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Curvature inducing macroion condensation driven shape changes of fluid vesicles

K. K. Sreeja,1,a) John H. Ipsen,2,b) and P. B. Sunil Kumar1,c)

1Department of Physics, Indian Institute of Technology Madras, Chennai 600036, India
2Department of Physics and Chemistry, MEMPHYS-Center for Biomembrane Physics, University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark

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We study the effect of curvature inducing macroion condensation on the shapes of charged deformable fluid interfaces using dynamically triangulated Monte Carlo simulations. In the weak electrostatic coupling regime, surface charges are weakly screened and the conformations of a vesicle, with fixed spherical topology, depend on the charge-charge interaction on the surface. While in the strong coupling regime, condensation driven curvature induction plays a dominant role in determining the conformations of these surfaces. Condensation itself is observed to be dependent on the induced curvature, with larger induced curvatures favoring increased condensation. We show that both curvature generation and curvature sensing, induced by the interplay of electrostatics and curvature energy, contribute to determination of the vesicle configurations. © 2015 AIP Publishing LLC. [http://dx.doi.org/10.1063/1.4935596]

I. INTRODUCTION

Understanding the effect of electrostatic interactions in shaping interfaces is of fundamental importance in the areas of biophysics and soft matter. Soft materials such as polymers, colloids, and membranes can acquire surface charges when dissolved in a polar solvent such as water, due to the dissociation of the surface chemical groups. In such systems, electrostatic interactions play a decisive role in determining the morphological behavior of the material, with entropy of the mobile ions contributing significantly. Competing interactions between the charged surface, the mobile counterions (CIs), and coions can lead to the condensation of CIs on the surface as a function of temperature and dielectric constant of the medium. In more complex systems, like lipid membranes, the condensed CIs or adsorption of oppositely charged ions on the interface can induce morphological changes in the surface. For example, the electrostatics mediated adsorption and activity of proteins and macromions on a membraneous system can significantly alter its morphology.1

One out of four main lipid groups in the plasma membrane, phosphatidylethanolamine (PS), is negatively charged.2 Plasma membranes of mammalian cells often consist of 10%–20% anionic lipids.3 In addition to PS, phosphatidylinositol (PI) and polyvalent PI species like PI(4)P, PI(4,5)P2, PI(3,4,5)P3 also contribute to the surface charges in membranes.3 Zwitter ionic lipid species (mainly phosphatidylcholine and phosphatidylethanolamine) can also contribute significantly to the electrostatic forces. Charge distribution on such surfaces determines the spatial localization and orientation of membrane associated proteins. Conversely, the surface charge can be sequestered by protein interactions.5 The selective binding or unbinding of such proteins can modulate local membrane shapes through the wide range of local curvature it can generate, depending on the stiffness of the membrane, and lead to new membrane conformations. For example, the positively charged membrane facing motifs of Bin-Amphiphysin-Rvs (BAR) domains interact with negatively charged lipids on the membrane surface leading to formation of tubular membrane.6–9 The acidic protein Myristoylated Alanine-Rich C-Kinase Substrate (MARCKS), which has significant role in the cell signaling and vesicle transport, produces lateral domains on charged membranes.4

While such ion induced morphological changes in polymers have been investigated in some detail,10–13 its two dimensional counterpart, the membrane is much less studied.14,15 The in-plane fluidity of these two dimensional objects makes it even more distinct from polymers. In the present paper, we focus on the equilibrium conformations of a charged vesicle placed in a solution containing oppositely charged macroions and different concentrations of salt. For simplicity, we consider cases wherein the charged lipids are only on the outer leaflet of the vesicle membrane, hence, restricting macroions to the region outside the vesicle surface. Here, by the term counterions we mean macroions, such as proteins, present in surrounding fluid carrying a charge opposite to that of membrane charges. We use the Dynamical Triangulation Monte Carlo (DTMC) method to explore the shape deformations under various electrostatic conditions.16 Similar methods have been previously used to study the shape changes charged vesicle and colloid membrane interactions.18

Electrostatics is incorporated either via bare Coulomb or screened Coulomb interactions. An explicit coupling between the electrostatics and the local membrane conformation is introduced through a change in the preferred local mean curvature $C_0$ on ion condensation. We investigate the implications of the coupling between elastic and electrostatic
interactions and show that the membrane conformations are dependent on both the mean and variance of the number of condensed macromolecules. In biology, the interaction between membrane surface charges and proteins takes place in a salty environment. With this in mind, we also looked at the membrane conformations when the electrostatics is through a screened Coulomb interaction. Another macroscopic parameter that affects the equilibrium shapes of membrane is the pressure difference between the inside and outside of the vesicle, an aspect which is well explored in the previous studies.\textsuperscript{19,20} In the case of charged membranes, electrostatic interactions can change the ion concentration near the vesicle surface resulting in pressure changes. We explore this aspect here. Finally, we compare the electrostatics mediated curvature fluctuations with that induced by equilibrium and non-equilibrium activities of uncharged curvature inducing proteins.

