Electrostatic Applied to Biological Systems

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Summary. — Polyelectrolytes are of great importance in different branches of chemistry and biology. DNA is a very good example of a polyelectrolyte of biological relevance. In this seminar, we describe DNA solutions in the presence of salt and amphiphiles. After thermodynamic equilibrium is reached, the amphiphiles and the electrolyte associate to DNA, leading to the formation of complexes. As the density of amphiphiles increases, we observe charge inversion of DNA complexes at not too high amphiphile densities. The formation of DNA-amphiphile complexes is essential for developing delivery systems in gene therapy, and we see that it is possible to reverse the charge of DNA even in the presence of multivalent salt.

1. – Description

Electrostatic plays a relevant hole in physics, chemistry and biology. Molecular biology functions in water at room temperature. This is quite a limited temperature range for a physicist if compared with low temperature physics where a large energy scales can be scanned. However, the presence of long-range interactions in the biological structures, give rise to a rich variety of scales in the problem that ultimately leads to very interesting phenomena. In this lecture, we will review the recent developing in understanding one of these phenomena.

More specifically, let us consider a charged rod, the macroion in the solution of its counterions. Due to the electrostatic interactions, the small ions are bound to the big ion

screening the interactions between like-charged rods. When the electrostatic interaction between polyion and the counterion is high enough, the counterions are bound to the polyion even in the infinite dilution limit. This is the case of segments of DNA, modeled as rigid rods, that exhibit condensation at room temperature in water.

This phenomena has many practical applications. Foremost among these is the technology of dressing DNA with cations [1], positively charged star-like polymers called dendrimers [2][3][4], liposomes in order to produce complexes with DNA. This facilitates the gene delivery through a negative cell surface membrane [5]. Also, one can manufacture nanowires by attaching positively silver or gold colloids to DNA [6].

Besides being strongly bound to DNA, multivalent ions repeal each other forming a correlated liquid. As a result, condensation is enhanced and in the presence of salt the charge of the polyion is reversed. Moreover, in dense polyion solutions, the counterions belonging to different macroions correlate so to create an effective attractive force between the like-charged polyions.

This like-charge attraction manifests itself in *in vitro* formation of toroidal aggregates of concentrated DNA [7]-[8], similar to the one observed in bacteriophasage heads [9], and in bundle formation of F-actin and tobacco mosaic virus [10]. The enhancement of condensation and consequent charge reversal [11]-[13] can be seen in the formation of the chromatic in eukariotic cells. Nature uses proteins having large positive charge, the histones. The histones are release from the protein and associate to the DNA in a necklace shape.

Although the phenomena described above are quite complex, we can go along describing them employing a surprisingly simple models and theories. Particularly, in this lecture we will show that a lot of physics and correlations are within the Debye-Hückel theory an old theory introduced primarily to study electrolytes 80 years ago [14].

2. – DNA in the Presence of Monovalent Counterions and Surfactants

Let us consider the following system, illustrated in Fig. 1, a solution of *DNA* strands of length L and diameter a_p, monovalent surfactant and monovalent salt. In aqueous solution, the polyions become ionized resulting in a negative charge $-Zq$ distributed uniformly distributed with separation $b = L/Z$. The solvent, water, is modeled as a continuous medium of dielectric constant D. The ions of the salt are completely dissociated, forming an equal number of positive and negative ions. Similarly, the surfactants are assumed to be fully dissociated producing negative monovalent coions and polymeric chains with a monovalent cationic head group. For simplicity, all the counterions and coions are treated as identical, independent of the molecules from which they were derived. The electrolytes are depicted as hard spheres of diameter a_c and charge $\pm q$ and the surfactant is modeled as a polymer of s monomers each one considered as a rigid sphere of diameter a_c with the head monomer carrying a charge of $+q$. The interaction between the hydrophobic tails is short ranged and characterized by the parameter χ. The density of DNA strands is $\rho_p = N_p/V$, the density of monovalent salt is $\rho_s = N_s/V$ and the density of amphiphiles is $\rho_a = N_a/V$.
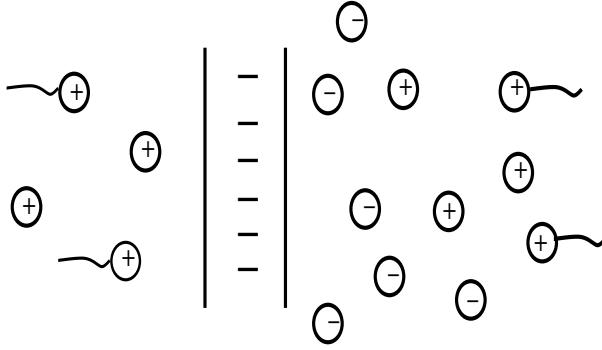
Fig. 1. – The model

