

A Two-State Model for the Multilamellar Structure of a DNA/Cationic Lipid Complex in the Bulk

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Polyanionic DNA can bind electrostatically with cationic lipids to form a complex used for gene delivery and nanostructure construction. Here, we reveal two multilamellar phases, L_I and L_{II}, characterized by distinct states of lipid packing and DNA conformation in a DNA/cationic lipid complex in the bulk state. The L_{II} phase, formed when the lipids are in excess of DNA in terms of overall ionic charge, is composed of B-DNA confined between the bilayers with the lipid tails aligning normal to the lamellar interface. When DNA becomes in excess of the lipids, the L_I phase in which the DNA is bound with the tilted lipid chains adopting the A conformation is favored because this configuration offers more economical electrostatic binding between these two components.

Engineering the molecular and higher-order self-assembly continues to be one of the main topics in the research of soft condensed matter for nanostructure construction. Among the wide spectrum of strategies adopted, the construction of "supramolecules" by binding different types of molecules through noncovalent interactions offers a simple pathway toward switchable structures and functions.¹ Under the influences of surface force and Brownian motion, these supramolecules may self-assemble into long-range ordered structures that may be switched thermally or tuned via the composition or the quality of solvent media. Biopolymers and lipids, for instance, may be jointed noncovalently to form materials that combine the lipid mesophase with the properties or functions of the biopolymers.^{2–4} This class of bioassembly offers a useful model for resolving the molecular mechanism of membrane-mediated biological functions⁵ in addition to its potential for medical and nonmedical applications.

Among the variety of biopolymer/lipid assemblies, the complexes of polyanionic DNA with cationic lipids (CLs) constitute a special system exhibiting rich self-assembled structures at different length scales.^{2,3,6} In the presence of excess CLs, the DNA/CL complex carries an overall

positive charge that favors its attachment to anionic animal cells; therefore, nonviral gene delivery for gene therapy has been considered as a promising application for this type of material.⁷

The lipid membrane typically used as the gene vector is not purely cationic but contains a portion of zwitterionic helper lipids. Such a lipid mixture may be processed into unilamellar vesicles or liposomes in aqueous media.⁶ Complexation with DNA induces a membrane ordering where the unilamellar vesicles aggregate and fuse into multilamellar vesicles or compact multilamellar condensates containing alternating lipid bilayers and DNA monolayers.^{2,6} DNA ordering is also accomplished where the DNA chains confined between the lipid bilayers organize into a smectic ordering characterized by a DNA–DNA correlation peak in the X-ray scattering profile.^{2,8}

Previous studies of DNA/CL complexes have centered almost exclusively on the systems in the presence of excess water. In this case, the water molecules entering the hydrophilic domains can mediate the DNA–lipid interaction, which then perturbs the structure from that which would have been induced by the interaction alone. Moreover, the prevalence of water molecules would allow sufficient hydration of the DNA phosphate groups such that the DNA chains adopt the B conformation in the complex.² In the present study, we mainly deal with the complex of DNA with a conformationally rigid CL in the *bulk state* to explore the structural patterns induced predominantly by the DNA–lipid interaction. One issue worth noticing here is whether the significant loss of water content in the system would allow the transformation of

