Supplementary Information

High Order Multi-Block Copolymers via Iterative Cu(0)-Mediated Radical Polymerizations (SET-LRP): towards Biological Precision

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1. Experimental

Materials:

Ethyl 2-bromoisobutrate (EbiB, Aldrich, 98 %), *tert*-butyl acrylate (*t*BA, Aldrich, 99%), copper (II) bromide (Sigma-Aldrich, 99 %), benzyl mercaptan (Aldrich, 98%), 4-hydroxy-2,2,6,6-tetramethylpiperidin-1-oxyl (4-Hydroxy TEMPO, Aldrich, 97%), triethylamine (TEA, Aldrich, >99%), toluene (Aldrich, 99.9 %), tetrahydrofuran (THF, Sigma, 99 %), dimethyl sulphoxide (DMSO, UNIVAR, AR) were all used as received.

Copper wire (diameter = 1.25 mm) was activated by washing in sulfuric acid for 10 min, and exhaustively rinsed with MiliQ Water and dried under nitrogen. The copper was used immediately. [Alternative methods exist][1]

Both *tris*(2-(dimethylamino)ethyl)amine (Me_6TREN)[2,3] and methanethiosulfonate[4] were synthesized according to literature procedures and stored under nitrogen prior to use.

Monomers methyl acrylate (MA, Aldrich, 99%), *n*-butyl acrylate (*n*BA, Sigma-Aldrich, 99 %), ethyl acrylate (EA, Sigma-Aldrich, 99 %), *tert*-butyl acrylate (*t*BA, Aldrich, 99%), 2-ethyl hexylacrylate (2-EHA, Sigma-Aldrich, 99 %) were de-inhibited by percolating over a column of basic alumina (Ajax, AR).

A typical Cu(0)-meditated polymerization for end-group analysis:

Methyl acrylate (MA, 7.5 mL, 83.3 mmol, 26 eq), DMSO (7.5 mL), EBiB (0.48 mL, 3.23 mmol, 1.00 eq), Me6TREN (0.125mL, 0.54 mmol, 0.18 eq), $CuBr_2$ (37.2 mg, 0.16 mmol, 0.05 eq) and a magnetic stir bar

were charged to a polymerization flask fitted with a rubber septum and the mixture degassed via nitrogen sparging for 20 min. A slight positive pressure of nitrogen was then applied and the pre-activated copper wire (5 cm) was carefully added under a nitrogen blanket. The polymerization flask was then resealed and polymerized at room temperature. After 24 and 72 h a sample of the reaction mixture was carefully removed for ^{1}H NMR, GPC and mass spectroscopy analysis. The sample for ^{1}H NMR was simply diluted with $CDCl_3$, while the sample for GPC and mass spectroscopy analysis was first diluted with THF then passed over an aluminum oxide column to remove metal salts.

A typical iterative Cu(0)-meditated polymerization:

Methyl acrylate (MA, 1 mL, 11.1 mmol, 2.5 eq), DMSO (1 mL), EBiB (0.64 mL, 4.31 mmol, 1.00 eq), Me6TREN (0.17 mL, 0.74 mmol, 0.18 eq), CuBr2 (50mg, 0.22 mmol, 0.05 eq) and a magnetic stir bar were charged to a polymerization flask fitted with a rubber septum and the mixture was degassed via nitrogen sparging for 10 min. A slight positive pressure of nitrogen was then applied and the pre-activated copper wire (0.5 cm) was carefully added under a nitrogen blanket. The polymerization flask was then resealed and polymerized at room temperature. After 24 h a sample of the reaction mixture was carefully removed for ^{1}H NMR, GPC and mass spectroscopy analysis. The sample for ^{1}H NMR was simply diluted with $CDCl_3$, while the sample for GPC and mass spectroscopy analysis was first diluted with THF then passed over an aluminum oxide column to remove metal salts.

For the iterative chain extension, a further 2 mL of a degassed monomer (in 50 vol-% DMSO) solution was carefully added via gas tight syringe and again the solution was allowed to polymerize at RT for another 24 h with stirring.

