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Attraction between similarly charged surfaces  
mediated by extended charges

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# 1 Introduction

Within the theory of the electrical double layer, the electrostatic properties of a system composed of charged surfaces and ionic solution are studied. Development of the electrical double layer theory began at the beginnings of the 20th century, with the intention to understand the stability of colloids and suspensions. Later on it became evident, that the double layer theory is applicable to many different systems and is essential in understanding different chemical and biological processes. Therefore since the beginnings of the theory development much effort has been put into improvement of the original Gouy-Chapman model [1], [2], which is subjected to many simplifications and restrictions.

In this seminar I will present a possible extension of the simplest double layer theory. First a simple model of solving linearized Poisson-Boltzman model for certain boundary conditions accounting for membrane bound protein will be presented. Then the interaction between two flat charged plates in the solution with point-like ions will be presented, followed by a description of a system with divalent spherical counterions where internal charge distribution of the particles will be taken into account. The free energy of such a system will reveal important consequences of taking into account the internal charge distribution of particles. We should bear in mind that in our case this work is aimed towards the understanding of interactions between negatively charged membranes and protein molecules with spatially distributed positive charge. Before we start a very brief presentation of the experiments will be done to see where the ideas for such a theoretical research came from.

## 2 Experiment

The adhesion of giant phospholipid vesicles in the presence of certain proteins and/or antibodies (Fig. 1) had been observed [3].

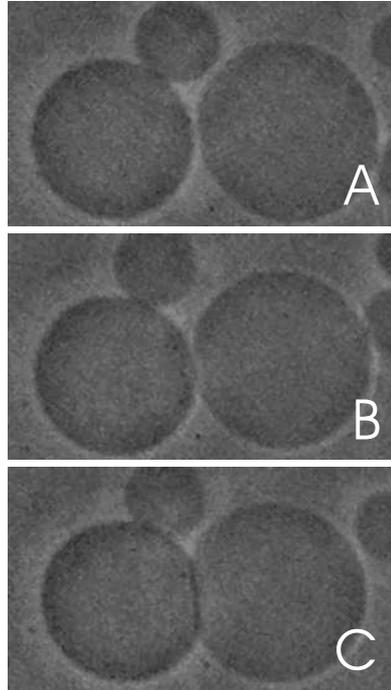


Figure 1: A sequence showing the process of vesicle adhesion in 30s intervals. The first picture is taken 14 minutes after the addition of HCAL monoclonal anti- $\beta$ 2GPI antibodies to the charged vesicles (POPC:cholesterol:cardiolipin=2:2:3).

The experiments were made in order to study the mechanisms involved in the antiphospholipid syndrome (APS). APS is an autoimmune disease characterized by thrombotic events and/or pregnancy morbidity. The etiology and underlying mechanisms of APS are not yet understood, however, activation of the coagulation system is evident. Antiphospholipid antibodies are present in the sera of patients with APS. These antibodies were found to interact directly with phospholipids constituting cell membranes (e.g. cardiolipin) or to bind to an antigen beta2-glycoproteinI ( $\beta$ 2GPI). The interactions between phospholipid membranes, protein cofactors and antibodies can be studied in a system of giant phospholipid vesicles. The change of vesicles shape and coalescence of vesicles due to the presence of protein cofactors and/or antibodies in the solution can be observed under phase contrast microscope. The giant phospholipid vesicles were prepared (by electroformation method) with three different lipids: POPC (1-Palmitoyl-2-Oleoyl-sn-Glycero-3-Phosphocholine), cholesterol and cardiolipin in different proportions. With such selection of lipids we obtain negatively charged surfaces in contact with electrolyte solution. The vesicles are stable for days. For comparison we also prepared uncharged vesicles with POPC and cholesterol only (in proportions POPC:cholesterol=4:1). Interactions between vesicles, antibodies and  $\beta$ 2GPI were studied under phase contrast microscope.

