PLANAR BILAYER MEMBRANES MADE FROM
PHOSPHOLIPID MONOLAYERS FORM
BY A THINNING PROCESS

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ABSTRACT We investigated the manner in which planar phospholipid membranes form when monolayers are sequentially raised. Simultaneous electrical and optical recordings showed that initially a thick film forms, and the capacitance of the film increases with the same time course as the observed thinning. The diameter of fully thinned membranes varies from membrane to membrane and a torus is readily observed. The frequency-dependent admittance of the membrane was measured using a wide-bandwidth voltage clamp whose frequency response is essentially independent of capacitative load. The membrane capacitance dominates the total admittance and the membrane dielectric is not lossy. The specific capacitance of membranes of several mixtures was measured. A schematic diagram of the formation of these membranes is presented.

INTRODUCTION
There are two basic methods for forming phospholipid bilayer membranes. In the first, lipid is dissolved in a nonvolatile hydrocarbon solvent. A small quantity of this membrane-forming solution is applied to a hole in a sheet, typically made of Teflon, separating two aqueous compartments. The hydrocarbon drains into a Gibbs-Plateau border (known as a torus), which surrounds the thin film that forms (Mueller et al., 1963). This thin film, referred to as a black lipid membrane (BLM), consists of a phospholipid bilayer with a considerable amount of dissolved solvent (Hanai et al., 1965; White, 1972). In the second method, bilayers are formed from monolayers as originally described by Takagi et al. (1965) and modified by Montal and Mueller (1972). Monolayers spread at two air-water interfaces are sequentially raised over a hole that has been pretreated with hydrocarbons such as Vaseline or squalene (Montal, 1974; Reyes and Latorre, 1979), and a phospholipid bilayer forms where the monolayers meet. These latter membranes, which we refer to as MM films, are relatively solvent-free. They are commonly described as forming via the direct apposition of the two monolayers (Montal, 1974; White et al., 1976).

Here we report simultaneous optical and electrical studies showing that contrary to the common view, sequential raising of the monolayers results initially in the formation of a thick hydrocarbon–phospholipid mixture within the hole. Only when the hydrocarbon drains does a bilayer form. When the membrane has fully thinned, a visually apparent torus surrounds the bilayer. By measuring the area of thinned MM membranes and using a specially constructed wide-bandwidth voltage clamp, we measured the specific capacitance of the bilayer.

METHODS AND MATERIALS
Planar membranes were formed by sequentially raising monolayers past a 50–300-μm diam hole in a partition separating the two aqueous compartments. Teflon partitions, varying in thickness from 1 to 5 ml, (Dielectric, Lockport, NY), were used when the hole was pretreated with squalene. A Saran wrap partition (Dow Chemical, Indianapolis, IN) was used when the hole was precoated with petroleum jelly (Vaseline; Cheesebrough-Ponds, Greenwich, CT). The chamber, milled from Teflon, had glass coverslips (0.17-mm thick) as front and rear walls, which provided an unobstructed light path. Ag/AgCl electrodes were placed in each compartment through holes bored in the Teflon chamber and connected to the headstage of the voltage clamp.

Membranes were formed either from asolectin (Type IVS; Sigma Chemical Co.) washed by the procedure of Kagawa and Racker (1971), bacterial phosphatidylethanolamine (BPE), PE prepared by transphosphatidylation of egg lecithin in the presence of ethanolamine (TPE), or diphytanoyl phosphatidylcholine (DPC). The latter three phospholipids were purchased from Avanti Polar Lipids, Inc. (Birmingham, AL) and used as received. Monolayers were spread by applying 10 and 5 μl of a 1% lipid/hexane (Burdick & Jackson Laboratories Inc., Muskegon, MI) solution (wt/vol) to the larger (1.5 ml) and smaller compartments (0.7 ml), respectively. Solvents were allowed to evaporate for up to 30 min before the monolayers were raised. Holes in Teflon partitions were pretreated with (typically) 4μl of 1% squalene (Sigma Chemical Co.) in pentane (Burdick & Jackson Laboratories Inc.) (vol/vol). The squalene was passed over an activated alumina column. Holes in Saran wrap were treated with the supernatant of 15% Vaseline in petroleum ether (Baker Co., Sanford, ME) (wt/vol). The membranes were bathed with symmetrical solutions of 400 mM KCl, 10 mM Hepes, 3 mM MgCl₂, 1mM EDTA, pH 7.5. Milli-Q water (Millipore Corp., Bedford, MA) was used for all solutions (18 M Ω-cm).

To observe the formation of membranes, we used a setup previously described in detail (Niles and Cohen, 1987). In brief, a microscope (Laborlux 11; E. Leitz, Inc., Rockleigh, NJ) without a stage was laid on its back brace and bolted to an air table. The chamber, placed where the stage would normally go, was mounted in a Lucite holder attached to a micromanipulator. The membrane was brought into focus with the micromanipulator and was illuminated by transmitted light from a brightfield source. This light was passed through a Nikon MPPlan.
Capacitance

Hours
Minutes
Seconds
100'ths of a Second

00:00 00:00:00:00

A

B

C

D

E

F

BIOPHYSICAL JOURNAL VOLUME 53 1988
The signal was displayed on a 4-k monitor (V20; Electrohome Ltd., Canada). The position of the 15× eyepiece was varied for each size hole so that the image of each hole filled the screen of the monitor. The total magnification of the system when a 150-µm hole was used was 1,000. The video signal was recorded on 3/4-in. U-matic videotape recorder.

The voltage clamp headstage amplifier consisted of a discrete junction field-effect transistor (JFET) (U430; Siliconix, Santa Clara, CA) differential input stage followed by a second stage comprised by an operational amplifier (OPA101; Burr-Brown Corp., Tucson, AZ). Switching between five feedback resistors (100 kΩ, 5 MΩ, 50 MΩ, 100 MΩ, and 10 GΩ) was accomplished using reed relays (Coto, Providence, RI) located in the headstage and configured to minimize their contribution to overall noise. The clamp was designed to have a wideband response (-3 dB bandwidth = 50 kHz for all resistors except 10 GΩ) and achieve a frequency response that would be essentially independent of capacitative loads at the input (i.e., the bilayer capacitance) up to ~500 pF. This was accomplished by deliberately increasing the time constant of each of the feedback resistors (except 10 GΩ) by adding a small parallel capacitor. The reduced frequency response was restored by a high frequency “boost” circuit of the type traditionally used in patch voltage clamps employing gigohm range feedback resistors (see, e.g., Rae and Levis, 1984). The utility of this procedure is demonstrated in the Appendix.

Two methods were used to measure the membrane capacitance. In the first, we used the quadrature technique of Neher and Marty (1982). A reference 10 mV peak-to-peak, 1,000 Hz sine-wave, was applied across the membrane and the membrane current converted to voltage by the clamp. This output voltage was passed to a two-channel lock-in amplifier (EG&G 5208; Princeton Applied Research, Princeton, NJ). The time constant of the lock-in output was 10 ms. The in-phase (conductance) and out-of-phase (capacitance) components from the lock-in were digitized and a display of each, generated by modified video date-time generators (Video Timer VTG-33; For-A Corp., West Newton, MA), was superimposed on the video image. In this way, the measured capacitance was synchronized to the observed degree of thinning on a field-by-field (60 Hz) basis. The capacitative component of the lock-in output was also sent to a chart recorder. The phase difference between the reference and clamp signals due to series resistance and stray capacitance was determined with calibrated capacitative loads at the input of the voltage clamp (Neher and Marty, 1982), and was only −0.5 to −0.8°. The signal from the clamp was advanced this amount at the input of the lock-in, minimizing contamination of the capacitative and conductance components by each other. Standard curves relating the output from the lock-in to the input capacitance were generated by using capacitors of known value.