The above *polymerization-sampling-extension* procedure was repeated as required.

End-group modification with benzyl mercaptan:

Poly(MA) “copolymers” obtained after 6 block chain extensions (0.10 g, 8.16 x 10^{-2} mmol, M_n = 1250 g/mol) were diluted in 2 mL of DMF in the presence of triethylamine (20 µL), and *benzyl mercaptan* (10

mg, 8.2 x 10^{-2} mmol) was subsequently added. The solution was stirred for 14 h at room temperature. An aliquot was carefully taken for ^{1}H NMR, GPC and mass spectroscopy analysis.

End-group modification with sodium methanethiosulfonate:

Poly(MA) "copolymers" obtained after 6 block chain extensions (0.1 g, 8.16 x 10^{-2} mmol, M_n = 1250 g/mol) were diluted with 2 mL of DMF, and sodium methanethiosulfonate (12 mg, 8.2 x 10^{-2} mmol) was added. The solution was stirred for 14 h at room temperature. An aliquot was carefully taken for ^{1}H NMR, GPC and mass spectroscopy analysis.

End-group modification with 4-hydroxy TEMPO:

Into 1 mL of DMSO was dissolved the following: purified Poly(MA), recovered after 6 block formation cycles (0.1g, 8.16 x 10^{-2} mmol), 4-hydroxy TEMPO (0.028g, 16.3 x 10^{-2} mmol, 2 eq.) and Me_6TREN (0.037g, 16.3 x 10^{-2} mmol, 1 eq.). The solution was degassed with nitrogen for 10 min. A slight positive pressure of nitrogen was then applied and CuBr was carefully added (0.023 g, 16.3 x 10^{-2} mmol, 2 eq.) was carefully added under a nitrogen blanket. The polymerization flask was then resealed and allowed to react at room temperature. After 2 h a sample of the reaction mixture was carefully removed for ^{1}H NMR, GPC and mass spectroscopy analysis.

2. Characterization

2.1 NMR Spectroscopy

^{1}H NMR spectra were recorded using a Bruker ACF300 (300 MHz) spectrometer employing $CDCl_3$ as solvent. Monomer conversions were determined via ^{1}H NMR spectroscopy, comparing the signal areas from the vinyl protons (δ ~ 6.50-6.00 ppm) 3H/mol to the signal area from the backbone polymer protons.

2.2 Gel Permeation Chromatography (GPC)

Gel permeation chromatography (GPC) was conducted using THF as the mobile phase. GPC analyses were performed at 40 ^{o}C (flow rate = 1 mL/min) using a Shimadzu modular system comprising an LC-20AT pump, SIL-10AD auto-injector, CTO-16AC column oven and RID-10A RI detector. Molecular weight

separation was achieved via a column set comprising a PL 5.0-mm bead-size guard column (50 × 7.8 mm) followed by four Phenominex PHENOLGEL GPC columns (300 × 7.8mm; 5µm; 10^{-2}, 10^{-3}, 10^{-4} and 10^{-6}A). A calibration curve was generated with commercial linear polystyrene standards ranging from 500 to 10^6 g/mol.

Note: *The analysis of the GPC data required extrapolation of the GPC calibration curve below the minimum calibration standard. The molecular weight and PDI data is presented in consideration of this fact.*

2.3 Electrospray Mass Spectrometry (ESI-MS)

All samples were analyzed using Thermo Scientific LTQ Orbitrap XL hybrid FTMS (Fourier Transform Mass Spectrometer) (San Jose, CA), equipped with a Nanospray source operating in the nebulizer assisted electrospray mode used in the positive ion mode. Mass calibration was performed using caffeine, Met-Arg-Phe-Ala acetate salt (MRFA, Sigma-Aldrich, 90 %), and Ultramark 1621 in the m/z range 195-1822. All spectra was acquired within the m/z range of 150 – 4000, and typical instrumental parameters were a spray voltage of 1 kV, a capillary voltage of 40 V, a capillary temperature of 275 °C. Nitrogen was used as sheath gas (flow: 5% maximum) and helium was used as auxiliary gas (flow: 5% maximum). Approximately 100 scans were signal averaged to obtain the final mass spectrum. Samples were introduced to the mass analyzer via a heated electrospray interface (HESI-II; Thermo Fischer Scientific, San Jose, CA, USA). The instrument can be run according to two different mode which are the ITMS and FTMS. In ITMS, the mass accuracy is 0.1Da while in FTMS the mass accuracy is 0.001 Da for the range that has been employed in this works.[5] FTMS mode was used to generate mass spectrum in Figure S4 while, ITMS mode was used for all other mass spectrum.

