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# Charge inversion in DNA–amphiphile complexes: possible application to gene therapy

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## Abstract

We study complex formation between the DNA and cationic amphiphilic molecules. As the amphiphile is added to the solution containing DNA, a cooperative binding of surfactants to the DNA molecules is found. This binding transition occurs at a specific density of amphiphile, which is strongly dependent on the concentration of the salt and on the hydrophobicity of the surfactant molecules. We find that for amphiphiles which are sufficiently hydrophobic, a charge neutralization, or even charge inversion of the complex is possible. This is of particular importance in applications to gene therapy, for which the functional delivery of specific base sequence into living cells remains an outstanding problem. The charge inversion could, in principle, allow the DNA–surfactant complexes to approach the negatively charged cell membranes permitting the transfection to take place. © 1999 Elsevier Science B.V. All rights reserved.

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## 1. Introduction

In the last few years gene therapy has received significant attention both from the scientific community and from the general public. The development of new techniques for transferring genes into living cells allowed for the potential treatment of several diseases of genetic origin [1–11]. The central problem of gene therapy lies in the development of safe and efficient gene delivery system. Since both the DNA and the cell membranes are negatively charged, the naked polynucleotides are electrostatically prevented from entering the cells. Furthermore, the unprotected DNA is rapidly degraded by nucleases present in plasma [11].

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Although, much effort has concentrated on viral transfection, non-viral methods have received increased attention. This is mostly due to the possible complications which can arise from recombinant viral structures, and the consequent risk of cancer. In the non-viral category, the DNA–liposome complexes have shown the most promise. Cationic liposomes can associate with the DNA segments, neutralizing or even inverting the electric charge of nucleotides, thus significantly increasing the efficiency of gene adsorption and transfection by cells.

In this paper we present a model of DNA–amphiphile solutions. We find that in equilibrium, solution consists of complexes composed of DNA and associated counterions and amphiphiles. As more amphiphiles are added to solution, a cooperative binding transition is found. At the transition point, a large fraction of the DNA’s charge is neutralized by the condensed surfactants. If the density of surfactant is increased beyond this point, a charge inversion of the DNA becomes possible. The necessary density of amphiphile needed to reach the charge inversion is strongly dependent on the characteristic hydrophobicity of surfactant molecules. In particular, we find that for sufficiently hydrophobic amphiphiles, such as for example some cationic lipids, the charge inversion can happen at extremely low densities.

## 2. The model

Our system consists of an aqueous solution of DNA segments, cationic surfactants, and monovalent salt. Water is modeled as a uniform medium of dielectric constant  $D$ . In an aqueous solution, the phosphate groups of the DNA molecules become ionized resulting in a net negative charge. The salt is completely ionized, forming an equal number of cations and anions. Similarly, the surfactant molecules are assumed to be fully dissociated producing negative anions and polymeric chains with cationic head groups.

Following the usual nomenclature, we shall call the ionized DNA molecules the “polyions”, the positively charged ions the “counterions”, and the negatively charged anions the “coions”. To simplify the calculations, all the counterions and coions will be treated as identical, independent of the molecules from which they were derived. The DNA strands will be modeled as long rigid cylinders of length  $L$  and diameter  $a_p$ , with the charge  $-Zq$  distributed uniformly, with separation  $b \equiv L/Z$ , along the major axis. The cations and anions will be depicted as hard spheres of diameter  $a_c$  and charge  $\pm q$ . For simplicity we shall also suppose that each one of the  $s$  surfactant monomers is a rigid sphere of diameter  $a_c$  with the “head” monomer carrying the charge  $+q$ . The interaction between the hydrophobic tails is short ranged and characterized by the hydrophobicity parameter  $\chi$  (see Fig. 1). The density of DNA segments is  $\rho_p = N_p/V$ , the density of monovalent salt is  $\rho_m = N_m/V$ , and the density of amphiphile is  $\rho_s = N_s/V$ , where  $N_i$  is the number of molecules of specie  $i$  and  $V$  is the volume of the system.

