Chiral glass of charged DNA rods, cavity loops

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Abstract
Recently, the collective phase behaviors of charged DNA-viruses (rods) are explored in various low ionic strengths, where much slowing down relaxations are observed in equilibrium. These phases are distinguished by crossing the critical ionic strength, where the salt-dependent effective microscopic dynamics are important with non-monotonic multi-phase boundaries. This is also interested in terms of phase transition and replica symmetry breaking (RSB), realized by the divergence of relaxation time and the exponent power of correlation lengths in dynamic disorderorder transitions. RSB occurs as the result of many-body interactions of randomized orientations. Here, in this paper, one of the chiral mesophases, the X-pattern is highlighted for its long-time metastable state consisting of cavity loops bounded within the planar nematic, as a new type of a glass, noted as a chiral glass. The chiral glass is discussed in terms of the divergences for relaxations in the characteristic time as well the inverse of an optical pitch length, which are analyzed in the long-time-lapsed orientation textures via image-time correlations. The outcome highlights interesting aspects of charge effect in the ‘twist’ elasticity energy for optimizing the long-ranged repulsive electrostatic interactions among charged DNA rods.

1. Introduction

The slow dynamics of collective phenomena is interesting itself for various thermodynamic systems. It has been already known that 3d Ising spin glass is generally driven by a strong and critical slowing down [1], shown by large-scale MC simulations of 3D short-ranged Heisenberg spin glass. The chiral susceptibility decreases with lowering temperature, but it increases again to reach an equilibrium [2]. The interest is a dynamic changes of local orientation orders approaching transition, at a higher temperature, which often causes the small energy dissipated in the mean-field approach of replica (entities) with the symmetry breaking, so called the replica symmetry breaking (RSB). In practice, reaching a zero temperature is not feasible, and interpreted by the ‘freezing’ of diffusion, seen in the system of anisotropic metallic alloy of spin-glass pseudobrookite (Fe2TiO5), magnetization states [3], and the plastic crystal phase of hybrid perovskites [4]. Different classes of glass states also exist in thermodynamic systems, as dipolar, quadrupolar glasses [5], and orientational glass of mixed cyanide for orientationally disordered crystals [6–8].

Since the glass transition in bulk phases is rather disordered in collective dynamics, mode-coupling approaches can be used to show dynamical transitions in slowly varying non-ergodicity, including structural correlations. Phase boundaries of a glass state are then often demonstrated by MD simulations between orientation glasses and structural glasses [9]. However, the situation becomes obscure when the decoupling of spin and chirality occurs in the case of many long-ranged interactions for an infinitely large system size. Thus, hierarchical defect structures are often expected to form in the microscopic evolution theory of plastic deformation [10], as seen in the spin glasses with dynamic susceptibility for decay in correlations. Furthermore, the nature of replica symmetry breaking (RBS) is based on the probability distribution of the order parameter, relating to the spin-glass phase coupled to the thermodynamic limit [11]. A valid concern here is how the
‘overlapping’ regime of an extended volume can be defined in the concentration for existing deformability of the systems. Thus RSB is a generic feature of crowded systems of particles in thermodynamic equilibrium, where the internal stress relaxation during equilibration exhibits a sudden jump or discontinuity. In the current system of charged DNA rods, such RSB is observed through mechanical ‘kinks’ of during the formation of orientational textures at intermediate rod-concentrations as quantified by means of image-time correlation [12].

The term ‘chiral glass’ appears, first suggested by Kawamura (1992), in an equilibrium thermodynamic phase with spontaneously broken symmetries, similar to the Ising spin glasses in high-temperature superconductors with the exponent powers [13, 14]. Compared to the physics of a ‘well-defined’ spin-glass state [15], the chirality is a disordered (and ill-defined) state [16]. The characteristics of chiral glass have been discussed further such that chiral symmetry breaking leading to the chiral-glass transition, in the wavevector space with the finite-size correlation length [17, 18] (see figure 21 in [17]). The chiral glass appears with a marginal difference in the long-range power-law interaction, with the exponent power slightly larger than the value of 1, in which the spin chirality eventually decouples. Possible examples of replica symmetry breaking (RSB) are in systems when the effective temperature cools down, such as the glass-forming liquids in growing cavity point-to-set correlations [19–21], as well as the mixed state of ferro- and anti-ferromagnets, and the phase transitions of the spin-glass state [22]. So far, there has been little experimental evidence for demonstrating chiral glasses of biologically relevant charged rods in 3D bulk solutions.

