

DNA at membrane surfaces: an experimental overview

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Atomic force microscopy studies of DNA attached to rigid surfaces were initially motivated by the development of methods which would stretch DNA for the purposes of rapid sequencing. Currently there is much interest in studies of multilayers of DNA chains self-assembled on membranes which form spontaneously when DNA adsorbs onto oppositely charged cationic liposomes (CLs). A major motivation for elucidating the structures and interactions in these CL–DNA complexes arises for two reasons. The first is that they are known to mimic certain characteristics of viruses by being efficient chemical carriers of genes (DNA sections) for delivery in cells. The second is that they are models of studies of DNA condensation phases in two dimensions. DNA–membrane interactions should also provide clues for the relevant molecular forces in the condensation of DNA in chromosomes and viral capsids. The particular complexes described in this review contain linear DNA forming a new ‘hybrid’ phase of matter; that is, the DNA chains form a finite size two dimensional smectic coupled to the three dimensional smectic phase of membranes.

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Current Opinion in Colloid & Interface Science 1998, 3:69–77

Electronic identifier: 1359-0294-003-00069

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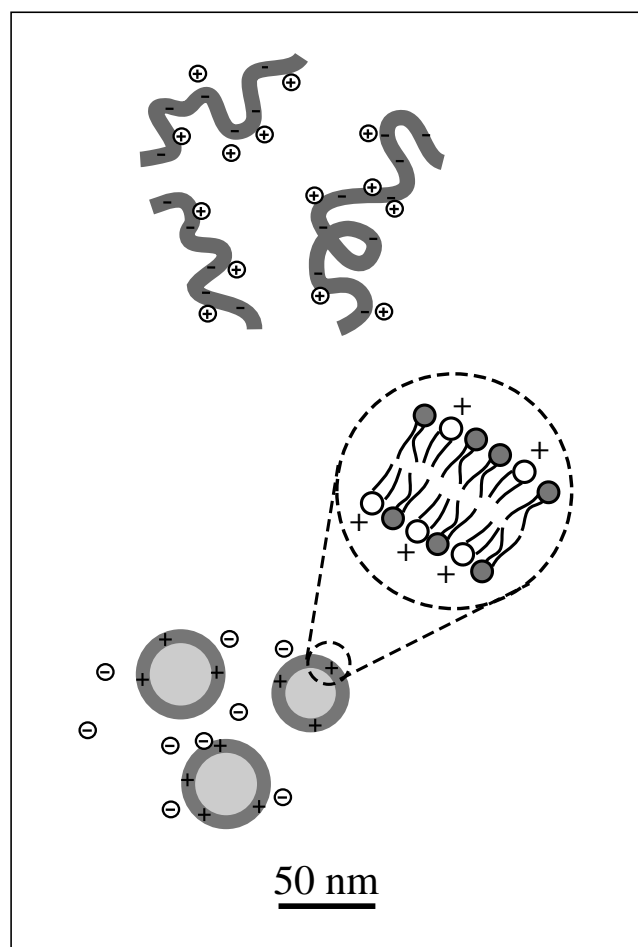
Abbreviations

AFM	atomic force microscopy
CL	cationic liposome
D	DNA (weight)
DIC	differential-interference-contrast
DOTAP	di-oleoyl trimethylammonium propane
DOPC	di-oleoyl phosphatidyl cholin
L	total lipid (weight)

Introduction

In this review we will be describing experiments which are centered around DNA electrostatically adsorbed at cationic lipid surfaces. Recently, it was discovered [1•,2] that a multilayer assembly of DNA adsorbed on bilayer membranes forms spontaneously when DNA is complexed with cationic membranes. The cationic membranes may either be comprised of a broad size distribution of vesicles (single or a few concentric closed bilayer shells, Figure 1) or more concentrated multilamellar (tens of layers) L_{α} phases.

Figure 1



(a) Schematic of anionic DNA polyelectrolytes with cationic counterions condensed on the backbone due to Manning condensation. (b) Schematic of cationic liposomes (or vesicles) which consist of topologically spherical membranes containing a bilayer of lipid molecules (e.g. a mixture of neutral and cationic lipids).

We will also briefly describe recent atomic force microscopy (AFM) experiments on DNA monolayers adsorbed to cationic lipids on rigid surfaces. There are numerous important reasons to study these systems. First, from a biomedical and biotechnological point of view, CLs (or vesicles) are empirically known to be efficient synthetic carriers of genes (i.e. sections of DNA) in gene delivery applications [3–7]. We shall be discussing later that the development of efficient synthetic carriers of genes is currently an area of intense research, and it is only recently that we are beginning to understand the structures of the carrier–gene complex in different lipid mixtures. Another

reason is that from a fundamental biological interest, DNA confined to the surface of membranes is a model for studies of DNA condensation and decondensation in two dimensions. DNA condensation and decondensation which happens, for example, during the cell-cycle in three dimensions is still very poorly understood [8]. *In vitro* studies of DNA in solution gives rise to a rich variety of multivalent-induced, condensed phases [9] and liquid crystalline phases at high concentrations [10]. A third reason is that from a perspective of biophysics and soft condensed matter, DNA-membrane assemblies are important model systems for understanding the statistical mechanics of polyelectrolytes adsorbed on two dimensional fluctuating membranes [11]. Finally, once methods have been developed to produce highly oriented single crystals, it will be possible to use DNA-membrane self-assemblies for the development of nano-scale masks in lithography and molecular sieves with nanometer scale cylindrical pores in separations technology. In all of the areas that we describe in this article the fundamental issues that we will address will revolve around understanding the self-assembled structures and the intermolecular interactions in DNA-membrane assemblies.

