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Binding of oppositely charged membranes and membrane reorganization

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Abstract. We consider the electrostatic interaction between two rigid membranes, with different surface charge densities of opposite sign, across an aqueous solution without added salt. Exact solutions to the nonlinear Poisson-Boltzmann equation are obtained and their physical meaning discussed. We also calculate the electrostatic contribution to the free energy and discuss the renormalization of the area per head group of the charged lipids arising from the Coulomb interaction.

PACS. 82.65.Dp Thermodynamics of surfaces and interfaces

1 Introduction

Electrostatics often plays an important role in determining the structure of macromolecules in aqueous solution, *e.g.* polyelectrolytes, charged membranes, and charged colloidal particles. For many physical systems, where charge densities on the surfaces are equal and of the same sign, the Poisson-Boltzmann equation provides quantitative descriptions of their electrostatic interactions [1]. However, many biological processes involve charge densities on the surfaces that are not equal and sometimes even have opposite sign [2]. Examples of this situation include protein association with DNA and membranes; the interaction between cationic liposomes and negatively charged cell membranes [3]. It also has significant biotechnological importance for the study of DNA association with artificial cationic liposomes [4]. In a recent experimental work on two oppositely charged membranes [5], it is found that the membrane, due to its fluidity, adjusts its area per head in response to the electrostatic interaction. Similar conclusion has been reached by Radler *et al.* [6] on DNA-cationic liposome complexes. Motivated by these experiments, in the present paper we study a model of two oppositely charged membranes in electrostatic interaction and quantify how the area per head renormalizes by using the Schulman-Montagne condition [7] for membrane self-assembly.

We consider a system composed of two oppositely charged rigid membranes [8] separated by a distance L across an aqueous solution with dielectric constant ϵ (see Fig. 1). One carries positively charged lipids with magnitude q per head group and area per head Σ_+. The other membrane is composed of negatively charged lipids of charge $-q$ with Σ_- area per head. Without loss of generality, we assume here that $\Sigma_-^{-1} > \Sigma_+^{-1}$. We imagine that the two membranes, each with its own counterions which we assume to be monovalent, initially infinitely far apart are brought into the vicinity of each other, where the electrostatic interaction dominates other interactions. Since the counterions can gain entropy by escaping to infinity, we only consider the positive counterions that are required to neutralize the system [9]. We also assume that there is no salt in the solution in to order to focus on the fundamental effects of the electrostatic interaction (see Conclusion

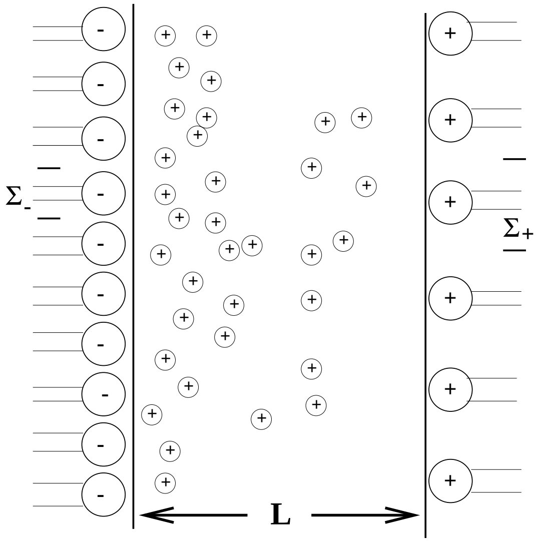

Fig. 1. Geometry of the problem: two membranes of unequal opposite charge, separated by a distance L with counterions between them.

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for a discussion of the effects of added salt.) The charged lipids in the membrane are modeled as a fluid with an effective surface tension γ, which typically has a value of $\sim 0.04\, k_{\rm B}T/\text{Å}^2$ for bilayers; thus in addition to the electrostatic energy, there is a surface free energy of the form $\beta F_{\rm s}/N = \gamma\, \Sigma$ for each membrane, where N is the number of lipids, $\beta = 1/k_{\rm B}T$, T is the temperature, and $k_{\rm B}$ is the Boltzmann constant. Note that energies are expressed in units of $k_{\rm B}T$. We are interested in the values of $\Sigma_\pm^*$ which minimize the total free energy – surface and electrostatic energy – as a function of L and γ.

