Supporting Information

Biophysical Mimicry of Lung Surfactant Protein B by Random Nylon-3 Copolymers

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Materials and Instrumentation

All commercially available compounds were purchased from Aldrich or ACROS and used as received unless otherwise noted. Tetrahydrofuran (THF, anhydrous, inhibitor-free) was purchased from Aldrich and used in the glove box without further treatment. Polymerization reactions were carried out in an MBraun UniLab double glove box under a nitrogen atmosphere. Yields refer to chromatographically and spectroscopically pure compounds, unless otherwise stated. Masses of final polymers were confirmed by MALDI-TOF mass spectrometry using a Bruker REFLEX II[™] (Billerica, MA) spectrometer in positive ion mode (acceleration voltage = 25 kV), equipped with a 337 nm laser, a reflectron, and delayed extraction. ¹H NMR spectra were obtained on a Bruker AC-300 spectrometer at 300 MHz. DMSO-d₆ and D₂O for NMR were purchased from Aldrich. ¹H chemical shifts are reported relative to HOD (δ 4.79) for D₂O. Gel permeation chromatography (GPC) was performed on an instrument composed of a Shimadzu LC-10AD liquid chromatography (HPLC) pump and a Wyatt Technology miniDAWN multi-angle light scattering (MALS) detector (690 nm, 30 mW) in series with a Wyatt Technology Optilab-Rex refractive index detector (690 nm). GPC was performed using two Waters columns (Styragel HR4E, particle size 5 µm) with THF (Fisher HPLC-grade, used without further treatment) as mobile phase at a flow rate of 1.0 mL min⁻¹ at 40 °C. The refractive index increment (dn/dc) of the polymers was measured in THF at 30 °C using the refractive index detector, and used to determine the number-average molecular weight (M_n) and polydispersity (PDI) values of each polymer. The data were processed using ASTRA 5.3.2.15 software (Wyatt Technology).

Polymer Synthesis

All monomers and copolymers were synthesized according to our previously published procedures.¹⁻³ Characterization data for the polymers in this report that have been previously studied can be found in the Supporting Information of those sources.

Characterization of Polymers

The methods for determination of molecular weights and polydispersities detailed below have been reported previously in the Supporting Information of reference 2^2 .

GPC calculations: The values derived from the GPC data detailed in the foregoing table are determined according to the following methods:

Target M_n : The target number-average molecular weight (M_n) is determined by the following formula:

Target
$$M_n = M_{eg} + a(M_{cat}) + b(M_{cy})$$

where M_{eg} is the mass of the N-terminal end group (e. g. *t*-BuC₆H₄-CO-), M_{cat} is the mass of the cationic monomer, M_{cy} is the mass of the cyclic monomer, *a* is the total number of cationic monomers, and *b* is the total number of cyclic monomers in the molecule, such that a + b = the intended degree of polymerization (DP). For example, for the Boc-precursor of polymer **1:1 MM:CO** (**Table S1**), the target M_n is 161.22 + 10(214.26) + 10(153.22) = 3836.0.

Obs. M_n and *PDI*: The observed M_n and polydispersity index (PDI) are determined from the gelpermeation chromatogram of the Boc-protected precursors as described above using Astra[®] software. *Obs. DP*: The observed degree of polymerization is calculated from the observed M_n value using the following formula:

$$DP = (M_n - M_{eg}) / [M_{cat}x + M_{cy}(1 - x)]$$

where *x* is the mole fraction of cationic monomer in the feed ratio. The reported number is rounded to the nearest integer. For example, the Boc-precursor of polymer **2:1 MM:CH** (**Table S1**) has an M_n value of 5830 Da by GPC. Its degree of polymerization is (5830-161.22) / [(214.26 * 0.67) + (125.17 * 0.33)] = 30.7, rounded off to 31 residues.