Let us first look briefly at the phenomenon of DNA condensation and decondensation in biological systems [8,9,10]. The DNA-lipid multilayers we describe in this article are a novel form of DNA condensation in two dimensions (closely resembling what occurs in some rod-shaped virus particles). DNA condensation occurs in viruses, in bacterial cells, and in eucaryotic (nucleus-containing) cells. In eucaryotic cells DNA condensation is required to ensure that daughter cells receive equal amounts of chromosomal material (the most condensed form of DNA) during cell division. Decondensation is necessary during much of the life of cells because proteins have to access the DNA template; for example, during transcription when RNA polymerase attaches to the template to form a protein, and in gene regulation when various transcription factors attach to specific DNA sequences. In bacteria, we know very little about how DNA packs *in vivo*. What we do know is that polyamine molecules such as spermine (4+) and spermidine (3+) occur in abundant quantities and probably enhance condensation. In fact, numerous *in vitro* studies have found that in the presence of multivalent cations with charge 3+ or larger, DNA condenses into compact objects with dimensions of approximately 50 nm [9]. These objects are either torus or rod shaped. In eucaryotes DNA condensation is most quantitatively understood at the smaller length scales. DNA is known to wrap twice around positively-charged histone proteins (an octameric self-assembly of four different histone particles H2A, H2B, H3, H4) forming a nucleosome unit which has a bead-on-string like structure. The nucleosome particle has a size of 10 nm. In the presence of a fifth linker histone particle (H1), and at physiological salt conditions, the nucleosomes further self-assembles into a helical pattern

forming what is referred to as the 30 nm chromatin fiber. On its pathway to forming ultra-condensed chromosomes with microscopic length scales readily visible in the light microscope, chromatin further self-assembles into a higher-ordered structure; the mechanisms involved and the structures at these length scales are currently not understood. In certain rod-shaped viruses, such as the tobacco mosaic virus, the RNA genome of the virus appears to form a template to allow the self-assembly of the protein of the capsid shell. The RNA-protein interactions then condense the RNA which is now adsorbed on the positively charged viral shell [8]. What is important to note is that DNA condensation *in vivo* and *in vitro* is controlled by multivalent cations, polyamines, peptides and proteins (e.g. histones), and that polyelectrolyte effects are among the dominant interactions.

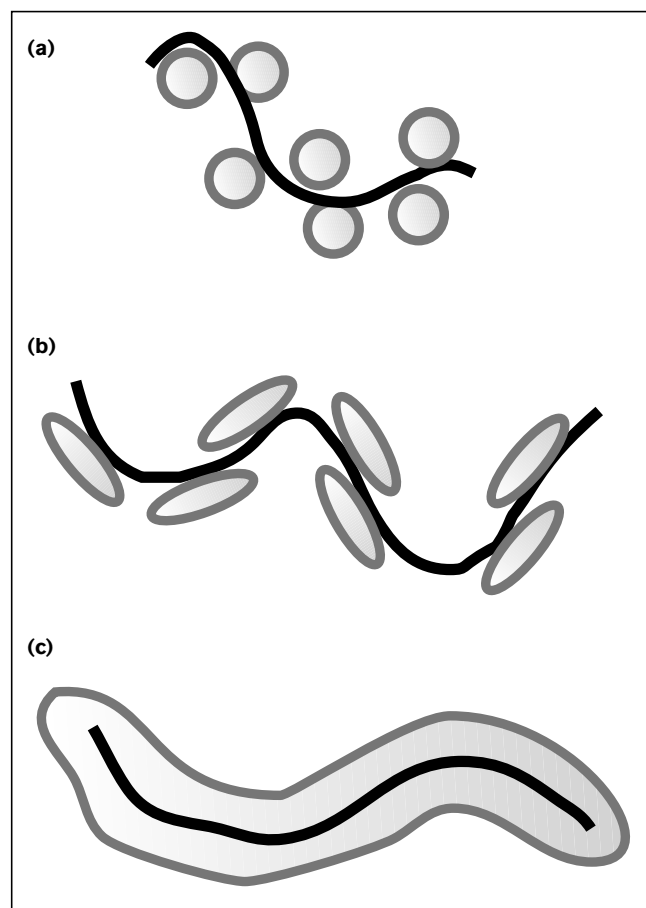
DNA adsorbed at cationic lipid surfaces

Aside from DNA condensation phenomenon, complexes of DNA-membrane assemblies are studied intensively because the multilayers, which form spontaneously when DNA is mixed with CLs [1], are known to be able to mimic certain characteristics of natural viruses. They are, for example, able to act as efficient chemical carriers of extracellular DNA across outer cell membranes and across nuclear membranes for gene delivery. Gene therapy depends on the successful transfer and expression of extracellular DNA to the nucleus of eucaryotic cells, with the aim of replacing a defective or adding a missing gene [3]. Viral-based carriers of DNA are presently the most common method of gene delivery, but there has been tremendous activity in developing synthetic nonviral carriers [3–5]. Gene delivery via replication-deficient viral methods has the advantage over nonviral-carried in that throughout the past millions of years extremely efficient viral machineries which allow viral genomes to reach the nuclear region through various, specific escape mechanisms against the cells natural immune defenses (e.g. enzymatic breakdown) have evolved. On the other hand the primary disadvantage of viral methods is the small size of the viral capsid which limits the amount of DNA base pairs that may be delivered to typically less than 10 Kilobase. Furthermore, viral methods almost always invoke the bodies natural immune system because of foreign proteins attached to the viral surfaces.

An important recent breakthrough [4–6] involves the use of ‘cationic’ liposomes as non-viral vectors of nucleic acids and recombinant DNA molecules. In this approach, it has been hypothesized [4] that the negatively charged nucleic acid binds electrostatically to the outer surface of the CLs (Figure 1) forming a complex consisting of beads (i.e. the liposome) on a string-like (nucleic acid) structure depicted schematically in Figure 2a. The ratio of lipid to nucleic acid is large enough so that there is an overall positive charge on the complex. In this manner, the cationic complexes are believed to improve transfection (gene delivery and expression) rates by enhancing the initial

step of binding electrostatically to negatively charged cell membranes.

Figure 2



Some of the structures which have been proposed for DNA–CL mixtures as described in the text. The DNA is represented by the thick black lines. **(a)** DNA is shown attached to the outer surface of cationic liposomes forming a stable bead-on-string structure. **(b)** The light cigars represent cationic cylindrical micelles (i.e. the lines are lipid monolayers). **(c)** A single bilayer lipid coats the DNA strand. Quantitative X-ray diffraction reveals that the dominant structure (for DOPC/DOTAP membranes) is multilamellar, even in very dilute solutions, as shown in Figure 4b.