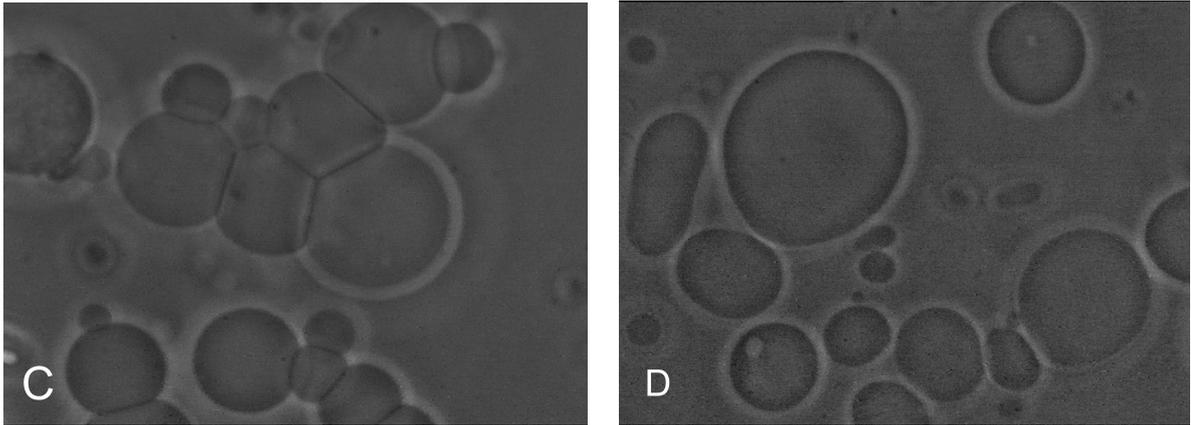


Figure 2: Adhesion of giant unilamellar vesicles 27 minutes after the insertion of HCAL monoclonal anti- $\beta$ 2GPI antibodies into the solution with negatively charged (C) and neutral (D) vesicles.

It turns out that antibodies alone cause the adhesion of charged vesicles, but not uncharged vesicles (Fig. 2). Adhesion also does not occur if vesicles are prepared only with very small amount of cardiolipin. Monoclonal antibodies (HCAL monoclonal anti- $\beta$ 2GPI antibodies) were used in the experiments in order to avoid the variability in the case of using different IgG fractions obtained from human sera. On the other hand the protein  $\beta$ 2GPI, which is known to insert into the membrane with its hydrophobic loop, caused adhesion of charged and also neutral vesicles. Some theoretical effort has been put into understanding of both phenomena and will be present in the following sections.

### 3 A very simple theory

We try to explain the attraction between two negatively charged phospholipid membranes mediated by protein  $\beta_2GPI$ .

$\beta_2GPI$  is a J-shaped molecule, composed of five domains. The fifth and the first domains are predominantly positively charged. Besides, there is a surface exposed hydrophobic loop on the fifth domain. If the membrane is negatively charged (as in our case), the fifth domain binds strongly to the membrane surface because of electrostatic attraction and also because of the insertion of hydrophobic loop into the membrane (Bouma et al. 1999; Schwarzenbacher et al. 1999). The positively first domain of the  $\beta_2GPI$  thus attracts the oppositely charged membranes of surrounding vesicles (see Fig.3).

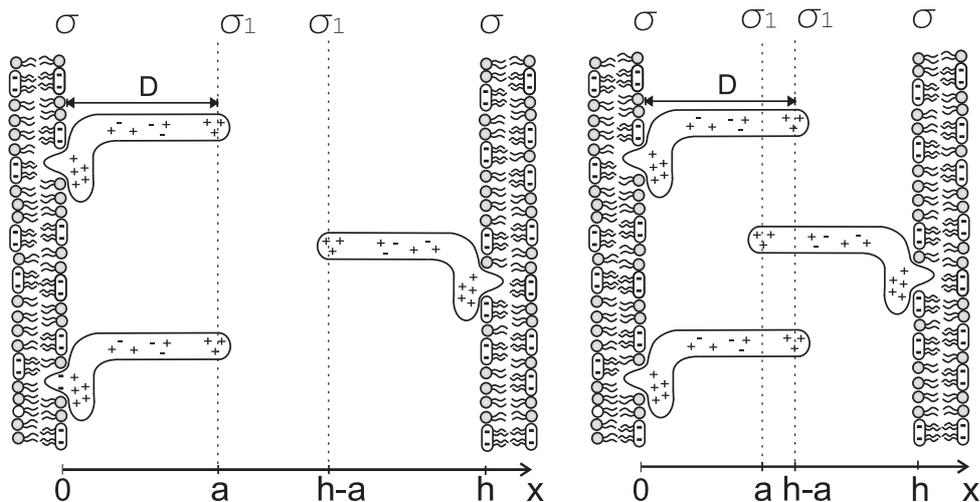


Figure 3: Schematic presentation shows two different regimes regarding the distance between the lipid surfaces:  $h \geq 2D$  (left) and  $h < 2D$  (right).

In our theoretical model we consider two planar charged lipid surfaces composed of POPC and cardiolipin (negatively charged lipid) and with electrolyte (salt) solution between the surfaces (Fig.3). Because of positively charged first domains of membrane-bound  $\beta_2GPI$  an apparent positively charged region approximately at the distance of  $\beta_2GPI$ 's length  $D$  away from each surface is created. In the model the positive charge of the tips of the  $\beta_2 - GPI$  molecules bound to both surfaces is represented by two charged surfaces (with the surface charge densities  $\sigma_1$ ) at the distance  $D$  from the each of lipid surfaces (Fig.3).

The electric charge distribution of both cardiolipin containing lipid surfaces is in the first approximation described by surface charge densities of both lipid surfaces ( $\sigma$ ), where the contribution due to 5-th domain of bound  $\beta_2 - GPI$  molecules is neglected.

