Lipid Flow Patterns in Vesicles

Rumiana Dimova 06.04.1971
1995: Diploma, Chemistry (Sofia University, Bulgaria), Major: Chemical Physics and Theoretical Chemistry, Thesis: Role of the Ionic-Correlation and the Hydration Surface Forces in the Stability of Thin Liquid Films
1997: Second MSc (Sofia University, Bulgaria)
Thesis: Interactions between Model Membranes and Micron-Sized Particles
1999: PhD, Physical Chemistry (Bordeaux University, France)
Thesis: Hydrodynamical Properties of Model Membranes Studied by Means of Optical Trapping Manipulation of Micron-Sized Particles
2000: Postdoc (Max Planck Institute of Colloids and Interfaces, Potsdam)
Since 2001: Group Leader (Max Planck Institute of Colloids and Interfaces, Potsdam)

When applied to colloidal systems, electric fields induce various phenomena, which have found wide application in micro- and nano-technologies in the past decade. Neutral particles (droplets, bubbles, lipid vesicles, solid beads) suspended in a medium of different polarizability acquire charge at their surfaces when exposed to electric fields. The interaction of the field with the surface charges results in a surface force that may cause particle deformation, kinetic effects, electro-osmotic fluid flows, etc.

In the case of uniform electric fields applied to lipid vesicles, the radial component of the electric surface force leads to a shape deformation at weak field strengths (see section "Morphological transitions of vesicles in AC electric fields" on page 122) or causes membrane rupture, at strong fields **[1]**. The lateral component, on the other hand, may induce fluid flows, analogous to the flows induced in liquid droplets. However, there is a fundamental difference between droplets and vesicles, which arises from the properties of the lipid bilayer. The membrane behaves as a two dimensional nearly incompressible fluid. It develops tension to keep its surface area constant.

Under homogeneous alternating (AC) fields, membrane flow in the vesicle is not expected because the lateral electric stress is counterbalanced by the resulting axially symmetric gradient in the membrane tension. In inhomogeneous fields however, this force balance is broken and a flow of lipids occurs in order to restore it. Note that in most experimental chambers and conditions used for electro-manipulation, vesicles, cells or other particles experience inhomogeneous fields, due to screening by neighbors, sedimentation, chamber geometry, or other factors.

To study the membrane dynamics in AC fields, we used giant vesicles made of lipid mixtures, which at room temperature phase separate into liquid ordered (Lo) and liquid disordered (Ld) phases. The membrane was composed of saturated and unsaturated lipids and cholesterol; for more information on the vesicle preparation see **[2]**. A tiny fraction of fluorescent dye was added, which preferentially partitions in the Ld phase. The lipid ratio was such that the Lo phase appeared as dark circular patches in the surrounding fluorescently labeled Ld phase.

The membrane flow pattern was resolved by following the motion of the Lo patches with confocal microscopy. The bottom and the top part of the vesicle were recorded as shown on the micrographs in **Fig. 1 a-c**. The inner and outer vesicle solutions were 0.1 M sucrose and glucose, respectively. This ensures an osmotic balance, i.e. constant vesicle volume, and causes the vesicles to sediment at the bottom of the chamber since sucrose solutions have higher density than glucose solutions with the same concentration. The electric field was applied between two parallel cylindrical electrodes. The proximity of the bottom glass to the vesicle leads to an asymmetric field distribution at its surface. The field strength is much higher at the lower vesicle part, facing the glass, than at the top part, see **Fig. 2**.

