Colloidal DNA

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For practical and fundamental reasons, DNA assemblies have become rewarding objects of study. Colloid physics is now learning to connect the structures of these assemblies with the measurable physical forces that organize them. The subject has ripened to the point where systematic inquiry and rigorous testing of theories is to be expected.

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Introduction

Because colloid physics deals with thermodynamic phases of and interactions between constituent macromolecules or macromolecular complexes, it creates a logic to relate molecular arrangements to the interactions that create them. During the past decade, following the thrust of DNA research connected with its nature as a carrier of genetic information, its physical properties are now being studied with renewed intensity. Because of the variety of its liquid-crystalline phases, the possibility to observe its assemblies by X-ray diffraction and the ability to measure its free energy of organization DNA is a surprisingly instructive material.

We shall concentrate on only some of the numerous investigations into the colloid state of DNA in solution and in condensed phases. There are already excellent reviews on some of the aspects omitted here [1,2]. Nor will we discuss the underlying molecular DNA–DNA interaction potentials that were treated in our previous review [3••]. Rather, we will describe the symmetries of different states of DNA assemblies and how they relate to DNA–DNA interactions. The elegance of recent theories and the rigor of recent experiments suggest that there are many instructive possibilities for examining the connection between DNA chirality and the different ways DNA molecules can pack.

DNA mesophases

The equivalence of one ordered DNA mesophase and the magnetic vortex phases in type II superconductors [4] has thrust the liquid crystallinity of DNA into the mainstream of socially acceptable physics-research topics. The sequence of mesophases as a function of increasing DNA concentration — isotropic, 'blue', cholesteric, line hexatic or hexagonal columnar, crystalline — has been elucidated [2]. The nature of the transitions between different mesophases, especially between the isotropic solution and the cholesteric (or the blue) phase and between the cholesteric and the line hexatic phase, still need explanation. Since use of a phase diagram means not only use of phase boundaries (between different phases as functions of salt species and concentration, DNA length, stiffness and temperature) but also a complete mapping of the equation of state in each of the phases [5], the phase diagram of DNA is still only partially determined.

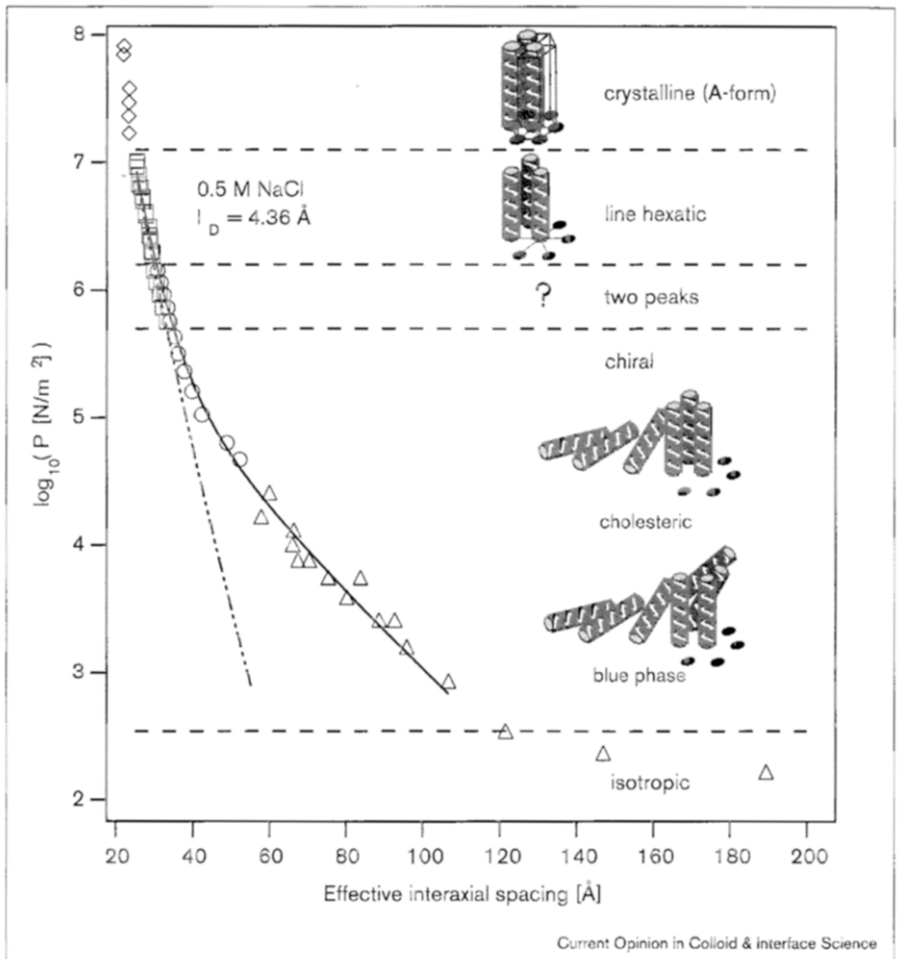
In our view applied osmotic stress [6], coupled to a structural probe such as X-rays, polarization microscopy or NMR spectroscopy, is the best method to determine simultaneously the equation of state and the structures of DNA mesophases. This method avoids the problems of multiple phase coexistence encountered in gravimetric mixtures because the water, salt and DNA activities are controllably set. In Figure 1 we show an experimental isoionic strength (0.5 M) univalent salt cut through the phase diagram going from the crystalline phase to the liquid phase at room temperature. The equation of state is phrased in terms of the DNA osmotic pressure dependence on DNA density, temperature, and salt concentration; it provides the information for quantitative theoretical analysis. Similar equations of state have been obtained for lipid multilayer phases [7,8] and have been successfully used for DNA [3••].

