(*Supporting Information*)

Synthetic Polymers Active Against *Clostridium difficile* Vegetative Cell Growth and Spore Outgrowth

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Materials and Methods

[1]H spectra were collected on a Bruker Avance III 400 spectrometer at 400 MHz using D_2O as the solvent. ^{1}H NMR chemical shifts were referenced to the resonance for residual protonated solvent (δ 4.79 for D_2O). MM-TM polymers were characterized by GPC in at the side-chain NHBoc protected stage using dn/dc of 0.1 ml/g for all polymers as described previously.[1,2] The GPC (Shimadzu) was equipped with two Waters columns (Styragel HR 4E, particle size 5 μm) linked in series, a multi-angle light scattering detector (Wyatt miniDAWN, 690 nm, 30 mW), and a refractive index detector (Wyatt Optilab-rEX, 690 nm). THF was used as the mobile phase at a flow rate of 1 mL/min at 40 ℃. The fully deprotected MM-TM polymers were characterized by ^{1}H NMR (see spectra below). All other polymers were reported and characterized previously.[2-4] All copolymers in this study were prepared in via anionic ring-opening copolymerization of a mixture of two β-lactam monomers. Polymers are named on the β-lactam proportion used for the polymerization reaction. Thus, "50:50 DM:TM" is the copolymer produced from copolymerization of β-lactams DMβ and TMβ in 50:50 (mol:mol) ratio. The actual ratio of two subunits within each polymer can vary from the β-lactam proportion, especially for some of the MM:TM copolymers described below (see Table S1).
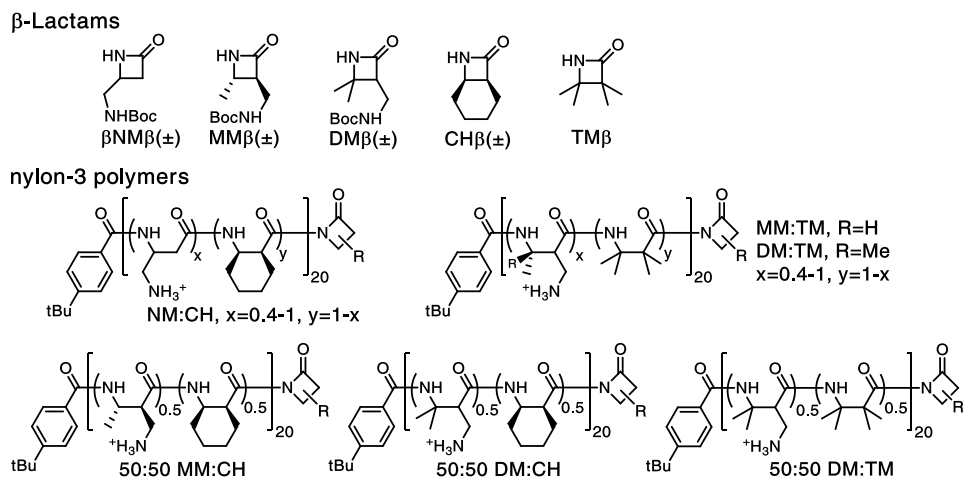
Figure S1. β-Lactams and nylon-3 polymers used in this study. β-Lactams βNMβ, MMβ and DMβ gave the corresponding cationic subunits within copolymers, whereas, CHβ and TMβ gave the corresponding hydrophobic subunits.
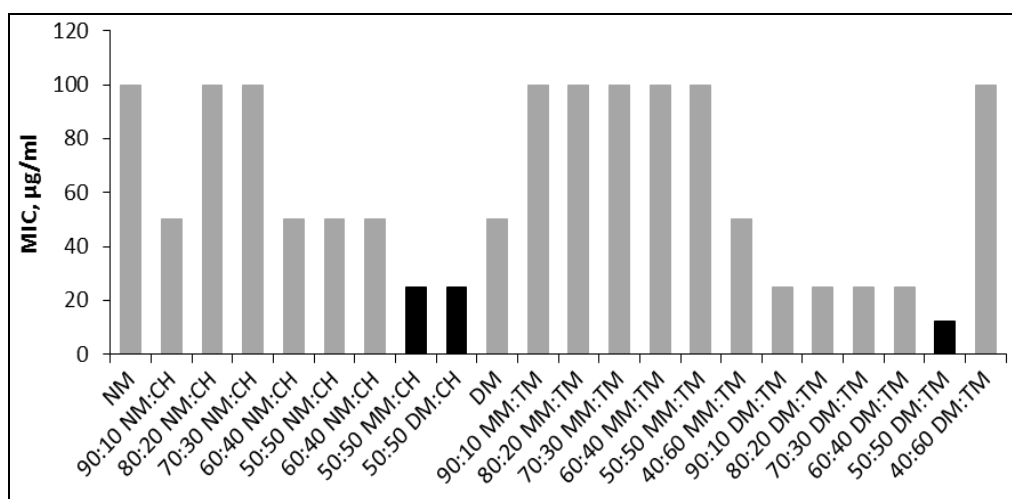

Figure S2. Initial screening of nylon-3 polymers against *C. difficile* vegetative cells (R20291 strain). Three of the most active co-polymers (50:50 MM:CH, 50:50 DM:CH, and 50:50 DM:TM; shown as the black bars) were selected for further study.

Table S1. Viability of spores recovered from outgrowth inhibition (OIC) assays.

| Compound	Concentration (µg/ml)	R20291 Mean Spore Viability	630ΔErm Mean Spore Viability
50:50 DM:TM	200	0.5% (±0.1%)	1.4% (±0.5%)
	100	0.4% (±0.2%)	0.7% (±0.2%)
	50	0.4% (±<0.1%)	0.5% (±0.1%)
	25	0.3% (±0.1%)	0.7% (±0.3%)
	12.5	0.4% (±<0.1%)	1.1% (±0.7%)
50:50 DM:CH	200	1.8% (±0.8%)	4.7% (±1.7%)
	100	1.0% (±0.6%)	3.1% (±0.9%)
	50	0.4% (±<0.1%)	3.5% (±2.3%)
	25	0.5% (±0.1%)	2.5% (±1.7%)
	12.5	0.9% (±0.2%)	ND
50:50 MM:CH	200	0.5% (±0.1%)	2.7% (±2.0%)
	100	0.6% (±0.2%)	1.4% (±0.5%)
	50	0.5% (±0.1%)	1.5% (±0.5%)
	25	0.7% (±0.2%)	6.0% (±5.2%)
	12.5	2.9% (±3.6%)	ND
LL-37	80	5.8% (±2.9%)	7.8% (±3.9%)
	40	3.6% (±1.8%)	4.0% (±4.0%)
	20	1.2% (±0.7%)	5.5% (±7.4%)
	10	0.4% (±0.7%)	ND
Nisin	360	0.4% (±<0.1%)	NI
	180	0.2% (±0.2%)	NI
	90	0.4% (±<0.1%)	NI
Vancomycin	8	0.9% (±0.2%)	NI
	4	1.3% (±0.2%)	NI
	2	3.4% (±2.4%)	NI
	1	7.7% (±7.5%)	NI
	0.5	21.1% (±21%)	NI

