyl POSS®	3347	NH-CO
		2954, 2927, 2906, 2870	C-H
		1731	C=O; ester
		1665	NH-CO-S
		1109	Si-O
j)	Furfuryl mercaptan	3326	NH-CO
		2987, 2939	C-H
		1728	C=O; ester
		1663	NH-CO-S
		1523	NH-CO (amide band II)
		1204, 1156	C-O (cyclic ether)
		1068	C-O-C (cyclic ethers: 5-membered ring)
k)	Hexyl amine	3344	NH-CO
		2954, 2930, 2856	C-H
		1731	C=O; ester
		1573	NH-CO-NH
		1460	$(CH_2)_n$
l)	Benzyl amine	3353	NH-CO
		3085, 3061, 3025	=C-H
		2933, 2868	C-H
		1728	C=O; ester
		1567	NH-CO-NH
		1565, 1493, 1451	C=C

Water Contact Angle Measurements (WCA).

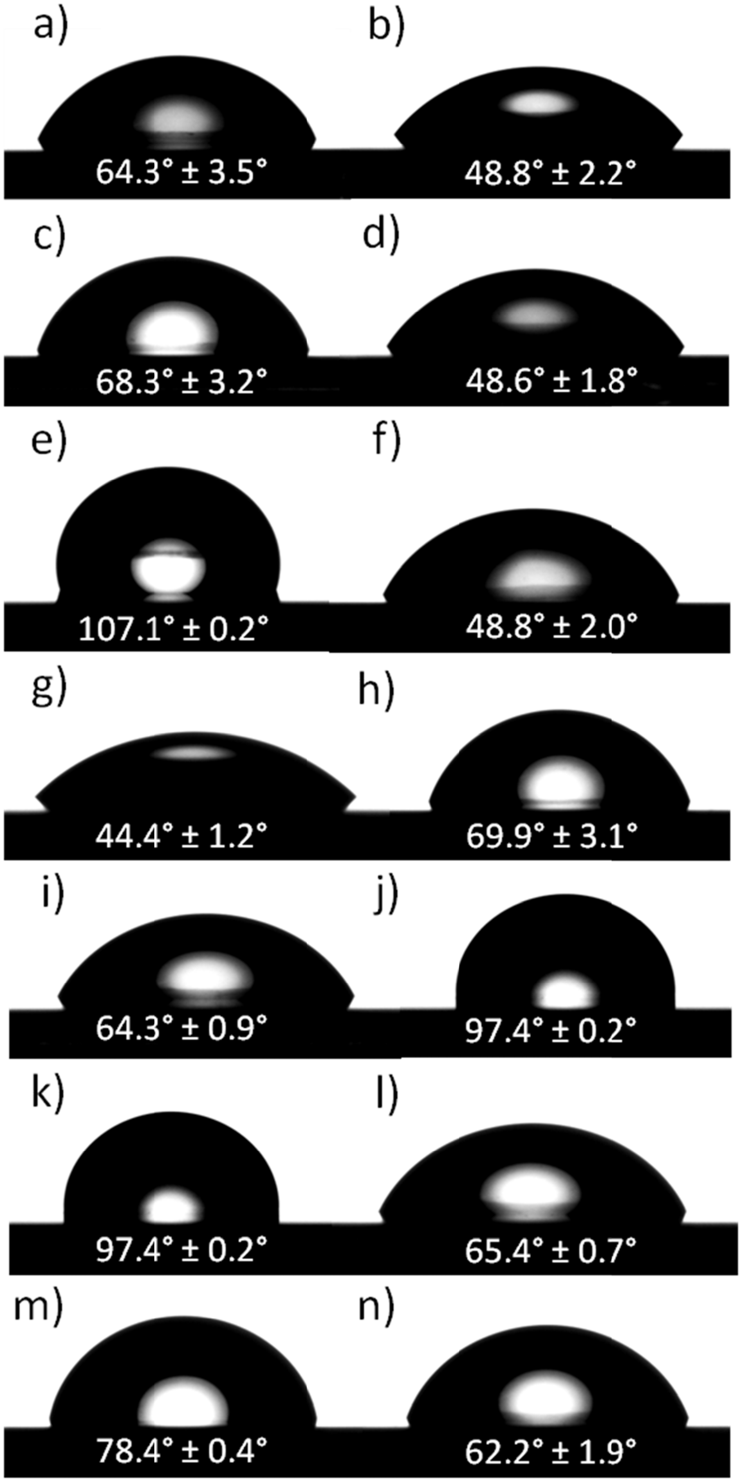


Figure SI.1: WCA: a) Protected initiator b) Deprotected initiator c) 2-isocyanatoethyl methacrylate polymer brush d) 3-mercaptopropionic acid e) 1-dodecanethiol f) 1-thioglycerol g) N-acetyl-L-cysteine h) Benzyl mercaptan i) 1-admantanethiol j) Thiocholesterol k) Mercaptopropylisobutyl POSS® l) Furfuryl mercaptan m) Hexyl amine n) Benzyl amine

Thickness measurements.

