

## Mesoscale Computer Modeling of Lipid-DNA Complexes for Gene Therapy

Oded Farago

*Department of Biomedical Engineering, Ben Gurion University, Be'er Sheva 84105, Israel*

Niels Grønbech-Jensen

*Department of Applied Science, University of California, Davis, California 95616, USA*

Philip Pincus

*Physics Department, University of California, Santa Barbara, California 93106, USA,  
and Physics Department, Korea Advanced Institute of Science and Technology (KAIST),  
373-1 Kusong-dong, Yusong-gu, Taejon 305-701, Korea*

(Received 1 August 2005; published 4 January 2006)

We report on a molecular simulation method, which captures the self-assembly of cationic lipid-DNA (CL-DNA) gene delivery complexes. Computational efficiency required for large length- and time-scale simulations is achieved through a coarse-grained representation of the intramolecular details and via intermolecular potentials, which effectively mimic the hydrophobic effect *without* an explicit solvent. The broad utility of the model is illustrated by demonstrating excellent agreement with x-ray diffraction experimental data for the dependence of the spacing between DNA chains on the concentration of CLs. At high concentrations, the large electrostatic pressure induces the formation of pores in the membranes through which the DNA molecules may escape the complex. We relate this observation to the origin of recently observed enhanced transfection efficiency of lamellar CL-DNA complexes at high charge densities.

DOI: [10.1103/PhysRevLett.96.018102](https://doi.org/10.1103/PhysRevLett.96.018102)

PACS numbers: 87.15.-v, 87.16.Ac, 87.16.Dg, 82.70.-y

Modeling and simulation of biological systems pose significant challenges due to the spatial complexity of such systems and by the accompanying range of temporal scales involved. In the case of lipid bilayers, the width of the bilayer is in the nanometer range, whereas the lateral dimension may extend up to several micrometers. The corresponding time scales span an even larger range of orders of magnitude. To address the multiscale nature of membranes, a variety of models have been devised which differ in the length and time scales of the phenomena of interest [1]. The two most extreme approaches are (i) atomistic computer simulations, in which the lipids and the embedding solvent are modeled explicitly in full detail [2], and (ii) phenomenological models such as the effective Helfrich Hamiltonian, where the bilayer membrane is treated as a smooth continuous surface with a small number of elastic coefficients [3].

The gap between those two approaches can be bridged by coarse-grained (CG) computer models, in which groups of several atoms are represented by unified particles interacting via effective potentials [4]. The essential benefit of such models is that they have condensed the most detailed variations in both time and space while retaining the fundamental properties of membrane self-assembly and elasticity. The level of detail employed by different CG models varies greatly. Some CG models are constructed to mimic specific lipidic systems by selecting simplified representations for water molecules and different chemical groups which constitute the lipids and by developing effective force fields that reproduce key structural features known

from experiments and atomistic simulations [5]. In coarser-grained models, the amphiphilic molecules are constructed from only two types of particles—hydrophilic and hydrophobic—which are attracted and repelled, respectively, from a third kind of particles representing the solvent [6]. Such models address more general features of self-assembly rather than specific systems.

More recently, a number of research groups have developed CG models in which bilayers are simulated without direct representation of an embedding solvent; this is accomplished by constructing intermolecular force fields that mimic effects of hydration [7–10]. The development of these “water-free” models is an important advance in large-scale membrane simulations, considering the fact that the number of solvent particles in conventional CG models is significantly larger than the number of lipids. Most of these models employ simple Lennard-Jones-type pair potentials [8–10] and exhibit spontaneous self-assembly to a membrane as demonstrated in Fig. 1(a).