NI = Not Inhibitory; ND = Not Determined

Table S2. GPC characterization of nylon-3 copolymers

polymer	expected subunit ratio (cationic:hydrophobic)	expected Mn	GPC characterization[a]			NMR characterization[b]	
			PDI_{GPC}	Mn_{GPC}	DP_{GPC}	DP_{NMR}	observed subunit ratio (cationic:hydrophobic)
40:60 **MM:TM**	40 : 60	3399	1.22	2338	14	12	68 : 32
50:50 **MM:TM**	50 : 50	3574	1.16	2825	17	13	76 : 24
60:40 **MM:TM**	60 : 40	3748	1.15	3274	18	14	83 : 17
70:30 **MM:TM**	70 : 30	3922	1.17	3423	18	15	85 : 15
80:20 **MM:TM**	80 : 20	4096	1.15	3774	19	16	88 : 12
90:10 **MM:TM**	90 : 10	4270	1.13	4370	21	18	92 : 8
50:50 **MM:CH**	50 : 50	3555	1.32	4938	28	24	46 :54
50:50 **DM:CH**	50 : 50	3694	1.05	4766	26	26	50 : 50
50:50 **DM:TM**	50 : 50	3714	1.13	3205	18	17	60 : 40

[a]PDI is the polydispersity index; Mn is the number average molecular weight at the side chain protected stage; DP is the degree of polymerization, i.e., the average number of subunits, calculated from GPC characterization of side chain protected (NHBoc) polymers using THF as the mobile phase. [b]DP and subunit ratio were calculated independently from NMR data using the integration of proton signals. Aromatic protons of *N*-terminal t-BuBz group were used for calibration and subunit ratio calculation. Polymers 50:50 MM:CH, 50:50 DM:CH and 50:50 DM:TM were reported previously;[3,4] the data are included here for comparison with the MM:TM series polymers.
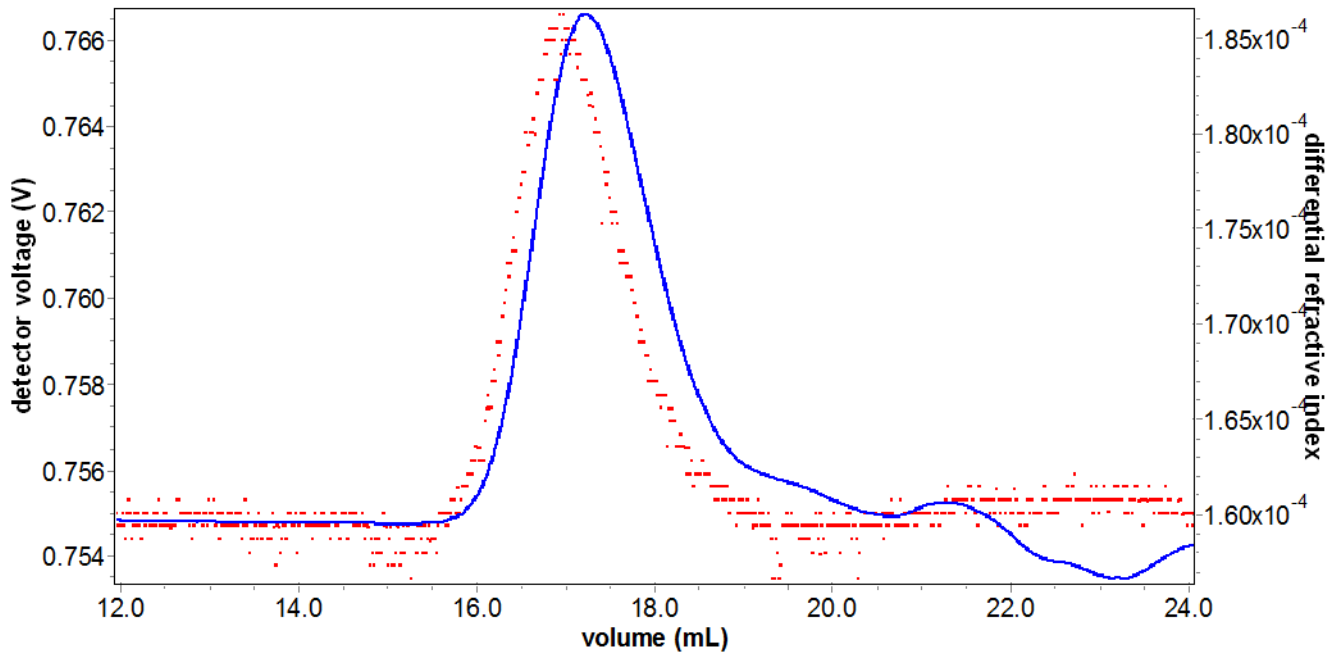
¹H NMR (500 MHz, D₂O)
40:60 **MM:TM** one-pot copolymer

