**Supporting Information**

**TRANSMEMBRANE POTENTIAL IN VESICLES FORMED BY CATANIONIC SURFACTANT MIXTURES IN AQUEOUS-SALT SOLUTION**

Ksenia A. Emelyanova, Polina O. Sorina, Alexey I. Victorov

## I.    THE ELECTROSTATIC FREE ENERGY OF A VESICLE

We use the process of charging both sides of the vesicle's membrane to obtain the electrostatic free energy of the system. We show that this free energy contains the contribution from the dielectric membrane subject to the resulting electric field.

Consider a vesicle of a given geometry and aggregation numbers inserted in a given volume of solution that is in equilibrium with a large external reservoir of mobile ions. Initially, both sides of the vesicle's membrane are set electrically neutral but as we add an infinitesimal charge $\delta Q$ to the surfaces of the membrane, the mobile ions are free to move inside and around the vesicle, forming the electrical double layers on both sides of the membrane. For an isothermal charging process, the reversible electrical work is equal to the change of the semi-grand thermodynamic potential [1,2]

$$d\Omega = \int_S \Psi \, d\sigma \, dS \tag{A1}$$

where $\Psi$ is the surface value of the electrostatic potential, integration is over all charged surfaces in the system, $\sigma$ is the surface charge density; the added infinitesimal charge $\delta Q = \int_S d\sigma \, dS$.

There are two charged surfaces in a vesicle, and hence

$$d\Omega = \int_{S_{in}} \Psi_{in} \, d\sigma_{in} \, dS + \int_{S_{out}} \Psi_{out} \, d\sigma_{out} \, dS \tag{A2}$$

where integration is performed separately for the inner and for the outer surfaces of the membrane.

Because the electric potentials and the surface charge densities are uniform on both sides of the spherical membrane, the integration over the surfaces gives

$$d\Omega = 4\pi R_{in}^2 \Psi_{in} \, d\sigma_{in} + 4\pi R_{out}^2 \Psi_{out} \, d\sigma_{out} \tag{A3}$$

where $R_{in}$ and $R_{out}$ are the curvature radii of the inner and the outer sides of the membrane, respectively. Integration of Eq.(A3) over the charge densities from zero to their final values gives the electrostatic contribution to the free energy of the vesicle:

$$\Omega = 4\pi R_{in}^2 \int_0^{\sigma_{in}} \Psi_{in} \left( \sigma_{in}, \sigma_{out} \right) d\sigma_{in} + 4\pi R_{out}^2 \int_0^{\sigma_{out}} \Psi_{out} \left( \sigma_{in}, \sigma_{out} \right) d\sigma_{out} \tag{A4}$$

The surface potentials depend on the charge densities of both surfaces; this dependence is linear within the LPB theory and is given by Eqs.(8a,b), (5a,b) and (9) of the main text. The

contour integral of Eq. (A4) along the path connecting points (0,0) and $(\sigma_{in},\sigma_{out})$ is then easily evaluated by introducing the charging parameter $\lambda \in [0,1]$ and simultaneously charging both surfaces: $\sigma_{in} \to \lambda\sigma_{in}$ , $d\sigma_{in} \to \sigma_{in}d\lambda$ and $\sigma_{out} \to \lambda\sigma_{out}$ , $d\sigma_{out} \to \sigma_{out}d\lambda$ . From Eq. (A4) we have

$$\Omega = 4\pi R_{in}^2 \int_0^1 \lambda\sigma_{in}\Psi_{in}(\sigma_{in},\sigma_{out})d\lambda + 4\pi R_{out}^2 \int_0^1 \lambda\sigma_{out}\Psi_{out}(\sigma_{in},\sigma_{out})d\lambda \tag{A6}$$

Integration over $\lambda$ yields

$$\Omega = 4\pi R_{in}^2 \frac{\sigma_{in}\Psi_{in}(\sigma_{in},\sigma_{out})}{2} + 4\pi R_{out}^2 \frac{\sigma_{out}\Psi_{out}(\sigma_{in},\sigma_{out})}{2} \tag{A7}$$

Expressing Eq. (A7) in reduced variables we find the electrostatic free energy of the vesicle per surfactant molecule, $g_{el}$ , Eq.(11) of the main text.

The electrostatic free energy given by the contour integral of Eq. (A4) may not depend on the path of integration. This is readily shown by taking another integration path, $(0,0) \to (\sigma_{in},0) \to (\sigma_{in},\sigma_{out})$, or, alternatively, $(0,0) \to (0,\sigma_{out}) \to (\sigma_{in},\sigma_{out})$ .

Eq. (A4) then gives

$$\Omega = 4\pi R_{in}^2 \int_{(0,0)}^{(\sigma_{in},0)} \Psi_{in}(\sigma_{in},0)d\sigma_{in} + 4\pi R_{out}^2 \int_{(\sigma_{in},0)}^{(\sigma_{in},\sigma_{out})} \Psi_{out}(\sigma_{in},\sigma_{out})d\sigma_{out} \tag{A8}$$

From Eqs. (8a,b), (5a,b) and (9) the dependence of the surface potentials on the surface charge densities may be expressed in the form

$$\Psi_{in}(\sigma_{in},\sigma_{out}) = \sigma_{in}a_{in} - \sigma_{in}b_{in} + \sigma_{out}c_{in}$$

$$\Psi_{out}(\sigma_{in},\sigma_{out}) = \sigma_{out}a_{out} - \sigma_{out}b_{out} + \sigma_{in}c_{out}$$

where the coefficients $a_{in/out}$ , $b_{in/out}$ , and $c_{in/out}$ depend on the geometry of the membrane but not on the charge density. Solving the integrals in Eq. (A8), we obtain

$$\Omega = 4\pi R_{in}^2 \left[ \frac{\sigma_{in}\Psi_{in}(\sigma_{in},\sigma_{out})}{2} - \frac{\sigma_{in}\sigma_{out}}{2}c_{in} \right] + 4\pi R_{out}^2 \left[ \frac{\sigma_{out}\Psi_{out}(\sigma_{in},\sigma_{out})}{2} + \frac{\sigma_{in}\sigma_{out}}{2}c_{out} \right] \tag{A9}$$

Eq. (A9) is identical to Eq. (A7) because the product $R_{in}^2 c_{in}$ is identical to $R_{out}^2 c_{out}$ and the extra terms in the square brackets of this equation cancel out. The identity of the products is verified by expressing $c_{in}$ and $c_{out}$ explicitly from Eqs. (8a,b), (5a,b), (9) and (1). The equivalence of Eqs. (A7) and (A9) proves the independence of the electrostatic free energy on the specific path of the charging process as it should be.


Below we demonstrate that the presence of the dielectric membrane in the vesicle has been taken into account by Eq. (A3).

The drop of the electric field across a charged surface is given by [1-3]

$$(\varepsilon\nabla\Psi)_+ \cdot \vec{n} - (\varepsilon\nabla\Psi)_- \cdot \vec{n} = -\sigma \tag{A10}$$

where $\vec{n}$ is the outward unit normal to the surface, the subscripts "+" and "-"denote the outer and inner sides of the surface, respectively. Here and below, the constant factor $\varepsilon_0$ has been included in the definition of $\varepsilon$. Substituting the differential of Eq. (A10) into Eq. (A2), we obtain for the contributions of the outer and inner surfaces of the membrane to the free energy

$$d\Omega = d\Omega_{in} + d\Omega_{out}$$

$$d\Omega_{out} = \int_{S_{out}} \Psi_{m_-} d\{\varepsilon_m\nabla\Psi_m\}_- \cdot \vec{n}dS - \int_{S_{out}} \Psi_+^{out} d\{\varepsilon_{out}\nabla\Psi^{out}\}_+ \cdot \vec{n}dS \tag{A11}$$

$$d\Omega_{in} = \int_{S_{in}} \Psi_-^{in} d\{\varepsilon_{in}\nabla\Psi^{in}\}_- \cdot \vec{n}dS - \int_{S_{in}} \Psi_{m_+} d\{\varepsilon_m\nabla\Psi_m\}_+ \cdot \vec{n}dS$$

We now transform the surface integrals of Eq. (A11) into the integrals over the volume. According to the Gauss theorem, the volume integral of the divergence $\nabla \cdot \vec{A}$ any vector $\vec{A}$ is equal to the integral of vector $\vec{A}$ over the surface enclosing that volume. We take $\vec{A} \equiv \Psi d(\varepsilon\nabla\Psi)$, the general term under the integral sings of Eq. (A11), and hence $\nabla \cdot \vec{A} = \nabla \cdot \{\Psi d(\varepsilon\nabla\Psi)\}$.

We divide the volume of our system into three parts and consider three closed surfaces that engulf these parts of volume (Fig. S1):

- the solution inside the vesicle enclosed by the inner surface of the vesicle membrane;
- the solution outside the vesicle enclosed by the outer surface of the membrane, by the outer boundary of our system far away in the bulk solution where the electric potential is zero, and by the surface constructed by a cut of space to connect these two surfaces;
- the membrane of the vesicle enclosed by its inner and outer surfaces and by the surface produced by a cut across the membrane to connect its inner and outer sides.

The surface integrals of vector $\vec{A}$ over the outer sides of these three closed surfaces reduce exactly to the four integrals of Eq. (A11) because the integrals over the cuts cancel out when integrating over their opposite sides and the integral over the outer boundary of the system also vanishes since the gradient of the electric potential and vector $\vec{A}$ are identically equal to zero far from the vesicle. The free energy given by Eq. (11) is thus equal to the sum of three volume integrals spanning the whole volume of the system:

$$d\Omega = \int_{V_{in}} \nabla \cdot \vec{A}_{in} d\vec{r} + \int_{V_m} \nabla \cdot \vec{A}_m d\vec{r} + \int_{V_{out}} \nabla \cdot \vec{A}_{out} d\vec{r} \tag{A12}$$

The integration variable in Eq. (A12) never crosses the charged surfaces of the membrane where $\nabla\Psi$ is singular. Keeping this in mind, we rewrite this equation in a more compact form, as a single integral over the whole volume of the system:

$$d\Omega = \int_V \nabla \cdot \vec{A} d\vec{r} = \int_V \nabla \cdot \{\Psi d(\varepsilon\nabla\Psi)\} d\vec{r} \tag{A13}$$
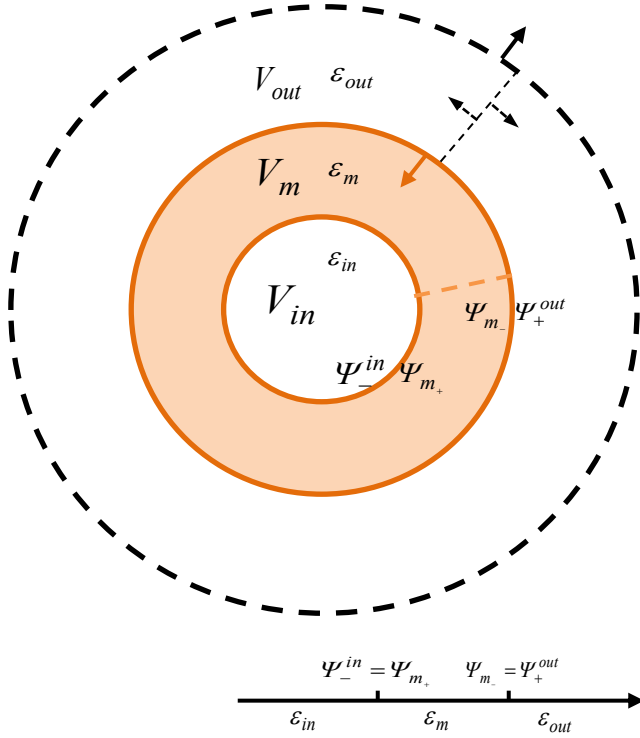
For the integrand of Eq. (A13), we have

Figure S1 Membrane of the vesicle (shaded) with its charged inner and outer surfaces divides the whole volume of the system into three parts: the solution in the inner compartment of the vesicle, the dielectric membrane itself, and the outside solution from the charged outer surface of the membrane to the outer boundaries of the system far in the bulk (dashed). Arrows show the outward normals to the closed surface that engulfs this outer part. Part of this closed surface that cuts this outside solution is also shown by dashes. The electric potential is continuous at the charged surfaces of the membrane.

$$\nabla \cdot \{\Psi d(\varepsilon \nabla \Psi)\} = \nabla \Psi \cdot d(\varepsilon \nabla \Psi) + \Psi \nabla \cdot \{d(\varepsilon \nabla \Psi)\} = \frac{1}{2} d\{\varepsilon(\nabla \Psi)^2\} + \Psi d\{\nabla(\varepsilon \nabla \Psi)\} \tag{A14}$$

where the second equality is justified because $\nabla$ and $d$ are differential operators and hence they are interchangeable.

Using Eq. (A14) in (A13) we obtain

$$d\Omega = \frac{1}{2} \int_V d\{\varepsilon(\nabla \Psi)^2\} d\vec{r} + \int_V \Psi d\{\nabla(\varepsilon \nabla \Psi)\} d\vec{r} \tag{A15}$$

The electrostatic free energy $\Omega$ is obtained by integrating the differential terms of Eq. (A15). Integrating the second term of this equation by parts, where

$$\left[ \int_V \Psi \cdot \nabla(\varepsilon \nabla \Psi) d\vec{r} \right] \Big|_0^\Psi = \int_V \Psi \cdot \nabla(\varepsilon \nabla \Psi) d\vec{r}$$

we finally have

$$\Omega = \int d\Omega = \frac{1}{2}\int_V \varepsilon(\nabla\Psi)^2 d\vec{r} + \int_V \Psi \cdot \nabla(\varepsilon\nabla\Psi)d\vec{r} - \int_V \int_0^\Psi \nabla \cdot (\varepsilon\nabla\Psi)d\Psi d\vec{r} \qquad \text{(A16)}$$

The integrands of this equation behave differently in different parts of the system. In the electric double layers inside and outside the vesicle $\nabla(\varepsilon\nabla\Psi) = -\rho$, from the Poisson equation. Inside the membrane $\nabla(\varepsilon\nabla\Psi) = 0$ by the Laplace equation and the contribution of this part of the volume to the free energy is given by

$$\frac{1}{2}\int_{V_m} (\vec{D} \cdot \vec{E})d\vec{r} \qquad \text{(A17)}$$

where $\vec{D} = -\varepsilon\nabla\Psi$ is the displacement field (and $\vec{E} = -\nabla\Psi$ is the electric field). Eq. (A17) gives the polarization energy [1,3] of the polarizable dielectric membrane in the electric field created by charging the vesicle's surfaces. Thus the electrostatic free energy of the vesicle calculated from Eqs. (A2)-(A4) automatically includes this polarization contribution.

## II.   NUMERICAL SOLUTION OF NLPB EQUATION FOR VESICLE AND THE ARTIFACTS FROM LPB THEORY

The electrostatic problem for the vesicle has also been solved numerically using NLPB equations for the interior and the exterior of the vesicle coupled through the boundary conditions on the inner and the outer sides of the vesicle's membrane. This system of coupled differential and algebraic equations has been solved iteratively with respect to the potential values at the inner and the outer sides of the membrane. For testing the FORTRAN software developed in this work, calculations have also been performed with the aid of COMSOL MULTIPHYSICS 4.3 software both using 3D Electrostatics and 1D Coefficient Form PDE models. Figure S2 illustrates excellent agreement between the calculated results. A minor systematic underestimation of the potential on the vesicle's external surface given by COMSOL 3D Electrostatics is related to a somewhat sparser mesh than that used in COMSOL 1D or our FORTRAN routines in the domains of rapidly changing potential (see the location of black points in Figure S2). Shown in Figure S3 is an example of two-dimensional distribution of the electric potential in the plane that contains the center of the vesicle.

The dependence of the transmembrane potential on salinity is shown in Figure S4 for vesicles of systematically increasing inner radii. At a given salinity the transmembrane potential from NLPB is a monotonically decreasing function of the curvature radius. Such a monotonic decrease is also illustrated by the profiles shown in Figure SI 5.

