Membrane flow patterns in multicomponent giant vesicles induced by alternating electric fields

Margarita Staykova, Reinhard Lipowsky and Rumiana Dimova*

Max Planck Institute of Colloids and Interfaces, Science Park Golm, 14424 Potsdam, Germany

Supplementary information

1. Preparation of giant lipid vesicles (GUVs):

GUVs were prepared using the electroformation method, introduced by Angelova and Dimitrov.1 Stock solutions of 4 mM lipid in chlorophorm were prepared from dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC) and cholesterol (Chol), and subsequently mixed to form solutions with 5.33:2.66:2, 2.66:5.3:3:2 and 4.8:3.2:2 DOPC:DPPC:Chol molar lipid ratios. The fluorescent dye N-(lysamine rhodamine B sulphonyl)-dipalmitoylphosphatidylethanolamine (Rh-DPPE) was added to the mixture (0.1 mol %). A small volume (12 μl) of the lipid solution was spread on the conductive surfaces of two glass plates coated with indium tin oxide. The latter were kept at around 60°C and under vacuum for approximately 2 h to remove all traces of the organic solvent. The two glasses were placed with their conductive sides facing each other and separated by a 2 mm-thick Teflon frame to form a chamber, which was sealed with silicon grease. The chamber was then gently filled with preheated sucrose solution (0.01 to 0.1 M) and placed in an oven at 60°C. The glass plates were connected to a function generator and an alternating current (700 mV, 10 Hz frequency) was applied for one hour. Vesicles with an average diameter of 50 μm and a large polydispersity were observed after about 2 hours. Then, a 5 Hz current of 2 V was applied for another hour to detach the vesicles from the glass surfaces. The vesicle solution was let to cool slowly, removed from the electroswellung chamber and diluted 40 times into an isotonic glucose solution. The osmolarities of the sucrose and glucose solutions were measured with cryoscopic osmometer Osmomat 030 (Gonotec, Berlin, Germany) and carefully matched to avoid an osmotic pressure difference. The conductivity of the glucose solution was adjusted by adding NaCl solution and measured with conductivity meter SevenEasy (Mettler Toledo, Greifensee, Switzerland).

Approximately 1 ml of the obtained vesicle solution was placed in a chamber for electromanipulation purchased from Eppendorf (Hamburg, Germany). Because of the differences in density and refractive index between the sucrose and glucose solutions, the vesicles were stabilized by gravity at the bottom of the chamber. At room temperature, lipid membranes with the above composition are in the two-phase coexistence region.2 The vesicles exhibit liquid ordered (l₂) and liquid disordered (l₁) domains. Rh-DPPE is known to partition in the l₁ phase.3 After equilibration, the vesicles typically contained one l₂ domain and one l₁ domain. We preheated them to obtain a larger number of smaller domains before subjecting them to electric fields.

2. Supplementary movie material

All movies were recorded with an inverted confocal laser scanning microscope Leica DM IRE2 (Leica Microsystems Heidelberg GmbH, Germany) using 40× Ph2 objective and laser excitation at 561 nm (DPSS laser). Emission light was detected by a photomultiplier tube in the spectral range 580- 650 nm. The l₁ domains are stained in red by Rh-DPPE, whereas the l₂ ones appear black. The direction of the field is the same in all movies and is denoted by an arrow.

Movie 1 Flow pattern in the lower part of a vesicle: The vesicle corresponds to the one in Fig. 1 in the article. The applied AC field is 360 V/cm, 80 kHz. The vesicle was prepared from 4.8:3.2:2 DOPC:DPPC:Chol solution. The conductivity of the external medium is 250 μS/cm. The vesicle diameter is 150 μm. The domains move from the polar regions (see Fig. 1 in the article) towards the lowest point of the vesicle (the middle of the image) and then towards the equatorial region. Domain fission can be also observed. The movie is speeded up 4.2 times.

