Supporting Information

Polymer Chain Length Effects on Fibroblast Attachment on Nylon-3-Modified Surfaces

Runhui Liu,†,‡ Kristyn S. Masters*,‡, and Samuel H. Gellman*,†

† Department of Chemistry, ‡ Department of Biomedical Engineering, University of Wisconsin, Madison, Wisconsin 53706, USA

E-mail: kmasters@wisc.edu; gellman@chem.wisc.edu

Content

Materials and Methods

Dulbecco's modified eagle medium (DMEM), cell culture supplies, mammalian cell LIVE/DEAD Viability/Cytotoxicity Kits (L3224), and NanoOrange Protein Quantitation Kits (N6666) were from Invitrogen (Carlsbad, CA); NIH 3T3 fibroblast cells were from the American Type Tissue Collection (ATCC, Manassas, VA); Peptide GRGDS (61107) was from AnaSpec (Fremont, CA); NHS ester-functionalized CodeLink microarray slides (DN01-0025) were from SurModics (Eden Prairie, MN); Multichannelled silicon coverslips containing 50 wells (103350) were from Grace Biolabs (Bend, OR); Fluorescein PEG amine (H_2N-PEG(10K)-FITC; PG2-AMFC-10K) was obtained from Nanocs (New York, NY) All other chemicals were purchased from Sigma-Aldrich and used without further purification. Polymers were dissolved in tetrahydrofuran (THF) and filtered through a 0.2 µm polytetrafluoroethylene (PTFE) filter before GPC analysis. Polymers at the side chain-protected stage were analyzed using two Waters columns (Styragel HR 4E, particle size 5 µm) linked in series on a Shimadzu gel-permeation chromatography (GPC) instrument equipped with a multiangle light scattering detector (Wyatt miniDAWN, 690 nm, 30 mW) and a refractive index detector (Wyatt Optilab-rEX, 690 nm). Number-average molecular weight (M_n), weight-average molecular weight (M_w) and polydispersity index (PDI) was measured using THF as mobile phase at a flow rate of 1 mL/min at 40 °C and calculated using ASTRA 5.3.3.15 software with a dn/dc value of 0.1 mL/g for all polymers.[1] The degree of polymerization (DP) for a particular polymer was calculated based on the deduced M_n value, the initial ratio of β-lactam monomers in the reaction, and the molecular weight of the β-lactam monomers, as described previously.[2] GPC chromatograms for side chain-protected polymers by light scattering and refractive index were obtained as EASI graph using ASTRA software and shown separately below in Figure S1 to Figure S10.

To evaluate surface modification efficiency for the CodeLink slides, different surfaces were exposed to solutions containing H_2N-PEG(10K)-FITC at a concentration of 4 mM, 2 mM, 1 mM, 0.5 mM, 0.25 mM, 0.125 mM, 0.063 mM, 0.031 mM or 0.016 mM in 100 mM $NaHCO_3$ aqueous buffer containing 15% glycerol. (As a control, we used a solution of this buffer that did not contain any amine.) A

CodeLink slide was covered with a 50-well silicone coverslip, and the wells were filled with above solutions, with three wells for each amine concentration. The glass slides were incubated in a water-loaded humidifying chamber for 12 h at room temperature, detached from the coverslip, rinsed thoroughly with Milli-Q water, and scanned on a GeneTAC UC 4 × 4 scanner for FITC. Data were analyzed using NIH MacBiophotonics ImageJ (version 1.43m) software; these data are summarized in Figure S11.
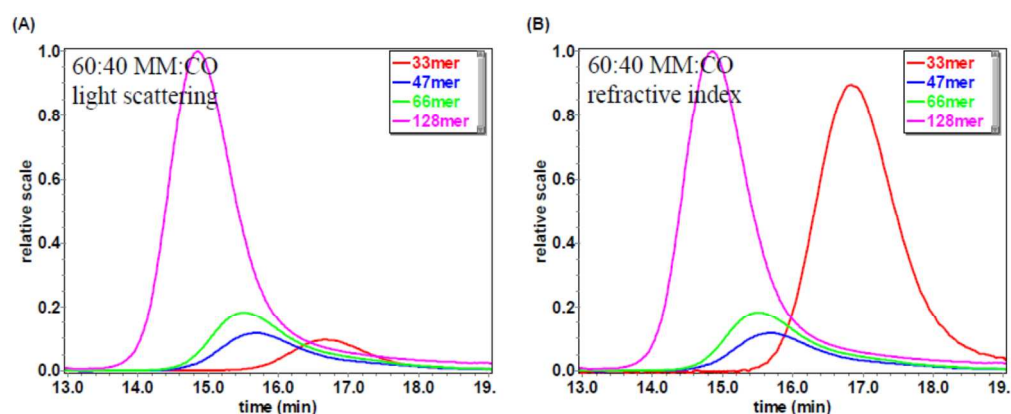

