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# Molecular Mechanism of Calcium-Induced Adsorption of DNA on Zwitterionic Phospholipid Membranes

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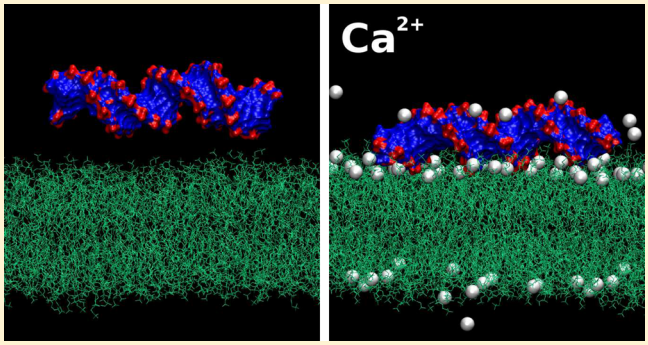
**ABSTRACT:** Interaction of DNA with zwitterionic phospholipids is an important long-standing problem in the field of liposome-based gene delivery. Although it is well-established that divalent cations can promote formation of stable DNA–phospholipid complexes, the underlying molecular mechanism remains largely unknown. Here we employ computer simulations to gain atomistically resolved insight into the kinetics of calcium-induced adsorption of DNA on zwitterionic phosphatidylcholine membranes as well as into the structure and stability of the resulting complexes. Overall, our findings show that calcium ions play a dual role in DNA–phospholipid systems. First, binding of divalent cations to the lipid–water interface turns the surface of the zwitterionic membrane positively charged, promoting thereby the initial electrostatic attraction of a polyanionic DNA molecule. Second, we show that calcium ions are crucial for stabilizing the DNA–lipid membrane complex as they bridge together phosphate groups of DNA and lipid molecules. In contrast to previous hypotheses, we demonstrate that direct interactions between choline groups of phospholipids and DNA phosphates play only a rudimentary role as they are relatively short-lived and unstable: typical residence times for such interactions are 2 orders of magnitude smaller than those for Ca-mediated bridges between DNA and lipid phosphate groups. The results of our study can serve as a basis for a deeper understanding of molecular mechanisms behind noncovalent binding of DNA and DNA-based nanodevices to complex surfaces such as cell membranes.




## 1. INTRODUCTION

Interactions of DNA with zwitterionic phospholipid molecules are essential from the point of view of numerous biomedical applications. One well-known example is related to lipid-based DNA delivery vectors for gene therapy.[1] Although the major components of such nonviral delivery vectors are cationic lipids that are capable of direct electrostatic attraction with anionic DNA molecules, zwitterionic (neutral) phospholipids are also involved in corresponding formulations as "helper lipids".[2,3] What is more, replacing cationic lipids with zwitterionic ones has recently attracted a great deal of attention.[4] Indeed, such an approach offers many potential advantages as the use of cationic species is known to be accompanied by cytotoxicity,[5] while zwitterionic phospholipids, being natural components of cellular membranes, are completely nontoxic. In addition to gene delivery vectors, the DNA–lipid interactions also play an important role in interactions of DNA-based nanodevices with plasma membranes of living cells[6] and can possibly be encountered in the cell nucleus.[7]

In contrast to cationic lipid systems, anionic DNA molecules do not adsorb spontaneously on electrically neutral, zwitterionic phospholipid membranes. However, many experimental studies have repeatedly demonstrated that DNA–zwitterionic lipid association can be triggered by divalent cations such as calcium.[4,8−12] It was hypothesized that $Ca^{2+}$ ions adsorb on a lamellar zwitterionic phospholipid structure making it effectively cationic.[9,11,12] As far as phosphatidylcholine (PC) lipids are concerned, two distinct binding modes for DNA and lipid molecules in the presence of calcium ions were identified: (i) $Ca^{2+}$ ions can bind to phosphate groups of lipid molecules and induce a reorientation of phospholipid polar head groups, so that their positively charged choline moieties interact with DNA's phosphate groups, and (ii) $Ca^{2+}$ ions can bridge phosphate groups of DNA and PC lipids.[9] It was suggested that direct interactions between positively charged moieties of lipid head groups and negatively charged DNA phosphate groups could play a dominant role in the stability of the DNA–lipid complexes.[9] However, to the best of our knowledge, no molecular-level support for this hypothesis has been provided by far. It should also be noted that interactions of choline groups of PC lipids and DNA phosphate groups [type (i) in the above classification] were witnessed in a computational study of complexes of DNA and mixed cationic/zwitterionic lipid bilayers.[13] Furthermore, several attempts to characterize such DNA-lipid-divalent cation complexes with the use of theoretical approaches have also been reported.[14,15] Unfortunately, the

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exact molecular mechanism of the Ca-mediated DNA adsorption on a zwitterionic phospholipid membrane as well as the detailed structure of the resulting DNA−lipid complexes remain poorly understood.

In the present study, we employ state-of-the-art computer simulations to unlock the molecular mechanism of Ca-mediated adsorption of DNA on zwitterionic phospholipid bilayer membranes. To this end, we explore interactions between a DNA fragment and a phosphatidylcholine lipid membrane in aqueous solution with and without $CaCl_2$ salt. The use of computational models of atomistic resolution allowed us to follow closely the kinetics of the DNA adsorption and to gain unprecedented insight into the structure of Ca-triggered DNA−lipid membrane complexes. In particular, our study is the first where a relative importance of various types of interactions between DNA, zwitterionic phospholipids, and divalent cations is evaluated.