In a dilute solution, the polyions will arrange themselves uniformly on the solution, while the microions (counterions, salt and surfactant) will stay in the vicinity of the polyion. This picture suggests that there is strong positional correlation between the polyions and strong positional correlation among the microions. In this sense,

The strong electrostatic interactions between the polyions, the counterions, salt cations and surfactant heads leads to the formation of complexes, which in thermodynamic equilibrium will be made up of one polyion, n_c monovalent counterions and n_a monovalent surfactant. We do not consider the effects of of polydispersity in the size of the complexes, since it does not affect the final result. Due to the association and to the charge conservation, there are only two free quantities and so,

$$(1) \quad \rho_c = (Z - n_c)\rho_p + \rho_s \quad \rho_{a+} = \rho_a - n_a\rho_p \quad \rho_- = \rho_s + \rho_a \quad \rho_f = \rho_c + \rho_{a+} + \rho_-$$

where ρ_c is the density of free monovalent counterions, ρ_{a+} is the density of free amphiphiles and ρ_- is the density of negative ion.

The objective of this theory is to determine the number of counterions n_c and surfactants n_a associated to each DNA strand. For this, we construct the Helmholtz free energy of the system and minimize it.The relevant contributions for the Helmoltz free energy density are two, the electrostatic and the entropic namely:

$$(2) \qquad \frac{F}{V} = f_{el} + f_{ent}$$

In F_{el} is the electrostatic free energy between the polyion and the counterions and can be derived in the spirit of the DH theory for electrolytes as follows. Suppose we choose a coordinate system so that it is centered on one of the polyions with the z-axis
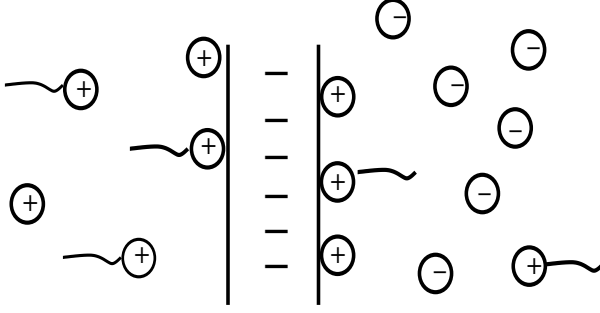
Fig. 2. – Association

along the polyion's major axis. The electrostatic potential for $r > a$ satisfies the Laplace equation:

$$(3) \qquad\qquad\qquad \nabla \phi(r) = 0$$

while the potential for $r < a$ satisfies the Poisson equation namely:

$$(4) \qquad\qquad\qquad \nabla^2 \phi(r) = \frac{4\pi}{\epsilon} \rho_q(r)$$

with a charge distribution given by:

$$(5) \qquad\qquad\qquad \rho_q = -Zq\rho_p + q\rho_q e^{-\beta q \phi(r)} \quad .$$

In order to solve this equations, we would like to linearize it. Since the electrostatic interactions are quite strong in the vicinity of the polyion, the polyion-counterions correlation in the vicinity of the polyion must be taken into account. This can be done by introducing the idea of polyion-counterion association. In this context the polyelectrolyte solution can be thought as composed of free unassociated counterions, salt and surfactant, ρ_q and clusters made of one polyion, n_c counterions and ρ_a surfactant molecules as illustrated in Fig. 2. Once the cluster concept is introduced in the theory, one can expand the exponential in Eq. 5 and use the linearized form in the Poisson Equation what gives raise to the Poisson Boltzmann Equation namely

$$(6) \qquad\qquad\qquad \nabla^2 \phi(r) = \kappa^2 \phi(r)$$

where

$$\kappa = \sqrt{\frac{4\pi q^2 \rho_f}{k_B T \epsilon}} \quad .$$
(7)

The solution of the linearized equation outside the cluster at $r > a$ is given by:

$$\phi(r) = \frac{2\lambda}{\epsilon} \frac{K_0(\kappa r)}{\kappa a K_\nu(\kappa a)} \quad .$$
(8)

where K_{nu} is the modified Bessel function of order ν and $\lambda = (Z - n_c - n_a)/L$. For $r < a$ the potential is given by:

$$\phi(r) = \ln\frac{r}{a} + \frac{2\lambda}{\epsilon} \frac{K_0(\kappa a)}{\kappa a K_\nu(\kappa a)} \quad .$$
(9)

The electrostatic free energy due to the free ions-cluster interaction can be obtained from the electrostatic energy

$$u_p = \frac{1}{2} \int [\rho_q(r) + q_p(r)]\phi(r) d^3 r$$
(10)

together with the Debye charging process, namely,

$$\beta f_{el} = \int_0^1 \frac{u_q}{2\lambda} d\lambda$$
$$= -\frac{(Z - n)^2 \xi}{Z} \left[\frac{2\gamma_E - 1}{2} + \ln(\kappa a)\right]\rho_p^*$$
(11)

where $\xi \equiv \beta q^2/Da$ adn $\rho^* = \rho a^3$.

The calculation of the entropic contribution can be obtained with the aid of Flory [15] theory of mixing. The free energy is a sum of ideal free energies of various species, namely

$$\beta f_{ent} = \sum \left[\rho_s - \rho_s \ln\left(\frac{\phi_s}{\zeta_s}\right)\right]$$
(12)

where s represents the different species and ζ_s is the internal partition of the species s. In the case of the particles without structure, the internal partition function $\zeta_- = \zeta_c = \zeta_{a+} = 1$. The volume fraction ϕ_s of the different species are:

$$\phi_p = \frac{\pi \rho_p^*}{4(a/L)}\left(\frac{a_p}{a}\right)^2 + \frac{Z\pi\rho_p^*}{6}(s_a m_a + m_c)\left(\frac{a_c}{a}\right)^3$$
$$\phi_c = \rho_c^* \frac{\pi}{6}\left(\frac{a_c}{a}\right)^3$$

$$\phi_{a+} = \frac{s_a \pi \rho_{a+}^*}{6} \left(\frac{a_c}{a}\right)^3$$

(13)
$$\phi_- = \frac{\pi \rho_-^*}{6} \left(\frac{a_c}{a}\right)^3 .$$

Here we introduce the fractions counterions, $m_c = n_c/Z$, and surfactant, $m_a = n_a/Z$, associated to each DNA strand. Accounting for all these terms, the entropic contribution becomes

$$\beta f^{ent} = \rho_p \ln \left(\frac{\phi_p(1 + m_c + m_a)}{\zeta_{cl}(1 + m_c + m_a s_a)}\right) - \rho_p$$

$$+ \rho_c \ln \phi_c - \rho_c + \rho_{a_+} \ln \frac{\phi_{a_+}}{s_a} - \rho_{a_+}$$

(14)
$$+ \rho_- \ln \phi_- - \rho_-$$

where the entropy for the free surfactants arises from the Flory theory for polymers [15] which is also the basis of the entropic contribution for the complex [16]. The internal partition function of the complex, ζ_{cl}, can be calculated by modeling the DNA by an one dimensional lattice with Z sites. If the number of associated ions to each site can be only zero or one, this problem becomes equivalent to finding the free energy of an one dimensional array with the three different states: empty, a surfactant associated, a counterion associated.

The solution of this model is not trivial, so we employ the mean-field Gibbs-Bogoliubov-Feymman inequality. The resulting partition function is given by

$$-ln\zeta_c[m_c, m_a] = \xi K \left[\frac{Z_c^{\,2}}{Z^2} - 1\right] + \beta \chi (Z - 1) m_a^2$$

(15)
$$+ Z m_c \ln m_c + Z m_a \ln m_a$$

$$+ Z (1 - m_c - m_a) \ln (1 - m_c - m_a)$$

where $\xi \equiv \beta q^2/Da$ is the Manning parameter, $K = Z[\psi(Z) - \psi(1)] - Z + 1$, and $\psi(n)$ is the digamma function.

The equilibrium configuration of the system is found by the minimization of the Helmoltz free energy, leading to two equations, namely

$$\frac{\partial f}{\partial m_c} \delta m_c = 0$$

(16)
$$\frac{\partial f}{\partial m_a} \delta m_a = 0 .$$

Solving this system of two equations, it is possible to obtain the values of m_c and m_a.

We define a "surfoplex" to be a complex in which almost all of the DNA's phosphate groups are neutralized by the associated surfactant molecules. As mentioned earlier, we are interested in the minimum amount of cationic surfactant needed to transform naked
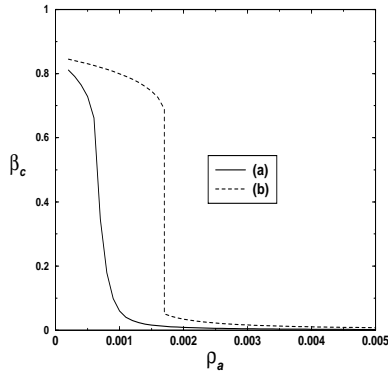
Fig. 3. – The DNA-counterion binding isotherm for various concentrations of added salt: (a) 5mM, (b) 18mM.
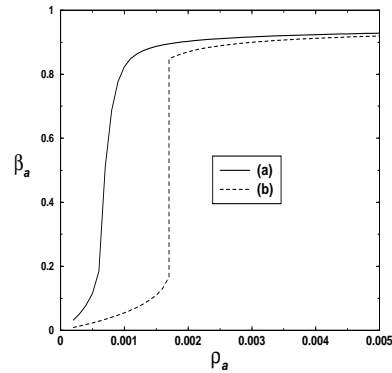
Fig. 4. – The DNA-surfactant binding counterions isotherm for various concentrations of added salt: (a) 5mM, (b) 18mM.

DNA into surfoplexes. To this effect, we study the dependence of the number of condensed surfactant molecules on the bulk concentration of surfactant ρ_a. In order to evaluate the relevance of hydrophobic interactions between the amphiphiles, the hydrophobic parameter was varied from 0 to $\beta\chi = -3.5$. The effect of addition of high concentrations of salt to the system was analyzed by varying the amount of salt added to the system ρ_s.

Figs.3a, b illustrate the surfactant binding isotherms, $\beta_a = m_a$ and $\beta_c = m_c$, as a function of total amphiphilic concentration for hydrophobicity parameter $\beta\chi = -3.5$, salt concentrations $\rho_s = 5mM, 18mM$, DNA concentration $\rho_p = 2\mu M$ for a DNA segment with 220 base pairs. At very low densities of surfactant, the condensation is dominated by the monovalent ions, since the amphiphilic molecules gain more entropy, and thus lower the total free energy, by staying free. However, as the concentration of surfactant increases, the gain in electrostatic energy due to condensation and to the hydrophobicity of the amphiphilic molecules wins over the entropy. Even though, the use of monovalent ions guarantee that the the decrease of the effective charge of the DNA, it does not revert its charge. In order to get charge reversal multi binding and multivalent ions are required. In the next session we will include these two ingredients.

3. – DNA in the Presence of Multivalent Ions

Let us now consider another system made of ρ_p DNA molecules, ρ_M monovalent, ρ_s divalent salt molecules, $\rho_c = Z\rho_p$ monovalent counterions and ρ_a monovalent surfactant. The strong electrostatic interactions between the polyions, the counterions, salt cations and surfactant heads leads to the formation of complexes, which in thermodynamic equilibrium will be made up of one polyion, n_c monovalent counterions, n_s divalent salt ions and n_a monovalent surfactant. We do not consider the effects of of polydispersity in the size of the complexes, since it does not affect the final result. Due to the association
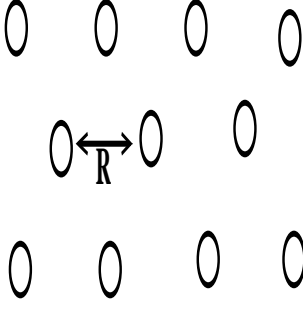
Fig. 5. – Two-dimensional Wigner Crystal

and to the charge conservation, there are only two free quantities and so,

$$\rho_c = (Z - n_c)\rho_p + \rho_p$$
$$\rho_{s+} = \rho_s - n_s\rho_p$$
$$\rho_{a+} = \rho_a - n_a\rho_p$$
$$\rho_- = 2\rho_s + \rho_a$$
(17)
$$\rho_f = \rho_c + \rho_{a+} + \rho_- + \rho_{s+}$$

The theory developed in the previous session did not account for the correlations between the ions. For dilute systems, the correlation between the free ions is irrelevant, however, the correlations between the associated ions is indeed important, particularly in the case of multivalent ions. So, it becomes important to compute the correlation between the divalent ions of salt. To begin with, let us assume that the n_s associated divalent salt ions are in a planar surface and not in the cylindrical geometry. The dependence on the shape of the polyions for this this contribution of the free energy is not dramatic, particularly if the polyion radius is bigger than the distance between the charged groups along the polymer. In this case, the two dimensional concentration of divalent ions is $(n_s)/(2\pi a_p L)$ and the surface area per ion can be characterized by R such that $\pi R^2 = 2\pi a_p L/(n_s)$ as illustrated in Fig. 5. Thus $R = \sqrt{2a_p L/(n_s)}$ and $\Gamma = 4q^2/(k_B T \epsilon R)$, where Γ is the plasma parameter, a Coulomb coupling constant that scales quadratically with the valence. For $\Gamma \approx 1$, correlations are not so relevant. This is the case of monovalent ions. For $\Gamma >> 1$ the screening atmosphere is narrowly confined as a two-dimensional liquid of classical charged particles in an uniform neutralizing background located at the surface of the polyion. This liquid, also called one component plasma, at zero temperature acquires the minimal energy state of a Wigner Crystal showed in Fig. (5). The correlational energy associated to this plasma made of n_s ions of valence v is given by:

(18)
$$e(n_s) = \frac{-3.92q^2}{\epsilon R} \ .$$

Applying the charging process to this energy one gets the free energy associated with the correlational energy of divalent salt ions given by:

(19)
$$\beta f(n_s) = -0.98\left(\frac{(n_s)L/a_p}{2\pi}\right)^{1/2}(n_s)\rho_p^* .$$

Once we have the proper correlational energy given by the above equation, we have to address another important point. In the case of surfactants with the high hydrophobicity parameter, the energy gain when two amphiphilic molecules are close is so high that instead of associating one surfactant per site it might occur that more than one amphiphilic molecule is close to a charge group [17]. In this case the strong electrostatic attraction between the polyions, counterions, and amphiphiles, leads to the formation of complexes consisting of one polyion, n_c counterions, and n_a amphiphilic molecules. We shall assume that to each phosphate group of the DNA molecule can be associated at most one counterion or l_{max} surfactants. This assumption seems to be quite reasonable in view of the fact that the electrostatic repulsion between the counterions will prevent more than one counterion from condensing onto a given monomer. On the other hand, the gain in hydrophobic energy resulting from the close packing of the surfactant molecules might be able to overcome the repulsive electrostatic interaction between the surfactant head groups, favoring condensation of more than one surfactant on a given monomer.The l amphiphilic molecules form a "ring" of radius a around the central negative monomer of the DNA (see Fig (6)) .If we assume that most of the hydrocarbon chain of the associated surfactants is hidden inside the DNA molecule, the maximum number of surfactants in a ring can be estimated from the excluded volume considerations,$l_{max} = 2a/a_c$, where $(a_p + a_c)/2$ is the radius of the exclusion cylinder around a polyion.

At equilibrium, each site (monomer) of a polyion can be free or have one counterion or a ring of $l = 1, ..., l_{max}$ surfactants associated to it. We define the surface coverage of counterions as $p_c = n_c/Z$, and the surface coverage of surfactant rings as $p_l = n_l/Z$, where n_c is the number of condensed counterions and nl is the number of rings containing l surfactants. Each polyion has a distribution of rings containing from one to l_{max} surfactants. We shall neglect the polydispersity in the size of the complexes, assuming that all the complexes have nc counterions and ns amphiphilic molecules – in rings of $\{p_l\}$. The aim of the theory is to determine the characteristic values of m_a, m_c, and the surface coverage by rings. To accomplish this, the free energy of the DNA-surfactant system given by Eq. (2)

(20)
$$\frac{F}{V} = f_{el} + f_{ent}^{new} + f_{WC}$$

has to be minimized.