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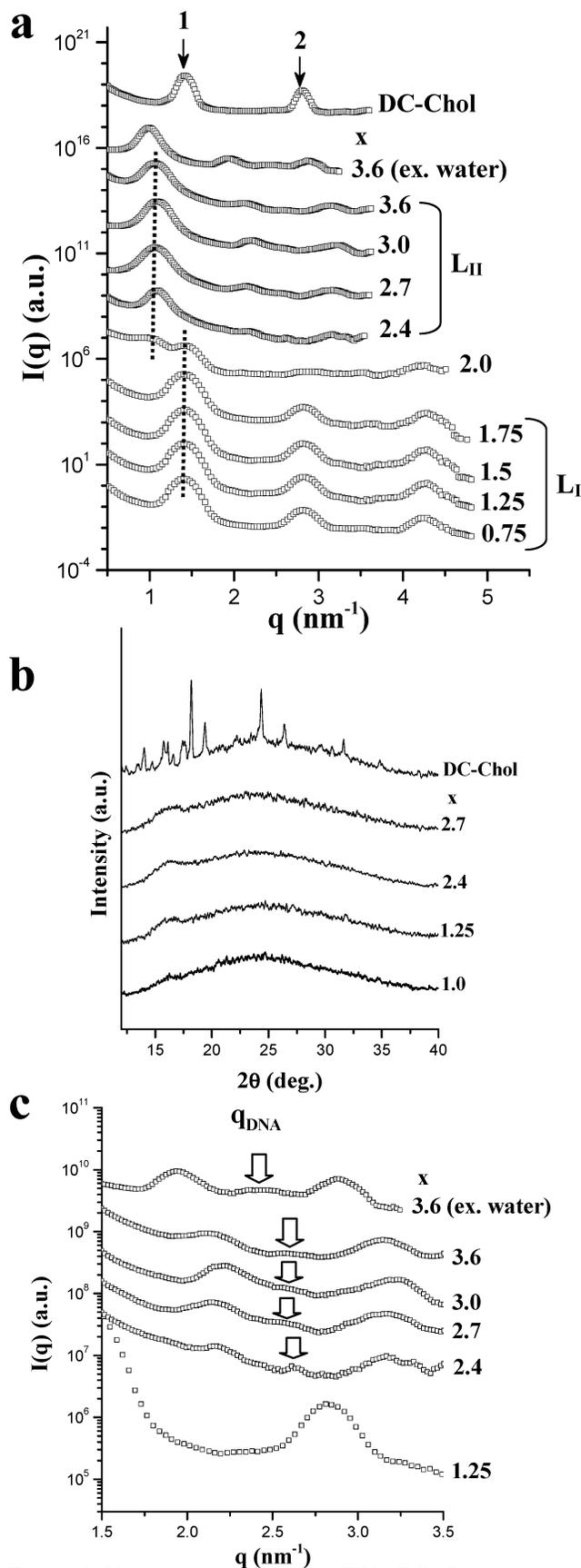


Figure 1. X-ray scattering profiles of DNA/DC-Chol complexes in the bulk state: (a) SAXS profiles. The multilamellar phase found below the stoichiometric composition is denoted by L_I , while that observed at $x > 2$ is denoted by L_{II} . The scattering pattern of the complex with $x = 3.6$ in excess water is also displayed for comparison. (b) Wide-angle profiles. (c) Enlarged plots of the SAXS profiles showing the presence of a DNA-DNA correlation peak at 2.61 nm^{-1} (marked by q_{DNA}) at $x > 2$.

the DNA duplex conformation to the A form⁹ under the influence of electrostatic interaction with the lipid membrane.

DNA type XIV from herring testes with an average molecular weight of ~ 700 bp and the CL cholesteryl 3β -*N*-((dimethylamino)ethyl) carbamate (DC-Chol), consisting of a rigid cholesteryl moiety in the lipid chain, were acquired from Sigma. DNA/DC-Chol complexes were prepared by adding a prescribed amount of 1.5 mg/mL DNA aqueous solution to the DC-Chol aqueous suspension, and the complexes formed spontaneously as manifested by precipitation. The solution containing the precipitates was dried by allowing water to evaporate for 2 weeks under ambient atmosphere. The complex composition is denoted by x , which expresses the molar ratio of DC-Chol to the base pair of DNA; $x = 2.0$ corresponds to the stoichiometric composition for charge neutralization because each DC-Chol molecule has a positive charge and a base pair of DNA carries two negative charges.

The structure of the DNA/DC-Chol complexes in the bulk state was probed by small-angle X-ray scattering (SAXS) at room temperature ($\sim 27^\circ\text{C}$). The SAXS apparatus consisted of an 18 kW rotating anode X-ray generator (Rigaku) operated at 200 mA and 40 kV, a pyrolytic graphite crystal for incident beam monochromatization, and a two-dimensional position sensitive detector (ORDELA, model 2201X, Oak Ridge Detector Laboratory Inc., U.S.A.) with 256×256 channels. The intensity profile was output as the plot of the scattering intensity (I) versus the scattering vector, $q = 4\pi/\lambda \sin(\theta/2)$ (θ , scattering angle).

