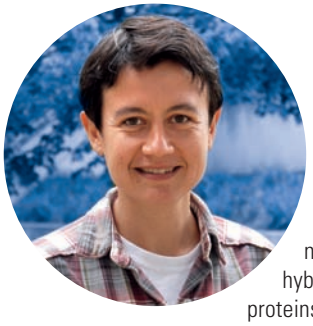
Electro-Deformation and -Poration of Vesicles



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)

The response of membranes to electric fields has been extensively studied in the last decades. The phenomena of electrodeformation, electroporation and electrofusion are of particular interest because of their widespread use in cell biology and biotechnology as means for cell manipulation, cell hybridization or for introducing molecules such as proteins, foreign genes (plasmids), antibodies, or drugs into cells. Giant vesicles are the simplest model of the cell membrane. Being of cell size, they are convenient for direct microscopy observations.

Deformation in AC Fields

When subjected to alternating electric fields, giant vesicles deform into elliptical shapes. The deformation depends on the AC field frequency and on the conductivities of the aqueous solution in the interior and exterior vesicle compartments **[1]**. When the interior solution has conductivity (σ_{in}) higher than the exterior one (σ_{out}), a quasispherical vesicle deforms into a prolate. This deformation is observed for a large range of AC frequencies, up to 10^6 Hz. Interestingly, whenever the internal conductivity is lower than the external one ($\sigma_{in} < \sigma_{out}$), as in **Fig. 1**, a prolate-oblate transition (**Fig. 1a** and **1b**) is observed for intermediate frequencies of a few kHz. This applies also to external conductivities close to physiological conditions. At higher frequencies, more than about 10^7 Hz, the vesicles attain a spherical shape (**Fig. 1c**) irrespective of conductivity conditions; see **Fig. 2**.

Fig. 1: A giant vesicle (phase contrast microscopy) subjected to an AC field of 10 V (2 kV/cm). The field direction is indicated with the arrow in (a). The external solution has a higher conductivity than the internal one ($\sigma_{in} > \sigma_{out}$). From (a) to (c) the field frequency increases causing shape transformations of the vesicle: (a) 5 kHz, prolate morphology; (b) 100 kHz, oblate shape; (c) 10 MHz, sphere.

Using giant unilamellar vesicles made of egg PC, we succeeded to map the morphological transitions as a function of AC frequency and conductivity ratios. The conductivities were varied by the addition of NaCl (leading to concentration of up to about 1 mM) in the exterior or interior vesicle solutions. A large interval of frequencies was studied (up to 10^8 Hz). The degree of vesicle deformation was quantitatively characterized from optical video microscopy images.

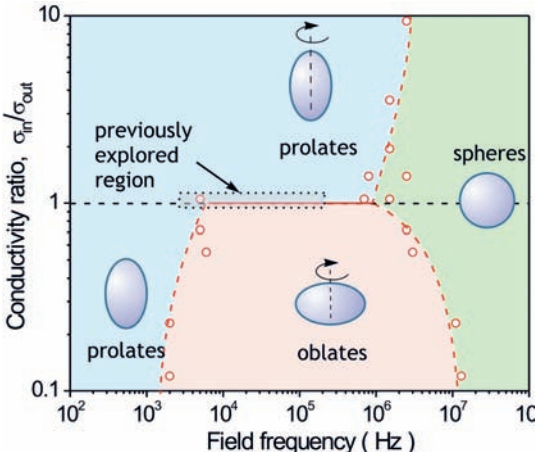


Fig. 2: Morphological diagram of the shape transformations of vesicles in different conductivity conditions and various field frequencies. When the conductivity of the solution inside the vesicles is larger than the one outside, ($\sigma_{in} > \sigma_{out}$), transitions from prolate to spherical vesicles are observed (upper part of the diagram). For internal conductivities lower than the external one ($\sigma_{in} > \sigma_{out}$), the vesicle undergoes prolate-to-oblate-to-sphere transitions depending on the field frequency (lower part of the diagram). The open circles are experimentally determined. The dashed lines are guides to the eye for the various region boundaries. The area surrounded by the dotted line shows the region previously explored in the literature.