MALDI calculations: The values derived from the MALDI data detailed in the foregoing table are determined from the highest-intensity mass peak of the spectrum according to the following methods. We do not calculate any average molecular weight or polydispersity information from the MALDI spectrum because the largest members of the polymer population are underrepresented, and the recorded spectrum cannot be assumed to be representative of the population as a whole. All information regarding polymer size is calculated *only* for the mass peak identified.

MALDI Target MW: The target MW value for the final compounds is calculated the same way as for the Boc-precursors, except using the mass of the deprotected cationic monomer for M_{cat} , and using exact masses.

MALDI Obs. DP: The degree of polymerization corresponding to the highest-intensity mass peak is calculated after determining the ratio of cationic to cyclic monomer corresponding to that mass. The ratio is determined as follows:

Obs.
$$m/z = M_{eg} + a(M_{cat}) + b(M_{cy}) + M_{ion}$$

where M_{ion} is the mass of the complexed ion in the volatile species. The integers *a* and *b* are varied until the calculated mass is obtained, and the mass of the complexed ion (or water) is added only if it results in a more exact mass than can be obtained without. For example, the highest-intensity mass of polymer **2:1 DM:CH** (**Table S1**) corresponds to a 22-mer: 161.10 + 14(128.10) + 8(125.08) + 38.964 (for K⁺) = 2994.1 (the nearest calculated value to the observed m/z). Masses corresponding to (M+H₂O)⁺ may indicate a polymer molecule where the C-terminal β -lactam has been hydrolyzed to a carboxylic acid.

For most of the MALDI masses reported in the foregoing tables, the ratio of monomers we indicate may not correlate with the ratio of monomers in the polymerization feed. We cannot assume that all polymer molecules are volatilized with the same intensity, nor can we assume that all polymer molecules possess the same ratio of monomers as the feed. The ratio, **2:1** MM:CH for example, refers to the ratio of monomers *in the entire population of molecules*; individual ratios may vary from molecule to molecule.

	GPC o	data for Bo	c-polyn	ners	MALDI data for final polymers ^a					
Polymer	Target	Obs.	DDIb	Obs.	Target	Highest	Obs. DP ^c			
	$\mathbf{M}_{\mathbf{n}}$	$M_n (Da)^b$	PDI	DP ^c	M _n	AI m/z	(cat:cycl)			
MM homopolymer	4447	Ins ^d			2443	2005	16 (16:0)			
DM homopolymer	4727	6380	1.08	~27	2723	4190	31 (31:0+K+H ₂ O)			
1:2 MM:DM	4640	6560	1.18	~29	2636	NS ^e				
2:1 MM:DM	4546	6510	1.14	~29	2542	NS ^e				
1:2 MM:CH	3259	5880	1.04	~37	2516	2649	20 (1:19)			
2:1 MM:CH	3852	5830	1.06	~31	2589	2335	18 (7:11)			
2:1 DM:CH	4040	6350	1.07	~32	2703	2993	22 (14:8+K)			
1:1 MM:CO	3836	5170	1.07	~27	2833	2678	20 (14:6+H)			
1:2 MM:CO a	3633	5250	1.06	~29	2963	2564	19 (13:6+H)			
1:2 MM:CO b	3633	6360	1.06	~36	2963	3197	22 (9:13+H ₂ O)			
1:2 MM:CO c	3633	4780	1.06	~26	2963	2814	21 (15:6+Na)			
C18-1:2 MM:CO	3739	5640	1.05	~31	3069	3050	$22 (16:6+H_2O+Na)$			
2:1 MM:CO a	4040	5110	1.07	~25	2703	2300	16 (9:7+Na+H ₂ O)			
2:1 MM:CO b	4040	5260	1.08	~26	2703	2300	16 (9:7+Na+H ₂ O)			
1:1 DM:CO a	3976	7100	1.06	~36	2973	3363	22 (7:15+Li)			
1:1 DM:CO b	3976	5590	1.09	~28	2973	2647	17 (5:12+Li)			
1:1 DM:CO c	3976	7340	1.06	~38	2973	2799	20 (17:3+H)			
C18-1:1 DM:CO	4083	6140	1.05	~31	3079	2817	18 (9:9+H ₂ O)			
2:1 DM:CO a	4227	6110	1.11	~29	2890	2965	21 (18:3+K)			
2:1 DM:CO b	4227	5670	1.15	~27	2890	3006	20 (9:11+Li)			

Table S1. Molecular Weights and Polydispersities of P-(t-Butyl)Benzamide Nylon-3 Copolymersand Their Boc-Protected Precursors.All polymers' target degree of polymerization was 20.

^a Molecular weight assignments by MALDI were based on the highest-intensity mass peaks from the spectrum.

 ${}^{b}M_{n}$ and PDI were calculated as described previously using Astra[®] software.²

^c DP = degree of polymerization.

^d Ins = insoluble in THF, therefore preventing collection of GPC data.

^e NS = no spectrum was produced.



Figure S1. The MALDI-TOF Mass Spectrum of Polymer 2:1 MM:CH.



Figure S2. The MALDI-TOF Mass Spectrum of Polymer 2:1 DM:CH.







Figure S4. The MALDI-TOF Mass Spectrum of Polymer 1:2 MM:CO b.



Figure S5. The MALDI-TOF Mass Spectrum of Polymer 1:2 MM:CO c.







Figure S7. The MALDI-TOF Mass Spectrum of Polymer 2:1 MM:CO b.









Figure S10. The MALDI-TOF Mass Spectrum of Polymer 1:1 DM:CO c.



Figure S11. The MALDI-TOF Mass Spectrum of Polymer C18-1:1 DM:CO.



Figure S12. The MALDI-TOF Mass Spectrum of Polymer 2:1 DM:CO a.



Figure S13. The MALDI-TOF Mass Spectrum of Polymer 2:1 DM:CO b.

















The MALDI and ¹H NMR spectra of the **MM** and **DM** homopolymers and of polymers **1:2 MM:CH**, **1:2 MM:CO a** and **2:1 MM:CO a** have been reported previously.¹

Pulsating Bubble Surfactometry Data

Batch-to-batch reproducibility. Polymer activities were re-evaluated in subsequent batches in PBS static- and dynamic-bubble mode, as well as for toxic effects against NIH 3T3 fibroblasts. It should be noted that even the largest inter-batch variations did not alter the conclusions of our study. The labor-intensive nature of sample preparation, the PBS assays, and the cytotoxicity experiments precluded us from evaluating inter-batch variation for every polymer. However, our observations, on the whole, indicate that inter-batch variation was minimal, and in almost every case, polymer activities were reproduced within the range of uncertainty for our experiments.

Film	γ* 1 min		γ 2.5 min		γ5 min		γ 10 min		γ_{eq} 20 min	
	Avg	$\boldsymbol{\sigma}^{\dagger}$	Avg	σ	Avg	σ	Avg	σ	Avg	σ
\mathbf{TL}^{\dagger}	61.8	1.6	58.5	1.7	55.8	1.8	53.2	1.7	50.7	1.7
$\mathbf{TL} + \mathbf{MM}^{\mathrm{F}}$	45.9	3.7	44.9	2.8	44.0	2.6	43.6	2.6	43.2	3.0
TL + DM	39.3	3.1	37.8	3.6	36.8	4.2	35.7	4.6	34.6	4.4
TL + 1:2 MM:DM	43.7	2.7	42.8	2.3	41.7	2.1	40.6	1.9	39.4	2.1
TL + 2:1 MM:DM	48.1	1.8	46.7	1.5	45.3	1.1	44.4	1.4	43.6	1.4
TL + 1:2 MM:CH	39.3	2.3	35.0	2.5	33.8	2.5	32.7	2.4	31.6	1.2
TL + 2:1 MM:CH	43.0	2.5	41.3	1.8	39.7	1.5	37.1	2.6	32.8	3.4
TL + 2:1 DM:CH	36.6	2.2	34.1	2.3	32.0	0.9	30.4	1.1	29.2	1.5
TL + 1:1 MM:CO	35.4	0.8	32.4	0.7	30.4	0.7	28.8	0.5	26.1	0.6
TL + 1:1 MM:CO	30.1	2.0	28.1	1.5	26.5	1.2	25.7	0.2	25.8	0.3
TL + 2:1 MM:CO	38.7	2.8	36.1	4.9	34.8	5.3	33.5	4.9	30.7	3.2
TL + 1:1 DM:CO	29.5	1.1	27.1	0.7	25.9	0.4	25.7	0.5	25.2	0.8
TL + 2:1 DM:CO	33.5	2.9	30.8	2.7	28.2	1.6	26.9	1.1	25.7	0.4
$TL + SP-B_{1-25}$	40.5	1.2	39.4	1.9	37.9	1.2	36.8	1.0	35.6	1.3
$TL + KL_4$	27.7	0.8	24.7	1.3	22.4	0.7	22.4	0.7	21.6	0.8
TL + Peptoid B1	44.6	3.0	42.5	2.8	40.7	2.3	39.2	1.9	38.1	1.2