GPC: 14 mer
NMR: 11.8 mer
MM: 8 subunits
TM: 3.8 subunits

t-BuBz+MM+TM

MM   TM

t-BuBz

MM

MM

2.00 2.01

8.06

27.00

78.28
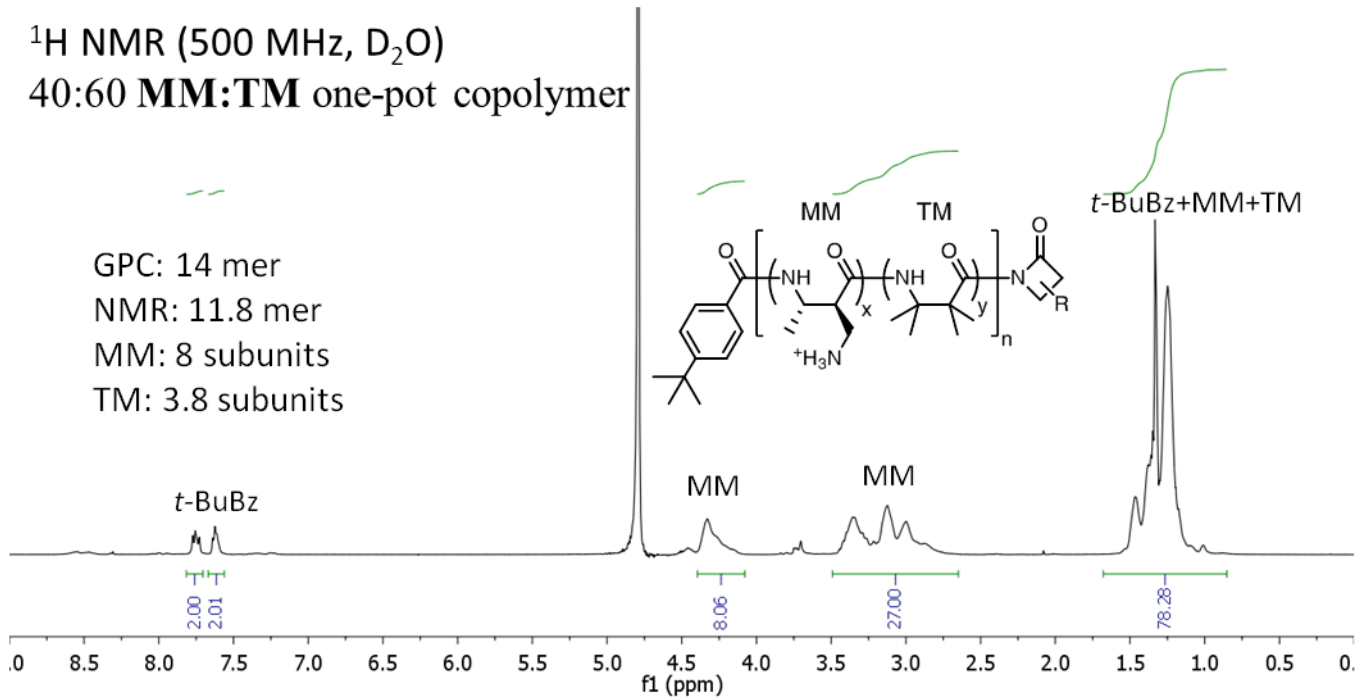
GPC Chromatogram (40:60 **MM:TM** copolymer)
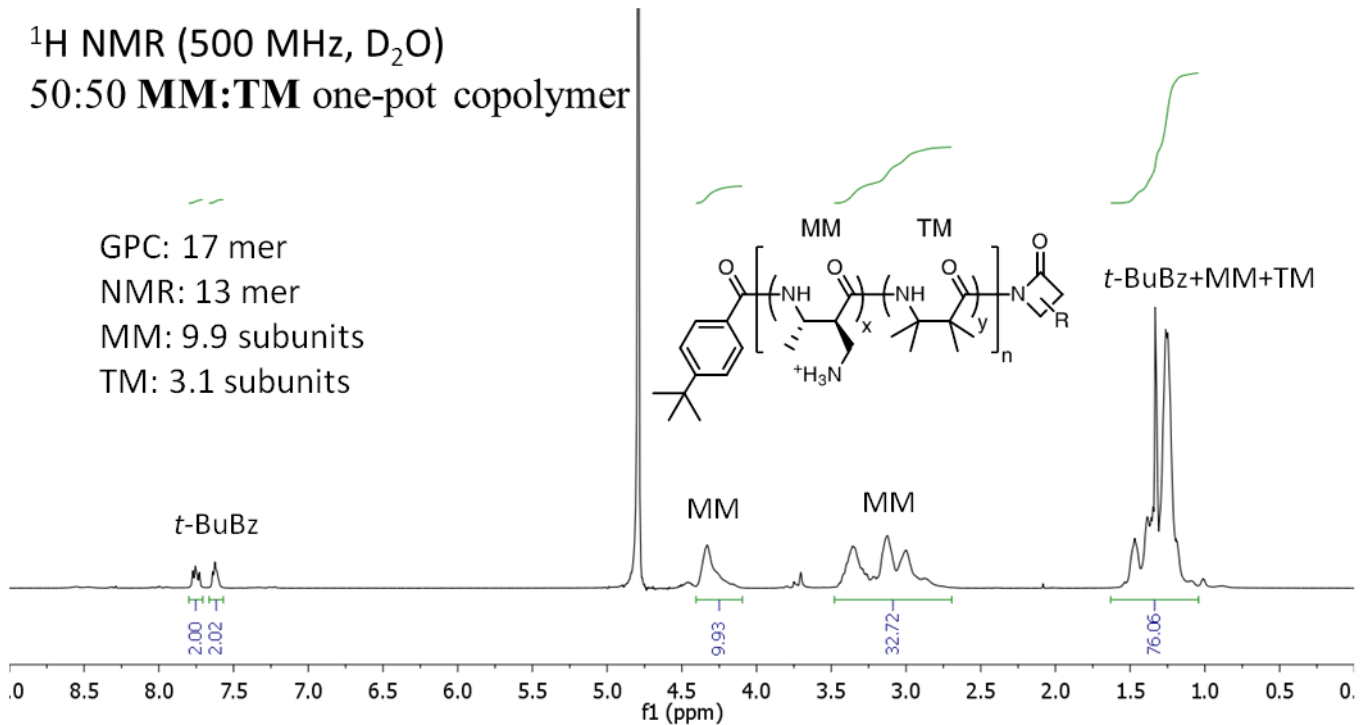mobile phase: THF   ☐ · LS 1   ☑ · LS 2   ☐ · LS 3   ☑ · dRI

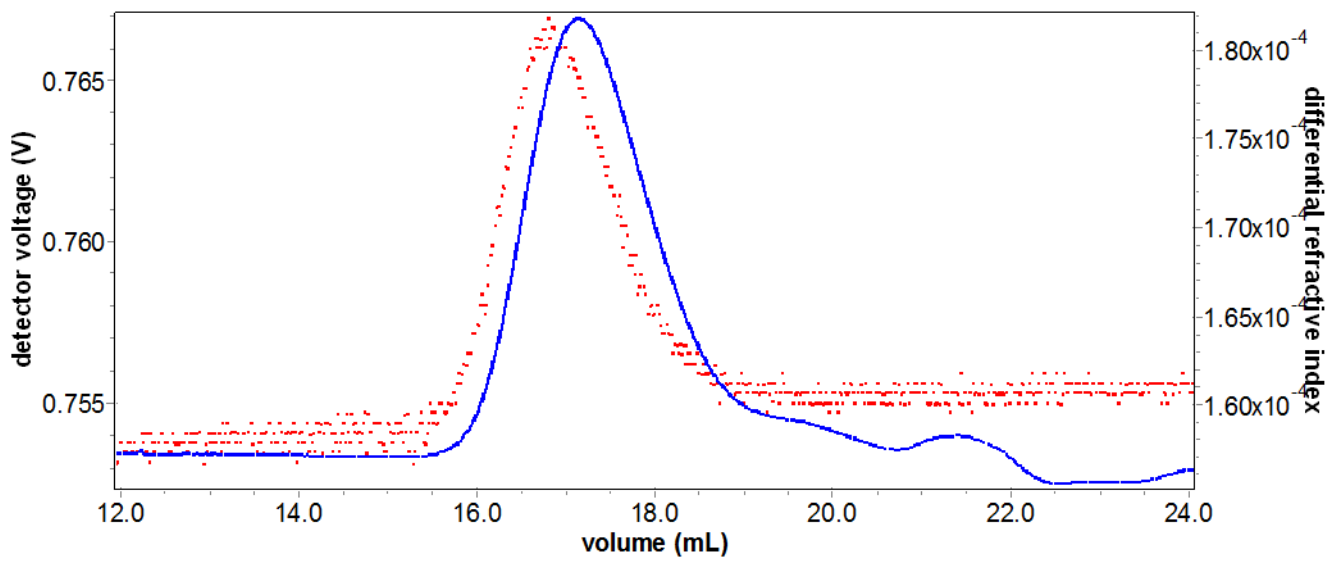## ^{1}H NMR (500 MHz, D$_2$O)
50:50 **MM:TM** one-pot copolymer

GPC: 17 mer
NMR: 13 mer
MM: 9.9 subunits
TM: 3.1 subunits

MM   TM

t-BuBz+MM+TM

t-BuBz

MM

MM

2.00
2.02

9.93

32.72

76.06

f1 (ppm)

GPC Chromatogram (50:50 **MM:TM** copolymer)
mobile phase: THF

☐ · LS 1   ☑ · LS 2   ☐ · LS 3   ☑ · dRI

detector voltage (V)

differential refractive index

volume (mL)