In all systems (to achieve an optimum spectral quality), the solvent used was a 3:1 mixture of dichloromethane: methanol with 0.3 µM sodium acetate added to the solvent prior to analysis to ensure that ionization would occur and to suppress potassium salt peaks. All data were processed using the XcaliburTM software included with Thermo Finnigan products. Theoretical molecular weights were calculated using the exact mass for the most abundant peak in any given isotopic pattern. Molecular weights

of the first peak isotope were calculated using the following values: C12 = 12.000000; H1 = 1.007825; O16 = 15.994915; Na23 = 22.989768; Br79 = 78.918336. The simulated spectra were generated using XcaliburTM software.

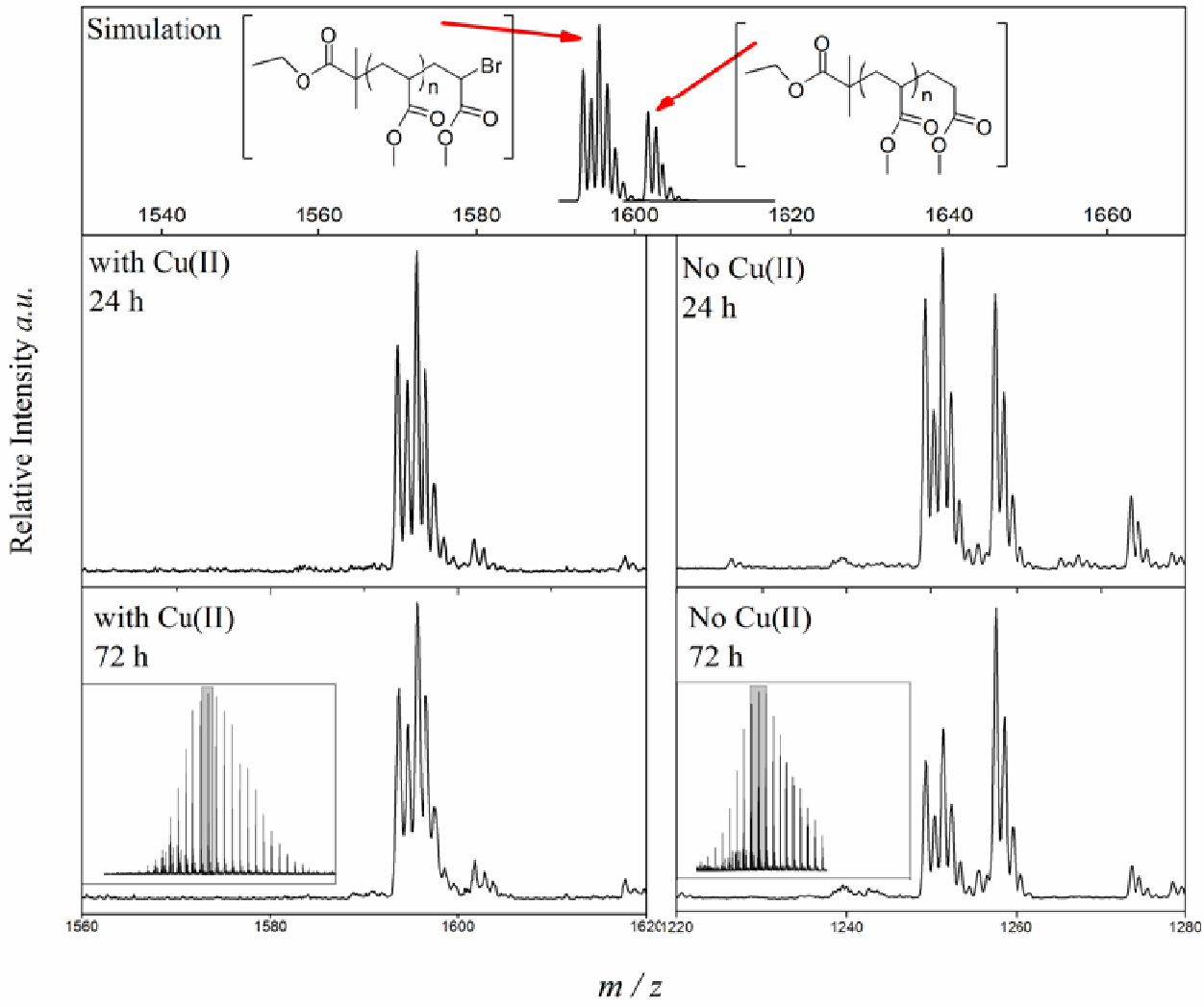

Figure S1. Simulated and experimental mass spectra of P(MA) prepared by Cu(0)-mediated radical polymerization (polymerization time: 3 days) with and without initially added Cu(II). Experimental peaks zoomed in to the most abundant peaks from each respective sample.