If the distance between the lipid surfaces ( $h$ ) is larger than  $2D$ , the space between both lipid surfaces can be divided into three different regions  $0 \leq x \leq a$ ,  $a < x \leq (h - a)$  and  $(h - a) < x \leq h$  (see Fig.3). If  $h \geq 2D$  the value of  $a = D$ , while in the case  $h < 2D$  the value of  $a$  depends on  $h$  and is always smaller than  $D$ .

It is well known that linearized Poisson-Boltzmann (PB) theory overestimates electrostatic free energies for lipid membranes. Nevertheless, to keep our model traceable we

adopt the result of linearized PB theory, i.e. the electrostatic potential  $\Psi(x)$  in the system was calculated from linearized PB (LPB) equation:

$$\nabla^2\phi = \kappa_d^2\phi , \quad (3.1)$$

where  $\phi = e_0\Psi/kT$  is dimensionless electrostatic potential and the Debye length  $\kappa_d^{-1} = \sqrt{\epsilon_w\epsilon_0kT/(2n_0N_Ae_0^2)}$ . Here  $\epsilon_w$  is the dielectric constant of the aqueous solution,  $\epsilon_0$  is the permittivity of free space,  $n_0$  is the ionic strength in the bulk solution (i.e. bulk salt concentration; assuming a 1:1 salt such as NaCl),  $N_A$  is Avogadro's number and  $e_0$  is the unit charge. The bulk solution (outside the space between the membrane) provides a suitable reference for the electric potential (i.e.  $y = 0$ ).

Due to the symmetry of the system we are searching for the solution of Eq.(3.1) only in the region  $0 \leq x \leq h/2$  (see Fig.(3)). The solution of Eq.(3.1) can be then written as:

$$\phi = A \exp(-\kappa_d x) + B \exp(\kappa_d x) , \quad 0 \leq x \leq a , \quad (3.2)$$

$$\phi = C \exp(-\kappa_d x) + D \exp(\kappa_d x) , \quad a \leq x \leq h/2 , \quad (3.3)$$

where the constant  $A$ ,  $B$ ,  $C$  and  $D$  were determined analytically from the boundary conditions  $\frac{d\phi}{dx}(x=0) = -\sigma e_0/\epsilon_w\epsilon_0kT$ ,  $\phi(x=a_-) = \phi(x=a_+)$ ,  $\frac{d\phi}{dx}(x=a_-) = \frac{d\phi}{dx}(x=a_+) + \sigma_1 e_0/\epsilon_w\epsilon_0kT$  and  $\frac{d\phi}{dx}(x=h/2) = 0$ .

Including also the configurational entropy of the anions ( $i = 1$ ) and cations ( $i = 2$ ) of the salt dissolved in the water between both planar lipid surfaces, we can write the free energy of the system in the form [5] :

$$F/A = \int_0^h \left( \frac{1}{2} \epsilon_w\epsilon_0 \left( \frac{d\phi}{dx} \frac{kT}{e_0} \right)^2 + kT \sum_{j=1}^2 \left( n_j \ln \left( \frac{n_j}{n_0} \right) - (n_j - n_0) \right) \right) dx , \quad (3.4)$$

where  $n_j$  are the number densities of anions ( $i = 1$ ) and cations ( $i = 2$ ) in the salt solution,  $n_0$  is the number density of the anions and cations in the bulk solution (i.e. outside the space between the planar lipid surfaces). The bulk solution provides a suitable reference also for electric potential  $\phi_{\text{bulk}} = 0$ .

Fig. 4 shows the free energy ( $F$ ) of the system as a function of the distance ( $h$ ) between the two adjacent membrane surfaces with attached  $\beta_2 - GPI$ . As it can be seen in the Fig. 4 for small values of  $\sigma_1$  the free energy  $F$  increases with decreasing inter membrane distance  $h$  while for larger values of  $\sigma_1$  the free energy decreases with decreasing  $h$  until the absolute minimum of  $F$  close to  $h \cong D$  is reached.

The results presented in Fig. 4 reflect the fact that two adjacent membranes without bound  $\beta_2 - GPI$  repel each other while for high enough concentration of membrane-bound  $\beta_2 - GPI$  the force between two negatively charged membranes becomes strongly attractive leading to the equilibrium distance at  $h \cong D$ . The origin of attractive interactions between two like-charged membrane surface is the electrostatic attraction between the positively charged first domain on the tip of the membrane-bound  $\beta_2 - GPI$  and negatively charged opposite membrane (Fig.3). The observed weak  $\beta_2 - GPI$ -induced interaction between zwitterionic POPC-cholesterol vesicles indicates that the proposed attractive Coulomb (charge-charge) interactions between positively charged part of the first domain of membrane bound  $\beta_2$ -GPI and the negatively charged phospholipids of the adjacent membrane