In the second method, the frequency-dependent admittance of the fully thinned membrane was measured with a spectrum analyzer (Dynamic Signal Analyzer 3523A; Hewlett-Packard Co., Palo Alto, CA). The membrane was clamped to a white noise voltage command (Noise Generator 3722A; Hewlett-Packard Co.). The magnitude and phase shift of each frequency component of the membrane current were computed by the analyzer and stored on disk.

RESULTS

The basic observation that MM membranes form via a thinning process is illustrated by Fig. 1. Both monolayers have been raised above the hole in B, but as determined visually the thinning process had not yet begun. The capacitance (the four left-most digits in the lower left-hand corner; see A for schematic and the figure legend for details) remained small. As the hydrocarbon drained out, a front separating the thin from the thick region advanced. Thin and thick regions were distinguished by an unambiguous difference in opalescence. In Fig. 1, the thinned region is advancing bottom to top.1 In C–E, a progressively larger region of thinned bilayer is observed. In F, a fully thinned membrane with a surrounding torus is shown. As the membrane thins, a correspondingly larger capacitance is measured as is seen from the progressively larger values of the digital capacitance display. The time course for the increase in membrane capacitance therefore reflects the degree of thinning. Fig. 2 shows a record for the capacitance as a function of time after two solutions have been raised above the hole. The time course for the rise in capacitance is on the order of seconds2 and has a sigmoidal

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1 Images observed on the monitor are optically inverted. Therefore, the membrane is really thinning top to bottom, not bottom to top as seen in Fig. 1.

2 The rise times for the capacitance increases varied from on the order of a second to tens of seconds.

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![Figure 2](image-url)  
**Figure 2** Time course for the increase in membrane capacitance. Asolecin monolayers were raised above a 160-µm hole pretreated with squalene.
shape, which is typical for the squalene-treated holes. When the holes were pretreated with Vaseline, the capacitance increase often exhibited a more complex time course, with two or more components evident.  

To quantify the specific capacitance, the area of bilayer membrane was evaluated. We focused through the membrane and located the bilayer at the center of the depth of field. As can be seen in Fig. 1 $F$, a visible boundary separated the thinned bilayer from the torus. At the center of the depth of field, small changes in focus caused contrast reversal in the banding pattern surrounding this visible boundary. We always verified that the thinned membrane was optically flat throughout its area (depth of focus 5–10 $\mu$m), thereby ensuring that the membrane was not bulged. This was routinely accomplished by raising the solution on the two sides of the partition to the same level. In control experiments, the membrane was bulged by altering the levels of the aqueous phases. As expected, this manipulation resulted in small increases in capacitance. However, we noted that such bulging also altered the position of the visible boundary within the hole, and the torus changed in size. The major and minor axes of the separating boundary were measured from the video monitor, and the area of the flat bilayer was computed assuming the membrane to be an ellipse. As we could locate each end of an axes to within 1 $\mu$m, the maximum error in the measured length of an axes was 2 $\mu$m. Therefore, for a 100-$\mu$m diam membrane, the maximum error in membrane area should not exceed 4%.

The net capacitance of the bilayer was taken to be the final total value of the measured capacitance minus the background capacitance. The background capacitance, typically 10–15% of the total capacitance, was measured when the first monolayer was raised above the hole, but the second monolayer was just below the hole. We thus assumed that the torus contributed negligible capacitance. In this manner, we determined the specific capacitance of the bilayer when the hole was pretreated with squalene to be $0.70 \pm 0.10 \mu F/cm^2$ mean ± SD for assolecin, 0.79 $\mu F/cm^2$ for bacterial PE, 0.65 ± 0.08 $\mu F/cm^2$ for TPE, and 0.94 ± 0.27 $\mu F/cm^2$ for DPC. These results are summarized in Table I.