Table SI.2: Thickness measurements before/after X-isocyanate "click" reactions.

	Thickness (nm)	
	Initiator*	
	Protected	Deprotected
	1.82 ± 0.56	0.92 ± 0.74
	Polymer Brush*	
	28.2 ± 6.05	
Thiol-Isocyanate "Click" Reactions		
Thiol Derivatives	**Polymer Brush****	**"Click" Rxn**
3-mercaptopropionic acid	37.8 ± 2.84	72.9 ± 1.58
1-dodecanethiol	18.3 ± 0.75	47.8 ± 1.52
1-thioglycerol	37.8 ± 2.84	71.3 ± 3.26
N-acetyl-L-cysteine	29.5 ± 1.94	69.5 ± 5.04
Benzyl mercaptan	19.2 ± 1.68	38.9 ± 2.15
1-admantanethiol	19.2 ± 1.68	44.5 ± 5.14
Thiocholesterol	28.9 ± 3.60	104.1 ± 1.05
Mercaptopropylisobutyl POSS®	28.9 ± 3.60	81.9 ± 1.10
Furfuryl mercaptan	29.5 ± 1.94	51.0 ± 3.12
Amine-Isocyanate "Click" Reactions		
Benzyl amine	33.8 ± 0.57	55.6 ± 2.29
Hexyl amine	28.3 ± 0.43	53.6 ± 1.58

* Values indicative of all substrates used before "Click" Reactions

** Values indicative of individual Polymer Brush uniformity before functionalization

Fluorescent Dye Functionalized Polymer Brush Preparation. Water contact angle measurements (Figure SI.2) were the only means of determining the covalent attachment of each molecule synthesized onto the surface. Microscope cover glass slides were cut into appropriate sized pieces and ultrasonically cleaned in acetone, ethanol, and toluene for 15 min. in each solvent. The substrates were dried under a stream of N_2 and treated with UV-ozone for 45 min. Surface-initiated polymerization of **1** was facilitated according to our recent work with p(PgMA) polymer brushes.[2] A 1:6 vol.% solution of **1** in dry THF was injected into a microfluidic channel containing the initiator-immobolized substrate and subsequently exposed to UV light for 20 minutes. After polymer brush formation, a thiol-isocyanate "click" reaction with 3-mercaptopropionic acid was performed with subsequent deprotonation with 0.1M KOH solution for 5 minutes. The deprotonated MPA functionalized polymer brush was allowed to form ionic interactions with acridine orange (0.10g, 0.38 mmol) in deionized water (10 mL) for 30 minutes with subsequent rinsing with deionized water before characterization.

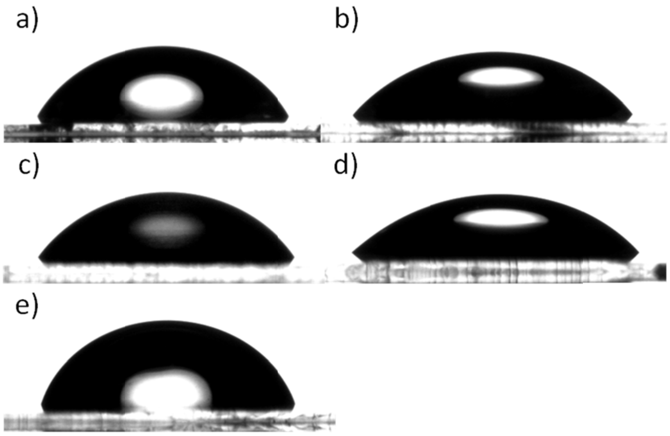


Figure SI.2: WCA: a) Protected initiator (64.3° ± 3.5°) b) Deprotected initiator (48.8° ± 2.2°) c) Protonated MPA polymer brush (48.9° ± 2.1°) d) Deprotonated MPA polymer brush (29.4° ± 1.7°) e) Acridine orange functionalized polymer brush (73.8° ± 3.5°)

Fluorescent microscopy and UV-Vis spectroscopy was performed before/after functionalization with the fluorescent dye, acridine orange, in order to monitor the absorbance of the polymer brush without and with incorporation of the fluorescent molecule.

