Formation of Two-Dimensional DNA Smectics Layered between Lipid Membranes

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In the past several years experimental results have observed a new structural formations of DNA and cationic lipid bilayer constructs. In vitro, two-dimensional smectics, consisting of the long DNA strands, embedded between liposome membranes that are themselves three-dimensional smectics, have shown to spontaneously self-assemble. These formations have been studied using standard x-ray diffraction methods. I will be discussing these experimental results and their interpretations as they pertain to the governing characteristics of these states of matter. A type of phase transition pertaining to this structure, its coupling between layers, and thermal fluctuations, has also been observed. Also there will be some discussion concerning theoretical models that attempt to describe properties of experimentally rare two-dimensional smectic phases.

Introduction

Due to their unquestionable importance in biological systems, the physiological properties of DNA molecules have been well studied and classified over several years of research. From charge distribution to persistence lengths, DNA is generically one of the most fundamentally researched and documented constructs in the biosciences. Now, with gene therapy on the horizon, there is pressure to discover effective means by which to extracellularly transport these polymer chains. The solution may lie within casings comprised of cellular or nuclear membrane material called liposomes, similar to vesicles that allow viruses mediate their genetic coding so efficiently throughout the cells of their host. Along the lines of these studies, a new form of biological structure has been observed to self-assemble in vitro comprising of long DNA chains molecules and bilayers comprised of neutral and cationic lipid proteins. This structure consists of rod-like DNA molecules aligned parallel to one another to form a two-dimensional smectic structure. These layers are sandwiched between lipid membrane sheets in a repeating fashion as shown in figure 1 taken from Salditt et al. (1997).

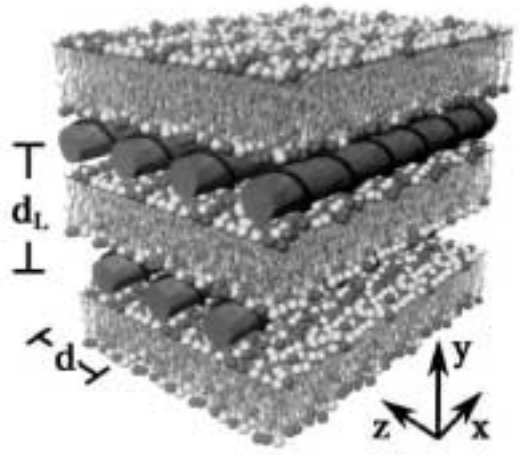


Figure 1

Looking at the formation of these structured layers, or most any protein-protein interaction in water, there are a number of effective forces to consider, some of which are not completely understood. The main ones that are discussed include the van der Waals attraction, electrostatics, and hydrophobic and hydrophilic effects. A balance in competition between these and perhaps other sources of free energy result in the system being able to create structures such as this. I will discuss these effective forces and how they pertain to this DNA-liposome construction.

Formation of the lipid bilayer membranes can be well accounted for by considering the hydrophobic effect. Water is a polar molecule and therefore has a

strong attraction to other polar, or hydrophilic, structures. Proteins and molecules with a large amount of exposed polar regions are quite soluble in water. Non-polar, or hydrophobic, regions feel and "attraction" to one another when submerged in water due to more entropic reasons. As two hydrophobic regions become close to one another, the water molecules between them are confined to the volume between them. The amount of phase space that they can now becomes limited compared to that of if they were far from the non-polar regions. As a consequence, the two hydrophobic surfaces expel the water between them to raise its entropy and come close together (Clegg). Lipid molecules are made up of a hydrophilic head group and two hydrophobic tails. In water the tails will aggregate together to expel the water between them. The result is the bilayer membrane that keeps the hydrophobic tail groups hidden from the water solvent and the hydrophilic head groups exposed. There are other structures that may form depending on the concentration of lipid proteins, such as small spheres or long cylindrical rods, but these are irrelevant to the topic of this paper.

Secondly, there is the van der Waals attraction, which brings together the lamellar membranes and the DNA rods. The van der Waals interaction is governed by induced-dipole interactions within the molecules themselves. An r^{-6} power law attraction is attributed to this when looking at two point-like molecules, which is very strong at short distances, but falls off rather quickly. However, this DNA-liposome mixture will deal with surface-cylinder and surface-surface interfaces, which have power law interactions that go as $r^{-3.5}$ and r^{-2} per unit area respectively (Clegg). These forces die of at a much slower rate and are more effective at longer distances to bring the membrane sheets and DNA polymers together.