The computational simplicity and efficiency of water-free CG models make them excellent tools for testing mesoscopic scale theories of membranes and gaining insight into the basic molecular mechanisms that govern these systems. More importantly, these models may serve as platforms for CG simulations of more complex systems including biopolymers and networks. This Letter reports on such a computational study of complexes of cationic lipid and DNA (CL-DNA) in the context of recent experiments on molecular assemblies. The molecular assemblies are formed spontaneously when DNA is mixed with cati-

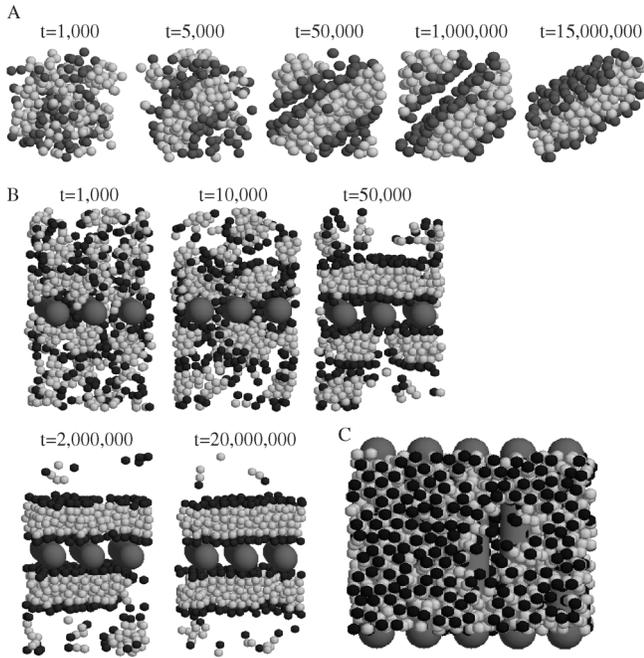


FIG. 1. (a) Self-assembly of a 100 lipid bilayer patch. Lipids are modeled as trimers with hydrophilic (black) and hydrophobic (light gray) particles. The membrane in the last figure has been rotated for a better viewing of its structure. (b) Self-assembly of a CL-DNA complex consisting of 240 lipids (of which 90 are charged). Dark gray rods represent DNA segments. (c) Equilibrium configuration of a complex with  $\phi_c \sim 0.9$  whose membranes develop pores.

onic and neutral lipids in an aqueous environment [11,12]. Their formation is driven by the electrostatic attraction between the negatively charged DNA and the CL headgroups and by the entropic gain associated with the concurrent release of the tightly bound counterions from the CL and the DNA [11–13]. X-ray diffraction experiments have revealed that CL-DNA complexes exist in a variety of mesoscopic structures [14], including a lamellar phase where the DNA is intercalated in between lipid bilayers [11]. The DNA strands form a 1D ordered array, where the DNA interaxial spacing  $d_{\text{DNA}}$  decreases with the charge density of the membranes. Isoelectric complexes, where the charges on the DNA exactly match those on the CL, are the most stable ones since they enable nearly complete counterion release [13].

CL-DNA complexes have attracted much attention because of their potential use as nonviral transfection vectors in gene therapy [14–17]. Cationic liposome transfer vectors exhibit low toxicity, nonimmunogenicity, and ease of production [15,18], but their transfection efficiency (TE) remains low compared to that of viral vectors [19,20]. This has spurred an intense research activity aimed at enhancing TE [16,19,20]. Recognizing that the structure of CL-DNA complexes may strongly influence their function and TE, much of the effort in theoretical and experimental studies has been devoted to understanding the mechanisms gov-

erning complex formation, structure, and phase behavior [13,14,21–23].

To our best knowledge, only a single attempt has so far been made to study CL-DNA complexes using molecular simulations [24]. That study employed a fully atomistic description of both the lipids and the DNA and has, consequently, been limited to a small system of 48 lipids (of which 20 were charged) and a short DNA segment of 10 base pairs. The duration of the simulations was of several nanoseconds. The mesoscopic regime encompassing the statistics and evolution of large molecular ensembles is, however, inaccessible to full atomistic simulations due to the computational requirements, and only continuum behavior of existing CL-DNA structures can be addressed based on free energy functionals which are insensitive to the fine molecular details [21]. Thus, our understanding of many important CL-DNA complex features, such as the process of self-assembly and membrane evolution, mesoscopic structure, and defects, can be addressed only through CG simulations.