The paper is organized as follows: Sec. II introduces the triangulated surface model for membranes, the Canham-Helfrich energy functional, and the inclusion of electrostatic interactions in the membrane model. Sec. III A describes the conformational changes in the vesicle when CI condensation does not induce a spontaneous curvature and Sec. III B when it induces a curvature. Sec. III C shows the effect of osmotic pressure on the CI condensation and resulting shape changes. In Sec. III D, the effect of salt in the solution is described through a screened Coulomb interaction. In Sec. III E, the equilibrium conformations of the charged vesicle system are compared to the steady state shapes of the vesicle caused by the stochastic binding and unbinding of curvature inducing proteins.

II. MODEL AND MONTE CARLO SIMULATIONS

The elastic energy of a vesicle is described by the well known Canham-Helfrich Hamiltonian, which assumes the membrane to be a thin two-dimensional elastic sheet embedded in three dimensions (Willmore surface).\textsuperscript{21,22} The energetics of thin fluid membranes are well described by this mathematical framework. In Monte Carlo simulations, this continuum surface is replaced by a triangulated mesh of \(N_T = 2(N_v - 2)\) triangles, with \(N_v\) vertices connected by \(3(N_v - 2)\) tethers. The self-avoidance of the surface is ensured by assigning a hard core spherical bead of diameter \(\sigma\) to each vertex and restricting the maximal tether length to \(\sqrt{3}\sigma\). Membrane fluidity is maintained by dynamically altering the triangulation by flipping the bonds. This model has been used extensively to study shapes and fluctuations of vesicles.\textsuperscript{23–25}

In the triangulated surface model, each vertex represents a patch of the membrane. The local surface charge density is set by assigning a charge \(-q\) to a fraction of the vertices. When the charge neutrality of the system is explicitly maintained, there are equal number of macromolecules (CIs) with charge \(+q\) in the simulation box. For simplicity, we set the size of the macromolecules to be the same as that of membrane vertices. This is set by a hardsphere interaction and will ensure that CIs do not penetrate into the vesicle. The CIs reside and diffuse in the bulk solution surrounding the membrane and can bind/unbind to the membrane surface. Unless otherwise mentioned, interactions between the various charges are assumed to be through a bare Coulomb potential in a medium with uniform dielectric constant.

The total Hamiltonian of the charged vesicle, with explicit macromolecules in the medium around, is given by

\[
\beta \mathcal{H} = \frac{k}{2} \sum_i \left( H_i - \mathcal{C}_0(i) \right)^2 a_i + A \sum_{l>k} s_i s_k - \Delta p V. \tag{1}
\]

Here, \(k_B T = 1/\beta\) is used as the unit of energy and all length are in units of \(\sigma\) which is set as typical lipid bilayer thickness. The first part in the above equation is the discretized form of Helfrich elastic energy, where \(\kappa\) is the bending stiffness in units of \(k_B T\), \(a_i\) is the area of vertex \(i\), and the summation is over all the vertices. \(H_i\) is the mean curvature and \(\mathcal{C}_0(i)\) is the spontaneous curvature of the membrane vertex \(i\). The second part of Eq. (1) is the electrostatic contribution, where \(A = \frac{\sigma^2}{\varepsilon_0 T} \) is the electrostatic coupling constant and \(s_i\) and \(s_k\) represent the sign of the charges \(l\) and \(k\), and the sum is over all pairs of charges in the system, i.e., both the charges at the vertices and the CIs in solution. The net charge at any vertex or on a CI is \(q = ze\), where \(z\) is the valency of the ions. The third term corresponds to the contribution from osmotic pressure where \(\Delta p = p_{\text{in}} - p_{\text{out}}\) is the pressure difference between inside and outside of the vesicle and \(V\) is the volume of the vesicle.