Earlier studies by Helfrich and collaborators (see e.g. Winterhalter and Helfrich, *J. Coll. Interf. Sci.* 122, 1987) report on prolate deformations of vesicles in AC fields, but conductivity asymmetry has not been studied and thus not taken into account in the theoretical modelling. Thus the transition observed in our system cannot be predicted by the existing theory. We extended these theories to include the effect of asymmetric conductivity conditions and the frequency dependence of the conductivity (PhD project of Said Aranda).

Electroporation of Vesicles Subjected to DC Pulses

When subjected to short and strong electric pulses (~100 µs, ~1 kV/cm) the vesicle response is qualitatively similar to the one in AC fields. However, microscopy observation of effects caused by electric pulses on giant vesicles is difficult because of the short duration of the pulses. To tackle this problem, recently in our group, imaging with a fast digital camera was used to record the pulse response of giant lipid vesicles with a high temporal resolution of up to 30 000 frames per second (one image every 33 microseconds) **[2]**. This approach helped record extraordinary cylindrical shapes on vesicles **[3]**. These unusual morphologies (cylinders or disks with spherical caps) have not been previously observed due to their short lifetime of a few milliseconds. The observation with the fast digital camera allowed resolving the pores on the vesicle and the dynamics of the vesicle response **[2]**. The lifetime of the pores, which was in the millisecond range, was found to depend on the membrane viscosity. In the fluid phase, the latter can be determined from optical manipulation of a probe attached to the membrane (optical dynamometry) **[4]**. When the membrane undergoes a fluid-to-gel transition, the membrane viscosity drastically increases. Thus, it is to be expected that the lifetime of pores formed on vesicles in the gel phase would be much longer. We attempted to visualize such pores using confocal microscopy on giant vesicles in the gel phase; see **Fig. 3**. Indeed, the time of these pores to reseal was orders of magnitude longer than the lifetime of pores in electroporated membranes in the fluid phase **[5]**.

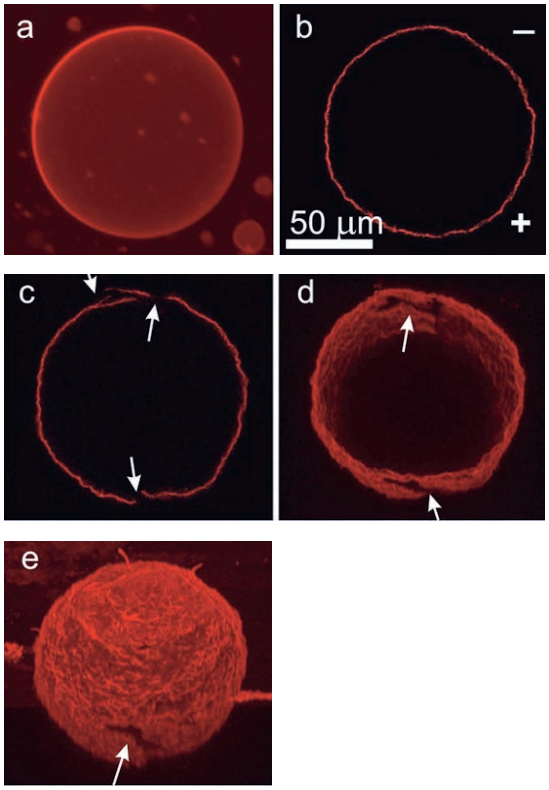


Fig. 3: Electroporation of a fluorescently labeled vesicle in the gel phase as imaged with confocal microscopy. (a) A 3d projection averaged image of a vesicle in the fluid phase. (b-e) Images of a vesicle in the gel phase: Equatorial sections of the vesicle before (b) and after poration (c) caused by an electric pulse of 300 V (6 KV/cm) and duration 300 microseconds. The electrode polarity is indicated with plus (+) and minus (-) signs in (b). The arrows in (c) show the ruptured zones at the vesicle poles. A 30 micrometer wide stripe from the equatorial area of the vesicle (slightly rotated around the horizontal axis) shows the ruptured places in the membrane at the north and south poles (d) as indicated with arrows. A complete 3d projection average image of the same vesicle (again rotated around the x-axis) shows better the crack on the southern pole of the vesicle (e) pointed by the arrow. Contrary to vesicles in the fluid phase (a), pores formed on vesicles in the gel phase (e) do not reseal over a period of at least ten minutes.

R. Dimova, S. Aranda, R. Knorr, K. A. Riske
Rumiana.Dimova@mpikg.mpg.de

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