In contrast to this result, the transmembrane potential obtained from the LPB theory for low salinity may exhibit non-monotonic dependence on curvature, as illustrated in Figure SI 6 for $C_{salt} = 0.1 mM$ $C_{salt} = 10^{-3} mM$ and $C_{salt} = 10^{-5} mM$. Such non-monotonic behavior is an artifact of the LPB approximation that may not be applied at low salinity where the electric potentials become large. Nevertheless, we add below a few comments on the asymptotic behavior of the LPB solution in the low salinity limit, stressing that this behavior is purely formal and is outside of the range of applicability of LPB theory.
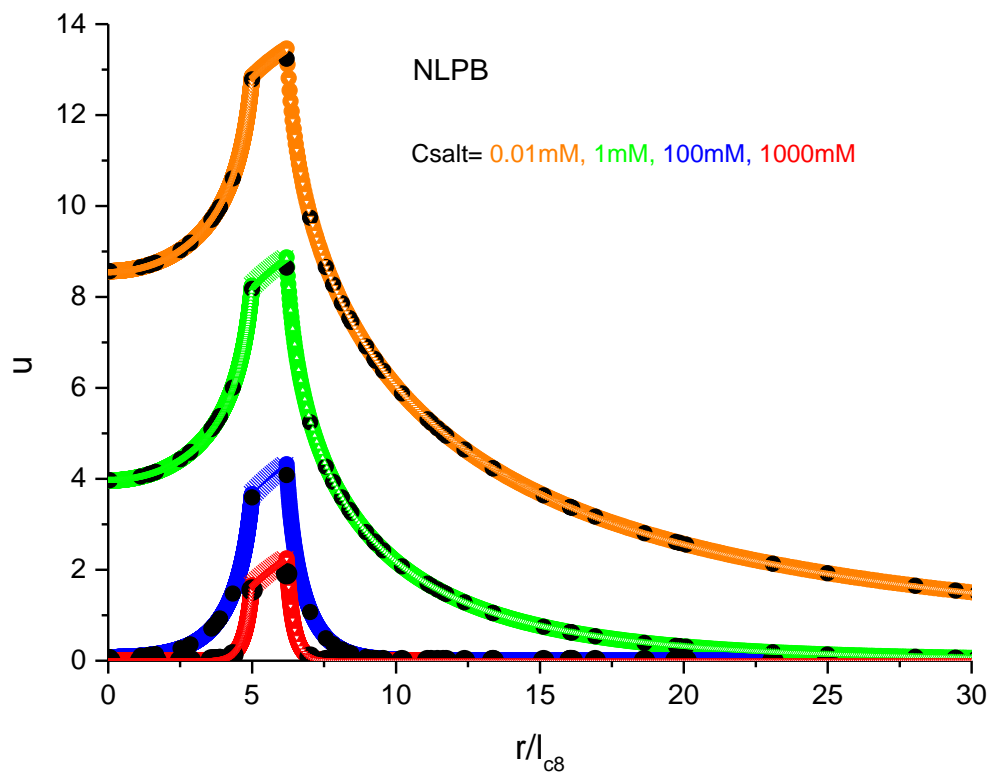
