



## Ordered phases of filamentous viruses

Zvonimir Dogic<sup>a,1</sup>, Seth Fraden<sup>b,\*</sup>

<sup>a</sup> Rowland Institute at Harvard, Harvard University, Cambridge, MA 02142, USA

<sup>b</sup> Complex Fluids Group, Brandeis University, Waltham, MA 02459, USA

### Abstract

This review will focus on the equilibrium and non-equilibrium phase behavior of colloidal suspensions of rod-like viruses. It will treat the simplest case where the rods are the sole colloidal component and also the more complex phase behavior that arises in mixtures of binary rods and mixtures of rods with spherical colloids, or with polymers. An enormous volume of literature is devoted to the theoretical treatment and computer simulation of the phase behavior of hard rod systems. Viruses, because of their monodispersity and well-characterized interparticle interactions, continue to be the best experimental system for exploring the issues raised by theory. These include elucidating the fundamental relationship between molecular properties, such as length and charge, and macroscopic properties, such as phase behavior. Furthermore, through genetic engineering of viruses, new colloids have been created that are interesting to materials science for their potential to form self-assembling functional materials, such as biosensors and transducers.

© 2005 Elsevier Ltd. All rights reserved.

*Keywords:* Liquid crystals; Colloids; Virus; Self-assembly; Nanoparticles

### 1. Introduction

We review the phase behavior of colloidal rods and mixtures of rods with hard spheres or polymers. This specialized subject [1] has considerable overlap with many topics of interest to readers of this journal, such as liquid crystals, colloids, nanoparticles, ordering in biopolymers and self-assembly of surfactants and block copolymers. The majority of the observed ordered phases in virus suspensions are of a liquid crystalline nature and, consequently, the same theoretical framework which successfully describes the hydrodynamic and elastic properties of low molecular weight liquid crystals [2] and polymer liquid crystals [3] also describes similar properties of the virus systems. However, because the viruses are large, it is experimentally possible to probe structure and dynamics at the molecular level with imaging techniques, such as optical microscopy. The statistical mechanical theory of the liquid crystalline behavior of viruses, which relates the

virus molecular structure to its phase behavior, was published by Onsager [4] in a paper for which no praise is too lavish. As Onsager noted, rod-like colloids can be of mineral, as well as of biological origin. A recent review in this journal is devoted to the topic of mineral liquid crystals [5]. Other biopolymers besides viruses can form liquid crystalline phases, such as DNA [6,7,8,9], actin [10], microtubules [11] and polysaccharides such as schizophyllan [12] and cellulose [13].

#### 1.1. Major recent advances

Liquid crystals composed of viruses possess the same phases as thermotropic liquid crystals, isotropic, nematic, cholesteric, smectic A, smectic C and crystalline phases [14,15,16]. Ten years ago, we reviewed work on the phase behavior of single component virus suspensions (virus plus solvent) [1]. Since then, the forefront of the field has moved to the study of the kinetics of phase transitions [17,18,19], molecular dynamics [20] and non-equilibrium phase transitions [20,21,22,23]. Another area of activity has been with mixtures of virus with polymers or spherical colloids [17,24,25,26]. Finally, an important advance has been the use of genetic engineering techniques to create binding sites for other molecules at specific locations on the virus

\* Corresponding author. Tel.: +1 781 736 2888; fax: +1 781 736 2915.

E-mail addresses: dogic@netrider.rowland.org (Z. Dogic), fraden@brandeis.edu (S. Fraden).

<sup>1</sup> Tel.: +1 617 497 4682; fax: +1 617 497 4627.

[27,28,29,30,31–37]. This enables the construction of designed particles, not found in nature, to use as building blocks for self-assembling systems.

## 2. Onsager model

Tobacco mosaic virus (TMV) was the first virus to be isolated and purified. Shortly after this accomplishment, it was observed that a 2% solution of virus formed a two-phase solution with one part optically isotropic and the other, denser part being birefringent [38]. TMV is composed of 2130 copies of an identical protein arranged in a helix around a hollow core, like bricks in a chimney. Each of the protein subunits is negatively charged at pH greater than 3. Onsager [4] showed that the birefringent phase of TMV solutions is a nematic liquid crystal, which forms for purely entropic reasons. Onsager begins by mapping the free energy of a solution of charged rods onto the free energy of hard rods with a charge and ionic strength dependent effective diameter,  $D_{\text{eff}}$ . The free energy of hard particle suspensions only depends on concentration and shape of the particles and, consequently, all phase transitions in hard particle suspensions are athermal. In the Onsager model, the free energy is treated as a virial expansion truncated at the second virial coefficient.

Onsager next showed how the nematic phase results as the competition between rotational and translational entropy. Rotational entropy describes the ideal gas behavior of rods and is a maximum when the rods point in all directions with equal probability. Translational entropy is a maximum when the free volume of the rod suspension is maximized, so that the particles have the most space to move in, which occurs when the rods all point in the same direction. The rotational entropy is proportional to particle density, while in the Onsager model the translational, or packing entropy, is a two-particle property that is a function of the angle between two rods. Thus, at low concentrations, rotational entropy dominates and suspensions of rods are isotropic, but above a critical concentration packing entropy dominates and the suspension maximizes total entropy by having the rods break orientation symmetry and pick a preferred direction to point in.

A number of excellent review articles have been written on the Onsager theory [39,40,41,42,43]. The importance of Onsager's work stems from the fact that it is one of the few examples in the entire field of liquid theory for which there is essentially an exact result for the free energy functional [44]. A measure of the influence of Onsager's paper is given by approximately 2000 papers in the Science Citation Index, which refer to it, and the breath of systems to which the Onsager theory is applicable. Although Onsager's work is over 60 years old, its impact is increasing with over 100 citations in 2005. Much of the motivation for the research covered in this review was derived from theoretical papers inspired by Onsager. However, due to space constraints, we give short shrift to the theory of hard rod suspensions. Perhaps, the most effective way to access this literature is to browse through the Science Citation Index and read the titles and abstracts of the papers that reference Onsager [4].

