Supporting Information

Fractional Polymerization of a Suspended Planar Bilayer Creates a Fluid, Highly Stable Membrane for Ion Channel Recordings

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UV Polymerization

Although the conversion of bis-DenPC monomer to polymer can be followed by UV-vis absorbance,[1-2] insufficient material is present in a BLM on a pipet to enable this measurement to be made with adequate sensitivity. To determine the irradiation time necessary to polymerize BLMs with a high degree of conversion, bis-DenPC vesicles were prepared with an average diameter of 100 nm using the vesicle extrusion method.[3] Vesicles suspended in recording buffer were then placed into the *cis* (bath) solution compartment used for alamethicin recordings and irradiated. This optical geometry was identical to that used to irradiate BLMs.

Aliquots were withdrawn periodically to measure UV-vis absorbance. Spectra of samples measured after irradiation for 0, 1, 5, and 15 minutes, respectively, are shown in Figure S1A. The band due to the dienoyl group at ~260 nm decreases over time, signifying a conversion of the monomeric bis-DenPC to polymerized bis-DenPC (poly(bis-DenPC)). The normalized absorbance values at 260 nm plotted versus time (in Figure S1B) show that greater than 95% conversion of monomer to polymer occurred in 15 min. These data agree with those obtained from other experiments in which the photopolymerization of bis-DenPC was monitored.[1-2] Thus 15 minutes of UV irradiation was determined to be sufficient for polymerization of BLMs.

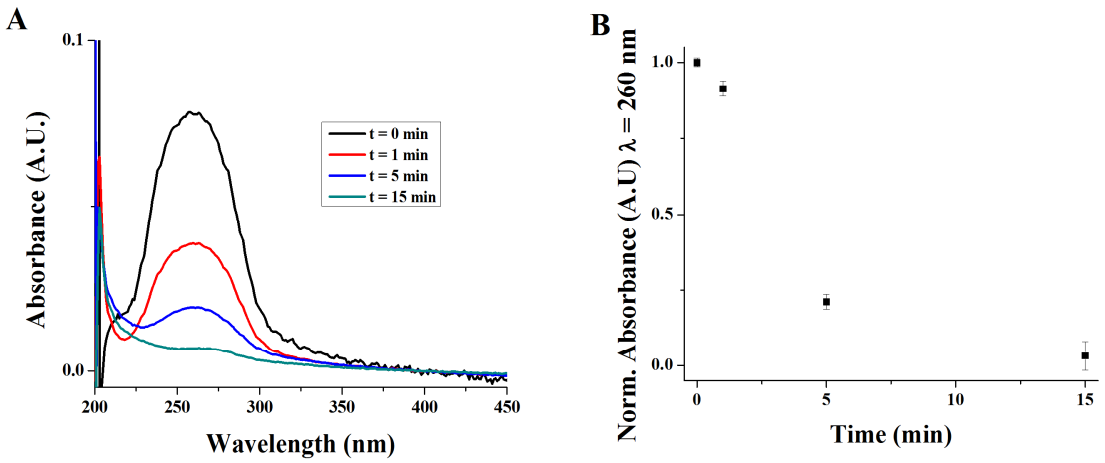

Figure S1. UV spectra of lipid vesicles were measured to verify conversion of bis-DenPC to poly(bis-DenPC). (A) Spectra were acquired after irradiation with UV light for 0, 1, 5, and 15 min. (B) Normalized absorbance values (λ = 260 nm).

***i*-V Plots of BLMs**

Current versus voltage (*i*-V) plots of BLMs were acquired by applying sequential steps in applied potential of 10 mV for 100 msec, from -150 to +150 mV. At each potential, the mean current was measured for a period of 20 msec following decay of the capacitive peak to <2% of its initial value. BLMs composed of DPhPC, bis-DenPC, and 1:1 DPhPC:bis-DenPC were formed and *i*-V curves were measured before and after UV irradiation. For each composition, 20 BLMs were characterized. Typical *i*-V plots are shown in Figure S2. Conductance and current data obtained from these experiments are presented in Table 1 in the article.