Table S2. PBS Adsorption Data at Selected Time Intervals of Originally Synthesized Polymers, 37 $^\circ\mathrm{C}.$

* Mean surface tension in mN m⁻¹

[‡] Tanaka lipid mixture, DPPC:POPG:PA 68:22:9 [wt]

¥ Mimics added at 10 wt% relative to the total lipid content

 $\dagger \sigma$ is the standard deviation of the mean

Film	γ* 1 min		γ 2.5 min		γ5 min		γ 10 min		$\gamma_{eq} \ 20 \ min$	
	Avg	$\boldsymbol{\sigma}^{\dagger}$	Avg	σ	Avg	σ	Avg	σ	Avg	σ
\mathbf{TL}^{\dagger}	61.8	1.6	58.5	1.7	55.8	1.8	53.2	1.7	50.7	1.7
TL + 1:2 MM:CO a^{F}	30.1	2.0	28.1	1.5	26.5	1.2	25.7	0.2	25.8	0.3
TL + 1:2 MM:CO b	30.5	1.2	28.8	1.2	26.2	1.5	25.0	0.5	24.3	0.2
TL + 1:1 MM:CO c	29.3	1.5	27.6	2.3	25.9	0.9	24.6	0.8	23.4	1.9
TL + C18-1:2 MM:CO	30.0	1.9	27.7	1.6	25.0	0.7	24.5	0.9	24.1	2.3
TL + 2:1 MM:CO a	38.7	2.8	36.1	4.9	34.8	5.3	33.5	4.9	30.7	3.2
TL + 2:1 MM:CO b	48.5	0.5	45.4	0.7	42.1	0.5	39.6	0.9	36.1	2.6
TL + 1:1 DM:CO a	29.5	1.1	27.1	0.7	25.9	0.4	25.7	0.5	25.2	0.8
TL + 1:1 DM:CO b	29.0	0.9	27.0	1.0	25.3	1.3	24.8	0.9	24.5	1.2
TL + 1:1 DM:CO c	29.6	0.9	26.9	0.8	25.1	0.5	24.4	0.7	22.8	1.5
TL + C18-1:1 DM:CO	28.6	0.5	26.4	0.3	24.6	0.4	24.2	0.2	23.9	0.3
TL + 2:1 DM:CO a	33.5	2.9	30.8	2.7	28.2	1.6	26.9	1.1	25.7	0.4
TL + 2:1 DM:CO b	33.7	1.5	31.0	1.0	28.8	0.7	26.1	0.2	24.2	0.4

Table S3. PBS Adsorption Data at Selected Time Intervals of Re-Synthesized Polymers, 37 °C.