Figure 1a displays the room-temperature SAXS profiles of DNA/DC-Chol complexes in the bulk state as a function of composition. Neat DC-Chol exhibits a multilamellar structure as manifested by the multiple scattering peaks with relative positions prescribed by a 1-D stacked lamellar morphology (i.e., 1:2:3...). A interlamellar distance (d) of 4.42 nm signifies that the membrane is in the bilayer form, as the fully extended length of a DC-Chol molecule is ~ 2.0 nm. The multiple diffraction peaks in the corresponding wide-angle X-ray scan in Figure 1b attest that the lipid chains in neat DC-Chol are in the crystalline state.¹⁰

Multilamellar structures are also observed in the complexes. Below the stoichiometric composition ($x < 2$), the lamellar phase denoted by L_I is characterized by an interlamellar distance of 4.36 nm irrespective of the composition. This structure transforms to another lamellar phase (denoted by L_{II}) with a larger d value of 5.7 nm right above the stoichiometric composition, and the interlamellar distance remains composition independent at least up to $x = 3.6$. The L_I and L_{II} phases coexist at $x = 2$, where the primary peaks associated with both lamellar structures are observed. We also note that the crystalline order of the lipid tails is lost in the complexes, as the wide-angle patterns of the complexes in Figure 1b display a broad peak. Disruption of the crystalline order may be due to the large mismatch between the cross-sectional area per lipid molecule in the crystalline state and the cross-sectional area per binding site on the DNA, where the latter dominates the lipid packing and hence prohibits the formation of crystalline order.

A close examination of the SAXS profiles reveals the presence of a small peak (marked by q_{DNA}) at $\sim 2.61 \text{ nm}^{-1}$

(9) Neat DNA tends to adopt the A conformation when the water activity is reduced for the sake of more economical hydration.¹⁶

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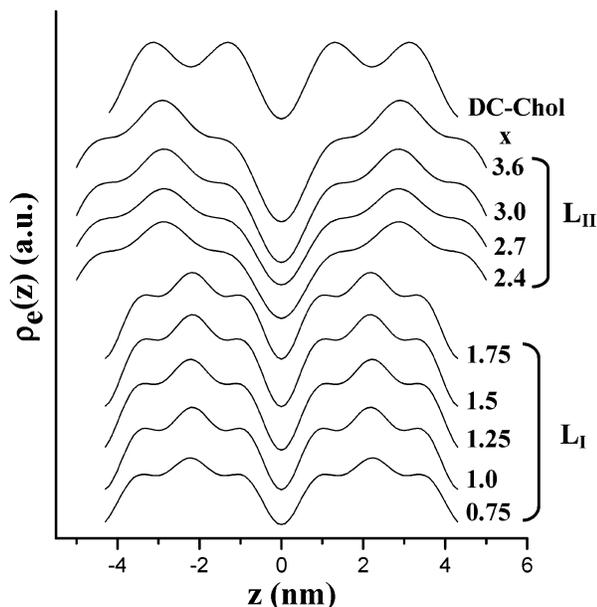


Figure 2. The relative electron density profiles of DNA/DC-Chol complexes in the bulk state.

for the L_{II} phase, as demonstrated by the enlarged SAXS plots in Figure 1c. This peak is attributed to the DNA-DNA correlation prescribed by the smectic ordering of the DNA chains in the hydrophilic layers.^{2,8} The overlap with the rather strong second-order lamellar peak may cause q_{DNA} to be unresolvable for the L_I phase. The observed q_{DNA} value corresponds to the interhelical distance of the DNA chains, $d_{DNA} = 2\pi/q_{DNA} = 2.41$ nm irrespective of x .² This length approaches the typical diameter of DNA,¹¹ implying that the DNA chains confined in the hydrophilic layers are nearly closet packed in the bulk state. The incorporation of water molecules slightly swells the interhelical distance, as Figure 1c shows that the q_{DNA} value of the complex with $x = 3.62$ shifts to ~ 2.5 nm⁻¹ ($d_{DNA} = 2.51$ nm) in the presence of excess water.¹²