The strong electrostatic attraction between the polyions, counterions, and amphiphiles, leads to the formation of complexes consisting of *one* polyion,  $n_c$  counterions, and  $n_s$

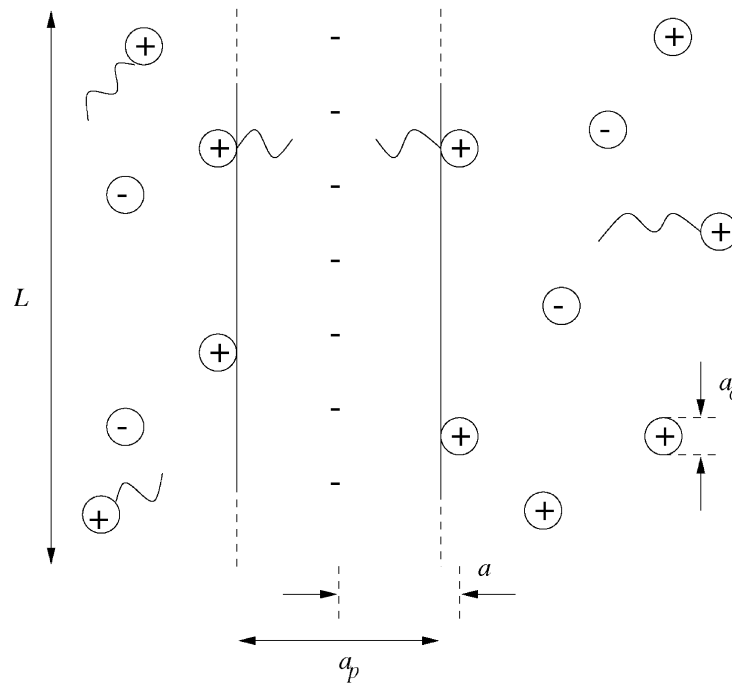


Fig. 1. A cylindrical polyeion of diameter  $a_p$ , length  $L$ , and charge  $-Zq$ , surrounded by spherical ions of radius  $a_c$  and amphiphilic molecules of  $s$  monomers. Each monomer of a macroion is free or has *one* counterion, or a ring made of  $l$  amphiphilic molecules associated with it.

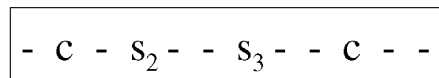


Fig. 2. Schematic representation of a complex. Empty sites (monomers) ( $-$ ), sites with associated counterion ( $c$ ), sites with  $l$  associated amphiphiles ( $s_l$ ).

amphiphilic molecules. We shall assume that to each phosphate group of the DNA molecule can be associated at most *one* counterion or  $l \leq l_{\max}$  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 (see Fig. 2). The  $l$  amphiphilic molecules form a “ring” of radius  $a$  around the central negative monomer of the DNA (see Fig. 3). 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,  $l_{\max} = 2\pi a/a_c$ , where  $a \equiv (a_p + a_c)/2$  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  $l=1, \dots, l_{\max}$  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