Figure S1. GPC chromatograms for side chain-protected 60:40 **MM:CO** series random copolymers by light scattering (A) and refractive index (B).

Table S1. GPC analysis of side chain-protected 60:40 **MM:CO** series random copolymers with varied chain length.

R represents side chain groups of MM or CO β-lactam
60:40 MM:CO, stereo- and sequence-random copolymers

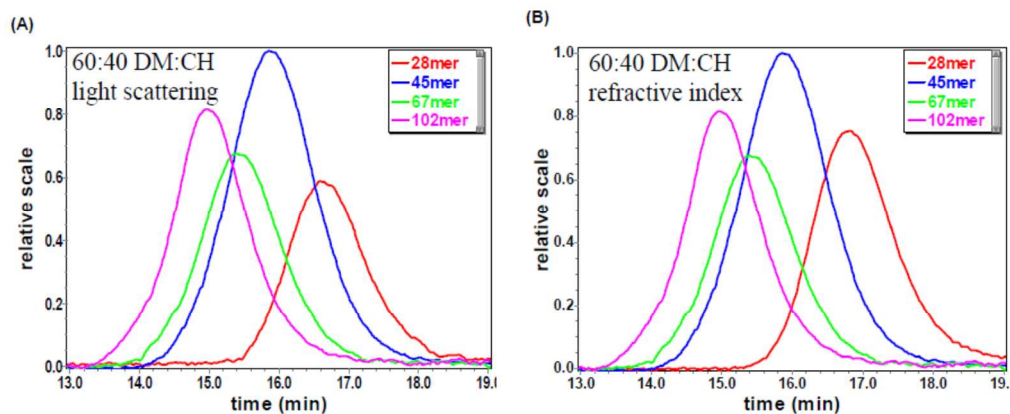| DP (Obs.)	Mn (Obs.)	PDI
33	6447	1.07
47	9176	1.07
66	12320	1.06
128	24190	1.07

Figure S2. GPC chromatograms for side chain-protected 60:40 **DM:CH** series random copolymers by light scattering (A) and refractive index (B).

Table S2. GPC analysis of side chain-protected 60:40 **DM:CH** series random copolymers with varied chain length.

R represents side chain groups of DM or CH β-lactam
60:40 DM:CH, stereo- and sequence-random copolymers

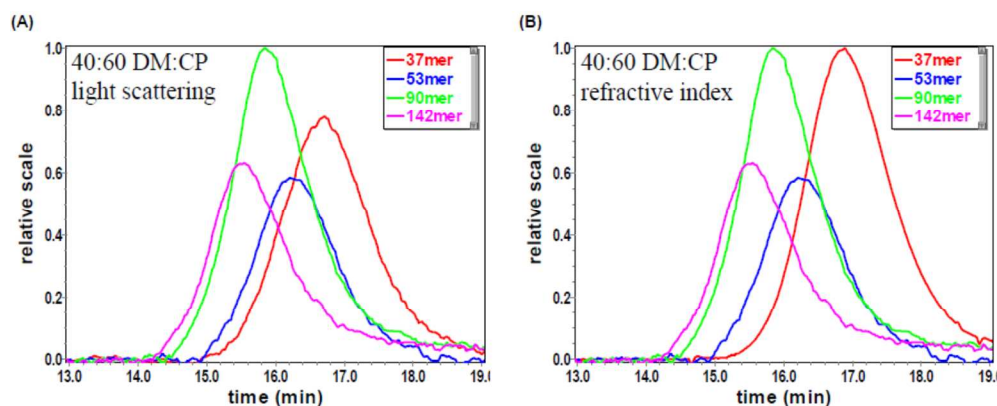| DP (Obs.)	Mn (Obs.)	PDI
28	5384	1.10
45	8658	1.10
67	12640	1.12
102	19190	1.07