In this case, the electrostatic contribution is given by Eq. (12) and the entropic contribution is given by

$$\beta f_{ent}^{new} = \rho_p \ln\left(\frac{\phi_p(1 + m_c + m_a + m_s)}{\zeta_{cl}^{new}(1 + m_c + m_a s_a + m_s)}\right) - \rho_p$$
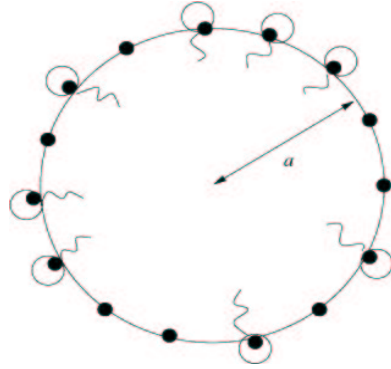
Fig. 6. – l surfactant molecules associated to one charged group forming a l-ring

$$+ \rho_c \ln \phi_c - \rho_c + \rho_{s+} \ln \phi_{s+} - \rho_{s+} + \rho_{a+} \ln \frac{\phi_{a+}}{s_a} - \rho_{a+}$$

(21) $$+ \rho_- \ln \phi_- - \rho_-$$

The internal partition function of the complex, ζ_{cl}, can be calculated by modeling the DNA by an one dimensional lattice with Z sites. If the number of associated ions to each site can be only zero or one, this problem becomes equivalent to finding the free energy of an one dimensional array with the four different states: empty, a surfactant associated, a counterion associated and a divalent salt associated.

The solution of this model is not trivial, so we employ the mean-field Gibbs-Bogoliubov-Feymman inequality. The resulting partition function can be found in reference [17].

The equilibrium configuration of the system is found by the minimization of the

Helmoltz free energy, leading to two equations, namely

$$\frac{\partial f}{\partial m_c} \delta m_c = 0$$

$$\frac{\partial f}{\partial m_s} \delta m_s = 0$$

(22)
$$\frac{\partial f}{\partial m_a} \delta m_a = 0$$

(23)
$$\frac{\partial f}{\partial p_l} \delta p_l = 0 \ .$$

Solving this system of two equations, it is possible to obtain the values of m_c and m_a and the distribution $\{p_l\}$. We define a "surfoplex" to be a complex in which almost all of the DNA's phosphate groups are neutralized by the associated surfactant molecules.As mentioned earlier, we are interested in the minimum amount of cationic surfactant needed to transform naked DNA into surfoplexes. To this effect, we study the dependence of the number of condensed surfactant molecules on the bulk concentration of surfactant ρ_a. In order to evaluate the relevance of hydrophobic interactions between the amphiphiles, the hydrophobic parameter was varied from 0 to $\beta\chi = -6$. The effect of addition of high concentrations of salt to the system was analyzed by varying the amount of salt added to the system ρ_s.

Fig. (7) illustrate the case of monovalent surfactant, monovalent and divalent salt. The first graph is the binding isotherms, $\beta_a = m_a$, $\beta_c = m_c$ and $\beta_s = m_s$ respectively, as a function of total amphiphilic concentration ρ_a for $\chi = --6k_BT$. The density of DNA is $\rho_p = 2\mu M$ and the number of base pairs is 220, giving $z = 440$. The concentrations of added salt are: $\rho_M = 510 - 4M$, $\rho_s = 110 - 4M$. The distribution of m-divalent ions on the ring in a complex with n_s divalent ions associated is given by the form:

(24)
$$p_n = \frac{e^{-\alpha n}}{n_s \sum_{l=0}^{l_{max}} l e^{-\alpha l}} \ .$$

The values of α for different surfactant concentrations are illustrated in Fig. (7). The average number of divalent ions , $<< n >>$ in each ring is also shown in Fig. (7). Differently from the pure monovalent case where the both the correlational term and the ring formation were not taken into account, one can observe from the charge of complex given by $1 - \beta_c - 2\beta_s - \beta_a$ that there is charge reversal. This means that for a certain density of surfactant the DNA is completely neutralized. Further increase of sufactant concentration revert the charge.

