ABSTRACT. The effect of applied voltage $V$ on local thickness fluctuations (LTF) in lipid bilayers is examined using the continuum model and accounting for non-locality of membrane elastic moduli. Ponderomotive forces, calculated from the linearized Poisson-Boltzmann equation, reduce LTF energy quadratically with $V$. Combined with a significant increase in the apex field gradient and perturbation of lipid packing at large LTF amplitudes, this creates a driving force for instability. The critical voltage, though less than Crowley's classical prediction, is still 2-3 times that experimentally observed. This, and the even larger voltages applied in recent molecular dynamics (MD) simulations, demonstrate the difficulty of fully understanding low-voltage breakdown. Some MD results are critically reviewed, including the paradox of negligible pre-breakdown membrane compression. Possible connections between MD results and the question of negative capacitance and related instabilities at electrified interfaces are outlined.

Key words: lipid bilayers, electroporation, continuum and molecular models, instabilities, electrified interfaces.

Short title: Low-voltage membrane electroporation

Introduction. For the last 30 years electroporation and field induced instability of lipid bilayers and cellular membranes have been heavily studied and widely exploited (see refs. 1-15 and references therein). Breakdown typically occurs at moderate voltages $\leq 0.5-1$ V, with little ($\leq 2\%$) prior membrane thinning. The electroelastic (ELE) model, a simple and attractive picture [1], predicts large voltage ($\approx 3-5$ V) instability preceded by significant ($\approx 30-40\%$) thinning. Modifying a phenomenological nucleation theory description of free liquid film rupture [16] effectively explains the properties of field-induced pores [2, 4, 9], but does not account for the onset of membrane instability or formation of the original (hydrophobic) pore (see ref. 10 for review). Recent simulations [11-15] show that a membrane's hydrophobic domain (the lipid tails) and forces exerted on water by the interfacial electric field much more strongly affect electroporation than do the lipid headgroups or the surrounding electrolyte. Simulations also demonstrate noticeable membrane fluctuations, both in shape and thickness, prior to breakdown. These findings, supported by experimental evidence, suggest that simplified continuum models accounting for general membrane properties while ignoring details, can provide insight into instability onset. Here we modify our non-local electroelastic model [17] to treat local membrane thickness fluctuations and analyze possible destabilization by an applied voltage. Despite recent progress, a self-consistent explanation of low-voltage membrane instability is still a distant goal. With this as background, we critically review simulational results and discuss the remaining challenges.
"Elastic capacitor" (EC) models provide useful insights into ELE instability. The energy of an EC connected to a battery is

\[ W = -\varepsilon_0 \varepsilon V^2/2d + K(d - d_0)^2/2, \]

where \( \varepsilon_0 \) is the vacuum permittivity, \( \varepsilon \) the dielectric constant, \( V \) the transmembrane potential drop, \( d \) the plate separation, \( d_0 \) the equilibrium separation at \( V = 0 \), and \( K \) the effective "spring" constant \([10]\). Equilibrium analysis shows that the EC becomes unstable at a critical voltage \( V = V_{cr} \)

\[ V_{cr} = \sqrt[3]{\frac{8Kd_0^3}{27\varepsilon_0 \varepsilon}} \]  

and that the critical separation preceding collapse is

\[ d_{cr} = \frac{2}{3} d_0 \]  

Now consider a symmetrically deformed membrane with midplane \( XY \) and unperturbed thickness \( d_0 \); its surfaces are shifted locally in opposing directions with thickness variation

\[ d(\rho) = d - 2u(\rho) \]

where \( u(\rho) \) is the vertical displacement profile, \( \rho \) a 2D-vector in the \( XY \) plane, and \( d \) the average membrane thickness. In continuum smectic modeling two elastic moduli, a stretching constant \( E_s \) and a bending constant \( K_e \), characterize membranes. The corresponding elastic deformation (d) energy \( W_d \) is

\[ W_d = \int [w_{ds}(\rho) + w_{db}(\rho)] d\rho; w_{ds} = 2E_s u(\rho)^2/d_0^2, w_{db} = \frac{1}{2} K_e (\nabla^2 u(\rho))^2. \]