* Mean surface tension in mN m⁻¹

[‡] Tanaka lipid mixture, DPPC:POPG:PA 68:22:9 [wt]

¥ Mimics added at 10 wt% relative to the total lipid content

 $\dagger \sigma$ is the standard deviation of the mean

Figure S29. PBS Data for Lipids + Positive Controls in Static- and Dynamic-Bubble Mode at 37 °C. Representative static-bubble adsorption traces (Panel A) and representative dynamic-bubble hysteresis loops at 5 minutes of cycling (20 cpm) (Panel B) for Tanaka lipids alone (TL) and TL + 10 relative weight % of each positive control in an aqueous buffer (150 mM NaCl, 10 mM HEPES, 5 mM CaCl₂, pH 6.9) suspension at 37 °C.



		min			2.5	min		10 min				
Film	γm	ax	γn	nin	γm	ax	γn	nin	γm	ax	γn	nin
	Avg	$\boldsymbol{\sigma}^{\dagger}$	Avg	σ	Avg	σ	Avg	σ	Avg	σ	Avg	σ
\mathbf{TL}^{\dagger}	60.8	2.8	15.0	2.5	61.0	2.4	14.3	2.9	60.1	2.3	12.0	2.9
$\mathbf{TL} + \mathbf{MM}^{\mathrm{F}}$	49.9	1.3	7.95	3.63	49.7	1.4	6.38	2.38	48.1	1.7	5.03	1.41
TL + DM	46.3	0.7	5.84	2.57	46.0	0.7	5.10	1.83	44.2	1.4	5.75	1.93
TL + 1:2 MM:DM	47.9	0.7	3.11	3.0	47.4	0.9	2.62	2.36	45.9	0.8	2.62	2.89
TL + 2:1 MM:DM	47.9	1.3	7.79	2.58	47.6	1.4	7.43	3.12	46.9	0.6	5.37	1.70
TL + 1:2 MM:CH	47.4	0.8	< 1	-	47.3	0.8	< 1	-	47.5	0.6	< 1	-
TL + 2:1 MM:CH	48.1	1.9	2.72	2.44	46.9	1.0	3.08	2.67	44.3	1.1	3.03	2.63
TL + 2:1 DM:CH	44.6	1.0	2.81	0.54	44.0	1.3	3.08	0.63	42.5	1.5	4.45	0.60
TL + 1:1 MM:CO	44.0	0.4	< 1	-	43.9	0.6	< 1	-	42.8	0.2	< 1	-
TL + 1:1 MM:CO	43.1	1.2	< 1	-	42.5	0.7	< 1	-	44.5	1.3	< 1	-
TL + 2:1 MM:CO	45.7	0.6	1.67	1.97	44.9	0.7	1.84	1.53	42.2	1.5	2.47	1.95
TL + 1:1 DM:CO	39.5	0.7	< 1	-	39.3	1.1	< 1	-	42.0	2.6	< 1	-
TL + 2:1 DM:CO	41.4	1.2	< 1	-	40.1	1.5	< 1	-	39.6	2.6	< 1	-
$TL + SP-B_{1-25}$	49.6	0.5	< 1	-	49.9	0.7	< 1	-	49.8	0.7	< 1	-
$TL + KL_4$	47.2	1.3	< 1	-	47.9	1.2	< 1	-	48.2	1.3	< 1	-
TL + Peptoid B1	48.9	1.7	< 1	-	49.2	1.6	< 1	-	49.0	1.6	< 1	-

Table S4. PBS Dynamic Data at Selected Time Intervals of Originally Synthesized Polymers, 37 $^{\circ}\mathrm{C}.$

* Mean surface tension in mN $\rm m^{-1}$

[‡] Tanaka lipid mixture, DPPC:POPG:PA 68:22:9 [wt]

¥ Mimics added at 10 wt% relative to the total lipid content

† σ is the standard deviation of the mean. No σ values are available for "< 1" table entries.

