

Exotic Phases of DNA-Lipid Complexes

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Abstract

It was found in 1997 that mixing DNA and lipid vesicles in water results in the formation of complexes comprised of bilipid layers with aligned DNA strands intercalated between the layers. In addition to having useful applications in gene therapy, these complexes exhibit unique physical properties and manifest phases that had been previously only of theoretical interest. In particular, these systems have an approximate expression of the 2D smectic phase. This paper provides a brief summary of the progress in both the theoretical and experimental study of these complexes.

1 Introduction

During the past decade, physics has provided the biological sciences with many new methods and techniques, which have generated far-reaching new insights and discoveries. In this way, physics has aided biology. The help has not gone unreciprocated, however. Biological systems have provided condensed-matter physicists with a host of excellent systems for the experimental study of fundamental physics principles.

One of the more recent examples of these systems are complexes of DNA and lipids. These systems exhibit a number of exotic phases that are accessible both to both theory and experiment. This article presents a summary of results from both aspects of the study of these systems.

1.1 The System

DNA-cationic-lipid (DNACL) complexes are prepared by mixing DNA with liposomes (lipid vesicles) in ultra-pure water. These complexes were first studied in detail by Rädler et al., and are described in [5]. The liposomes have a hydrophobic and a cationic end. This end is attracted to and is neutralized by the negatively charged phosphate groups on the DNA. The resulting self-assembled complexes contain aligned strands of DNA sandwiched between planar lipid bilayers similar in structure to the phospholipid layers comprising cell membranes. A section of the structure is shown schematically in Figure 1.

1.2 Biological Significance

The similarity between these complexes and the phospholipid bilayers in cell membranes has important practical implications in biology. In particular, these complexes can be used to transport DNA across cell and nuclear membranes *in vivo*. Hence, they provide an alternative to viral-based carriers in applications such as gene therapy. They have low toxicity and do not trigger an immune response as viral carriers do [5]. Furthermore, their production involves little more than the mixing of the DNA and liposomes in water, as opposed to complicated gene splicing protocols required for viral carriers. Unfortunately, this new method of transport is not well understood, and its effectiveness varies up to two orders of magnitude between different types of animal cells.

1.3 Physical Significance

While of important practical value to biologists, the DNACL complexes are also of distinct theoretical interest to condensed matter physicists. In these complexes, the

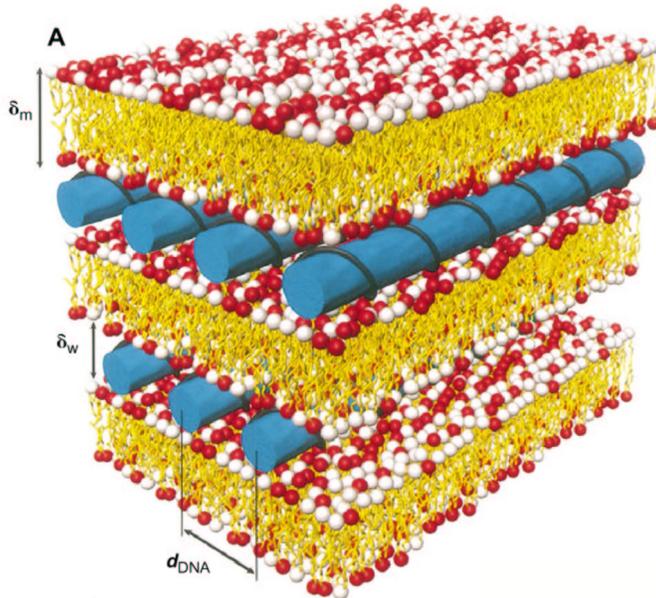


Figure 1: A schematic drawing of the interior of a lipid-DNA complex. The blue rods represent DNA helices between the layers of lipid complex. This picture was borrowed from [5].

lipid bilayers effectively restrict the motion of the DNA strands to a plane. When self-assembly is complete, the strands become aligned and form what is known as a two-dimensional smectic.

In three spatial dimensions, a smectic consists of fluctuating planes of material. Perhaps the canonical examples of this phase occur in some liquid crystals [8]. By analogy, the two-dimensional form of this phase consists of fluctuating lines of material in a plane. This phase is expressed in extremely few experimentally-accessible physical systems, although close analogies exist with quantum Hall phases [2].