In fact, the recent excitement in the field of drug and gene delivery via non-viral methods stems from the fact that the transfection rates and reproducibility in many cells is significantly enhanced by using CLs as compared to the other traditional chemical based non-viral vectors of anionic liposomes, calcium phosphate, diethylaminoethyl dextran [7]. In particular, CL–DNA complexes have shown gene expression *in vivo* in targeted organs [5], and human clinical protocols are ongoing [6]. The correlation, however, between structure and cellular uptake and subsequent transfection remain unknown. In particular, the mechanisms involved in the route of cell

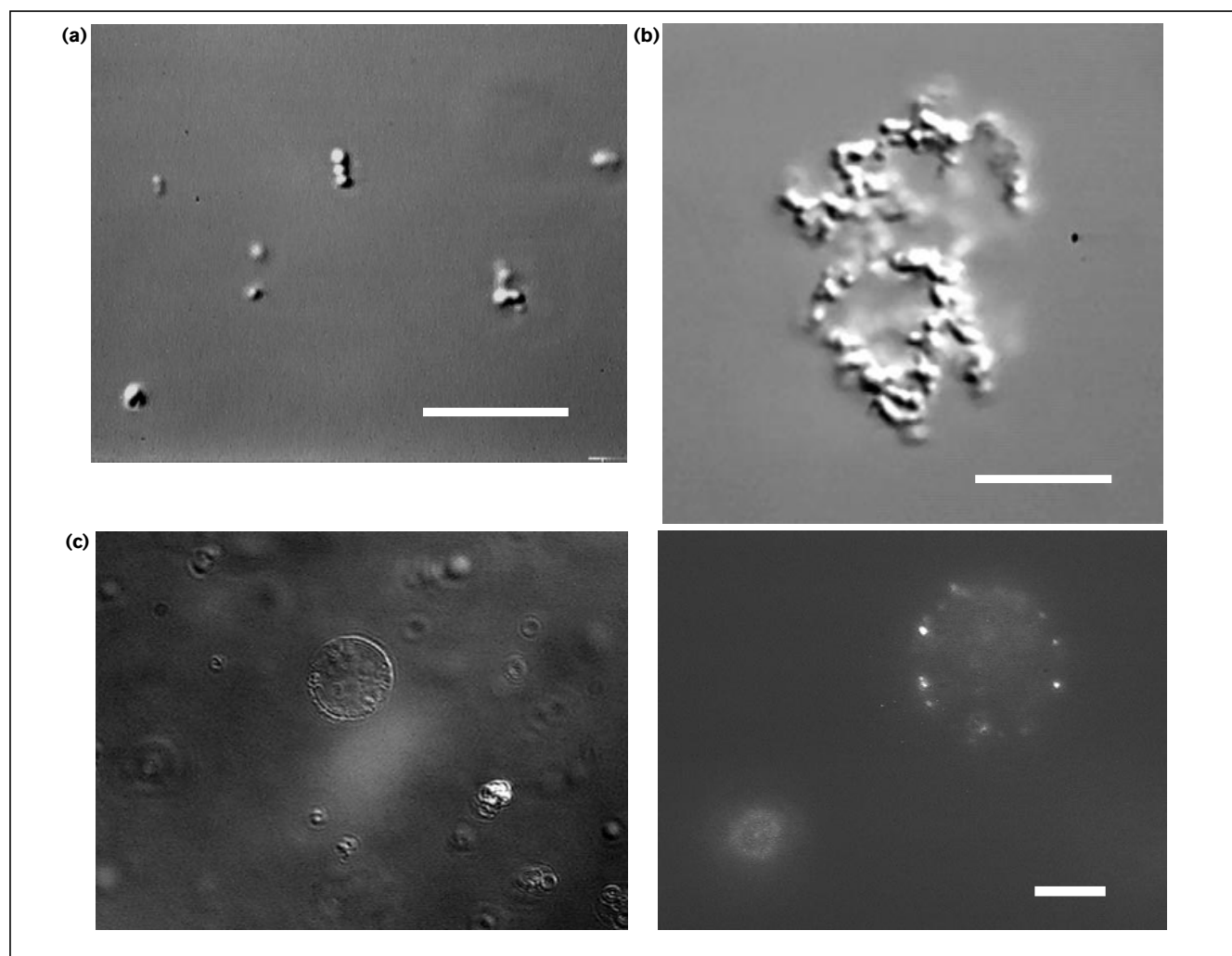
entry, the transfer to the nuclear region, and the delivery of DNA within the nucleus are still under question. For example, depending on the cell type, the transfection rate improvement ranges from 5 to 300 [7]. Furthermore, in most cell types studied, a further transfection rate increase of a factor of 100 (i.e. to 30,000) is needed for synthetic carriers to become competitive with replication-deficient viral carriers.

Aside from the hypothesized beads on string structure (Figure 2a) for the complex, some possible alternative structures, which have been proposed to result in solution as a consequence of the electrostatic attractive forces between the nucleic acid and the cationic lipids, are shown in Figure 2 (b,c). In Figure 2b, the lipid mixture forms cylindrical cationic micelles, while in Figure 2c the nucleic acid is wetted completely by a bilayer coat. The structure in Figure 2c has been suggested in a recent freeze-fracture electron microscopy study [12]. Oligo-lamellar structures have also been reported previously in cryo-TEM studies [13]. It seems, however, that cryo-TEM images of complexes as presented in existing publications [13,14] are not able to detect the DNA chains intercalated between membranes.

DNA self-assembled on cationic membranes: multilayers

Recent work using high resolution small angle X-ray diffraction has revealed distinct states of the CL–DNA complexes in water [1••]. This data shows that the structure is significantly different from the previously hypothesized structures depicted in Figure 2. We show in Figure 3a,b differential-interference-contrast (DIC) images of linear DNA (48 Kilobase) from bacteriophage λ mixed in and complexed with CLs (mean diameter of 70 nm) for two lipids (L) to DNA (D) ratios (L = DOTAP + DOPC [1:1]). Stoichiometric charge neutrality occurs around $L/D = 4.4$ (weight/weight). The complexes in Figure 3a with $L/D = 10$ are positive and are observed to strongly repel each other, thus remaining as individual or a few linked globules [1••]. In Figure 3b the complexes are near the isoelectric point ($L/D = 4.4$) and the individual globules tend to stick when they collide (due to van der Waals attractions overcoming weak electrostatic repulsions) leading to larger aggregates of globules. Fluorescent microscopy (not shown) of the DNA (labeled with YoYo) and the lipid (labeled with TexasRed-DHPE) also showed that the individual globules contain both lipid and DNA. The charge reversal of the complexes can be observed optically by looking to see whether they attach to oppositely charged membrane surfaces. For example, Figure 3c, left, shows a giant anionic liposome, imaged by DIC, (modeling a negative animal plasma membrane) which is seen to be decorated with cationic CL–DNA complexes (anionic complexes with $L/D < 4$ are seen not to attach). The same liposome is shown in green fluorescence (Figure 3c, right) where the complexes which contain DNA are clearly visualized.