Figure S2 Profiles of electrostatic potentials for different salinities of the external solution calculated from NLPB theory. The vesicle is composed of symmetrical cationic and anionic $C_8$ – surfactants. The inner radius $R = 5l_s$; the mole fraction of the cationic surfactant $\alpha_{in} = 0.6$ in the inner and $\alpha_{out} = 0.7$ in the outer monolayers of the vesicle's membrane, respectively; the monolayers' thickness $\tau_{in} = \tau_{out} = 0.6l_s$; the dielectric permittivity $\varepsilon_m = 2.1$ in the hydrocarbon membrane and $\varepsilon_w = 78.2$ in water. Colored symbols: from FORTRAN routine; black points: COMSOL 3D; white triangles: COMSOL 1D.
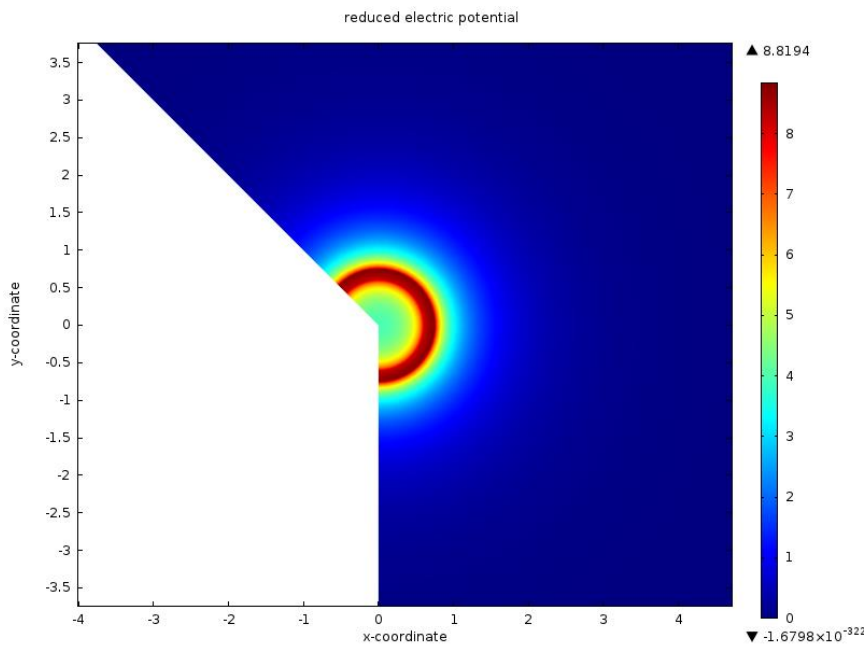
Figure S3 The map of electrostatic potential for the model vesicle of Figure S2 at 1.0 mM salt. Calculated using COMSOL 1D model.
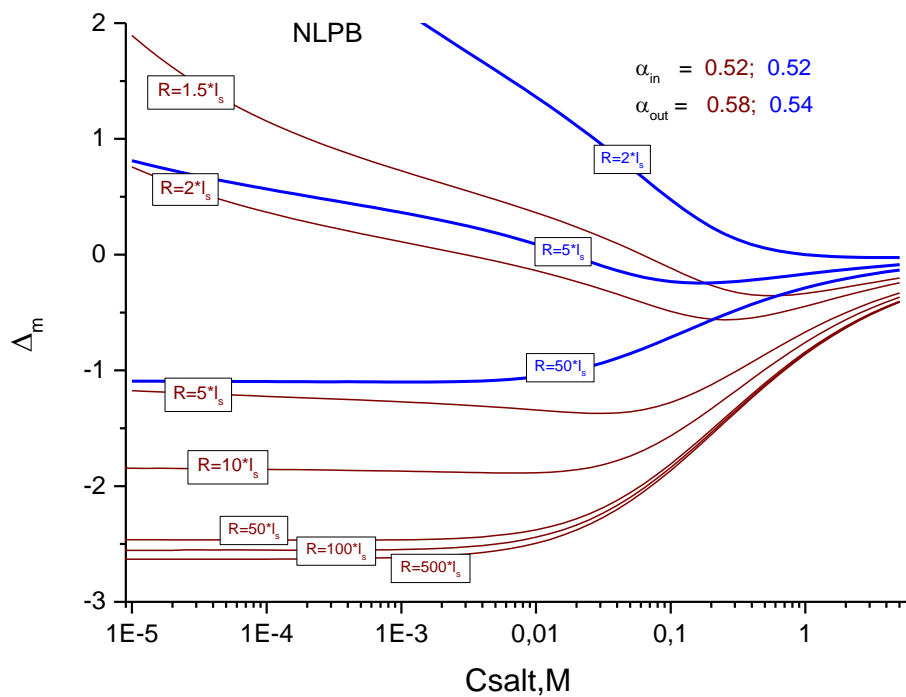