Movie 2 Flow pattern in the upper part of a vesicle: This movie is recorded at the same exposure conditions as Movie 1 and for the same vesicle. The flow at the top part of the vesicle is in opposite direction and slower than the flow at the bottom part (Movie 1). The movie is speeded up 4.2 times.

Movie 3 Frequency dependence of the flow in the lower part of the vesicle: The vesicle was prepared from 5.33:2.66:2 DOPC:DPPC:Chol solution. An AC field at a fixed strength of 260 V/cm and for three different frequencies (500 kHz, 1 MHz and 3 MHz) was applied. The conductivity of the external medium is 360 μS/cm and the diameter of the vesicle is approximately 120 μm. For the low frequency (500 KHz), the direction of the membrane flow is as described in Movie 1. At 1 MHz the flow almost ceases and at 3 MHz its direction reverses. Between the field frequency switches, the vesicle is not exposed to an AC field. The movie is speeded up 8.4 times.

Movie 4 AC-field induced lipid mixing: The movie shows the bottom part of a vesicle (180 μm in diameter) that has been prepared from 2.66:5:3:2 DOPC:DPPC:Chol. Such membrane composition favors lipid segregation in
two large domains – \( l_d \), stained in red and \( l_o \), in black. At the beginning of the movie, before the field application, the bottom part of the vesicle is occupied mainly by the red \( l_d \) domain. The membrane on the upper part of the vesicle is in the \( l_o \) phase (dark). The small black domains in the red \( l_d \) phase are due to mixing from previous exposures to electric field. The AC field exposure (500 V/cm, 80 kHz) at an external conductivity of 250 \( \mu \)S/cm causes a strong membrane flow, observed as dark protrusions of the \( l_o \) phase in the red \( l_d \) phase. The movie is speeded up 4.2 times.

3. Numerical simulations

The calculations were done in 3D and in the quasi-static approximation, using the commercially available modeling package COMSOL (Multiphysics, Germany). The parameters for the calculations, as given in Table 1, matched the experimental conditions.

<table>
<thead>
<tr>
<th>Parameter name</th>
<th>Symbol notation</th>
<th>Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Internal dielectric constant</td>
<td>( \varepsilon_{in} )</td>
<td>( 7.08 \times 10^{-10} )</td>
<td>C(^2) J(^{-1}) m(^{-1})</td>
</tr>
<tr>
<td>External dielectric constant</td>
<td>( \varepsilon_{ex} )</td>
<td>( 7.08 \times 10^{-10} )</td>
<td>C(^2) J(^{-1}) m(^{-1})</td>
</tr>
<tr>
<td>Membrane dielectric constant</td>
<td>( \varepsilon_{me} )</td>
<td>( 2.66 \times 10^{-11} )</td>
<td>C(^2) J(^{-1}) m(^{-1})</td>
</tr>
<tr>
<td>Internal electric conductivity</td>
<td>( \lambda_{in} )</td>
<td>( 3 \times 10^{-4} )</td>
<td>S m(^{-1})</td>
</tr>
<tr>
<td>External electric conductivity</td>
<td>( \lambda_{ex} )</td>
<td>( 3 \times 10^{-7} )</td>
<td>S m(^{-1})</td>
</tr>
<tr>
<td>Membrane electric conductivity</td>
<td>( \lambda_{me} )</td>
<td>( 10 \times 10^{-10} )</td>
<td>S m(^{-1})</td>
</tr>
<tr>
<td>External vesicle radius</td>
<td>( R )</td>
<td>( 40 \times 10^{-6} )</td>
<td>m</td>
</tr>
<tr>
<td>Membrane thickness</td>
<td>( d )</td>
<td>( 5 \times 10^{-9} )</td>
<td>m</td>
</tr>
<tr>
<td>Angular frequency</td>
<td>( \omega )</td>
<td>( 628 \times 10^{3} )</td>
<td>Rad s(^{-1})</td>
</tr>
</tbody>
</table>

Table 1 Parameters used for the numerical calculations.