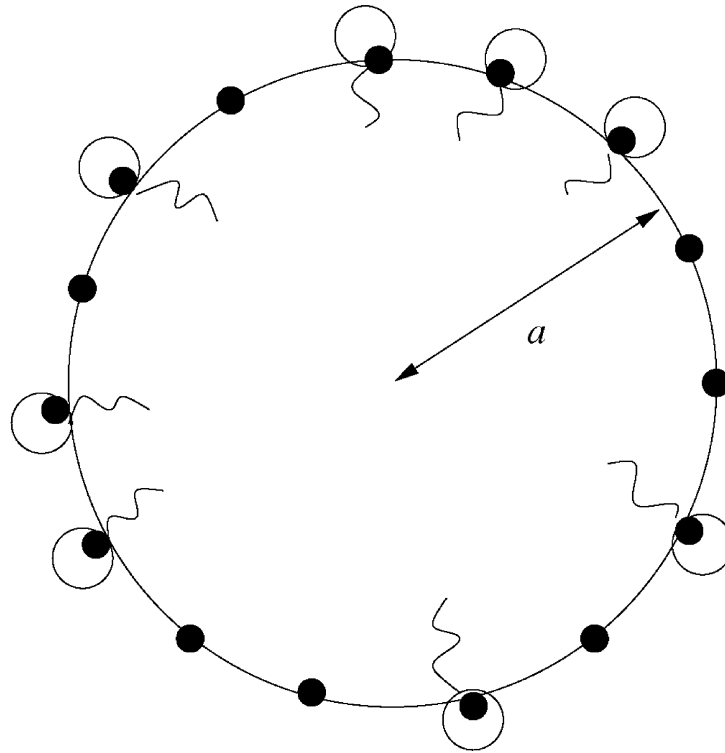


Fig. 3. Ring composed of  $l$  surfactant molecules,  $l_{\max} = 15$ .

$n_c$  is the number of condensed counterions and  $n_l$  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  $n_c$  counterions and  $n_s$  amphiphilic molecules — in rings of  $\{p_l\}$  — with

$$n_s = \sum_{l=1}^{l_{\max}} Zl p_l . \tag{1}$$

The total charge of each polyion is, therefore, renormalized from  $-Zq$  to  $-Z_{\text{eff}}q$ , with  $Z_{\text{eff}} \equiv Z - n_c - n_s$  [12–16]. From overall charge neutrality, the density of free cations is  $\rho_+ = \rho_m + (Z - n_c)\rho_p$ , the density of free anions is  $\rho_- = \rho_m + \rho_s$ , and the density of free surfactants is  $\rho_s^f = \rho_s - n_s\rho_p$ . We shall restrict our attention to the limit of low surfactant densities, so as to prevent micellar formation in the bulk.

The aim of the theory is to determine the characteristic values of  $n_c$ ,  $n_s$ , and the surface coverage by rings  $\{p_l\}$ . To accomplish this, the free energy of the DNA–surfactant solution will be constructed and minimized.

### 3. The Helmholtz free energy

The free energy is composed of three contributions,

$$F = F_{\text{complex}} + F_{\text{electrostatic}} + F_{\text{mixing}} . \tag{2}$$

The first term is the free energy needed to form the isolated complexes. The second term accounts for the electrostatic interaction between the counterions, coions, surfactants and complexes. Finally, the third term is the result of entropic mixing of various species.

To calculate the free energy required to construct an isolated complex composed of one polyion,  $n_c$  condensed counterions, and  $n_s$  condensed surfactants, we employ the following simplified model. Each monomer of a polyion can be free or occupied by a counterion, or by  $1 \leq l \leq l_{\max}$  amphiphiles (see Fig. 2). Therefore, to each monomer  $i$  we associate occupation variables  $\sigma_c(i)$  and  $\{\sigma_l(i)\}$ , which are nonzero if that particular monomer is occupied by a condensed counterion or a ring with  $l$  surfactants, respectively. The free energy of  $N_p$  isolated complexes can then be written as

$$\beta F_{\text{complex}} = -N_p \ln \sum_v e^{-\beta E_v}, \quad (3)$$

where the sum is over all possible configurations of counterions and surfactants along a complex. For a particular configuration  $v$ , the energy can be expressed as the sum of three terms,  $E_v = E_1 + E_2 + E_3$ . The first one is the electrostatic contribution arising from the Coulombic interactions between all charged sites of a complex,

$$E_1 = \frac{q^2}{2} \sum_{i \neq j}^Z \frac{[-1 + \sigma_c(i) + \sum_{l=1}^{l_{\max}} l \sigma_l(i)][-1 + \sigma_c(j) + \sum_{l=1}^{l_{\max}} l \sigma_l(j)]}{D|r(i) - r(j)|}, \quad (4)$$