## 2. METHODS

We have performed atomic-scale molecular dynamics simulations of a palmitoyl-oleoyl-phosphatidylcholine (POPC) lipid membrane with a short double helix of DNA that was placed in the aqueous phase parallel to the membrane surface. The initial distance between a DNA fragment and a lipid membrane was defined as the distance between closest DNA and lipid atoms along the membrane normal. Three initial distances were considered: 0.18, 0.5, and 1 nm. For each distance, MD simulations of DNA−membrane systems with 100 mM of $CaCl_2$ salt and without divalent salt were performed. Furthermore, for the Ca-DNA-membrane system with the initial distance of 0.5 nm, three additional simulations (with different starting DNA orientations as well as under the presence of NaCl salt) were carried out. A complete set of simulated systems is presented in Table 1.

**Table 1. Simulated DNA-Lipid Membrane Systems**

| DNA-membrane system | initial distance (nm) | $CaCl_2$ salt (mM) | simulation time (ns) |
|---|---|---|---|
| DNA-POPC-0.18 | 0.18 | – | 100 |
| DNA-POPC-0.5 | 0.5 | – | 150 |
| DNA-POPC-1.0 | 1.0 | – | 100 |
| DNA-POPC-Ca-0.18 | 0.18 | 100 | 150 |
| DNA-POPC-Ca-0.5[a] | 0.5 | 100 | 150 |
| DNA-POPC-Ca-0.5[b] | 0.5 | 100 | 150 |
| DNA-POPC-Ca-0.5-NaCl[c] | 0.5 | 100 | 150 |
| DNA-POPC-Ca-0.5 | 0.5 | 100 | 600 |
| DNA-POPC-Ca-1.0 | 1.0 | 100 | 600 |

[a]The initial configuration of the system differs from that of the DNA-POPC-Ca-0.5 system by 90 deg DNA rotation in the membrane plane. [b]The initial configuration of the system differs from that of the DNA-POPC-Ca-0.5 system by 90 deg DNA rotation around the main DNA axis. [c]The initial configuration of the system differs from that of the DNA-POPC-Ca-0.5 system by the presence of 100 mM of NaCl.

A typical DNA-membrane system consists of a DNA helix (a double Dickerson's dodecamer[16,17] of 24 base pairs with the total charge of −46e), 46 $Na^+$ counterions, a POPC lipid membrane of 288 lipids, and ∼25000 water molecules; the total number of atoms in the system amounts to ∼115000. The systems with $CaCl_2$ salt additionally contains 45 $Ca^{2+}$ ions and 90 $Cl^-$ ions, which corresponds to ∼100 mM of $CaCl_2$ (the salt concentration was calculated with respect to the total number of water molecules in the system). To evaluate the effect of NaCl salt, 45 $Na^+$ ions and 45 $Cl^-$ ions (∼100 mM of NaCl) were added to one of the simulated systems (see Table 1). The initial structure of a double Dickerson's dodecamer in the canonical B-form was generated with the use of the 3DNA suite.[18]

DNA was described in the framework of an extensively validated AMBER parmbsc0 force-field,[19] the AMBER lipid force-field Lipid14 was used for POPC lipids.[20] Water was represented by the TIP3P model.[21] A proper choice of parameters for ions, especially for monovalent salt ions,[22,23] can be rather challenging. In particular, it was demonstrated that the impact of monovalent ions on the structure of phospholipid bilayers[24−28] can vary considerably for different parameter sets of monovalent ions. However, in this study, we chose to use the standard AMBER parameters for $Na^+$ and $Cl^-$ ions as their effects on lipid bilayers and DNA are largely suppressed by divalent calcium ions. As far as $Ca^{2+}$ ions are concerned, most improvements of their interaction parameters (especially for charged objects such as DNA considered here) imply deviations from the standard 12−6 Lennard-Jones potential through introducing additional terms into the potential or adding dummy sites into the ion model.[29−31] In order to describe divalent ions on the same footing as monovalent ones, the standard AMBER parameters for $Ca^{2+}$ ions were used.

The systems were simulated in the $NpT$ ensemble at $T$ = 303 K and $P$ = 1 bar. Temperature was kept constant with the use of the velocity-rescaling thermostat.[32] Pressure was controlled anisotropically through the Berendsen scheme.[33] The Lennard−Jones interactions were cut off at 1 nm; the particle-mesh Ewald method (PME) was employed for handling the electrostatic interactions.[34] The time step was 2 fs. The Gromacs 4.5.6 suite was used in all simulations.[35]

Most DNA-membrane systems were simulated for 100−150 ns (see Table 1). Prior actual simulations of DNA-membrane systems, each component (a 24-bp DNA fragment and a POPC lipid bilayer) was simulated separately in aqueous solution for 200 ns for the sake of initial equilibration and force-field implementation testing. Simulations of two selected Ca-DNA-membrane systems (DNA-POPC-Ca-0.5 and DNA-POPC-Ca-1.0) were extended up to 600 ns. These simulations were used for a subsequent analysis of the adsorption kinetics and the equilibrium structure of stable Ca-mediated DNA−lipid complexes. Most structural characteristics were averaged over the last 200 ns (out of 600 ns trajectories) and over two selected Ca−DNA−membrane systems (DNA−POPC−Ca-0.5 and DNA−POPC−Ca−1.0).