## 3. Phase behavior of single component virus

Currently, our favored experimental system of rod-like colloids is a family of viruses related to filamentous phage *fd*, first discovered in the sewers of New York City [45]. *fd* exhibits the following phase sequence with increasing concentration: isotropic, chiral nematic (cholesteric), smectic A and smectic C [14,16,33,46,47–49,50,51]. A number of features render filamentous phage a particularly attractive experimental system for fundamental studies of liquid crystals and self-assembling systems. First, genetic engineering allows the systematic modification of the most important physical properties of the virus such as its length and charge per unit length [45]. Some mutants are present naturally (M13, *fd*), while others were created in the laboratory [19,52]. A second important feature is the monodispersity of the viruses. No commercially available rod-like colloid or polymer approaches viruses in this regard. Even when the effects of polydispersity are studied, it can be done systematically by first creating bidisperse samples and subsequently increasing the polydispersity in a controlled manner. Third is the ability to modify the virus chemically after it has been expressed. For example, we sterically stabilized the virus by grafting poly(ethylene)glycol (PEG) to the virus [19]. We also have fluorescently labeled the virus allowing visualization of single viruses moving inside equilibrated samples [20]. Fourth is the ability to use phage display to genetically engineer the virus to have highly specific binding sites located at the ends and side of the virus [29]. Fifth, because *fd* is a micron in length, single fluorescently labeled molecules can be visualized in optical microscopes. This is an experimental advantage of colloidal systems over low molecular weight systems because single molecule dynamics and mesoscopic structure can be visualized in real time in equilibrated samples [20], which is not experimentally possible for small molecule systems. This fact is one of the main reasons why colloids in general are experimental systems of fundamental interest.

Properties of *fd* and wild type M13 include length  $L=0.88$   $\mu\text{m}$ , diameter  $D=6.6$  nm, persistence length  $P=2.2$   $\mu\text{m}$  and molecular weight  $M=1.6 \times 10^7$  g/mol [1]. Each virus consists of approximately 2700 coat proteins (pVIII) wrapped around a circular loop of single stranded DNA. The two structurally identical [53] viruses differ only by one amino acid per coat protein; *fd* has one more charge than M13, which results in a net charge difference of 30% [54]. At pH 8.2, *fd* has 3.4 negative charges per protein subunit, while M13 has 2.4 negative charges. This translates to a linear charge density of  $\sim 10$  charges/nm, which is high enough to condense free ions onto the virus. The Onsager theory predicts that the effective diameter is independent of charge for such high charge densities [46,51].

### 3.1. Genetic engineering

Viruses of different lengths were developed using genetic engineering [45] to make M13 viruses longer [52] or shorter [19,51] than the wild type. These mutants share the same

properties as wild type M13, varying only in length ranging from 0.39  $\mu\text{m}$  to 1.2  $\mu\text{m}$  (Fig. 1).

Phage display was used by Belcher to functionalize the surface of the virus to have highly specific binding sites on the side and two ends of virus. In one example, different binding sites were engineered at each end of the virus and a complimentary hetero-functional linker was synthesized, which allowed the virus to form rings [27]. In another example, binding sites were selected for semiconducting nanoparticles [32]. Quantum dots were attached at one end of the virus and then the virus/dot was concentrated and spontaneously formed the smectic phase [31]. This body of work has the goal of developing a ‘bottoms up’ approach to directed assembly, where tailored particles with designed functionality self-assemble to form useful devices and materials [28,30,33,34,55]. Using viruses for templates for biomineralization has been an active field for some time [29,35–37] and this work promises to produce a new class of colloids combining the best of both the biological and chemical synthetic worlds.

### 3.2. Nematic and cholesteric phases

In a nematic, the rods point in a preferred direction with a Gaussian-like distribution and neighboring rods fluctuate about the average alignment. Cholesterics have similar local order to the nematic, but on average the rods fluctuate a fraction of a degree more in one direction than in the other so that the preferred alignment direction slowly rotates in a helical

fashion. The cholesteric pitch is the distance it takes for the average alignment to rotate  $2\pi$  (Fig. 1). Because the net cholesteric alignment is small compared to the magnitude of the angular fluctuations, the free energy difference between a nematic and cholesteric is negligible and the Onsager theory should apply to *fd* [46,50,51].

In the Onsager theory, the free energy and coexistence concentrations are integral functionals of the angular distribution function and, consequently, the most sensitive test of the Onsager theory is a measurement of the angular distribution function itself, and not the I–N coexistence concentrations [46,56,57]. Magnetic fields were used to unwind the cholesteric pitch and transform the cholesteric *fd* into a nematic. With the sample remaining in the field, the angular distribution in the nematic phase was determined using X-ray scattering [50,58]. Measurements at high ionic strength demonstrate that the order parameter is in agreement with Onsager theory when it is extended to treat high particle concentration and flexibility. However, at low ionic strength, the experimental order parameter was higher than Onsager’s prediction.

Liquid crystalline behavior was studied using genetically engineered and naturally occurring mutants of filamentous bacteriophage as a function of virus length and surface charge. We measured the isotropic and cholesteric coexistence concentrations and the nematic order parameter after unwinding the cholesteric phase in a magnetic field. The viruses are structurally identical to M13 virus, but varied either in contour length or surface charge. While varying contour length ( $L$ ), we assume that the persistence length ( $P$ ) remains constant and thus we alter the rod flexibility ( $L/P$ ). Surface charge is altered both by changing solution pH and by comparing two viruses *fd* and M13, which differ only by the substitution of one charged for one neutral amino acid per virus coat protein. For all ionic strengths and all concentrations for which the nematic phase existed, the measured order parameter and coexistence concentrations of the differently charged *fd* and M13 were indistinguishable, as predicted by the Onsager theory. Furthermore, the isotropic-cholesteric transition experimental results agree semiquantitatively with theoretical predictions for semiflexible, charged rods at high ionic strength. However, at low ionic strength, the order parameter and coexistence concentrations were systematically higher than predicted by theory [50,51].

Sterically stabilized virus were created by grafting poly(ethylene)glycol (PEG) to the coat protein pVIII [19,49]. We used a commercially available PEG that was end-functionalized with *N*-hydroxylsuccinimide (mPEG-SPA, Nektar Therapeutics), which covalently bonds to the solvent accessible primary amines on the N-terminus of coat protein pVIII. As shown in Fig. 2, the phase transition of the virus coated with 20,000 MW PEG becomes independent of ionic strength above 3 mM, which corresponds to the bare virus’ electrostatic diameter,  $D_{\text{eff}}$ , becoming less than 35 nm. In a good solvent, the 20,000 MW PEG has a diameter of  $D_{\text{PEG20k}}=14$  nm. The transition to sterically stabilized behavior of the PEGylated virus suggests that the grafted virus has its bare diameter increased by two PEG diameters,