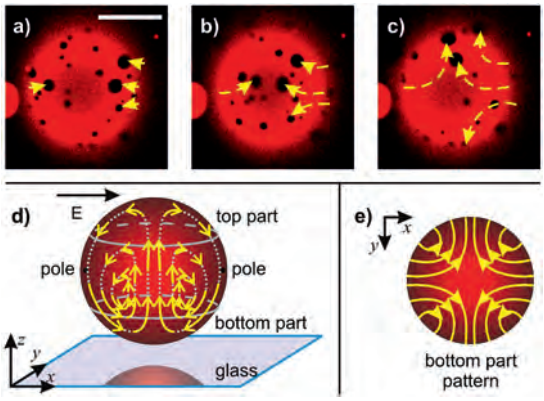

Fig. 1: Confocal micrographs illustrating the membrane flow on the bottom part (a-c) of a giant vesicle about 150 µm in diameter induced by an AC field (360 V/cm, 80 KHz), at an external conductivity of 25 mS/m. The vesicle was prepared from a mixture of 4.8 : 3.2 : 2 dioleoylphosphatidylcholine : dipalmitoylphosphatidylcholine : cholesterol. The time between the consecutive snapshots is approximately 1.3 s. The yellow dashed arrows indicate the trajectories of selected domains in the consecutive snapshots. The scale bar corresponds to 50 µm. The vesicle is located close to the bottom of the observation chamber as illustrated in (d), where the vesicle top and bottom parts, the poles and the field direction are indicated. The side and the bottom views of the flow lines are sketched in (d) and (e), respectively. The length of the arrows in (d) indicates the flow velocity.

Such asymmetric field distribution leads to a special membrane flow pattern consisting of concentric closed trajectories, organized in four symmetric quadrants, each extending from the bottom to the top of the vesicle as in **Fig. 1d, e**. The flow is fastest at the periphery of the quadrant and at the bottom of the vesicle. The top and the bottom of the vesicle are stagnation points. The velocity of the domains reaches about 30 µm/sec corresponding to laminar flows. It can be further enhanced by the field strength and the conductivity of the external solution. Interesting effects are observed when the field frequency is varied. At frequencies less than about 3 MHz, the motion in the circular trajectories is directed downwards past the poles and upwards along the equator as sketched on **Fig. 1d** but at higher frequencies it reverses its direction.

The calculation of the lateral electric stress or surface force density on the membrane suggests that the vesicle experiences larger stress in the vicinity of the solid substrate **[2]**. As a result, a non-uniform and non-symmetric membrane tension builds up. It triggers lipid flow towards the regions of highest tension, similarly to Marangoni flows in monolayers. Interestingly, the frequency at which we observe reversal of the flow direction, i.e. around 3 MHz, coincides with the Maxwell-Wagner frequency, above which the polarization of the vesicle is determined by the media permittivities. Thus, the reversal of the flow direction may arise from the difference between the permittivities of the glucose and the sucrose solutions.

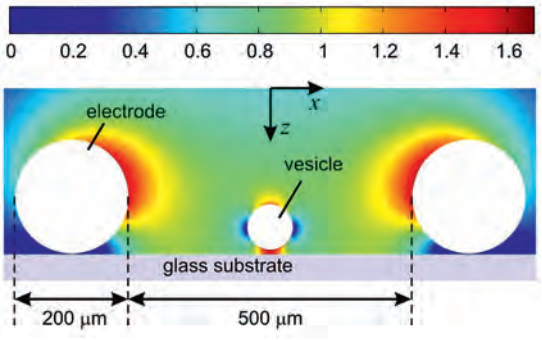

Fig. 2: Electric field distribution at 100 kHz in a cross section of the chamber consisting of two parallel cylindrical electrodes fixed to a glass substrate. The image displays a cross section passing through the centre of a vesicle located in the middle between the electrodes. The vesicle is 40 µm in radius and is located at 8 µm above the glass. The media conductivity is 0.3 mS/m. The field inside the vesicle is not shown. The data are rescaled with the strength of a field, which would be induced between two parallel planar electrodes at a distance of 500 µm.

To investigate the lipid flow driven by the electric field, we theoretically model the dielectric polarization of the vesicle by placing image electric dipoles and quadrupoles at the center of the vesicle. The calculated Maxwell stresses arising from asymmetric electric fields are found to induce flow patterns, which agree with the experimental observations **[3]**.

The flow in the membrane is coupled to fluid flows in the internal and external media. To visualize the effect of the membrane flow on the internal medium we used vesicles containing aqueous solution of the water-soluble polymers poly-(ethylene glycol) (PEG) and dextran (see section "Morphologies of Vesicles loaded with Aqueous Polymer Solutions" on page 120). At specific polymer concentration, this solution can undergo phase separation **[4]** producing droplets of dextran-rich phase, which can be visualized e.g. by fluorescently labeled dextran. The droplets tend to coarsen slowly. Before the coarsening is completed we subject such vesicles to asymmetric AC fields. As expected, the droplets start to move because of the coupling to the membrane flow. Therefore, when a cross section of the vesicle is observed with confocal microscopy as in **Fig. 3**, the droplets are observed to come into focus and to go out of focus again.

