CONTENTS

LIST OF FIGURES

I. MATERIALS AND METHODS

Potassium salts of $H_2PO_4^-$, Cl^-, Br^-, NO_3^-, ClO_4^-, and SCN^- (Sigma) were used to prepare 0.2 M electrolyte solutions for bilayer experiments. All solutions were buffered by 5 mM HEPES to maintain pH 7.4.

Bilayer lipid membranes were prepared from diphytanoyl phosphatidylcholine (DPhPC), using a modification of the monolayer-opposition technique by Montal and Mueller [1, 2]. A Teflon chamber, with two (cis and trans) compartments, was divided by a 15-μm-thick Teflon film with a 50 μm diameter aperture, pretreated with hexadecane. Lipid monolayers were prepared by spreading a few μl of 2 mg/ml lipid solutions in pentane on top of electrolyte solution in each chamber compartment. After bilayer formation, gramicidin A (a generous gift of O. S. Andersen, Weill Cornell University Medical College) was added from 1-10 nM ethanol stock solution to both electrolyte-containing chamber compartments at the amount sufficient to produce a single-channel activity.

The applied transmembrane voltage was maintained using Ag/AgCl electrodes with 2 M KCl and 15% (w/v) agarose bridges. Voltage was designated as positive if the potential was higher on the cis side of the membrane. All bilayer current measurements were performed using an Axopatch 200B amplifier (Molecular Devices, Foster City, CA) in voltage clamp mode. The membrane-holding chamber and the amplifier headstage were isolated from external noises with a double metal screen (Amuneal Manufacturing, Philadelphia, PA). Data were filtered by a low-pass 8-pole Bessel filter (model 9002, Frequency Devices, Haverhill, MA) at 1 kHz and directly saved into the computer memory with a sampling frequency of 5 kHz, and analyzed using pClamp 10 software. Gramicidin A amplitudes at a given transmembrane voltage were collected from individual single-channel events and calculated by Gaussian fitting of a histogram of 50 single events.

Liposomes were prepared by sonication of DPhPC water solutions according to the Morrissey Laboratory protocol [3]. Liposomes diameter (75-120 nm) was checked with light scattering. Measurement of the liposome ζ-potential and sizing of the liposomes were performed with Zeta-Plus ζ-potential analyzer (Brookhaven Instruments Corporation, Holtsville, NY). Diluted (1:10) liposome solutions in 1.5 mL cuvettes were injected with electrolyte solutions of potassium salts of anions of interest to result in 0.2 M concentration (same as for the bilayer measurements) and transferred into the analyzer. All measurements were performed at room temperature, T = (23 $\pm$ 1) °C.

II. THEORY: CHANGE IN MEMBRANE ELESTIC PROPERTIES WITH SURFACE PRESSURE

The following model follows Evans' [4] development of the polymer brush model applied to predict changes in K_A and k_c with monolayer surface pressure Π, and relating their magnitudes to leaflet thickness.

The available space for a chain in a lipid leaflet is parameterized by

$$x = \frac{A_c}{A}, \tag{1}$$

with x ranging from zero to one, where A is the average area of the chain at a particular condition, and A_c is the area of the chain at full (all-*trans*) extension. Here n_s is the number of independent polymer chain segments, a constant that is folded into K_A. For x near one the chain has limited conformational freedom and thus high free energy. According to Flory, the free energy of such a confined freely joined chain is:

$$F_{\text{c.e.}} \approx \frac{3n_s k_B T}{2} x^2, \tag{2}$$

an approximation valid to high chain extension (≈ 0.9).

At equilibrium the change in chain free energy with area is balanced by the surface pressure, thus:

$$\Pi = -\frac{\mathrm{d}F_{\text{c.e.}}}{\mathrm{d}A} = 3\frac{n_s k_B T}{A_c} x^3 \tag{3}$$

Derivatives with respect to A, for example, can be computed by the chain rule with differentiation with respect to x and $\frac{\mathrm{d}x}{\mathrm{d}A} = \frac{A_c}{A^2} = \frac{x^2}{A_c}$.