To illustrate the effect of the electrostatic interaction on the structure of membranes, let us consider the simplest case in which $\Sigma_+ = \Sigma_- = \Sigma$; in this situation there are no counterions between the charged membranes. The electrostatic contribution to the free energy can be calculated using Gauss' law to give [10]

$$\beta F_{\rm el} = N\left(\frac{2\pi l_{\rm B} L}{\Sigma}\right), \tag{1}$$

where $l_{\rm B} \equiv q^2/\epsilon k_{\rm B}T \approx 7$ Å, is the Bjerrum length for an aqueous solution of dielectric constant $\epsilon = 80$ and N is the total number of lipids in the membrane. Minimizing $F_{\rm s} + F_{\rm el}$ with respect to Σ, we find $\Sigma^* = (\pi l_{\rm B} L/\gamma)^{1/2}$. Hence, the optimal area per head group depends on the square root of the distance separating the two membranes, and for a separation of $\sim$ 10 Å, we find $\Sigma^* \sim 75$ Å^2. In contrast, the electrostatic free energy for an isolated charged membrane with its own counterions is given by [11]

$$\beta F_{\rm el} \cong -2N \ln \Sigma, \tag{2}$$

to within an additive constant. Therefore, $\Sigma^* = 2/\gamma \sim 50$ Å^2. Thus, the optimal area per molecule with electrostatic interactions may deviate substantially from that of an isolated charged membrane. Note that while the order of magnitude is the same in both cases, the functional dependences on the parameters are quite distinct.

In order to take the counterions into account for unequally charged membranes, let us suppose that the negatively charged membrane is located at $x = 0$ and the positively charged one at $x = L$. One constraint is that of charge neutrality – the number of counterions must be equal to the difference between the number of molecules on the two membranes:

$$\int_0^L n(x){\rm d}x = \frac{1}{\Sigma_-} - \frac{1}{\Sigma_+}, \tag{3}$$

where $n(x)$ is the counterion density which is, in the mean field approximation, related to the potential ϕ by the Boltzmann factor:

$$n(x) = n_0\, {\rm e}^{-\beta q \phi(x)}, \tag{4}$$

where q is the unit of charge and the prefactor n_0 is fixed by equation (3). Combining equation (4) with the Poisson equation from electrostatics

$$-\nabla^2 \phi(x) = \frac{4\pi q}{\epsilon}\, n(x), \tag{5}$$

we arrive at the Poisson-Boltzmann (PB) equation:

$$-\frac{{\rm d}^2\psi}{{\rm d}x^2} = 4\pi l_{\rm B} n_0\, {\rm e}^{-\psi}, \tag{6}$$

where we have defined $\psi(x) \equiv \beta\, q\, \phi(x)$.

Equation (6) encapsulates a mean field approach to the many-body problem. It assumes that the counterions are point-like and collectively generate an average potential $\phi(x)$ which governs how the counterions are themselves distributed. Furthermore, the PB equation, which neglects correlations among counterions, is valid only for sufficiently high temperatures or low surface charge densities [1,12]. Therefore, within the mean-field approximation, our task is to solve the PB equation subject to the boundary conditions:

$$\left.\frac{{\rm d}\psi}{{\rm d}x}\right|_0 = +\frac{4\pi l_{\rm B}}{\Sigma_-} \tag{7}$$

and

$$\left.\frac{{\rm d}\psi}{{\rm d}x}\right|_L = +\frac{4\pi l_{\rm B}}{\Sigma_+}\,\cdot \tag{8}$$

In the next section, we present the solutions to equation (6) and discuss the equilibrium configurations of the counterions. In Section 3 we analyze the electrostatic contribution to the free energy and pressure of the system. In Section 4, the phase diagram of the system is presented and followed by a discussion of the equilibrium value of the area per head.

2 Nonlinear Poisson-Boltzmann solutions

Equation (6) can be solved using the "energy" method of classical mechanics, where we obtain a useful constant of motion, defined by

$$E \equiv \left(\frac{{\rm d}\psi}{{\rm d}x}\right)^2 - 8\pi l_{\rm B} n_0 {\rm e}^{-\psi(x)}. \tag{9}$$

This constant can be physically interpreted as being proportional to the difference between the electrostatic stress and thermal pressure of the counterions ($\sim n(x) k_{\rm B}T$). There are three cases to consider: i) $E > 0$, ii) $E = 0$, iii) $E < 0$.

For $E > 0$, we have for the normalized potential

$$\psi(x) = \ln\left[\frac{8\pi l_{\rm B} n_0}{E} \sinh^2\left(\frac{\sqrt{E}}{2}(x - x')\right)\right] \tag{10}$$

and the counterion distribution

$$n(x) = \frac{E}{8\pi l_{\rm B} \sinh^2(\frac{\sqrt{E}(x-x')}{2})}\,\cdot \tag{11}$$

We choose the normalized potential to be zero at $x = 0$. This determines the value of $n_0 = n(0)$. Using the boundary conditions, one can show that the counterion densities at the surface of the membranes are given by:

$$n(0) = \frac{2\pi l_{\mathrm{B}}}{\Sigma_-^2} - \frac{E}{8\pi l_{\mathrm{B}}} \tag{12}$$

and

$$n(L) = \frac{2\pi l_{\mathrm{B}}}{\Sigma_+^2} - \frac{E}{8\pi l_{\mathrm{B}}}, \tag{13}$$

where E satisfies

$$E = \frac{(4\pi l_{\mathrm{B}})^2}{\Sigma_+\Sigma_-} - \frac{4\pi l_{\mathrm{B}}(\Sigma_+ - \Sigma -)}{\Sigma_+\Sigma_-}\sqrt{E}\coth\frac{\sqrt{E}L}{2}\,\cdot \tag{14}$$

For $E = 0$, we have for the normalized potential

$$\psi(x) = \ln\left[2\pi l_{\mathrm{B}} n_0 (x - x')^2\right] \tag{15}$$

and the counterion distribution

$$n(x) = \frac{1}{2\pi l_{\mathrm{B}}}(x - x')^{-2}. \tag{16}$$

The boundary conditions determine the value of L:

$$L = \frac{\Sigma_+ - \Sigma_-}{2\pi l_{\mathrm{B}}}\,\cdot \tag{17}$$

The density at each surface can be obtained using equations (12, 13) by setting $E = 0$.

For $E = -E_- < 0$, we have for the normalized potential

$$\psi(x) = \ln\left[\frac{8\pi l_{\mathrm{B}} n_0}{E_-}\cos^2\left(\frac{\sqrt{E_-}}{2}(x - x')\right)\right] \tag{18}$$

and counterion density

$$n(x) = \frac{E_-}{8\pi l_{\mathrm{B}}}\sec^2\frac{\sqrt{E_-}(x - x')}{2}\,\cdot \tag{19}$$

The boundary conditions give

$$E_- = \frac{4\pi l_{\mathrm{B}}(\Sigma_+ - \Sigma_-)}{\Sigma_+\Sigma_-}\sqrt{E_-}\cot\frac{\sqrt{E_-}L}{2} - \frac{(4\pi l_{\mathrm{B}})^2}{\Sigma_+\Sigma_-}\,\cdot \tag{20}$$

Equations (12, 13) are still valid with the replacement $E \to -E_-$.

We observe that for the dilute counterion limit, $E > 0$, the equilibrium density distribution is essentially exponential. However, as the counterion density increases, the collective effect of mutual repulsion of the counterions leads to a $\sec^2$ dependence of the counterion distribution as shown in Figure 2. Note that even for small separations, the counterions density is not uniform, in contrast to the case of electric double layers of like charges.

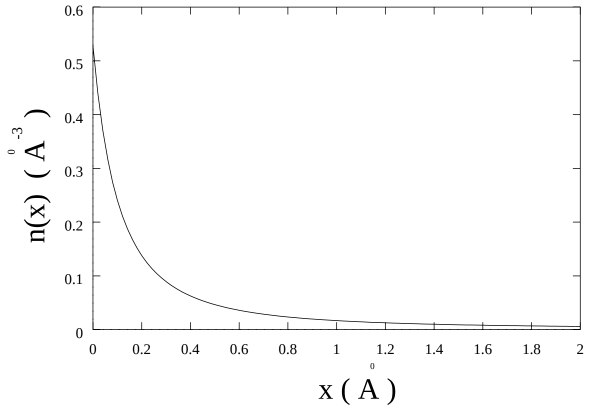

Fig. 2. The counterion distribution for the case $E < 0$ given by equation (19). It is not uniform for small distance in contrast to the case of two electric double layers of equal charge density of the same sign.