The number of contacts (non-normalized coordination numbers) were calculated in line with ref 25. First, the radial distribution functions for the pair of atoms in question were calculated; the following pairs were considered: Npc (nitrogen atoms of choline PC groups)−Pdna (DNA's phosphate atoms), Ca−Ppc (phosphate atoms of PC head groups), Ca−Pdna, Na−Ppc, and Na−Pdna. The positions of first minima of the radial distribution functions were then identified; these minima provided us with the radii of the first coordination spheres. The corresponding radii were found to be 0.45 nm for Ca−Ppc and Ca−Pdna pairs, 0.42 nm for Na−Ppc and Na−Pdna pairs, and 0.6 nm for the Npc−Pdna pair. The number of contacts between A and B atoms were calculated by counting the number of B atoms within the first coordination sphere of A atoms. The ions were considered to be adsorbed on the


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membrane surface when they were within the first coordination shell of a lipid phosphate atom Ppc. Furthermore, a Ca-mediated (Na-mediated) bridge between phosphate groups of DNA and lipid molecules was considered to exist when both Ppc and Pdna atoms were within the first coordination shell of the same $Ca^{2+}$ ($Na^{+}$) ion.

The deuterium order parameter $S_{CD}$ for hydrocarbon groups of lipid acyl chains was calculated from simulations according to $S_{CD} = (1/2)\langle 3\cos^2\theta - 1\rangle$, where $\theta$ is the angle between a CH bond and the membrane normal and the angular brackets denote averaging over a MD trajectory.

## 3. RESULTS

**3.1. Kinetics of Calcium-Induced Adsorption of DNA on Zwitterionic Phospholipid Membranes.** To demonstrate that the presence of calcium ions indeed can induce spontaneous adsorption of DNA on a zwitterionic phospholipid membrane, we have performed a series of computer simulations in which a short (24 bp) double helix of DNA was placed in close juxtaposition with the surface of a palmitoyl-oleoylphosphatidylcholine (POPC) lipid membrane. The initial distance between closest DNA and lipid atoms was varied from 0.18 to 1 nm. Most systems were simulated for 100−150 ns, which was long enough to come to a conclusion of whether DNA adsorption was observed.

When no divalent cations were present in aqueous solution, DNA did not bind to the membrane surface in all Ca-free simulations. In contrast, adding 100 mM of $CaCl_2$ to the "DNA-membrane" system changes the situation drastically: $Ca^{2+}$ ions trigger DNA adsorption on the membrane on the timescale of 100 ns in all systems with $CaCl_2$ (see Table 1). In Figure 1, we present typical time behavior of the relative

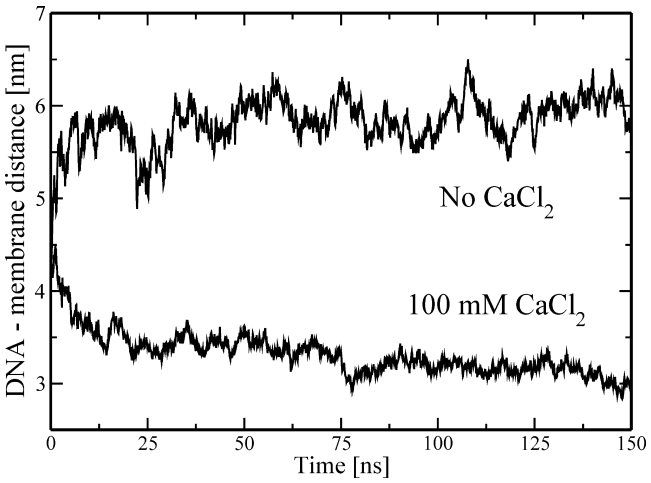


**Figure 1.** Time evolution of the distance between centers of mass of a DNA fragment and a phospholipid membrane along the membrane normal (results for DNA−membrane systems DNA-POPC-Ca-0.5 and DNA-POPC-0.5 are shown).

distance between centers of mass of a DNA molecule and a phospholipid membrane in the direction along the membrane normal. In the beginning of simulations, this distance is the same for both DNA−POPC−Ca−0.5 and DNA−POPC−0.5 systems and equals ∼4.5 nm, the corresponding initial distance between closest DNA and lipid atoms being set to 0.5 nm (note that the two distances can unambiguously be related only at $t$ = 0 when a DNA helix is parallel to the membrane surface). It is seen that the presence of $Ca^{2+}$ ions leads to a steep drop of the distance between the lipid membrane and DNA, while in the Ca-free case, a DNA molecule does not tend to approach the membrane surface. As a result of the DNA adsorption under the presence of divalent cations, one can observe formation of a stable lipid−DNA complex (see Figure 2). Thus, our atomistic