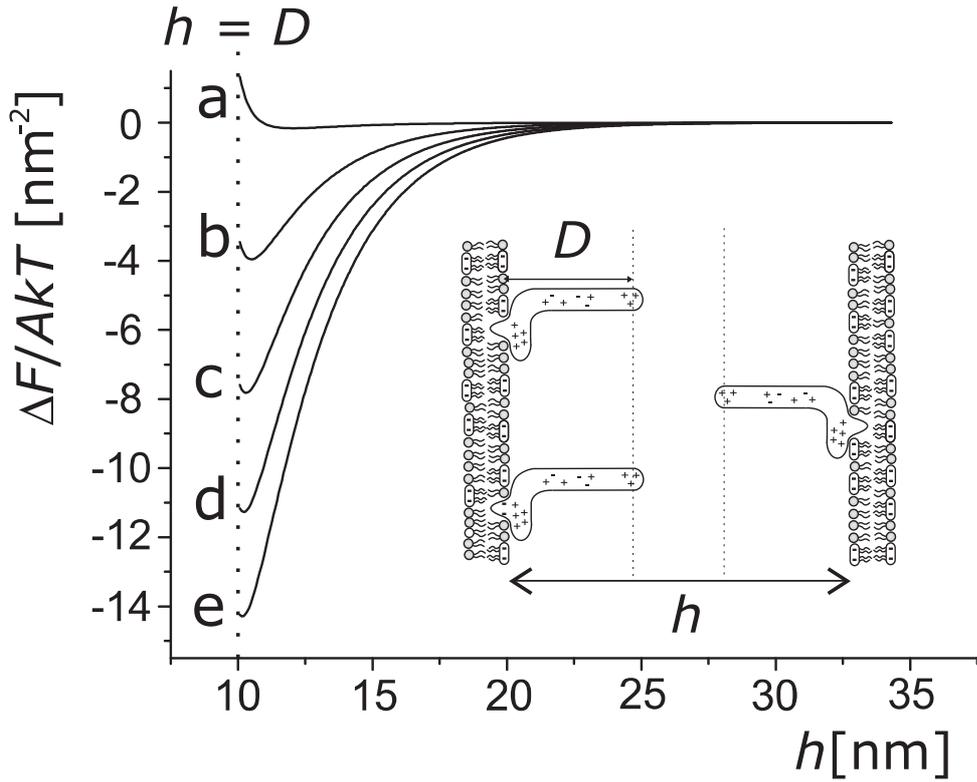


Figure 4: Free energy  $\Delta F = F - F(h \rightarrow \infty)$  as a function of  $x$  for five different values of  $\sigma_1$ :  $0.002 \text{ As/m}^2$  (a),  $0.003 \text{ As/m}^2$  (b),  $0.004 \text{ As/m}^2$  (c),  $0.005 \text{ As/m}^2$  (d),  $0.006 \text{ As/m}^2$  (e). Length of the  $\beta_2\text{GPI}$  molecule  $D=10 \text{ nm}$ . Values of surface charge density at lipid surface and buffer concentration are constant:  $\sigma = -0.05 \text{ As/m}^2$ . Salt concentration in the bulk solution  $n_0/N_A = 15 \text{ mmol/l}$ , where  $N_A$  is Avogadro's number.

is not the only possible interaction responsible for  $\beta_2$ -GPI-mediated agglutination between vesicle membrane surfaces. In general, also the charge-dipole and dipole-dipole electrostatic interaction [6] between the lipid headgroup electric dipole moment and  $\beta_2$ -GPI may contribute to the  $\beta_2$ -GPI-induced agglutination between like-charged membrane surfaces.

## 4 A more sophisticated theory

### 4.1 Double layer free energy for counterions only

In this section we will introduce the theory of electrical double layer for a system of two equally charged surfaces and oppositely charged ions between them. We will use this as a basis for the more sophisticated theory which will follow. Our aim here is to compute the free energy of a system of two double layers. The electrolyte solution contains point-like counterions only (see figure 5).

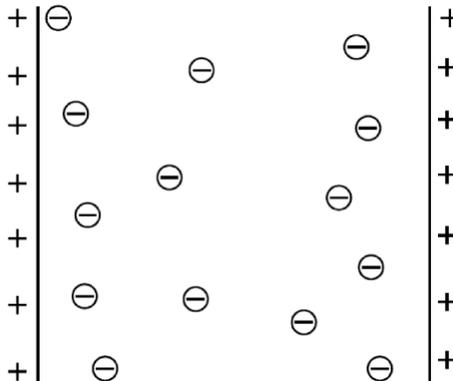


Figure 5: Schematic illustration of two equally charged planar surfaces, interacting through an electrolyte solution that contains monovalent counterions.