Figure S4 The transmembrane potential from NLPB theory as function of external salinity for C8+C8 catanionic vesicles of varying inner radii. $\tau_{in} = \tau_{out} = 0.6 l_s$ Different colors correspond to different charges of the inner and outer surfaces of the vesicle's membrane.
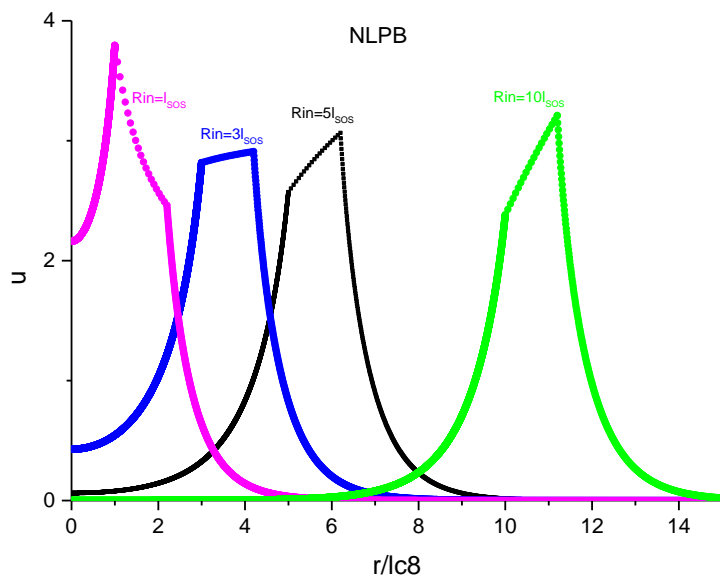
Figure SI 5 Profile of the electric potential calculated from NLPB theory for CTAB+SOS vesicles of varying curvature at 100mM external salinity. $\tau_{in} = \tau_{out} = 0.6 l_s$ ; CTAB mole fractions in the membrane $\alpha_{in} = 0.6$ and $\alpha_{out} = 0.7$ .
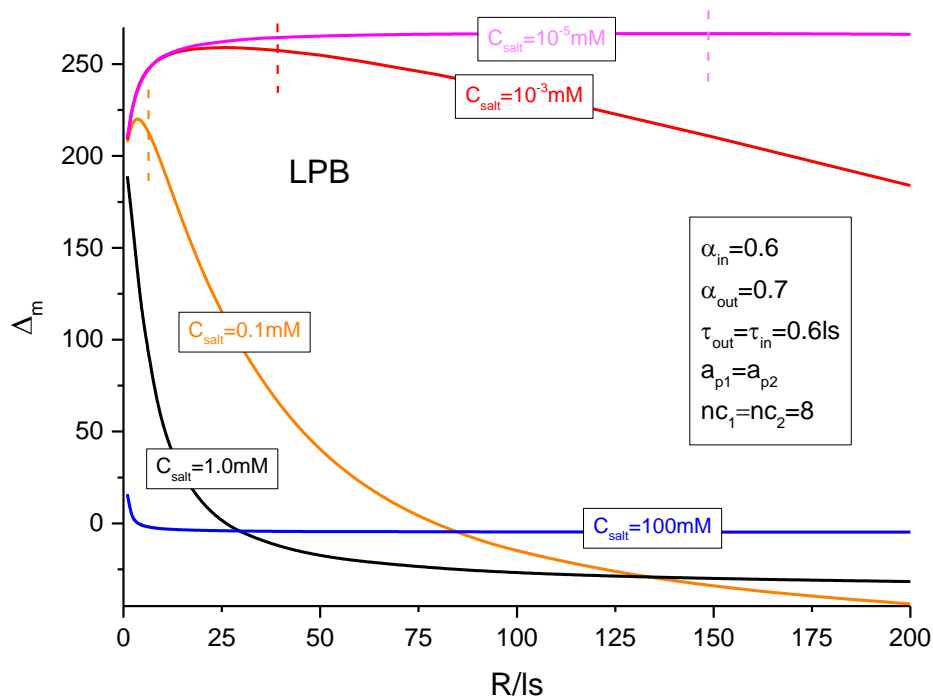