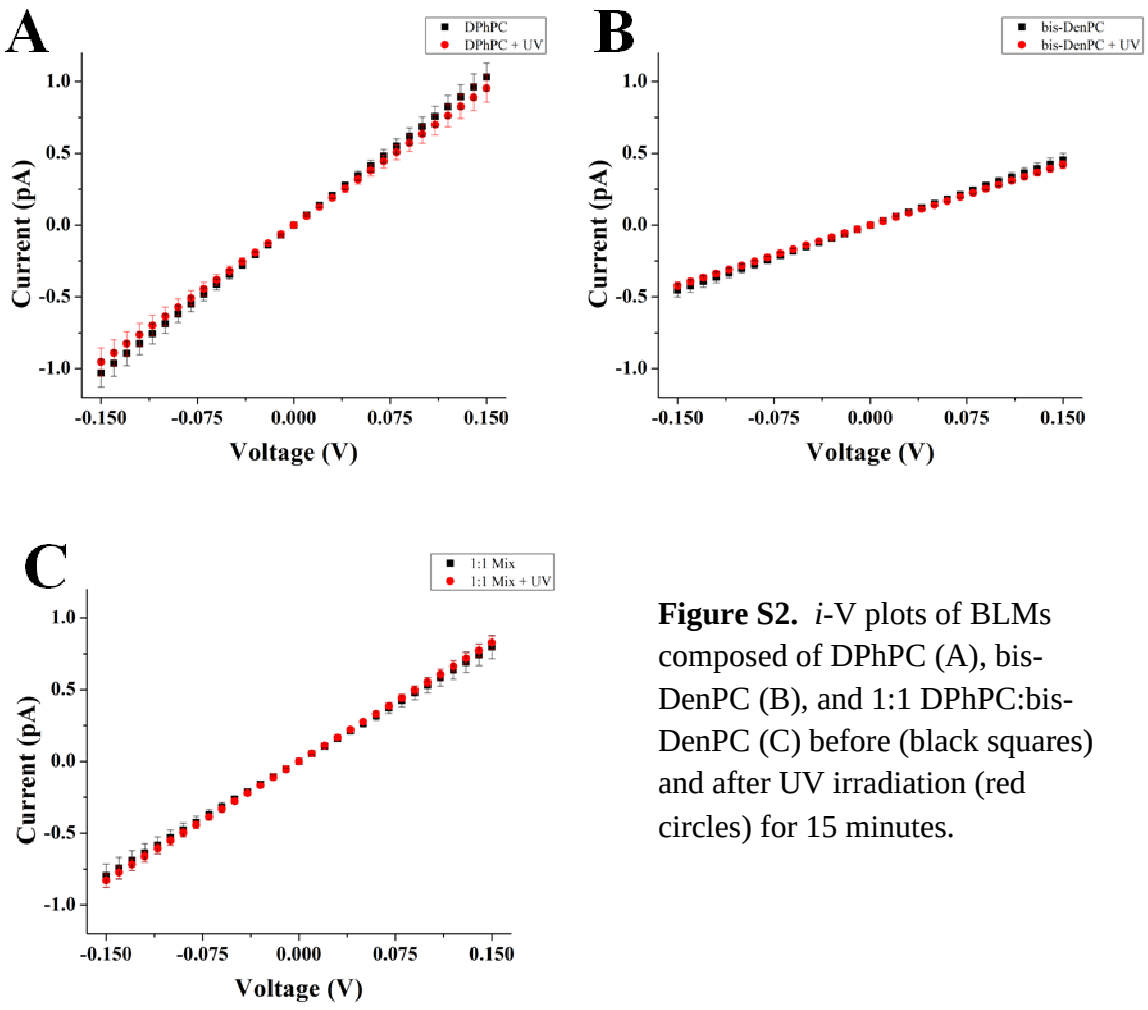

Figure S2. *i*-V plots of BLMs composed of DPhPC (A), bis-DenPC (B), and 1:1 DPhPC:bis-DenPC (C) before (black squares) and after UV irradiation (red circles) for 15 minutes.