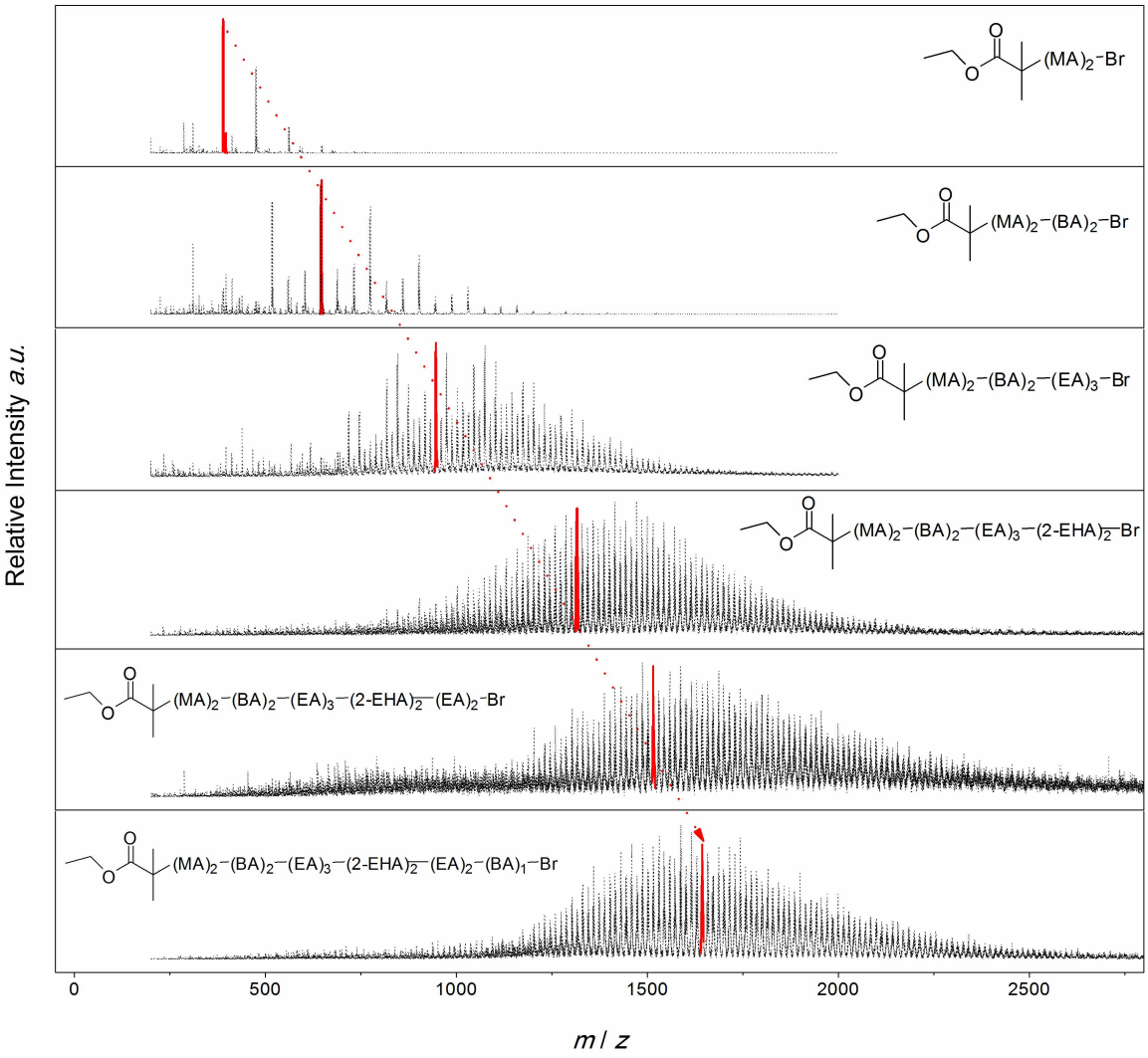

Figure S2. Mass spectra of multi-block copolymers obtained in successive cycles by Cu(0)-mediated radical polymerization. The highlighted peaks correspond to the structures shown.