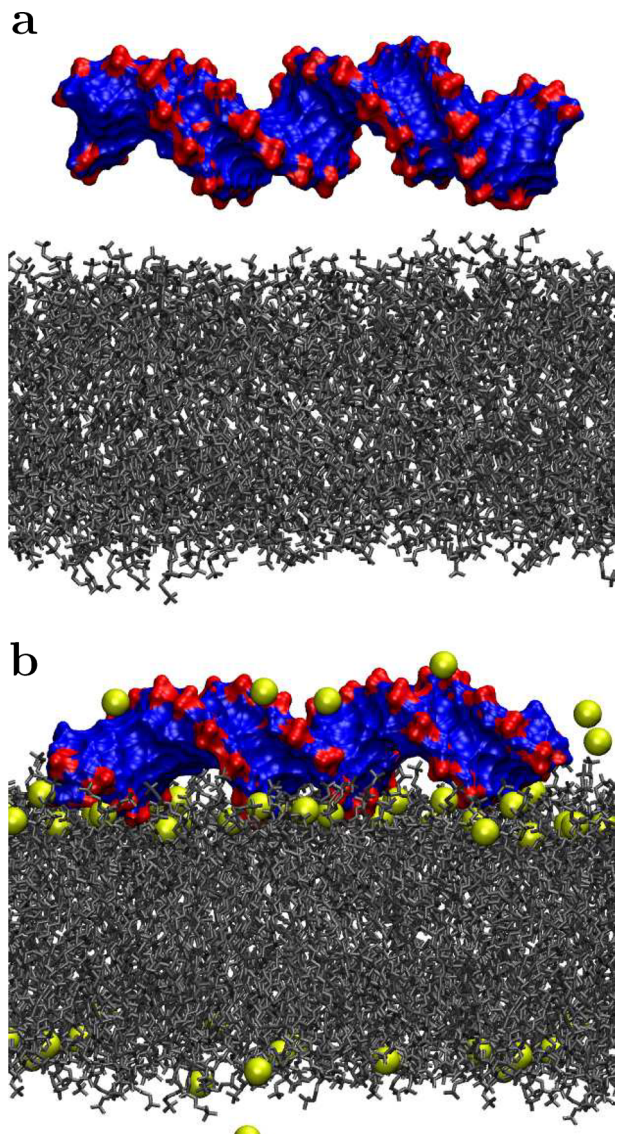


**Figure 2.** Typical snapshots of DNA-membrane systems (a) without and (b) with $Ca^{2+}$ ions. DNA is shown in blue and red, lipids in gray, and $Ca^{2+}$ ions in yellow.

computer simulations successfully reproduced earlier experimental observations of Ca-mediated binding DNA to phosphatidylcholine bilayer membranes.[8,9,12] Now we can take advantage of our high-resolution computational model and gain a microscopic insight into the kinetics of the Ca-induced DNA adsorption.

As mentioned in the Introduction, one can think of two binding modes between DNA and PC lipids in the presence of calcium ions: (i) direct interactions between choline lipid groups and DNA's phosphate groups and (ii) Ca-mediated bridges between phosphate groups of DNA and lipids.[9] To identify the role of both types of interactions upon DNA adsorption, we calculated the numbers of various contacts


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between DNA, lipids, and $Ca^{2+}$ ions as a function of time (see Figure 3). First of all, it is seen that one of the fastest processes during initial stages of DNA adsorption is related to binding of $Ca^{2+}$ ions to phosphate groups of PC lipids. This binding starts immediately after the beginning of the simulation, making the membrane surface positively charged: a monolayer patch of 144 lipids acquires a positive charge of +30e (15 $Ca^{2+}$ ions adsorbed) within just 50 ns (see Figure 3c). Apparently, the positive charge of the membrane surface triggers electrostatic attraction of DNA to the membrane surface and subsequent adsorption, the latter can be witnessed through a steep increase of the direct contacts between DNA phosphates and choline moieties of PC lipids (Figure 3a). We note that complete adsorption of $Ca^{2+}$ ions on the membrane surface is not observed so that several divalent cations are always present in the water phase where they interact with DNA. Interestingly, $Na^+$ counterions also contribute to the initial charging of the membrane surface to some (very small) extent, see Figure 3c. However, their binding is rather unstable and seems to be suppressed by adsorption of $Ca^{2+}$ ions.

As far as Ca-mediated bridges between DNA and lipid phosphates are concerned, their formation develops with a delay of ~10−15 ns with respect to DNA−choline contacts. This is most likely due to the fact that the average angle between PN vectors of phosphatidylcholine lipids and the outward membrane normal equals 69.2 ± 0.1 degrees, so that choline groups are located closer to the water phase and therefore easier accessible for DNA during its adsorption. It is also noteworthy that initial formation of Ca-mediated P−P bridges is a much slower process as compared to that of choline−DNA contacts (see Figure 3a) as it requires three (and not two) groups of atoms to come in close contact. As for $Na^+$ counterions, we witnessed formation of a few single Na-mediated P−P bridges with lifetimes shorter than 600 ps (for the DNA-POPC−Ca−0.5 system).

The overall process of DNA adsorption on a zwitterionic PC membrane turns out to be relatively fast: after adding $Ca^{2+}$ ions to the DNA−lipid membrane system, the number of direct DNA phosphate−PC choline contacts reaches its near-equilibrium value within the first 100 ns. In contrast, the number of Ca-mediated P−P bridges continues to increase for more than 100−150 ns, indicating the existence of a slow structural reorganization in the resulting Ca-mediated DNA−lipid complex. These structural changes are related to multistep formation of aggregates of calcium ions and phosphate groups of lipids and DNA: initially one can witness Ca-mediated bridges between a DNA phosphate and a single PC phosphate group while later, in several hundreds of nanoseconds the majority of the Ca-mediated aggregates involve one DNA phosphate and phosphate groups of three lipids (see Figure 3b). The observed mechanism of formation of Ca-bridged aggregates resembles the one reported earlier for Ca binding to phosphatidylcholine membranes.[36] It should be stressed that the multistep formation of Ca-mediated P−P bridges is a consequence of the fact that $Ca^{2+}$ ions and DNA are simultaneously added to the water phase next to a lipid membrane. If DNA was solvated in the system after adsorption of $Ca^{2+}$ ions has taken place, one can expect different kinetics of DNA binding, although the equilibrium structural properties of the Ca−DNA−membrane system should remain largely the same. This problem will be addressed in detail in our forthcoming publication.