Voltage Screens of Alamethicin in BLMs

Alamethicin channel activity at -150, -100, -50, 0, 50, 100, and 150 mV was measured for each of the three lipid compositions (DPhPC, bis-DenPC, and 1:1 DPhPC:bis-DenPC) to investigate whether voltage gating was affected by differences in BLM composition. Figure S3 shows representative recordings for a DPhPC BLM incubated with 60 ng/mL of alamethicin. Voltage gating is observed in both the positive and negative directions which agrees with published data.[4-5] Recordings for bis-DenPC and 1:1 DPhPC:bis-DenPC BLMs incubated with 60 ng/mL of alamethicin also exhibited both positive and negative voltage gating (data not shown), and no significant difference in voltage dependence relative to DPhPC were observed. Small differences in onset voltage and current magnitude were apparent, e.g., a shift to more positive voltages occurs with 1:1 DPhPC:bis-DenPC. One likely source of these differences is that placing the micropipet near the Ag/AgCl electrode and sometimes contacting it caused the electrode to drift and resulted in some uncertainty in the applied potential. Other potential sources include differences in the structure of the chains of DPhPC and bis-DenPC, differences in the concentration of the peptide in each type of BLM, and the proximity of the measurement temperature (22±2 ˚C) to the T_m of bis-DenPC (20.2 ˚C);[6-11] these are discussed further in the next section.

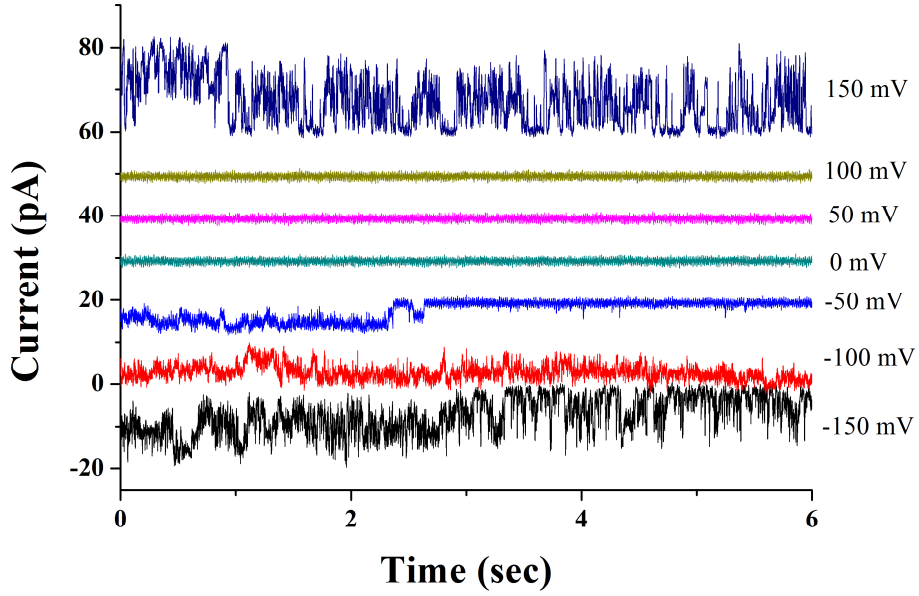

Figure S3. Ion channel activity in a DPhPC BLM that was incubated with 60 ng/mL of alamethicin. Recordings were acquired at 50 mV intervals over a potential range of -150 to +150 mV. For clarity in presentation, each trace is offset from the one below it by 10 pA.