3.1. Dielectric model

Lipid vesicles are commonly modeled as single shell objects with confocal geometry, i.e., the two surfaces bounding the shell are ellipsoids with the same foci. The internal vesicle solution, the membrane and the external medium are leaky dielectrics defined by complex permittivity (\( \varepsilon \)), which comprises the influence of both their electric conductivity and dielectric permittivity:

\[
\varepsilon_k = \sigma_k + i\omega \varepsilon_k, \quad \text{with} \quad k = \text{in, me, and ex,}
\]

where \( i \) is the imaginary number (see Table 1 for symbol definition).

The single shell model can be further simplified using the Maxwell body approximation, according to which, any spherical confocal single shell object may be represented as a homogenous sphere of the same external radius \( R \), having an effective permittivity (\( \varepsilon_{eff} \)) and the same polarization as the one of the shell (Fig. 1S). The effective permittivity of the equivalent body is given by:

\[
\varepsilon_{eff} = \frac{2(1-v)\varepsilon_{me} + (1+2v)\varepsilon_{in}}{(2+v)\varepsilon_{me} + (1-v)\varepsilon_{in}}, \quad \text{where} \quad v = \frac{(1-d/R)^3}{1-d/R} \quad \text{is a geometric factor and} \quad d \quad \text{is the membrane thickness.}
\]

Fig. 1S. Schematic illustration of dielectric models of a lipid vesicle.

The electric potential is a solution of the Laplace equation. The normal component of the electric current density across the vesicle interface is continuous, whereas at the external boundaries, i.e. the box walls, it is zero (insulators). The external field is generated between two cylindrical electrodes with voltages of 1 and -1 V at their boundaries.
3.2. Lateral electric stress for a vesicle in a homogeneous AC field.

As a test case, the lateral stress induced on the surface of a vesicle was calculated for a homogeneous electric field (Fig. 2S). The parameters for the calculation (field conditions, media properties, vesicle size) are analogous to the parameters used to obtain Fig. 3 in the article. The only difference is that the electrodes are far from any solid substrate and the vesicle is situated in the middle between them, i.e. it is subject to a homogenous AC field.

![Fig. 2S](image)

**Fig. 2S.** Lateral stress distribution on a vesicle in homogeneous AC field.

The numerical results show axially symmetric distribution of the lateral electric stress on the surface of the vesicle with maxima at $\theta = \pm \pi/4$, similar to results reported in the literature.\(^5\) The symmetry axis is provided by the field direction. Note that the same stress distribution is observed also in droplets.\(^6\) However, since the molecules at the droplet surface are not bound to the interface but are free to flow in the droplet interior, such stress distribution gives rise to a toroidal flow inside (as well as outside) as sketched in Fig. 3S. In vesicles, the lipids are bound to the vesicle surface. For an axially symmetric stress distribution (Fig. 2S), the gradient in the membrane tension on one side of the vesicle (e.g. the lower part) would be exactly balanced by identical gradient on the other (upper) side of the vesicle. Thus, no flow in the membrane is initiated. Note that in inhomogeneous fields, this balance is broken (see Fig. 3 in the article) giving rise to the flow described in the article.

![Fig. 3S](image)

**Fig. 3S.** Sketch of the toroidal flow inside droplets subjected to homogeneous AC fields. The system has rotational symmetry along the azimuthal angle $\phi$.

It is interesting to draw a parallel between the stress distribution on membranes in homogeneous electric fields and the concentration gradients observed in supported lipid bilayer. In the studies reporting the latter\(^7,8\), the filed is applied along the surface of the planar supported bilayer and concentration gradients of the fluorescent marker are observed. These gradients, however, do not illustrate the stress profile in the supported membrane and is thus of entirely different nature as compared to the stress gradients driving the domains in vesicles in inhomogeneous fields as reported here.

References: