What Is the Origin of Chirality in the Cholesteric Phase of Virus Suspensions?

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We report a study of the cholesteric phase in monodisperse suspensions of the rodlike virus fd sterically stabilized with the polymer polyethylene glycol (PEG). After coating the virus with neutral polymers, the phase diagram and nematic order parameter of the fd-PEG system then become independent of ionic strength. Surprisingly, the fd-PEG suspensions not only continue to exhibit a cholesteric phase, which means that the grafted polymer does not screen all chiral interactions between rods, but paradoxically the cholesteric pitch of this sterically stabilized fd-PEG system varies with ionic strength. Furthermore, we observe that the cholesteric pitch decreases with increasing viral contour length, in contrast to theories which predict the opposite trend. Different models of the origin of chirality in colloidal liquid crystals are discussed.

Chirality leads to a wonderful variety of liquid crystalline phases [1], from the cholesteric state to very complex structures such as smectic blue phases [2]. In spite of its importance, the connection between chirality at the molecular scale and the macroscopic chiral structure of liquid crystalline phases is not yet understood, and quantitative prediction of the twist periodicity, also called cholesteric pitch, based on molecular features remains an unsolved problem. The elaboration of a rigorous statistical theory for the chiral interaction is one of the greatest challenges in the physics of liquid crystals.

We report and discuss experimental studies of the cholesteric phase of colloidal suspensions of filamentous bacteriophages, denoted fd. The rodlike virus fd is a micron-length semiflexible polyelectrolyte formed by a single stranded DNA around which coat proteins are helicoidally wrapped. Therefore fd is a chiral object exhibiting a helical charge distribution. Suspensions of fd rods in aqueous solution form several liquid crystalline phases with increasing concentration, and especially a cholesteric or chiral nematic phase which lies between the isotropic and smectic phases [3]. However, molecular chirality does not guarantee the existence of a macroscopic chiral structure such as a cholesteric phase. For instance other chiral viruses such as tobacco mosaic virus [4] or Pf1 [5] with a helical structure extremely similar to fd exhibit only a nematic phase. Up to now, theory has failed to answer even the simple qualitative question: When does macroscopic chirality, such as the cholesteric phase, occur in liquid crystals formed by helical molecules? In this Letter, by studying a well-characterized system of a rodlike virus stabilized by a grafted neutral polymer, we highlight that counterions play a crucial role. More specifically we show that ionic strength is one of the major physical parameters controlling the chirality of colloidal liquid crystalline suspensions.

The bacteriophage fd is a polyelectrolyte with a contour length of $L = 880$ nm, a diameter of $D_{\text{bare}} = 6.6$ nm, a persistence length of $\xi = 2200$ nm, a molecular weight

of $M_W = 1.64 \times 10^7$ g/mol, and a charge density of $10e/\text{nm}$ in water at $p\text{H} = 8.1$ [3,6]. About 2700 copies of a single protein form the coat helicoidally wrapped following a fivefold rotation axis combined with an twofold screw axis around the inner DNA [7]. The fd virus was grown and purified following standard biological protocols [6]. To vary ionic strength (I), viruses were extensively dialyzed against a 20 mM TRIS-HCl buffer at $p\text{H} = 8.1$ with an adjusted amount of NaCl. All measurements were done at room temperature and the virus concentrations were determined using spectrophotometry with an absorption coefficient of 3.84 cm²/mg at 269 nm.

Although the fd virus is charged in solution, it is possible to prepare sterically stabilized colloids by irreversibly binding neutral polyethylene glycol (PEG) of different molecular weights (5000 or 20 000 g/mol) to the surface of the virus [8]. End functionalized PEG molecules (Shearwater Corp.) have been used to covalently attach the water soluble polymer to the amino groups on the coat proteins of fd that are exposed to the solution. The refractive index increment, dn/dc, has been measured to estimate the degree of coverage of the fd-PEG virus [9], and 195 ± 30 polymers PEG-20k (400 ± 40 for PEG-5k) are attached to each virus. This result corresponds to nearly complete coverage of the virus by the polymer. Indeed, by considering grafted polymers as spheres of radius of gyration R_g, about $\pi(1 + D_{\text{bare}}/2R_g) \times L/2R_g$ polymers can be close packed around a virus of diameter D_{bare} (Fig. 1). In the case of PEG-20k ($R_g = 7$ nm [10]), this model predicts about 250 grafted polymers per virus, which is close to the measured value.

The isotropic-cholesteric (I-N*) transition has been investigated for this system of fd virus grafted with PEG [8]. According to Onsager [11], an isotropic to nematic phase transition occurs in suspensions of hard rods when the rod number density n reaches
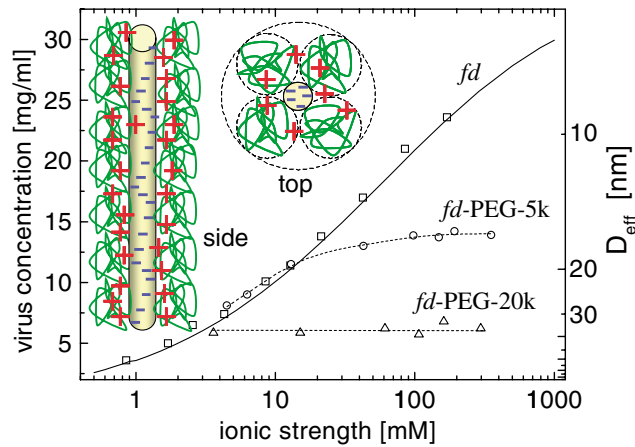
$$n\pi L^2 D \simeq 16. \qquad (1)$$

FIG. 1 (color online). Isotropic-cholesteric phase boundary as a function of ionic strength for a 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. Squares and circles indicate the I-N* transition in fd and in fd coated with PEG-5k respectively [8], while triangles refer to the fd virus coated with PEG-20k. The concentration of the I-N* transition for bare fd increases as a function of ionic strength as $1/D_{eff}$ (solid line), where D_{eff} is the effective diameter taking into account the electrostatic repulsion between rods at the I-N* transition [11]. fd with grafted PEG shows identical behavior to nongrafted, up to an ionic strength corresponding to a D_{eff} that matches the size of the stabilizing polymer layer. For a higher amount of added salt, the phase diagram becomes independent of ionic strength, and the fd-PEG system behaves as a sterically stabilized suspension. This is schematically represented by the cartoon of a virus with polymers grafted on its surface where $D_{eff}^{electrostatic} < D_{eff}^{polymer}$.

This is experimentally observed for bare fd (without any grafted polymer) over a wide range of ionic strengths as shown with square symbols in Fig. 1. Note that there is no difference in Onsager's theory between the isotropic-nematic and isotropic-cholesteric phase transitions. The free energy difference between the nematic and the cholesteric phase is negligible compared to the free energy difference between the isotropic and the nematic phase [12].

Because the fd virus is charged, it is necessary to account for electrostatic repulsion by considering the colloids as having an effective diameter (D_{eff}) larger than their bare diameter (D_{bare}) with D_{eff} roughly proportional to the Debye screening length [11]. D_{eff} therefore decreases with increasing ionic strength [13] and approaches D_{bare} at high ionic strength. By substituting D_{bare} with D_{eff} in Eq. (1), it follows that the concentration of fd at the I-N* transition is inversely proportional to D_{eff}. For fd-PEG rods, there is an ionic strength at which the coexistence concentrations of the I-N* transition become independent of ionic strength. The crossover from the regime where the rods are electrostatically stabilized to where they are sterically stabilized by "soft" repulsion between virus-bound polymers is shown in

Fig. 1. This corresponds to an ionic strength of $I = 3$ mM for fd-PEG-20k and $I = 20$ mM for fd-PEG-5k. At this ionic strength, the diameter associated with electrostatic interactions (D_{eff}) equals the diameter of the virus plus grafted polymer. It is therefore possible to extract an effective diameter for the polymer coated virus. In this range of ionic strengths, between 15 and 200 mM, the polymer is in a good solvent and the effects of NaCl on PEG properties are negligible in terms of the radius of gyration and viscosity [14]. From Fig. 1, the effective diameter of fd-PEG-20k at $I = 3$ mM is 37 nm, which is approximately equal to the hard diameter of fd ($D_{bare} = 6.6$ nm) plus twice the diameter of PEG ($R_g = 7$ nm [10]): $D_{bare} + 4R_g = 35$ nm. The fd-PEG-5k complex has $D_{eff} = 17$ nm at high ionic strength, while $D_{bare} + 4R_g = 19$ nm ($R_g = 3$ nm for PEG-5k [10]).

The good agreement between the measured and estimated value of the fd-PEG effective diameter supports the model of polymer behaving mainly as a sphere attached to the surface of the virus. Thus, the independence of the phase diagram on ionic strength reported in Fig. 1 shows that the fd virus coated with PEG-20k is a system whose stability is entirely determined by polymer repulsion. This result has been confirmed by measuring the nematic order parameter of fd-PEG-20k close to the I-N* transition. Figure 2 indicates that the order parameter, determined by birefringence under magnetic field [15], is independent of ionic strength just as is the phase diagram. Therefore, fd-PEG-20k liquid crystal turns out to be a sterically stabilized system, that, surprisingly, continues to exhibit a cholesteric phase as shown in Fig. 3. Indeed, one could expect that grafted polymers on a virus surface would screen chiral interactions, leading thus only to a nematic phase. To our knowledge, fd virus coated with PEG is the first completely sterically stabilized system which still has chiral features.
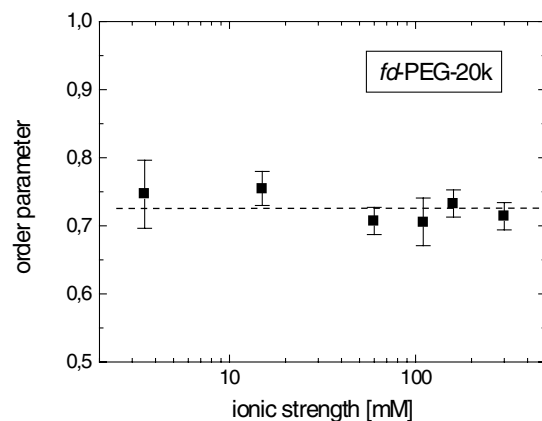

FIG. 2. Ionic strength dependence of the nematic order parameter of fd virus coated with PEG-20k measured close to the I-N* transition (sample concentrations around $c \sim 9$ mg/ml). The order parameter is obtained from birefringence measurements after unwinding the cholesteric phase with a magnetic field. The dashed line is a guide for the eye.