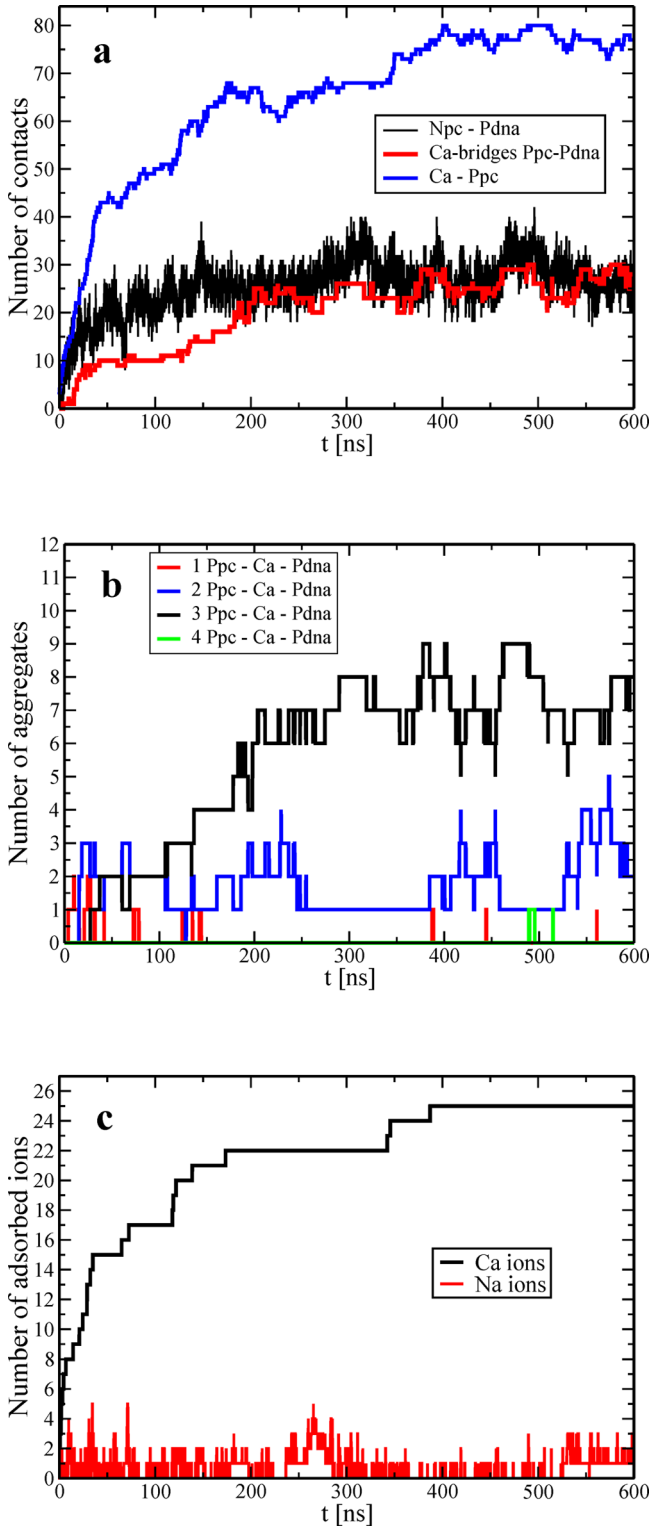


**Figure 3.** (a) The number of contacts of lipid choline groups with DNA phosphates (black line), the number of Ca-mediated bridges between lipid and DNA phosphates (red line), and the number of contacts of $Ca^{2+}$ ions with lipid phosphate groups (blue line), as a function of time. (b) The number of different Ca-mediated aggregates between DNA and lipid phosphates as a function of time. Shown are the aggregates composed of a DNA phosphate and one (red line), two (blue line), three (black line), and four (green line) phosphate groups of lipid molecules. (c) The total number of $Ca^{2+}$ (black line) and $Na^+$ (red line) ions adsorbed on the membrane leaflet next to DNA. Shown are results for the DNA-POPC-Ca-0.5 system.



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As suggested in earlier experimental studies,[9] the contacts between choline moieties of PC lipids and DNA's phosphate groups can be facilitated by more vertical reorientation of polar lipid head groups with respect to the outward membrane normal. This reorientation is indeed observed in Ca–DNA–lipid membrane systems: the average angle between PN vectors of phospholipids and the outward membrane normal gradually decreases from 69.2 to 60.0 degrees (see Figure 4). Such

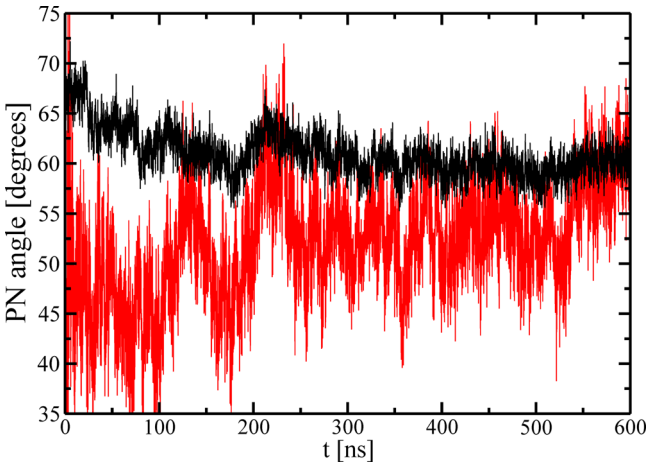


**Figure 4.** Time evolution of the average angle between the P–N vector of phospholipid head groups and the outward normal of the membrane leaflet next to DNA for the DNA–POPC–Ca-0.5 system (black line). Also shown is the P–N angle for the lipids whose choline groups are in direct contact with DNA phosphates (red line).

behavior is mostly due to adsorption of calcium ions and can also be observed in a DNA-free lipid membrane system under the presence of divalent cations:[36] formation of tight Ca–lipid aggregates brings lipid phosphate groups closer to each other, pushing choline groups toward the water phase and promoting therefore more vertical orientation of PC head groups. Interestingly, adsorption of DNA on the membrane surface induces additional, more vertical headgroup reorientation mostly among the lipids whose choline moieties come in contact with DNA phosphates during the initial stage of adsorption (i.e., when most DNA's atoms are still located in the water phase) (see Figure 4). On later stages, when DNA fuses deeper and deeper into the lipid–water interface, it pushes choline groups toward the membrane interior, so that the additional reorientation of lipid heads diminishes.