Figure S3. GPC chromatograms for side chain-protected 40:60 **DM:CP** series random copolymers by light scattering (A) and refractive index (B).

Table S3. GPC analysis of side chain-protected 40:60 **DM:CP** series random copolymers with varied chain length.

R represents side chain groups of DM or CP β-lactam
40:60 DM:CP, stereo- and sequence-random copolymers

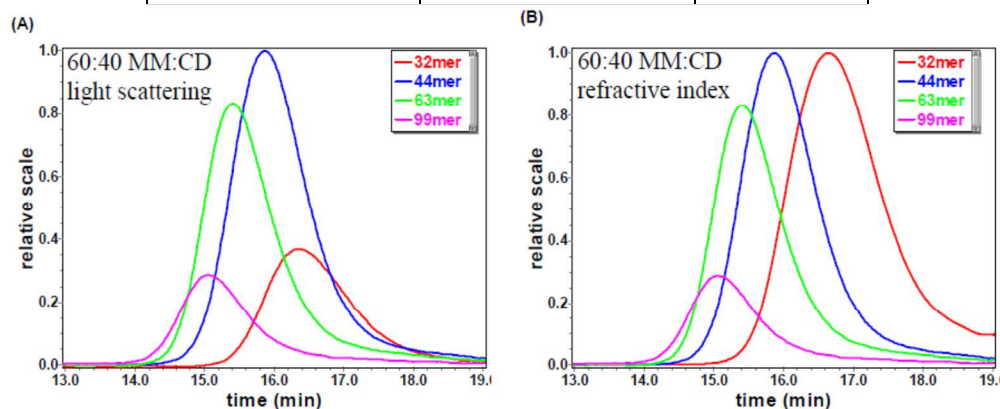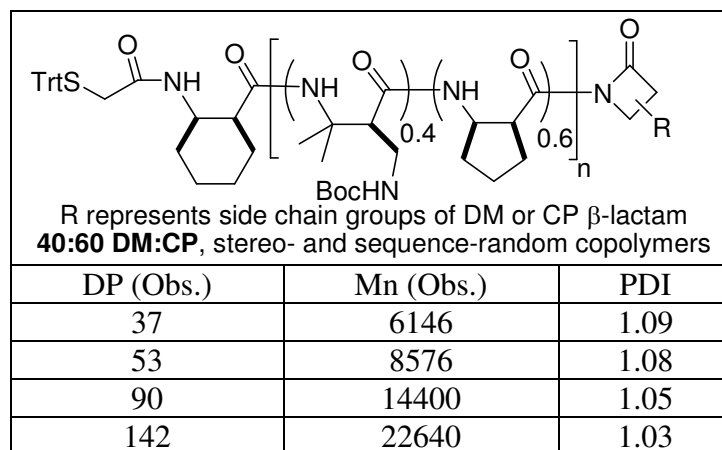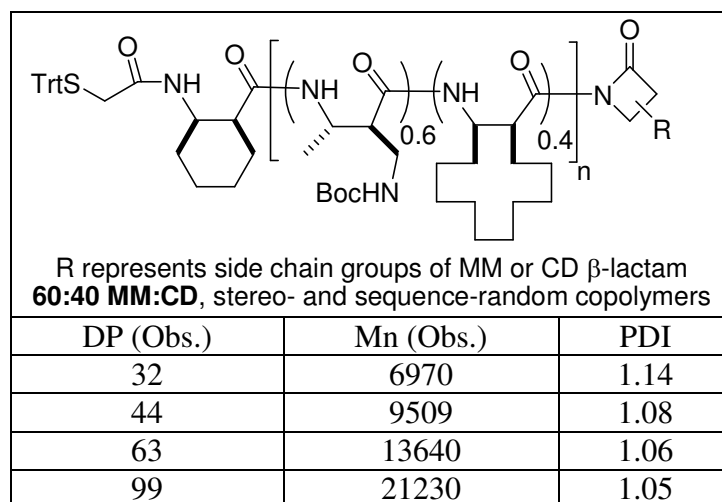| DP (Obs.)	Mn (Obs.)	PDI
37	6146	1.09
53	8576	1.08
90	14400	1.05
142	22640	1.03

