SUPPORTING INFORMATION

Enhanced long-term stability for single ion channel recordings using suspended poly(lipid) bilayers

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EXPERIMENTAL

Chemicals. Ion channel recording buffer (1 M KCl, 5 mM HEPES, pH 7.5; also referred to as bath solution) was prepared with KCl and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (Sigma-Aldrich, St. Louis, MO, USA) in deionized (DI) water (18 MΩ•cm) and filtered through a 0.2 µm filter. 3-Cyanopropyldimethylchlorosilane and n-decane were purchased from Alfa Aesar (Ward Hill, MA, USA) and used as received. Acetonitrile and ethanol were purchased from EMD (Gibbstown, NJ, USA). α-Hemolysin (α-HL) was purchased from Sigma-Aldrich and diluted to 0.5 mg/mL in recording buffer. 1,2-Diphytanoyl-sn-glycero-3-phosphocholine (DPhPC) was purchased in chloroform from Avanti Polar Lipids, Inc. (Birmingham, AL, USA). Bis-dienoylphosphatidylcholine18,18 (bis-DenPC) was synthesized according to Dorn et al.1

Pipette fabrication. Borosilicate pipettes were purchased from Sutter Instrument, Co. (Novato, CA) or World Precision Instruments, Inc. (Sarasota, FL) with a 1.5 mm outer diameter and 1.0 mm inner diameter. The pipettes were pulled to a sharp tip using a Sutter Instrument Co. Model P-97 micropipette puller. Pulled pipettes were then broken to an opening of ca. 200 µm and fire polished using a microforge (Narishige MF-900) to obtain an aperture ranging from 5-20
µm in diameter. The resulting pipettes were rinsed with 0.1 M HNO$_3$ for 30 minutes followed by H$_2$O then acetonitrile. Pipettes were then filled with 2% (v/v) 3-cyanopropyl dimethylchlorosilane in acetonitrile and stored overnight. After 12 hours, pipettes were rinsed with acetonitrile, ethanol, and water to remove excess silane reagent, then air dried for storage until use.

**BLM formation.** Lipids were dried under Ar in a glass vial, followed by vacuum for a minimum of 4 hours. Dried lipids were resuspended to a concentration of 10 mg/mL in $n$-decane. Formation and subsequent analysis of BLMs (see below), both with and without α-HL, was performed at 22 ± 2 °C. BLMs were formed by applying a small volume (ca. 3 µL) of lipid solution to the tip of the silanized pipette and allowing evaporation of the solvent. Pipettes were then back-filled with recording buffer and the tip was recoated with lipid solution and placed in a bath containing recording buffer. A clean disposable pipette tip was dragged across the glass pipette tip while monitoring resistance using a patch clamp amplifier, until an increase of ca. 10$^6$ Ω was observed, signifying BLM formation. If no increase in resistance was observed, the tip dragging process was repeated until the increase occurred. BLM formation was further verified by applying a 1 V potential across the pipette and observing rupture of the BLM as indicated by a return (decrease) to open pipette resistance. The BLM was then easily reformed by dragging a clean tip across the pipette.$^2$ The success rate for forming bis-DenPC BLMs by this method was three- to five-fold lower than that obtained using DPhPC. A contributing factor may be that the main phase transition temperature of bis-DenPC is 20 °C,$^3$ which is slightly lower than the temperature at which BLMs were prepared (22 ± 2 °C).

**Functionalization of BLMs with ion channels.** While monitoring the current passing through the BLM at a holding potential of -40 mV, a small amount of α-HL (3 µL of 0.5 mg/mL) was added to the bath solution (~500 µL) into which the pipette was immersed (i.e., the exterior
surface of the BLM was in contact with the solution containing α-HL). Insertion of an α-HL pore was observed as a stepwise, negative decrease in the measured current. To perform single ion channel recordings, the bath solution was exchanged with fresh recording buffer immediately upon insertion of the first pore to minimize the probability of multiple channel insertions. For multiple ion channel recordings, the insertion time was lengthened prior to bath exchange.

**UV polymerization of bis-DenPC BLMs.** Bis-DenPC BLMs with and without α-HL were prepared as described. The resulting BLM was irradiated with a UV pen lamp (UVP, Upland, CA, USA, Model 90-0012-01) placed a few centimeters from the pipette tip for 15 minutes. The manufacturer’s specified intensity is 4750 µW/cm² at 254 nm at a distance of 0.75” from the lamp; the actual intensity was not measured. Previous work has shown that under similar irradiation conditions, ≥95% lipid polymerization is achieved. In all cases, α-HL was inserted prior to UV polymerization. Attempts to insert α-HL after polymerization were unsuccessful.

**Investigation of BLM stability.** Long-term BLM stability was examined by preparing BLMs composed of DPhPC, bis-DenPC, or poly(bis-DenPC). Following BLM formation, initial conductances were measured and then pipettes were transferred into a storage container filled with recording buffer. After 24 hours, the BLM conductance was measured again. This process was repeated every 24 hours until a rise in the conductance to the open pipette value, signifying BLM failure, was observed. After seven days, BLMs that were still intact were measured every third day.