The cholesteric phase

The transition from isotropic DNA solution to the cholesteric phase has been studied experimentally both for short (~50 nm) [9] and for very long (~2700 nm) molecules [10•]. It appears that the transition is first-order. Both studies [9,10•], however, used gravimetric mixtures of salt, DNA, and water. These gravimetric studies leave unresolved questions. In order to avoid the pitfalls created by the redistribution of different components of the system, one can set all chemical potentials by equilibrating against large reservoirs [6]. The chemical potential of water in this setup gives the osmotic stress on the sample. The dependence of osmotic pressure on the concentrations for very long DNA molecules (> 1 micron) suggests a weak first-order transition in going from an isotropic solution to a cholesteric phase [5].

Cholesteric phases are thought to occur in a wide variety of biological systems [11]. The chiral nature of the DNA molecule is clearly visible in this phase through its polarization texture [2]. Theorists have tried to relate the microscopic chirality of the molecule and the macroscopic pitch of the cholesteric phase, but theoretical explanation of the connection has been refractory for many reasons. Chiral interactions appear to be inextricably

Figure 1



The equation of state for DNA at 0.5 M NaCl in the whole range of DNA densities, plotted as effective interaxial spacing between the molecules (assumed to be locally hexagonally ordered). This plot is analogous to a single isotherm of a van der Waals'-like phase diagram. The two fits represent the equation of state, firstly without any conformational fluctuations and, secondly, with conformational fluctuations leading to fluctuation-enhanced interactions. The phase boundaries between the crystalline and line hexatic phases, as well as the line hexatic and chiral phases are also depicted. There is a region of densities in which X-rays show two peaks, which have not yet been assigned to any of the known phases. The phase boundary between the cholesteric and blue phases has not been unequivocally detected either. Data points: bold line, fit to theory with thermal fluctuations; broken line, fit to theory without any thermal fluctuations; diamonds and squares, Bragg X-ray peaks; circles, diffuse X-ray peaks; triangles, effective spacing obtained via direct density measurements. Data taken from [61].

connected with biaxial correlations between molecules [12••]; this condition makes the problem of calculating the macroscopic properties of the cholesteric phase starting from microscopic interactions difficult [13]. Chirality also implies the absence of mirror symmetry. Because of this, it is not a property associated with a single order parameter, such as those used in the Landau theories of phase transitions [14].

The line hexatic phase

There is a continuous or weak first-order [5] transition from the cholesteric phase to a denser phase characterized by short-range positional order and long range bond-orientational order. Before its discovery, this line hexatic phase [15] was hypothesized to exist on purely theoretical grounds [16]. Unfortunately the cholesteric→line hexatic transition is even less characterized than the isotropic→cholesteric transition. The approaches to this transition suggested up until now [17,18] have relied heavily on the use of the semi-empirical Lindemann criterion coupled to fluctuation-enhanced interactions. The Lindemann criterion *sensu stricto*, however, can be invoked for this transition only by assuming that it is from a crystalline-like (hexagonal columnar) to a liquid-like (cholesteric) phase. Therefore, for the liquid→liquid transition, which the cholesteric→line hexatic transition appears to be, the Lindemann criterion does not apply. The non-crystalline nature of this high density DNA phase is reflected also in its free energy that can be well rationalized within a nematic-like [19••] elastic theory valid in the hexatic as well as in the cholesteric phase.