The SAXS patterns demonstrate that two multilamellar phases characterized by distinct interlamellar distances exist in DNA/DC-Chol complexes and the transition between L_I and L_{II} occurs near the stoichiometric composition. Such a structural transition only exists in the bulk state; according to our recent study, the complexes display one lamellar structure with $d = 6.28$ nm in the presence of excess water.¹² To gain further insight into the structures of the L_I and L_{II} phases, the electron density profiles along the lamellar normal [$\rho_e(z)$] are constructed from the SAXS data via¹³

$$\rho_e(z) \sim \sum_{k=1}^n \sqrt{I(q_k)q_k^2} \varphi_k \cos(q_k z) \quad (1)$$

where n is the total number of diffraction order, q_k is the scattering vector of the k th diffraction order, $[I(q_k)q_k^2]^{1/2}$ is the magnitude of the k th amplitude, and φ_k is the corresponding phase which is either $+1$ or -1 for the centrosymmetrical lamellar structure.¹³

Figure 2 presents the relative electron density profiles calculated using the intensities of the first three scattering

peaks and the most reasonable phase combination of $(-1, -1, -1)$.¹⁴ For DC-Chol, the valley centered at $z = 0$ corresponds to the hydrophobic layer, while the two humps at $z = \pm 1.27$ and ± 3.13 nm represent the headgroup regions. For the hydrophilic layers in the complexes, a peak beside the humps is identified irrespective of the composition. This peak is ascribed to the DNA sandwiched between the lipid headgroups because DNA, having a mass density of 1.7 g/cm³,² has a higher electron density than the headgroup of DC-Chol.

The thickness of the hydrophobic layer (d_m) in the L_I phase estimated from Figure 2 is ~ 1.5 nm, which is thinner than that in neat DC-Chol ($d_m \approx 1.92$ nm). Consequently, complexation with DNA induces a "bilayer thinning" (along the lamellar normal) below the stoichiometric composition. The hydrophobic layer recovers to a thickness comparable to that of neat DC-Chol right above the stoichiometric composition as the system enters the L_{II} phase. The hydrophilic layer in which DNA is contained also undergoes an abrupt thickening across the transition. The diameter of DNA in the L_{II} phase estimated from the width of the DNA peak is around 2.0 nm, which agrees with the diameter of B-DNA.¹¹ In the L_I regime, however, the corresponding peak width is only 1.5 nm, which is obviously smaller than the diameter of DNA in any conformational form.¹¹ It should be noted that the fine details of the electron density profiles are not resolved in Figure 2 because only three diffraction orders were considered in the $\rho_e(z)$ construction. This however does not affect the conclusion that the DNA peak is narrower in the L_I phase. As a test, we included the fourth- and fifth-order peaks with intensities varying in such a way to maintain the $\rho_e(z)$ profile in plausible shape (but with finer detail); then, the width of the DNA peak was found to vary from 1.2 to 1.5 nm and from 1.7 to 2.0 nm for the L_I and L_{II} phases, respectively.

The distinction between the L_I and L_{II} phases becomes clear in light of the electron density profiles. The L_{II} phase is composed of B-DNA intercalated in the multilamellar membrane with the lipid tails aligning normal to the lamellar interface. In this case, the B conformation of DNA persists from excess water into the bulk state under the electrostatic binding with the cationic lipid membrane. Figure 3a illustrates the packing mode of B-DNA and DC-Chol in the L_{II} phase. Since the average cross-sectional area of a DNA-bound DC-Chol molecule measured from the self-assembled monolayer technique is around 50 – 55 Å² (see ref 15), the cross section of each DC-Chol molecule with the tail aligning normal to the paper plane is represented by a square of 7 Å \times 7 Å in the figure. The side length of the square is close to the distance (≈ 6.6 Å) between the adjacent phosphate groups on B-DNA.¹⁶ The squares drawn by the black lines signify the ionically bound lipids and are hence placed on top of the anionic phosphate groups located at the surface of the B-DNA (see the top view of the B-DNA cross section in the inset) for charge matching. Placing these bound lipids alone however results in density dips under the groove regions of DNA; therefore, additional (unbound) lipids represented by the blue squares must be placed over the grooves to attain the normal density for the lipid. Four

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(15) The cross-sectional area of DNA-bound DC-Chol was measured from the surface pressure-area (π -A) isotherm using a Langmuir-Blodgett trough (Nima Technology, model 622D1). We spread 22 μ L of DC-Chol solution in chloroform (2.5 mg/mL) on a water solution of 1.0 μ M DNA, and the monolayer was compressed for recording the π -A isotherm after 1 h. The cross-sectional area per DC-Chol molecule was estimated from the region where π started to rise sharply.