Figure 3



In **(a)** and **(b)** the CL-DNA complexes are visualized with differential interference contrast microscopy for two different lipid/DNA ratios (which controls the charge of the complex). The complexes in **(a)** are weakly positive while in **(b)** they are at the isoelectric point. **(c)** The left panel shows a giant anionic liposome (diameter=10 micron) visualized with differential-interference-microscopy. The rough condensates which appear to be attached to the liposome consist of CL-DNA complexes. The right-hand panel shows the same giant liposome as in left. Green fluorescence of YoYo labeled λ -DNA shows the location of the complexes. The bars in **(a)** **(b)** and **(c)** are 10 microns.

To probe the interior structure of the complexes we have to use a transmission structural probe such as X-ray or neutron diffraction. Because we are interested in probing the structures under even very dilute conditions (e.g. those used in gene delivery applications) the natural probe to use is X-rays produced by powerful synchrotron radiation sources. Figure 4a shows a typical representative X-ray diffraction spectrum probing the interior of the complex under extremely dilute DNA-CL mixtures in water (99% water) for positive complexes with $L/D=5.5$. Unexpectedly, the data reveal a layered structure for the lipid-DNA mixture shown schematically in Figure 4b. This is seen by the presence of the two narrow peaks (at $q=0.096 \text{ \AA}^{-1}$ and 0.192 \AA^{-1}) which correspond to the

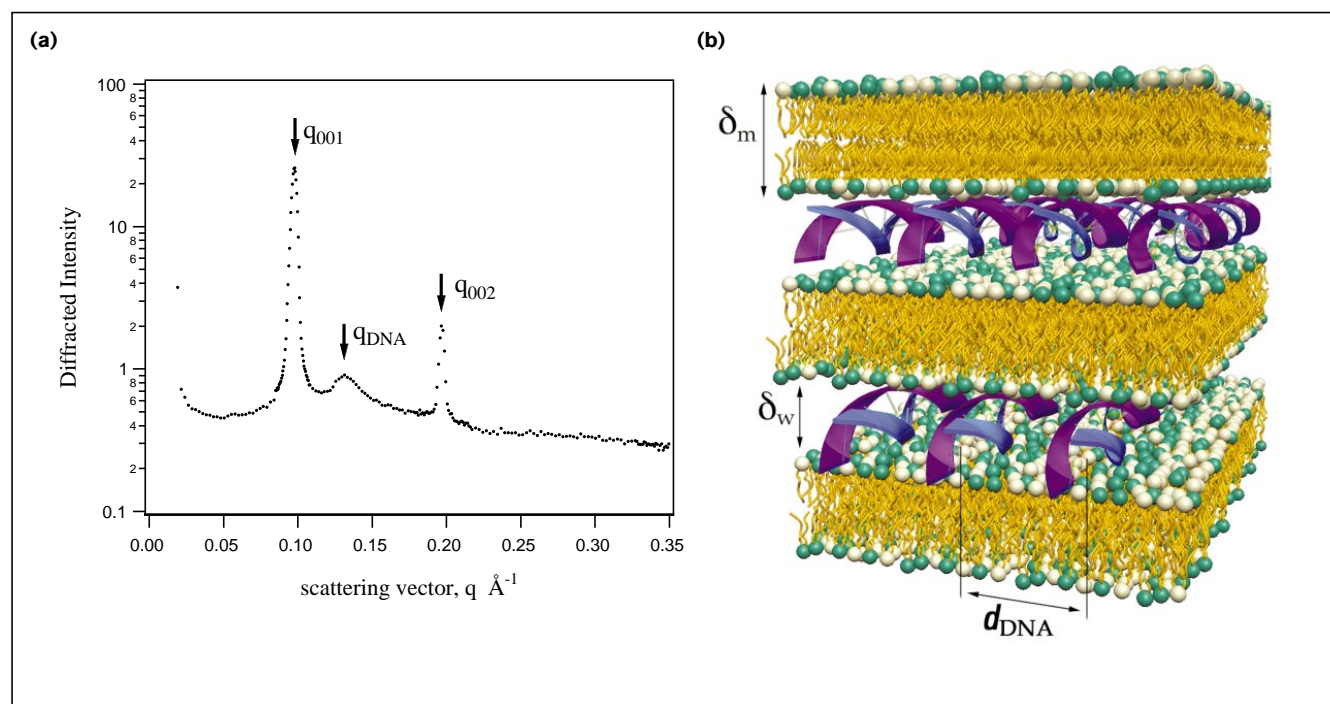
lamellar (00L) peaks of a layered structure with an interlayer spacing $d=65.4 \text{ \AA}$ equal to the sum of thickness of the lipid bilayer (around 40.5 \AA , which is measured in the absence of DNA by X-rays) and the DNA monolayer (25 \AA , corresponding to B-form DNA with a hydration layer). The middle broad peak arises from DNA-DNA correlations and gives an average DNA interchain spacing of 48 \AA . In the absence of DNA, the multilamellar L_{α} phase of this charged lipid-water mixture gives rise to very large interlayer spacings of order 1000 \AA (at 99% water) due to long range electrostatic interactions. The DNA which appears to condense on the cationic lipid layer strongly screens this interaction leading to a collapse of bilayers into condensed multi-layers. The individual

globules (i.e. similar to what is shown in Figure 3) thus consist of finite-sized, lipid multilamellar structures with DNA sandwiched between the bilayers (Figure 4b).

The DNA–lipid condensation can be understood to occur as a result of the release of bound counterions in solution. DNA in solution (Figure 5a) has, on average, a bare length between negative charges (phosphate groups) equal to $b_0=1.7\text{Å}$. This is substantially less than the Bjerrum length in water $b_j=7.1\text{Å}$ which corresponds to the distance where the Coulomb energy between two unit charges is equal to the thermal energy $k_B T$. Therefore, from a nonlinear Poisson-Boltzmann analysis we expect that counterions will condense on the DNA backbone until the Manning parameter, $x=b_j/b$, approaches 1. (Here, b is the renormalized distance between negative charges after counterion condensation.) Through DNA–lipid condensation the cationic lipid tends to fully neutralize the phosphate groups on the DNA, in effect replacing and releasing the originally condensed counterions in solution (Figure 5b). Thus, the driving force for higher-order-self-assembly is the release of counterions into solution, which were one dimensionally bound to DNA and two dimensionally bound to cationic membranes.