^{1}H NMR (500 MHz, D$_2$O)
60:40 **MM:TM** one-pot copolymer

GPC: 18 mer
NMR: 14 mer
MM: 11.6 subunits
TM: 2.4 subunits

2.00
2.02
11.58
37.89
72.98

f1 (ppm)

.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.
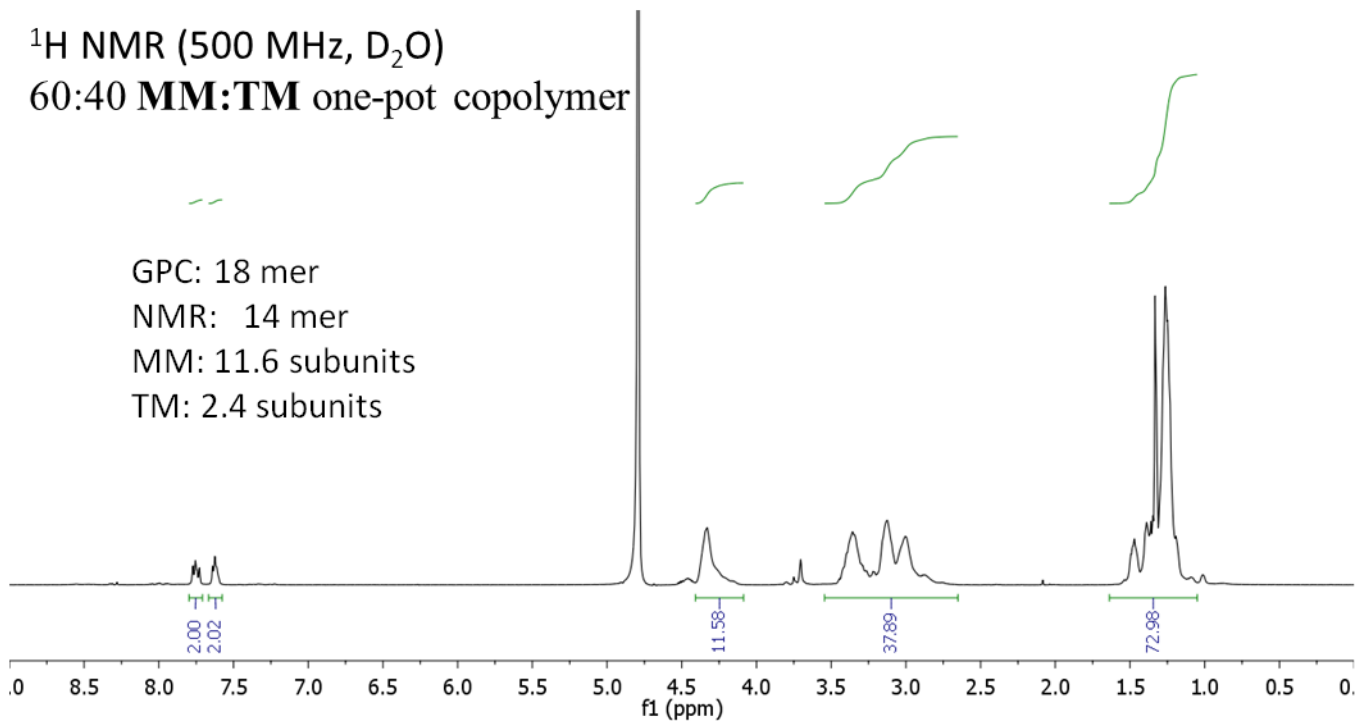
GPC Chromatogram (60:40 **MM:TM** copolymer)
mobile phase: THF   ☐ · LS 1   ☑ · LS 2   ☐ · LS 3   ☑ · dRI

detector voltage (V)

0.770

0.765

0.760

differential refractive index

1.40x10^{-4}
1.35x10^{-4}
1.30x10^{-4}
1.25x10^{-4}
1.20x10^{-4}

12.0   14.0   16.0   18.0   20.0   22.0   24.0

volume (mL)
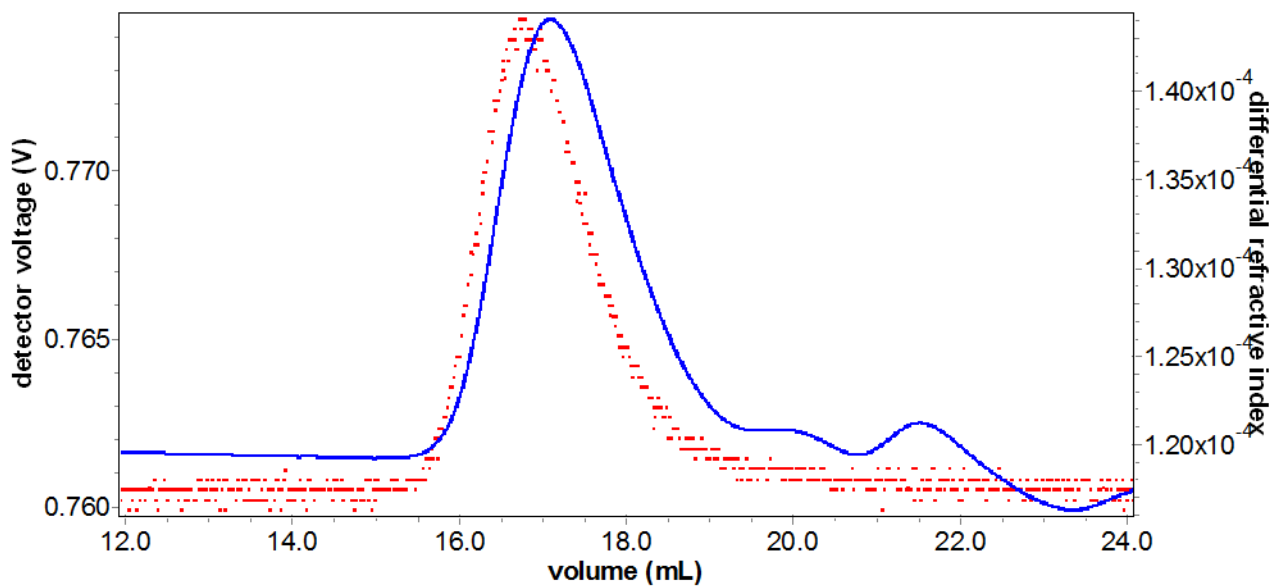
^{1}H NMR (500 MHz, D$_2$O)
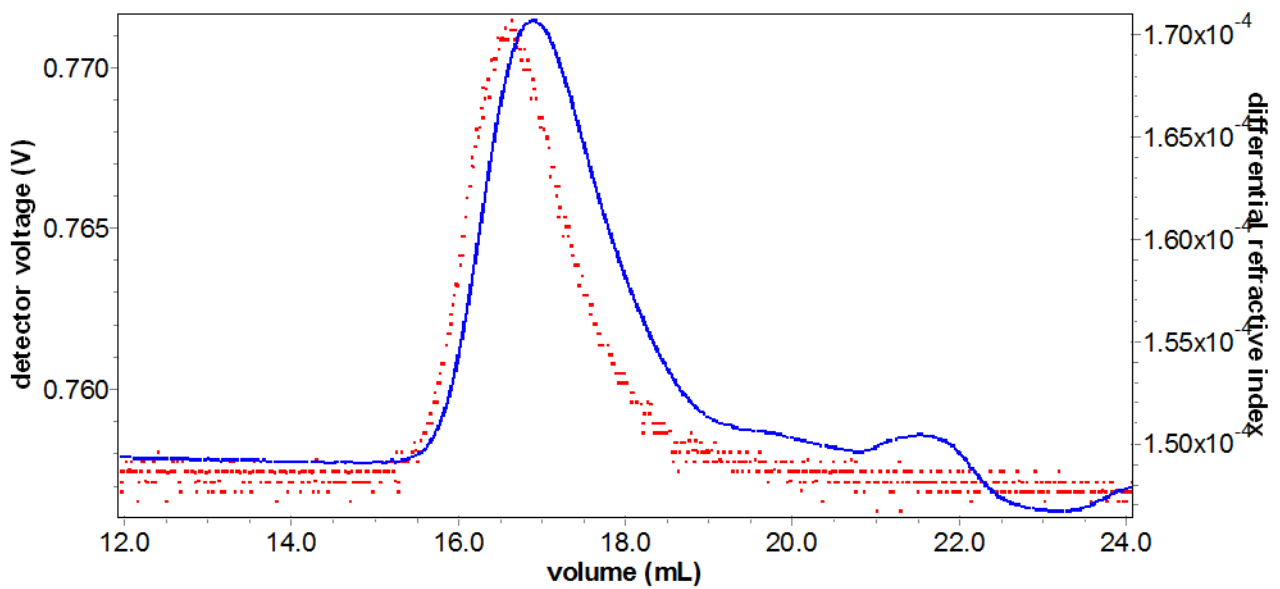70:30 **MM:TM** one-pot copolymer