Of course, electrostatics plays a large role in these structures. DNA molecules have a large negative charge from the phosphate groups that comprise their backbone. Therefore there will be some repulsion between adjacent strands. However, the bilayer is composed of neutral and cationic (positively charged) lipids that will repelled from one another, yet be attracted to the DNA. The relative density ratio between these positive and negative charges will be discussed later. These electrostatic interactions are comparatively strong to begin with, yet if salt or free ions are present in the solution, a screening effect takes place effectively decreasing the amount of charge that one molecule sees of another according to Debye-Hueckel theory. One can imagine two large heavily positively charged molecules in solution filled with tiny charged particles. Several of the negative point charges will cluster around each large molecule while the positive ions will be dispersed. Clustering of negative ions will "screen" the amount of charge one molecule detects of the other, and as a consequence, the molecules will not repel one another as intently as before (Clegg).

There also exists two other membrane-membrane repulsion interactions that exist spawning mainly from entropic arguments. First is something called the "hydration force." It remains unclear, but has to do with the free energy costs associated with water concentration gradients at very close molecular separation lengths and decays exponentially with a characteristic length of ~3Å. The second

is repulsion due to the thermal undulations of the membranes themselves. Imagine a flexible membrane trapped between two other rigid sheets. Fluctuations in the membrane are confined by the separation length of the two sheets and closing this length translates into a loss in configurational entropy. From this arises and effective repulsion interaction with an r^{-2} power law, which is on the same order as van der Waals (Gelbart et al.).

Attractive forces from electrostatic, hydrophobic, and van der Waals interactions as well as the repulsive forces from "hydration," thermal fluctuations, and like-charge repulsion are phenomena that are present in most all biological protein interactions. The extent at which these force interactions seems to be understood far to little compared to their omnipresence in experimental procedures. Theories at this time are far from complete, but perhaps further study of this system and others like it, may lead to deeper understandings of the way in which these free energy contributions interplay with one another in other systems.

Experimental Observations

These layered DNA smectics inset between lipid membranes were self assemble from mixtures of neutral and positively charged lipids and negatively charged DNA strands. This paper will cover the experimental results of two labs. One is from the University of California, Santa Barbara as discussed in Salditt et al (1997), as well as those from the European Molecular Biology Laboratory as discussed in Artzner et al. (1998). The first looks at a few variations of experimental parameters, namely the ratio of neutral to charged lipids. While the later focuses on trying to observe DNA positional correlations across the lipid bilayer, from one two-dimensional smectic to another, and various temperature conditions. In the end, they find a first order phase transition with lowering temperatures as the thermal fluctuations decrease and trans-membrane interactions between DNA strands becomes more dominant. Both labs observed these DNA-liposome structures using x-ray diffraction methods. The former utilized facilities at the Stanford Synchrotron Radiation Laboratory, and the other at the EMBL beam line X13 at DESY Hamburg, Germany.

First off, the University of California team was able to identify the nature of this structure through sufficient x-ray scattering line shape analysis. The results showed a clear spacing of lipid layers as well as tell signs of the DNA ordering. All findings were characteristic of the DNA being arranged into a two-dimensional smectic formation encased within the three-dimensional lamellar construct of membranes.

With intent to vary the one-dimensional DNA lattice spacing, samples of varying concentrations of cationic lipid proteins were created and observed. The idea being that bilayers with different surface charge densities would align the DNA correspondingly closer or farther apart. Values of $\nu = 0$, 0.67, 1.5, 2.33, and 5.25 we studied where ν is the mass ratio of neutral lipid molecules divided by that of the positively charged lipids. Results showed that the highly negative DNA strands were arranged to exactly neutralize the positively charged

membrane surface. The intermolecular spacing of DNA went from nearly closed packed form at d = 26Å when ν = 0, to fairly dispersed at d = 54 Å at high ν. Experimental results agreed well with the functional form

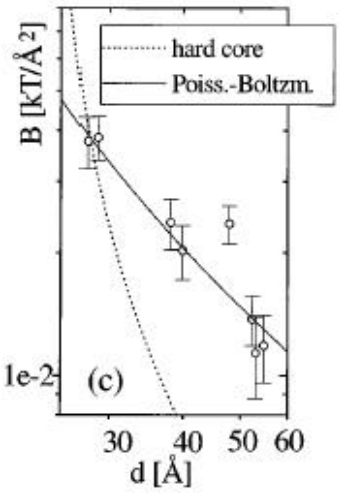
$$d = (A_D/\rho_D)/(\delta_m/\rho_l)(L/D)$$

Here, A_D is the cross sectional area of the DNA, ρ_D and ρ_l are the densities of DNA and liposome, δ_m is the membrane thickness, and (L/D) is the total lipid over DNA mass ratio. Throughout this separation variation data set, the distance between membrane faces remained relatively the same. It consisted of the DNA diameter thickness (20Å) plus a small "hydration" layer.