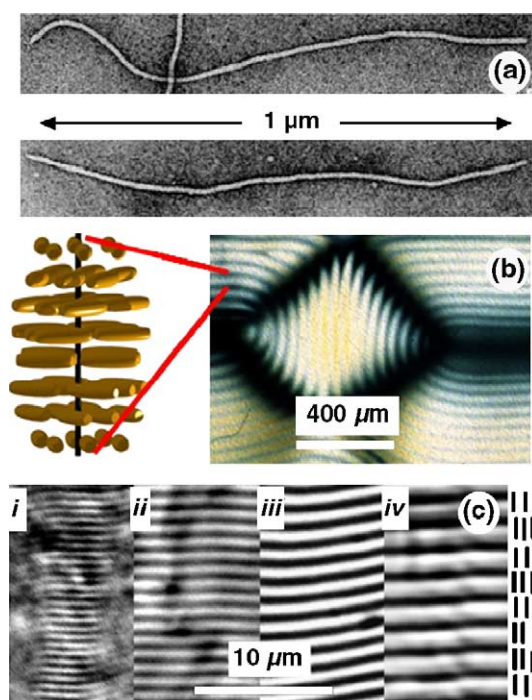


Fig. 1. (a) Electron micrograph of *fd*. (b) Polarized optical micrograph of the cholesteric phase of *fd* and a cartoon schematically illustrating the helical arrangement of molecules. Unlike in the figure, there are about 1000 molecules per cholesteric pitch [48]. (c) Micrographs of the smectic phase taken using differential interference contrast of four species of m13/*fd*. (i) phagemid LITMUS38, 0.39  $\mu\text{m}$ ; (ii) phagemid PGTN28, 0.64  $\mu\text{m}$ ; (iii) wild type *fd*, 0.88  $\mu\text{m}$ ; (iv) M13K07, 1.2  $\mu\text{m}$  [51].

$D_{\text{PEGylated}} = D_{\text{bare}} + 2D_{\text{PEG20k}} = 35$  nm, implying that the grafted PEG adopts a configuration similar to a free polymer in solution.

The origin of chirality in the cholesteric phase of virus suspensions [49\*\*] is an open question. Viruses are composed of chiral amino acids (primary structure), which are assembled into proteins that consist of alpha helices (secondary structure). In turn, the proteins are helically arranged into filamentous phages (tertiary structure). At the still higher level of mesoscopic organization, suspensions of viruses order into either the achiral nematic or chiral cholesteric phase. TMV and pfl, filamentous phages with chirality at the primary, secondary and tertiary levels form a nematic phase, while *fd* structurally extremely similar to pfl is cholesteric. In order to probe the range of the chiral interaction responsible for the cholesteric phase, we PEGylated *fd* to create a sterically stabilized suspension, which prevented the viruses from approaching closer than four virus diameters. For ionic strengths greater than 3 mM, the virus–virus interactions were dominated by the grafted polymer layers, and the phase diagram and order parameter were independent of ionic strength (Fig. 2), implying that the viruses were decoupled electrostatically. However, the sterically stabilized virus had the baffling feature of a cholesteric phase whose pitch depends on ionic strength. The hypotheses postulated by Loren Day and Robert B. Meyer explaining this observation are that (1) the virus assumes a ‘cork screw’ shape illustrated in Fig. 3, which is responsible for the cholesteric phase; (2) the pitch of the cork screw varies with ionic strength; and (3) the microscopic origin of the cork screw distortion resides in the protein–DNA interaction. The filamentous phage has positive amino acid residues located on the part of coat protein pVIII that are next to the DNA. The hypothesis is that a lack of stoichiometry between the DNA and protein leads to electrostatic induced torsion of the protein coat [59].

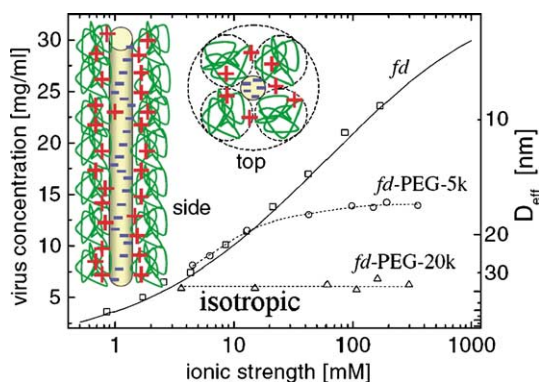


Fig. 2. Isotropic–cholesteric phase boundary as a function of ionic strength for bare virus (*fd*) and for virus coated with neutral polymer (*fd*-PEG-5k and *fd*-PEG-20k). The lines represent the highest concentration for which the isotropic phase, I, is stable (below line), whereas the range of the cholesteric phase, N\*, is above each line. The concentration of the I–N\* transition for bare *fd* increases as a function of ionic strength as  $\rho$  (mg/ml) =  $222/D_{\text{eff}}$  (nm) (solid line), where  $D_{\text{eff}}$  is the effective diameter taking into account the electrostatic repulsion between rods [46]. PEGylated *fd* shows identical behavior to bare *fd* up to an ionic strength corresponding to a  $D_{\text{eff}}$  that matches the size of the stabilizing polymer layer [19].

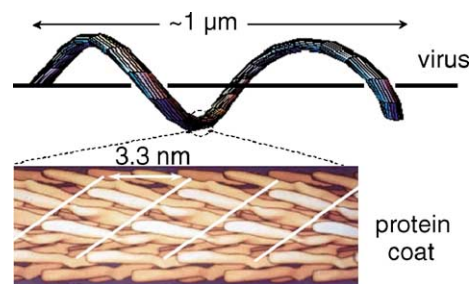


Fig. 3. Schematic representation of the ‘corkscrew’ model: the chirality is transmitted between viruses by the helical shape of the whole virus at the micron length scale and not by the helical structure of the protein coat [49\*\*].

### 3.3. Smectic phases

Using the length mutants [51\*], the nematic–smectic (N–S) phase diagram was determined as a function of length, surface charge and ionic strength. In work soon to be published, we observed that in the semiflexible rod limit the N–S phase boundary is independent of rod flexibility in contrast to the strong flexibility dependence seen in the isotropic–nematic transition [51\*]. By studying the ionic strength dependence of the N–S transition, we observed that renormalizing the volume fraction by Onsager’s effective diameter does not produce an ionic strength independent phase transition concentration, which would be necessary to map the flexible rod phase behavior onto hard particle theories. Currently, no theory is able to account for the ionic strength dependence of the phase transition. Furthermore, in contrast to the I–N transition, significant differences were measured between M13 and *fd* N–S phase behavior, even under conditions when they shared the same average surface charge. These results indicate that the electrostatic interactions between the viruses at the high concentrations of the N–S transition (i.e. at short interparticle separations) are more complicated than can be accounted for by calculating the interparticle potential assuming a uniform surface charge, even though such calculations are accurate for describing the I–N transition. We hypothesize that the electrostatic interactions between rods are influenced by the configuration of the charged amino acids on the rod surface.