The intellectually appealing realization of the line hexatic phase in DNA provokes fundamental questions. Should this phase be able to exist only in a twisted conformation (twisted hexatic) [20] because DNA molecules are chiral? In a twisted hexatic the coupling between chirality and bond orientational order would lead to bond order in a macroscopic sample that rotates around the long axes of the molecules, making the system periodic in only one direction. The observed hexatic X-ray fingerprint [15], however, is evidence against any strong twist of the bond orientational order in this phase. The reasons for such a straight structure have been frustratingly elusive. One possibility might lie in the connection between macroscopic chirality and short-range biaxial correlations [12••]. If biaxial correlations at high density were, for some reason, small, then macroscopic manifestations of chirality would be suppressed [21].

Free energy

Paradoxically, though of biological origin, DNA provides physical information that is not readily available from most liquid-crystal forming materials. The osmotic stress used to measure intermolecular forces can be integrated over the phase diagram to give free energies of phases for all densities of DNA over a wide range of temperatures, salt concentrations and salt types, and particularly for those salts that effect DNA condensation [8]. It is apparently taking theorists some time to realize that their theories can be compared to precisely known free energies. There is no place or justification for the still-fashionable qualitative speculation about DNA liquid-crystals and condensates seen in various precipitates or gravimetric mixtures. We have accurate free energies now. Why not use them in order to assess directly the effective charge on DNA molecules [19••] or even more ambitiously to assess any changes in the persistence length of DNA due to long range intersegment forces?

Experimentally, the interpretation of the osmotic pressure data has been well worked out in terms of microscopic potentials between DNAs [19••] and is recently reviewed in [3••]. This is true both for line hexatic and for cholesteric phases. The magnitude of the hydration force that dominates the energetics of the line hexatic phase (where interaxial spacings are ≤ 32 Å) is similar to hydration forces seen with lipids, polysaccharides, and proteins [22]. There is little likelihood of more far-fetched force models [23] that would put these forces in DNA arrays on a completely and fundamentally different footing than in other systems where they have also been measured [22]. In uni-univalent salt solutions devoid of condensing counterions [1], DNA–DNA interactions show no evidence of significant attractive forces either through counterion correlation or other charge fluctuations [3••].

Fluctuation-enhanced interactions

In cholesteric phases (where interaxial spacings are ≥ 32 Å), rapidly decaying hydration forces lose sway to electrostatic double layer interactions [24]. The interactions weaken expectant with increasing salt concentration in the bathing solution but often show exponential decay lengths much longer than those expected from the double layer theory [5,25]. This expansion of the DNA–DNA force, quantified through a renormalized value of the exponential decay length has been ascribed to the enhancing action of DNA fluctuations. A renormalization of the electrostatic decay length can be rationalized through several theoretical models [19••,26]. After correction for fluctuations, forces measured in this regime [5] basically conform to electrostatic double layer models [24]. This exponentially varying interaction differs qualitatively from the power-law variation expected to occur between fluctuating hard-rods [27,28].

Renormalized decay lengths

Though the original idea [19••,26] has remained the same, the theory of fluctuation-enhanced interactions in flexible polymer arrays has gone through various amendments. Odijk [29] proposed a self-consistent theory of interactions in hexagonal polyelectrolyte arrays. It was supposed to give a better estimate for the fluctuation contribution to the free energy but it instead led to algebraically decaying fluctuation forces with no direct renormalization of the decay length. Undulation enhanced forces appear to play a fundamental role in many soft condensed matter systems. They have been studied in lamellar phases and hexagonal gels to describe the transition from a hexagonal columnar to a cholesteric phase [17]. Fluctuation-enhanced forces were invoked to approach the nematic→hexagonal liquid crystalline transition [30]. Here, too, it was concluded that conformational fluctuations lead to a renormalization of the interaction decay length. Fluctuations might produce a sequence of regimes where the renormalization of the decay is two-fold, then four-fold; at longer distances there is finally a regime with an interaction free energy that, like steric fluctuation forces, decays algebraically with interaxial separation [27].