In this model the external pressure Π is considered as a constraint that does not fluctuate with x, and thus the area compressibility modulus K_A is determined by $F_{\text{c.e.}}$. A model of the free energy as a function of area considering the chain free energy and surface pressure is:

$$F = F_{\text{cons.}} + A_{\min}\frac{1}{2}K_A(1 - \frac{A}{A_{\min}})^2 + \Pi A \tag{4}$$

with

$$K_A = 2A_{\min}\frac{\mathrm{d}^2F_{\text{c.e.}}}{\mathrm{d}A^2}\Big|_{A=A_{\min}} = \frac{18n_sk_BT}{A_c}x^3 = 6\Pi \tag{5}$$

where the leading factor of two is for the two leaflets of the bilayer, $A_{\min}$ is the area with minimum free energy at the chosen conditions, and $F_{\text{cons.}}$ is constant with respect to area. Note that the factor of six originates from the quadratic expansion of the free energy with extension (using $\frac{\mathrm{d}x}{\mathrm{d}A} = \frac{x^2}{A_c}$):

$$F \propto x^n \tag{6}$$

$$\frac{\mathrm{d}F}{\mathrm{d}A} \propto -nx^{n-1}\frac{x^2}{A_c} = -nx^{n+1}\frac{1}{A_c} \tag{7}$$

$$A\frac{\mathrm{d}^2F}{\mathrm{d}^2A} \propto n(n+1)x^{n+2}\frac{1}{A_c^2}\frac{A_c}{x} = n(n+1)x^{n+1}\frac{1}{A_c} \tag{8}$$

With $n = 2$ there is a factor of three difference between $\Pi = -\frac{\mathrm{d}F}{\mathrm{d}A}$ and $K_A = A\frac{\mathrm{d}^2F}{\mathrm{d}^2A}$. The value of K_A is defined for bilayers (not leaflets) and so requires an additional factor of two to compare to leaflet surface pressure, giving $K_A = 6\Pi$.

Through this polymer theory Rawicz et al. also relate the value of the bending modulus to K_A, using similar arguments but now with the curvature-dependence of chain-extension. They find:

$$k_c = \frac{K_Ah^2}{24} \tag{9}$$

where h is the height of the leaflet. The proportionality to K_A and scaling with h is consistent with a continuum material description of the bilayer.

Solving for the area in terms of the pressure change from Eq. 3 gives:

$$A = A_0 - \frac{2A_0}{K_{A,0}}\Delta\Pi + \mathcal{O}[\Delta\Pi^2] \tag{10}$$

Here, all quantities labeled with a subscript 0 indicate quantities at a "standard" value of $\Pi = \Pi_0$, with non-zero $\Delta\Pi = \Pi - \Pi_0$ resulting from a change in solvent conditions, ionic-adsorption or lipid-composition. Conceptually, if the amplitude of the surface pressure increases, the area decreases. Highly contracted bilayers with x nearer one have large surface pressures (and thus high $K_{A,0}$) as small changes in x yield large relative changes in chain entropy. Similarly, small changes in the pressure of these contracted bilayers have little effect on the area, suppresed by the value of $K_{A,0}$ in the denominator.

Relative to the soft bending and area change moduli the bilayer's volume is incompressible. Thus, thickness is directly related to the area:

$$h = h_0 + \frac{2h_0}{K_{A,0}}\Delta\Pi + \mathcal{O}[\Delta\Pi^2] \tag{11}$$

In a typical bilayer patch measurement the lipids of the patch are in equilibrium with a reservoir whose chemical potential might also depend on Π in some complex way. After the condition Π changes, these lipids will exchange with the reservoir, with parameter $\lambda_e = 0$ indicating no transfer of lipids, and $\lambda_e = 1$ indicating complete exchange of lipids such that the tension is unchanged. Values of λ_e outside this range would be possible given an extreme change in the chemical potential. The resulting change in the bilayer surface tension is:

$$\Delta\gamma = K_A(A - A_0)(1 - \lambda_e) = 2\Delta\Pi(1 - \lambda_e) \tag{12}$$