### 4. Novel structures and phase behavior of mixtures

With the qualitative understanding of the phase behavior of pure colloidal suspensions of hard rods, recent efforts have shifted towards studying the phase behavior of colloidal mixtures. The concept of the depletion interaction, first put forward by Asakura and Oosawa [60\*\*], forms an important stepping stone for understanding the phase behavior of highly asymmetric mixtures. In the depletion picture, the free energy of the binary mixture is integrated over the degrees of freedom of the smaller species. Therefore, the behavior of a binary mixture is equivalent to a suspension of large colloids with effective attractive interactions. The range and the strength of attraction are proportional to the size and concentration of smaller species, respectively. The phase behavior of a mixture of colloidal spheres and polymers is well understood [61,62].

Adding small non-adsorbing polymer to a colloidal suspension of spheres widens the liquid to colloidal crystal coexistence. If the size of the polymer is increased, a stable coexistence between a colloidal gas and a liquid will be observed in addition to a direct transition from a colloidal liquid to a colloidal crystal.

#### 4.1. Rod–polymer mixtures

Returning to the case of colloidal rods, we consider extending the second virial approximation to include attractive interactions, in a spirit similar to work on sphere/polymer mixtures. This presents a theoretical challenge because attractions favor the parallel configurations of rods. These are exactly configurations that need to be avoided in order for the free energy to converge at the second virial level. One way to avoid this is to approximate higher virial coefficients using scaled particle theory [63<sup>\*</sup>]. The phase diagrams obtained from this calculation show I–I and N–N phase coexistence in addition to I–N phase coexistence. Experimentally, the attractions can be introduced by adding non-adsorbing polymers to hard rod suspensions. The phase diagram, together with associated tie-lines of *fd*/Dextran mixtures, has been measured for different ionic strengths [64]. The addition of polymers widens the I–N coexistence with polymer preferentially partitioning in the isotropic phase (Fig. 4), which is in qualitative agreement with theoretical predictions. Our measurements indicate that the suspension of rods can be thought of as a van der Waals liquid, where the order parameter of the nematic phase is determined by repulsive interactions, while attractive interactions provide a structureless cohesive energy. Within the admittedly noisy experimental data, we find that the order parameter is determined solely by the rod concentration and not by the polymer concentration, or equivalently, the strength of attraction, as assumed by theory. Experimentally, no I–I or N–N phase coexistence is observed, but it is also not theoretically predicted for the range of experimentally accessible parameters.

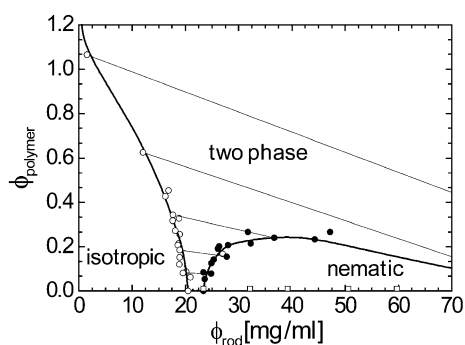


Fig. 4. Phase diagram of a mixture of *fd* virus and Dextran polymer. Available evidence indicates that Dextran is a non-adsorbing polymer and induces effective depletion attraction interactions between *fd* virus. Thick full lines indicate the phase boundary between two-phase isotropic–nematic (cholesteric) phase coexistence and single stable phase, while tie lines are indicated with thin lines [64].

#### 4.2. Rod–rod mixtures

Nematic phase transitions were also mapped out in mixtures of thin and thick colloidal filaments composed of thin, charged semiflexible *fd* virus and thick *fd* virus sterically stabilized with covalently bound poly(ethylene) glycol (*fd*-PEG) [65<sup>\*</sup>]. Because the *fd*-PEG are sterically stabilized, their diameter is independent of ionic strength (Fig. 2). But the effective diameter of the bare *fd* rods can be changed by varying the ionic strength, allowing the effective diameter ratio,  $D_{\text{fd-PEG}}/D_{\text{fd}}$ , to vary from 3.7 to 1. In solution, binary mixtures of *fd* and *fd*-PEG exhibit two-phase isotropic–nematic and nematic–nematic coexistence, as well as three-phase isotropic–nematic–nematic coexistence. We measured the binary phase diagrams as a function of composition, total concentration and ionic strength. A theoretical controversy concerning the topology of the phase diagram has risen in the literature because the phase behavior of the more concentrated phases (N–N and I–N–N) is sensitive to the virial approximation, which is only accurate for the I–N transition where the differences in excluded volumes are large. In contrast, N–N transitions involve small differences in the excluded volume and theories must account for many body terms to even qualitatively describe the phase behavior. For example, the virial theories predicted an upper critical point for the N–N phase [66], while we predicted and observed a lower critical point [65<sup>\*</sup>].

#### 4.3. Lamellar and columnar phases

In addition to coexistence between positionally disordered isotropic and nematic phases, a number of unexpected structures have been observed (Fig. 5), which are associated with the presence of the underlying layered (smectic/lamellar) phases. Historically, the first such phase to be studied was a one-dimensional phase of liquid-like layers of 0.1  $\mu\text{m}$  polystyrene spheres intercalated with smectic-like layers of 0.9  $\mu\text{m}$  long *fd* virus [24<sup>\*\*</sup>25,67<sup>\*</sup>]. While the symmetry of this microphase separated state, named the lamellar phase, is the same as those of more common layered structures found in lipids and block-copolymers, there are two important differences. First, the formation of the microphase separated states in amphiphilic molecules is a result of the covalent bond between two immiscible components. In the absence of this covalent bond, the individual components would presumably undergo bulk phase separation. In contrast, in a mixture of hard rods and spheres, there is no covalent bond between immiscible components. The system is free to bulk phase separate at all times, yet it chooses to microscopically phase separate under certain conditions. This is surprising because normally the creation of an interface raises the free energy, either through the energy term because of surface tension or through the entropy term, which in the ideal case favors uniform structures. Second, the microphase separation in amphiphilic systems is driven by enthalpic forces, while hard rod/sphere mixtures are entirely entropic.

In addition to lamellar phase, structures with more complex geometry, namely a columnar phase of spheres in

a nematic background of rods, have been observed in mixtures of virus and polystyrene latex spheres. Individual columns are composed of spheres such that the diameter of a column is three spheres across and the length of the column is bounded by the sample dimensions. In turn, the columns are packed in a hexagonal lattice, which with increasing sphere concentration continuously transforms to the lamellar phase. No explanation of this phase has been made. Perhaps simulations can address the unanswered question of whether or not purely hard particle suspensions will exhibit these columnar phases.