Figure S4. GPC chromatograms for side chain-protected 60:40 **MM:CD** series random copolymers by light scattering (A) and refractive index (B).

Table S4. GPC analysis of side chain-protected 60:40 **MM:CD** series random copolymers with varied chain length .

R represents side chain groups of MM or CD β-lactam
60:40 MM:CD, stereo- and sequence-random copolymers

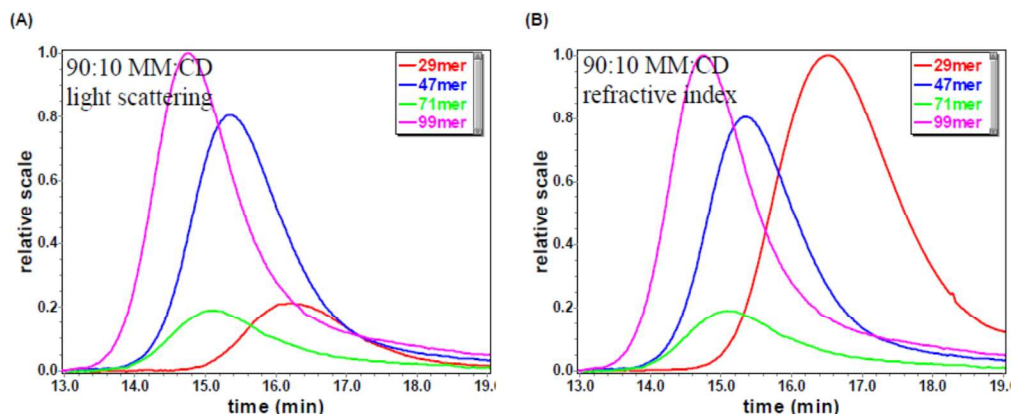| DP (Obs.)	Mn (Obs.)	PDI
32	6970	1.14
44	9509	1.08
63	13640	1.06
99	21230	1.05

Figure S5. GPC chromatograms for side chain-protected 90:10 **MM:CD** series random copolymers by light scattering (A) and refractive index (B).

Table S5. GPC analysis of side chain-protected 90:10 **MM:CD** series random copolymers with varied chain length.

R represents side chain groups of MM or CD β-lactam
90:10 MM:CD, stereo- and sequence-random copolymers

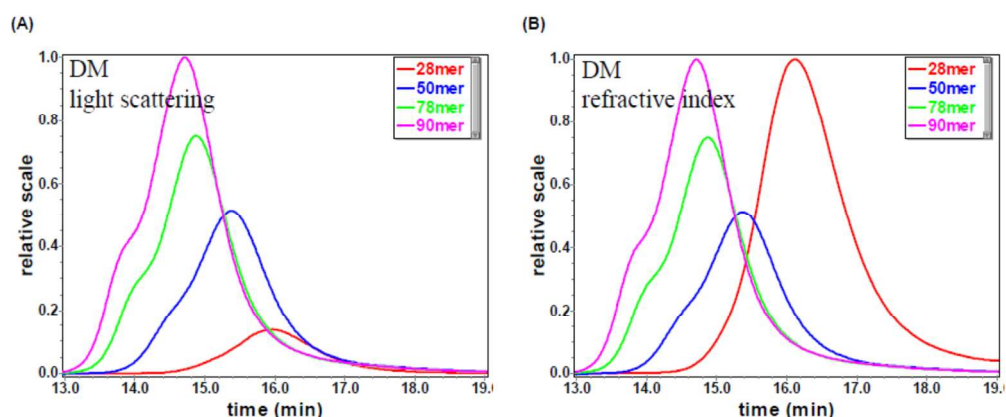| DP (Obs.)	Mn (Obs.)	PDI
29	6006	1.11
47	9857	1.13
71	15030	1.05
99	20980	1.06

Figure S6. GPC chromatograms for side chain-protected **DM** series homopolymers by light scattering (A) and refractive index (B). The fast-running shoulder on 50mer, 78mer and 90mer polymers was considered as part of the polymer peak and included to calculate M_n, etc..