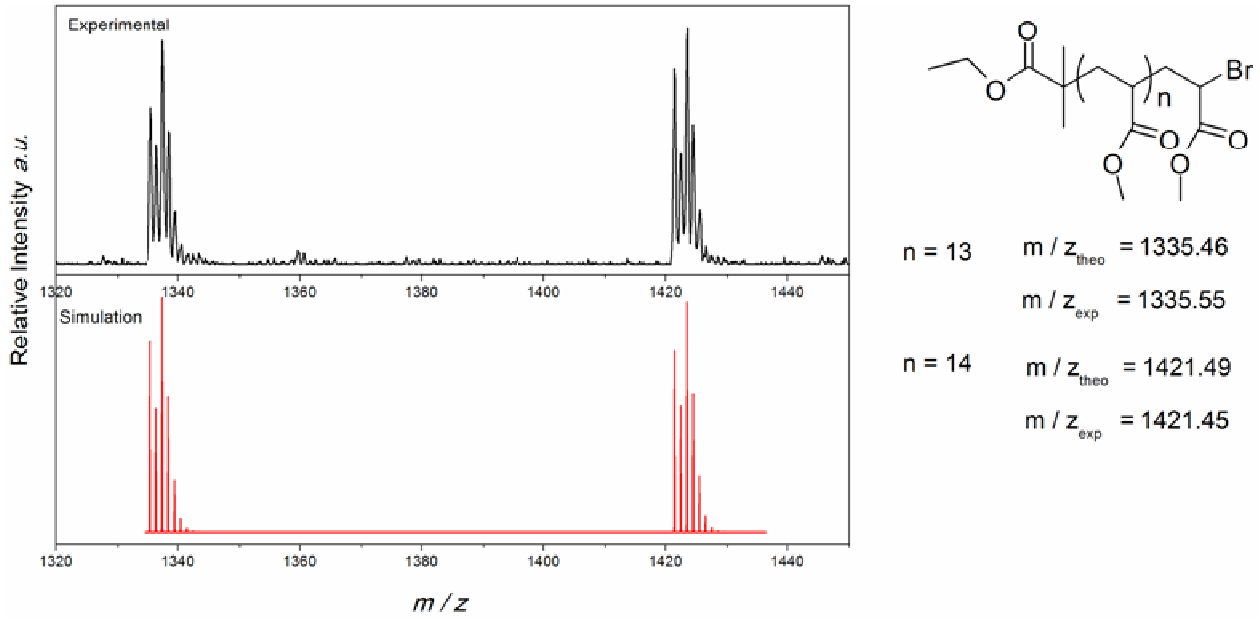

Figure S3. Experimental and simulated mass spectra of P(MA) prepared by Cu(0)-mediated radical polymerization (6 cycles).