3 The electrostatic free energy and pressure

The electrostatic free energy per unit area, which is the sum of the electrostatic energy and the entropy of the counterions, can be written as

$$\beta f_{\mathrm{el}} = \int_0^L \mathrm{d}x \left\{\frac{E}{8\pi l_{\mathrm{B}}} - n(x)\,\left[\,\psi(x) - \ln(n_0 v_0)\,\right]\right\}, \tag{21}$$

up to an additive constant, where v_0 is the volume per counterion. From equation (21) or using a known expression for the pressure [12],

$$\beta P = n(0) - (\nabla\psi)^2/8\pi l_{\mathrm{B}},$$

we obtain

$$P = -\frac{k_{\mathrm{B}}T}{8\pi l_{\mathrm{B}}}E. \tag{22}$$

Here we have the simple result that the pressure is proportional to $-E$. Hence the solutions with $E > 0$ and $E < 0$ describe membranes that attract and repel each other, respectively. For the solution of $E = 0$, the membranes exert no net force on each other. Figure 3 shows how the pressure varies with the distance. Using equation (17), we can determine the equilibrium distance L^*:

$$L^* = \frac{\Sigma_+ - \Sigma_-}{2\pi l_{\mathrm{B}}}\cdot$$

Therefore, the result in equation (22) leads to the following picture. For large separation, $L > L^*$, the counterion concentration is dilute and the electrostatic attraction dominates. On the other hand, the counterions are dense when the separation is small, $L < L^*$. Hence thermal pressure dominates. When $L = L^*$ the electrostatic and thermal pressure balance, leaving zero net pressure. Similar results have also been obtained from numerical solutions to the PB equation for other systems [13]. We note that since $\partial^2 f_{\mathrm{el}}/\partial L^2\big|_{L^*} > 0$ as suggested in Figure 3, the system is in a stable equilibrium at $L = L^*$.

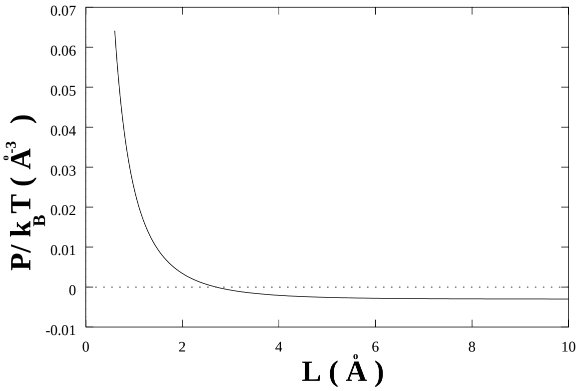

Fig. 3. A plot of pressure *vs.* separation distance for the case of $\mathit{\Delta} = 0.1$ and $\mathit{\Sigma}_+ = 130$ Å^2 from equation (22).

Upon explicit evaluation of equation (21) and multiplying by the area of the membrane, the electrostatic free energy becomes:

$$\beta F_{\rm el}/N_+ = -y(z)^2 z - 2\,(\mathit{\Delta}^{-1}-1)\ln z + \mathit{\Delta}^{-1}\ln\left[1-(\mathit{\Delta}\,z\,y(z))^2\right] - \ln\left[1-(zy(z))^2\right], \tag{23}$$

where $y_\pm \equiv \frac{\sqrt{E_\pm}L}{2}$, $z \equiv \frac{\mathit{\Sigma}_+}{2\pi l_{\rm B} L}$, and $\mathit{\Delta} \equiv \frac{N_+}{N_-}$. The function $y(z)$ is defined as

$$y(z) = \begin{cases} y_+ & \text{if } 0 < z < (1-\mathit{\Delta})^{-1} \\ 0 & z = (1-\mathit{\Delta})^{-1} \\ {\rm i}y_- & z > (1-\mathit{\Delta})^{-1} \;. \end{cases} \tag{24}$$