#### 4.4. Colloidal membranes and ribbons

Other surprising structure, shown in Fig. 6, are isolated lamellae (colloidal membranes) suspended in a background suspension of non-adsorbing polymer that can have either liquid or crystalline order [18<sup>\*\*</sup>,19<sup>\*</sup>]. We recently found that the amplitude of a typical fluctuation of a colloidal membrane scales as  $1/q^4$  where  $q$  is the wavelength of the fluctuation. Equivalent scaling is found in lipid membranes and is indicative of sheets whose fluctuations are determined by curvature energy as opposed to interfacial tension [68]. While most membrane forming materials such as lipids and block-copolymer are composed from amphiphilic molecules [68], colloidal membranes are built from components with very simple interaction potentials, which are distinctly non-amphiphilic. It seems likely that isolated colloidal membranes are just a special case of highly swollen lamellar phase. A swelling transition between these two states has indeed been observed in thermosensitive *fd*/NIPA mixtures [17<sup>\*</sup>]. Finally, the 2D colloidal membranes exhibit a remarkable structural transition

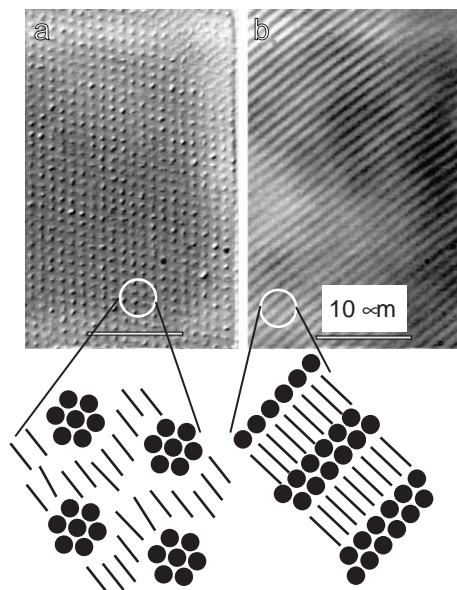


Fig. 5. Optical microscopy images of microphase separated states in a mixture of *fd* and polystyrene spheres ( $D=0.1 \mu\text{m}$ ). (a) For concentration of spheres below 1%, a stable columnar phase is observed. As the concentration of spheres is increased, the columnar phase is transformed into a lamellar phase as shown in (b) [24<sup>\*\*</sup>].

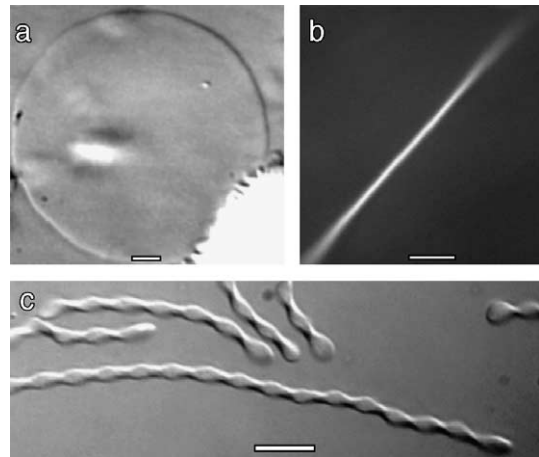


Fig. 6. (a) DIC micrograph of a 2D membranes in which the viruses lie perpendicular to the image plane. (b) 2D membranes taken with polarization microscope in which viruses lie in the plane of the image. (c) Under certain conditions, a flat 2D membrane undergoes a polymorphic transition to a 1D twisted ribbons [18<sup>\*\*</sup>,19<sup>\*</sup>]. Scale bar is  $5 \mu\text{m}$ .

into 1D twisted ribbons. It seems that the polymorphic transition of colloidal membranes is driven by the chirality of individual *fd* molecules. While 2D membranes are incompatible with twist, 1D ribbons can partially accommodate twist.

Another relatively unexplored area is related to the kinetics of phase transitions between the liquid crystalline phases described in previous sections. Even for the simplest case of isotropic–nematic phase transitions, the size and shape of the critical nucleus remain undetermined [70,71]. For more complex nucleation and growth of the smectic phase from a isotropic solution, a complex kinetic pathway has been uncovered [18<sup>\*\*</sup>].

## 5. Dynamics of rod-like viruses

The dynamics of long and thin rod-like colloids is described by the equation of motion of the orientational probability distribution of rods, called the Doi-Edwards equation. In the appropriate limit, the dynamical Doi-Edwards equation reduces to the thermodynamics Onsager description of the isotropic–nematic phase transition. The same reasons that make *fd* an ideal model system to test Onsager theory also make it a good system to examine validity of the dynamical Doi-Edwards equation [22,72<sup>\*</sup>]. Work along these lines has been pursued both theoretically and experimentally by the group of Jan Dhont in Juelich.

### 5.1. Dynamics of quiescent nematics

The dynamics of *fd* and TMV suspensions can be studied using birefringence and light scattering measurements [23,73]. Recently, individual fluorescently labeled viruses have been tracked with optical microscopy. This experimental technique provides a new powerful probe with which it is possible to uncover rich dynamics of rod-like colloids. For example, in quiescent nematics, the broken orientational symmetry results

in an anisotropic diffusion coefficient with rods preferentially diffusing along the nematic director. The concentration dependence of this anisotropic diffusion has been directly measured using particle tracking [20]. The diffusion along the nematic director is enhanced, while diffusion perpendicular to the director is suppressed when compared to diffusion in the isotropic phase just below I–N coexistence. Using fluorescence microscopy, it is also possible to study the fluctuations of long polymers suspended in a background nematic [26\*\*]. These polymers undergo a conformation transition from random coils to extended rod as the background liquid goes through isotropic–nematic phase transition. By analyzing the fluctuations of a polymer in a nematic background, it is possible to measure quantities such as the coupling strength of a polymer to a nematic field and Odijk persistence length [42\*\*]. These quantities are difficult to extract from more conventional scattering experiments.