Figure SI 6 The transmembrane potential from LPB theory vs the inner radius of the vesicle (in units of surfactant's tail length $l_s$ ) at varying salinity. The parameters of the vesicle are given in Figure SI 2. Vertical dashes show locations of maxima estimated from Eq. (A21)

For sufficiently low salinity we may have $R_{in}\kappa << 1$ and $R_{out}\kappa << 1$ even for vesicles about hundred nanometers large. Expanding the transmembrane potential to second order in $\kappa$ we obtain the effective charge densities from Eqs. (9), (5a) and (5b)

$$t_{out}^{eff}(\Delta_m) = \frac{t_{out}R_{out}^2 + t_{in}R_{in}^2}{\varepsilon_w R_{out}^2} \tag{A18a}$$

$$t_{in}^{eff}(\Delta_m) = \frac{\kappa^2 R_{in}}{3\varepsilon_w}\left[t_{out}R_{out} + t_{in}R_{in}\left(\frac{R_{in}}{R_{out}} + \frac{\varepsilon_w}{\varepsilon_m}\frac{\tau}{R_{out}}\right)\right] \tag{A18b}$$

These equations and Eqs (8a), (8b) give the surface potentials in the low salinity limit

$$u_{out}(x_{out}) = \frac{\sigma_{out}R_{out}^2 + \sigma_{in}R_{in}^2}{\varepsilon_w R_{out}}\frac{e_0}{\varepsilon_0 kT} \tag{A19a}$$

$$u_{in}(x_{in}) = \frac{1}{\varepsilon_w}\left[\sigma_{out}R_{out} + \sigma_{in}R_{in}\left(\frac{R_{in}}{R_{out}} + \frac{\varepsilon_w}{\varepsilon_m}\frac{\tau}{R_{out}}\right)\right]\frac{e_0}{\varepsilon_0 kT} \tag{A19b}$$

and the limiting value of the transmembrane potential

$$\Delta_m = \frac{\sigma_{in}R_{in}\tau}{\varepsilon_m R_{out}}\frac{e_0}{\varepsilon_0 kT} \tag{A20}$$

In Eqs. (A18)-(A20), the vesicle dimensions $R_{in}$, $R_{out}$ and $\tau$ have been expressed in units of length. Eqs. (A19)-(A20) show that the surface potentials and their transmembrane difference remain finite in the zero salinity limit. Remarkably, in this limit, the transmembrane potential does not depend on the charge of the outer surface of the membrane.

For low salinity, there is a maximum of the transmembrane potential vs curvature. The location of this maximum may be estimated from an approximate equation obtained by expanding the transmembrane potential to the second order in $\kappa$ and the surface charge densities to the first order in $\tau_{in}/R_{in}$. Equating to zero the derivative of $\Delta_m$ with respect to $R_{in}$ and performing tedious algebra we obtain

$$\tau_{out} + \frac{(\tau_{out} + \tau_{in})}{3}\frac{\tau_{in}}{R_{in}} \cong \frac{2}{3}\frac{\tau_{out}}{\tau_{in}}\frac{(2\alpha_{out}-1)}{(2\alpha_{in}-1)}\kappa^2 R_{in}^3$$

Neglecting the linear in curvature term in this equation, we finally have

$$R_{in} \approx \left[\frac{3}{2}\frac{(2\alpha_{in}-1)}{(2\alpha_{out}-1)}\tau_{in}\kappa^{-2}\right]^{\frac{1}{3}} \tag{A21}$$

The location of the maximum estimated from this equation is shown in Figure SI6 that displays an example of such maxima for a model vesicle. These maxima first appear at salinity of ca 0.1mM and shift to larger radii as the salinity is diminished. The accuracy of Eq.(A21) improves with decreasing salinity.

**REFERENCES**

[1] L D Landau, E.M. Lifshitz. Course on Theoretical Physics, Vol.8, L D Landau L. P. Pitaevskii E.M. Lifshitz. Electrodynamics of Continuous Media. 2nd ed., Imprint: Butterworth-Heinemann, 1984, page 72.

[2] V.A. Andreev, A.I. Victorov. Molecular thermodynamics for micellar branching in solutions of ionic surfactants. *Langmuir*, 2006, **22**, 8298-8310.

[3] J.D. Jackson. Classical Electrodynamics. Wiley and Sons Inc., N.Y., 1962, page 124.