Since the DNA strands in DNACL complexes are effectively confined to a plane, the parallel strands form an effective 2D smectic phase. This gives rise to interesting physical properties, such as a strand-strand correlation function for the DNA that decays as a power law. This indicates that there is no true long-range order in the in-plane direction perpendicular to the strands. In the next section, we discuss these properties, and those of smectics in general, in more detail.

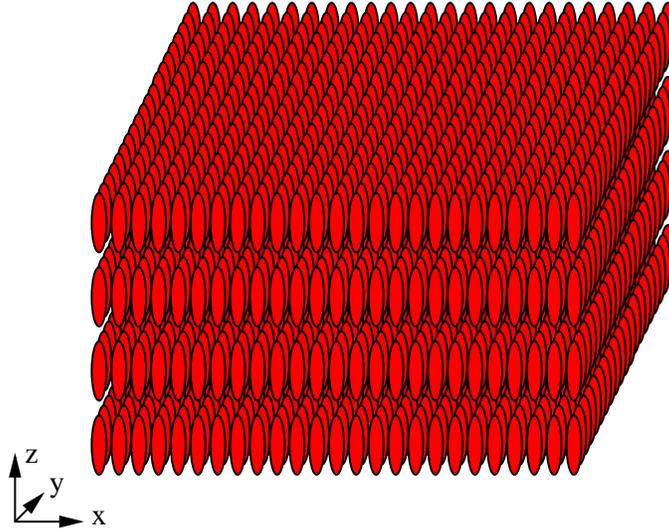


Figure 2: Schematic diagram of the smectic A phase in a liquid crystal. The rod-like molecules align into parallel sheets, resulting in unusual anisotropic behavior.

2 Smectics

2.1 Three Dimensions

Early experiments in x-ray crystallography decades ago revealed that periodic arrays of atoms in three-dimensional crystals have what is known as “long-range order”. X-ray diffraction experiments directly measure the structure factor, $S(\mathbf{q})$, which is the Fourier transform of the real-space correlation function between atoms, $g(\mathbf{r})$. Long range order implies that $S(\mathbf{q})$ has δ -function peaks at the \mathbf{q} ’s corresponding to lattice displacements. In an infinite crystal, this implies that $g(\mathbf{r})$ does not decay with distance, and that spatial correlations exist between infinitely distant atoms. In three dimensions, this is true even in the presence of thermal fluctuations. It is commonly demonstrated in introductory solid-state physics courses that these fluctuations reduce the magnitude of the δ -function peaks, but do not broaden them [4]. This reduction is known as the Debye-Waller factor.

In two dimensions, however, it can be shown that the Debye-Waller factor becomes zero, corresponding to a total suppression of δ -function peaks in $S(\mathbf{q})$. That implies that thermal fluctuations destroy the long range order – that distant atoms are no longer correlated in a 2D crystal. As a result, $S(\mathbf{q})$ has power-law singularities instead of δ -functions at the inverse lattice spacings. Furthermore, the peaks are greatly suppressed at successive harmonics. In real space, $g(\mathbf{r})$ decays with a power-law form. For a number of technical reasons beyond the scope of this paper,

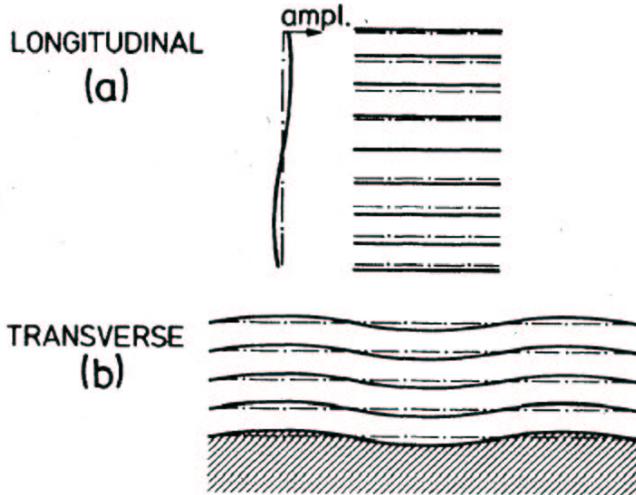


Figure 3: The two fundamental fluctuation modes of a three-dimensional smectic. The longitudinal modes reflect the compression of sheets against each other, while the transverse modes reflect the bending of the layers. This figure taken from [3].

these properties are extremely difficult to observe experimentally in 2D crystals.

A very similar phenomenon, which *is* experimentally observable[3] is known to occur in the smectic phases of liquid crystals. Liquid crystals, which consist of weakly-interacting rod-like molecules, are so named because they exhibit characteristics of an intermediate between solids and liquids. In particular, in the smectic phases, the rod-like molecules align into lamellar sheets, as shown in Figure 2. Within the planes of these sheets, the molecules exhibit liquid-like properties, with no long-range order. In the direction normal to the sheets, however, order does exist, although it is not long-ranged. $S(\mathbf{q})$ decays as a power law in these systems. In particular, for an ideal system with no external field, the energy density is given by

$$F_{3D}(\mathbf{q}) = \frac{1}{2} (Bq_{\parallel}^2 + Kq_{\perp}^4) u^2(\mathbf{q}). \quad (1)$$

Here, $u(\mathbf{q})$ represents a displacement field corresponding to the wavevector \mathbf{q} , and B and K are empirically determined constants. q_{\parallel} represents a longitudinal mode in the \hat{z} direction, while q_{\perp} represents transverse perturbations of the smectic layers as shown in Figure 3. It can be shown that the scattering amplitude has the form

$$S(0, 0, q_{\parallel}) \sim (q_{\parallel} - q_0)^{-2+\eta} \quad (2)$$

$$S(q_{\perp}, 0, q_0) \sim q_{\perp}^{-4+2\eta} \quad (3)$$

in the long wavelength limit, with $\eta = kT(q_0^2/8\pi)(BK)^{-\frac{1}{2}}$, and $q_0 = 2\pi/d$, where d is the average spacing between layers. This is derived from the correlation function,

which is given by

$$g(x, y, z) \sim e^{-2\eta\gamma} \left(\frac{4d^2}{x^2 + y^2} \right) e^{-\eta E_1((x^2+y^2)/4\lambda z)}, \quad (4)$$

where γ is Euler's constant and

$$E_1(-x) \equiv \int_x^\infty \frac{e^{-t}}{t} dt \quad (5)$$

The calculations leading to these forms are given in [3].

2.2 Two Dimensions

In two dimensions, a smectic consists of a stack of flexible rods confined to a plane. For consistency with [3] and [9], we adopt the coordinate system in which \hat{x} is parallel to the direction of the rods, and \hat{z} is normal to it. The simplest free energy density can then be given by

$$F_{2D} = \frac{1}{2}B \left(\frac{\partial u(x, z)}{\partial z} \right)^2 + \frac{1}{2}K \left(\frac{\partial^2 u(x, z)}{\partial x^2} \right)^2. \quad (6)$$

A simple Fourier transform shows that this form is equivalent to that given in the 3D case in (1). The first term gives the elastic energy for the compression or rarification of neighboring rods against each other, while the second term gives the elastic energy for the bending of individual rods. This free energy leads to a correlation function of the form

$$g(x, z) = \exp \left[-\frac{2\pi\eta}{\lambda} \sqrt{\pi\lambda|z|} \exp \left(\frac{x^2}{4\lambda|z|} \right) - \frac{\pi^2\eta}{\lambda} |x| \operatorname{erf} \left(\frac{|x|}{2\sqrt{\lambda|z|}} \right) \right]. \quad (7)$$

as given in [9]. This form does not have a simple Fourier transform and requires the structure factor to be calculated numerically.

3 Modeling DNACL Complexes

The free energy density given in (6), while capturing some of the most interesting physics of DNACL complexes, does not account for all of the relevant degrees of freedom in those systems. In particular, this free energy neglects at least the following terms:

- The out-of-plane fluctuations of the DNA against the lipid walls.

- The interaction energy between DNA strands separated by lipid layers.
- The interaction energy between DNA strands and the lipid layers.
- The elastic energy resulting from deformations of the lipid layers (which is anisotropic due to the stiffening in the \hat{x} direction due to the presence of the DNA).
- The compressional energy due to longitudinal density fluctuations within each lipid layer.