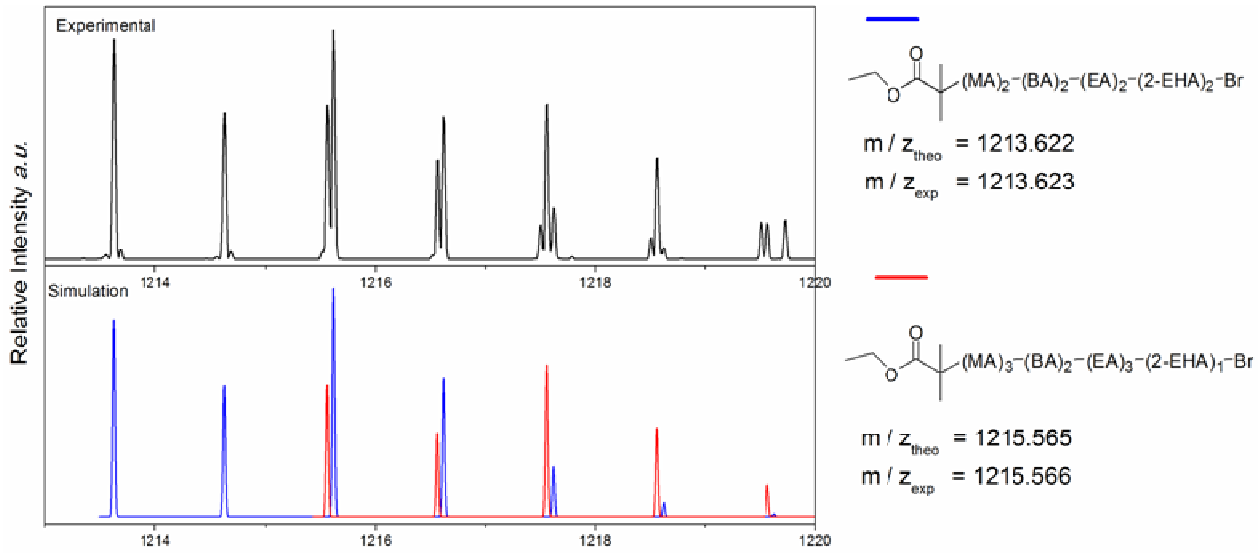

Figure S4. Comparison between experimental and simulated mass spectrum of copolymer taken from a population in **Figure S2** (Entry 4). Experimental mass spectrum was taken using FTMS mode to gain maximum resolution and mass accuracy as explained in experimental section of the Supplementary Information.