The spontaneous curvature \(\mathcal{C}_0\) at any vertex is induced by the presence of CI and is nonzero only when a CI is bound to that vertex. This sets the explicit coupling between electrostatic interactions and membrane curvature, which is modeled as follows. When the CI distance to a charged vertex is below a fixed distance (set to be 1.1\(\sigma\)), that CI is considered as condensed and the spontaneous curvature at the vertex is then set to \(\mathcal{C}_0\).

In the following discussions, we consider a system with \(\sigma = 1\) and \(N_v = 1202\). The simulations are carried out for a

FIG. 1. Snapshots of the equilibrium configurations of a charged vesicle with \(\mathcal{C}_0 = 0\), \(\Delta p = 0\), at different values of electrostatic constant. The shape of the vesicle changes from quasi-spherical (at \(A = 0\)) to dumbbell (from \(A = 0.2\) to 8) and then back to quasi-spherical (at \(A > 8\)). In the snapshots shown above, red color represents charged vertices which are not bound to CI and green color for vertices with condensed CIs. CIs are not shown for clarity.
membrane with fixed spherical topology, with 50% of vertices charged and fixed osmotic pressure $\Delta p$. The vesicle-macroion system is bounded by a cubic box having a fixed length $L = 58$. Periodic boundary conditions are applied in all directions, $\kappa$ is chosen to be 10, which is comparable with that of lipid membranes.

One Monte Carlo Step (MCS) involves $N_v$ attempts to change the position of the vertices, $N_L$ attempts for flipping of bonds, and $N_c$ attempts to change the position of macroions, where $N_c$ is the number of CIs. In a vertex move, a vertex is chosen randomly and its position is updated by displacing it to a randomly chosen position within a 3D box centered at its current position without changing the triangulation. The attempted moves are accepted according to Metropolis scheme. In the bond flip move, a randomly chosen bond is flipped to connect the previously unconnected vertices.

$\Delta p = -0.001 \quad \Delta p = -0.003 \quad \Delta p = -0.05 \quad \Delta p = -0.1$

$A = 0.5$

$A = 2$

$A = 5$

$c_0 = 0.1 \quad c_0 = 0.3 \quad c_0 = 0.5$

FIG. 2. A comparison of charged and uncharged vesicles with explicit deflating pressure. Panel (a) represents uncharged vesicles and Panel (b) represents the charged vesicle with $A = 1$, without CIs.

FIG. 3. Equilibrium vesicle configurations at $\Delta p = 0$ and $c_0 > 0$. As a function of $c_0$ and $A$, the vesicle transforms from a beaded tubular conformation to branched structures.
of its adjacent triangles. Such flipping of links changes the neighborhood of vertices and hence the triangulation of surface.

Unscreened electrostatic interactions used here require summation over a large number of periodic boxes to converge. We use the standard Ewald summation technique\(^{26}\) to achieve this. However, in the regime where the electrostatics is strong and large number of CIs condensing on the membrane surface, we find that the summation over minimum image is sufficient to get comparable results.

### III. RESULTS AND DISCUSSIONS

#### A. Vesicle without induced curvature

The Monte Carlo procedure followed here allows us to find the equilibrium/long-lived configurations of fluid membranes, that correspond to a minimum in the free energy landscape, for the given set of parameters. In Fig. 1, the change in the equilibrium shape of the vesicle as a function of the electrostatic coupling parameter \(A\), when the curvature coupling parameter \(C_0 = 0\), is shown. We choose the elastic parameters such that when there are no electrostatic interactions, i.e., when \(A = 0\), the vesicle is quasi-spherical. On increasing the value of \(A\), the vesicle first transforms to a dumbbell shape. As can be seen from Fig. 4(a), there is very little macroion condensation at this point. On further increase of \(A\), the macroions in the box begin to condense on the vesicle surface and the vesicle begins to swell again. When \(A = 10\), a large fraction of the macroions are condensed and the shape transforms back to quasi-spherical.

The observed change in the conformations of the vesicle, even in the absence of any explicit coupling between the curvature and electrostatics, is driven by the delicate interplay of electrostatics and membrane elasticity, involving the osmotic pressure induced by the macroions, electrostatic interaction between the surface charges, and curvature elasticity of the flexible membrane. In order to understand the effect of ion pressure on the surface better, we studied the phase diagram of a charged vesicle with explicit pressure and no CIs. Fig. 2 compares the effect of a deflating pressure in a uncharged and charged vesicle. The effect of pressure on an uncharged vesicle has been explored previously,\(^{19,20}\) where a negative pressure changes vesicle shape from quasi-spherical to disc and then to surface with inner layers, see Fig. 2(a). The conformations of the charged vesicles are different from that of their uncharged counterpart and tend to elongate upon reduction in the pressure inside, which is in agreement with previous studies of charged membranes.\(^{17,27}\) From Fig. 2(b), it is clear that a pressure difference of \(\Delta p = -0.003\) is sufficient to induce shape transformations in charged vesicles.

