Supplemental Data for

Close mimicry of lung surfactant protein B by "clicked" dimers of helical, cationic peptoids

Michelle T. Dohm, Shannon L. Seurynck-Servoss, Jiwon Seo, Ronald N. Zuckermann, and Annelise E. Barron

Surfactant Activity Comparisons

To estimate the extent of biomimicry provided by the peptoids, their *in vitro* surface activity characteristics in a mixed lipid film were compared with those known for the natural protein or relevant peptide mimics. Several tools for estimating *in vitro* surface activity exist, and the two chosen for this study were the Langmuir-Wilhelmy surface balance (LWSB) with epifluorescent (FM) imaging, and the pulsating bubble surfactometer (PBS). The effectiveness of these and other tools have been evaluated in the literature, and both are still continually used in both the academic and pharmaceutical development settings. In this work, these tools were efficiently and reliably used to enable the needed comparisons with repeatable results.

Similarly, the choice of lipid mixture, the Tanaka lipids (TL)[1], 1,2-diacyl-*sn*-glycero-3-phosphocholine (DPPC): 1-palmitoyl-2-oleoyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)] (POPG): palmitic acid (PA) 68:22:9 by weight, stemmed from a need for comparison. The complete elimination of PA from lipid formulations has recently been advocated because it increases the viscosity of the lipid film and causes it to reach lower γ than in its absence. Though the fraction of PA in natural LS is small, it is still commonly added to clinical LS formulations due to its added benefit *in vivo*, and in a trial of different lipid formulations, we found that *in vitro* surface

activity characteristics of peptoids in the physiologically relevant γ regime were most easily identifiable and compared with this particular formulation[2]. Once an optimal mimic has been developed, other lipid formulations that more closely represent physiological conditions *in vivo* will be utilized[3]. Where relevant, selected temperatures reflected the phase behavior of the lipid film well below and roughly at or above the T_c of the lipid mixture, 25 and 37 °C, respectively, where the latter is more physiologically relevant. The addition of mimics at 10 wt% relative to the total lipid content was also chosen to standardize the formulations and facilitate comparison. Experiments at different wt%'s have revealed small increases and decreases (± 10%) to activity with corresponding increases and decreases in peptoid content.

Azide Submonomer Synthesis and Azide- and Alkyne-Containing Peptoid Monomer Synthesis and Purification

The azide submonomer 3-azidopropylamine was synthesized according to the work of Carboni *et al*[4]. This submonomer was then used in peptoid synthesis of the azide-containing peptoid monomer (first precursor of **dB2c**). The alkyne submonomer propargylamine is commercially available (Aldrich), and was used in alkyne-containing peptoid monomer synthesis (second precursor of **dB2c**) without further purification. After the azide-containing and alkyne-containing peptoid "monomers" were individually synthesized, they were purified by RP-HPLC, and masses were confirmed *via* MS(HR-MALDI).

Peptoid Dimerization *via* Microwave-assisted 1,3-dipolar Cycloaddition ('Click-Chemistry')

All solvents were degassed with N_2 streaming for 15 minutes. To a microwave reaction tube equipped with a magnetic stirrer were successively added stock solutions of the azide-containing peptoid monomer in *t*-BuOH/H_2O 1:1 (0.3 mL, 1.23 μmol, 4.1 mM stock solution), the alkyne-containing peptoid monomer in *t*-BuOH/H_2O 1:1 (0.6 mL, 1.08 μmol, 1.8 mM stock solution), L-(+)sodium ascorbate in *t*-BuOH/H_2O 1:1 (2 μL, 0.30 μmol, 197 mM stock solution), and $CuSO_4 \cdot 5H_2O$ in water (4 μL, 0.09 μmol, 23 mM stock solution). The tube was sealed and heated in the microwave reactor for 1 h (100 °C, 38 W, absorption level:high). Reaction completion was determined by RP-HPLC, and then the mixture was dialyzed as described in the main text. The product (**dB2c**) was then purified by RP-HPLC, mass confirmed by MS(HR-MALDI), and lyophilized to a powder.

Circular Dichroism Methods

Circular dichroism (CD) spectra were acquired on a Jasco J-715 spectropolarimeter (Easton, MD) in a cylindrical quartz cuvette (Hellma model 121-QS, Forest Hills, NY) with a scan rate of 100 nm min^{-1}, 0.02-cm path length, 0.2 nm data pitch, 1 nm bandwith, 2 s response, and 100 mdeg sensitivity. Samples were dissolved in methanol from lyophilized powder to a concentration of ~ 60 μM and run at RT. Each presented CD spectrum represents the average of 40 accumulations.

Figure 1 SD. CD spectra of peptoid mimics at 60 μM in methanol at room temperature. Each spectrum represents the average of 40 accumulations. λ is Wavelength (nm) and θ is Per Residue Molar Ellipticity (deg cm^2 $dmol^{-1}$). **B1** (red), **dB1** (red, open circles), **mB2** (blue), **dB2** (blue, open squares), **dB2c** (green), **mB3** (orange), **dB3** (orange, open triangles), **dB4** (black, open diamonds).