The lattice model has been chosen in our case to obtain the free energy of the system. The lattice model is any system of particles attached to a set of lattice sites. In our case these particles are positive and negative ions. We restrict the discussion here to the case in which the binding on any one site is independent of the binding on the remaining sites. Actually we begin with a system of  $N$  molecules bound not more than one per site to a set of  $M$  equivalent, distinguishable, and independent sites. The free energy of a cell is obtained from the canonical partition function:

$$F = -kT \ln Q. \quad (4.5)$$

And the cell partition function can be written as a product of the particles partition functions  $Q = \prod_m^N q_m$ , with an additional factor due to the configurational degeneracy [4]:

$$Q = \frac{M! \prod_m q_m}{N!(M - N)!} \quad (4.6)$$

The single particle canonical partition function  $q_m$  is:

$$q_m = \sum_i e^{-\varepsilon_{mi}/kT} \quad (4.7)$$

where  $i$  runs through all possible energy states of the particle. The product of single particle partition functions is connected with electrostatic part of the free energy. With

the use of the Stirling approximation for large  $n$  ( $\ln n! \simeq n \ln n - n$ ), by supposing that the number of the attached molecules is much smaller than the number of sites ( $N/M \ll 1$ ), and summing up the contributions of the whole system composed of charged surfaces and the electrolyte solution, the entropic part of the free energy is obtained (see Appendix A):

The total free energy  $F$  per area  $A$  of the system is thus:

$$F/A = kT \int_0^D [n(x) \ln(n(x)v_0) - n(x)] dx + \frac{1}{2} \int_0^D \varepsilon \varepsilon_0 \left( \frac{d\psi}{dx} \right)^2 dx, \quad (4.8)$$

where  $v_0$  is a volume of one particle. The electric potential  $\psi(x)$  and the density of the number of counterions  $n(x)$  are computed by using the Poisson-Boltzmann equation to obtain the desired free energy.

The Poisson-Boltzmann equation:

$$\frac{d^2\psi(x)}{dx^2} = -\frac{\rho}{\varepsilon \varepsilon_0}, \quad (4.9)$$

is solved by taking into account the relevant boundary conditions. The electroneutrality condition for the system reads:

$$\left. \frac{dy}{dx} \right|_{x=0} = \frac{\sigma e_0}{\varepsilon \varepsilon_0 kT}, \quad (4.10)$$

where  $\sigma$  is charge density of the surface. We define:

$$y = Ze\psi/kT \quad \kappa^2 = \frac{2N_A c e^2 Z^2}{\varepsilon \varepsilon_0 kT} \quad (4.11)$$

The function  $y$  reaches an extreme in the middle between both charged surface because of the symmetry of the problem, giving us the condition:

$$\left. \frac{dy}{dx} \right|_{x=D/2} = 0 \quad (4.12)$$

Because we now treat the solution that contains counterions only, the Poisson-Boltzmann equation reads:

$$\frac{d^2y}{dx^2} = -\frac{\kappa^2}{2} e^{-y} \quad (4.13)$$

The analytical solution of Eq. (4.13) is:

$$y = y_0 - \ln \left( 1 + \operatorname{tg}^2 \left[ \frac{\kappa}{2} e^{-\frac{y_0}{2}} \left( \frac{D}{2} - x \right) \right] \right), \quad (4.14)$$

where  $y_0 \equiv y(x = D/2)$ . We can calculate the density distribution from the potential:

$$n(x) = n_0 e^{-y}, \quad (4.15)$$

$n_0$  being a bulk concentration of counterions. Inserting the electric potential (4.14) and the density function (4.15) of the number of counterions into the Eq. (4.8) gives us the desired free energy in dependence of the distance between the two surfaces (Fig. 6).

The total free energy results in repulsive interaction. We can see (Fig. 6) that the entropic energy contribution at small distances by far exceeds the electrostatic contribution which is attractive by itself. The main part of the free energy is thus of entropic origin.

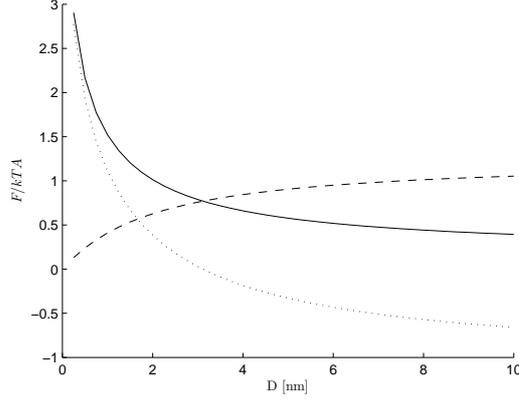


Figure 6: The free energy (full line), electrostatic energy (dashed line) and entropic part of the free energy (dotted line) as functions of the distance between the equally charged surfaces. The model parameters are  $\sigma = 0.1As/m^2$ .