### 5.2. Transitions in shear flow

The Doi-Edwards equation predicts that the dynamics of nematic rods in ‘non-equilibrium’ dissipative fields, such as shear flow, exhibit rich behavior such as director tumbling, wagging or flow aligning. Experimentally, the transitions between these flow regimes were identified using rheological measurements and quantitatively compared to Doi-Edwards predictions [21\*]. Shear flow also has a strong influence on the alignment of isotropic rods and it is expected that the nature and the location of the isotropic–nematic phase transition will be greatly affected by shear flow. Theoretically, predicting the location of the shear dependent isotropic–nematic binodals is a difficult task. For equilibrium systems, there is a well-defined prescription for locating first order phase transitions based on principles of equality of mechanical and chemical equilibrium. No equivalent principle exists for non-equilibrium systems and this remains an area of vigorous research. Experimentally, the shear dependence of the I–N binodal and spinodal have been determined in *fd* suspensions [21\*,23]. Associated with the isotropic–nematic phase transition is the occurrence of shear-banding transitions which have been studied extensively in solutions of worm-like micelles [74] and have been recently found in suspensions of *fd* [21\*]. Unlike the shear induced isotropic–nematic phase transition, shear banding transitions have no equilibrium analog.

## 6. Conclusions, outstanding problems and future directions

Viruses are unique colloidal suspensions because they are large enough to visualize at the single molecule level in the optical microscope, are monodisperse and are stable to 20% volume fraction. They are the only anisotropically shaped colloids for which experiments show quantitative comparison with Onsager theory for isotropic–nematic transition in the limit of high ionic strength where the particles are weakly repulsive and approximate hard rods.

While the Onsager theory is successful for hard rods, it fails for moderate repulsion and even weak interparticle attraction

(Section 3.2). Future progress in the understanding of colloidal suspensions of anisotropic particles calls for generalizing the Onsager theory to go beyond the hard rod limit. A number of outstanding issues regarding the phase behavior of the viruses (and all liquid crystals) remain to be solved. For example, the question of how chirality on the molecular scale transmits itself to the mesoscopic scale of the cholesteric pitch has stayed shrouded in mystery (Section 3.2).

Understanding non-equilibrium phenomena is at the forefront of research in statistical mechanics and studies of the kinetics of phase transitions (Section 4.4) and phase transitions in non-equilibrium systems (Section 5.2) have recently been undertaken with colloidal viruses.

The Onsager [4\*\*\*] hard rod theory and the Asakura–Oosawa [60\*\*] depletion theory are examples of how the shape of hard particles influences the entropy of colloidal suspensions and can lead to ordered phases. Even under conditions where the viruses approximate hard rods, mixtures of rods and spheres lead to a variety of bafflingly complex phases whose understanding remains a serious challenge [24\*\*] (Section 4.3). A promising direction of research, vigorously pursued by Belcher [29\*\*], is to systematically reshape the virus molecules in controlled ways in order to create new building blocks for the entropically driven self-assembly of ordered phases (Section 3.1). The advances and opportunities described in this review indicate that the future promises to be bright for this fledgling field of ‘entropy engineering’.

## Acknowledgments

SF acknowledges support from the National Science Foundation-DMR. ZD is supported by a junior fellowship from Rowland Institute at Harvard.

## References and recommended readings

- [1] Fraden S. Phase transitions in colloidal suspensions of virus particles. In: • Baus M, Rull LF, Ryckaert JP, editors. Observation, prediction, and simulation of phase transitions in complex fluids NATO-ASI Series C, vol. 460. Kluwer Academic Publishers. p. 113–64. Comprehensive review of the phase behavior of TMV and *fd*.
- [2] de Gennes P, Prost J. The physics of liquid crystals, 2nd edn. Oxford: Clarendon Press, 1992.
- [3] Cifferi A, Krigbaum WR, Meyer RB, editors. Polymer liquid crystals. New York: Academic Press, 1982.
- [4] Onsager L. The effects of shape on the interaction of colloidal particles. \*\*\* Ann N Y Acad Sci 1949;51:627. If you read only one paper, pick Onsager’s. With over 2000 citations, this paper launched an entire field.
- [5] Davidson P, Gabriel JCP. Mineral liquid crystals. Curr Opin Colloid Interface Sci 2005;9:377–83.
- [6] Livolant F, Levelut AM, Doucet J, Benoit JP. The highly concentrated • liquid-crystalline phase of DNA is columnar hexagonal. Nature 1989;339:724–6. Thorough treatment on how to identify phases correctly. Livolant’s work has consistently been the best there is on ordered phases of DNA.

• Of special interest.

\*\* Of outstanding interest.