Note also that $y(z) \neq 0$ satisfies

$$zy(z) = \tanh[y(z)] + \mathit{\Delta}\,z\,y(z)\;(1 - z\,y(z)\,\tanh[y(z)])\,. \tag{25}$$

In deriving equation (23), we have made an implicit assumption that the areas of the two membranes are the same, namely, $N_+\mathit{\Sigma}_+ = N_-\mathit{\Sigma}_-$. The validity of this assumption is justified, since the edge effect, which arises when the areas of the two membranes are different, adds to equation (23) a correction term of order $O(A^{-2})$. Therefore, in the limit of large surface areas, this contribution is negligible. Note that this assumption implies that the ratio $\mathit{\Delta} \equiv \mathit{\Sigma}_-/\mathit{\Sigma}_+$ is fixed. Physically, the asymmetric parameter $\mathit{\Delta}$ gives the ratio between the number of particles in each membrane. Equations (23, 24, 25) are the final results from which the equilibrium properties are derived in the next section.

4 Equilibrium properties

In this section, we determine the equilibrium value of $\mathit{\Sigma}_+$, using equation (23) for the electrostatic contribution to the total free energy, which is given by

$$\beta F_{\rm tot}/N_+ = 2\gamma\mathit{\Sigma}_+ + \beta F_{\rm el}[L, \mathit{\Sigma}_+; \mathit{\Delta}]/N_+, \tag{26}$$

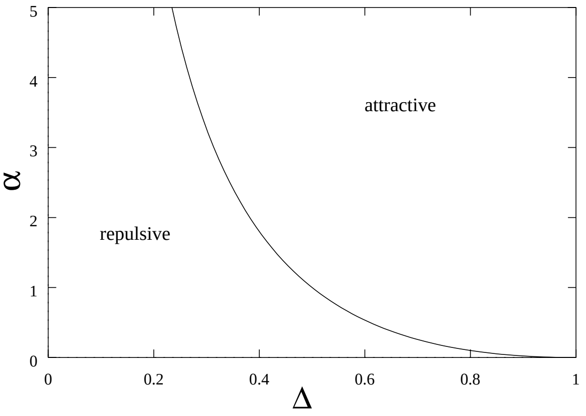

Fig. 4. The phase diagram as determined by equation (28): $\alpha \equiv 4\pi l_{\rm B} L\gamma$ *vs.* $\mathit{\Delta}$. The region above the line is the attractive region of the membranes and the region below repulsive.

where γ is the surface tension. Let us first consider the case in which the system is at equilibrium ($P = 0$). By setting $y(z) = 0$ in equation (23), it can be shown that the optimal area per head group is given by

$$\mathit{\Sigma}_+^* = \frac{\mathit{\Delta}^{-1}-1}{\gamma}, \tag{27}$$

and the equilibrium distance can be calculated using equation (17) to give

$$L^* = \gamma^{-1}\,\frac{(1-\mathit{\Delta})^2}{2\pi l_{\rm B}\,\mathit{\Delta}}\,\cdot \tag{28}$$

Note that we must restrict $\mathit{\Delta}$ to the range of $0 \le \mathit{\Delta} < 1$, since the case of $\mathit{\Delta} = 1$ corresponds to zero counterion density where the membranes attract for all separations. For the case of $\mathit{\Delta} = 0.1$, we find $\mathit{\Sigma}_+^* \sim 200$ Å^2 and $L^* \sim 5$ Å. Therefore, the counterions have yet a stronger effect on the stretching of the membrane compared to the single charged membrane case where we have shown $\mathit{\Sigma} \sim 50$ Å^2. According to the discussion of Section 3, equation (28) represents a line which separates the repulsive region and the attractive region in the phase diagram, which is shown in Figure 4.