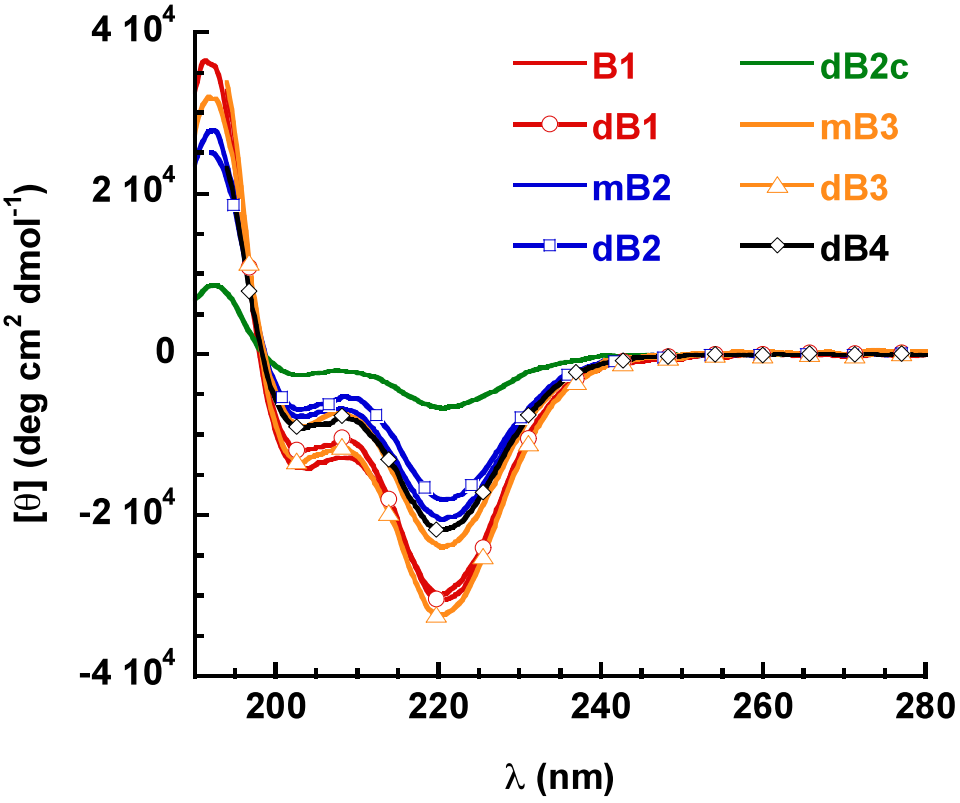

Table I SD. Additional Sequence Information and Quantitative Content in the Lipid Film.

Molecule	Sequence	Absolute wt% in Film	Mol % in Film
B1	H-*N*spe$_2$-(*N*Lys[a]-*N*spe$_2$)$_5$-CO*N*H$_2$	9.1	2.37
dB1	[H-***N*Cys**-*N*spe$_2$-(*N*Lys-*N*spe$_2$)$_5$-CO*N*H$_2$]$_2$	8.6	1.16
mB2	H-*N*spe$_2$-(*N*Lys-*N*spe$_2$)$_5$-*N*meg$_5$-CO*N*H$_2$	9.1	1.96
dB2	[H-*N*spe$_2$-(*N*Lys-*N*spe$_2$)$_5$-*N*meg$_4$-***N*Cys**[b]-CO*N*H$_2$]$_2$	10.0	0.99
dB2c	[H-*N*spe$_2$-(*N*Lys-*N*spe$_2$)$_5$-*N*meg$_4$-***N*'click'**-CO*N*H$_2$]$_2$	9.1	0.99
mB3	H-*N*spe$_2$-(*N*Lys-*N*spe$_2$)$_5$-(*N*meg-*N*prp)$_2$-*N*me-CO*N*H$_2$	9.1	1.98
dB3	[H-*N*spe$_2$-(*N*Lys-*N*spe$_2$)$_5$-(*N*meg-*N*prp)$_2$-***N*Cys**-CO*N*H$_2$]$_2$	9.9	1.01
dB4	[H-*N*spe$_2$-(*N*Lys-*N*spe$_2$)$_5$-(*N*meg-*N*pm)$_2$-***N*Cys**-CO*N*H$_2$]$_2$	10.1	0.97
(KL)$_4$K	H$_2$*N*-KLLLLKLLLLKLLLLKLLLLK-COOH	9.1	2.49
SP-B$_{1\text{-}25}$	H$_2$*N*-FPIPLPYAWLARALIKRIQAMIPKG-COOH	9.1	2.16

[a] Underlined side chains are cationic.

[b] Bold side chains are dimerization points.

Table II SD. PBS Adsorption Data at Selected Time Intervals, 37 °C.

Film	γ^a 1 min		γ 2.5 min		γ 5 min		γ 10 min		γ_{eq} 20 min	
	Avg	σ^c	Avg	σ	Avg	σ	Avg	σ	Avg	σ
TL	63.8	1.7	61.2	1.7	58.8	1.6	56.5	1.6	54.2	2.0
TL + B1[b]	49.5	1.9	46.3	2.3	43.2	1.4	39.9	1.7	39.0	1.5
TL + dB1	46.2	1.7	44.1	1.4	42.5	1.0	41.7	1.4	41.4	2.1
TL + mB2	49.4	1.5	43.3	1.2	41.1	0.7	40.2	0.6	40.0	0.5
TL + dB2	57.4	0.8	53.8	0.7	50.2	0.7	47.6	0.8	45.5	0.7
TL + dB2c	35.1	2.3	30.6	2.9	25.4	2.3	22.5	1.1	22.1	0.5
TL + mB3	43.7	2.2	40.1	1.4	38.4	1.0	37.4	0.9	36.7	0.8
TL + dB3	43.8	2.5	41.4	2.2	40.0	2.1	38.7	2.5	38.2	2.9
TL + dB4	52.6	4.1	50.3	3.5	48.6	3.1	47.3	2.5	46.4	2.4
TL + SP-$B_{1\text{-}25}$	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	21.9	0.5	21.6	0.8

[a] Mean surface tension in mN m^{-1}.

[b] Mimics added at 10 wt% relative to the total lipid content.

[c] σ is the standard deviation of the mean.

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