Ice-like melting of hexagonal columnar crystals was also described on the basis of fluctuation-enhanced interactions [31]. The Einstein model of a stiff polymer in the mean field of its neighbors leads to a four-fold renormalization of the decay length of the underlying soft (screened electrostatic) repulsion potential [32]. The four-fold renormalization of the decay length emerges naturally also from the theories of nematic polymers based on the underlying Frank elastic energy with minimal assumptions for elastic constants [5,19••]. It is this theory that should be applicable for DNA in the hexatic as well as in the cholesteric phase; happily, the theoretically predicted four-fold renormalization of the decay length is strongly borne out in experiments covering a very extensive range of DNA density [19••].

Colloidal aspects of gene therapy

Curing diseases by correcting errant genetic code, or gene therapy, has attracted much attention. Many obstacles, however, have yet to be overcome before gene therapy can become a practical alternative to conventional treatments. In gene therapy one has to deliver a large piece of DNA into the cell nucleus instead of delivering a small chemical drug. Strategies involve viral and non-viral based delivery systems (see [33•,34••] for an introduction). Here we will discuss only one of the non-viral approaches [35], in particular DNA-cationic lipid complexes [36], pioneered by Felgner *et al.* [37]. We feel that many questions about how these complexes form can be addressed using the language of colloidal interaction and complex fluids. Systematic studies of the physical chemistry and colloidal properties have just begun.

For the application of gene therapy several requirements have to be met. The aim is to pack as few copies of plasmid DNA as possible into virus-size complexes to increase the efficiency of transformation, and to use fewer plasmids

to transfect a large number of cells. At the same time these complexes have to be stable in serum while protecting the DNA from nucleases and the immune system [38,39•]. Once in circulation, complexes have to penetrate the target cells membrane either by fusion or endocytosis (the process by which cells take up molecules bound to its surfaces), and following that, in order for the DNA to be expressed it has to get into the cell nucleus [40•]. Sometimes more efficient endocytosis of complexes into cells does not lead to more expression because the DNA cannot reach the nucleus [40•]. All steps are necessary for successful transfection!

Several attempts have been made to create efficient small, virus-size complexes. This can be done by controlling lipid vesicle size by extrusion techniques before mixing them with DNA [41•]. Complexes seem to work best when there are more charges on the available lipid than charges on DNA [42]. When mixed, this positive overcharge produces small metastable complexes which can be used for transfection [41•]. In most cases helper lipids like DOPE or cholesterol that mix with the cationic lipid improve efficiency [42]. Most probably, they do this by being able to match better the surface charge density to the charge density on DNA than the cationic lipids on their own. Sometimes the lipid itself forms small micelles which eliminates lipid extrusion [43]. Another interesting approach uses single chain surfactants that associate with DNA into small, virus-size particles [44]. After this initial assembly the surfactant is dimerized by two cysteines (sulfur-containing amino acids) to form double-chained lipids that stabilize the complexes.

Typically colloidal interactions are taken to be nonspecific. Using these concepts when dealing with a complex system like a cell requires caution. Most of a cell's interactions with its environment are very specific. Therefore it is not surprising that endocytosis and transport to the nucleus is more dependent on the particular chemical composition than on the structure of the complexes [42]. More studies of these specific mechanisms are needed to design better delivery systems.

DNA–cationic lipid complexes

Complexes of DNA with cationic lipids are now widely used for *in vitro* transformation of cells with recombinant plasmid DNA [45•]. Various lipid formulations are commercially available for *in vitro* delivery [46•]. The equilibrium DNA–cationic lipid phase diagram was investigated only recently. DNA–cationic lipid complexes can exist in a variety of forms with different packing symmetries. Explicit structural studies (small angle X-ray spectroscopy) have confirmed the existence of honeycomb (I Koltover, T Salditt, JO Rädler, CR Safinya, personal communication; see note added in proof) and lamellar [47•,48••] structures. A careful X-ray scattering study of the lamellar phase revealed a fascinating new liquid crystalline phase with orientationally ordered DNA layers intercalated between lipid bilayers as reviewed in [49].