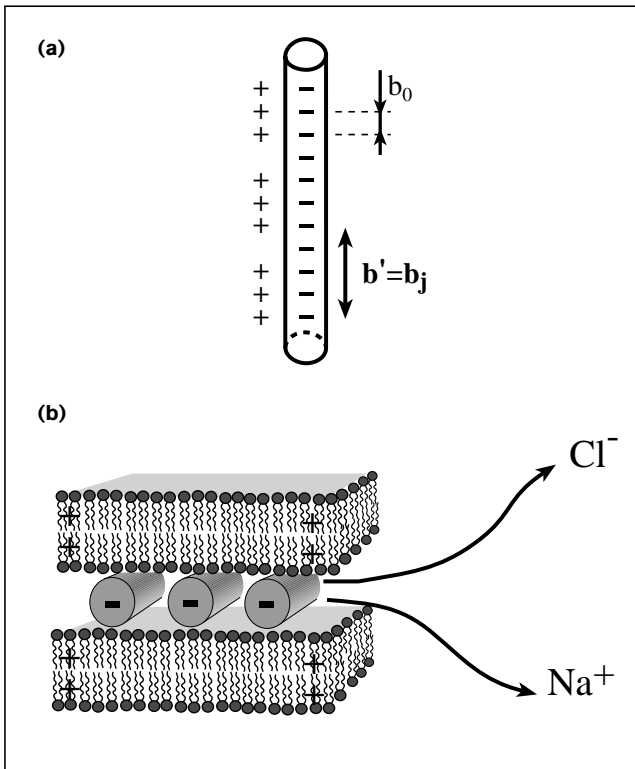
Because the largest energy in the system is the entropy gain by counterion release, all DNA is expected to remain fully attached to all of the available cationic membrane as long as we maintain overall charge neutrality at the isoelectric point of the complex. Therefore, the interaxial distance between the DNA chains can be controlled simply by changing the lipid charge density at the isoelectric point. This corresponds to keeping the ratio $L_{\text{DOTAP}}/\text{DNA}=2.2$ constant while increasing (or decreasing) L/D ratio by the addition (or removal) of the charge neutral lipid DOPC to the complex (see Figure 6a). Figure 6b shows schematically that as we add neutral lipid (at the isoelectric point) and therefore expand the total cationic surface we expect the DNA chains to also expand and increase their interaxial spacing. The solid line in Figure 6a is derived from the simple geometric packing relationship of DNA–DNA spacing, $d_{\text{DNA}}=(A_D/\rho_D)/(\delta_m/\rho_L)$ (L/D) which equates the cationic charge density (due to the mixture of DOTAP+ and DOPC) with the anionic charge density (due to DNA⁻). Here, $\rho_D=1.7$ (g/cc) and $\rho_L=1.07$ (g/cc) denote the densities of the DNA and the lipid respectively, δ_m the membrane thickness, and A_D the DNA area. $A_D=Wt(\lambda)/(\rho_D L(\lambda))=186\text{Å}^2$, $Wt(\lambda)$ = weight of λ -DNA = $31.5 \times 10^6/(6.02210^{23})$ g and $L(\lambda)$ = contour length

Figure 4



(a) Synchrotron small-angle X-ray diffraction data on a DNA/cationic liposome/water mixture ($L/D=5.5$ (wt./wt.); $L=\text{DOPC}+\text{DOTAP}$, $D=\text{DNA}$). The Bragg-diffraction at $q_{001}=0.096\text{Å}^{-1}$ and $q_{002}=1.92\text{Å}^{-1}$ reveal a multilamellar structure with DNA sandwiched between bilayers. The broad peak at q_{DNA} is due to the DNA-interaxial spacing d_{DNA} as shown schematically in Figure 4b. (b) Schematic of the DNA–cationic membrane structure showing the alternating lipid bilayer–DNA monolayer structure which results spontaneously when DNA is mixed with cationic lipid as seen in the DIC image in Figure 3 (the lipid mixture is DOPC/DOTAP).

Figure 5



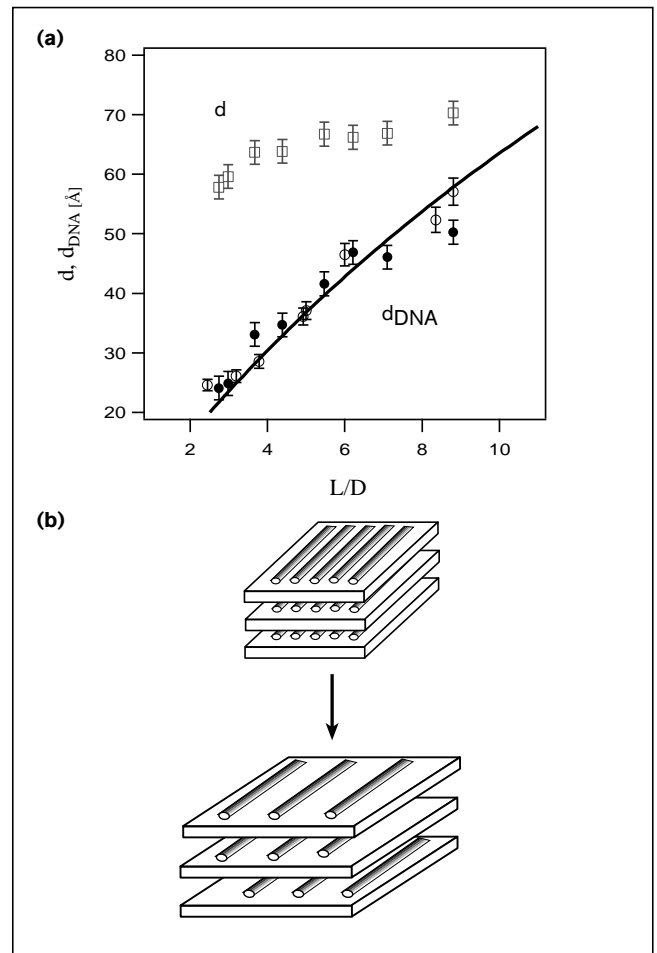
(a) Schematic of double stranded DNA molecule with a bare distance between negative charges of $b_0 = 1.7 \text{ \AA}$. From non-linear Poisson-Boltzmann we know that positive counterions condense on DNA until the renormalized distance between the negative charges b equals the Bjerrum length which is $b_j = 7.1 \text{ \AA}$ in water. **(b)** Schematic drawing showing that as DNA condenses onto the cationic membrane there is a simultaneous release of counterions and a gain in the entropy of solution when the previously condensed counterions (Na^+ on DNA and Cl^- near the cationic liposome membrane) leave the immediate vicinity of DNA and the cationic membrane respectively.

of $\lambda\text{-DNA} = 48502 \times 3.4 \text{ \AA}$. The agreement between the packing relationship (solid line) with the data over the measured interaxial distance from 24.5 \AA to 57.1 \AA (Figure 6a) is quite remarkable given the fact that there are no adjustable parameters.