GPC: 18 mer
NMR: 14.6 mer
MM: 12.4 subunits
TM: 2.2 subunits
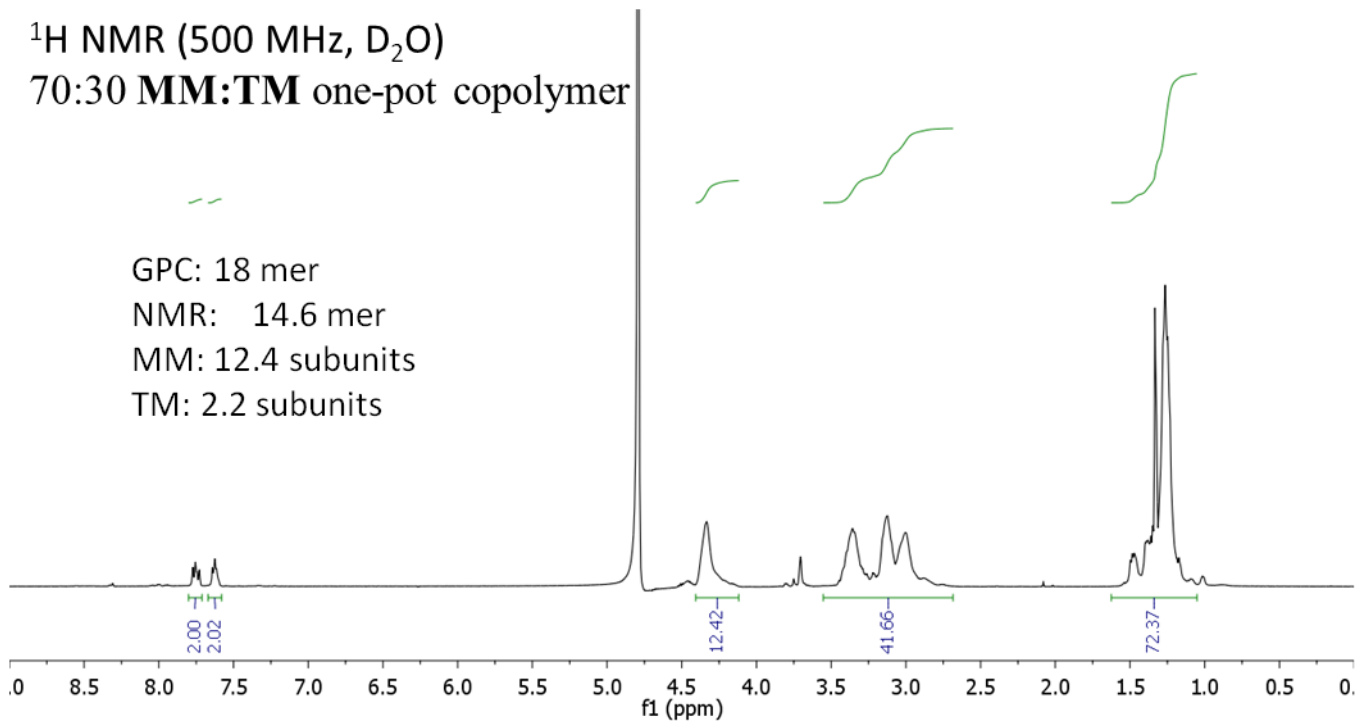
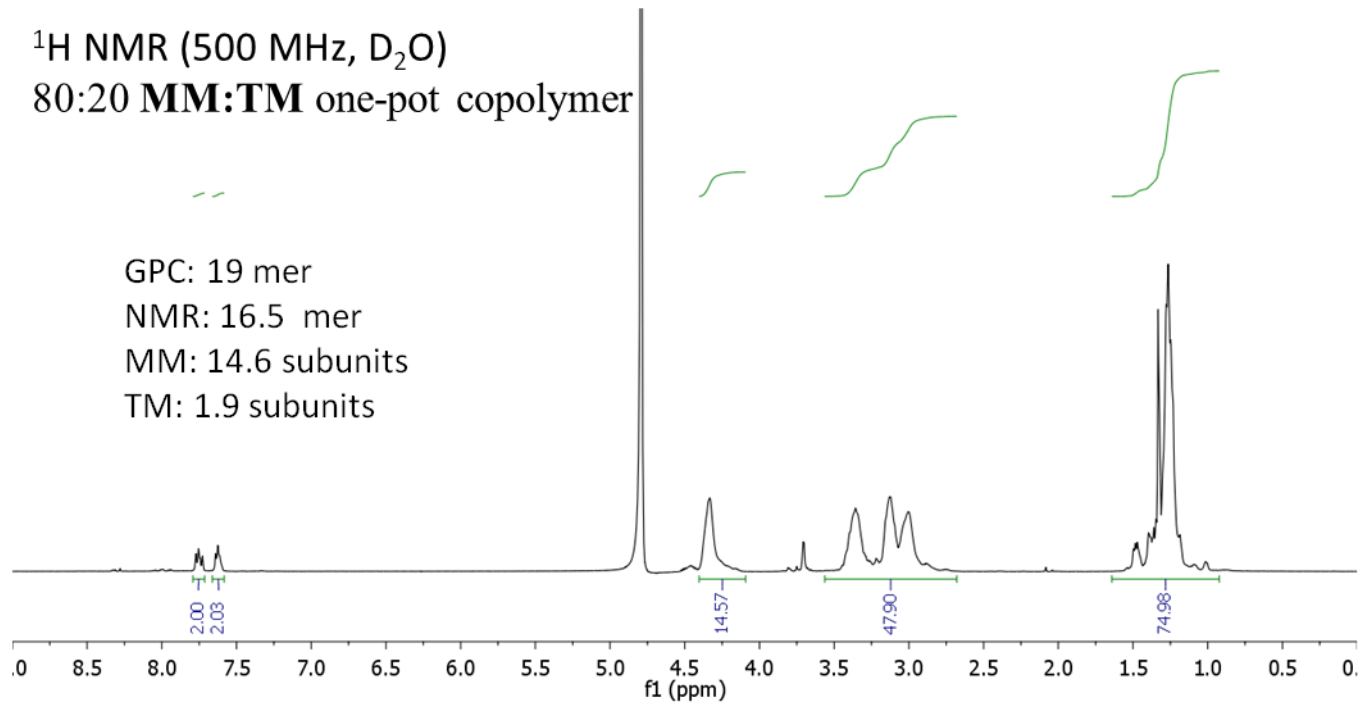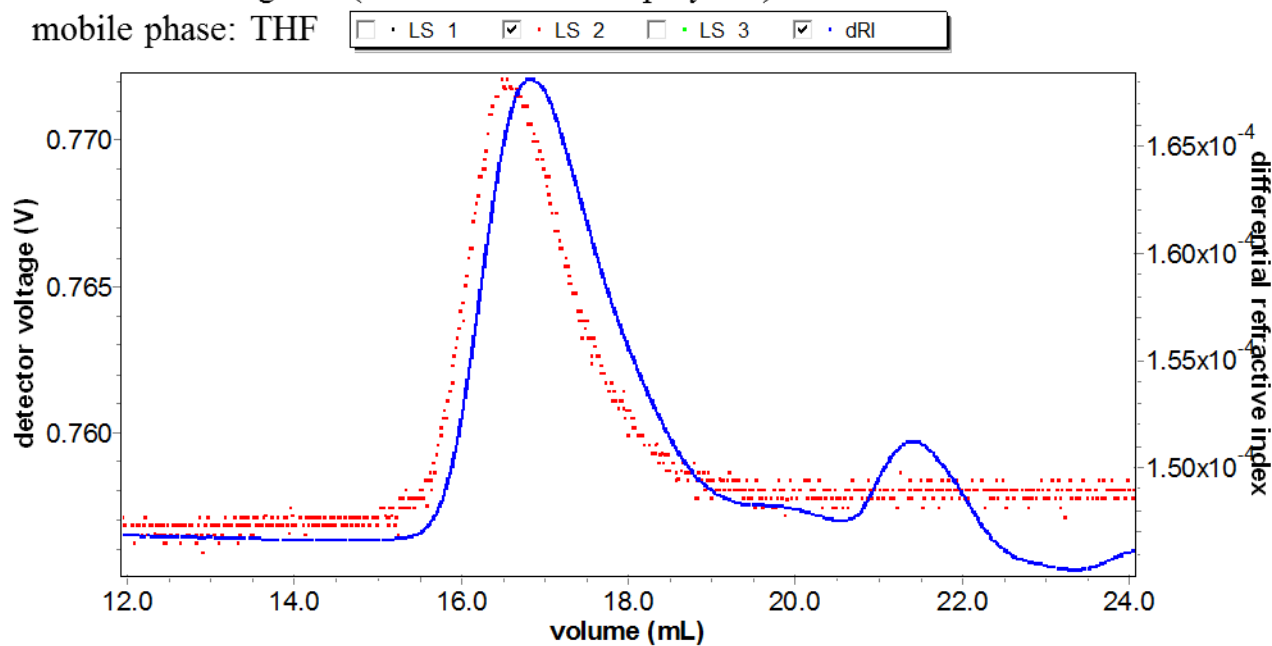GPC