The critical voltage (in Volts), analogous to Eq. 1, is

\[ V_{cr} = 0.082(E_s d_0/\varepsilon_m)^{1/2} \]

with \( E_s \) in erg/cm\(^2\) and \( d_0 \) in Å. In electroelastic models instability onset is associated with significant compression, typically 30-40% (≈ 33% in Eq. 2). Remarkably, the electroelastic instability arises at \( V_{cr} \) not only for uniform deformations but also for harmonic peristaltic modes \([1, 17]\), \( u(\rho) \approx \exp(-i\mathbf{q} \cdot \rho) \) where \( \mathbf{q} \) is the wave vector in the \( XY \) plane. Earlier analysis \([11]\) ignored the bending contribution \( w_{db} \), resulting in stability loss at all wavelengths (\( \lambda \)) simultaneously. Accounting for bending energy limits the unstable range to comparatively long \( \lambda \), \( \geq 5d_0 \) \([17]\). Instability at \( \lambda \)'s typical of membrane thicknesses \([1]\), needed here to create molecular scale pores, naturally suggests non-locality, i.e. the \( q \)-dependence of elastic moduli \([17]\).
EQUATIONS

Two opposing forces account for local membrane thickness fluctuations, modeled as water "dimples," illustrated in Fig. 1.

(1) Elastic forces which perturb the membrane from planarity exact an energy penalty, describable by modifying elastic theory to describe spatial dispersion of the elastic moduli [17].

(2) Ponderomotive forces, which promote deformation ala electrocapillarity and electrowetting. Similar molecular level effects were discussed in ref. 12.

The total dimple energy is the sum of elastic deformation and electrostatic (el) contributions

$$W_{\text{dim}} = W_d + W_{el}$$  \hspace{1cm} (6)

![Fig. 1. Model computational system, illustrating a water dimple fluctuation. Maximum dimple indentation is $u_0$.](image)

**The Elastic Term - Effect Of Non-Locality**

With $q$-dependent elastic moduli the elastic energy must converge to the classical expression, Eq. 4, at large $\lambda$ but deviate from it at $\lambda \leq d_0$ [10]. Short wavelength softening is not uncommon, observed in liquid surface tension [18, 19], in membrane density fluctuations [20], and in lipid bilayer simulation [21]. The elastic energy of an harmonic peristaltic mode is [17]

$$w(q) = \frac{2E_i u(q)u(-q)}{d_0^2} f(q)$$  \hspace{1cm} (7)

where $f(q)$ is the dimensionless mode energy, and

$$u(q) = \int d\mathbf{p} u(\mathbf{p}) \exp[-i\mathbf{q}\mathbf{p}]$$  \hspace{1cm} (8)

is the Fourier amplitude of the surface perturbation $u(\mathbf{p})$. In the long $\lambda$ limit,

$$f(q) \rightarrow f_*(q, \alpha) = \frac{1}{\alpha} + \zeta q^4$$  \hspace{1cm} (9)

where $f_*(q, \alpha)$ arises directly from Eq. 4. Here $\zeta \equiv K_i d_0^2/4E_i$, $\alpha = |q|$ and $\alpha$, which
equals $h/h_0$, describes uniform membrane compression [17]. At short $\lambda$ non-locality leads to the modification $f(q, \alpha) \rightarrow f_3(q, \alpha)$. Following ref. 17, $f_3(q, \alpha)$ takes the same functional form as $f_1(q)$

$$f_3(q, \alpha) = r f_1(q, \alpha)$$

(10)

(An alternate description of short $\lambda$ dispersion was suggested in ref. 21 based on MD analysis of membrane fluctuations; it takes the form of a surface tension contribution $f_5(q) = \gamma q^2$)

where $r$ is the softening parameter. While the condition of linear instability at $V \approx 1$ V imposed in ref. 17 required significant softening ($r \approx 0.1$) we now consider a moderate range, $0.5 \leq r \leq 1$ and show that $V_{cr}$ is quite insensitive to $r$.

From Eq. 7, a cylindrically symmetric perturbation, $u(\rho)$, has an elastic energy

$$W = \frac{4 \pi E_L}{d_0^2} \int_0^{d_{\max}} |u(q)|^2 f(q)q dq - \frac{4E_L}{d_0^2} \Omega_{dimple}$$

(11)

where $u = (\bar{d} - d)/2$ describes uniform membrane compression and $\Omega_{dimple} = u(q)|_{q=0}$ (see Eq. 8) is the dimple volume. Prompted by solution of the Hertz "pinned" membrane problem (see ref. 22 and references therein), we describe fluctuations of amplitude $u_0(\rho) = u_0 Kei[\rho/a]$ and characteristic decay length $a$, determined by minimizing energy at fixed amplitude $u_0$.