From the width of the DNA peak in the X-ray diffraction spectrum one obtains the domain size of the one dimensional lattice of DNA chains which turns out to be of the order of eight to ten unit cells. This turns out to be about 500 \AA , which is close to the persistence length of highly screened DNA chains. A schematic of the interior of the CL-DNA complex is shown in Figure 7b. The complex thus consists of a new hybrid phase of matter; that is, the DNA chains form a finite size two dimensional smectic which is coupled to the three dimensional smectic phase of lipid membranes.

To understand the nature of the long-range interactions between DNA chains one needs to carry out a line-shape

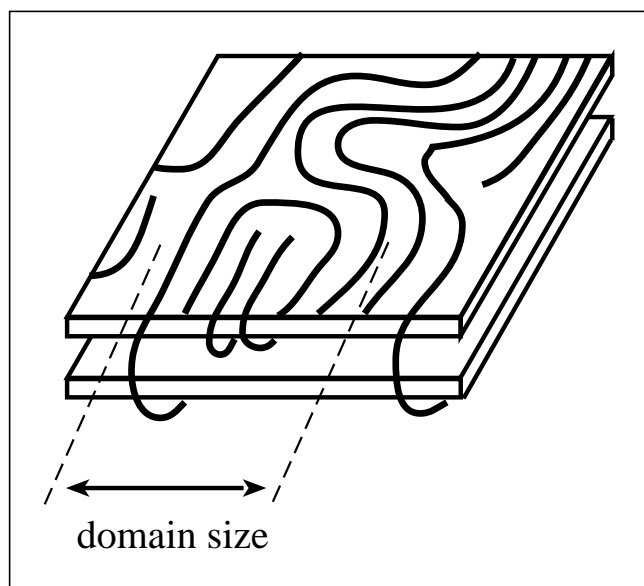
Figure 6



(a) The DNA interaxial distance d_{DNA} and the interlayer distance d plotted as a function of lipid/DNA (L/D) (wt/wt) ratio at the isoelectric point of the complex DOTAP/DNA = 2.4 ± 0.1 . d_{DNA} is seen to expand from 24.5 \AA to 57.1 \AA . The line through the data is the prediction of a packing calculation where the DNA chains form a space filling one dimensional lattice (i.e. a two dimensional smectic phase). **(b)** Schematic drawing of DNA-membrane multilayers showing the increase in distance between DNA chains as the membrane charge density is decreased at the isoelectric point.

analysis of the X-ray diffraction and scattering peak [15]. As we can see from Figure 8a the profiles are found to fit nicely to a simple harmonic Hamiltonian description of the one dimensional lattice of chains (i.e. a two dimensional smectic) in terms of the smectic lattice compressibility modulus, B [erg/ 2], and the splay modulus, $K = k/d$ [erg], derived from the bending modulus of a single chain, k [erg \cdot cm]. Because $B(d) = d_{\text{DNA}} dP/d(d_{\text{DNA}})$, where $P(d_{\text{DNA}})$ is the DNA-DNA interchain pressure (force/length), then the lineshape analysis allows us to access the chain-chain interactions. The compression modulus, B , derived from the data as a function of d_{DNA} (Figure 8b) decreases slowly with respect to $1/d_{\text{DNA}}$ and shows clearly that the interactions between DNA chains are repulsive and long-range. For further information,

Figure 7

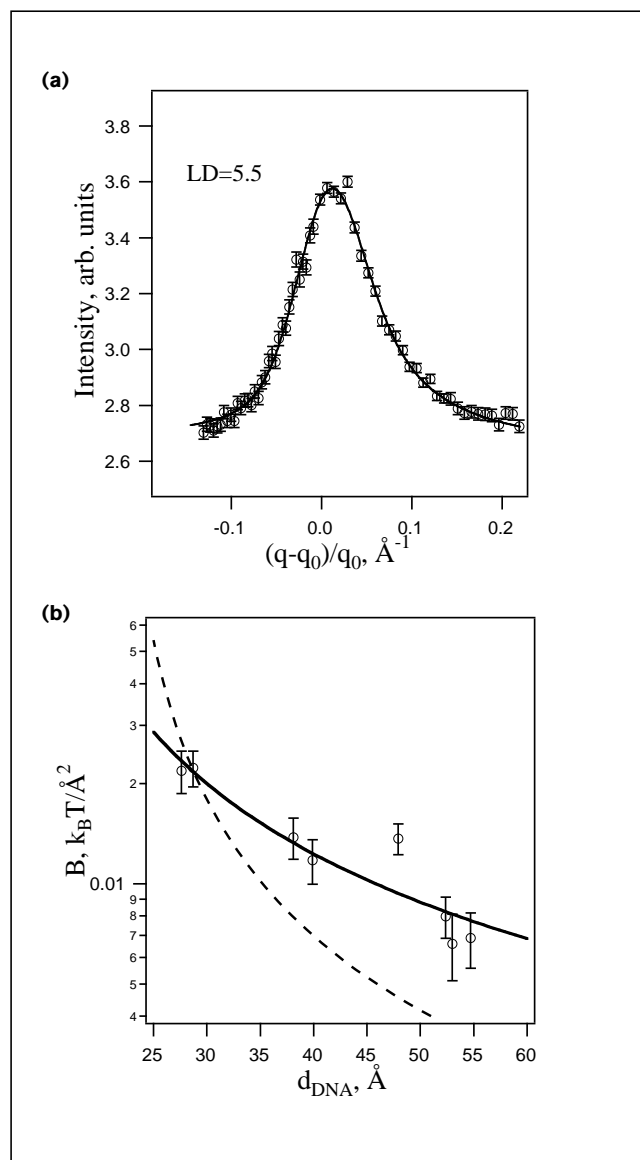


A schematic drawing of the multilamellar cationic lipid-DNA complex consistent with the X-ray diffraction data taking into account the broad width of the DNA peak due to the finite coherent domain size of the DNA chains adsorbed on lipid bilayers in complex.

please refer to [15]. Studies in these unoriented multilayers only allow one to measure $B(d_{\text{DNA}})$. Future studies in aligned samples will also allow an accurate measurement of K and will open up a novel way to determine the persistence length of surface adsorbed polyelectrolytes.