Chromatogram (70:30 **MM:TM** copolymer)
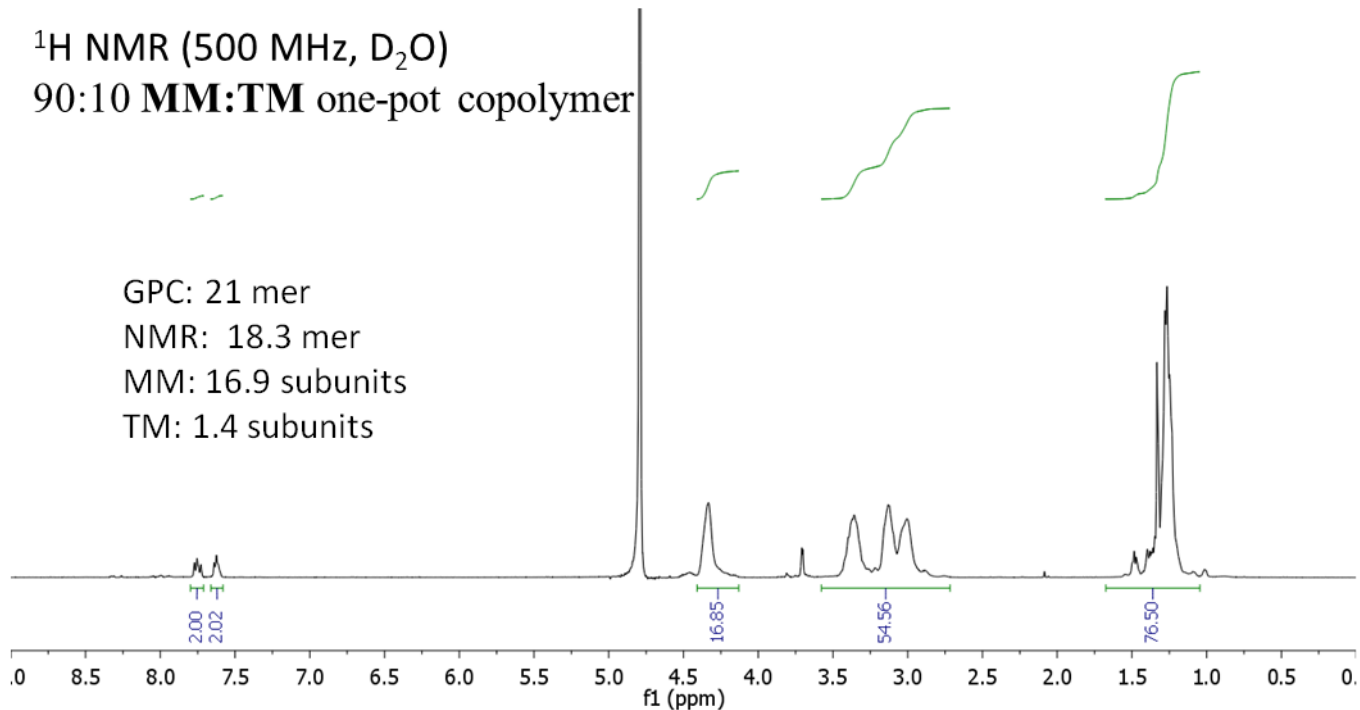mobile phase: THF   □ · LS 1   ☑ · LS 2   □ · LS 3   ☑ · dRI