**The Electrostatic Contribution**

The increase in electrostatic energy due to membrane perturbation was calculated from the linearized Poisson-Boltzmann equation (LPBE). The computational cell (Fig. 1) is a cylinder with radius $R$ (typically 12 nm) and width $L = d + 2h_w$ where $d \approx 3$ nm is the membrane thickness and $h_w \approx 1$ nm the thickness of the water region far from the dimple. The potential was fixed on the upper and lower boundaries ("electrodes"): $\phi(\pm L/2, \rho) = \pm V/2$. The potential drop across the membrane was only insignificantly lower than the total applied voltage $V$. On the external cylindrical surface we chose $\phi(z, R) = \phi_b(z)$ where $\phi_b(z)$ is the piecewise linear potential profile for a planar (unperturbed) membrane. The LPBE is solved on a cylindrically symmetric grid by modifying the finite-difference algorithm described in ref. 23. Details will be described elsewhere. The total electrostatic energy is

$$W_{elec} = \frac{1}{2} \int \! d\mathbf{r} \, \mathbf{D}(\mathbf{r}) \mathbf{E}(\mathbf{r}) - qV$$

where $q$ is the total charge on the upper "electrode".
RESULTS

Fig. 2. Amplitude dependence of electrostatic energy profiles for a dimple with \( a = 0.5, 0.7 \) and \( 0.9 \) nm (1 V transmembrane potential).

Fig. 2 demonstrates that the ponderomotive force promotes an increase in the dimple's amplitude and "width," both in a strongly non-linear fashion. It is also instructive to consider the electric field near the dimple's apex.

Fig. 3. Ratio of the interfacial electric field, \( E_z \), in the membrane at the peak of the dimple, to the field at the flat interface far from the dimple.

As our description is linear in \( V \), this ratio is voltage independent.

Fig. 3 shows that the field at maximal dimple indentation is greatly enhanced. With \( u_0 = 0.5 \) nm and a typical \( a \approx 0.7-0.9 \) nm, this field corresponds to a voltage \( \approx \)
Electroporation is very dependent on the interface field gradient, \( \partial_z E_t(z, \rho = 0) \)\textsuperscript{top}, the force attracting water into the membrane\textsuperscript{12}. This gradient increases roughly as fast as the field. Thus, the region near the dimple apex promotes water defect formation. In addition, the large local fluctuation disturbs lipid packing, also promoting breakdown. Continuum analysis only describes the onset of breakdown, identified by the appearance of "sufficiently large" voltage induced local fluctuations. Defining criticality in this context is somewhat arbitrary; we choose \( u_0^{cr} = 0.5 \) nm. The critical voltage occurs when the energy for creating a fluctuation approaches zero. This condition is softer than the absolute loss of stability required by Crowley's model\textsuperscript{1}. Choosing a smaller critical amplitude (e.g. \( u_0 = 0.4 \) nm) has little effect on \( V_{cr} \), because the two competing forces, ponderomotive destabilization and elastic stabilization depend similarly on dimple amplitude.

![Graph](image)

**Fig. 4.** The energy of a dimple, \( u_0 = 0.5 \) nm, in a dioleoylphosphocholine (DOPC) membrane, minimized with respect to \( a \) for two elastic models: local and moderately softened (\( r = 0.5 \)) non-local.

Fig. 4 displays the optimized energy of a dimple for \( u_0 = 0.5 \) nm in the local model and in the non-local model with moderate softening factor \( r = 0.5 \). We assume that voltage can be applied instantaneously (as in the treatment given in refs. 21 and 11-13) so that the reference energy is the energy of the unperturbed membrane with \( d = d_0 \). Although short-\( \lambda \) softening reduces the energy, it does not crucially alter the critical voltage for dimple formation. This reflects two opposing tendencies. While softening is more important for the sharper dimples, applied voltages tend to increase \( a \) (see Fig. 2) reducing the influence of non-locality.

**DISCUSSION**

Our model predicts critical voltages of 1.8-2.2 V, still 2-3 times those observed experimentally. MD simulations also do not resolve the problem of low voltage electroporation [12]. The electric fields used in MD studies [12-14] were...
chosen to trigger electroporation on a time scale of a few nanoseconds. Table 1 compares typical voltages applied in the MD treatment [12-14], with $V_{cr}$ of the Crowley model. In each case the simulational voltages are either near to or greater than Crowley's $V_{cr}$, Eq. 1. Put differently, the MD studies were conducted in voltage ranges where the bilayer is absolutely unstable from the electroelastic point of view. In this context the following should be noted. The $V_{cr}$ of Table 1 presume $\varepsilon_m = 2$, typical of membrane interiors. However, simulational force fields are vacuum based, with $\varepsilon = 1$. Were $\varepsilon_m = 1$, Eq. 5 would imply $V_{cr} \approx 3.9$ and $4.3$ V for dimyristoylphosphatidylcholine (DMPC) and DOPC respectively. It is far from obvious that other membrane parameters affecting $V_{cr}$ are independent of the choice of $\varepsilon_m$ and that using a more realistic polarizable force field would not affect both the effective value of $\varepsilon_m$ and the membrane material constants. Direct comparison of the continuum and MD approaches is currently impossible because elastic properties of the simulated membranes were not reported in refs. 12-14.