The inset of Fig. 3 presents a typical "fingerprint" texture of fd-PEG suspensions characteristic of a cholesteric phase observed by optical microscopy between crossed polarizers. After a few days of equilibration following the preparation of the samples, we have measured the dependence of the cholesteric pitch on concentration and ionic strength (Fig. 3). Paradoxically, fd-PEG-20k liquid crystals have a cholesteric pitch which depends on ionic strength. Close to the I-N* transition where both the phase diagram and the order parameter are independent of ionic strength (Figs. 1 and 2), the pitch varies by a factor of 2 from 100 μm at $I = 15$ mM to 200 μm at $I = 110$ mM. In this range of low rod concentrations the mean interparticle distance (d_{inter}) is about 60 nm: the fd-PEG surfaces are then separated by typically three bare diameters [15]. The corresponding volume fraction at the I-N* transition is $\Phi = 0.2$ where Φ is defined by $\Phi = c N_A L \pi (D_{\mathrm{eff}}/2)^2 / M_W$ with N_A being the Avogadro's number. We can then calculate the concentration (c^*) for which the grafted polymers of fd-PEG-20k begin to overlap (corresponding to $\Phi^* = 1$): this gives $c^* \simeq 35$ mg/ml, which is close to the concentration $c_0 = 28$ mg/ml where the pitch is independent of ionic strength (Fig. 3). The concentration c^* separates two regimes: we mainly focus on the dilute regime ($c < c^*$), where chiral rods do not overlap. Complexity increases for $c > c^*$ because of short-range interactions between the polymers as well as electrostatic interactions between the viruses, which can induce intricate behaviors similar to those seen for instance in DNA condensation [16].
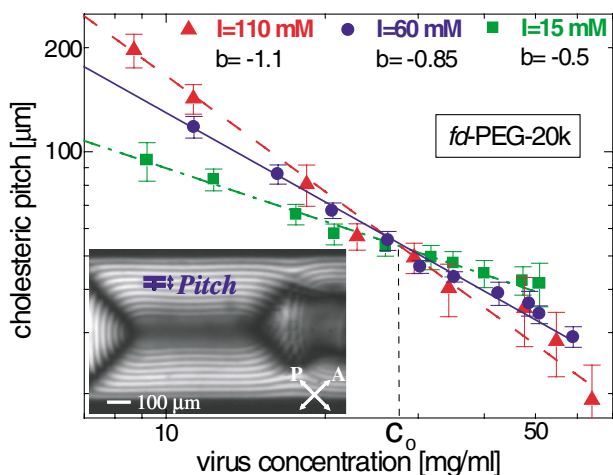
What then is the mechanism of transmission of chiral interactions between sterically stabilized viruses? The first model of the origin of intermolecular twist was proposed by Straley who considered two screwlike molecules with excluded-volume interactions [17]. He predicted the pitch to be independent of the concentration, which is not observed experimentally. Moreover, if chirality is transmitted by some microscopic detail of the virus, we then expect that the chiral effects will be diminished for fd grafted with polymers. However, the same range of pitch has been seen for the fd-PEG system as for bare fd (between 10 and 200 μm) [5]. That seems to exclude the strict steric model of the packing of screws, because the thread of the screws of the fd virus is covered by a 30 nm thick polymer coat. Even with the assumption that the polymers grafted to the helical protein coat replicate the chiral symmetry of the underlying bare virus, it does not explain why the cholesteric pitch should depend on ionic strength.

If the chirality is not transmitted at the microscopic length scale of the helical periodicity of the protein coat (with an axial repeat of 3.3 nm for the fd virus [7]), the existence of a *superhelical* twist, where chirality occurs at a much larger length scale, was proposed by Meyer [18]. In this model, Brownian motion causes the virus to bend back and forth, and due to the coupling between flexibility and the underlying structural chirality imposed by the helical arrangement of coat proteins, the virus could twist more in one direction than in another leading to the creation of a "corkscrew" shape with a helical pitch of the order of a micron (Fig. 4). However, the corkscrew model cannot be the whole story as Pf1, a chiral and flexible virus with a structure very similar to fd, has only a nematic phase, which implies that not all chiral flexible molecules form corkscrews. Additionally, completely rigid rods like cellulose [19] and chitin [20] form a cholesteric phase implying that the corkscrew distortion is not necessary for cholesteric production. An interesting experiment would be to coat these rigid
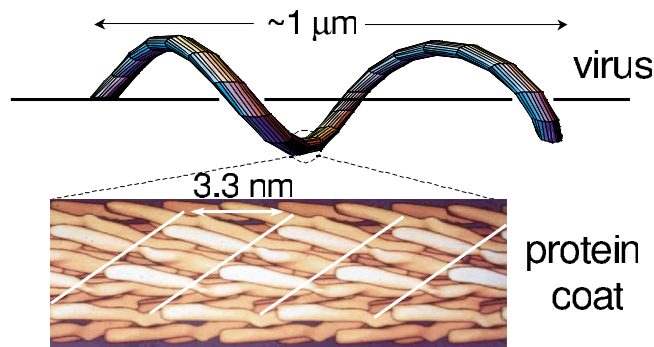

FIG. 3 (color online).   Evolution of the cholesteric pitch (P) with the concentration (c) of fd virus grafted with PEG-20k, for three different ionic strengths ($I = 15, 60,$ and 110 mM). Cholesteric pitch was measured from the "fingerprint" texture, as shown in the inset. The sample is contained in a cylindrical glass capillary of diameter 0.7 mm and several cm long. Error bars have been obtained by repeating the same measurement along the full length of the sample. The scaling exponent of the fit to $P \propto c^b$ is indicated for each ionic strength.