		min			2.5	min		10 min				
Film	γm	ax	γn	nin	γm	ax	γr	nin	γm	ax	γn	nin
	Avg	$\boldsymbol{\sigma}^{\dagger}$	Avg	σ	Avg	σ	Avg	σ	Avg	σ	Avg	σ
\mathbf{TL}^{\dagger}	60.8	2.8	15.0	2.5	61.0	2.4	14.3	2.9	60.1	2.3	12.0	2.9
TL + 1:2 MM:CO a^{a}	43.1	1.2	< 1	-	42.5	0.7	< 1	-	44.5	1.3	< 1	-
TL + 1:2 MM:CO b	42.6	0.8	< 1	-	41.8	0.2	< 1	-	40.9	0.3	< 1	-
TL + 1:1 MM:CO c	43.6	0.7	< 1	-	43.7	1.3	< 1	-	44.2	0.8	< 1	-
TL + C18-1:2 MM:CO	42.1	0.8	< 1	-	41.2	0.3	< 1	-	40.1	0.0	< 1	-
TL + 2:1 MM:CO a	45.7	0.6	1.67	1.97	44.9	0.7	1.84	1.53	42.2	1.5	2.47	1.95
TL + 2:1 MM:CO b	48.3	0.8	< 1	-	47.5	0.8	< 1	0.8	45.8	1.5	< 1	-
TL + 1:1 DM:CO a	39.5	0.7	< 1	-	39.3	1.1	< 1	-	42.0	2.6	< 1	-
TL + 1:1 DM:CO b	43.0	0.4	< 1	-	41.9	0.2	< 1	-	42.0	0.5	< 1	-
TL + 1:1 DM:CO c	42.8	0.9	< 1	-	42.5	0.7	< 1	-	42.7	0.4	< 1	-
TL + C18-1:1 DM:CO	38.3	1.2	< 1	-	37.6	0.9	< 1	-	37.5	1.0	< 1	-
TL + 2:1 DM:CO a	41.4	1.2	< 1	-	40.1	1.5	< 1	-	39.6	2.6	< 1	-
TL + 2:1 DM:CO b	43.4	0.5	< 1	-	42.7	0.7	< 1	-	41.3	0.5	< 1	-

Table S5. PBS Dynamic Data at Selected Time Intervals of Re-Synthesized Polymers, 37 °C.

* Mean surface tension in mN m⁻¹

[‡] Tanaka lipid mixture, DPPC:POPG:PA 68:22:9 [wt]

¥ Mimics added at 10 wt% relative to the total lipid content

† σ is the standard deviation of the mean. No σ values are available for "< 1" table entries.

Film	Avg % Comp	$\boldsymbol{\sigma}^{\dagger}$
TL	42.5	2.9
TL + MM	31.1	4.6
TL + DM	27.0	1.5
TL + 1:2 MM:DM	28.8	1.3
TL + 2:1 MM:DM	29.5	2.5
TL + 1:2 MM:CH	28.3	1.4
TL + 2:1 MM:CH	30.5	1.3
TL + 2:1 DM:CH	28.3	2.2
TL + 1:1 MM:CO	23.9	2.0
TL + 1:2 MM:CO a	22.4	3.0
TL + 1:2 MM:CO b	16.4	0.7
TL + 1:2 MM:CO c	22.0	3.8
TL + C18-1:2 MM:CO	13.9	1.5
TL + 2:1 MM:CO a	26.4	1.8
TL + 2:1 MM:CO b	29.5	2.1
TL + 1:1 DM:CO a	17.5	3.1
TL + 1:1 DM:CO b	21.1	1.5
TL + 1:1 DM:CO c	20.9	3.8
TL + C18-1:1 DM:CO	11.6	2.1
TL + 2:1 DM:CO a	18.4	1.3
TL + 2:1 DM:CO b	23.2	2.0
$TL + SP-B_{1-25}$	33.2	2.7
$TL + KL_4$	24.9	3.7
TL + Peptoid B1	31.4	1.2

Table S6. Percent Bubble Surface Area Compression Required to Reach 20 mN m⁻¹ Upon Compression, at 5 minutes of Dynamic-Bubble Pulsation on the PBS, 37 °C.

 $\dagger \, \sigma$ is the standard deviation of the mean



Figure S30. PBS Hysteresis Pulsation Loops for Lipid-Polymer Films in Dynamic-Bubble Mode, 5 Minutes, 37 $^{\circ}\mathrm{C}.$

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