¹H NMR (500 MHz, D₂O)

^{1}H NMR (500 MHz, D$_2$O)
80:20 **MM:TM** one-pot copolymer

GPC: 19 mer
NMR: 16.5 mer
MM: 14.6 subunits
TM: 1.9 subunits

2.00
2.03
14.57
47.90
74.98

f1 (ppm)

GPC Chromatogram (80:20 **MM:TM** copolymer)
mobile phase: THF ☐ · LS 1 ☑ · LS 2 ☐ · LS 3 ☑ · dRI
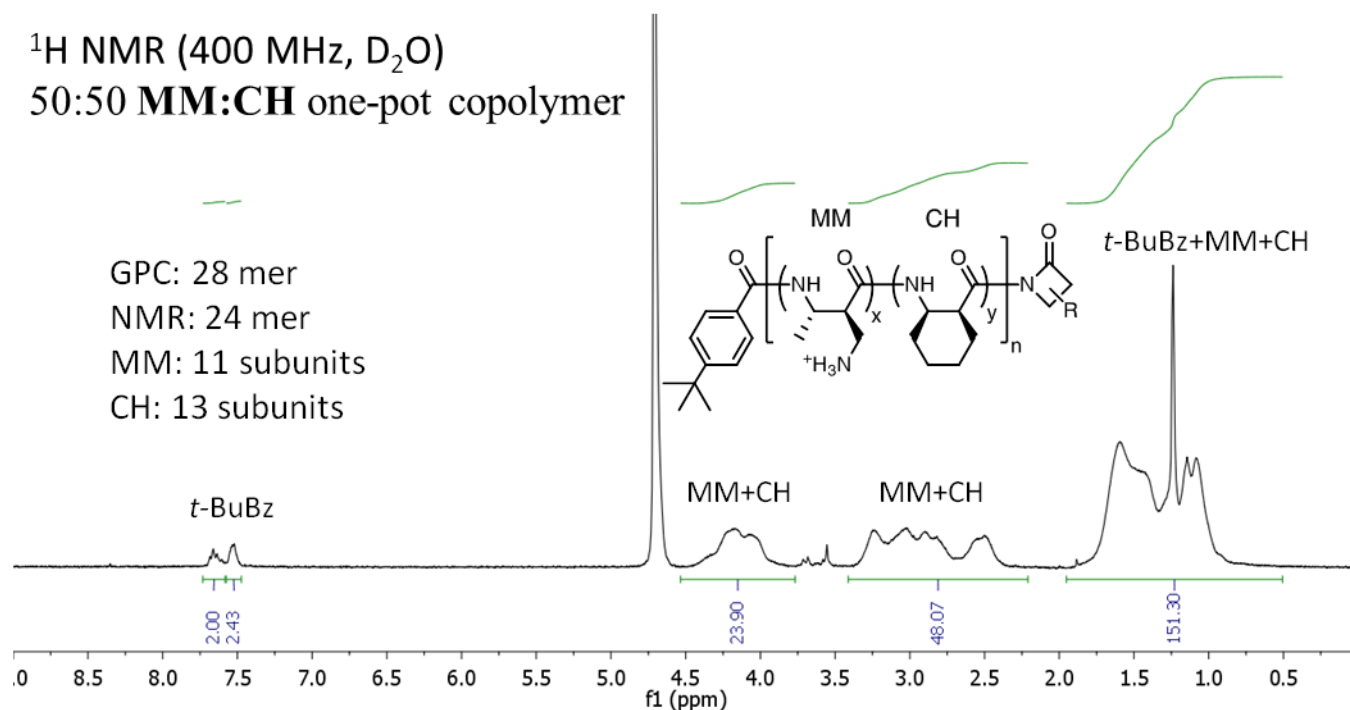
¹H NMR (500 MHz, D₂O)
90:10 **MM:TM** one-pot copolymer

GPC: 21 mer
NMR: 18.3 mer
MM: 16.9 subunits
TM: 1.4 subunits

GPC Chromatogram (90:10 **MM:TM** copolymer)
mobile phase: THF

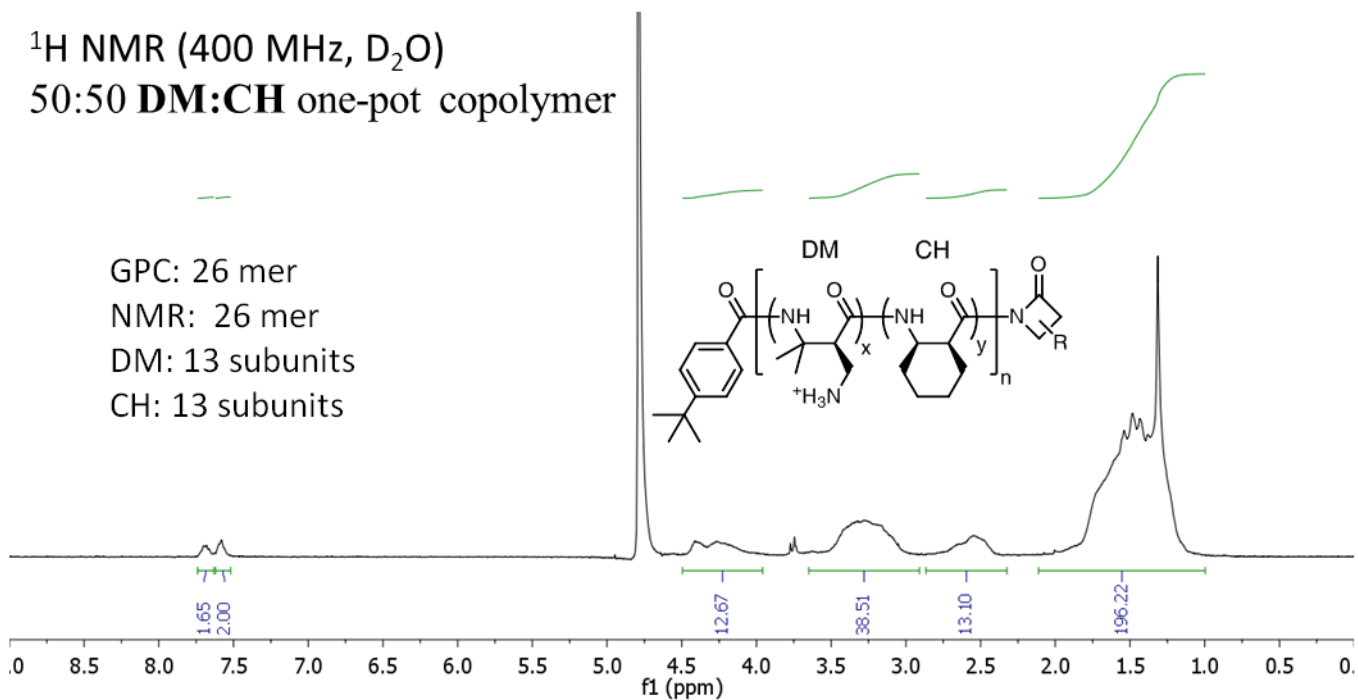☐ · LS 1   ☑ · LS 2   ☐ · LS 3   ☑ · dRI
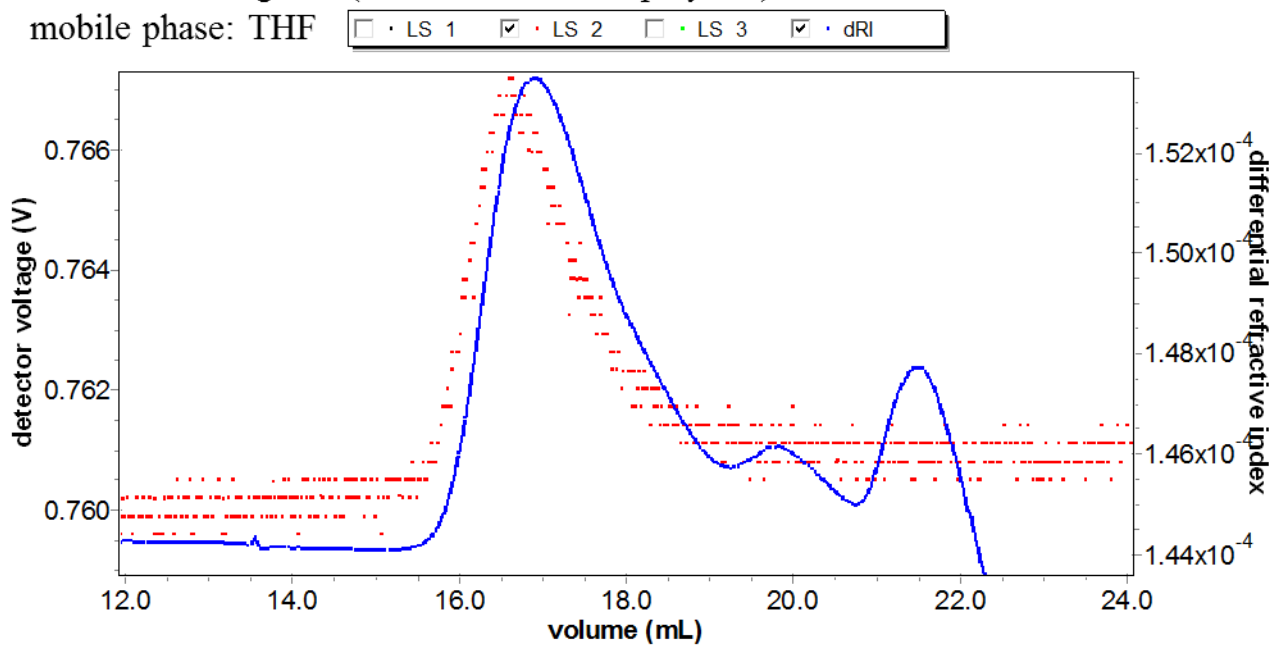
¹H NMR (400 MHz, D₂O)
50:50 **MM:CH** one-pot copolymer