Lamellar structures of DNA lipid complexes are only superficially similar to smectics. In reality, coupling between intercalated two-dimensional DNA [50] layers leads to a new 'sliding columnar' phase with long range correlations that are quite different from those seen in two-dimensional smectics [51•,52•]. The nature of the molecular interactions in these phases is just as important as in the case of their counterparts (see above) in the bulk. For example, electrostatic interactions between DNAs intercalated between oppositely charged lipid bilayers in the complexes are, in fact, a lower dimensional analog of the counterion mediated forces between charged surfaces in the bulk [53]. The stable separation between DNAs could be due to the coupling between direct electrostatic forces and the (cationic) lipid membrane mediated elastic attractive force [54]. The DNA–DNA equilibrium spacing in the complex seen in experiments [48••] has also been explained by a rather different model [55•,56] based purely on electrostatics of DNA, counterions, and inhomogeneous charge distribution along the membrane surface. The corresponding Poisson–Boltzmann equation has been solved both approximately [56] and numerically [55•]. The isoelectric point of the complex appears to be unstable against adsorption of extra DNA or lipid while the instability is due to released counterion entropy [56].

Analysis of the stability of honeycomb-like and spaghetti-like structures [57] concludes that honeycomb-like aggregates should be more stable. Lamellar bilayer aggregates, however, seem to be even more stable than cylindrical ones [58]. Stable monophasic suspensions of DNA–cationic lipid complexes are obtained only near charge neutralization. For asymmetric adsorption to cationic lipid bilayers DNA adsorption is coupled to a shape transition of the supporting lipid bilayer, leading to encapsulation of DNA [59].

Conclusions

DNA assemblies have many properties in common with those of liquid crystals in less accessible structures. Lessons learned from DNA are likely to carry over to those other materials.

Osmotic stress measurements have mapped the entire phase diagram of DNA assemblies. Derived equations of state of these phases compel quantitative tests of models of phase structure and formation.

Intermolecular forces can be systematically varied as functions of temperature, salt activity, and salt type. In this way, statistical mechanical theories may be tested over a wide range of conditions.

The symmetries of different states are beginning to be reconciled with DNA–DNA interactions. The elegance of

recent theories suggests that there are many instructive possibilities examining the connection between chiral DNA and the different ways it can pack.

Practical applications in gene therapy can use ideas derived from the study of well-prepared thermodynamic phases of pure DNA or DNA–lipid mixtures.

In the future we expect to see ever more detailed analyses of the crystal-line hexatic transition and of the nature of the line hexatic phase itself in terms of the underlying interactions. Our understanding of the phases and interactions in DNA arrays will become a model also for other biologically relevant polyelectrolytes.

Note added in proof

The data referred as I Koltover *et al.*, personal communication, has now been published [60].

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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. Bloomfield VA: **DNA condensation.** *Curr Opin Struct Biol* 1996, **6**:334-341.

2. Livolant F, Leforestier A: **Condensed phases of DNA: structures and phase transitions.** *Prog Polym Sci* 1996, **21**:1115-1164.

3. •• Strey HH, Podgornik R, Rau DC, Parsegian VA: **DNA-DNA interactions.** *Curr Opin Struct Biol* 1998, **8**:309-313.
An up to date summary of the state of our knowledge regarding the nature of microscopic potentials, repulsive as well as attractive, between DNA molecules in condensed phases and solutions.

4. Nelson DR: **Vortex lattice melts like ice.** *Nature* 1995, **375**:356-357.

5. Strey HH, Parsegian VA, Podgornik R: **Equation of state for DNA liquid crystals: fluctuation enhanced electrostatic repulsion.** *Phys Rev Lett* 1997, **78**:895-898.

6. Parsegian VA, Rand RP, Fuller NL, Rau DC: **Osmotic stress for the direct measurement of intermolecular forces.** In *Methods Enzymol.* Edited by Packer L. New York: Academic Press, 1986:400-416.

7. Dubois M, Zemb T, Fuller N, Rand RP, Parsegian VA: **Equation of state of a charged bilayer system: Measure of the entropy of the lamellar-lamellar transition in DDABr.** *J Chem Phys* 1998, **108**:7855-7869.

8. Parsegian VA, Evans EA: **Long and short range intermolecular and intercolloidal forces.** *Curr Opin Colloid Interface Sci* 1996, **1**:53-60.