For the above measurements to be reliable, it is necessary that the clamp has sufficient bandwidth and that the frequency characteristics be independent of the capacitive load. We determined the frequency characteristics of the clamp by passing white noise through an integrator and sending this integrated noise via a capacitor to the negative input of the headstage. The magnitude of the admittance thus determined (input current/output voltage) was independent of frequency out to 50 kHz (-3 dB attenuation ≥ 50 kHz), and in this respect the clamp was close to ideal. When white noise (not integrated) was applied across a solid sheet of Teflon bathed by buffer, the transfer function shown in Fig. 3 $A$ was obtained. The magnitude of the transfer function increased linearly with frequency, as theoretically expected for a pure capacitor, although there was a small but noticeable rolloff at higher frequencies. The phase shift was 45° for the Teflon partition at 42 kHz. But the frequency at which the phase shift was 45° varied somewhat with the load capacitance, due to the nonideality of the clamp. The 45° phase shift occurred at 29 kHz for the bilayer whose transfer function is shown in Fig. 3 $B$. For the bilayer, the magnitude increased linearly with frequency, showing that the transfer function was dominated by the capacitance at frequencies above a few hertz. This occurred because the capacitative admittance, $2\pi f/C_m$, was much larger than the membrane conductance, $10^{-8}$ S/cm². Deviations from linearity due to the voltage clamp frequency response are, however, evident for high frequencies. For practical purposes, the bandwidth of the clamp is adequate and sufficiently insensitive to load capacitance to allow reliable measurements of capacitance to be performed with a lock-in amplifier at 1 kHz.

As the Teflon is electrically in parallel with the membrane, the admittance of the membrane, $Y_m$, was obtained by subtracting the admittance when one monolayer was above the hole and the other just below the hole from the total admittance in the presence of a thinned membrane. The specific capacitance of the membrane, $C_m$, was calculated from the equation

$$C_m = \frac{Y_m}{2\pi f R_f A},$$

where $Y_m$ was evaluated in its linear range, $f$ is the frequency, $R_f$ is the resistance of the feedback resistor of the head stage, and $A$ is the area of the membrane. By this method, the specific capacitances for membranes formed

### Table I

<table>
<thead>
<tr>
<th>Phospholipid</th>
<th>$C_m$ (lock-in)</th>
<th>$d$</th>
<th>$C_m$ (transfer)</th>
<th>$n$</th>
<th>$d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asolecin</td>
<td>$0.70 \pm 0.10$</td>
<td>7</td>
<td>$2.8 \pm 0.13$</td>
<td>5</td>
<td>2.3</td>
</tr>
<tr>
<td>BPE</td>
<td>0.79</td>
<td>1</td>
<td>$2.5 \pm 0.32$</td>
<td>3</td>
<td>2.5</td>
</tr>
<tr>
<td>TPE</td>
<td>$0.65 \pm 0.08$</td>
<td>7</td>
<td>$3.0 \pm 0.34$</td>
<td>4</td>
<td>2.2</td>
</tr>
<tr>
<td>DPC</td>
<td>$0.94 \pm 0.27$</td>
<td>6</td>
<td>$2.1 \pm 0.16$</td>
<td>5</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Values are the mean and SD for $n$ measurements.

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3Petroleum jelly (Vaseline) consists of nonstraight-chain solid and liquid hydrocarbons held within micelles (Schindler, 1961). We contacted Cheesebrough-Ponds, explained how we were using the product, that it had interesting properties, and requested more detailed information on its content. After repeated inquiries, we obtained a letter explaining that the company is not responsible for any harm their product might cause when applied in ways other than for the intended use. A further request for product information resulted in our receiving a one dollar refund, which we used to purchase generic petroleum jelly. It too exhibited a complex time course for thinning.
from various lipid mixtures were obtained and are shown in Table I.

**DISCUSSION**

The formation of MM membranes is customarily described and depicted (Montal and Mueller, 1972; Montal, 1974; White et al., 1976) to be the result of two monolayers directly collapsing onto each other as they are sequentially raised above an aperture. By observing the formation of these membranes with high magnification microscopy (rather than with dissecting microscopes which are normally employed), it is apparent that contrary to the above view, these membranes form by basically the same thinning procedure that occurs with BLMs. It is a common observation that the growth in capacitance lags, rather than occurs simultaneously with, the raising of both monolayers above the hole. The thinning process naturally explains this observation. When squalene or Vaseline are used as coating solvents, the thinning times are on the order of a few seconds, which is faster than usually occurs with

**Figure 3** Transfer functions. (A) The transfer function of 1-mL Teflon bathed by buffer. (B) Transfer function of an asolectin membrane in a 160-μm diam hole pretreated with squalene. The calculated capacitance of the membrane is 149 pF. Note the different scales for the magnitude in A and B.
alkane-based BLMs. The MM membranes contain less solvent as is apparent from their larger capacitances.

The thickness of the hydrocarbon core, \( d \), was computed from \( C_m = \epsilon_s / d \), where \( \epsilon_s = 8.85 \times 10^{-12} \) F/m is the permittivity of free space and \( \epsilon \) is the dielectric constant of the core. \( \epsilon \) has been estimated to range from 2.1 to 2.2 by a variety of independent techniques (White, 1978; Requena and Haydon, 1975b; Dilger et al., 1982). We assumed \( \epsilon = 2.2 \). The calculated thicknesses, which correspond to the thickness of the hydrocarbon core, are shown in Table I. The variability in the measured capacitance leads to variabilities in thicknesses. Determining the thickness of the hydrocarbon core is of some practical importance as this thickness is sometimes used to establish detailed models of membrane structure on the molecular level. For instance, recently the depth water penetrates within the headgroup has been estimated by subtracting the thickness of the low dielectric region (determined from capacitance measurements) from the distance between head groups (determined by x-ray diffraction) (Simon and McIntosh, 1986). As extensive capacitance measurements of MM films have assumed the area of the orifice to be that of the membrane, specific capacitance has been underestimated and thicknesses overestimated.4 A torus was previously inferred to exist in MM films on theoretical grounds (White et al., 1976), and in this study we directly observed such a structure.

The variability in measured capacitance was due to variation between membranes, and not due to the measurements. Lock-in and transfer function measurements were reproducible and agreed with each other to within 4% when fixed capacitors were used. Furthermore, a one-way analysis of variance of the specific membrane capacitances showed that data obtained by the two approaches were identically distributed at an attained level of significance >40%. Variations in measuring capacitance combined with the errors in determining membrane areas are therefore less than the measured variations in specific capacitance. Our observations of specific capacitance are normally distributed for each phospholipid as determined at a 5% level of significance (two-tailed), indicating that with sufficient measurements the mean specific capacitance of a membrane with a given phospholipid composition can be obtained. A complicating factor is, however, that after the initial rapid increase in capacitance due to thinning (Fig. 2), we usually observed a small but steady increase in capacitance (without a concomitant increase in membrane area) over the course of ~1 h. This variable capacitance is symptomatic of lack of thermodynamic equilibrium. That it is difficult to reach equilibrium with diaclyphospholid/...
than that of a water–air interface. In practice, sufficient squalene is added to ensure that the configurations illustrated by Fig. 4, B and C occur.

In the above scheme for membrane formation, one does not expect, a priori, that the thinned membrane be devoid of solvent. This depends solely on the choice of hydrocarbon that is used to coat the partition. As has been discussed previously (Benz et al., 1975; White et al., 1976; Alvarez and Latorre, 1978), the measured capacitances of MM membranes that use petroleum jelly or squalene as the coating solvent correspond to virtually solvent-free bilayers. This is consistent with the finding that the exclusion of a hydrocarbon from the bilayer region increases as its chain-length increases (Benz et al., 1975, White, 1975), that squalene partitions poorly into phospholipid bilayers (Simon et al., 1977), and with theoretical considerations (Gruen, 1981). Furthermore, it is established that asymmetric membranes can be formed by the method of raising monolayers (Montal, 1973; Hall and Latorre, 1976). This indicates that there is little mixing of lipid between the two monolayers during the configuration of Fig. 4 C and any such mixing is negligible compared with lipid mixing within a monolayer due to the lateral diffusion.

APPENDIX

In this Appendix, we elaborate on the use of a capacitor across each feedback resistor to achieve a frequency response that is independent of capacitative load. Consider the circuit shown in Fig. A1. While this figure is somewhat oversimplified, it demonstrates the principles involved. If the open-loop frequency response of the amplifier is approximated by a single pole roll-off, then the transfer function is given by

$$V_o \approx \frac{iR_f}{1 + s(\tau_f + \tau_s) + s^2 \tau_s(\tau_L + \tau_f)}.$$  \tag{A1}

where \(s\) is the Laplace transform variable \((s = jw)\), \(\tau_s = 1/(2\pi f_s)\), and \(f_s\) is the open loop unity gain frequency in hertz. \(\tau_f = R_f C_f\) and \(\tau_L = R_L C_L\). Note that \(C_s\) is the parallel combination of load capacitance, and the amplifier input capacitance plus strays; it can vary from a minimum of \(\approx 10 \, \text{pF}\) (with the input open circuited) to as much as \(1,000 \, \text{pF}\) (with a 300–400-\(\mu\text{m}\) diam bilayer).

We illustrate the consequence of Eq. A1 with examples. Consider an amplifier with \(\tau_s = 10^{-4} \, s (f_s = 16 \, \text{MHz})\) and a 50-M\(\Omega\) feedback resistor. The minimum value of \(C_s\) is \(\approx 0.2 \, \text{pF}\), arising simply from stray capacitance around the resistor. For \(R_L = 50 \, \text{M}\Omega\) and \(C_L = 0.2 \, \text{pF}\), \(\tau = 10 \, \mu\text{s}\); with \(C_s = 10 \, \text{pF}\), the transfer function has two real poles located at 16.8 and 301 kHz. The lower frequency pole is located near \(1/2\pi f_t\) as intuitively expected. However, as \(C_s\) increases, the frequency of the first pole increases and that of the second pole decreases until for \(C_s > 50 \, \text{pF}\) both poles are located at 31.8 kHz. For all values of \(C_s > 50 \, \text{pF}\), the poles form a complex conjugate pair and therefore the output will “ring” in response to a step change of current. Moreover, as \(C_s\) increases, both the natural frequency and damping factor of the poles decrease, i.e., the response becomes slower and the ringing becomes more severe. Indeed, by the time \(C_s\) has increased to 1,000 pF, the natural frequency has declined to 7.1 kHz and the damping factor is 0.2; the response to a current step will overshoot by 50% and require about half a millisecond to settle to within 1% of its final value. This situation is clearly unacceptable unless the highest frequency of interest is substantially \(< 7 \, \text{kHz}\).

If \(C_s\) is deliberately increased to 2 pF, \(\tau_s\) becomes 100 \(\mu\text{s}\), and it is found that with \(C_s = 10 \, \text{pF}\), the transfer function now has two real poles located at 1.59 kHz \((\approx 1/2\pi f_t)\) and 2.65 MHz. With \(C_s = 100 \, \text{pF}\), the pole frequencies become 1.60 and 155 kHz, and with \(C_s = 1,000 \, \text{pF}\), the pole frequencies become 1.68 and 30 kHz. Thus, although the first pole has moved to a lower frequency in comparison with the result for \(C_s = 0.2 \, \text{pF}\),
its location is more stable with changes in C1. The performance achieved with C1 = 2 pF is thus considerably improved, but the desired independence of frequency response has not been fully achieved. The problem is that with C1 = 1,000 pF, the secondary pole (located at 30 kHz) will restrict the final boosted bandwidth, and the small (5%) increase in the first pole frequency will mean that the setting of the boost circuit established in the absence of a bilayer membrane will no longer be appropriate, resulting in a step response with a small overshoot (~5%), decaying with a 100-μs time constant for C1 = 1,000 pF.

Therefore, consider the consequence of increasing C1 to 20 pF (τ1 = 1 ms). With C1 = 10 pF, the transfer function has two real poles at 159.16 Hz and 10.6 MHz. With C1 = 100 pF, the pole frequencies become 159.17 Hz and 2.65 MHz, and with C1 = 1,000 pF, they become 159.24 Hz and 312 kHz. In this situation, the frequency of the first pole varies by only ~0.05% as C1 changes from 10 to 1,000 pF. The frequency of the second pole declines dramatically as C1 increases, but even with a 1,000 pF load, it remains at a sufficiently high frequency to have no significant effect in the frequency range of interest. A 20-MHz boost amplifier could theoretically increase the bandwidth from 159 Hz to ~56 kHz; in actual practice ~35 kHz can readily be achieved.

The consequences arising from capacitive loading of the input of the current to voltage converter (“headstage”) that was illustrated in the above examples can be understood more generally by referring to Eq. A1. For any given value of C0, the value of the coefficient of s (call this coefficient A) is fixed; however, as C1 increases, the coefficient of s2 (call this coefficient B2) increases. To avoid a complex conjugate pair of poles (with a “ringing” step response), it is required that A > 2B. Moreover, if both poles are real and if the lower frequency pole is to remain at a more or less constant location, it is necessary that A > B for all anticipated values of B (i.e., of C1). In the above example, it was assumed that τ1 was 10-4 s (f1 = 16 MHz). Examination of Eq. A1 shows that the condition A > B can be achieved with smaller values of τ1 if τ1 is decreased (f1 is increased). Gain-bandwidth products substantially larger than 16 MHz can readily be achieved, although it is difficult for a unity gain stable amplifier to do much better.6 It should also be noted that B varies at τ1-1/2. Thus, although it is desirable to use the fastest possible amplifier (because of this square root relation), decreasing the value of τ1 within practical limits cannot completely solve the problem. Our approach simply increases the value of A (i.e., of τ1) to a value that is sufficient to achieve A > B for all values of C1 that are expected. In our particular implementation, we sometimes used values of C1 that were smaller than the above considerations would indicate. This was done as a compromise between maintaining a high boosted bandwidth and adequate independence of capacitive loads.

It should be noted that for frequency domain measurements of the type considered in this paper, it is quite possible to calibrate the frequency response for any constant value of C1. However, we did not do this because the bilayer capacitance varied during the thinning process. Moreover, such calibration would be of little help for time domain measurements from various sized bilayers.

We implemented switching between feedback resistors with reed

6The frequency response of the amplifier, reduced by C0, can be increased. A single stage high frequency “boost” circuit employing an amplifier with a unity gain frequency denoted by fA can theoretically increase the bandwidth from the “unboosted” headstage output (unboosted bandwidth of frequency response f) to (fA)2 (see Lewis, 1981; Rae and Lewis, 1984). Thus, for f1 = 1.6 kHz, a boost amplifier with fA = 20 MHz could theoretically produce a boosted bandwidth (natural frequency) of ~180 kHz. Due to higher frequency poles not considered in this analysis, actual performance is somewhat worse, leading to a final bandwidth of ~100 kHz.

4Unity gain stability is not necessary in this situation, but to analyze the results with such an amplifier, it is necessary to at least consider the secondary pole in the open loop frequency response. The location of this pole is usually determined by the JFET input stage.

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