The model presented here is based on the CG water-free membrane model, which produces self-assembled bilayer membranes such as the one depicted in Fig. 1(a); model details are given in Ref. [8]. The lipids consist of one hydrophilic (representing the headgroup) and two hydrophobic (representing the tail) spherical particles with short-range pair interactions between them. We set the diameter of these particles  $\sigma \approx 6.3 \text{ \AA}$  (see the definition of  $\sigma$  in Ref. [8]). For this value of  $\sigma$ , the area per lipid in the original model  $a_{\text{lipid}} \approx 70 \text{ \AA}^2$ . In the present model, a fraction  $\phi_c$  of the hydrophilic headgroups carry charge  $+e$ . DNA is modeled as a rigid rod with a uniform axial charge density  $\lambda_{\text{DNA}} = -e/1.7 \text{ \AA}^2$  and radius  $R_{\text{DNA}} = 10 \text{ \AA}$ . Excluded volume interactions between rods ( $R$ ) and spheres ( $S$ ) are introduced via a truncated and shifted potential of the form:  $U_{RS}/k_B T = 50\{[(\sigma/2 + R_{\text{DNA}})/r]^{12} - 1\}$ , where  $r$  is the distance between the center of the sphere and the axis of symmetry of the rod. The distance between nearest-neighbor rods is restricted to  $d_{\text{DNA}} \geq 2R_{\text{DNA}}$ .

We study isoelectric complexes where the total charges of the DNA and the CLs neutralize each other, with no added counterions. Simulations of the quasi-2D complex are conducted in a rectangular system of size  $L_x \times L_y \times L_z$ , with full periodic boundaries along the  $x$  and  $y$  directions and periodicity with respect to only lipid mobility and short-range interactions in the  $z$  direction. Electrostatic interactions and their periodic images are accounted for by full resummation [25]. Simulations were performed at room temperature and with a bulk water uniform dielectric constant  $\epsilon = 78$  [26]. The rods are arranged in a 1D array with equal spacing in the  $xy$  plane along the  $y$  direction. We first examined the ability of the complex to self-assemble spontaneously: 240 lipids, of which 90 are charged, were randomly distributed in a box of size  $L_x = 94 \text{ \AA}$ ,  $L_y = 51 \text{ \AA}$ ,  $L_z = 157 \text{ \AA}$ . Three equally spaced DNA strands, each carrying a total charge  $-30e$ ,

were placed at the midplane  $z = L_z/2$  of the box. Canonical ensemble Monte Carlo (MC) simulations were used to generate the temporal evolution of the lipids, while the positions of the rods were fixed. Each MC step consists of (on average) an attempt to translate (and make some minute changes in the relative locations of the three particles with respect to each other) and rotate each lipid. The typical evolution of the system is depicted in Fig. 1(b). The figure demonstrates that a complex is formed quite rapidly, but full association of the lipids is much slower and has not been accomplished by the end of the MC run. The large majority of CLs (except for, typically, as few as 3–4 molecules) tend to reside in the two inner monolayers facing the DNA array, as expected in view of the strong electrostatic attraction between the CLs and DNA. The final snapshot in Fig. 1(b) is typical for an equilibrium distribution of free and complexed lipids. On the other hand, preassembled complexes appear to be stable with respect to dissociation and lipids loss, even in long MC runs.

One of the parameters which is believed to strongly influence the TE is the membrane charge density  $\sigma_M = 2e\phi_c/a_{\text{lipid}}$  [20]. It was found in x-ray diffraction experiments that, for isoelectric complexes, the DNA interaxial spacing  $d_{\text{DNA}}$  is related to  $\sigma_M$  by [11,14]

$$d_{\text{DNA}} = \frac{\lambda_{\text{DNA}}}{\sigma_M} = \left( \frac{a_{\text{lipid}} \lambda_{\text{DNA}}}{2e} \right) \frac{1}{\phi_c}. \quad (1)$$