where we have assumed that the only effect of association is the renormalization of the effective charge of each monomer. The second term  $E_2$ , is due to hydrophobic interactions between the surfactant molecules,

$$E_2 = \frac{\chi}{2} \sum_{\langle i,j \rangle}^Z \sum_{l,l'=1}^{l_{\max}} \frac{(l+l')}{2} \sigma_l(i) \sigma_{l'}(j), \quad (5)$$

where in order to simulate the short-ranged nature of hydrophobic interactions, the first sum is constrained to run over the nearest neighbors. The hydrophobicity parameter  $\chi$  is negative, representing the tendency of the two adjacent surfactant molecules to expel water. We can estimate its value from the experimental measurement of the energy necessary to remove an amphiphile from a monolayer and place it in the bulk [17].

The third contribution  $E_3$ , accounts for the internal energy of each ring,

$$E_3 = \sum_i^Z \sum_{l=2}^{l_{\max}} \sigma_l(i) E_l. \quad (6)$$

$E_l$  is the interaction energy between  $l$  surfactants forming a ring. Each ring contains a maximum of  $l_{\max}$  sites, which can be occupied by surfactants. To each one of these sites we associate an occupation variable  $\tau(j)$ , which is zero if site  $j$  is unoccupied by a surfactant and is one if it is occupied (see Fig. 3). The interaction energy of surfactants forming a ring can then be written as

$$E_l = \frac{q^2}{2D} \sum_{i \neq j}^{l_{\max}} \frac{\tau(i)\tau(j)}{2a \sin(\pi|i-j|/l_{\max})} + \frac{\chi}{2} \sum_{\langle i,j \rangle}^{l_{\max}} \tau(i)\tau(j). \quad (7)$$

The first term of Eq. (7) is due to electrostatic repulsion between the surfactant head groups, while the second is the result of attraction between the adjacent hydrocarbon tails.

The exact solution of even this simpler sub-problem (i.e. evaluation of the sum in Eq. (3)) is very difficult due to the long ranged electrostatic interactions. We shall, therefore, resort to mean-field theory, which works particularly well for long-ranged potentials. Evaluating the upper bound for the free energy, given by the Gibbs–Bogoliubov inequality, and neglecting the end effects we obtain,

$$\beta F_{\text{complex}} = \beta N_p [f_{\text{el}} + f_{\text{hyd}} + f_{\text{ring}} + f_{\text{mix}}]. \quad (8)$$

The first term,

$$\beta f_{\text{el}} = \xi S \left[ -1 + p_c + \sum_{l=1}^{l_{\text{max}}} l p_l \right]^2 - \xi S N_p, \quad (9)$$

is the electrostatic interaction between the sites along one rod and is related to  $E_1$ .  $S$  is expressed in terms of the digamma function [18],

$$S = Z[\Psi(Z) - \Psi(1)] - Z + 1, \quad (10)$$

and  $\xi \equiv \beta q^2 / Db$  is the Manning parameter [19,20]. The second term in Eq. (8),

$$\beta f_{\text{hyd}} = \beta \chi (Z - 1) \sum_{n,m} \frac{(n+m)}{2} p_n p_m, \quad (11)$$

is the hydrophobic attraction between the rings inside a complex.<sup>1</sup> The third term is the free energy due to the electrostatic and hydrophobic interactions between the surfactants forming a ring,

$$\begin{aligned} \beta f_{\text{ring}} = & \frac{2 \ln l_{\text{max}} + v_0}{4\pi T^*} \sum_{l=2}^{l_{\text{max}}} Z p_l l^2 + \frac{\beta \chi}{l_{\text{max}}} \sum_{l=2}^{l_{\text{max}}} Z p_l l^2 \\ & + \sum_{l=1}^{l_{\text{max}}} Z p_l l \ln \left( \frac{l}{l_{\text{max}}} \right) + \sum_{l=1}^{l_{\text{max}}} Z p_l l_{\text{max}} \left( 1 - \frac{l}{l_{\text{max}}} \right) \ln \left( 1 - \frac{l}{l_{\text{max}}} \right), \end{aligned} \quad (12)$$