Observations were also done on the interaction forces between neighboring DNA chains within the same smectic layer. Through certain fitting analysis applied to the x-ray scattering peaks, Salditt et al. were able to extract correlation lengths along the x- and z-axes. Using this and assumptions that the system's splay modulus K is governed by the persistence length ξ_p of the DNA chains (a measure on which length scale a DNA rod remains rigid ≈500Å), one can calculate the experimental compressional modulus B(d). By definition B(d) = -d ∂P/∂d, where P(d) is a pressure between the DNA molecules. With low concentrations of salt, it was assumed that this pressure was governed by electrostatic repulsion between adjacent DNA chains. Following a Poisson-Boltzmann model for counterion interaction in two-dimensions, the pressure is proportional to $(\pi k_B T) / [2l_B(d - \rho)]$. Here ρ = 4.4Å is an exclusion radius determined by the radius of DNA and lipid head group size, l_B is the Bjerrum length $e^2 / (\varepsilon k_B T)$, e is a charge unit and ε is the dielectric constant of water = 80.

$$B(d) \propto \frac{\varepsilon\pi(k_B T)^2}{2e^2}\frac{d}{(d-\rho)^2}$$

Figure 2



This gave a reasonable fit to the experimental B(d) results (fig. 2) demonstrating that indeed the structuring is consistent with a description of dominating electrostatic forces. Also shown in the figure is a curve that corresponds to the compressional modulus governed strictly by a pressure related to one-dimensional hard-core repulsions of the DNA rods (Salditt et al 1997).

According to Artzner et al., there are theoretically three different phases allowed with this structure of DNA-liposome construct. Their difference lies within the correlations between the inset DNA rods across the cationic lipid bilayers. One phase is described as an ultimately decoupled phase where strong fluctuations in the two-dimensional smectics dominate the

positioning. The second is what was more than likely observed by Salditt where there is long-range orientational order (inter-layer DNA molecules retain the same directionality), yet only contain short-range positional order (positions along the z-axis are weakly correlated with those in neighboring smectics). Finally, Artzner describes and observes the third phase, which is a columnar phase they call a "lipid-gel" phase where there is strong coupling positionally as well as orientationally across lipid membranes. This phase is observed at lower temperatures (15 °C) under conditions of low ν (high charged lipid concentrations). Observations of the weakly coupled phase were done at 55°C.

Figure 3

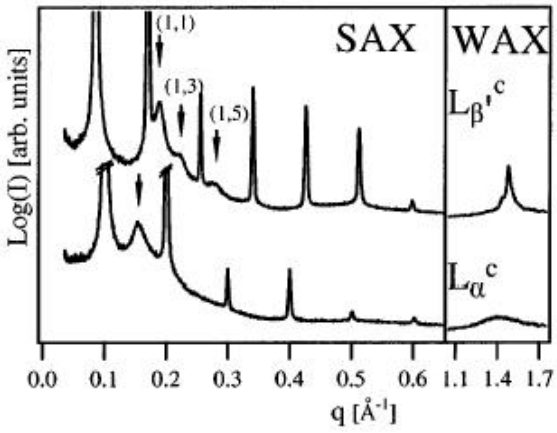
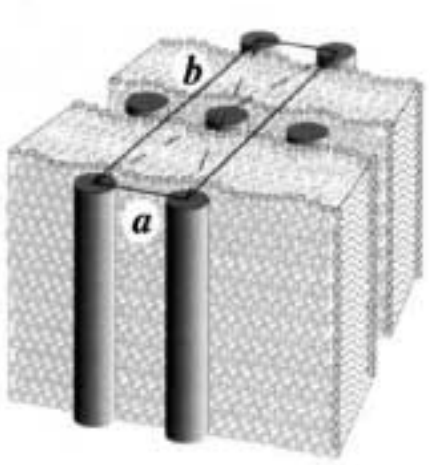


Figure 4



Bragg peaks observed from the columnar phases (shown in figure 3) that are absent in the weakly correlated phase can be attributed to a rectangular centered lattice arising in the DNA positioning (figure 4). (In figure 3 the top line corresponds to the "lipid-gel" phase, and the lower corresponds to the weakly coupled phase. The (1,1) (1,3) and (1,5) labeled peaks are the ones discussed.) These peaks were present in a number of samples with varying DNA spacing d. Artzner et al. discuss two interactions that could account for this stacking phenomenon. First, the membranes may slightly deform around the DNA rods and this bending may propagate across the lipid layer creating an outward protrusion on the other side. Second, the DNA electrostatic repulsions across the layers would also lend itself to such an ordering. Both of these effects would lead to a "washboardlike" energy potential within the two-dimensional smectic arising from the neighboring layers (Artzner et al. 1998).