The progressive fusion of DNA into the lipid bilayer can promote contacts of positively charged choline groups not only with DNA phosphates but also with atoms of DNA grooves (see Figure 2b). Inspection of radial distribution functions of lipid choline groups with various electronegative atoms of DNA bases shows that this is indeed the case (data not shown). However, it turns out that choline moieties do not demonstrate preferences in binding to any particular DNA grooves, highlighting thereby the fact that the PC lipid–DNA base interactions are not groove-specific and caused mostly by steric restrictions due to DNA fusion into the membrane.

**3.2. Direct DNA-Lipid Contacts versus Ca-Mediated Bridges.** The most important problem that has not been addressed so far is a relative contribution of the two types of DNA–lipid interactions into the stability of the resulting complex. To examine this, we calculated typical residence times for both types of interactions in the system. The residence time for choline–DNA contacts was estimated as the average amount of time that a phosphorus atom of DNA spends in the first coordination shell of a nitrogen atom of a choline lipid group. In turn, the residence time of a Ca-mediated P–P bridge was related to the period of time that both phosphate atoms of a DNA molecule and a lipid spend in the first coordination shell of the same calcium ion.

Overall, we found that the residence time for Ca-mediated P–P bridges equals 98 ± 14 ns, while the residence time for choline–DNA phosphate contacts equals 3.2 ± 1.5 ns (the averaging was performed over last 200 ns of MD trajectories when DNA adsorption has already taken place (see Figure 3). Therefore, our simulations provide compelling evidence that Ca-mediated P–P bridges represent a dominant factor in stabilizing DNA–lipid membrane complexes as their residence time is almost 2 orders of magnitude larger than the corresponding quantity of direct lipid–DNA contacts.

We found that some of the DNA and lipid phosphate groups stay calcium-bridged for more than half a microsecond, which is close to the total simulation time in our study. Furthermore, the Ca-mediated P–P bridges seem to strengthen the contacts between choline lipid groups and DNA phosphates. Careful inspection shows that most stable choline-DNA contacts are due to combination of two factors: (i) the phosphate group of a particular lipid molecule is linked via $Ca^{2+}$ ion to a DNA phosphate group and (ii) the lipid is located just beyond the adsorbed DNA which immobilizes the lipid by pushing it toward the membrane center.

To evaluate the impact of Ca-mediated P–P bridges on stability of choline–DNA contacts, we calculated the residence time of these contacts during the initial stage of DNA adsorption (first 100 ns of MD trajectories): as one can expect from Figure 3, Ca-mediated P–P bridges should have minimal influence on choline–DNA contacts within this initial time interval as the formation of stable, multilipid P–P bridges requires a much longer time. Indeed, in this case, we found that the residence time for direct lipid–DNA contacts drops to 0.66 ± 0.07 ns (i.e., by a factor of 5), highlighting thereby the importance of Ca-mediated P–P bridges on later stages of DNA adsorption. What is more, this finding leads to another important conclusion: unstable and short-lived direct interactions between lipid choline groups and DNA phosphate groups can hardly serve as a driving force for DNA adsorption on a zwitterionic lipid membrane. Instead, it is the positive charge of $Ca^{2+}$ ions bound to the lipid membrane that is mainly responsible for the initial steps of DNA adsorption.

Remarkably, a single calcium ion bridges a DNA phosphate group with phosphate moieties of several lipid molecules, so that one can witness formation of aggregates of phosphate groups. The average number of lipids involved in such Ca-mediated aggregates was estimated to be 2.88 ± 0.02 (the averaging was performed over the last 200 ns when the stable adsorption of DNA was observed). Figure 5a emphasizes the existence of the distribution over aggregates of different types. As illustrated in Figure 5b, the majority of the Ca-mediated lipid–DNA aggregates consists of a DNA phosphate and phosphate groups of three zwitterionic PC lipids. The observed distribution over aggregate sizes is reminiscent to earlier findings related to adsorption of $Ca^{2+}$ ions on mixed zwitterionic/anionic lipid membranes.[37] Therefore, it may be of interest to extend the present study on phospholipid membranes of complex lipid composition.