- [7] Livolant F. Ordered phases of DNA in vivo and in vitro. *Physica A* 1991;176:117–37.
- Provocative presentation of biological manifestations of liquid crystallinity.
- [8] Livolant F, Leforestier A. Condensed phases of DNA: structures and phase transitions. *Prog Polym Sci* 1996;21:1115–64.
- [9] Podgornik R, Strey HH, Parsegian VA. Colloidal DNA. *Curr Opin Colloid Interface Sci* 1998;3:534–9.
- [10] Helfer E, Panine P, Carlier MF, Davidson P. The interplay between viscoelastic and thermodynamic properties determines the birefringence of F-actin gels. *Biophys J* 2005;89:543–53.
- [11] Hitt AL, Cross AR, Williams RC. Microtubule solutions display nematic liquid-crystalline structure. *J Biol Chem* 1990;265:1639–47.
- [12] Sato T, Teramoto A. Concentrated solutions of liquid-crystalline polymers. *Adv Polym Sci* 1996;126:85–161.
- This is a leading group in the field of colloidal liquid crystals and their work merits a search through Science Citations. This review article is particularly well written and comprehensive.
- [13] Yoshida K, Teramoto A, Nakamura N, Sato T. Chiral interactions in polymer liquid crystals reflecting polymer conformations: triple-helical polysaccharide schizophyllan and poly( $\gamma$ -benzyl L-glutamate). *Macromolecules* 2003;36:2108–13.
- [14] Lapointe J, Marvin DA. Filamentous bacterial viruses: 8. Liquid-crystals of Fd. *Mol Cryst Liq Cryst* 1973;19:269.
- Marvin is a structural biologist specializing in fd. This paper, which has the first reports of liquid crystallinity of fd, is incredibly complete.
- [15] Kreibitz U, Wetter C. Light-diffraction of in vitro crystals of 6 tobacco mosaic-viruses. *Z Naturforsch [C]* 1980;35:750–62.
- TMV has a propensity to aggregate and Wetter shows clearly how to make monodisperse TMV. This paper is full of beautiful pictures of iridescent colloidal crystalline TMV.
- [16] Welsh LC, Symmons MF, Nave C, Perham RN, Marseglia EA, Marvin DA. Evidence for tilted smectic liquid crystalline packing of fd Inovirus from X-ray fiber diffraction. *Macromolecules* 1996;29:7075–83.
- [17] Alsayed AM, Dogic Z, Yodh AG. Melting of lamellar phases in temperature sensitive colloid-polymer suspensions. *Phys Rev Lett* 2004;93:057801.
- Yodh's group is at the forefront of soft matter research and has recently started studying *fd*. This paper is full of insight explaining the kinetics of complex phase transition pathways.
- [18] Dogic Z. Surface freezing and a two-step pathway of the isotropic–smectic phase transition in colloidal rods. *Phys Rev Lett* 2003;91:165701.
- A mysterious phase—the surface smectic—is explained.
- [19] Dogic Z, Fraden S. Development of model colloidal liquid crystals and the kinetics of the isotropic–smectic transition. *Philos Trans R Soc Lond Ser A Math Phys Eng Sci* 2001;359:997–1014.
- Reports of genetically engineered and sterically stabilized viruses. Descriptions of incredibly complex phase transition kinetics in virus/polymer mixtures.
- [20] Lettinga MP, Barry E, Dogic Z. Self-diffusion of rod-like viruses in the nematic phase. *Europhys Lett* 2005;71:692–8.
- [21] Lettinga MP, Dogic Z, Wang H, Vermant J. Flow behavior of colloidal rodlike viruses in the nematic phase. *Langmuir* 2005;21:8048–57.
- [22] Dhont JKG, Lettinga MP, Dogic Z, Lenstra TAJ, Wang H, Rathgeber S, et al. Shear-banding and microstructure of colloids in shear flow. *Faraday Discuss* 2003;123:157–72.
- [23] Lenstra TAJ, Dogic Z, Dhont JKG. Shear-induced displacement of isotropic–nematic spinodals. *J Chem Phys* 2001;114:10151–62.
- [24] Adams M, Dogic Z, Keller SL, Fraden S. Entropically driven microphase transitions in mixtures of colloidal rods and spheres. *Nature* 1998;393:349–52.
- Entropy produces phases of surprising complexity. Some of the observed phases remain unexplained.
- [25] Dogic Z, Frenkel D, Fraden S. Enhanced stability of layered phases in parallel hard spherocylinders due to addition of hard spheres. *Phys Rev E* 2000;62:3925–33.
- [26] Dogic Z, Zhang J, Lau AWC, Aranda-Espinoza H, Dalhaimer P, Discher DE, et al. Elongation and fluctuations of semiflexible polymers in a nematic solvent. *Phys Rev Lett* 2004;92:125503.
- Nematics exert an effective field on polymers, straightening them out if they are not too flexible.
- [27] Nam KT, Peelle BR, Lee SW, Belcher AM. Genetically driven assembly of nanorings based on the M13 virus. *Nano Lett* 2004;4:23–7.
- How to genetically engineer filamentous virus for bottoms up self-assembly.
- [28] Mao CB, Flynn CE, Hayhurst A, Sweeney R, Qi JF, Georgiou G, et al. Viral assembly of oriented quantum dot nanowires. *Proc Natl Acad Sci U S A* 2003;100:6946–51.
- [29] Flynn CE, Lee SW, Peelle BR, Belcher AM. Viruses as vehicles for growth, organization and assembly of materials. *Acta Mater* 2003;51:5867–80.
- Review article on genetic engineering of viruses for materials science application.
- [30] Seeman NC, Belcher AM. Emulating biology: building nanostructures from the bottom up. *Proc Natl Acad Sci U S A* 2002;99:6451–5.
- Review of virus and DNA based nanotechnology by two of the leaders of the field.
- [31] Lee SW, Mao CB, Flynn CE, Belcher AM. Ordering of quantum dots using genetically engineered viruses. *Science* 2002;296:892–5.
- [32] Whaley SR, English DS, Hu EL, Barbara PF, Belcher AM. Selection of peptides with semiconductor binding specificity for directed nanocrystal assembly. *Nature* 2000;405:665–8.
- [33] Lee SW, Wood BM, Belcher AM. Chiral smectic C structures of virus-based films. *Langmuir* 2003;19:1592–8.
- [34] Lee SW, Lee SK, Belcher AM. Virus-based alignment of inorganic, organic, and biological nanosized materials. *Adv Mater* 2003;15:689–92.
- [35] Lee SY, Royston E, Culver JN, Harris MT. Improved metal cluster deposition on a genetically engineered tobacco mosaic virus template. *Nanotechnology* 2005;16:S435.
- [36] Fowler CE, Shenton W, Stubbs G, Mann S. Tobacco mosaic virus liquid crystals as templates for the interior design of silica mesophases and nanoparticles. *Adv Mater* 2001;13:1266–9.
- [37] Dujardin E, Peet C, Stubbs G, Culver JN, Mann S. Organization of metallic nanoparticles using tobacco mosaic virus templates. *Nano Lett* 2003;3:413–7.
- [38] Bawden FC, Pirie NW, Bernal JD, Fankuchen I. Liquid crystalline substances from virus-infected plants. *Nature* 1936;138:1051–2.
- [39] Straley JP. Gas of long rods as a model for lyotropic liquid-crystals. *Mol Cryst Liq Cryst* 1973;22:333–57.
- First review of Onsager theory and full of insight and suggestions for experiments (not all yet performed).
- [40] Forsyth PA, Marcelja S, Mitchell DJ, Ninham BW. Ordering in colloidal systems. *Adv Colloid Interface Sci* 1978;9:37–60.
- Our favorite review. One of the first to point out the importance of entropy in colloidal suspensions. Builds analogies between the two quintessential entropic systems: hard rods and hard spheres.
- [41] Gelbart WM. Molecular theory of nematic liquid-crystals. *J Phys Chem* 1982;86:4298–307.
- Thoughtful discussion of the relative contribution of entropy and attraction in nematics.
- [42] Odijk T. Theory of lyotropic polymer liquid-crystals. *Macromolecules* 1986;19:2314–29.
- Excellent review of a series of extensions of Onsager theory by Odijk and collaborators. Odijk is one of the leading theorists in this field and it is worthwhile to search his name in Science Citations.
- [43] Vroege GJ, Lekkerkerker HNW. Phase-transitions in lyotropic colloidal and polymer liquid-crystals. *Rep Prog Phys* 1992;55:1241–309.
- Review of Onsager theory and some experiments. Lekkerkerker is a leader in both experiment and theory in the field of liquid crystal colloids. It is recommended to search his name in Science Citation Index.
- [44] Frenkel D. Perspective on “The effect of shape on the interaction of colloidal particles”—Onsager L (1949) *Ann NY Acad Sci* 51: 627. *Theor Chem Acc* 2000;103:212–3.
- On the importance of Onsager's paper. Frenkel himself is the leading computer simulator on hard particle systems and on hard rods in particular. It is recommended to search his name in Science Citation Index.
- [45] Sambrook J, Russell DW. *Molecular cloning: a laboratory manual*, 3rd edn. Cold Spring Harbor Laboratory Press; 2001.