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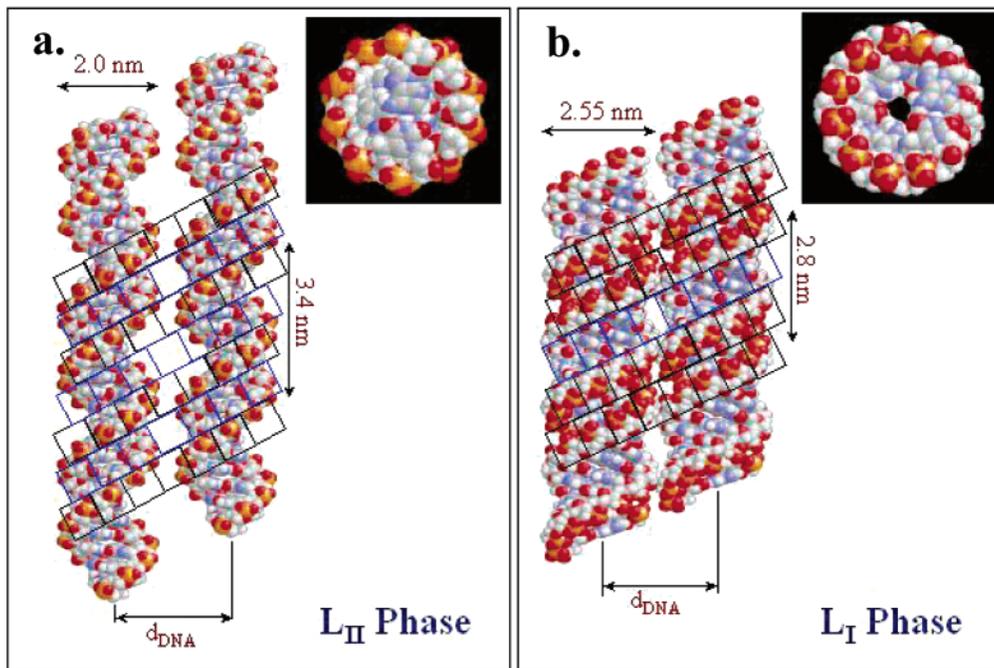


Figure 3. Schematic illustrations of the packing modes of DC-Chol and DNA packing in the (a) L_{II} and (b) L_I phases. The DNA conformations are of the B form and the A form in the L_{II} and L_I phases, respectively.

rows of lipids are needed to cover one helical turn of 34 Å in length for B-DNA. Due to the presence of the unbound lipid molecules, the lipid-to-base-pair molar ratio, x_c , for complete lipid coverage on a B-DNA chain is higher than the stoichiometric composition (i.e., $x = 2$) calculated by simple charge balance. The x_c value for the L_{II} phase may be estimated by $x_c \approx (\text{projected area per pitch of the duplex}) \div (\text{cross-sectional area per DC-Chol molecule}) \div 10 \text{ base pairs per pitch} = (20 \times 34 \times 2 \text{ \AA}^2) \div 50 \text{ \AA}^2 \div 10 = 2.7$, which is close to the composition of $x = 2.4$ from which the system predominantly adopts the L_{II} structure. For the compositions with $x > 2.4$, the excess free lipids may situate at the boundaries between the grains consisting of the complex lamellar domains or aggregate to form the neat DC-Chol lamellar phase. The fact that the diffraction peaks associated with the latter are absent in the SAXS profiles up to $x = 3.6$ indicates that the former is the dominant case.