## 4.2 Extended charges

Now our model is improved so, that instead of point like counterions we put into the solution spheres of diameter  $R$  within which two positive effective charges of a valency  $Z$  are separated by a distance  $l$  (Fig.7). This solution of spheres with extended charges is still confined between two flat surfaces, each of the area  $A$ , separated by a distance  $D$ . The surface area  $A$  is taken to be large comparing to the distance between surfaces  $D$  so that the end effects are neglected. Each surface bears uniformly distributed charge with the surface charge density  $\sigma$ . The positively charged spheres between the negatively charged surfaces represent the solution of monoclonal antibodies and two phospholipid vesicles. For simplicity, it is taken that  $l = 2R$ . The electrostatic field varies only perpendicularly to the charged surfaces (in the direction of the  $x$  axis) while we take that there is no electric field outside the electrolyte solution. The spherical counterions are now subject to positional and also orientational degrees of freedom. The center of the distribution of charge is located at  $x$  while the two point charges within the counterion attain the positions at  $x + s$  and  $x - s$ , respectively, with a certain probability density  $p(s|x)$ , where  $\frac{1}{l} \int_{-l/2}^{l/2} ds p(s|x) = 1$  and  $p(s|x) = 0$  for any  $x$  and  $|s| > l/2$ . The free energy of the system consists of three contributions: the electrostatic energy  $F_{\text{el}}$ , the configurational entropy of counterions  $F_{\text{ent}}$  and the orientational entropy of counterions  $F_{\text{o}}$ . The free energy contributions are given per  $AkT$  where  $k$  is the Boltzmann constant and  $T$  is the temperature of the system,  $f_i = F_i/AkT, i = \text{el}, \text{ent}, \text{o}$ . The electrostatic contribution to the free energy  $f_{\text{el}}$  is given by

$$f_{\text{el}} = \frac{1}{8\pi l_B} \int_0^D \Psi'(x)^2 dx, \quad (4.16)$$

where  $\Psi = e_0\psi(x)/kT$ ,  $\psi$  is the electrostatic potential,  $e_0$  is the elementary charge,  $l_B = e_0^2/4\pi\epsilon\epsilon_0kT$  is the Bjerrum length,  $\epsilon$  is the dielectric constant of the solution and  $\epsilon_0$

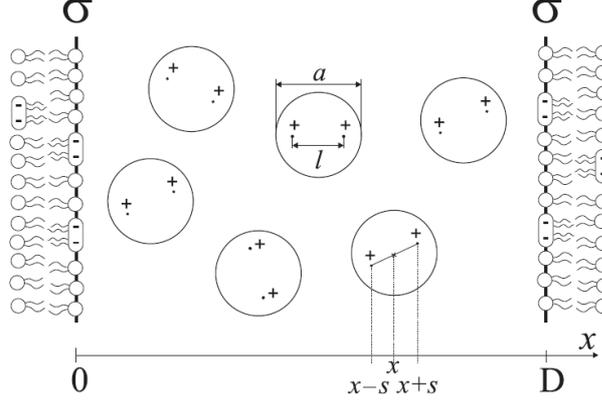


Figure 7: Schematic illustration of two negatively charged planar surfaces separated by a solution containing spherical counterions with spatially distributed positive charge. The charges are separated by a fixed distance  $l$ .

is the permittivity of the vacuum. Configurational entropy of the system is obtained as in previous chapter:

$$f_{\text{ent}} \simeq \int_0^D [n \ln(nv_0) - n] dx . \quad (4.17)$$

The contribution of the orientational ordering of the charges constrained into pairs to the free energy of the system  $f_o$  is

$$f_o = \int_0^D n(x) \left( \frac{1}{l} \int_{-l/2}^{l/2} p(s|x) [\ln p(s|x) + U(x, s)] ds \right) dx . \quad (4.18)$$

The function

$$U(x) = \left( \begin{array}{l} 0, \quad \frac{l}{2} \leq x \leq D - \frac{l}{2} \\ \infty, \quad \text{elsewhere} \end{array} \right) . \quad (4.19)$$

is introduced in order to ensure that the counterions cannot penetrate into the charged wall. The equilibrium state of the system is determined by the minimum of the free energy of the system

$$f = f_{\text{ent}} + f_{\text{el}} + f_o \quad (4.20)$$

subject to the constraint requiring the electroneutrality of the system,

$$2e_0Z \int_0^D n(x) dx = 2\sigma, \quad (4.21)$$

and the constraint requiring that the integral of all possible orientations of the counterions should be equal to zero,

$$\int_0^D n(x) \left( \frac{1}{l} \int_{-l/2}^{l/2} p(s|x) ds - 1 \right) dx = 0. \quad (4.22)$$

While performing the variation, the number of counterions is kept constant which is ensured by considering Eq.(4.21). To solve this variational problem, a functional  $\int_0^D \mathcal{L} dx$  is constructed,

$$\begin{aligned} \int_0^D \mathcal{L} dx &= f + \mu 2Z \int_0^D n(x) dx \\ &+ \int_0^D n(x) \lambda(x) \left( \frac{1}{l} \int_{-l/2}^{l/2} p(s|x) ds \right) dx, \end{aligned} \quad (4.23)$$

where  $f$  is the free energy of the system (Eq.(4.20)) while  $\mu(x)$  and  $\lambda$  are the Lagrange multipliers. In equilibrium,  $\delta \int_0^D \mathcal{L} dx = 0$  which yields the equilibrium volume density of counterions  $n(x)$  :

$$n(x) = \frac{\exp(-2Z\mu)}{v_0 l} \int_{-l/2}^{l/2} \exp(-Z\Psi(x+s) - Z\Psi(x-s)) ds \quad (4.24)$$

and the equilibrium probability density

$$p(s|x) = \frac{\exp(-Z\Psi(x+s) - Z\Psi(x-s))}{\frac{1}{l} \int_{-l/2}^{l/2} \exp(-Z\Psi(x+\bar{s}) - Z\Psi(x-\bar{s})) d\bar{s}}. \quad (4.25)$$