Next, we consider two membranes separated by a fixed distance. Equation (23) can be regarded as a function of only z with the help of equation (24). Thus, we can imagine solving $y(z)$ in terms of z, and by substituting the result into equation (23), minimization of the free energy can be carried out explicitly. Unfortunately, this cannot be done analytically in general. However, our estimate of L^* above indicates that for $L < L^*$, which is of atomic size, other effects such as thermal fluctuations and van der Waals attraction that have not been taking into account, may become significant. Therefore, we focus on the large separation limit, $L \gg L^*$, where $y(z) \gg 1$. With this approximation, we obtain

$$\mathit{\Sigma}_+^* \simeq \frac{1}{2\mathit{\Delta}\gamma}\left\{1 - \mathit{\Delta} + \left((1-\mathit{\Delta})^2 + 4\pi l_{\rm B}\mathit{\Delta}^2\gamma L\right)^{1/2}\right\}. \tag{29}$$

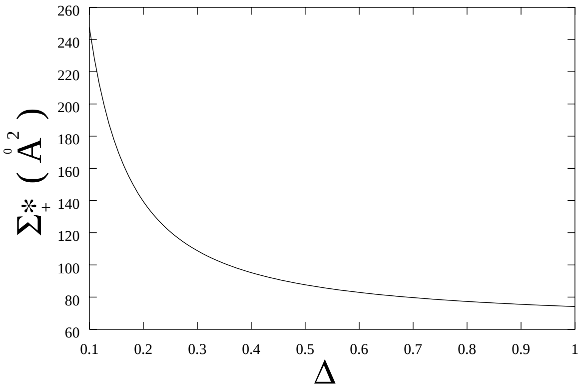

Fig. 5. The optimal value of the area per headgroup Σ_+^* as a function of the charge asymmetric parameter $\varDelta$ for $L = 10$ Å.

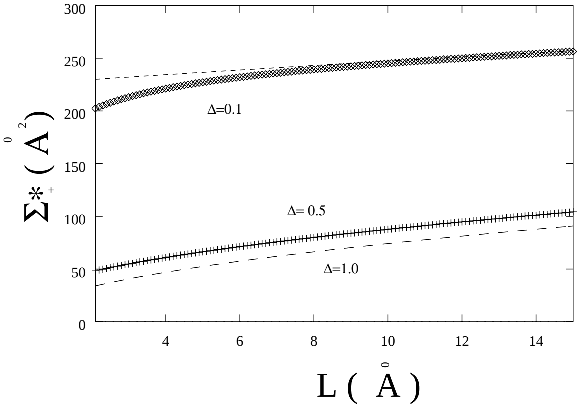

Fig. 6. The optimal value of the area per headgroup Σ_+^* as a function of distance, obtained numerically for $\varDelta = 0.1$, 0.5. The curves are plots of equation (29) for their respective values of $\varDelta$.

Here, we observe that Σ_+^* decreases as $\varDelta \to 1$ monotonically as shown in Figure 5. This is a reflection of the fact that the counterions have a strong effect on the stretching of the membrane. The presence of the counterions enhances the electrostatic energy; therefore, lowering of the charge density is energetically favorable. Hence the stretching of the membrane. For $\varDelta = 0.1$ and $L = 10$ Å, we find $\Sigma_+^* \sim 250$ Å^2 which is five times the value of an isolated charged membrane!

For completeness, we have performed the minimization of the free energy numerically for physically relevant separations ($L > 2$ Å). The results for the cases $\varDelta = 0.1, 0.5$ are shown in Figure 6. We see that equation (29) is indeed not a bad approximation for large distances. Furthermore, we observe that Σ_+^* increases monotonically with distance for all values of $\varDelta$, as also suggested by equation (29). Note that even for $L < L^*$, Σ_+^* bears the same qualitative dependence on $\varDelta$.

5 Conclusion

In this paper, we have considered two asymmetrically charged membranes of opposite sign. The counterion distribution is obtained by solving the PB equation. The membranes can either attract, repel, or exert no force on each other, depending on the difference in the area per head group of the membranes. We have also made an attempt to understand the collective effects of the counterions on the structure of the membranes. Due to the electrostatic interaction of the counterions and the membranes, the stretching of the membranes becomes energetically favorable. In the high counterion concentration limit ($\varDelta \ll 1$) the area per head can reach a value few times as large as an isolated charged membrane. We note here that since the volume of a lipid bilayer is fixed, due to the incompressibility of lipid molecules, the stretching of the membrane implies a decrease in its thickness. This effect may be observed in real experimental settings by *e.g.* X-ray scattering.