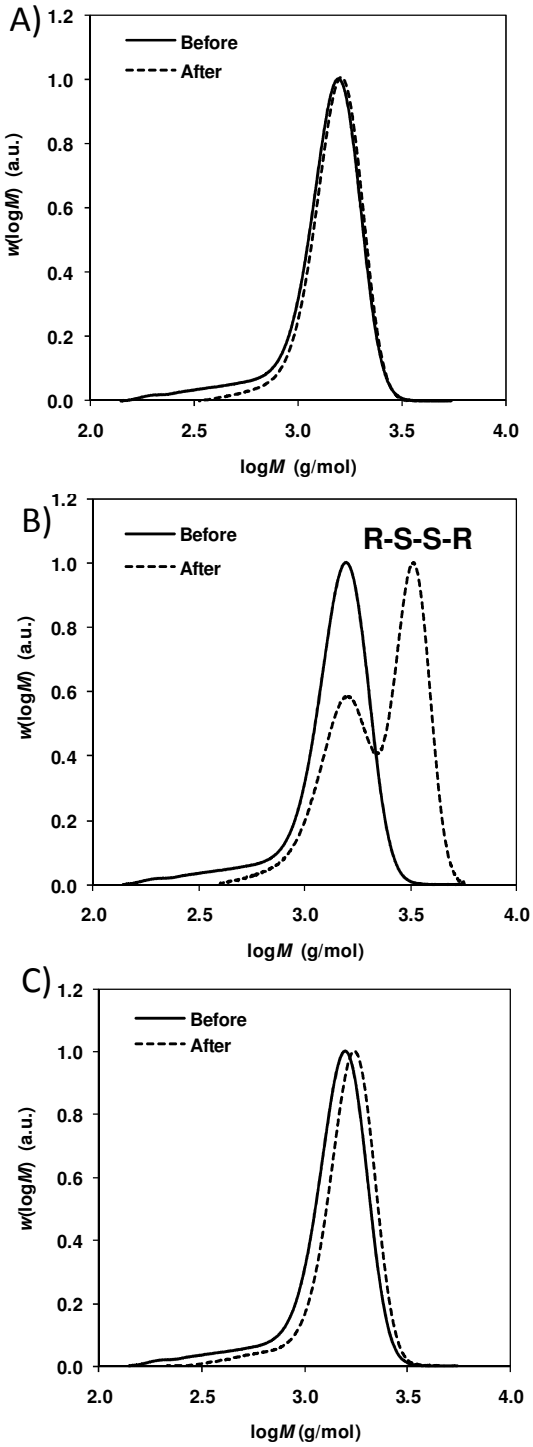

Figure S5 Molecular weight distributions (normalized to peak height) of post-functionalized multiblock PMA polymers obtained after 6 chain extension cycles.; A) by nucleophilic substitution using benzyl mercaptan, B) sodium methane thiosulfonate and C) atom transfer radical coupling (ATRC) in the presence of nitroxide.

Table S1. Peak assignments of P(MA) homopolymer prepared by iterative Cu(0)-mediated radical polymerization. The masses shown correspond to the areas highlighted in Figure 2.

Cycle	Homopolymer structures	m/z theoretical	m/z experimental
1	I-$(MA)_2$-Br	389.1	389.1
2	I-$(MA)_2$-$(MA)_5$-Br	819.2	819.2
3	I-$(MA)_2$-$(MA)_5$-$(MA)_2$-Br	991.3	991.2
4	I-$(MA)_2$-$(MA)_5$-$(MA)_2$-$(MA)_2$-Br	1163.4	1163.3
5	I-$(MA)_2$-$(MA)_5$-$(MA)_2$-$(MA)_2$-$(MA)_1$-Br	1249.4	1249.3
6	I-$(MA)_2$-$(MA)_5$-$(MA)_3$-$(MA)_2$-$(MA)_2$-$(MA)_1$-Br	1421.5	1421.3

Note: I = initiator moiety.

Table S2. Peak assignments for multi-block copolymers prepared by iterative Cu(0)-mediated radical polymerization. The masses shown correspond to areas highlighted in Figure S1.

Cycle	Copolymer structures	m/z theoritical	m/z experimental
1	I-$(MA)_2$-Br	389.1	389.1
2	I-$(MA)_2$-$(BA)_2$-Br	645.2	645.2
3	I-$(MA)_2$-$(BA)_2$-$(EA)_3$-Br	945.4	945.3
4	I-$(MA)_2$-$(BA)_2$-$(EA)_3$-$(2\text{-}EHA)_2$-Br	1313.7	1313.7
5	I-$(MA)_2$-$(BA)_2$-$(EA)_3$-$(2\text{-}EHA)_2$-$(EA)_2$-Br	1513.8	1513.8
6	I-$(MA)_2$-$(BA)_2$-$(EA)_3$-$(2\text{-}EHA)_2$-$(EA)_2$-$(BA)_1$Br	1641.9	1641.9

Note: I = initiator moiety.

Table S3. Summary of characteristics of multi-block P(MA) copolymer prepared by iterative Cu(0)-mediated radical polymerization.