The function  $n(x)$  is defined only in the interval  $l/2 \leq x \leq D - l/2$ . Using Eq. (4.24) the volume density of charge  $\rho(x)$  can be calculated and inserted into the Poisson equation  $\psi''(x) = -4\pi l_B \rho(x)/e_0$  to yield a integro-differential equation

$$\begin{aligned} \Psi''(x) &= -8\pi l_B \frac{Z}{v_0} \exp(-Z\Psi(x) - 2Z\mu) \\ &\times \frac{1}{l} \int_{-l/2}^{l/2} \exp(-Z\Psi(x+2s) - U(x+s)) ds \end{aligned} \quad (4.26)$$

which is valid in the region  $0 \leq x \leq D$ . The boundary conditions at the charged surfaces are

$$\Psi'(x=0) = -\frac{4\pi\sigma}{l_B e_0}, \quad \Psi'(x=D) = \frac{4\pi\sigma}{l_B e_0}. \quad (4.27)$$

The finite size of counterions is taken into account by the distance of closest approach of the center of the counterion to the charged surface ( $R = l/2$ ). The above integro-differential equation (4.26) was solved numerically.

The solution of the integro-differential equation (4.26) yielded the equilibrium potential  $\Psi(x)$ , consistently related equilibrium distribution  $n(x)$  and consistently related

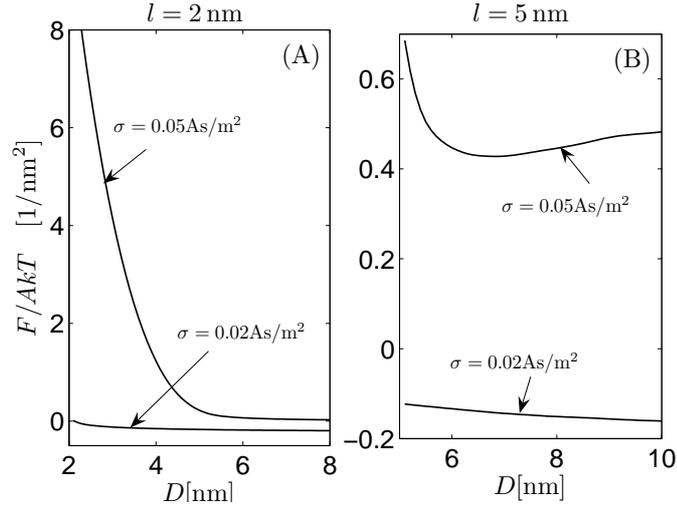


Figure 8: The electrostatic free energy  $F$  per unit area of the charged surface  $A$  and per thermal energy  $kT$ , as a function of the distance between the negatively charged surfaces  $D$ , for valency  $Z=2$  of the spheres and two different surface charge densities and two different distances as marked on the graph.

probability density  $p(s|x)$ . The equilibrium free energy of the system  $F$  was then calculated by taking into account these optimal functions. The free energy was calculated with respect to the free energy of the reference state in which the same number of counterions is uniformly distributed between the charged surfaces. The free energy of the reference state was proven to be equal for all distances between the charged surfaces and was therefore taken as a reference value in determining the values of the free energy.

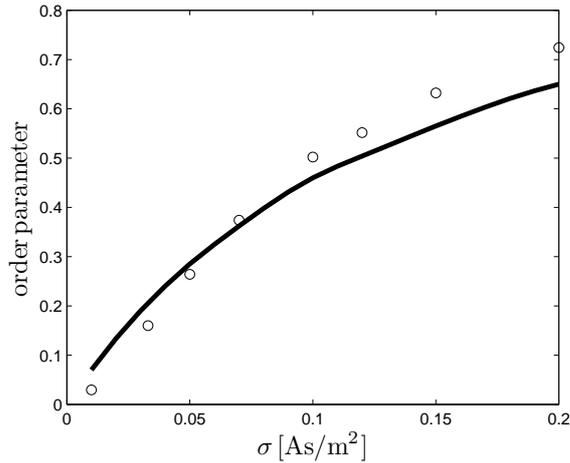


Figure 9: Average order parameter as a function of surface charge density of the plates  $\sigma$ . Points (open spheres) are obtained by Monte Carlo simulation of the same system.