A ‘cook book’, but also full of informative and interesting background material as well.

[46] Tang JX, Fraden S. Isotropic–cholesteric phase-transition in colloidal suspensions of filamentous bacteriophage fd. *Liq Cryst* 1995;19:459–67. There is excellent agreement between theory and experiment when flexibility is taken into account.

[47] Dogic Z, Fraden S. Smectic phase in a colloidal suspension of semiflexible virus particles. *Phys Rev Lett* 1997;78:2417–20.

[48] Dogic Z, Fraden S. Cholesteric phase in virus suspensions. *Langmuir* 2000;16:7820–4.

[49] Grelet E, Fraden S. What is the origin of chirality in the cholesteric phase of virus suspensions? *Phys Rev Lett* 2003;90:198302.

The relationship between molecular and macroscopic chirality is puzzling!

[50] Purdy KR, Dogic Z, Fraden S, Ruhm A, Lurio L, Mochrie SGJ. Measuring the nematic order of suspensions of colloidal fd virus by x-ray diffraction and optical birefringence. *Phys Rev E* 2003;67:031708.

[51] Purdy KR, Fraden S. Isotropic–cholesteric phase transition of filamentous virus suspensions as a function of rod length and charge. *Phys Rev E* 2004;70:061703.

Clear evidence of the breakdown of Onsager theory at low ionic strength.

[52] Maguire JF, McTague JP, Rondelez F. Rotational diffusion of sterically interacting rodlike macromolecules. *Phys Rev Lett* 1980;45:1891–4.

Pioneering paper in its first use of genetically engineered molecules for condensed matter research.

[53] Glucksman MJ, Bhattacharjee S, Makowski L. 3-dimensional structure of a cloning vector—X-ray-diffraction studies of filamentous bacteriophage-M13 at 7-Angstrom resolution. *J Mol Biol* 1992;226:455–70.

[54] Zimmermann K, Hagedorn H, Heuck CC, Hinrichsen M, Ludwig H. The ionic properties of the filamentous bacteriophages Pfl and Fd. *J Biol Chem* 1986;261:1653–5.

[55] Huang Y, Chiang CY, Lee SK, Gao Y, Hu EL, De Yoreo J, et al. Programmable assembly of nanoarchitectures using genetically engineered viruses. *Nano Lett* 2005;5:1429–34.

[56] Fraden S, Maret G, Caspar DLD, Meyer RB. Isotropic–nematic phase-transition and angular-correlations in isotropic suspensions of tobacco mosaic-virus. *Phys Rev Lett* 1989;63:2068–71.

[57] Fraden S, Maret G, Caspar DLD. Angular-correlations and the isotropic–nematic phase-transition in suspensions of tobacco mosaic-virus. *Phys Rev E* 1993;48:2816–37.

[58] Oldenbourg R, Wen X, Meyer RB, Caspar DLD. Orientational distribution function in nematic tobacco-mosaic-virus liquid-crystals measured by X-ray-diffraction. *Phys Rev Lett* 1988;61:1851–4.

Exploiting the structure of TMV to measure the angular distribution function which is the most sensitive test of the Onsager theory.

[59] Day LA, Marzec CJ, Reisberg SA, Casadevall A. DNA packing in filamentous bacteriophages. *Annu Rev Biophys Biophys Chem* 1988;17:509–39.

[60] Asakura S, Oosawa F. Interaction between particles suspended in solutions of macromolecules. *J Polym Sci* 1958;33:183–92.

How to build ‘attraction through repulsion’.

[61] Lekkerkerker HNW, Poon WCK, Pusey PN, Stroobants A, Warren PB. Phase-behavior of colloid plus polymer mixtures. *Europhys Lett* 1992;20:559–64.

[62] Gast AP, Russel WB, Hall CK. An experimental and theoretical-study of phase-transitions in the polystyrene latex and hydroxyethylcellulose system. *J Colloid Interface Sci* 1986;109:161–71.

[63] Lekkerkerker HNW, Stroobants A. Phase-behavior of rod-like colloid plus flexible polymer mixtures. *Nuovo Cim Soc Ital Fis D Condens Matter* 1994;16:949–62.

Important extension of depletion theory to treat the isotropic–nematic transition. Warning: some typographic errors are in the equations.

[64] Dogic Z, Purdy KR, Grelet E, Adams M, Fraden S. Isotropic–nematic phase transition in suspensions of filamentous virus and the neutral polymer Dextran. *Phys Rev E* 2004;69:051702.

[65] Purdy KR, Varga S, Galindo A, Jackson G, Fraden S. Nematic phase transitions in mixtures of thin and thick colloidal rods. *Phys Rev Lett* 2005;94:057801.

Experiments with virus demonstrate that the nematic–nematic transition is sensitive to the second virial approximation.

[66] Vroege GJ, Lekkerkerker HNW. Theory of the isotropic–nematic–nematic phase-separation for a solution of bidisperse rodlike particles. *J Phys Chem* 1993;97:3601–5.

[67] Koda T, Numajiri M, Ikeda S. Smectic-A phase of a bidisperse system of parallel hard rods and hard spheres. *J Phys Soc Jpn* 1996;65:3551–6.

Rare example of theory predicting complex entropically driven phase transitions ahead of experiment.

[68] Goetz R, Gompper G, Lipowsky R. Mobility and elasticity of self-assembled membranes. *Phys Rev Lett* 1999;82:221–4.

[70] Shimada T, Doi M, Okano K. Concentration fluctuation of stiff polymers: 3. Spinodal decomposition. *J Chem Phys* 1988;88:7181–6.

[71] Winters JW, Odijk T, van der Schoot P. Spinodal decomposition in a semidilute suspension of rodlike macromolecules. *Phys Rev E* 2000;63:011501.

[72] Doi M, Edwards SF. *The theory of polymer dynamics*. Oxford: Clarendon, 1986.

Doi is a leading theorist in the field of hard rods. It is recommended to search his name in Science Citation Index.

[73] Cush RC, Russo PS. Self-diffusion of a rodlike virus in the isotropic phase. *Macromolecules* 2002;35:8659–62.

[74] Boltzenhagen P, Hu YT, Matthys EF, Pine DJ. Observation of bulk phase separation and coexistence in a sheared micellar solution. *Phys Rev Lett* 1997;79:2359–62.