where  $v_0 \approx 0.25126591$ , and the reduced temperature is  $T^* = k_B T D a / q^2$ . Finally, the free energy of mixing for rings and counterions of a complex is,

$$\begin{aligned} \beta f_{\text{mix}} = & Z \left( 1 - p_c - \sum_l^{l_{\text{max}}} p_l \right) + \ln \left( 1 - p_c - \sum_{l=1}^{l_{\text{max}}} p_l \right) + Z p_c \ln p_c \\ & + Z \sum_{l=1}^{l_{\text{max}}} p_l \ln p_l - Z p \ln l_{\text{max}} + Z p l_{\text{max}} \left( 1 - \frac{1}{l_{\text{max}}} \right) \ln \left( 1 - \frac{1}{l_{\text{max}}} \right), \end{aligned} \quad (13)$$

<sup>1</sup> For the present calculation we shall neglect the additional hydrophobic contribution which arises from the interaction of amphiphiles with the backbone of the DNA.

where to be consistent with expression (12), we have included a contribution to the free energy arising from the azimuthal motion of condensed counterions around the polyion, i.e. the last two terms of Eq. (13).

Once a cluster, constructed in isolation, is introduced into solution, it gains an additional solvation energy due to its interaction with other clusters, free counterions, free coions, and free surfactants. The electrostatic repulsion between the complexes is screened by the ionic atmosphere, producing an effective short-ranged potential of DLVO form [21–25]. The electrostatic free energy due to interactions between various clusters can be estimated from the second virial coefficient,

$$\beta F^{cc} = (Z - n_c - n_s)^2 \frac{2\pi N_p^2 a^3 e^{-2\kappa a}}{VT^*(\kappa a)^4 K_1^2(\kappa a)}, \quad (14)$$

where  $(\kappa a)^2 \equiv 4\pi\rho_1^*/T^*$  and  $\rho_1^* \equiv a^3[\rho_p(Z - n_s - n_c) + 2\rho_m + 2\rho_s]$  is the reduced density of free ions. The free energy due to interaction between the complexes and free ions and surfactants can be obtained following the general methodology of the Debye–Hückel–Bjerrum theory [13,14,26–32],

$$\beta F^{ci} = N_p(Z - n_c - n_s)^2 \frac{(a/L)}{T^*(\kappa a)^2} \left[ -2 \ln(\kappa a K_1(\kappa a)) + I(\kappa a) - \frac{(\kappa a)^2}{2} \right] \quad (15)$$

with

$$I(\kappa a) = \int_0^{\kappa a} \frac{x K_0^2(x)}{K_1^2(x)} dx, \quad (16)$$

where  $K_n$  is the modified Bessel function of order  $n$ . The contribution to the total free energy arising from the interactions between the free ions and surfactants is given by the usual Debye–Hückel expression [26,27]

$$\beta F^{ii} = -\frac{V}{4\pi a_c^3} \left[ \ln(1 + \kappa a_c) - \kappa a_c + \frac{(\kappa a_c)^2}{2} \right]. \quad (17)$$

This term is very small and is included only for completeness.

The last contribution to the total free energy, Eq. (2), results from the entropic mixing of the counterions, coions, surfactant and complexes,

$$F_{\text{mixing}} = F_{m+} + F_{m-} + F_s + F_c. \quad (18)$$

The free energy of mixing is obtained following the general ideas introduced by Flory [33],

$$\begin{aligned} \beta F_{m+} &= N_{m+} \ln \phi_{m+} - N_{m+}, \\ \beta F_{m-} &= N_{m-} \ln \phi_{m-} - N_{m-}, \\ \beta F_s &= N_s \ln(\phi_s/n_s) - N_s, \\ \beta F_c &= N_p \ln \left( \frac{(Z + n_c + n_s)\phi_c}{Z + n_c + n_s} \right) - N_p. \end{aligned} \quad (19)$$