For a channel with thickness h_c and bilayer with total thickness $h + h_a$ (h_a is the thickness of alkane in the bilayer interior), Andersen casts the energy of the bilayer deformation around the channel using the phenomenological constant H:

$$F = H(\Delta h)^2 \tag{13}$$

where $\Delta h = h_a + h - h_c$. The value of H is a result of energetic terms that depend linearly on K_A and k_c. Thus according to Eqs. 5 and 9, and with $K_A = 6\Pi = K_{A,0} + 6\Delta\Pi$, the value of H should also vary with $\Delta\Pi$:

$$H = H_0\frac{K_A}{K_{A,0}} = H_0\frac{K_{A,0} + 6\Delta\Pi}{K_{A,0}} = H_0 + 6\frac{H_0}{K_{A,0}}\Delta\Pi \tag{14}$$

where H_0 and $K_{A,0}$ are measured experimentally for the same system.

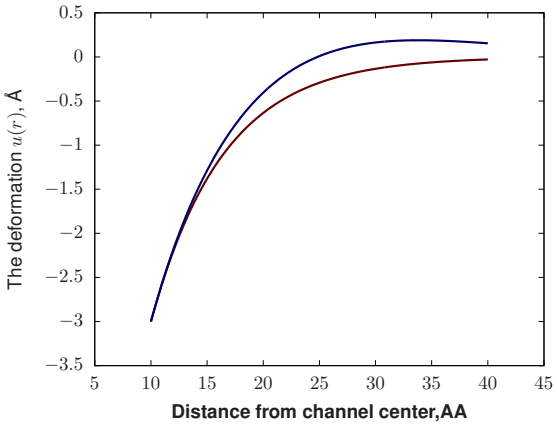

FIG. 1. A comparison of the exact model deformation solution $u(r)$, Eq. 16, and a simplification amenable to analytical analysis, Eq. 17.

A. Effect of external tension on the membrane deformation energy

Following Ref. [5], the deformation that minimizes the Hamiltonian

$$\bar{E} = \frac{K_A}{2}[\frac{2u(r)}{h_0}]^2 + \frac{k_c}{2}[\nabla^2 u(\mathbf{r})]^2 \tag{15}$$

for a cylindrically symmetric inclusion is:

$$u(r) = c\ \mathrm{kei}(\frac{r}{\sqrt{\xi h_0/2}}) + d\ \mathrm{ker}(\frac{r}{\sqrt{\xi h_0/2}}) \tag{16}$$

where $u(r)$ is the deformation of the membrane from its bulk thickness, $\xi = \sqrt{k_c/K_A}$, and kei and ker are zeroth order Kelvin functions. The constants c and d are fixed by the system boundary conditions, Δh and $u'(r_0)$, where r_0 is the channel radius. There is considerable literature debate regarding the appropriate boundary condition $u'(r_0)$, the slope of the leaflet where it meets the channel. The most influential experimental evidence is that large values of H observed implied from gramicidin lifetimes with varying Δh require nearly zero values of $u'(r_0)$ [5]. For example, zero values of the slope mean that first shell lipids are compressed to the thickness of the channel. Allowing the slope to be a free parameter, allowing compression to relax quickly (and even decreasing curvature) yields values of H reduced by approximately a fifth to a quarter [6, 7]. However, to infer the value of H from lifetime measurements requires assuming a form for the monomer-dimer dissociation path, specifically, the effective Δh of the *transition state*. Recent simulations, theory, and experiments of lipid redistribution around gramicidin (not relying on the form of the transition state) were shown to be mutually consistent when the slope is nearly free [7, 8].

For K_A and k_c considered below, the effective lengthscale $\lambda = \sqrt{\xi h_0/2} = 9.1$Å is comparable to the radius of the channel (ca. 10 Å). Thus, consider the following simplification, $u_s(r)$, of Eq. 16:

$$u_s(r) = (\frac{1}{2}\Delta h)\exp(-\frac{r - r_0}{\lambda}) \tag{17}$$

The total curvature $\nabla^2 u(\mathbf{r_0})$ is zero at the channel boundary, for $\lambda \approx r_0$. Not coincidentally, the value of $u_s'(r_0) = -\frac{\Delta h}{\lambda}$ is close to that to minimize the deformation energy. Note that for the exact solution, the characteristic length-scale of the deformation for a particular channel depends only on $\xi = \sqrt{k_c/K_A}$. Shown in Figure 1 is a comparison between Eq. 16 and Eq. 17 for matching values of $u'(r_0)$ with $\delta h/2 = 3$.