In many cases, the proper inclusion of these terms simply results in additional peaks in the structure factor, $S(\mathbf{q})$. For example, the inclusion of the elastic terms for the bending and longitudinal compression of the lipid layers results in peaks expected in a traditional 3D smectic, although the shapes of the peaks are slightly different because of the anisotropy introduced by the direction of the DNA strands. The resultant scattering profile is just a combination of peaks expected from 2D and 3D smectics. The out-of-plane oscillations of the DNA strands can be properly accounted for by integrating out these degrees of freedom from the partition function, resulting in an effective free energy for the in-plane oscillations, in which the constant B (specifying the compressional energy cost) is modified [7].

3.1 Alternative Phases

3.1.1 Columnar Phase

In other cases, these terms can result in phases which are qualitatively different, in which the 2D smectic behavior is suppressed. For example, the interactions between DNA strands across lipid membranes, if sufficiently strong, can cause the strands to form a columnar lattice, similar to the lattice of flux penetration in a high- T_c superconductor. The formation of the lattice in this *columnar phase* would result in true long-range order, i.e. correlation functions between the DNA strands which extend to infinity.

Thermal fluctuations can overcome these trans-membrane interaction energies, effectively “melting” the lattice without destroying the whole complex. This melted lattice gives rise to a number of phases ranging in the amount of relative order. At one extreme is the columnar phase just discussed. At the other extreme is the decoupled smectic phase, described above, in which the rows of DNA behave as if infinitely separated from each other, and hence totally uncorrelated.

3.1.2 Sliding Columnar Phase

In an intermediate regime between the columnar and smectic phases, the arrays of strands are correlated with arrays across membranes which have correlated directions, but not positions, as described by O’Hern and Lubensky in [1]. Formally, if we consider the displacement field, $u(\mathbf{r})$, the derivative $\frac{\partial u}{\partial x}|_{\mathbf{r}}$ at \mathbf{r} is correlated with the derivative $\frac{\partial u}{\partial x}|_{\mathbf{r}'}$ at a distant \mathbf{r}' , while $u(\mathbf{r})$ and $u(\mathbf{r}')$ are uncorrelated. It is also distinguished from the smectic phase by virtue of the fact that the energy of compressional modes of DNA fluctuations does not vanish as $q \rightarrow 0$. This phase exhibits a very unusual form of the correlation function: $g(r) \sim \exp(-\text{const} \times \ln^2 |\mathbf{r}|)$. This behavior is intermediate between a power law, which has no characteristic length scale, and an exponential, which has a characteristic length scale. This form is essentially a power law with an exponent that changes slowly with a characteristic length scale. This phase, known as the *sliding columnar phase*, is theoretically possible, but has not yet been observed experimentally. These phases were also studied independently by Golubović and Golubović and can be found in [7]. A collaboration between both author pairs later led to [6].

3.1.3 Nematic Lamellar Phase

The *nematic lamellar phase* is very similar to the sliding columnar phase, but is characterized by a compression modulus for in-plane strands of DNA which vanishes in the long-wavelength limit. This results in a positional correlation function for DNA strands which decays exponentially with distance, rather than the power-law form exhibited in 2D smectics and the exotic $\exp(\ln^2)$ form exhibited in the sliding columnar phase.

It should be noted that in the macroscopic limit, a truly decoupled smectic state does not exist unless the coupling constant governing trans-membrane DNA interactions is identically zero. In the limit of weak coupling, these complexes are actually in the nematic lamellar phase, which can appear indistinguishable from the smectic phase at relatively short distances [6].

4 Experimental Results

The theoretical investigations described above were first motivated by the experimental discovery and subsequent X-ray study of the DNACL complexes. Unfortunately, in the intervening time, it appears that the theoretical work has outpaced the experimental work, which has left a relative paucity of experimental data. The available data that has been published is summarized in this section.