Recent theoretical work appears to lead to a variety of novel new phases in DNA-lipid complexes (TC Lubensky and C O'Hern, personal communication). The ground state of these systems is considered to be a mixed columnar-lamellar phase in which the lipid forms a regular smectic phase and the DNA forms a periodic, aligned, columnar lattice with a single periodic row between each pair of lipid lamellae. This phase would have the symmetry of an anisotropic columnar phase. At higher temperatures it is possible both for positional coherence to be lost between DNA columns in adjacent layers and for orientational coherence between layers to remain, or for both positional and orientational coherence between layers to be lost. Recent calculations (TC Lubensky and C O'Hern, personal communication; L Golubovic and M Golubovic, personal communication) indicate that the orientationally ordered but positionally disordered phase can exist and that there can be a phase transition between it and the columnar phase. This would be a remarkable new phase of matter if it exists. Orientational coherence between layers can also be lost at sufficiently long length scales where nonlinearities and dislocations and disclinations in the 2D smectic lattice of DNA become important. This system shares many fascinating similarities with flux lattices in superconductors.

Figure 8



(a) The result of a line-shape analysis of the DNA peak measured in X-rays as discussed in the text. The solid line is a model describing a one dimensional lattice of chains at a finite temperature (i.e. a two dimensional smectic). (b) The compression modulus B of the one dimensional lattice of DNA chains measured from the X-ray lineshape of the DNA peak. B is found to decrease slowly and inversely proportional to d_{DNA} which is qualitatively consistent with long-range electrostatic interactions between DNA chains. Solid and dashed lines are predictions of different models of DNA-DNA interactions. Reproduced with permission from [15].

DNA adsorbed on rigid surfaces

In a different area, researchers [16–18] have been working for almost a decade on developing methods to align DNA onto ultra-smooth mica surfaces (rms roughness at the subnanometer range). One of the main motivations of research has been caused by the belief that highly aligned DNA molecules may allow for the rapid sequencing of DNA bases associated, for example, with the Human

Genome project. We see in Figure 9 a beautiful, atomic-force-microscope image of plasmid DNA adsorbed onto a cationic bilayer supported on freshly cleaved mica surfaces. The success of this recent preparation is in the direct observation of the periodic helical modulation of the double-stranded DNA molecules, which is measured to be 3.4 nm, consistent with the pitch of B-DNA [19]. The handedness of the DNA molecules is also clearly visible. The approach seems to be very promising and we can expect to see AFM studies of DNA sequencing and also DNA interacting with DNA-binding-proteins in the near future. More recent AFM studies of DNA adsorbed onto membrane supports using similar methods developed earlier [19] have shown a periodic spacing between DNA chains in some detail [20]. The interaction between DNA rods electrostatically adsorbed onto a deformable two dimensional lipid membrane has been theoretically studied by Dan [21]. The model, which balances the electrostatic repulsion between DNA molecules with an attractive interaction induced by the local membrane deformations, predicts the existence of a membrane-bound DNA condensed phase with fixed interaxial spacing.

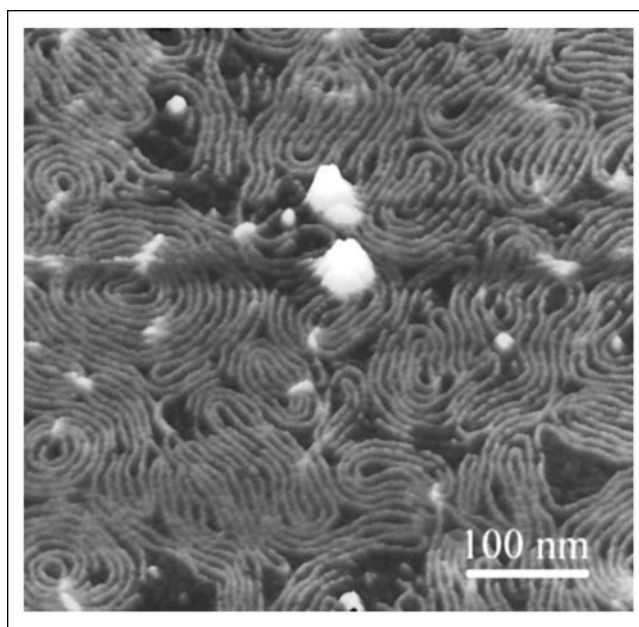
Along the same lines, but in three dimensional scale, there have been some interesting, new, theoretical developments in understanding the interactions between charged macromolecules. The effective interaction between DNA embedded in a solution of their counterions was studied numerically by Brownian particle dynamics by Gronbech-Jensen *et al.* [22]. A significant range of attraction between the rods was identified as arising from the microscopic ordering of counterions in the vicinity of the macromolecules for physically relevant parameters.

Finally, one other area involving DNA at rigid surfaces deserves mention. This involves the development of high density DNA arrays or so-called gene chips [23,24]. These biochips, which result from interdisciplinary work combining molecular biology with microfabrication and nanotechnology, are produced by combining photolithography techniques and combinatorial light-directed synthesis of oligonucleotide (single-stranded) DNA probes and are well on the way to revolutionizing DNA sequence analysis through the sequence hybridization methods. One can imagine that the future generation of gene-chips will take advantage of the rapid developments ongoing in self-assembly to produce higher-ordered-self-assembled gene chips consisting of multilayers of DNA probes, thus enhancing the signal to noise properties of these devices which at present remains a major technical obstacle.

Conclusion

The quantitative synchrotron-based X-ray diffraction data on the structure of CL-DNA complexes [1], has led to the discovery of new and distinct structural regimes of packing of linear DNA. The solution structure in CL-DNA complexes consists of a higher ordered multilamellar structure with DNA sandwiched between cationic

Figure 9



High resolution atomic-force-microscopy image of plasmid DNA adsorbed on a cationic bilayer (DPTAP) coating a freshly cleaved mica surface. The highly packed DNA chains are clearly visible. The measured width of DNA is close to 2 nm, the diameter of B-DNA. Adapted from [19] with permission.

bilayers. This structure is observed not only in higher concentration precipitates but also, by using the high brightness of the synchrotron sources, in extremely dilute suspensions of globules used in gene therapy applications. Linear λ -phage DNA intercalates between membranes in distinct interhelical packing regimes below, above, and at the isoelectric point of the complex. In the isoelectric regime, the DNA interaxial distance increases from 24.5 to 57.1 Å as a function of lipid dilution and is quantitatively consistent with an expanding one dimensional lattice of DNA chains (i.e. a novel 2D smectic phase) resulting from long-range electrostatic repulsions between DNA chains. It is only the outcome of these purposefully designed series of X-ray diffraction experiments, in other words the observation [1], of a variation in the DNA interaxial distance as a function of the lipid to DNA (L/D) ratio in multilayers (i.e. the 'moving' DNA peak in the X-ray spectrum), which unambiguously demonstrates that X-ray diffraction directly probes the DNA behavior in multilayer assemblies, and that the linear DNA chains confined between bilayers form a 2D smectic phase.