In the above expression  $m+$  denotes free counterions,  $m-$  free coions,  $s$  free surfactant molecules, and  $c$  complexes. The

$$\begin{aligned}\phi_{m+} &= \frac{\pi\rho_+^*}{6} \left(\frac{a_c}{a}\right)^3, \\ \phi_{m-} &= \frac{\pi\rho_-^*}{6} \left(\frac{a_c}{a}\right)^3, \\ \phi_s &= \frac{s\pi\rho_s^{f*}}{6} \left(\frac{a_c}{a}\right)^3, \\ \phi_c &= \pi\rho_p^* \left[ \frac{1}{4(a/L)} \left(\frac{a_p}{a}\right)^2 + \frac{1}{6}(n_c + n_s s) \left(\frac{a_c}{a}\right)^3 \right]\end{aligned}\quad (20)$$

are the volume fractions occupied by the free counterions, coions, surfactants, and complexes, respectively.

#### 4. Results and conclusions

The equilibrium configuration of the polyelectrolyte–surfactant solution is determined by the requirement that the Helmholtz free energy be minimum. Since  $F$  is the function of  $n_s, n_c$ , and the surface coverage by rings  $\{p_l\}$ , minimization of  $F$  implies that

$$\delta F = \frac{\partial F}{\partial n_s} \delta n_s + \frac{\partial F}{\partial n_c} \delta n_c + \sum_{l=1}^{l_{\max}} \frac{\partial F}{\partial p_l} \delta p_l = 0. \quad (21)$$

Using the constraint Eqs. (1) and (2) can be separated into  $l_{\max} + 1$  equations,

$$\frac{\partial F}{\partial n_c} = 0 \quad (22)$$

and

$$\frac{\partial F}{\partial n_s} Zl + \frac{\partial F}{\partial p_l} = 0, \quad l = 1, \dots, l_{\max}. \quad (23)$$

The system of Eqs. (22) and (23) can, in principle, be solved numerically. However, for reasonable values of  $l_{\max}$  this requires a significant numerical effort. Instead of pursuing this brute force method, we note that to a reasonable accuracy, the surface coverage by rings,  $\{p_l\}$ , can be approximated by an exponential distribution [34],

$$p_l = \frac{n_s e^{\alpha l}}{Z \sum_{l=1}^{l_{\max}} l e^{\alpha l}}. \quad (24)$$

We have checked that this is, indeed, a good approximation by numerically solving Eq. (23) for an isolated complex. Using ansatz (24), the total free energy becomes a function of  $n_c, n_s$ , and  $\alpha$ . For a fixed volume and number of particles, the equilibrium



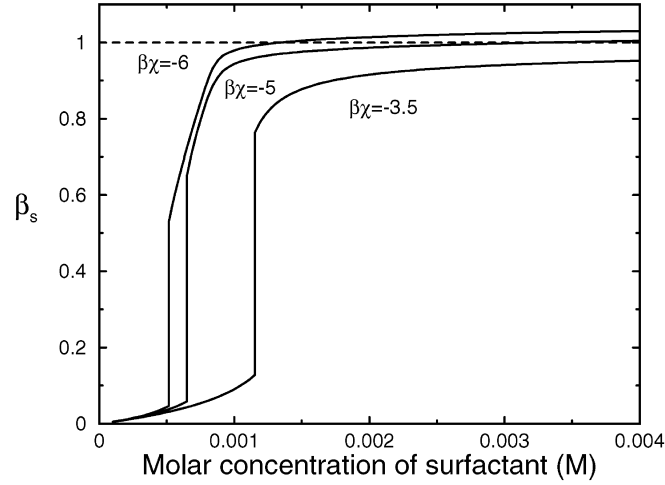


Fig. 4. Effective binding fraction of amphiphiles  $\beta_s \equiv n_s/Z$ , as a function of amphiphile concentration  $\rho_s$ . The concentrations of DNA and of added salt is  $2 \times 10^{-6}$  M and 18 mM, respectively. The length of the DNA segments is 220 base pairs. The solvent is water at room temperature, so that  $\zeta = 4.17$ .