Theoretical Models

Two papers, one by O'Hern and Lubensky (1998) and one by Leonardo and Mirjana Golubović (1998), discuss theories concerning the fluctuations and coupling of the smectic layers. Both begin by looking at energy descriptions and continue on to look at positional and orientational correlations between interlayer

DNA rods. In the end they discover interesting behavior of the characteristic fluctuations of the smectics.

O'Hern and Lubensky begin with a Hamiltonian $H = H^{el} + \sum_n \left(H_n^u + H_n^\theta\right)$ where

$$H^{el} = \frac{1}{2}\sum_n \int d^2 r \left[B_2 \left(u_{zz}^n\right)^2 + K_2 \left(\partial_x^2 u^n\right)^2\right]$$
$$H_n^\theta = -V_\theta \int d^2 r \cos\left[2\left(\theta^n - \theta^{n+1}\right)\right]$$
$$H_n^u = -V_u \int d^2 r \cos\left[k_0 \left(u^n - u^{n+1}\right)\right]$$

H_n^θ and H_n^u relate to the displacement and orientational dependent terms, respectively. B_2 and K_2 are the 2D compression and bending moduli, *u* is the displacement vector of each DNA, *n* is the index of each smectic layer, $k_0 = 2\pi/d$ describing rest molecular spacing, and V_θ and V_u are interaction amplitudes. If V_u is large, the system will align as a center rectangular 2D lattice, as described earlier. As V_u is decreased (or, effectively, temperature increases) fluctuations dominate and this columnar phase will melt into the weakly coupled phase. If both V_u and V_θ become sufficiently small, then we fall into the completely decoupled phase. It is argued that the coupling energies H_n^θ and H_n^u are "irrelevant" if the tend to zero for large system sizes L_x and L_z. Under this assumption H_n^u becomes irrelevant and the Hamiltonian takes the form

$$H = \frac{1}{2}\int d^3 x \left[B u_{zz} + K\left(\partial_x^2 u\right)^2 + K_y \left(\partial_y \partial_x u\right)^2\right]$$

Note that this is invariant under the transformation $u(r) \to u(r) + f(y)$ that describes a rigid transformation from one smectic layer to another. Therefore, there is no free energy cost associated with this y-directional translation. Through this description, one eventually arises at fluctuations characterized by $\left\langle u^2 \right\rangle \sim C \ln^2 L$ (O'Hern et al. 1998).

Leonardo and Mirjana Golubović report that samples of two-dimensional liquid crystals are characterized by $\left\langle u^2 \right\rangle \sim L_x$ for $L_x \ll L_z^{1/2}$ and $\left\langle u^2 \right\rangle \sim L_z^{1/2}$ for $L_x \gg L_z^{1/2}$. However, after deriving the following

$$\left\langle \left|u(q)\right|^2 \right\rangle = \frac{k_B T}{\tilde{B}_{sm}^{(2D)} q_z^2 + K_{sm}^{(2D)} q_x^4 + q_y^2 \left(K_{yx} q_x^2 + K_{yz} q_z^2\right)}$$

(*2D* and *sm* for 2D-smectics and $\tilde{B}$ is a renormalized compression modulus) they arise with the result reported previously $\left\langle u^2 \right\rangle \sim \ln^2 L_x$ for $L_x \ll L_z$ and $\left\langle u^2 \right\rangle \sim \ln^2 L_z$ for $L_x \gg L_z$. Their results are based on a similar interaction energy

description. They note that the fluctuations described by the equation above give rise to a "soft axis," being along q_y. Fluctuations diverge along this entire axis as $q_x = q_y = 0$, which is a result of the gauge symmetry $u(r) \rightarrow u(r) + f(y)$ that was noted earlier (Golubović et al. 1998).

Conclusions

In this paper I have shown experimental proof of the novel state of matter constructed of galleries of two-dimensional DNA rod smectics, intercalated between lipid membranes. X-ray scattering shows Bragg peaks that are telltale of these orderings within the sample. They even display signs of separate phases corresponding to weak and strong coupling between layers highly dependent upon the degree of thermal undulations. These couplings can be well interpreted by elasticity theories despite the complexity of the interplay between forces within the biological samples. From these theories eventually arises a novel classification of the characteristic fluctuations that is unseen in three-dimensional smectics or other experimental 2D smectic structures. Future studies may give further insight into other biological aggregation interactions properties as well as interesting theoretical models of coupled layered systems.

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