9. Kassapidou K, Jesse W, van Dijk JAPP, van der Maarel JRC: **Liquid crystal formation in DNA fragment solutions.** *Biopolymers* 1998, **46**:31-37.

10. • Merchant K, Rill RL: **DNA length and concentration dependencies of anisotropic phase transitions of DNA solutions.** *Biophys J* 1997, **73**:3154-3163.
This study addresses the important polymer liquid crystal question on how the isotropic→anisotropic transition concentration depends on DNA length.

11. Giraud-Guille M-M: **Twisted liquid crystalline supramolecular arrangements in morphogenesis.** *Int Rev Cytol* 1996, **166**:59-101.

12. •• Harris AB, Kamien RD, Lubensky TC: **On the macroscopic origin of cholesteric pitch.** *Phys Rev Lett* 1997, **78**:1476.
The first in-depth analysis of the chiral interactions in condensed phases of nematic polymers. The previously neglected connection between chiral interactions and biaxial correlations between interacting molecules is demonstrated to be essential for macroscopic manifestations of chirality.

13. Sato T, Nakamura J, Teramoto A: **Cholesteric pitch of lyotropic polymer liquid crystals.** *Macromolecules* 1998, **31**:1398-1405.

14. Lubensky TC, Harris AB, Kamien RD, Yan G: **Chirality in liquid crystals: from microscopic origins to macroscopic structure.** In *Proceedings of the 6th International Conference on Ferroelectric Liquid Crystals.* 1998, in press.

15. Podgornik R, Strey HH, Gawrisch K, Rau DC, Rupprecht A, Parsegian VA: **Bond orientational order, molecular motion and free energy of high density DNA mesophases.** *Proc Natl Acad Sci USA* 1996, **93**:4261-4266.

16. Toner J: **Bond-orientationally-ordered phases that scatter light strongly.** *Phys Rev A* 1983, **27**:1157-1163.

17. Odijk T: **Evidence for undulation-enhanced electrostatic forces from melting curves of a lamellar phase and a hexagonal polyelectrolye gel.** *Europhys Lett* 1993, **24**:177-182.

18. Kassapidou K, van der Maarel JRC: **Melting of columnar hexagonal DNA liquid crystals.** *Eur Phys J B* 1998, **3**:471-476.

19. •• Strey HH, Parsegian VA, Podgornik R: **Equation of state for polymer liquid crystals: theory and experiment.** *Phys Rev E* 1998, in press.
Theoretical analysis, based on the Frank elastic energy, of the equation of state for line hexatic as well as cholesteric DNA mesophases. In-depth discussion also occurs on the connection between liquid crystalline elastic and Einstein particle-in-a-cage models. The parameters of the microscopic interaction potentials are derived for the whole set of equation of state data. A definitive work on the equation of state for DNA in the line hexatic and cholesteric phases.

20. Kamien RD: **Liquids with chiral bond order.** *J Phys II* 1996, **6**:461-475.

21. Kamien RD: **Weak chirality in ordered DNA phases.** *Mol Cryst Liq Cryst Sci Tecnol A* 1997, **299**:265-275.

22. Leikin S, Parsegian VA, Rau DC, Rand RP: **Hydration Forces.** *Annu Rev Phys Chem* 1993, **44**:369-395.

23. Starikov EB: **Chemical physics of solid-state nucleic acids: new intriguing horizons.** *Phys Rep* 1997, **284**:1-89.

24. Stigter D: **Evaluation of the counterion condensation theory of polyelectrolytes.** *Biophys J* 1995, **69**:380-388.

25. Podgornik R, Rau DC, Parsegian VA: **The action of interhelical forces on the organization of DNA double helices: fluctuation-enhanced decay of electrostatic double-layer and hydration forces.** *Macromolecules* 1989, **22**:1780-1786.

26. Podgornik R, Parsegian VA: **Molecular fluctuations in the packing of polymeric liquid crystals.** *Macromolecules* 1990, **23**:2265-2269.

27. Odijk T: **On the statistics and dynamics of confined or entangled stiff polymers.** *Macromolecules* 1983, **16**:1340-1344.

28. Helfrich W, Harbich W: **Adhesion and cohesion of tubular vesicles.** *Chem Scr (UK)* 1985, **25**:32-36.

29. Odijk T: **Undulation-enhanced electrostatic forces in hexagonal polyelectrolyte gels.** *Biophys Chem* 1993, **46**:69-75.