Measuring  $d_{\text{DNA}}$  and verifying this result can serve as a test for our model's ability to mimic the mesoscale behavior of CL-DNA complexes. We have, therefore, performed a set of MC simulations of preassembled isoelectric complexes consisting of 300 charged lipids, an array of five equally spaced DNA molecules of length  $L_y = 102 \text{ \AA}$  (i.e., each carrying a total charge  $-60e$ ), and an additional number of neutral lipids. Simulations have been performed using the constant surface tension ensemble ( $N, \gamma, T$ ), with  $\gamma = 0$  [9,27]. The area of the complex has been changed by allowing flexibility in  $L_x$  (rescaling the  $x$  coordinates of the particles accordingly), keeping  $L_y$  fixed. Attempts to change the area of the system were made, on average, twice every MC time unit. Two flat bilayers, each consisting of 150 cationic and  $(2N - 150)$  neutral lipids, were initially set up on both sides of the DNA array with the headgroups in the inner (outer) monolayers at distance  $0.5\sigma + R_{\text{DNA}}$  ( $5.5\sigma + R_{\text{DNA}}$ ) from the complex midplane. Each monolayer consisted of  $N$  lipids, with the CLs occupying the two inner monolayers while the outer monolayers consist of neutral lipids only. This distribution of lipids remained unchanged over the course of the simulations (i.e., there was no diffusion of lipids between the monolayers on the time scale of the simulations). The fraction of charged lipids is defined as the number ratio of charged to neutral lipids in the inner monolayers,  $\phi_c = 150/N$ . The preassembled complexes were allowed to equilibrate for  $5 \times 10^5$  steps prior to data collections over at least  $2 \times 10^6$  additional steps. Membrane fluidity

was verified by lateral diffusion of both neutral and charged lipids.

The average spacing between adjacent DNA rods  $d_{\text{DNA}}$  is plotted in Fig. 2 as a function of  $1/\phi_c$ . The numerical data is in excellent agreement with the experimental results reported in Ref. [11]. The solid line is obtained from Eq. (1) with  $a_{\text{lipid}} = 69 \text{ \AA}^2$ . The deviation from linear behavior at high charge densities arises from the increase in the area per lipid as  $\phi_c$  (see Fig. 3: left y axis, solid circles). Similar deviation is observed in the experiment [28]. The assumption underlying Eq. (1) is that the effective interactions between the DNA are repulsive and balanced by the elastic membrane forces. According to Hooke's law, the elastic stress acting on a membrane is related to  $a_{\text{lipid}}$  and its equilibrium value  $a_{\text{lipid}}^0$  by  $\tau = K_A(a_{\text{lipid}} - a_{\text{lipid}}^0)/a_{\text{lipid}}^0$ , where  $K_A$  is the 2D stretching modulus, which for lipid bilayers is typically in the range  $K_A \geq 10^2 \text{ ergs/cm}^2$ . At high charge densities, the electrostatic stress is sufficiently large to eliminate the membrane thermal undulations and increase  $a_{\text{lipid}}$  [29]. In the present study, we find for complexes with  $\phi_c \sim 0.85$  that the strain  $\varepsilon \equiv (a_{\text{lipid}} - a_{\text{lipid}}^0)/a_{\text{lipid}}^0 \sim 0.1$ , which is the typical strain lipid bilayers withstand before rupture [30]. Membranes with higher  $\phi_c$  have indeed been found to be susceptible to pore formation, as illustrated by the configuration in Fig. 1(c) of a complex with  $\phi_c \sim 0.9$ . The loss of mechanical stability is also evident from the rapid decrease in the effective stretching modulus of the complex  $K_A^*$  for  $\phi_c > \sim 0.7$  (Fig. 3: right y axis, open squares), which has been extracted from the mean square of fluctuations in  $a_{\text{lipid}}$ :  $K_A^* = k_B T a_{\text{lipid}}^0 / [N \langle (a_{\text{lipid}} - a_{\text{lipid}}^0)^2 \rangle]$ . The larger area fluctuations at high  $\phi_c$  increase the probability of pore opening, which, in turn, may lead to disassociation of the complex. We suggest that this may be the origin of the recently observed enhanced TE of lamellar CL-DNA complexes at high charge densities [20]. Transfection is viewed as a two-stage process: (i) cellular uptake via endocytosis and (ii) escape of the complex from the endosome, pre-