Fig. 8 shows the electrostatic free energy as a function of the distance between two negatively charged surfaces. The interaction is attractive if the free energy increases with increasing distance between the surfaces  $D$  while it is repulsive if the free energy decreases with increasing  $D$ . For small surface density of charge  $|\sigma|$  and small separation between charges within the counterion  $l$  the interaction is found to be repulsive for any distance between the charged surfaces. Large enough  $\sigma$  and  $l$  yield a nonmonotonous behavior of the free energy with a minimum representing the equilibrium distance between the surfaces.

The counterions are orientationally ordered (Fig. 9), the order being stronger for higher surface charge  $|\sigma|$ . It could be interpreted that the orientationally ordered counterions form a layer which represents bridging between two like-charged surfaces.

## 5 Discussion and conclusion

Mean field theory was used to describe double layer interactions in all previously described models. Other basic approximations of Gouy-Chapman model are: homogenous surface charge distribution described by surface charge density  $\sigma$ , ion-ion interactions are not taken into account directly and solution is described by dielectric constant which is independent of the electric field variation and ion concentration, electric field behind the charged plates is not taken into account. At short separations of the two charged surfaces further factors come into play: ion correlation effects, image forces arising on the boundaries with different dielectric constants, solvation forces and discreteness of surface charges.

Regarding surfaces of spherical particles, surface curvature might also play an important role in interaction energies. But we do not expect this to play a major role in experiments with giant phospholipid vesicles because the membrane thickness and counterion sizes (some nm) are much smaller than an average size of the vesicle (some  $\mu\text{m}$ )

The problem of finite ion size was only partially resolved in our model. Ion size near the charged surface and charge distribution within an ion are taken into account. Other approximations remain unsolved. Different theories (hypernetted chain theory, modified Poisson-Boltzmann etc.) were made trying to incorporate additional factors, but because of their complexity in practice Gouy-Chapman model still remains the most useful tool for fast and lucid estimation of the forces in the electrical double layer. From this point of view our model is an upgrade of the Gouy-Chapman model, but remains transparent.

The Gouy-Chapman model predicts repulsion between equally charged surfaces [?]. We have shown, that taking into account spatial distribution of charges may lead to the attractive interaction due to the orientational ordering of counterions with spatially distributed charge. However, only for large enough surface charge densities the minimum of the free energy was obtained. Experimental observations can be explained with the results of developed theory.

Systems where interactions between charged membranes are mediated by particles of different shapes and with internal charge distribution are common in different biological and chemical environments and thus still represents a challenging problem in electrical double layer studies. Many similar, at first glance simple, but actually complex and poorly understood phenomena in the "bio" field are still waiting to be explored in the future.

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## Appendix A: Derivation of the entropic part of free energy from the partition function

We derive the entropic contribution to the free energy:

$$F^{\text{entropic}} = kT \int [n \ln(nv_0) - n] dV \quad (\text{A.28})$$

from the partition function:

$$Q = \frac{M!}{N!(M-N)!}. \quad (\text{A.29})$$

Using the Stirling approximation for large  $n$  ( $\ln n! \simeq n \ln n - n$ ) in Eq. (A.29) gives:

$$\ln Q = M \ln M - M - N \ln N + N - (M-N) \ln(M-N) + (M-N), \quad (\text{A.30})$$

Rewriting the last term we get:

$$\begin{aligned} \ln Q &= M \ln M - M - N \ln N + N - (M-N) \ln\left(M\left(1 - \frac{N}{M}\right)\right) = \\ &= M \ln M - N \ln N + N - (M-N) \ln M - (M-N) \ln\left(1 - \frac{N}{M}\right) \end{aligned} \quad (\text{A.31})$$

Finally:

$$\ln Q = -N \ln\left(\frac{N}{M}\right) - (M-N) \ln\left(1 - \frac{N}{M}\right). \quad (\text{A.32})$$

Now we introduce new variables  $v_0$  and  $n$ . We define  $v_0$  as volume of one particle so that  $Mv_0 = V_{\text{cell}}$ , where  $V_{\text{cell}}$  is the volume of the cell with  $M$  sites of volume  $v_0$ .  $n$  is the number density of counterions defined as  $n = N/V_{\text{cell}}$ . Total free energy is defined as:

$$F^{\text{tot}} = \int F_{\text{cell}} \frac{dV}{V_{\text{cell}}}, \quad (\text{A.33})$$

and  $F_{\text{cell}} = -kT \ln Q$ . Equation A.32 is inserted into the equation A.33:

$$\frac{F^{\text{entropic}}}{kT} = \int dV \left[ n \ln(nv_0) + \frac{1}{v_0} \left(1 - \frac{N}{M}\right) \ln\left(1 - \frac{N}{M}\right) \right] \quad (\text{A.34})$$

If we now assume that  $N/M \ll 1$ , the second term can be approximated by  $\ln(1+x) \approx x$ , giving us the final version of the entropic part of the free energy that we wanted to derive (Eq. A.28).