FIG. 4 (color online).   Schematic representation of the "corkscrew" model: the chirality is transmitted between viruses by the helical shape (accentuated for illustration) of the whole virus at the micron length scale and not by the helical structure of the protein coat.
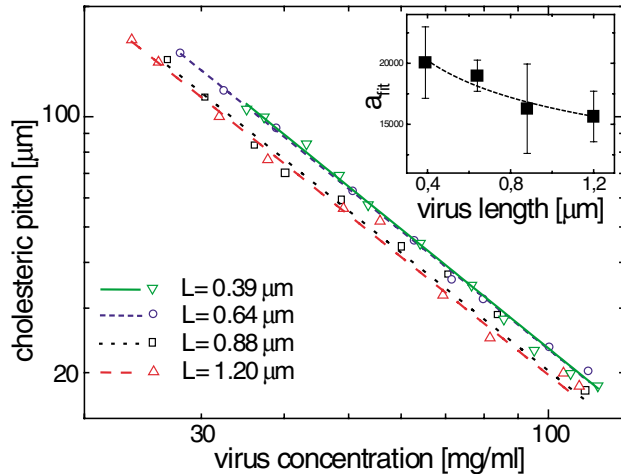
FIG. 5 (color online). Graph of the cholesteric pitch (P) versus the concentration (c) for mutant viruses of different contour lengths (L). The ionic strength is 60 mM. The result of the fit $P = a_{\mathrm{fit}}c^b$ indicates the same scaling exponent $b = -1.45$ for all mutants. The inset indicates the dependence of the pitch with the length of the virus at a given concentration: $P \propto a_{\mathrm{fit}} \propto 1/L^{0.25}$. This result contradicts the main existing theories of chirality in liquid crystals which predict $P \propto L^2$ [21,22].

molecules with long polymer. If they still form the cholesteric phase then the corkscrew model would be rejected.

One of the most advanced theories of cholesteric assemblies of charged rods has been proposed by Kornyshev *et al.* for DNA. They suggest that various details of the helical symmetry of macromolecules surface charge pattern may be responsible for macroscopic twist [21]. The main idea is based on direct chiral electrostatic interaction between long helical molecules. Their attempt is promising, but how can such a theory account, even qualitatively, for our results on the fd virus stabilized with polymer? Moreover, the prediction of a cholesteric pitch *increasing* with the square of the viral contour length ($P \propto L^2$) shown both by Kornyshev *et al.* [21] and by Harris *et al.* [22] is in contradiction to our results obtained with mutant viruses (without any polymer grafted). Indeed, we are able to grow monodisperse mutants [8] where only the contour length varies, keeping unchanged the local structure of the viruses. We find that the cholesteric pitch *decreases* with increasing viral contour length as shown in Fig. 5. These experiments suggest that current theories do not capture all the essential elements of the connection between microscopic and macroscopic chiralities in colloidal liquid crystals.

In conclusion, we have presented in this Letter a system of chiral colloidal rods coated with neutral polymer, which behaves as a sterically stabilized suspension because the phase diagram and order parameter are independent of ionic strength. However, this liquid crystal has the baffling feature that it exhibits a cholesteric phase whose pitch depends on ionic strength. If the cholesteric phase is induced by the charges on the virus which are buried by a polymer coat of thickness corresponding to several Debye screening lengths, then we conclude that the cholesteric phase arises from very weak and subtle forces. Our result highlights the fundamental role of counterions in the existence of a macroscopic chirality and should stimulate new theoretical investigations on this fundamental topic of the origin of chirality in liquid crystalline phases.

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