Alamethicin Activity at Concentrations ≥ 60 ng/mL

Representative recordings of alamethicin activity in non-irradiated BLMs composed of DPhPC, bis-DenPC, and 1:1 DPhPC:bis-DenPC are shown in Figures S4A, B, and C, respectively. The alamethicin concentration was 60 ng/mL and the applied potential was +150 mV. The corresponding all-points histograms are presented in Figures S5A, B, and C. The frequency of current bursts was comparable among the three lipid compositions while the number of subconductance states as well as their respective mean currents and widths showed some differences. For example, bis-DenPC exhibited fewer and slightly broader states relative to DPhPC and the 1:1 mixture. Previous studies have shown that alamethicin activity is affected by the lipid structure and composition of the BLM, including acyl chain length and degree of saturation, head group charge, and cholesterol content.[8-10,12-13] The proximity of the temperature of the measurement (here it was 21-24 ˚C) to the main phase transition temperature of the lipid (T_m for bis-DenPC is 20.2 ˚C[14]) is also known to affect IC activity.[13] In addition, the concentration of peptide in these BLMs may vary with the lipid structure (i.e., the partition coefficient may not be equal for DPhPC and bis-DenPC). Thus some differences in alamethicin activity such as those observed here are not unexpected, although these differences appear to be concentration-dependent because at peptide concentrations <60 ng/mL, these differences were not observed (as described in the article).

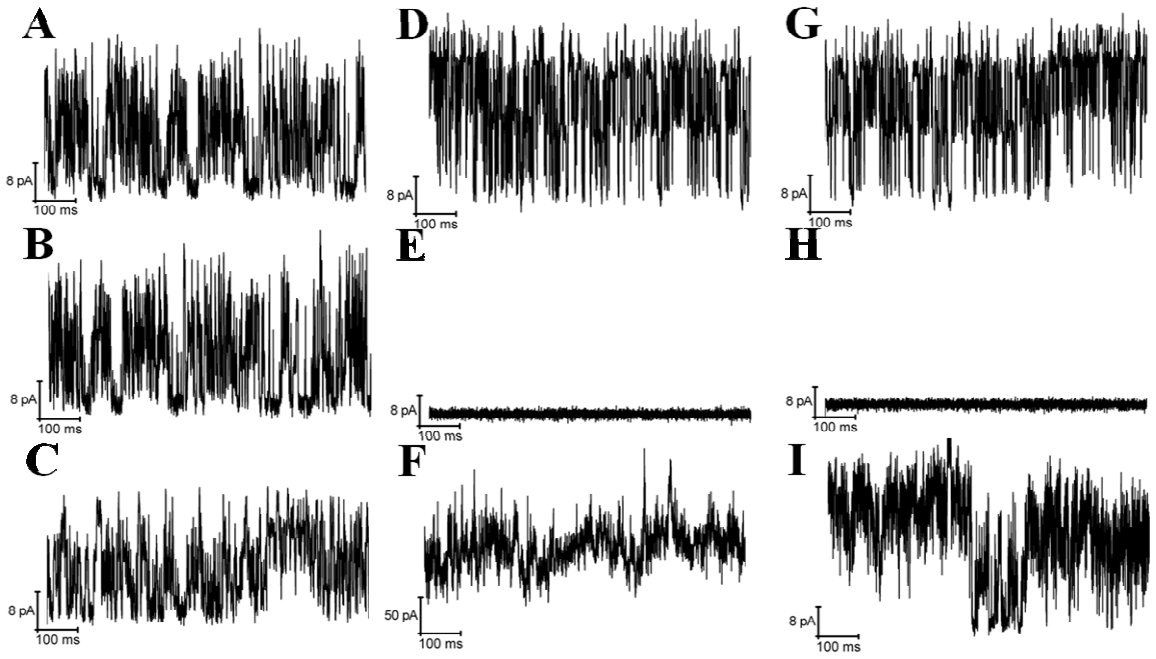

Figure S4. Typical recordings of BLMs composed of DPhPC (A, D, G), bis-DenPC (B, E, H), and 1:1 DPhPC:bis-DenPC (C, F, I) that were incubated with alamethicin at 60 ng/mL: non-irradiated (A, B, C), incubation before UV irradiation (D, E, F), and incubation after UV irradiation (G, H, I). All current scales are equal except for (F).