For the charged vesicle-CI system shown in Fig. 1, the ideal gas pressure due to CIs is maximum at \(A = 0\). As \(A\) increases CIs condense and as a result, pressure decreases. The pressure contribution from CIs when no ions are condensed is \(\sim -0.003\), which was earlier shown to be the pressure required for shape changes in charged vesicles. \(A = 0\) being an uncharged vesicle needs higher pressure to deform. In the strong coupling limit \(A > 8\), most of the ions are condensed and there is considerable reduction in the pressure; hence, the vesicle shape remains quasi-spherical.

At this point, a comparison of the charge densities considered here and that in physiological conditions may be relevant. If we assume the dielectric constant to be the same as that of water and vertex diameter to be \(\sigma = 5\) nm, the parameter \(A = 0.2\) to 10 will correspond to a valency of ions \(z = 1.2\) to 8.5. Hence, for a vesicle with equilateral triangles of side \(\sqrt{3}\sigma\), the values of \(A\) correspond to the variation of surface charge density from \(\sim 0.15\) to 1.1 \(\mu\)C/cm\(^2\). Similarly, the range of concentration of ions in the box can be obtained as \(-0.05\) to 0.35 mM. In the physiological conditions of surface.

![FIG. 4. (a) Percentage of condensed CIs as a function of \(A\) and \(C_0\). Condensation number increases with both \(A\) and \(C_0\). (b) Probability of CI binding at different mean curvatures at \(A = 1\). At each value of \(C_0\), there is a preference for association at vertices with particular mean curvature. The inset shows the distribution of charged vertices without condensed CIs as a function of mean curvature. (c) \(\sigma_{CI}\): Variance of CI condensation number/mean condensation number as a function of \(A\) and \(C_0\). \(\sigma_{CI}\) is decreasing with both \(A\) and \(C_0\).](image-url)
conditions, surface charge density of organelle membranes varies from 0.2 µC/cm² to 5.0 µC/cm², which indicates a higher range of charged density compared to the values considered here. Similarly, the concentration of the CIs in the solution considered here is lower than in physiological conditions; the concentrations of aminoacids from proteins are known to be greater than 100mM in cytosol. However, at the physiological conditions, the electrostatic interactions are heavily screened and interactions discussed in Sec. III D alone will be relevant.

**B. Vesicle with CI induced curvature**

The change in the equilibrium conformations of a charged vesicle due to the change in values of the coupling parameters $C_0$ and $A$ is shown in Fig. 3. The equilibrium configurations of the vesicle with no electrostatic interactions (see Fig. 1, $A = 0$ case) have a quasi-spherical shape with radius $\sim 12$, which implies an average curvature $\sim 0.08$. We see substantial change in the shape of the vesicle even for small values of the induced curvature, $C_0 > 0.08$. When $A = 0.5$ and $C_0 = 0.1$, we observe beaded tubular conformations (see top panel in Fig. 3), which are significantly different from the dumbbell shape seen when $C_0 = 0$, though the number of condensed CI here is as small as 5%. As $C_0$ is increased, we see this shape changing to a more uniform tube. Increasing the electrostatic interaction strength $A$ leads to branching for all nonzero values of $C_0$. Vesicle shapes up to $A = 5$ are shown in Fig. 3. When $5 < A \leq 10$, also the vesicle equilibrates to similar branched conformations. Thus, we see that curvature induced by CIs has a strong influence on the vesicle shape, with even small deviation in the preferred curvature of the CIs leading to large transformations in the overall vesicle shape.

The configuration of the membrane in this regime is controlled by the number of condensed macroions, through the spontaneous curvature that they induce, and the electrostatic interaction strength. It is important to note that the number of condensed CIs itself increases with $A$ and $C_0$. In Fig. 4(a), we see that the number of ions condensed as a function of $A$ increases and saturates to a value which is a function of $C_0$. While the increase in the number of condensed CIs with $A$ is expected, we also see an increase with the spontaneous curvature. The enhancement in the local membrane curvature on CI condensation leading to an increase in the local electric field and increase in CI’s accessibility to vertices is the primary reason for the increase in CI condensation with $C_0$. This would also mean that negative value for $C_0$ will create craters on the surface and limit the accessibility of CIs to nearby vertices, resulting in a lower number of condensed CIs (this has been checked for a vesicle with lower number of vertices).