For the simplified deformation, the change in the area of the deformed leaflet, measured near the hydrophobic surface (an adequate estimate of the leaflet neutral surface of bending where area deformations should be measured [9]) is:

$$\Delta A \approx \int \mathrm{d}r 2\pi r(\frac{1}{2}u_s'(r)^2) = \frac{3}{8}\pi(\frac{1}{2}\Delta h)^2 \tag{18}$$

B. Change in spontaneous curvature with surface pressure

The Helfrich Hamiltonian for curvature is:

$$E_{\text{curvature}} = \frac{1}{2}k_c[c(r) - c_s]^2 = k_c[\frac{1}{2}c(r)^2 - c(r)c_s + \frac{1}{2}c_s^2] \tag{19}$$

where c_s is the spontaneous curvature (conventionally denoted c_0 but here the subscript 0 is used instead to indicate a quantity at $\Pi = \Pi_0$).

The term $k_c c_s^2$ does not contribute to the energy barrier as it is the same for both the dimer and transition state. The surface pressure dependence of $k_c c(r)^2$ is already included in the value of H. The following accounts for the surface pressure dependence of

$$E_{\text{curvature}} = -k_c c_s \int c(r) = -k_c c_s c_{\text{total}}. \tag{20}$$

From Ref [10], the integral of the curvature over a leaflet of a bilayer deformed by a cylindrical inclusion is equal to (in the Monge gauge):

$$\int c(r) = \int_{r_0}^{\infty} 2\pi r[u''(r) + u'(r)/r]\mathrm{d}r \tag{21}$$

$$= 2\pi r_0 u'(r_0) \tag{22}$$

As discussed previously, the channel-lipid boundary condition specified by $u'(r_0)$ has a dramatic influence on H. In Ref. [7] molecular simulations verify that values of $u'(r_0)$ consistent with an unconstrained slope are also consistent with observables of simulations, including both the shape of the boundary and curvature stress. Using the simplified assumption of the deformation, $u_s(r)$ from Eq. 17, $u_s(r0) = \frac{\Delta h}{2r_0}$ yields $c_{\text{total}} = \pi\Delta h$ for the integrated change in curvature from the deformation. The difference in Δh between the dimer and transition state is δ, so that

$$\Delta c_{\text{total}} = \pi\delta \tag{23}$$

The effect of $\Delta\Pi$ on spontaneous curvature is not straightforward to estimate, because it depends on precisely where the cohesive or expansive effect of the ions is located, in the form of the lateral pressure profile, $p(z)$ [11]. An estimate of the effect is to assume that surface pressure is isolated at the hydrophobic interface, and that, at zero tension, this is balanced perfectly by the effect of acyl chain expansion or contraction, exerting lateral pressure $p(z) = \frac{2\Delta\Pi}{h_0}$ between 0 and $h_0/2$. In this case, for a single leaflet:

$$k_c\Delta c_s = \int_0^{h_0/2} \mathrm{d}z z\frac{-2\Delta\Pi}{h_0} + \lim_{\epsilon\to 0}\int_{h_0/2}^{h_0/2+\epsilon} \mathrm{d}z\frac{\Delta\Pi}{\epsilon} \tag{24}$$

$$= \frac{\Delta P i h_0}{4} \tag{25}$$

The individual terms can now be assembled to yield an estimate for the influence of on curvature stress. Combining Eqns. 20, 23 and 25 yields:

$$\Delta E_{\text{curv.}} = -\frac{1}{4}\pi h_0 \delta\Delta\Pi \tag{26}$$

C. Membrane contribution to the energetic barrier to gramicidin channel dissociation

In Andersen's model for the dissociation of gramicidin A in a bilayer that is too thick, the channel monomers must dissociate vertically a distance δ to reach the transition state. Using Eq. 18, The deformed leaflet area A_u in this process is lowered by:

$$\Delta A_u = \frac{3}{16}\pi(\Delta h - \delta)^2 - \frac{3}{16}\pi(\Delta h^2)$$

$$= \frac{3\pi}{16}\delta[\delta - 2\Delta h] \tag{27}$$

The change in the phenomenological deformation energy (Eq. 13) between the transition state (t.s.) and dimer state is:

$$\begin{aligned}\Delta E_{\text{def., t.s.}} &= H(\Delta h - \delta)^2 - H(\Delta h)^2 \\ &= H\delta(\delta - 2\Delta h)\end{aligned} \tag{28}$$