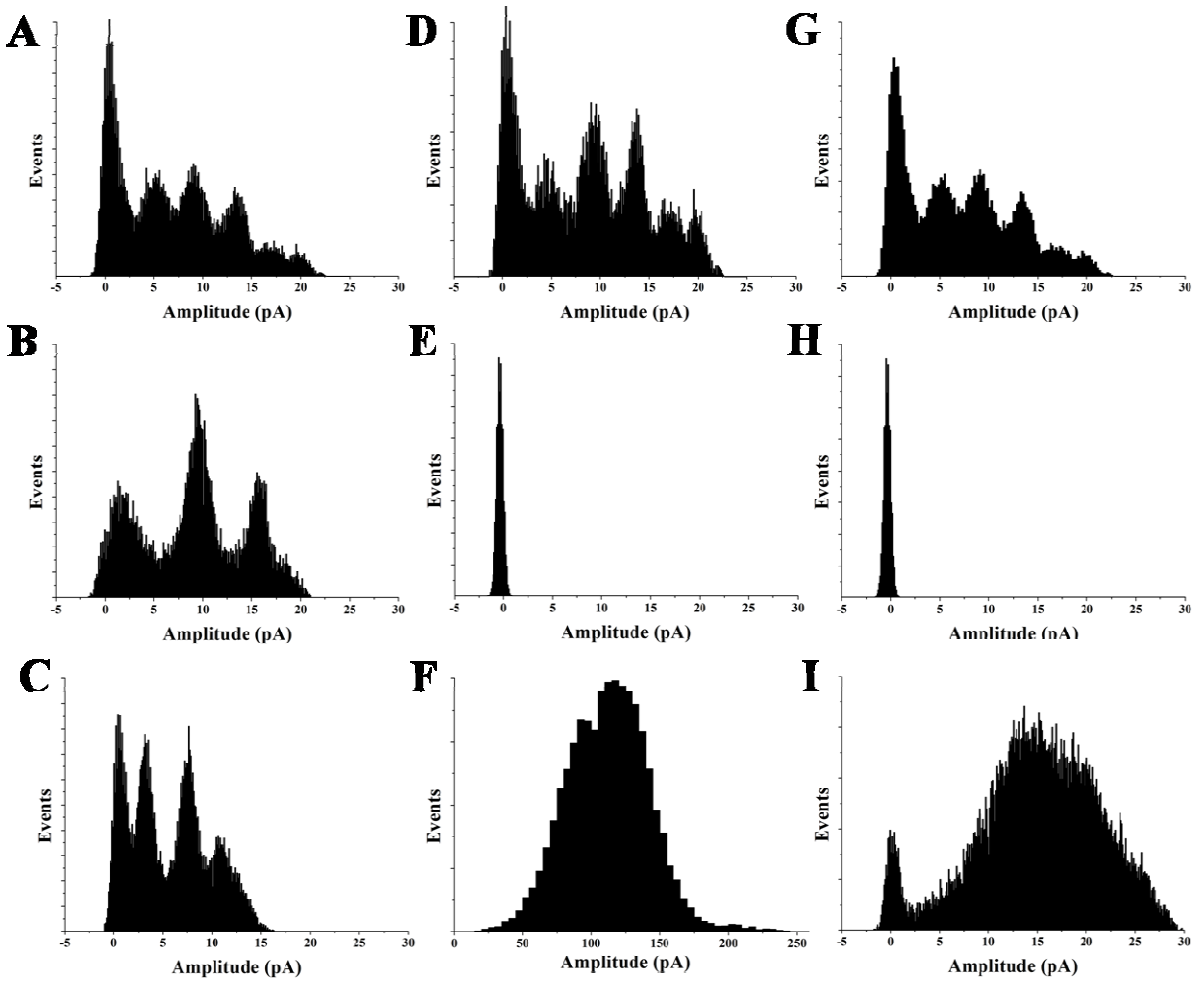

Figure S5. All-points histograms, obtained from the recordings in Figure S4, from BLMs composed of DPhPC (A, D, G), bis-DenPC (B, E, H), and 1:1 DPhPC:bis-DenPC (C, F, I) that were incubated with alamethicin at 60 ng/mL: non-irradiated (A, B, C), incubation before UV irradiation (D, E, F), and incubation after UV irradiation (G, H, I).

Representative recordings of IC activity in DPhPC, bis-DenPC, and 1:1 DPhPC:bis-DenPC BLMs that were incubated with alamethicin at 60 ng/mL followed by UV irradiation for 15 minutes are shown in Figure S4D, E, and F, respectively. The corresponding all-points histograms are presented in Figures S5D, E, and F. No noticeable differences in activity were seen in DPhPC before and after irradiation, showing that the UV exposure did not measurably affect the peptide. In contrast, irradiation of bis-DenPC completely eliminated alamethicin activity, as expected because lipid cross-linking significantly attenuates bilayer fluidity. In the case of 1:1 DPhPC:bis-DenPC, the current due to IC activity significantly increased and discrete subconductance states were absent. As discussed in the article, this result is due to concentration of the peptide in fluid DPhPC domains. Finally, BLMs were UV irradiated followed by incubation with alamethicin at 60 ng/mL. Representative recordings of IC activity in DPhPC,

bis-DenPC, and 1:1 DPhPC:bis-DenPC are shown in Figures S4G, H, and I. The corresponding all-points histograms are presented in Figures S5G, H, and I. The IC activity in DPhPC was indistinguishable from non-irradiated BLMs. In bis-DenPC, no activity was observed. The activity in 1:1 DPhPC:bis-DenPC was greater than for non-irradiated BLMs, but less than that observed when incubation preceded photopolymerization. The reasons for the latter observation are discussed in the article.

Experiments were also performed on non-irradiated BLMs incubated with alamethicin at 120 ng/mL. A representative recording and all-points histogram for DPhPC is shown in Figure S6. Near-continuous IC activity was observed and as a consequence, discrete subconductance states could not be resolved. Similar data were obtained for 1:1 DPhPC:bis-DenPC and bis-DenPC (data not shown).

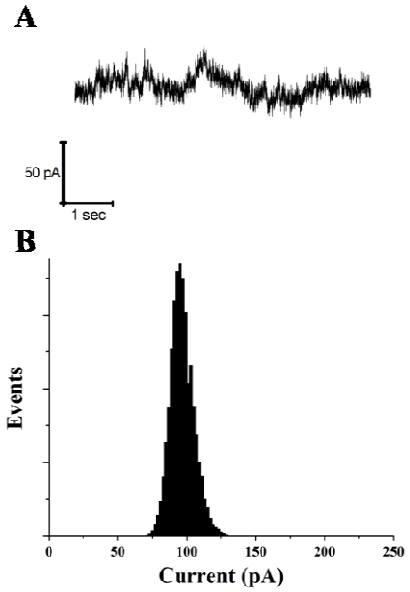

Figure S6. Recording (A) and all-points histogram (B) of a DPhPC BLM incubated with 120 ng/mL of alamethicin.

Analysis of Lifetimes of Alamethicin Subconductance States

Lifetimes of subconductance states of alamethicin in BLMs were analyzed by constructing a histogram of open times for each state formed by a transition from a previous state. For example, alamethicin channels in state O1 are formed by a transition from either the closed state (C) or from state O2; thus two histograms were constructed for state O1. Lifetimes for each state were determined by fitting the histograms to a single-exponential decay (minimum $R^2 > 0.99$). Lifetimes were determined as a function of lipid composition, BLM polymerization, alamethicin concentration, and whether the peptide was incubated before or after the BLM was UV irradiated. Lifetime data are listed in Table S1 in which row designations (in the left-most column) are used to assist in comparing the data sets.

Comparing non-irradiated BLMs incubated with 30 ng/mL alamethicin (rows 1-3), the lifetimes of the O1-O4 states in bis-DenPC were slightly less than the respective lifetimes in DPhPC, and in 1:1 DPhPC:bis-DenPC, the respective lifetimes were intermediate between those in the single lipid BLMs. The lack of significant lifetime differences indicates that lipid structure had a minimal effect on IC activity.

UV irradiation of DPhPC BLMs did not result in any detectable changes in O1-O4 lifetimes (compare rows 1, 4, and 7), whereas irradiation of bis-DenPC BLMs eliminated alamethicin activity, regardless of whether incubation was performed before or after UV irradiation (rows 5 and 8). These findings are consistent with those presented in the article. UV irradiation of 1:1 DPhPC:bis-DenPC, both before or after alamethicin incubation, produced significantly shorter lifetimes for all subconductance states (compare rows 6 and 9 to row 3). This result is likely due to an increase in peptide concentration in fluid DPhPC domains, due to exclusion of the peptide from the poly(bis-DenPC) domains.

To further investigate this hypothesis, two experiments were performed:

1) Lifetimes of subconductance states in non-irradiated 1:1 DPhPC:bis-DenPC: BLMs that were incubated with 60 ng/mL alamethicin were measured. These lifetimes (row 11) are much shorter than those obtained when the peptide concentration was 30 ng/mL (row 3), showing that the O1-O4 lifetimes are inversely correlated with alamethicin concentration. Thus the relatively short lifetimes obtained for polymerized 1:1 DPhPC:bis-DenPC BLMs at 30 ng/mL (rows 6 and 9) are consistent with the hypothesis that alamethicin is concentrated into fluid domains upon polymerization.

2) 1:1 DPhPC:bis-DenPC BLMs were incubated with 15 ng/mL alamethicin and then UV irradiated. The lifetimes (row 10) are shorter than those obtained for non-irradiated, 1:1 DPhPC:bis-DenPC BLMs at 30 ng/mL (row 3). The shorter lifetimes in the polymerized membrane indicate that the peptide concentration in the DPhPC domains is higher than that in an unpolymerized membrane, even when the incubation concentration is doubled to 30 ng/mL.

Table S1. Lifetimes of alamethicin subconductance states in BLMs as a function of lipid composition, peptide concentration, UV irradiation, and sequence of peptide incubation and irradiation steps.

Row	Lipid(s) [a]	Alamethicin (ng/mL)	BLM Condition [b]	Lifetimes of Subconductance States (ms) [c, d]							
				O1 (from C)	O2 (from O1)	O3 (from O2)	O4 (from O3)	O4 (from >O4)	O3 (from O4)	O2 (from O3)	O1 (from O2)
1	DPhPC	30	no UV	21.9	19.8	15.3	10.3	7.4	14.0	17.8	21.9
2	bis-DenPC	30	no UV	21.0	17.6	13.5	9.2	7.1	13.5	16.8	20.8
3	1:1	30	no UV	21.3	18.8	14.5	9.8	7.4	13.8	17.3	21.5
4	DPhPC	30	inc, +UV	22.0	19.9	15.4	10.3	7.5	14.0	17.8	22.0
5	bis-DenPC	30	inc, +UV	NA	NA	NA	NA	NA	NA	NA	NA
6	1:1	30	inc, +UV	6.1	4.1	4.5	5.5	4.9	4.6	5.1	5.5
7	DPhPC	30	+UV, inc	21.9	19.8	15.3	10.3	7.4	14.0	17.8	21.9
8	bis-DenPC	30	+UV, inc	NA	NA	NA	NA	NA	NA	NA	NA
9	1:1	30	+UV, inc	10.9	9.3	8.7	7.7	6.1	8.5	10.8	10.7
10	1:1	15	inc, +UV	15.0	14.6	13.2	8.0	6.0	11.7	14.8	17.0
11	1:1	60	no UV	6.0	4.0	5.0	NA	NA	4.5	5.0	4.8

[a] 1:1 refers to equimolar DPhPC:bis-DenPC.

[b] BLM conditions are alamethicin incubation without UV irradiation (no UV), alamethicin incubation followed by UV irradiation (inc, +UV), and UV irradiation followed by alamethicin incubation (inc, +UV).

[c] At alamethicin concentrations of 15 and 30 ng/mL, the subconductance states O1-O4 correspond to conductances of 40, 93, 147, and 173 pS. At 60 ng/mL, the subconductance states O1-O3 correspond to conductances of 20, 53, and 80 pS, and O4 was not observed.

[d] Two lifetimes were determined for each subconductance state. For example, state O1 can be formed by a transition from the closed (C) state or from O2. An entry of NA means that no IC activity was observed.

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