Table S6. GPC analysis of side chain-protected **DM** series homopolymers with varied chain length.

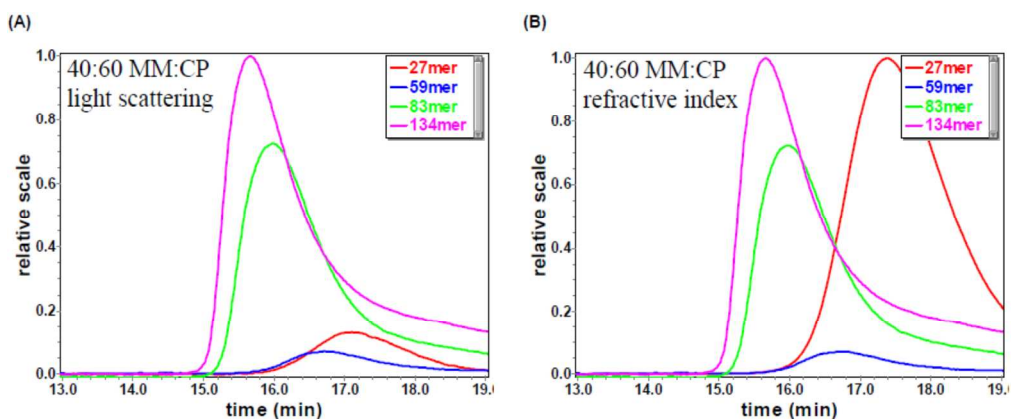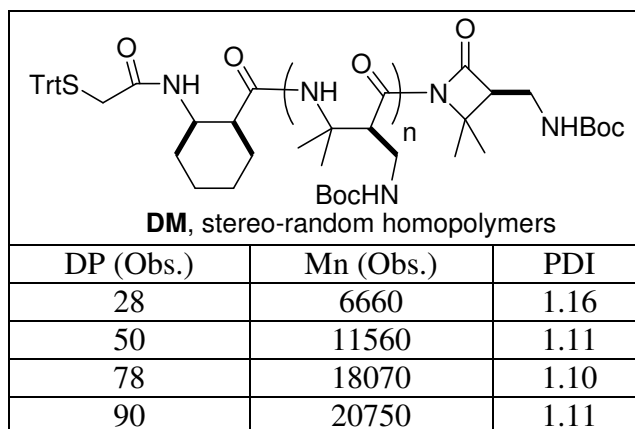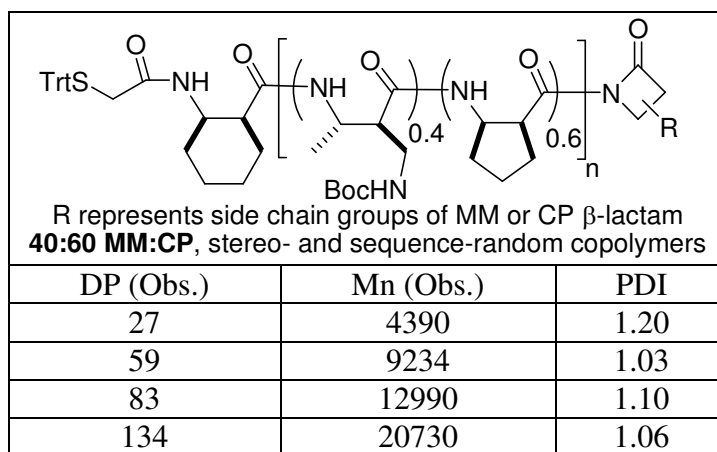| DP (Obs.)	Mn (Obs.)	PDI
28	6660	1.16
50	11560	1.11
78	18070	1.10
90	20750	1.11

DM, stereo-random homopolymers

Figure S7. GPC chromatograms for side chain-protected 40:60 **MM:CP** series random copolymers by light scattering (A) and refractive index (B).