GPC: 28 mer
NMR: 24 mer
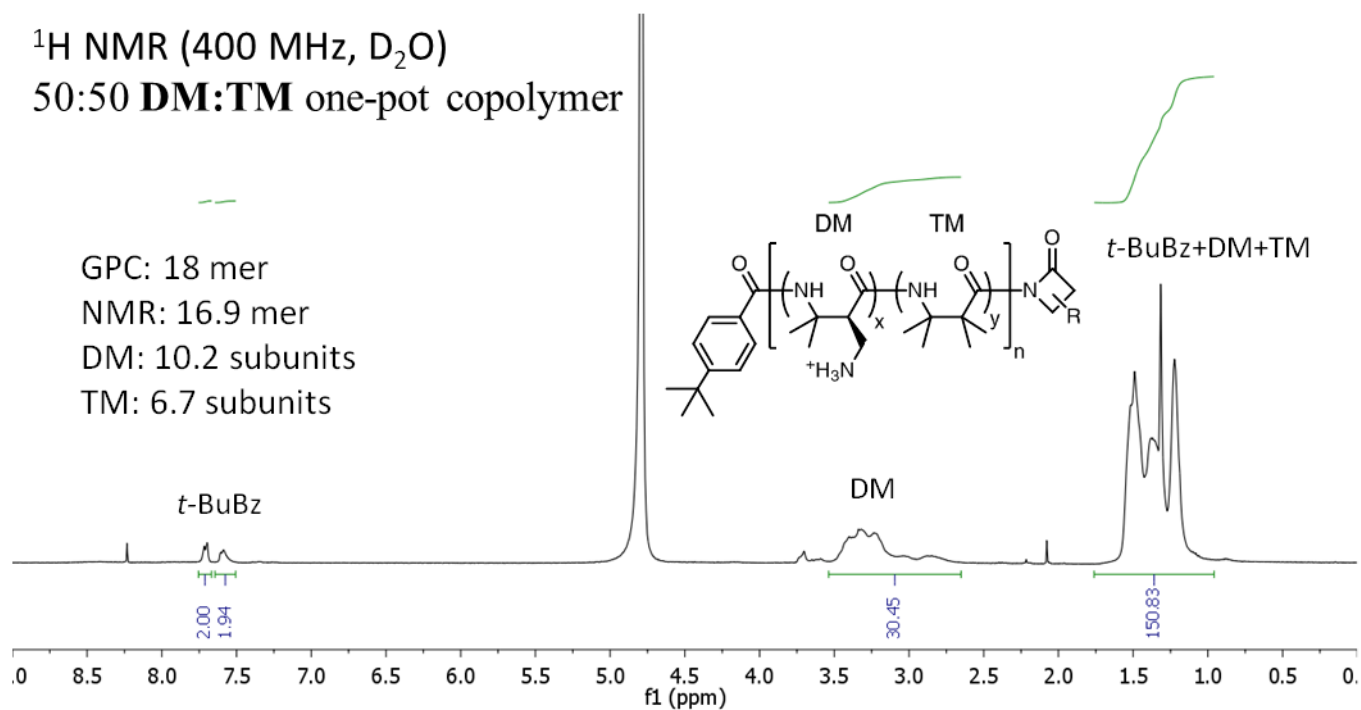MM: 11 subunits
CH: 13 subunits

¹H NMR (400 MHz, D₂O)
50:50 **DM:CH** one-pot copolymer

GPC: 26 mer
NMR: 26 mer
DM: 13 subunits
CH: 13 subunits

^{1}H NMR (400 MHz, D$_2$O)
50:50 **DM:TM** one-pot copolymer

GPC: 18 mer
NMR: 16.9 mer
DM: 10.2 subunits
TM: 6.7 subunits

t-BuBz

DM

TM

t-BuBz+DM+TM

DM

2.00
1.94

30.45

150.83

References

1   Lee, M. R., Stahl, S. S., Gellman, S. H. & Masters, K. S. Nylon-3 Copolymers that Generate Cell-Adhesive Surfaces Identified by Library Screening. *J Am Chem Soc* **131**, 16779-16789, (2009).
2   Liu, R. H. *et al.* Nylon-3 Polymers with Selective Antifungal Activity. *J Am Chem Soc* **135**, 5270-5273, (2013).
3   Liu, R. *et al.* Tuning the Biological Activity Profile of Antibacterial Polymers via Subunit Substitution Pattern. *J Am Chem Soc* **136**, 4410-4418, (2014).
4   Liu, R. *et al.* Structure–Activity Relationships among Antifungal Nylon-3 Polymers: Identification of Materials Active against Drug-Resistant Strains of *Candida albicans*. *J Am Chem Soc* **136**, 4333-4342, (2014).