These AC field-induced flows in giant vesicles have possible applications in microfluidic technologies. Giant vesicles in inhomogeneous AC fields or in hydrodynamic flows mimicking, e.g., the situation of red blood cells in capillaries may be used as nano-reactors for fluid manipulation, i.e. displacing, mixing, trapping, etc. To demonstrate lipid mixing, we performed experiments where lipid vesicles composed of only one Lo and one Ld domains, are exposed to an AC field for a certain period of time. One example is shown in **Fig. 4**. The field-induced membrane flow causes domain fission leading to the appearance of a large number of smaller domains. For sufficiently strong membrane flows, the number of domains grows with the time of exposure. The growing number of domains, on the other hand, increases the probability of domain encounter and fusion. Domain fusion counterbalances the fission and therefore, the domains will reach a stationary state characterized by a certain size distribution after a certain time.

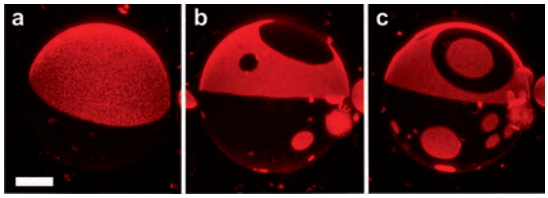

Fig. 4: 3D confocal scans of the lower vesicle hemisphere illustrating lipid mixing induced by AC field (80 kHz, 500 V/cm), at an external conductivity of 0.25 mS/m. The vesicle with a diameter about 95 µm was prepared from a mixture of 2.66 : 5.33 : 2 dioleoylphosphatidylcholine : dipalmitoylphosphatidylcholine : cholesterol. (a) Before applying the field, the vesicle has only two domains, which break apart after continuous field exposure of (b) 2 min and (c) 3 min. The scale bar corresponds to 25 µm.

We were not able to achieve similar mixing of dextran-rich and PEG-rich phases starting from a state, where the two phase are fully separated, because the pressure of the internal flow was not large enough to overcome the surface tension between the two polymer phases. However, in other systems, e.g. vesicles enclosing suspensions of micro-particles, the induced flows may be used to homogenize the internal vesicle content.

R. Dimova, M. Staykova, T. Yamamoto, Y. Li
rumiana.dimova@mpikg.mpg.de

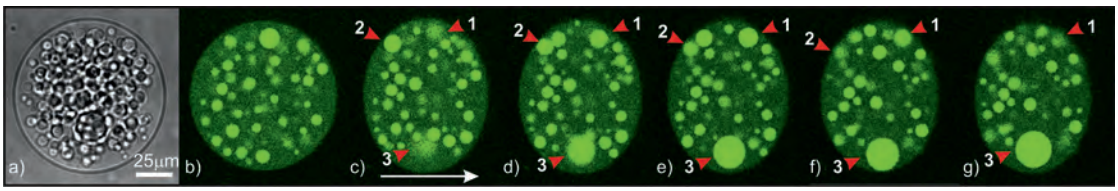

Fig. 3: (a) A phase-contrast images and (b-g) confocal cross sections of a giant lipid vesicle enclosing dextran-rich droplets (green fluorescence) in a PEG-rich phase. The cross section in (b), corresponding to the image in (a), is taken close to the equatorial plane of the vesicle and shows only the droplets in focus. Application of an inhomogeneous AC field (460 V/cm, 80 KHz) at an external conductivity of 40 mS/m leads to a vesicle shape deformation and an internal flow, in the direction perpendicular to the plane of the image in (c-d). The flow is visualized by following the motion of droplets 1, 2 and 3, which come in focus and go out of focus. The time period is 2.5 s between images c)-d) and d)-e), and 5 s between e)-f) and f)-g). The field direction is indicated by the arrow in (c).

References:

[1] Dimova, R., Riske, K., Aranda, S., Bezlyepkina, N., Knorr, R. and Lipowsky, R.: Giant vesicles in electric fields. Soft Matter **3**, 817-927 (2007).
[2] Staykova, M., Lipowsky, R. and Dimova, R.: Membrane flow patterns in multicomponent giant vesicles induced by alternating electric fields. Soft Matter **4**, 2168-2171 (2008).
[3] Yamamtoto, T., Staykova, M., Dimova, R., Lipowsky, R.: in preparation.
[4] Li, Y., Lipowsky, R. and Dimova, R.: Transition from complete to partial wetting within membrane compartments. J. Am. Chem. Soc. **130**, 12252-12253 (2008).