Short-term BLM stability was monitored on BLMs that failed in less than one day (DPhPC and unpolymerized bis-DenPC). BLMs were formed and the conductances measured every 15 minutes for the first hour and every hour thereafter. BLMs were maintained in
recording buffer during the entire experiment to avoid BLM failure due to passage across multiple air-buffer interfaces. The capability of BLMs to withstand transfer from the bath solution to the storage container was also investigated. Pipettes with BLMs were repeatedly passed across the air-buffer interface and conductances were measured as a function of the number of transfers.

**Data collection and analysis.** Ion channel recordings were acquired using a HEKA Electronik EPC-8 patch clamp amplifier configured with an ITC-16 A/D converter controlled with HEKA Pulse data acquisition software. Recordings were processed using either the HEKA PulseFit or QuB analysis software. Current versus voltage measurements were obtained by holding the potential at 0 mV and stepping the potential from -100 to +100 mV in 10 mV increments at 50 ms intervals. Signals were acquired at 2 kHz and filtered at 500 Hz and the average values recorded for each step. Conductances of BLMs were calculated based on the slope of the $i$-$V$ curves.\(^6\) Single ion channel recordings were acquired at 2 kHz and filtered at 500 Hz at a typical holding potential of -40 mV. All potentials are versus a Ag/AgCl reference electrode. In some cases, higher potentials (up to +1000 mV) were applied to determine breakdown voltages of BLMs.

**RESULTS**

**Membrane conductance and capacitance.** Typical $i$-$V$ curves for bis-DenPC and poly(bis-DenPC) BLMs are shown in Figure S1. A comparison of the conductances shows that an increase of ca. ~5-10% occurred upon polymerization. This difference is likely due to the alterations in lipid packing arising from polymerization of the dienoyl functionalities. The effect of UV exposure was investigated using DPhPC by measuring the conductance of the BLM prior to and immediately following 15 minutes of UV light irradiation. As shown in Figure S2, no
discernable differences were observed. Capacitance values of bis-DenPC and poly(bis-DenPC) BLMs were 0.27 (±0.03) µF/cm² ($n = 5$) and 0.29 (±0.04) µF/cm² ($n = 5$), respectively. Upon functionalization with multiple α-HL pores, the conductance of the BLM increased further; for example, shown in Figure S1 is an $i$-$V$ curve for a poly(bis-DenPC) BLM into which ca. 20 α-HL channels were inserted.

![Figure S1](image1.png)

**Figure S1.** (A) Current versus voltage plots for bis-DenPC BLMs with and without α-hemolysin. Poly(bis-DenPC) + α-HL shows an increase in conductivity corresponding to ca. 20 α-HL channels. (B) Data from A for bis-DenPC and poly(bis-DenPC) plotted on an expanded scale. bis-DenPC has a conductance ca. 5% lower than poly(bis-DenPC).

![Figure S2](image2.png)

**Figure S2.** Current versus voltage plots for DPhPC prior to (DPhPC) and immediately following UV irradiation for 15 minutes (UV DPhPC).
**α-HL channel insertions.** Evidence of ion channel insertion into the BLM was obtained by monitoring the change in current versus time. Individual insertions are identified by a stepwise decrease in current of ca. 30 pA per channel. The noise in the current signal before and after channel insertion was not noticeably different. Multiple insertions occurred and could be halted when the desired channel density was reached. Figure S3 shows the stepwise decrease in current corresponding to the opening of one and two channels. The initial value of the current is near 0 pA, which is attributable to the intact BLM containing closed channels. The all-points histograms are noticeably non-Gaussian. The asymmetric distributions result from an asymmetric distribution of noise in the measurement (e.g., see the noise in the current traces in Figure S3A). This appears to arise from capacitive charging and discharging of the pipette which in effect acts as a filter.

![Figure S3](image.png)

*Figure S3.* (A) Recording of sequential opening of two α-HL channels in a poly(bis-DenPC) BLM. (B) All-points histogram of channel opening events corresponding to zero (0 pA), one (-30 pA) and two (-60 pA) open channels.
Effect of UV light on α-HL activity. The effect of UV irradiation on α-HL activity was investigated by inserting channels into DPhPC BLMs and observing the ion channel activity prior to and following UV irradiation (Figure S4). No significant differences in the activity of the ion channel before and after irradiation were observed.

Investigation of BLM stability. Representative long-term stability data for BLMs formed using bis-DenPC, DPhPC, and poly(bis-DenPC) are listed in Table S1 for a single pipette of each type. Averaged data (for $n = 5$ pipettes, $n = 5$ pipettes, and $n = 4$ pipettes, respectively) are presented in the manuscript. A significant increase in stability and longevity was observed for poly(bis-DenPC) BLMs compared to unpolymerized bis-DenPC and DPhPC. This was further confirmed by BLM failure that occurred within the first 4 hours after BLM formation using unpolymerized bis-DenPC and DPhPC (Table S2).
The capability of BLMs to withstand removal from the recording buffer to allow long-term storage was investigated by repeated passage of BLMs across the air-liquid interface of the bath solution. Unpolymerized BLMs failed within the first three passes (Table S3). In contrast, poly(bis-DenPC) BLMs remained intact after 30 passes, at which point the evaluation was terminated.

Finally, higher potentials were applied to determine breakdown voltages of BLMs. Rupture of unpolymerized BLMs typically occurred between +500 and +800 mV. Polymerized BLMs did not rupture at the highest applied potential which was +1000 mV.

Table S1. Long-term stability of representative BLMs formed on pipettes for each type of bilayer. Conductance (κ) was measured prior to (bare pipette) and following BLM formation.

| Sample | bis-DenPC \( \kappa \) (S/cm) | DPhPC \( \kappa \) (S/cm) | poly(bis-DenPC) \( \kappa \) (S/cm) |
|--------|-------------------------------|---------------------------|-------------------------------------|
| Bare   | \( 2.48 \times 10^{-6} \)  | \( 5.39 \times 10^{-6} \)  | \( 2.21 \times 10^{-6} \)          |
| Day 1  | \( 1.62 \times 10^{-11} \) | \( 1.29 \times 10^{-15} \) | \( 8.69 \times 10^{-12} \)        |
| Day 2  | \( 2.44 \times 10^{-6} \)  | \( 5.31 \times 10^{-6} \)  | \( 8.39 \times 10^{-12} \)        |
| Day 3  | -                             | -                         | \( 7.21 \times 10^{-12} \)         |
| Day 4  | -                             | -                         | \( 9.65 \times 10^{-12} \)         |
| Day 5  | -                             | -                         | \( 8.67 \times 10^{-12} \)         |
| Day 6  | -                             | -                         | \( 9.65 \times 10^{-12} \)         |
| Day 7  | -                             | -                         | \( 1.07 \times 10^{-11} \)         |
| Day 10 | -                             | -                         | \( 8.31 \times 10^{-12} \)         |
| Day 13 | -                             | -                         | \( 9.38 \times 10^{-12} \)         |
| Day 16 | -                             | -                         | \( 9.45 \times 10^{-12} \)         |
| Day 19 | -                             | -                         | \( 8.31 \times 10^{-12} \)         |
| Day 22 | -                             | -                         | \( 2.43 \times 10^{-6} \)          |
Table S2. Short-term stability of representative BLMs formed on pipettes using unpolymerized bis-DenPC and DPhPC and maintained in recordings buffer for the duration of the experiment. Conductance (κ) was measured prior to (bare pipette) and following BLM formation.

| Sample     | bis-DenPC          | DPhPC          |
|------------|--------------------|----------------|
|            | κ (S/cm)           | κ (S/cm)       |
| Bare       | 3.62 X 10^{-6}     | 3.02 X 10^{-6} |
| t = 0      | 1.92 X 10^{-11}    | 4.28 X 10^{-14}|
| t = 15 mins| 2.02 X 10^{-11}    | 1.77 X 10^{-14}|
| t = 30 mins| 1.75 X 10^{-11}    | 1.70 X 10^{-14}|
| t = 45 mins| 1.67 X 10^{-11}    | 1.49 X 10^{-14}|
| t = 60 mins| 1.99 X 10^{-11}    | 1.71 X 10^{-14}|
| t = 120 mins| 3.72 X 10^{-6}  | 1.71 X 10^{-14}|
| t = 180 mins| -                 | 1.70 X 10^{-14}|
| t = 240 mins| -                 | 3.03 X 10^{-6}  |

Table S3. Stability of BLMs subjected to repeated withdrawal and reinsertion in recording buffer for representative pipettes. Conductance (κ) was measured prior to (bare pipette) and following BLM formation.

| Sample | bis-DenPC       | DPhPC       | poly(bis-DenPC) |
|--------|-----------------|-------------|-----------------|
|        | κ (S/cm)        | κ (S/cm)    | κ (S/cm)        |
| Bare   | 2.62 X 10^{-6}  | 4.62 X 10^{-6} | 2.62 X 10^{-6} |
| BLM    | 1.92 X 10^{-11} | 1.77 X 10^{-14} | 9.65 X 10^{-12} |
| 1 Pass | 1.99 X 10^{-11} | 1.71 X 10^{-14} | 9.38 X 10^{-12} |
| 2 Pass | 1.62 X 10^{-11} | 3.03 X 10^{-6}  | 9.99 X 10^{-12} |
| 3 Pass | 4.72 X 10^{-6}  | 4.72 X 10^{-6}  | 8.69 X 10^{-12} |
| 4 Pass | -               | -            | 8.39 X 10^{-12} |
| 10 Pass| -               | -            | 8.67 X 10^{-12} |
| 20 Pass| -               | -            | 8.69 X 10^{-12} |
| 30 Pass| -               | -            | 9.99 X 10^{-12} |

References

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