Table S7. GPC analysis of side chain-protected 40:60 **MM:CP** series random copolymers with varied chain length.

R represents side chain groups of MM or CP β-lactam
40:60 MM:CP, stereo- and sequence-random copolymers

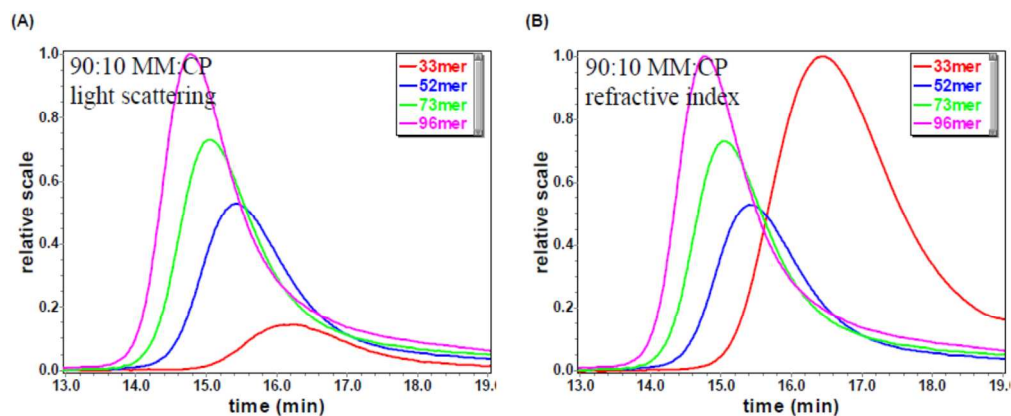| DP (Obs.)	Mn (Obs.)	PDI
27	4390	1.20
59	9234	1.03
83	12990	1.10
134	20730	1.06

Figure S8. GPC chromatograms for side chain-protected 90:10 **MM:CP** series random copolymers by light scattering (A) and refractive index (B).

Table S8. GPC analysis of side chain-protected 90:10 **MM:CP** series random copolymers with varied chain length.

R represents side chain groups of MM or CP β-lactam
90:10 MM:CP, stereo- and sequence-random copolymers

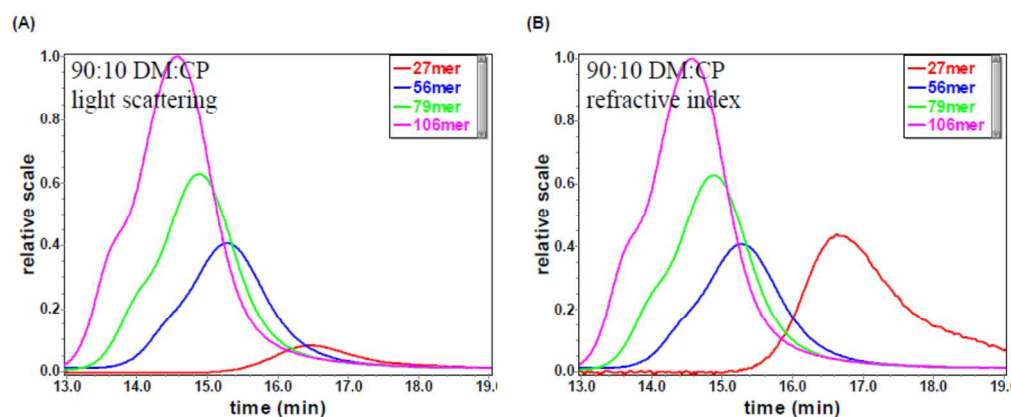| DP (Obs.)	Mn (Obs.)	PDI
33	6933	1.09
52	10820	1.08
73	15130	1.06
96	19840	1.05

Figure S9. GPC chromatograms for side chain-protected 90:10 **DM:CP** series random copolymers by light scattering (A) and refractive index (B). The fast-running shoulder on 79mer and 106mer polymers was considered as part of the polymer peak and included to calculate M_n, etc..