To understand the curvature dependence of CI association, we looked at the curvatures at the vertices where binding of CIs happens, at equilibrium. Fig. 4(b) shows the distribution of binding at different mean curvatures. It is clear from the figure that at each $C_0$, the CI association has a preference for vertices with a particular curvature. It has been also observed that the preferred curvature for binding is independent of the electrostatic constant $A$ for a given value of $C_0$. The inset to Fig. 4(b) shows the charged vertex distribution on curvatures to make sure that the average mean curvatures at charged vertices are not the reason for the peaks in the association. This curvature selection, which is similar to the curvature...
sensing mechanism shown by many membrane proteins, results from a combination of the gain in elastic energy through the term \((H(i) - C_0(i))^2a_i\), increased accessibility of macroions to the outward curved regions and larger electric field around highly curved regions.

Another feature of the charged vesicles is the fluctuation in the bound CI number. Since the mean number of condensed CIs, \(\text{N}_{\text{mean condensed}}\), is a function of \(A\) and \(C_0\), we use the variance in the number of condensed CIs divided by its mean, \(\sigma_{CI} = \frac{1}{\text{N}_{\text{mean condensed}}} \sum_i (\text{N}_{\text{condensed}} - \text{N}_{\text{mean condensed}})^2\), to characterize this fluctuation. Fig. 4(c) shows \(\sigma_{CI}\) as a function of \(A\). As \(A\) increases, \(\sigma_{CI}\) dips, indicating tighter electrostatic binding of the ions to the membrane surface. The detachment/dissociation of a condensed CI from the region of high curvature leads to a higher penalty on the bending energy.

An increase in \(A\) will increase the condensation number which means that the membrane will have more vertices with induced curvature. This implies that the total mean curvature \((C_{\text{mean}} = \sum_i H_i)\) is a function of \(A\) and \(C_0\), see Fig. 5(a). The maximum area of the vesicle is constrained by the allowed bond length fluctuation, which means an increase in mean curvature will lead to a decrease in the volume, see Fig. 5(b). This can also be seen from Fig. 5(b) inset, where the vesicle shapes are quantified by the reduced volume \(v\), defined as \(v = \frac{1}{4\pi R_s^3}\), where \(V\) is the volume of vesicle and \(R_s\) is the radius of a sphere with the same surface area as that of the vesicle.

In this section, we showed that local curvature generation associated with binding of CIs is another contribution to the tubulation (elongation) phenomena described in Sec. III A, as it introduces a new length scale \(C_0\) to the curvature of the membrane. In addition, it promotes branching of tubular shapes which may be caused by destabilization of membranes’ structures by diffusing or fluctuating local curvatures. It should be noted here that a value of spontaneous curvature \(C_0 = 0.1\) would correspond to a curvature 0.02 nm\(^{-1}\) when we set \(\sigma = 5\) nm. Hence, \(C_0\) used in the simulations can be compared to the curvatures generated by proteins consisting of N-BAR domains and Epsin N-terminal homology (ENTH) domains which is in a range of 0.02–0.07 nm\(^{-1}\).

C. Effect of pressure

So far we looked at cases where the vesicle volume is allowed to change without any energy cost, within the maximum allowed volume for a given surface area. We will now study the equilibrium shapes of charged vesicle, in the presence of curvature inducing mobiles ions in solution, when an explicit pressure difference \(\Delta p\) is applied. Effects of inflating \((\Delta p > 0)\) and deflating \((\Delta p < 0)\) pressures are considered. Equilibrium conformations at different values of \(\Delta p\) are shown in Fig. 6. From the figure, it is clear that shape deformations induced by \(A\) and \(C_0\) are significant only below a certain pressure difference \(\Delta p\), which we find to be 0.025 for \(C_0 = 0.3\) and \(A = 2\).

Fig. 7 shows that a decrease in pressure below \(\Delta p = 0\) increases the CI condensation, indicating that deformability of membrane enhances the binding of macroions. As \(\Delta p\)}
increases, the ability of CIs to induce curvature reduces, which in turn reduces the accessibility of CIs to the charged surface, and hence, CI condensation decreases. Understandably, this effect of $\Delta p$ is more prominent at higher $C_0$ values. This implies that, even in the presence of curvature inducing macroions, the deformations in the vesicle can be controlled by applying an inflating osmotic pressure, and this pressure will depend on $C_0$ and $A$.