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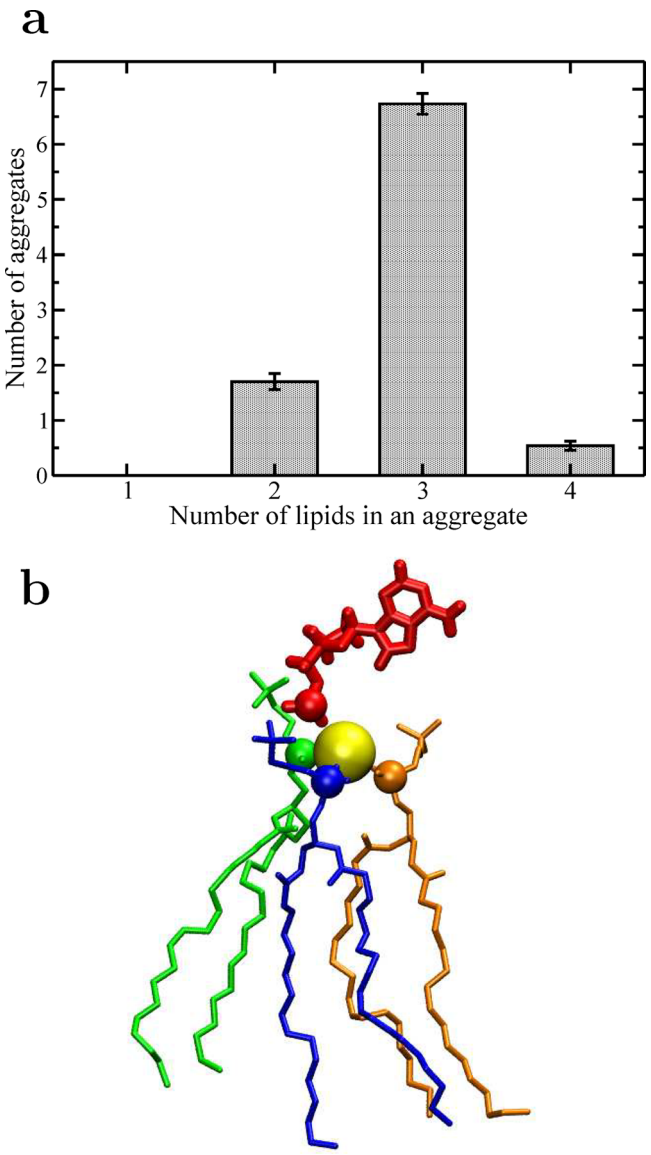


**Figure 5.** (a) The average number of Ca-mediated lipid−DNA aggregates of different types for a DNA fragment of 24 bp. An aggregate consists of a DNA phosphate group, a $Ca^{2+}$ ion, and several (from 1 to 4) phosphate groups of PC lipids. The averaging was performed over the last 200 ns of the MD trajectories of two selected Ca-DNA-membrane systems (DNA-POPC-Ca-0.5 and DNA-POPC-Ca-1.0). The error bars are standard errors of the mean. (b) A typical representative of the Ca-mediated lipid−DNA aggregates. Shown are three lipids (green, orange, and blue) that are bridged via a calcium ion (yellow) to a DNA base (red). Phosphate groups of lipids and DNA are highlighted by spheres.

## 4. DISCUSSION AND CONCLUSIONS

Interaction of DNA with zwitterionic (neutral) phospholipids represents an important long-standing problem in the field of liposome-based DNA delivery and, more generally, DNA-based nanodevices interacting with cellular membranes. Although it has been well-established that the presence of $Ca^{2+}$ ions is able to promote formation of stable complexes of DNA with zwitterionic phospholipids,[4,8−12] the precise molecular mechanism of the complex formation has not been unlocked yet. Previous experimental studies[9] suggested that direct interactions between positively charged moieties of lipid head groups and negatively charged DNA phosphate groups could play a dominant role in formation of the DNA−lipid complexes. However, our computational findings clearly demonstrate that such direct contacts between lipids and DNA are relatively short-lived and unstable. On the other hand, we found that calcium ions are largely responsible for the stability of the DNA−lipid complex: it is the $Ca^{2+}$ cations that tightly bind together phosphate groups of DNA and lipid molecules.

These Ca-mediated membrane−DNA bridges are relatively strong as a calcium ion bridges one DNA phosphate group with phosphate groups of (on average) three lipid molecules (see Figure 5). In fact, the P−P bridges turn out to be strong enough to change the local structural properties of the lipid membrane. We found that acyl chains of lipids bridged via calcium ions to DNA are more disordered compared to the rest of the lipids: the deuterium order parameter for saturated sn-1 acyl chains of the Ca-bridged POPC lipids is smaller than that of their unbridged counterparts, the effect being most pronounced for the lower part of the acyl chains (see Figure 6).

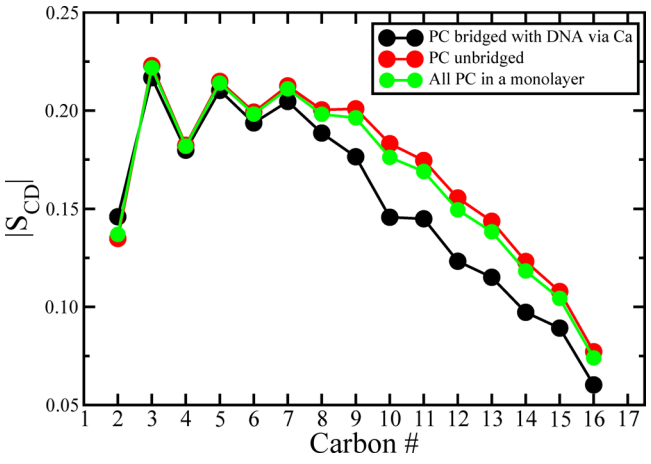


**Figure 6.** Deuterium order parameters for lipids that are Ca-bridged to DNA phosphates (black line), their counterparts with no Ca bridges (red line), and all lipids in a membrane monolayer adjoint to DNA (green line) for the DNA-POPC-Ca-0.5 system. Shown are results for sn-1 lipid chains; the averaging is performed over a period of time (from 465 to 485 ns) when the number of Ca-mediated lipid-DNA bridges stays constant (see Figure 3).