Figure 4 shows the results of 8 keV x-ray diffraction experiments carried out

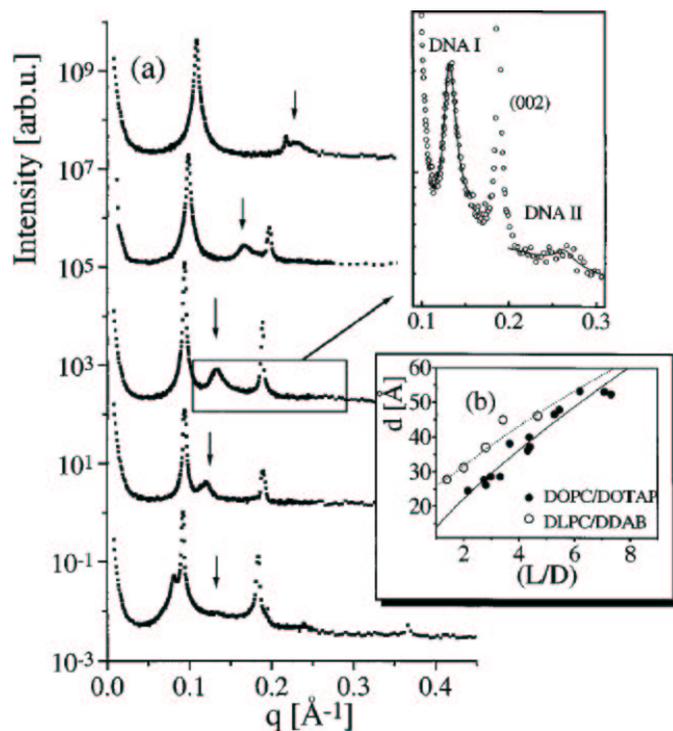


Figure 4: (a) X-ray scattering from DNACL complexes in water for small scattering angles. The various curves in the main graph represent different values for the ratio, ν , of neutral to cationic lipids. The curves have been shifted multiplicatively by arbitrary factors to separate them on the graph. From top to bottom, the curves represent systems for values of $\nu = 0, 0.67, 1.5, 2.33,$ and 5.25 . The large, sharp peaks show the usual power-law decay of Bragg-reflections from the multilamellar lipid bilayers. The smaller peaks, marked with arrows, show the peaks resulting from DNA-DNA correlation functions. In some samples, a second harmonic is visible, corresponding to strands separated by twice the fundamental spacing, as shown in the magnified inset. (b) The average distance between chains, d , is calculated from the position of the peaks as a function of ν . This figure was taken from [9].

at the Stanford Synchrotron Radiation Laboratory [9]. The samples were prepared by mixing λ -phage DNA ($\approx 16.5 \mu\text{m}$ in length) with a suspension of liposomes, which are essentially globules of lipid (fat) molecules. In particular, these liposomes consisted of a mixture of neutral and cationic (positively charged) lipids, with a weight ratio $\nu \equiv \text{mass neutral}/\text{mass cationic}$, which was varied as an experimental parameter. The various curves represent different values for ν , and have been shifted by arbitrary multiplicative factors to make the curves separately visible.

Perhaps the most prominent features in these curves are the sharp, power-law Bragg peaks. This results from reflections from the lamellar lipid membranes. We note a small shift in these peaks outward with increasing concentration of cationic lipids. This implies that the intermembrane spacing, d_L , decreases with increasing cationic concentration. The tails of these peaks decay algebraically, but the shape differs from a simple 3D smectic. This can be understood by considering the anisotropy of the membrane bending fluctuations, which result from the stiffening of the membrane in the \hat{x} direction of the DNA strands.

Marked with arrows are much smaller peaks corresponding to the scattering from the DNA chains themselves. We note that these peaks also shift outward with increasing cationic concentration, but much more markedly, indicating a strong decrease of the average strand-to-strand spacing, d , of the DNA.

Of greater relevance to the concepts discussed in this article are the specific shapes of the DNA correlation peaks. These are shown in an expanded view in Figure 5. In this plot, the background has been subtracted and the intensity plotted as a function of q scaled and shifted by q_0 . Of particular interest in these plots are solid curves representing fits of the data to curves expected from the theory of 2D smectics. In truth, these curves are not calculated from consideration of completely decoupled smectic planes of DNA, but take into account correlations between neighboring planes.