Combining the external tension contribution with the bilayer deformation energy yields the total contribution to the transition state energy change:

$$\Delta E_{\text{t.s.}} = (H + \frac{3\pi}{16}\Delta\gamma)\delta(\delta - 2\Delta h) \tag{29}$$

where $\Delta\gamma$, h, H, and ΔA_u are estimated from Eqs. 12, 11, 14, and 27, respectively.

D. Flexoelectricity

A surface can develop electrical polarization as a result of curvature [12]. With the bilayer normal and field aligned along z, the polarization per unit area $P_z(r)$ is

$$P_z(r) = \frac{1}{2}f[c_u(r) - c_l(r)], \tag{30}$$

where here $c_u(r)$ and $c_l(r)$ are the curvature of the upper and lower surfaces measured with respect to their individual normals (hence the negative sign for $c_l(r)$). The bilayer area flexoelectric coefficient, f, depends on lipid and solvent conditions. The leading factor of $\frac{1}{2}$ accounts for the average of the two leaflets.

Under symmetric conditions $c_u(r) = c_l(r)$ and thus the polarization is zero. However, an applied field breaks this symmetry. The effect is that the deformation itself may be asymmetric, with one leaflet having a larger deformation than the other. Applying Eq. 23, a change in the deformation of magnitude Δz results in the area-weighted curvature change $\pi\Delta z$. With $f = 0.31 \times 10^{-19}$C [13], and an electric field of $\frac{100\text{mV}}{5\text{nm}}$, the change in energy,

$$\int 2\pi r E_z P_z(r) = E_z f \delta\pi \tag{31}$$

is only 0.03 kcal/mol per Å deformation. Moreover, the flexoeletric effect is expected to perturb both dimer and transition states equally, and so differences will occur only at second order, that is, considering the curvature of dimer and transition states beyond the approximations given herein. Therefore, a difference in f due to a change in anionic conditions is unlikely to substantially affect the difference in channel lifetime.

E. Estimate of ΔP necessary for a 20-fold timescale change.

Consider that the effect of electrolytes near the bilayer surface may be to change the value of the surface pressure, consistent with the proposed mechanism of the Hofmeister effect. A 20-fold difference between two conditions corresponds to an approximately 1.8 kcal/mol energy difference between the energy barrier for the dissociation process.

Parameter values are available for the system considered here. DPhPC monolayers have area compressibility (122 mN/m [14], bilayer $K_{A,0}$=244 mN/m) very similar to standard-tail glycerophospholipids reported to have H_0=93 mN/m [15] and $K_{A,0}$=265 mN/m [4]. The area per lipid of DPhPC has been measured to have $A_0 = 80.6$ Å^2, while the hydrocarbon chain thickness is 27.2 Å at 30°C [16]. The value of $\delta = 1.6$ Å is the same as used in Ref. [15] used to determine H_0. The height of alkane is taken to be 5.8 Å, the maximum value from the bilayer capacitance measurement. Two channel thicknesses will be considered, 26 Å, and 22 Å. The former, longer channel, is consistent with molecular dynamics simulations [17]. The latter was inferred by the perturbation to the observed thickness of a bilayer by x-ray scattering, albeit at a 1:10 peptide to lipid ratio. The initial data given is for a channel length of 26 Å.