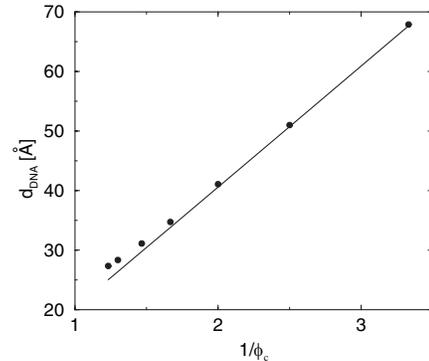


FIG. 2. Average DNA spacing  $d_{\text{DNA}}$  as a function of the inverse of the fraction of charged lipids  $1/\phi_c$ . Markers—numerical results (uncertainties are smaller than symbols); solid line—fit to Eq. (1) with  $a_{\text{lipid}} = 69 \text{ \AA}^2$ .

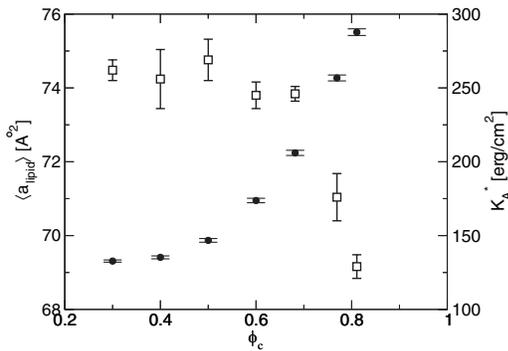


FIG. 3. (a) Left y axis, solid circles—average area per lipid  $a_{\text{lipid}}$  as a function of the fraction of charged lipids  $\phi_c$ . (b) Right y axis, open squares—effective stretching modulus  $K_A^*$  of the complex as a function of  $\phi_c$ .

sumably via fusion of the lipids with the endosomal membrane and release of the DNA into the cytoplasm. TE of lamellar complexes is limited by the rate of the second stage and, hence, increases with the decrease of mechanical stability, i.e., with increase of charge density.

In summary, the structural-mechanical properties of CL-DNA complexes have been studied using a coarse-grained water-free model. Despite the highly simplified representation of lipids and DNA, the model reliably demonstrates self-assembly of both a charge neutral bilayer lipid membrane and a lipid membrane DNA complex. The model reproduces accurately the measured interaxial spacing  $d_{\text{DNA}}$  over a wide range of charge densities. At high charge densities, we observe that the increasing electrostatic pressure exerted on the membranes leads to pore opening, through which the DNA may be released from the complex. The DNA release to the cytoplasm is a necessary step for successful transfection, and, thus, our results explain the enhancement of transfection efficiency at high concentrations of CLs. Given the consistent agreement between our course-grained molecular approach and observed experimental features, we suggest that the presented model is an appropriate and promising tool for investigating the statistics and dynamics of lipid-DNA complexes on spatial and temporal scales relevant for biological and biomedical applications.

We are grateful to C.R. Safinya for many useful discussions. O.F. and P.P. acknowledge the support of the MRL Program of the National Science Foundation under Grant No. DMR00-80034 and NSF Grant No. DMR-0503347.

- 
- [1] M. Müller, K. Katsov, and M. Schick, *J. Polym. Sci., Part B: Polym. Phys.* **41**, 1441 (2003), and references therein.  
 [2] L. Saiz, S. Bandyopadhyay, and M.L. Klein, *Biosci. Rep.* **22**, 151 (2002), and references therein.  
 [3] *Structure and Dynamics of Membranes*, edited by R. Lipowsky and E. Sackmann (Elsevier, Amsterdam, 1995).