30. Selinger JV, Bruinsma RF: **Hexagonal and nematic phases of chains. II. Phase transitions.** *Phys Rev A* 1991, **43**:2922-2931.

31. Jain S, Nelson DR: **Icelike melting of hexagonal columnar crystals.** *Macromolecules* 1996, **29**:8523-8529.

32. Nelson DR: **Statistical mechanics of directed polymers.** In *Observation, Prediction and Simulation of Phase Transitions in Complex Fluids.* Edited by Baus M. Amsterdam: Kluwer Academic Publishers; 1995:293-335.

33. • Felgner PL: **Nonviral strategies for gene therapy.** *Sci Am* 1997, **276**:102-106.
Introduction to nonviral gene delivery systems and why they might be superior to viral systems.

34. •• Friedmann T: **Making gene therapy work: overcoming the obstacles.** *Sci Am* 1997, **276**:102-106.
Recommended read for beginners in gene therapy. Comprehensive introduction to the concepts and methods for gene delivery. The article describes the most promising delivery systems and what problems have to be overcome to make them work.

35. Cotten M, Wagner E: **Non-viral approaches to gene therapy.** *Curr Opin Biotechnol* 1993, **4**:705-710.

36. Gao X, Huang L: **Cationic liposome-mediated gene transfer.** *Gene Therapy* 1995, 2:710-722.

37. Felgner PL, Gadek TR, Holm M, Roman R, Chan HW, Wenz M, Northrop JP, Ringold GM, Danielsen M: **Lipofection: a highly efficient, lipid-mediated DNA-transfection procedure.** *Proc Natl Acad Sci USA* 1987, 84:7413-7417.

38. Yang JP, Huang L: **Time-dependent maturation of cationic liposome-DNA complex for serum resistance.** *Gene Therapy* 1998, 5:380-387.

39. • Zuidam NJ, Barenholz Y: **Electrostatic and structural properties of complexes involving plasmid DNA and cationic lipids commonly used for gene delivery.** *BBA-Biomembranes* 1998, **1368**:115-128.
First study to follow cationic lipid charge neutralization while complexing it with DNA using a fluorescent probe. The addition of neutral helper lipids seem to help DNA encapsulation.

40. • Escriou V, Ciolina C, Helbling-Leclerc A, Wils P, Scherman D: **Cationic lipid-mediated gene transfer: analysis of cellular uptake and nuclear import of plasmid DNA.** *Cell Biol Toxicol* 1998, 14:95-104.
This study addresses some open questions on how DNA–cationic lipid complexes get into cells and how the DNA is then transported into the nucleus. The authors use various fluorescent probes to track plasmid DNA and nuclear expression.

41. • Templeton NS, Lasic DD, Frederik PM, Strey HH, Roberts DD, Pavlakis GN: **Improved DNA: liposome complexes for increased systemic delivery and gene expression.** *Nat Biotechnol* 1997, 15:647-652.
A systematic study on the factors that determine *in vivo* gene delivery and gene expression. Factors include DNA:liposome ratio, liposome size, liposome composition. Under optimal conditions DNA was encapsulated between two lipid bilayers as seen in electron microscopy. *In vivo* gene expression was improved 75 to 150-fold as compared to other studies.

42. Hope MJ, Mui B, Ansell S, Ahkong QF: **Cationic lipids, phosphatidylethanolamine and the intracellular delivery of polymeric, nucleic acid-based drugs (review).** *Mol Membr Biol* 1998, 15:1-14.

43. Pitard B, Aguerre O, Airiau M, Lachages AM, Boukhnikachvili T, Byk G, Dubertret C, Herviou C, Scherman D, Mayaux JF, Crouzet J: **Virus-sized self-assembling lamellar complexes between plasmid DNA and cationic micelles promote gene transfer.** *Proc Natl Acad Sci USA* 1997, 94:14412-14417.

44. Blessing T, Remy J-S, Behr J-P: **Monomolecular collapse of plasmid DNA into stable virus-like particles.** *Proc Natl Acad Sci USA* 1998, 95:1427-1431.