Finally, we wish to comment on the relevance of the model studied in this paper to other systems. First, for the case of permeable membranes, where counterions may freely permeate behind the charged membranes, the electrostatic interaction can be shown to be attractive for all separations, since the thermal pressure exerted on the membrane by the counterions is much weaker. In this case, we have verified that this problem is almost identical to our model for the solution $E > 0$. Therefore, the renormalization of area per head group as given by equation (29) should hold at least qualitatively. Secondly, we also consider the presence of a finite amount of salt in our system. This problem may be described at the PB level by an equation similar to equation (6),

$$-\frac{\mathrm{d}^2\psi}{\mathrm{d}x^2} = \kappa^2 \sinh\psi + 4\pi l_{\mathrm{B}} n_0\, \mathrm{e}^{-\psi},$$

where κ is the inverse of the screening length. The above equation can still be solved in principle but involves rather complicated mathematical functions. This is a subject for further study. However, it can be inferred that our model corresponds to the case where the separation between the membranes is small compared to the screening length, *i.e.* $L < \kappa^{-1}$. Typically, for $\kappa^{-1} \sim 20$ Å for 0.05 M of salt, our model should provide a good picture for the binding of oppositely charged membranes for a distance of the order of few Å. For $L \geq \kappa^{-1}$, the electrostatic interaction is exponentially screened. Nevertheless, according to reference [2] even with a finite amount of salt, the *linearized* PB theory also predicts regions of repulsion and attraction between membranes, similar to what we have found here. Therefore, we believe our model captures the essential physics associated with the electrostatic interaction of oppositely charged membranes.

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References

1. J.N. Israelachvili, *Intermolecular and Surface Forces* (Academic Press Inc., San Diego, 1992); B. Honig, A. Nicholls, Science **268**, 1144 (1995).
2. V.A. Parsegian, D. Gingell, Biophys. J. **12**, 1192 (1972).
3. B. Alberts, D. Bray, J. Lewis, *Molecular Biology of the Cell* (Garland Publishing, New York, 1994).
4. R.G. Crystal, Science **270**, 404 (1995).
5. J. Nardi, T. Feder, R. Bruinsma, E. Sackmann, Europhys. Lett. **37**, 371 (1997).
6. J.O. Radler, L. Koltover, T. Salditt, C.R. Safinya, Science **275**, 810 (1997).
7. J.H. Schulman, J.B. Montagne, Ann. N.Y. Acad. Sci. **92**, 366 (1961); P.-G. de Gennes, C. Taupin, J. Phys. Chem. **86**, 2294 (1982).
8. For a discussion on the stiffening of charged membranes, see D. Andelman, in *Handbook of Biological physics*, edited by R. Libowsky, E. Sackman (Elsevier Science Publishers, Amsterdam, 1995) and references therein.
9. It should be pointed out that the justification to this statement could be quite subtle and is an area for further study. However, consider an experimentally relevant scenario where the positively charged membrane is composed of acidic lipids and negatively charged membrane basic. The counterions released by the membranes are H^+ and OH^-. Therefore, assuming the system has had time to equilibrate, the counterions eventually form H_2O upon recombination and can be considered as escaping to infinity.
10. Note that this expression is infinite for infinite separation. Thus, we have to add to this expression a distance dependent term, in association with the entropy gained by the counterions. However, this constant does not play any role in what follows.
11. P. Pincus, in *Phase Transitions in Soft Condensed Matter*, edited by E. Riste, D. Sherrington (Plenum Publishing Corporation, 1989).
12. S.A. Safran, *Statistical Thermodynamics of Surfaces, Interfaces, and Membranes* (Addison-Wesley Publishing Com., Reading, 1994).
13. N. Ben-Tal, B. Honig, R.M. Peitzsch, G. Denisov, S. McLaughlin, Biophys. J. **71**, 561 (1996); N. Ben-Tal, B. Honig, C. Miller, S. McLaughlin, Biophys. J. **73**, 1717 (1997).