Table S9. GPC analysis of side chain-protected 90:10 **DM:CP** series random copolymers with varied chain length.

R represents side chain groups of DM or CP β-lactam
90:10 DM:CP, stereo- and sequence-random copolymers

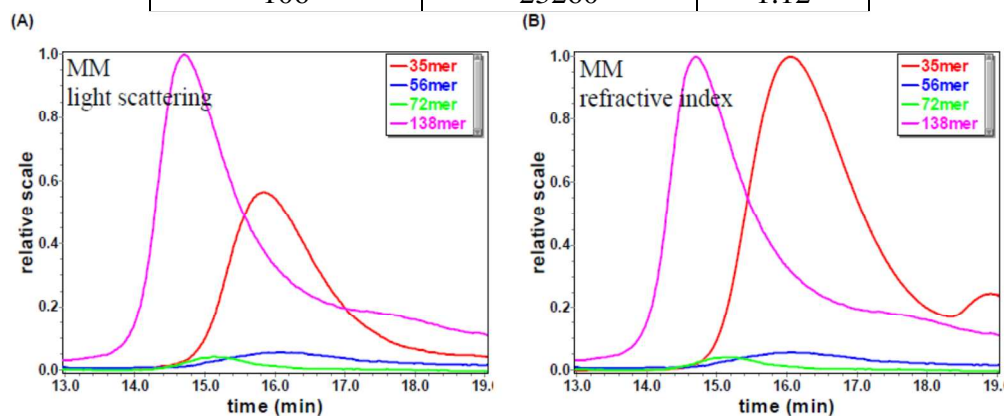| DP (Obs.)	Mn (Obs.)	PDI
27	6039	1.09
56	12270	1.13
79	17360	1.11
106	23260	1.12

Figure S10. GPC chromatograms for side-chain protected **MM** series homopolymers by light scattering (A) and refractive index (B). 56mer, 72mer and 138 mer MM homopolymers have a limited solubility in THF. About 10% methanol was added to THF to help dissolve the 138mer MM homopolymer.

Table S10. GPC analysis of side chain-protected **MM** series homopolymers with varied chain length.

MM, stereo-random homopolymers

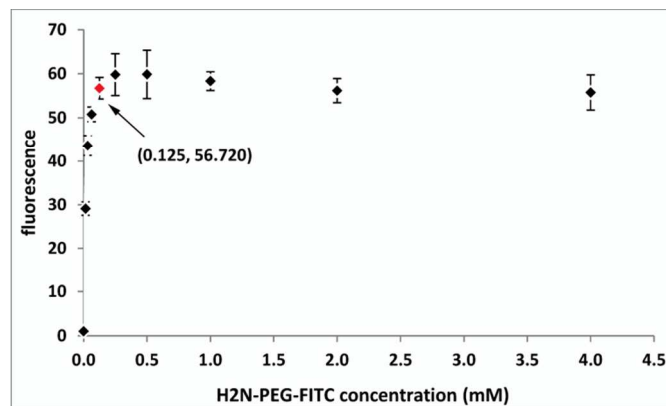| DP (Obs.)	Mn (Obs.)	PDI
35	7780	1.09
56	12290	1.11
72	15570	1.11
138	29830	1.03

Figure S11. CodeLink slide surface modification efficiency test using H$_2$N-PEG(10K)-FITC. Y axis is FITC fluorescence intensity, which should represent the surface density of covalently attached FITC groups on the slide surface.

Reference:

(1)   Lee, M. R.; Stahl, S. S.; Gellman, S. H.; Masters, K. S. *J Am Chem Soc* **2009**, *131*, 16779.

(2)   Mowery, B. P.; Lindner, A. H.; Weisblum, B.; Stahl, S. S.; Gellman, S. H. *J Am Chem Soc* **2009**, *131*, 9735.