- [4] S. O. Nielsen, C. F. Lopez, G. Srinivas, and M. L. Klein, *J. Phys. Condens. Matter* **16**, R481 (2004).  
 [5] E. Lindahl and O. Edholm, *J. Chem. Phys.* **113**, 3882 (2000); S. J. Marrink and A. E. Mark, *J. Phys. Chem. B* **105**, 6122 (2001).  
 [6] R. Goetz and R. Lipowsky, *J. Chem. Phys.* **108**, 7397 (1998).  
 [7] H. Noguchi and M. Takasu, *Phys. Rev. E* **64**, 041913 (2001).  
 [8] O. Farago, *J. Chem. Phys.* **119**, 596 (2003); O. Farago and P. Pincus, *J. Chem. Phys.* **120**, 2934 (2004).  
 [9] G. Brannigan, A. C. Tamboli, and F. L. H. Brown, *J. Chem. Phys.* **121**, 3259 (2004).  
 [10] I. R. Cooke, K. Kremer, and M. Deserno, *Phys. Rev. E* **72**, 011506 (2005).  
 [11] I. Koltover, T. Salditt, J. O. Rädler, and C. R. Safinya, *Science* **275**, 810 (1997).  
 [12] J. O. Rädler, I. Koltover, T. Salditt, and C. R. Safinya, *Science* **281**, 78 (1998).  
 [13] D. Harries, S. May, W. M. Gelbart, and A. Ben-Shaul, *Biophys. J.* **75**, 159 (1998).  
 [14] C. R. Safinya, *Curr. Opin. Struct. Biol.* **11**, 440 (2001).  
 [15] P. L. Felgner, *Sci. Am.* **276**, No. 06, 102 (1997).  
 [16] P. R. Clark and E. M. Hersh, *Curr. Opin. Mol. Ther.* **1**, 158 (1999).  
 [17] S. Li and L. Huang, *Gene Therapy* **7**, 31 (2000).  
 [18] H. Kamiya, H. Tsuchiya, J. Yamazaki, and H. Harashima, *Adv. Drug Deliv. Rev.* **52**, 153 (2001).  
 [19] H. F. Willard, *Science* **290**, 1308 (2000).  
 [20] K. Evert *et al.*, *Curr. Med. Chem.* **11**, 133 (2004).  
 [21] S. May and A. Ben-Shaul, *Curr. Med. Chem.* **11**, 151 (2004).  
 [22] R. Bruinsma, *Eur. Phys. J. B* **4**, 75 (1998).  
 [23] D. Harries, S. May, and A. Ben-Shaul, *J. Phys. Chem. B* **107**, 3624 (2003).  
 [24] S. Bandyopadhyay, M. Tarek, and M. L. Klein, *J. Phys. Chem. B* **103**, 10 075 (1999).  
 [25] N. Grønbech-Jensen, G. Hummer, and K. M. Beardmore, *Mol. Phys.* **92**, 941 (1997).  
 [26] Simulations of electrostatics in water-free models are usually conducted with a uniform dielectric constant due to the complexity of including, e.g., mirror charges in a disordered molecular ensemble. Studies of electrostatics near simple geometric interfaces indicate a minor net effect of mirror charges; see, e.g., R. J. Mashl and N. Grønbech-Jensen, *J. Chem. Phys.* **109**, 4617 (1998), and references therein.  
 [27] An alternative scheme [O. Farago (to be published)] for sampling the  $(N, \gamma, T)$  ensemble has been used in this work.  
 [28] The experimental results reported in Ref. [11] [see Fig. 4(B)] show agreement with Eq. (1) at low charge densities (with a value of  $a_{\text{lipid}}$  which is slightly different than the one defined here) and a similar deviation trend at high charge densities. Note that our Eq. (1) and the comparable one in Ref. [11] express the same relationship in different forms. See discussion in I. Koltover, T. Salditt, and C. R. Safinya, *Biophys. J.* **77**, 915 (1999).  
 [29] A. W. C. Lau, and P. Pincus, *Eur. Phys. J. B* **10**, 175 (1999).  
 [30] D. Boal, *Mechanics of the Cell* (Cambridge University Press, Cambridge, England, 2002).