45. • Papahadjopoulos D, Lasic DD, Podgornik R: **Polymorphism of lipids, nucleic acids and their interactions.** In *Self-Assembling Complexes for Gene Delivery: from Laboratory to Clinical Trial.* Edited by Kabanov AV, Felgner PL, Seymour LW. John Wiley; 1998:21-44.
A review on the colloidal and liquid crystalline properties of DNA, lipids and their complexes. The differences and the similarities of DNA and lipid phase behavior are discussed in detail.

46. • Lasic DD: *Liposomes in gene delivery.* Boca Raton: CRC Press; 1997
The first book that systematically reviews the properties of liposomes and their connection with the gene therapy issues. Colloidal aspects of the DNA–lipid interactions feature prominently.

47. • Lasic DD, Strey H, Podgornik R, R.Stuart MCA, Frederik PM: **DNA cationic liposome complexes: structure and structure-activity.** *J Am Chem Soc* 1997, 119:832-833.
Using evidence from electron microscopy and small angle X-ray scattering this study suggests a lamellar structure for DNA–cationic lipid complexes. DNA molecules are intercalated between lipid bilayers and show in-plane order.

48. •• 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.
Systematic high resolution small angle X-ray scattering study on DNA–cationic lipid complexes. The authors show that complexes have a lamellar structure which consists of alternating DNA layers and lipid bilayers. Additional X-ray peaks were associated to DNA–DNA in-plane spacings suggesting a novel liquid crystalline phase.

49. Safinya CR, Koltover I, Rädler J: **DNA at membrane surfaces: an experimental overview.** *Curr Opin Colloid Interface Sci* 1998, 3:69-77.

50. Podgornik R, Zeks B: **Coupling between smectic and twist modes in polymer intercalated smectics.** *Phys Rev Lett* 1998, 80:305-308.

51. • O'Hern CS, Lubensky TC: **Sliding columnar phase of DNA lipid complexes.** *Phys Rev Lett* 1998, 80:4345-4348.
This and [52•] consider the lamellar phase of the DNA–cationic lipid complexes as a new liquid crystalline phase with a novel fluctuation behavior of the smectic displacement field. In this phase there appears to be long-range orientational correlations between intercalated DNA lattices but with short-range positional correlations.

52. • Golubovic L, Golubovic M: **Fluctuations of quasi-two-dimensional smectics intercalated between membranes in multilamellar phases of DNA cationic lipid complexes.** *Phys Rev Lett* 1998, 80:4341-4344.
See annotation to [51•].

53. Bruinsma R, Mashl J: **Long-range electrostatic interaction in DNA cationic lipid complexes.** *Europhys Lett* 1998, 41:165-170.

54. Dan NL: **Multilamellar structures of DNA complexes with cationic liposomes.** *Biophys J* 1997, 73:1842-1846.

55. • Harries D, May S, Gelbart WM, Ben-Shaul A: **Structure, stability and thermodynamics of lamellar DNA-lipid complexes.** *Biophys J* 1998, 75:159-173.
A statistical thermodynamic analysis of the stability of lamellar DNA–cationic lipid complexes. The authors solve the Poisson–Boltzmann equation for the electrostatic potential of DNAs between the lipid bilayers. The stability of the DNA–lipid complexes is shown to depend on the competition between counterion entropy, inter-DNA and inter-bilayer interactions.

56. Bruinsma R: **Electrotatics of DNA-cationic lipid complexes: isoelectric instability.** *Eur Phys J B* 1998, 4:75-88.

57. May S, BenShaul A: **DNA-lipid complexes: stability of honeycomb-like and spaghetti-like structures.** *Biophys J* 1997, 73:2427-2440.

58. Dan N: **The structure of DNA complexes with cationic liposomes - cylindrical or flat bilayers?** *BBA-Biomembranes* 1998, 1369:34-38.

59. Podgornik R: **Supporting membrane shape instability in the presence of strongly adsorbed flexible polymers.** *Langmuir* 1997, 13:4791-4794.

60. Koltover I, Salditt T, Rädler JO, Safinya CR: **An inverted hexagonal phase of cationic liposome-DNA complexes related to DNA release and delivery.** *Science* 1998, 281:78-81.

61. Lindsay SM, Lee SA, Powell JW, Weidlich T, Demarco C, Lewen GD, Tao NJ, Rupprecht A: **The origin of the A to B transition in DNA fibers and films.** *Biopolymers* 1988, 27:1015-1043.