4. – Conclusions

In resume, we have presented a simple theory of DNA, for monovalent and multivalent salt and surfactant solutions. Our results should be of direct interest to researchers working on the design of improved gene delivery systems. In particular, we find that
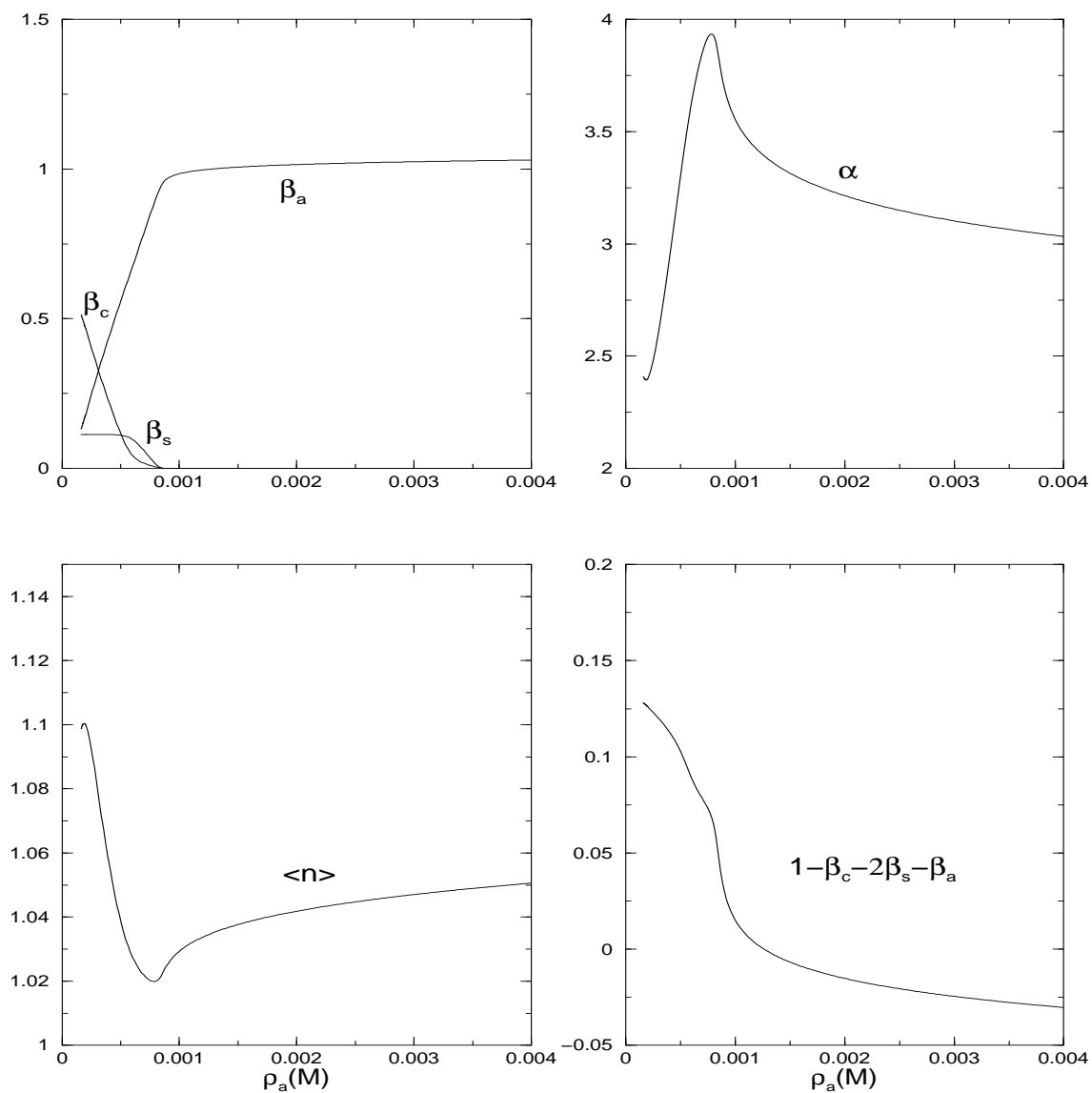
Fig. 7. – The adsorption isotherms for DNA solution from Ref.[17].

addition of cationic surfactants leads to a cooperative binding. This binding happens far below the critical micell concentration. The effect is more strong for divalent ions where overcharing appears.

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