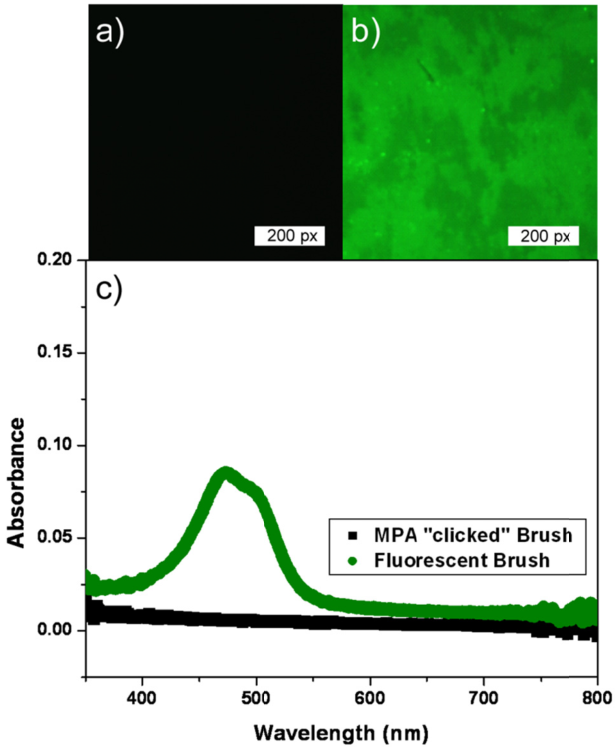


Figure SI.3: a) Fluorescent microscopy before functionalization with fluorescent dye b) Fluorescent microscopy after functionalization with fluorescent dye c) UV-Vis spectroscopy of "MPA" clicked polymer brush vs. polymer brush after functionalization with fluorescent dye.

Micropatterning and Sequential Thiol-Isocyanate Reactions. For patterning, a PDMS stamp was made from a master nanostamp (linewidth 15.0µm). Details of the procedure are given below.

PDMS stamp fabrication. 3 – 4 drops of (1H, 1H, 2H, 2H-perfluorooctyl) silane was added to the bottom of a small vacuum chamber containing a single crystal nanostamp. Vacuum was applied for 1 hour to create a monolayer of silane onto the nanostamp. A 10:1 mixture of Sylgard 184 silicone elastomer base (28.73g) and Sylgard silicone elastomer curing agent (2.87g) was mixed well in a disposable beaker followed by removing air bubbles by applying vacuum. Once the air bubbles are removed, the viscous Sylgard solution is poured over the silanated nanostamp in a plastic petri dish. The stamp is allowed to cure for 2 hours at room temperature followed by overnight exposure in an oven for 50°C.

Preparation of 3-mercapto-1-propanesulfonic acid/1-dodecanethiol micropatterns. The PDMS stamp (linewidth 15.0µm) previously made was placed onto an isocyanate-containing polymer brush and slight pressure was applied to ensure intimate contact between the stamp and surface. A solution of 3-mercapto-1-propanesulfonic acid (0.25g, 1.04 mmol) in methanol (10.0 mL) was prepared. A 300:1 (mol/mol) ratio of thiol:DBU (0.70µL DBU) was used to catalyze the reaction. Once prepared, the thiol solution containing the catalyst was wicked into the crevices of the stamp and allowed to react for 12 minutes. The stamp was then removed and the sample was washed with methanol and toluene followed by a sequential thiol-isocyanate reaction with 1-dodecanethiol to backfill the unexposed portion of the pattern. 1-dodecanethiol (1.5mL, 6.26 mmol) in THF (0.375 mL) with a 500:1 (mol/mol) ratio of thiol:DBU (1.91 µL DBU) was used for backfilling. Optical microscopy and AFM was used to analyze the micropatterned polymer brushes.

Polymer Brush Thickness as a Function of Polymerization Time.

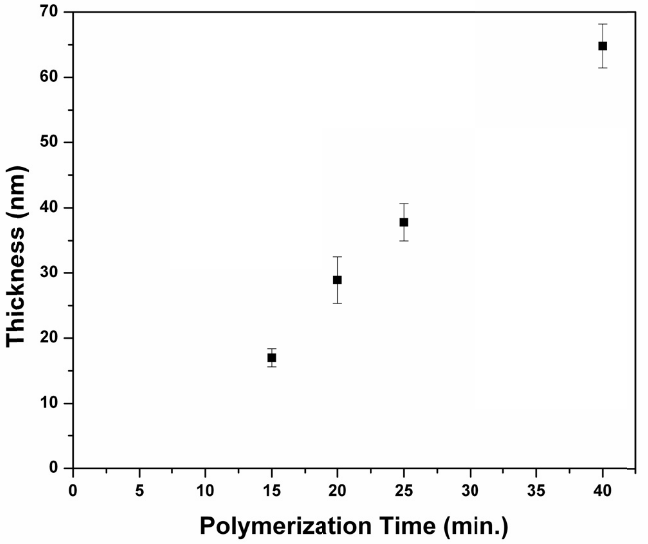


Figure SI.4: Isocyanate functionalized polymer brush thickness as a function of polymerization time.

The non-linear response of the thickness vs. polymerization time (or UV irradiation time) is the typical behavior observed for conventional free-radical surface-initiated photopolymerization. The behavior is also consistent with results reported by Schuh and coworkers[3] for other vinyl monomers using similar photoinitiator monolayers.

References

1. G. Socrates, *Infrared and Raman Characteristics Group Frequencies*, John Wiley & Sons Ltd., Chichester, 2001.
2. R. M. Hensarling, V. A. Doughty, J. W. Chan, D. L. Patton, *J. Am. Chem. Soc.*, 2009, **131**, 14673-14675.
3. C. Schuh, S. Santer, O. Prucker, J. Ruhe, *Adv. Mater.*, 2009, **21**, 4706 - 4710.