Cycle	Structure	Monomer conversion (%)[a]	$M_n^{theo.}$ (g/mol)[b]	M_n^{GPC} (g/mol)[c]	PDI^{GPC}	M_n^{NMR} (g/mol)[d]	$M_n^{Mass\ Spect.}$ (g/mol)
1	P(MA)	100	353	250	1.11	350	450
2	P(MA)-*b*-(MA)	100	525	480	1.22	500	600
3	$P(MA)_2$-*b*-(MA)	100	697	620	1.23	650	750
4	$P(MA)_3$-*b*-(MA)	100	869	900	1.18	900	925
5	$P(MA)_4$-*b*-(MA)	100	1041	1050	1.23	1050	1200
6	$P(MA)_5$-*b*-(MA)	>95	1213	1200	1.20	1200	1400

Note: a- monomer conversion (α^M, %) was determined by ^{1}H NMR analysis, using $\alpha^M = (I^{5.6\text{-}6.2ppm}/I^{3.6ppm}) \times 100$; b- theoretical molecular weight calculated using the following equation: $M_n^{theo.} = MW^{initiator} + [Monomer]/[Initiator]_0 \times MW^{monomer}$; c- molecular weight assessed by THF GPC analysis. NOTE: The analysis of the GPC data required extrapolation of the GPC calibration curve below the minimum calibration standard. The molecular weight and PDI data is presented in consideration of this fact.; d- experimental molecular weight assessed by ^{1}H NMR analysis: $M_n^{NMR} = MW^{initiator} + 2/3 \times (I^{3.6ppm}/I^{4.1ppm}) \times MW^{monomer,}$ with $I^{3.6ppm}$ and $I^{4.1ppm}$ correspond to integral of CH_3O- and CH_2O-, respectively.

Table S4. Summary of characteristics of multi-block copolymer prepared by iterative Cu(0)-mediated radical polymerization.

Cycle	Structure	Monomer conversion (%)	$M_n^{theo.}$ (g/mol)	M_n^{GPC} (g/mol)	PDI^{GPC}	M_n^{NMR} (g/mol)
1	P(MA)	100	353	250	1.11	350
2	P(MA)-*b*-(BA)	100	609	560	1.19	600
3	P(MA)-*b*-(BA)-*b*-(EA)	100	809	700	1.26	800
4	P(MA)-*b*-(BA)- *b*-(EA)-*b*-(2HEA)	100	1177	1250	1.13	1200
5	P(MA)-*b*-(BA)- *b*-(EA)-*b*-(2HEA)-*b*-(EA)	100	1377	1550	1.11	1650
6	P(MA)-*b*-(BA)- *b*-(EA)-*b*-(2HEA)-*b*-(EA)-*b*-PBA	100	1633	1750	1.12	1700

Note: a- monomer conversion (α^M, %) was determined by ^{1}H NMR analysis, using $\alpha^M = (I^{5.6\text{-}6.2ppm}/I^{3.6ppm}) \times 100$; b- theoretical molecular weight calculated using the following equation: $M_n^{theo.} = MW^{initiator} + \sum i\ [Monomer^i]/[Initiator]_0 \times MW^{monomer\ (i)}$, with $[Monomer^i]$, $[Initiator]_0$, $MW^{monomer\ (i)}$, $MW^{initiator}$ correspond to different monomer concentrations, initial initiator concentration, mass molr of monomer and initiator; c- molecular weight assessed by THF GPC analysis. NOTE: The analysis of the GPC data required extrapolation of the GPC calibration curve below the minimum calibration standard. The molecular weight and PDI data is presented in consideration of this fact.; d- experimental molecular weight assessed by ^{1}H NMR analysis.

References for SI:

(1) Nguyen, N. H.; Percec, V. *J. Polym. Sci. Part A: Polym. Chem.* **2010**, *48*, 5109.

(2) Queffelec, J.; Gaynor, S. G.; Matyjaszewski, K. *Macromolecules* **2000**, *33*, 8629.

(3) Ciampolini, M.; Nardi, N. *Inorg. Chem.* **1966**, *5*, 41.

(4) Boyer, C.; Soeriyadi, A. H.; Roth, P. J.; Whittaker, M. R.; Davis, T. P. *Chem. Comm.* **2011**, *47*, 1318.

(5) Makarov, A.; Denisov, E.; Kholomeev, A.; Balschun, W.; Lange, O.; Strupat, K.; Horning, S. *Anal. Chem.* **2006**, *78*, 2113.