In particular, these fitting curves are constructed from theory as follows. First, the 2D structure factor is computed by numerically Fourier transforming the correlation function, $g(x, z)$ given in (4),

$$S_{2\text{D}} = \frac{1}{2\pi} \int_{-\pi}^{\pi} d\phi \int_{-\infty}^{\infty} dx \int_{-\infty}^{\infty} dz g(x, z) \exp\left(-\frac{\pi r^2}{L^2}\right) e^{i(\mathbf{q}-q_0\hat{z})\cdot\mathbf{r}}. \quad (8)$$

Here, $\mathbf{r} = (x, z)$, $\mathbf{q} = (q_x, q_z)$, and the Gaussian term has been included as a convenient way to account for the finite size, L , of the DNACL complexes. X-ray scattering effectively samples complexes in all possible orientations with respect to the incident beam. As a result, to compute scalar structure factor, we must integrate over these orientational degrees of freedom,

$$S_{3\text{D}}(q) \propto \int_{-\frac{\pi}{2}}^{\frac{\pi}{2}} d\theta \sin(\theta) S_{2\text{D}}(q \sin(\theta)) \left[\frac{J_1(r_D q \cos(\theta))}{r_0 q \cos(\theta)} \right]^2 f_y(q \cos(\theta)). \quad (9)$$

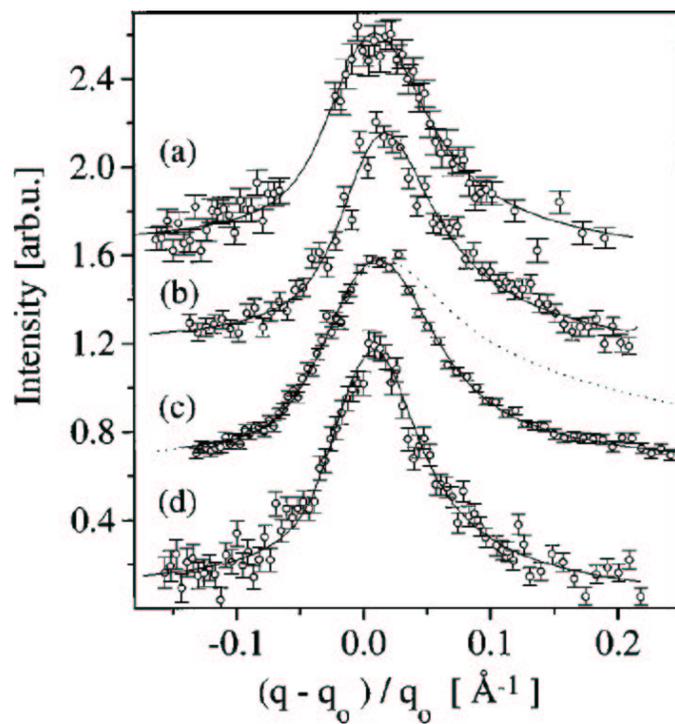


Figure 5: The DNA correlation peaks from Figure 4 have been expanded and the background subtracted to show the characteristics of the curves. The smooth curves through the data points represent fits to the 2D smectic structure factor. See text for a description of the construction of this fit. This figure was taken from [9].

The term in square braces accounts for the finite width of the DNA helices, while the final term, f_y , represents the correlations between planar lattices of DNA separated by membranes. If strands were truly uncorrelated across membranes, as one would expect in the decoupled smectic phase, then f_y would be equal to unity. This form does not properly fit the data, indicating correlations are present. The authors of [9] find that the form $f_y(q_y) = (\xi^2 + q_y^2)^{-1}$, which corresponds to an exponentially decaying correlation function in real space with characteristic length scale $1/\xi$, fits the data well, as can be seen in the figure. This implies that these complexes are not truly in the totally-decoupled smectic phase, but rather in the weakly-coupled limit of the nematic lamellar phase. Nonetheless, the form used for the in-plane DNA-DNA correlation functions, which was derived from a smectic free energy, fits the data well. This indicates that the local in-plane behavior of DNACL complexes is well described by the smectic phase, although theoretical considerations suggest that at large distances, the correlation functions must ultimately take on the asymptotic form of an exponential, rather than a power law. A full resolution of these issues may require experimentation with higher resolution.

Although much has been learned from both theoretical and experimental investigations into these complexes, it is clear that additional study is warranted. In particular, whether or not the sliding columnar phase is exhibited in these complexes for suitable values of the experimental variables is still in question. In addition to providing new insights in fundamental physics, an in-depth understanding of the behavior of these complex may ultimately have practical medical application in gene therapy.

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