Table 1. Comparison of voltages used in MD simulation with the critical voltages for electroelastic instability.

<table>
<thead>
<tr>
<th>Simulation</th>
<th>Bilayer</th>
<th>Applied potential (V)</th>
<th>$V_{cr}$ from Eq. 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ref. 12</td>
<td>DOPC</td>
<td>3</td>
<td>$\leq 3.2$</td>
</tr>
<tr>
<td>Ref. 13</td>
<td>DMPC</td>
<td>3-6</td>
<td>$\leq 2.8$</td>
</tr>
<tr>
<td>Ref. 14</td>
<td>DMPC</td>
<td>2.85 (average)</td>
<td>$\leq 2.8$</td>
</tr>
</tbody>
</table>

As the critical voltage is neared fluctuations rapidly lead to instability, and breakdown is very fast. At smaller voltages there is a significant lag time before pores form. Applying an Arrhenius treatment, our model yields estimates of the relative "lag times" for electroporation

$$\tau(V) = \tau_0 \cdot \text{Exp}[\frac{-W_{\text{dim}}(V)}{k_B T}]$$  

where $W_{\text{dim}}(V)$ is the energy of the dimple of "critical" amplitude. First, we choose a reference voltage $V^*$ where the "lag time" $\tau^* = \tau(V^*)$ is fairly small. As an example consider critical values $V^*_r \approx 2.2$ V and $V^*_{NL} = 1.8$ V for the local and non-local ($r = 0.5$) models respectively. How does decreasing $V$ affect "lag time"? Ratios $\Delta = \tau(V)/\tau(V^*)$ are presented in Table 2.

Table 2. Effect of voltage reduction on the relative increase in "lag time" for electroporation in local and non-local treatments.

<table>
<thead>
<tr>
<th>model</th>
<th>$V^*$, V</th>
<th>$\Delta$</th>
<th>0.25</th>
<th>0.5</th>
<th>0.75</th>
</tr>
</thead>
<tbody>
<tr>
<td>Local (r = 1.0)</td>
<td>2.2</td>
<td></td>
<td>2247</td>
<td>849</td>
<td>108</td>
</tr>
<tr>
<td>Non-local (r = 0.5)</td>
<td>1.85</td>
<td></td>
<td>65</td>
<td>37</td>
<td>12</td>
</tr>
</tbody>
</table>

Reducing the applied voltage by only 25% increases "lag times" by factors of 12 or 108, depending on the elastic model. This may explain why simulating very near the critical voltage is so important in time intensive MD studies.

Refs. 12-14 stress that electroporation is not accompanied by detectable
membrane compression-stretching. This is apparently viewed as an indication of model fitness, given the insignificant experimentally observed compression (≈1%). We find this conclusion quite surprising. Limited experimental compression is totally consistent with membrane elastic properties as the corresponding voltages are rather low, ≈0.5 V. However, voltages of ≈3 V used in simulations produce enormous electric pressures, ≈36 times stronger than the experimental ones. How can such huge forces have almost no effect on the membrane prior to electroporation? One possible explanation is implicit in our analysis. Instantaneously applying high voltages promotes strong local fluctuations that can overwhelm the uniform compression, resulting in membrane breakdown without noticeable thinning. The importance of this observation for MD should be studied. For instance, at a slightly (≈25%) lower voltage, when the lag time is much longer (Table 2) and pore formation quite unlikely, it would be possible for MD simulations to exhibit mechanical relaxation in response to the substantial electric pressure, still ≈20 times that used experimentally. How a simulated membrane accommodates such a stress during the long waiting period (before breakdown) needs study. In fact it is a corollary to the more practical question of how comparatively small electric voltages can break real bilayers. So far, neither molecular nor continuum models provide an answer.

Finally, we address the results of ref. 14, where charge (q) rather than potential (V) control was used to induce electroporation. This study may have general statistical mechanical importance in its relation to the well known problem of the admissible sign of double layer capacitance C and related instabilities (see refs. 24, 10, 25 for review and references). Refs. 25, 26 showed, using the membrane capacitor example, that there is a critical value of the average charge density \( \sigma = q/A \) (A is the membrane area) at which a uniform charge distribution becomes unstable, leading to a phase transition or to electrical breakdown. It would be important to establish if the membrane breakdown in MD simulation\(^{14}\), associated with a very non-uniform ionic distribution, is due to this general criticality. This possibility is suggested by the observation that the average \( \sigma \) chosen in ref. 14 is very close to the critical value predicted in refs. 25, 26. We believe that answers to the questions we have posed can assist in the design and interpretation of membrane simulations and aid in understanding instabilities at electrified interfaces.

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