corresponds to the minimum of Helmholtz free energy,

$$\frac{\partial F}{\partial n_c} = 0, \quad (25)$$

$$\frac{\partial F}{\partial n_s} = 0, \quad (26)$$

$$\frac{\partial F}{\partial \alpha} = 0. \quad (27)$$

These are three coupled algebraic equations, which can be easily solved numerically to yield the characteristic number of condensed counterions, surfactants, as well as the shape of the distribution of ring sizes ( $\alpha$ ). In Figs. 4 and 5 we present a numerical solution of these equations. As a specific example we consider a cationic surfactant with an alkyl chain of  $s = 12$  groups. In this case the hydrophobicity parameter can be estimated [30] to be in the range of  $\chi \approx -3,5k_B T$ . To explore the dependence of condensation on the hydrophobicity of surfactant, we shall vary this value within reason. The density of monovalent salt and the DNA is taken to be 18 and  $2 \times 10^{-3}$  mM, respectively.

The resulting binding isotherms are illustrated in Fig. 4. The fraction of associated amphiphilic molecules  $\beta_s = n_s/Z$ , is plotted against the density of surfactant for a fixed amount of monovalent salt,  $\rho_m$ . For small concentrations of cationic surfactant, few amphiphilic molecules associate with the DNA segments. At a certain critical concentration, however, the system forms surfoplexes [30,32] — complexes in which the charge of the DNA is almost completely neutralized by the associated amphiphiles. If the density is increased further, on average, more than one surfactant molecule will associate to each phosphate group, leading to charge inversion of the surfoplexes. For highly hydrophobic surfactants the charge inversion can happen very close to the

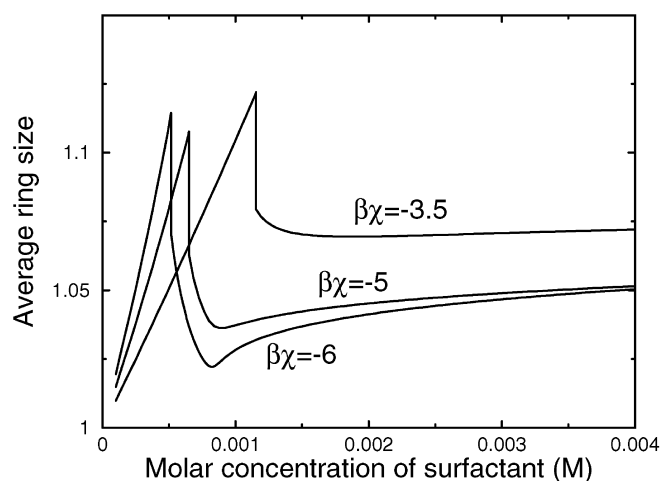


Fig. 5. Average size of rings in a complex (parameters are the same as in Fig. 4).

cooperative binding transition. We note that our theory predicts the binding transition to be discontinuous, this, most likely, is an artifact of the mean-field approximation [32].

We have presented a simple theory of DNA–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 addition of cationic surfactants leads to a strong cooperative binding transition. This transition happens far below the critical micell concentration. A further increase of amphiphile density can result in charge inversion of the DNA–surfactant complexes. This regime should be particularly useful in designing gene or oligonucleotide delivery systems. Until now most of nonviral gene–delivery systems were in the form of lipoplexes — complexes formed by DNA and cationic liposomes. To form the liposomes, however, is required a significant concentration of cationic lipid. Unfortunately, at high concentrations both lipids and surfactants are toxic to organism. Our model suggests that the charge inversion can be achieved with quite a small concentration of cationic amphiphile, *if* it is sufficiently hydrophobic. This should reduce the risk of unnecessary medical complications.

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