Such disordering of lipid chains can be explained by a considerable fusion of a DNA fragment into the lipid/water interface, which pushes DNA-bound lipids toward the membrane interior. Indeed, inspection of the component-wise density profiles of phospholipids shows that phosphate groups (as well as sn-2 glycerol carbon atoms) of DNA-bound lipids are closer by ∼0.35 nm to the center of the membrane as compared to the rest of the lipids (see Figure 7). As the overall lipid density gradually decreases toward the membrane center, the DNA-bound lipids have more free space to move and correspondingly become more disordered.

The tight electrostatic binding of DNA to the lipid membrane by calcium ions also affects the structure and properties of DNA. In particular, the root-mean-square deviation (RMSD) of the DNA structure from the initial canonical B-form turns out to decrease after DNA adsorption occurs. The RMSD fluctuations also become suppressed, so that the DNA fragment is partly immobilized by $Ca^{2+}$ ions on the surface of the lipid membrane. This DNA immobilization


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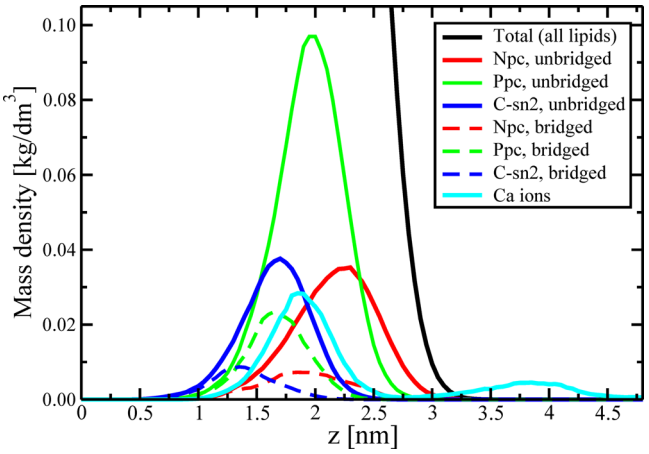


**Figure 7.** Component-wise density profiles for lipid molecules and $Ca^{2+}$ ions (the averaging was performed over the last 200 ns of the MD trajectory for the DNA-POPC-Ca-0.5 system). Shown are results for all lipids in the system (black line), $Ca^{2+}$ ions (cyan line), and several principal atoms of lipids that are unbridged (solid lines) and Ca-bridged to DNA phosphates (dashed lines): nitrogen atoms of choline groups (red lines), phosphorus atoms (green lines), and sn-2 glycerol carbon atoms (blue lines). The position $z = 0$ corresponds to the center of mass of the bilayer membrane.

could be a key to the experimentally observed increase of the melting temperature of DNA upon Ca-induced complexation with phospholipids:[38] to melt such DNA molecules one needs to additionally destroy tight Ca-mediated bridges between DNA and lipid phosphate groups.

Finally, we repeated one of the simulations of Ca-DNA-membrane systems with additional 100 mM of NaCl salt (see Table 1) and found that the overall picture stayed the same. This is mostly due to the fact that monovalent $Na^+$ ions cannot compete with divalent $Ca^{2+}$ ions for binding sites in DNA and lipid molecules, see also Figure 3c. However, in the absence of divalent salt sodium ions of relatively large concentration can adsorb on the membrane surface to a noticeable extent and might potentially induce DNA adsorption in line with experimental data.[12] We are currently exploring the feasibility of this scenario. As far as other divalent (and even trivalent) cations are concerned, one can anticipate that their effects will be similar to the effect of calcium ions as binding of multivalent ions to phospholipid bilayers and DNA is in most cases quite substantial. For instance, one can expect that another biologically relevant cation $Mg^{2+}$ also induces adsorption of DNA on zwitterionic lipid membranes although to a somewhat lesser extent: the affinity of $Mg^{2+}$ ions to both DNA[39] and zwitterionic phospholipids[40] is smaller as compared to that of $Ca^{2+}$, most likely due to tighter coordination of $Mg^{2+}$ with water molecules in the first hydration shell. As a result, $Mg^{2+}$ ions interact with phosphate groups through water bridges.[41]

Summarizing, our computer simulations shed light on a possible molecular mechanism behind calcium-induced adsorption of DNA on zwitterionic phospholipid membranes. The use of atomic-scale computational models allowed us for a first time to follow closely the process of Ca-triggered DNA adsorption and to study in detail the structure and stability of Ca–DNA–lipid complexes. In contrast to previous hypotheses, our simulations provide compelling evidence that direct interactions of DNA with lipid polar headgroups are relatively short-lived and unstable, so that they cannot be considered as a driving force for DNA adsorption as well as for the stability of the resulting supramolecular complexes. On the other hand, we found that calcium ions play a crucial role. In particular, the initial electrostatic attraction between DNA and the membrane surface is triggered by $Ca^{2+}$ cations that adsorb on the lipid–water interface and turn the zwitterionic membrane cationic. What is more, we show that calcium ions electrostatically bridge together phosphate groups of DNA and lipid molecules, stabilizing thereby the DNA–membrane complex. The discovered mechanism of calcium-induced formation of stable DNA–lipid complexes can be used for optimization of existing liposome-based gene delivery vectors. It can also serve as a guide in exploring alternative agents for noncovalent binding of DNA and DNA-based nanodevices to cell membranes and other complex surfaces.

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### Notes

The authors declare no competing financial interest.

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