The precise structural nature of DNA-lipid (i.e. gene-carrier) complexes is just beginning to be characterized quantitatively through X-ray diffraction in different synthetic systems. Distinct new types of self-assembled structures are emerging (I Koltover, T Salditt, JO Raedler, CR Safinya, unpublished data; A Lin, N Slack, C George, C Samuel, CR Safinya, unpublished data; N Clark, private communication) and we can expect that in the near future

specific trends in the nature of these self-assemblies will become evident. Clearly, the next step is to correlate these structures to transfection efficiencies for improved gene therapy applications.

Acknowledgement

The X-ray and optical microscopies described in this review were supported by National Science Foundation Division of Materials Research-9624091, the Petroleum Research Fund (31352-AC7), and a Los Alamos CULAR grant STB/UC:96-108. The Materials Research Laboratory at Santa Barbara is supported by National Science Foundation Division of Materials Research-9632716. The synchrotron experiments were carried out at Stanford (Stanford Synchrotron Radiation Laboratory) supported by the United States Department of Energy. Tim Salditts collaboration is gratefully acknowledged.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
 - of outstanding interest
1. Rädler JO, Koltover I, Salditt T, Safinya CR: **Structure of DNA-cationic liposome complexes: DNA intercalation in multilamellar membranes in distinct interhelical packing regimes.** *Science* 1997, **275**:810-814.
The paper describes the first structure of a DNA-cationic lipid self-assembly (in DOTAP/DOPC mixtures) which was derived using quantitative X-ray diffraction methods. This system is used in nonviral gene therapy applications as described in [5,6].
 2. Spector MS, Schnur JM: **DNA ordering on a lipid membrane.** *Science* 1997, **275**:791-792.
 3. Crystal RG: **Transfer of genes to humans: early lessons and obstacles to success.** *Science* 1995, **270**:404-410.
 4. Felgner PL, Rhodes G: **Gene therapeutics.** *Nature* 1991, **349**:351-352.
 5. Zhu N, Liggitt D, Liu Y, Debs R: **Systemic gene expression after intravenous DNA delivery into adult mice.** *Science* 1993, **261**:209-211.
 6. Nabel GJ, Nabel EG, Yang ZY, Fox BA, Plautz GE, Gao X, Huang L, Shu S, Gordon D, Chang AE: **Direct gene transfer with DNA liposome complexes in melanoma expression, biological activity, and lack of toxicity in humans.** *Proc Natl Acad Sci USA* 1993, **90**:11307-11311.
 7. Lasic D, Templeton NS: **Liposomes in gene therapy.** *Adv Drug Deliv Rev* 1996, **20**:221-266.
 8. Lewin B: *Genes VI.* Oxford: Oxford University Press; 1997.
 9. Bloomfield VA: **Condensation of DNA by multivalent cations: considerations on mechanism.** *Biopolymers* 1991, **31**:1471-1482.
 10. Livolant F, Leforestier A: **Condensed phases of DNA: structure and phase transitions.** *Prog Polym Sci* 1996, **21**:1115-1164.
A review of liquid crystalline phases of DNA in highly concentrated systems.
 11. Masanori H: *Polyelectrolytes Science and Technology.* New York: Marcel Dekker, Inc.; 1992.
 12. Sternberg B, Sorgi FL, Huang L: **New structures in complex formation between DNA and cationic liposomes visualized by freeze-fracture electron microscopy.** *FEBS Lett* 1994, **356**:361-366.
 13. Gustafsson J, Arvidson G, Karlsson G, Almgren M: **Complexes between cationic liposomes and DNA visualized by cryo-TEM.** *BBA-Biomembranes* 1995, **1235**:305-312.
 14. Lasic DD, Strey HH, Stuart MCA, Podgornik R, Frederik PM: **The structure of DNA-liposome complexes.** *J Am Chem Soc* 1997, **119**:832-833.
 15. Salditt T, Koltover I, Raedler JO, Safinya CR: **Two-dimensional smectic ordering of linear DNA chains in self-assembled DNA-cationic liposome mixtures.** *Phys Rev Lett* 1997, **79**:2582-2585.
 16. Bustamante C, Keller D, Yang GL: **Scanning force microscopy of nucleic acids and nucleoprotein assemblies.** *Curr Opin Struct Biol* 1993, **3**:363-372.
 17. Hansma HG, Hoh J: **Biomolecular imaging with the atomic force microscope.** *Annu Rev Biophys Biomol Struct* 1994, **23**:115-128.
 18. Bensimon D, Simon AJ, Croquette V, Bensimon A: **Stretching DNA with a receding meniscus – experiments and models.** *Phys Rev Lett* 1995, **74**:4754-4757.
 19. Mou J, Czajkowsky DM, Zhang Y, Shao Z: **High-resolution atomic-force microscopy of DNA: the pitch of the double helix.** *FEBS Lett* 1995 **371**:279-282.
 20. Fang Y, Yang J: **Two-dimensional condensation of DNA molecules on cationic lipid membranes.** *J Phys Chem B* 1997, **101**:441-449.
 21. Dan D: **Formation of ordered domains in membrane-bound DNA.** *Biophys J* 1996, **71**:1267-1272.
 22. Gronbeck-Jensen N, Mashl R, Bruinsma RF, Gelbart WM: **Counterion-induced attraction between rigid polyelectrolytes.** *Phys Rev Lett* 1997, **78**:2477-2480.
 23. Chee M, Yang R, Hubbell E, Berno A, Huang XC, Stern D, Winkler J, Lockhart DJ, Morris MS, Fodor SPA: **Accessing genetic information with high density DNA arrays.** *Science* 1996, **274**:610-614.
An important breakthrough in DNA sequence analysis done by using high density DNA arrays
 24. Stripp D: **Gene chip breakthrough.** *Fortune Magazine* March 31 1997, 56-73.