The lamellar structure in the L_I phase is characterized by a thinner hydrophobic layer (0.42 nm thinner than that in the L_{II} phase) and by a DNA electron density peak of only 1.5 nm in width (see Figure 2). Given that the volume of the lipid membrane is conserved, the observed bilayer thinning signals an expansion of the membrane planar surface by 28% or, more specifically, an increase of the average cross-sectional area per lipid molecule to $\sim 64 \text{ \AA}^2$. For DC-Chol consisting of an intrinsically rigid tail, such an enlargement of cross section can be achieved by tilting the lipid tails away from the lamellar normal. The cross section of each lipid molecule in this case is approximately represented by a rectangle of $7 \text{ \AA} \times 9 \text{ \AA}$ (corresponding to a tilt angle of 39°). When placed on top of B-DNA as was done for the L_{II} phase in Figure 3a, these rectangles do not match the charges on these DNA chains well once the normal lipid density is maintained. Therefore, the conformation of the DNA chains in the L_I phase may not be of the B form. As a matter of fact, if the tilted lipids were bound to B-DNA, the DNA electron density peak would have been 2.0 nm instead of 1.5 nm in width because the negatively charged oxygens on the phosphate groups situate at the outer surface of B-DNA.

The 1.5 nm width of the DNA electron density peak may be accommodated by postulating that the tilted lipids are bound to A-DNA, a conformation found under low water activity for neat DNA.¹⁶ The phosphate groups on A-DNA are inclined and displaced from the helix axis,¹⁷ such that the negatively charged oxygens appear to situate at the interior of the chain from the top view of the DNA cross section (see the inset in Figure 3b). The lipid headgroups bound to these sites would then appear to immerse in the chain from the top view. In this case, the humps beside the DNA peak in the corresponding electron density profile represent the average electron density of the headgroup and the portion of DNA embedding the headgroup, while the peak signifies the core region of DNA (excluding the phosphate groups) with a diameter smaller than the overall diameter of DNA. Such a core region of A-DNA is $\sim 1.3 \text{ nm}$ in diameter, which is close to the width of the DNA electron density peak associated with the L_I phase. The postulate of electrostatic binding of A-DNA with tilted DC-Chol in the L_I phase is supported by the successful packing of these two components under the premise of good charge matching while maintaining normal lipid density, as illustrated in Figure 3b. Again, the rectangles drawn by the black lines in the figure represent the bound lipids, while the blue ones signify the unbound lipids placed over the grooves to remove the density dips.

The critical lipid-to-base-pair molar ratio for complete lipid coverage on an A-DNA chain in the L_I phase is estimated by $x_c \approx (25.5 \times 28 \times 2 \text{ \AA}^2) \div 64 \text{ \AA}^2 \div (11 \text{ base pairs per pitch in A-DNA}) = 2.06$. This value is obviously smaller than the value 2.7 obtained for the L_{II} phase, because the enlargement of the lipid cross section by chain tilting effectively reduces the population of the unbound lipids for removing the density dips. Therefore, the L_I phase is favored when DNA is in excess in that the lipids are used efficiently for DNA binding rather than just filling into the regions with density dips. The efficiency could of course be enhanced (particularly for x lying far below x_c)

by tilting the lipid chains further; however, this will cost more energy and, more importantly, due to the limited conformational states accessible to double-helical DNA, there will not be any form of DNA conformation in which the spatial arrangement of the phosphate groups allows successful lipid packing under the conditions of good charge matching and attainment of normal lipid density. In this sense, the combination of tilted lipids and A-DNA in the L_1 phase represents the prescription for achieving economical electrostatic binding.

In conclusion, we have identified two distinct multi-lamellar phases characterized by distinct states of lipid packing (i.e., tilted vs untilted chains) and DNA conformation (A form vs B form) in the electrostatic DNA/DC-Chol complex in the bulk state. While the DNA duplex conformation is known to be governed by the local water activity and its base sequence, our finding discloses another pathway for altering the conformational structure through electrostatic binding with the lipid membrane in the bulk state. We have also demonstrated that the self-assembled architecture in terms of the DNA smectic

ordering and the long-range lamellar ordering of the DNA/CL complex in excess water can persist into the bulk state. The dry complex thus constitutes a special class of nanostructured bulk material with ordering at two length scales (i.e., the so-called "order-within-order"). Through the incorporation of inorganic substances on DNA,¹⁸ for example, this type of material may serve as a unique soft template for producing spatially ordered nanowires with longitudinal spacing between the nanowires switchable by the lipid-to-base-pair molar ratio.

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