D. Screened Coulomb interactions

The presence of salt is another factor that can alter the interaction between curvature inducing macroions in solution and the charged membrane. Presence of highly mobile ions of dissociated salt induces electrostatic screening and alters the interaction between charges on the membrane surface and the macroions in the solution. We assume uniform distribution of ions around all the charges and incorporate salt effects by replacing the bare Coulomb interaction between charges with an effective interaction of the Debye-Huckel form,

$$\mathcal{H}_{\text{electrostatic}} = A \sum_{i>j} s_i s_j e^{-\frac{r_{ij}}{\lambda}}$$

(2)

where $\lambda$ is the Debye screening length which depends inversely on the square root of the salt concentration. We assume free passage of salt through the membrane so that there is no osmotic pressure induced by salt. Fig. 8 shows the equilibrium shapes of the vesicle with increasing $\lambda$. At high $\lambda$, shapes are similar to the ones obtained using bare Coulomb interactions. Similar trends are also seen in the percentage of condensed ions as a function of $\lambda$, shown in Fig. 9. For small values $\lambda$ (high salt), the condensation number is small even at high values of $A$. The condensation number increases monotonically as a function of $\lambda$ saturating at its salt free value for all values of $A$, see Fig. 4(a) ($C_0 = 0.3$) and Fig. 7 ($\Delta p = 0$). However, the $\lambda$ at which the condensation number saturates is strongly reduced with $A$.

E. A comparison to non-equilibrium binding

In the current simulation, we have looked at the equilibrium conformations of the vesicle with curvature inducing macroions (CIs) binding on the vesicle through electrostatic interactions. In a recent work, it has been shown that a non-equilibrium stochastic binding/unbinding of curvature inducing components can induce large scale

![FIG. 9. Condensation number as a function of screening length at $C_0 = 0.3$ and $A = 0.5, 2,$ and 5. As $\lambda$ increases, the condensation number increases and becomes equal to that seen with bare Coulomb interactions.](image1)

![FIG. 10. Vesicle configurations with electrostatic and non-equilibrium binding of curvature inducing components at $C_0 = 0.3$ and $\Delta p = 0$. Panel (a) shows uncharged vesicle and (b) vesicle with surface charges. The condensation number and binding rate obtained for each electrostatic constant in (b) are used as curvature inducing parameters for corresponding configurations in (a). Green color represents vertices with $C_0$.](image2)
deformation of membranes resulting in a variety of steady state shapes. Here, we compare these two cases by choosing the $C_0$ induced by CIs in the charged vesicle to be the same as that of the curvature inducing active components in the non-equilibrium binding study. The parameters for non-equilibrium simulations are the mean number of vertices with induced curvature and the rate of inducing/disappearing curvature. These parameters are mapped to the average number of condensed CIs and the binding/unbinding rate of CIs, respectively. The observed conformations are compared in Fig. 10.

It is interesting to note that both cases show a deflating effect on the vesicle, as a result of binding of curvature inducing components. However, there is a significant difference with nonequilibrium binding inducing spherical budding with neck like shapes and equilibrium charged vesicle forming tubes with branches.

IV. SUMMARY AND CONCLUSIONS

We have studied the effect of counterion condensation, counterion induced pressure, counterion induced local curvature, and salt on the conformational properties of charged vesicles with spherical topology by use of a triangulated random surface model. Overall, we find that all these contributions have significant effect on the equilibrium shapes of charged membranes. Although, our results indicate that these effects are interrelated in a complex way, we have also revealed some mechanisms which may simplify the description.

For a semi-flexible, charged membrane, the surface shape tends to elongate and form tube-like structures. This feature is strongly enhanced if the membrane is subject to a deflating pressure. If non-penetrable CIs are present ambient to the vesicle, the surface charge is partly neutralized by CI condensation, while the remaining CI contributes to a deflating electrostatic pressure. Furthermore, a similar destabilization of the vesicle shape into elongated, tubular structures is found for local curvature inducing CIs. These tubular membranes show substantial branching, enhanced CI condensation with accompanying decrease in condensed CI number fluctuations. These results also hold at low salt content. However, as salt concentration is increased, a distinct crossover into a high salt regime takes place, where the effects of surface charge are fading away.

With these computer simulation results, we have uncovered some of the phenomena and building blocks which must be included for a comprehensive description of semi-flexible charged membranes. For biological membranes, the results show that local electrostatics can have profound effects on the membrane conformations in multiple ways available for cellular membrane remodeling processes.