Morphological transitions of vesicles induced by AC electric fields
Supplementary material

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Vesicle preparation and observation
Giant unilamellar vesicles were prepared from egg yolk L-α-Phosphatidylcholine (Avanti Polar Lipids, Alabaster, AL) using a procedure described in detail in (1). Briefly, 12μl of a 2mg/ml lipid solution in chloroform were spread on the surfaces of two conductive glasses (coated with Indium Tin Oxide). The latter were kept under vacuum for about 2h to remove all traces of the organic solvent. The two glasses separated by a 2mm thick Teflon frame were assembled into a chamber sealed with silicon grease. The glass plates were connected to an AC field function generator and an alternating current of 1V at 10Hz frequency was applied. The chamber was filled with 0.2M sucrose solution and the voltage was gradually increased to about 1V in 0.3V steps every 20min. Vesicles with an average size of 20μm and a large polydispersity were observed to form. The vesicles were subsequently diluted 40 times into 0.2M glucose solution, thus creating sugar asymmetry between the interior and the exterior of the vesicles. Up to 3mM NaCl was added to the sucrose solution (prior to electroformation) and up to 100mM NaCl to the glucose solutions to set the desired conductivity asymmetry across the vesicle membrane. Measurements of the refractive index of the salt solutions (RE50 Refractometer, Mettler Toledo GmbH, Germany) showed no significant change, suggesting that the solutions permittivity does not alter significantly. The osmolarities of the inner and the outer solutions were measured with a cryoscopic osmometer Osmomat 030 (Gonotec, Germany) and carefully matched with glucose to avoid osmotic stress. The solutions conductivities were measured with a conductivity meter SevenEasy (Mettler Toledo, Switzerland). Due to the density difference between the sucrose and glucose solutions, the vesicles settled at the bottom of the observation chamber where they were easy to locate. The refractive index difference produced a good optical contrast of the phase contrast images (the vesicles appear as dark objects on a light gray background). The osmotic stabilization of the vesicles ensured volume conservation throughout the measurements.

Analytical expressions for the vesicle deformation according to the model of Winterhalter-Helfrich
The relative vesicle deformation $a/R$ is estimated from
$$\frac{a}{R} = 1 + s_{2}/R,$$
where $R$ is the radius of the vesicle and $s_{2}$ is the deformation of the vesicles predicted by Winterhalter and Helfrich (2) and given by:
$$s_{2}=\varepsilon_{w}R^{2}[1+R(\beta)]/d/\left[32\kappa(1-Rc_{0}/6)\right],$$
where
$$\beta=(\lambda_{m}-i\omega \varepsilon_{m})/(\lambda_{w}-i\omega \varepsilon_{w}).$$

Here $E$ is the field amplitude, $\lambda_{w}$ and $\varepsilon_{w}$ are the conductivity and the dielectric constant of the water media respectively, and $\lambda_{m}$ and $\varepsilon_{m}$ are the conductivity and the dielectric constant of the membrane, $d$ is the bilayer thickness, $\kappa$ the bending stiffness, $c_{0}$ the spontaneous curvature ($c_{0} \approx 0$), and $\omega$ the field frequency. Re($\beta$) indicates the real part of $\beta$.

We used the above expressions to calculate $a/R$ for a vesicle with radius $R=1\mu$m (this example is given in (2)) as a function of the field frequency (see solid and dashed lines in Fig.S1). We did not plot a calculation for a larger vesicle because of the unrealistic degree of deformation predicted (for example, for $R=10\mu$m the calculated $a/R$ is more than three orders of magnitude higher than the experimental observation). The following values for the various parameters were used: $R=1\mu$m, $E=0.2kV/cm$, $\varepsilon_{w}=80\varepsilon_{0}$, $\varepsilon_{m}=2\varepsilon_{0}$, $\varepsilon_{0}=8.854\times10^{-12}C^{2}J^{-1}m^{-1}$, $\lambda_{m}=10^{-10}\mu S/cm$, $\lambda_{w}=1\mu S/cm$ or $\lambda_{w}=15\mu S/cm$, $d=4nm$, $\kappa=2\times10^{-19}J$, $c_{0}=0$. For the experimentally measured example (open circles in Fig. S1) $R=27.5\mu$m, $E=0.2kV/cm$, $\lambda_{w}=\lambda_{m} \lambda_{w}=15\mu S/cm$.

![FIGURE S1 Experimentally measured vesicle deformation (open circles), $R=27.5\mu$m $\lambda_{w}=15\mu S/cm$, $x=1$; and theoretical prediction for a vesicle with $R=1\mu$m according to the model of Winterhalter-Helfrich for media conductivity $\lambda_{w}=1\mu S/cm$ (solid line) and $\lambda_{w}=15\mu S/cm$ (dashed line). The field strength is $E=0.2kV/cm$.](Image)
REFERENCES