Three contributions to the height of the energy barrier can be isolated. First, the change in surface tension due to contraction or expansion of the bilayer, combined with incomplete lipid exchange with the peripheral reservoir ($\lambda_e < 1$):

$$\begin{aligned}\Delta A\Delta\gamma &= \frac{3\pi}{16}\delta(\delta - 2\Delta h)2(1 - \lambda_e)\Delta\Pi \\ &= (-23.4\text{ Å}^2)(1 - \lambda_e)\Delta\Pi\end{aligned} \tag{32}$$

Second, the change in the deformation due to changing thickness of the leaflets from contraction or expansion of the bilayer:

$$\begin{aligned}\Delta E_{\mathrm{def,h}} &= -4H_0\delta\frac{h_0}{K_{A,0}}\Delta\Pi \\ &= (-60.6\ \text{Å}^2)\Delta\Pi \end{aligned} \tag{33}$$

Third, the change in the value of H_0 due to contraction or expansion of the lipid acyl chains of the leaflets, and their new resistance to deformation:

$$\begin{aligned}\Delta E_{\mathrm{def,H}} &= 6\frac{H_0}{K_{A,0}}\delta(\delta - 2\Delta h)\Delta\Pi \\ &= (-41.5\ \text{Å}^2)\Delta\Pi \end{aligned} \tag{34}$$

If λ_e is one, implying complete exchange of lipids with the reservoir, only $\Delta E_{\mathrm{def,h}}$ and $\Delta E_{\mathrm{def,H}}$ contribute. In this case $\Delta\Pi = \frac{1.8\ \text{kcal/mol}}{-102.0\ \text{Å}^2} = 12.1$ mN. The effect of surface tension is a lesser contribution, although it would act to lower this estimate. Expanding the terms beyond first order reduces the effect by less than 10%. For the values chosen, the total bilayer thickness decreases by 2.3 Å. For the shorter value of the channel (22 Å) the required change in the surface pressure is reduced from 12.1 mN to 9.6 mN because the effects are larger with greater mismatch.

The effect of lateral stress coupling to curvature, estimated by Eq. 26, yields:

$$\begin{aligned}\Delta E_{\mathrm{curv.}} &= -\frac{1}{4}\pi h_0\delta\Delta\Pi \\ &= (-34.2\text{Å}^2)\Delta\Pi, \end{aligned} \tag{35}$$

which would lower the estimated $\Delta\Pi$ to 9.1 mN/m (7.6 mN/m with a channel length of 22 Å).

[1] S. M. Bezrukov and I. Vodyanoy, Biophys. J. **64**, 16 (1993).
[2] M. Montal and P. Mueller, Proc. Natl. Acad. Sci. USA **69**, 3561 (1972).
[3] J. H. Morrissey, "Morrissey laboratory protocol for preparing phospholipid vesicles (suv) by sonication," `http://tf7.org/suv.pdf` (2001), [Online; accessed Nov 16, 2016].
[4] W. Rawicz, K. C. Olbrich, T. McIntosh, D. Needham, and E. Evans, Biophys. J. **79**, 328 (2000).
[5] H. W. Huang, Biophys. J. **1986**, 1061 (1986).
[6] P. Helfrich and E. Jakobsson, Biophys. J. **57**, 1075 (1990).
[7] A. J. Sodt, A. Beaven, O. S. Andersen, W. Im, and R. W. Pastor, in preparation (2016).
[8] A. Beaven, A. Maer, A. J. Sodt, H. Rui, R. W. Pastor, O. S. Andersen, and W. Im, in preparation (2016).
[9] S. Leikin, M. M. Kozlov, N. L. Fuller, and R. P. Rand, Biophys. J. **71**, 2623 (1996).
[10] A. Ring, Biophys. Biochim. Acta **1278**, 147 (1996).
[11] R. Goetz and R. Lipowsky, J. Chem. Phys. **108**, 7397 (1998).
[12] A. G. Petrov, Biochim. Biophys. Acta **1561**, 1 (2001).
[13] N. I. Hristova, "Master Thesis, St. Kliment Ochridski Univ., Sofia," (1994).
[14] A. Yasmann and S. Sukharev, Langmuir **31**, 350 (2015).
[15] J. A. Lundbaek, S. A. Collingwood, H. I. Ingolfsson, R. Kapoor, and O. S. Andersen, J. R. Soc. Interface **7**, 373 (2010).
[16] N. Kučerka, M.-P. Nieh, and J. Katsaras, Biochim. Biophys. Acta **1808**, 2761 (2011).
[17] T. Kim, K. Lee, P. Morris, R. W. Pastor, O. S. Andersen, and W. Im, Biophys. J. **102**, 1551 (2012).