Previously, I have reported the self-similarity and replica symmetry breaking (RSB) that occurred in the equilibrium phase diagram for crowded suspensions of charged chiral DNA virus (fd) suspensions as a function of ionic strength [23]. Several distinguishable chiral-mesophases were observed above the isotropicnematic coexistence concentration (in equilibrium) and lower than the critical ionic strength of 12 mM. In contrast, at a higher ionic strength, the planar nematics are stable with a marginal isotropic-nematic coexistence concentration. The delicate interplay between the short-ranged helical corecore interactions and the long-ranged electrostatic interactions leads to variations of an optical pitch (from a chiral-nematic, N*, to a helical domain, H0) and hierarchical chirality (in X-pattern and helical domain, H0). In terms of RSB near the structural glass, both helical domains and X-patterns are stable at high concentrations, exploited by the long-time kinetic arrests (LTKAs) in the phase diagram. However, at higher ionic strengths, the glass state faces towards frozen in with the nematic (see LTKA23). Phase boundaries of different pathways are also shown with twist interactions of charged DNA viruses (fd rods) at low ionic strengths. This work is motivated by both experiments and semi-empirical theory concerning the phase behavior and glassy states of charged DNA rods. As compared to the work previously published [23], the main focus is on the existence of different morphologies of a chiral glass and their very slow rotational motion. Observations are quantified by means of image-time correlation in real space, which reveals the divergence of a characteristic length and time.

2. Materials and methods

2.1. Sample preparation and polarized images

The suspensions of charged DNA-viruses (fd) are dialyzed by using a commercial cassette (Slide-A-Lyzer Dialysis Cassette, 10,000 MWCO 0.5–3 ml capacity) from Thermo Scientific (USA), such that the ionic strength of each Tris/HCl buffer is balanced with the osmotic reservoir, followed the same protocol in [24]. The activity of H+ ions will be the same in the reservoir and fd suspension, so that also the pH of the fd suspensions will be the same, despite the fact that relatively many H+ ions are dissociated from the surface of DNA rods. The suspensions and osmotic reservoirs with the surrounding air and to account for the amounts of dissolved hydrated carbon dioxide is provided in the appendix D in [24]). The molecular structure of fd coat proteins consist of an open N-terminal forming 5-fold symmetry [25], as compared to the closed C-terminal in opposite. The electrostatic Debye length of the DNA fd-rod is varied by an ionic strength of the reservoir, as

\[ \kappa^{-1} [nm] = 0.304/\sqrt{c_0 [M]} \] (at 25°C), where \( c_0 [M] \) is the ionic strength. A commercially available round glass Hellma sample cell is used with a circular diameter of 20 mM and a thickness of 1 mM. The total sample volume is about 380 \( \mu \)l. The cuvette is placed between crossed polarizers to visualize the birefringent domain texture through a telescopic lens, and the resulting images are recorded using a CCD camera (AxioCam Color A12–312). The sample cell for the image-time correlation spectroscopy is arranged as follows: light source, polarizer, sample stage, analyzer, beam shutter, and the CCD camera connected to the PC interface (see figure 1 in [12]). The time evolution of depolarized optical morphologies for different fd concentrations is captured with varying ionic strengths.

For the time-resolved images, a larger field view of the textures was taken with an 18 mm diameter via a telescopic lens. For the image-time data analysis, various fields of view and time bins are available. Due to the slow dynamics of the system, each data point in the phase diagram requires 170 up to 300–500 hours, during which time a CCD camera takes an image every 20–30 min. The marginal variation occurs in approaching
towards the critical ionic strength, where the instability of nematic becomes dominant, compared to the stable chiral-nematic $N^*$- phase and the X-pattern at low ionic strengths. A determination of the location of phase boundaries as given in the phase diagram is taken already such long-lasting experiments. The quantification for the chiral-nematic (and helical) domain boundaries are now further analyzed in terms of Fourier space image-time correlations, where time-dependent averages over sectors corresponding to three characteristic length scales are performed: domains oriented either parallel or perpendicular to the direction of the chiral pitch, and the length of the chiral-nematic pitch. Fourier transformed images for equilibrated orientational morphologies for the chiral-nematic ($N^*$) domains are shown in figure 2(a). Fourier transformation of real-space images is used to distinguish between the occurrence and dynamics of structures on different length scales. Large structures that exist will give rise to peaks in the Fourier image near the center of that image, while structures corresponding to a smaller length scale appear as lobes away from the center. A Fourier-transformed image of a domain texture of chiral-nematic domains typically consists of peaks near the center of the Fourier image originating from the domains and lobes away from the center correspond to the chiral pitch. The image Fourier transform represents the averaged random orientational distributions of chiral-nematic $N^*$-domains, which may resemble the spectral intensity distributions appeared as the RSB in the spin-glass system [26]. Here, clearly lower rod DNA rod concentration (in the left of figure 2(a), smaller FT-ring is shown for the chiral-nematic domain, compared to the higher concentration for helical domains ($H_{hel}$) with the chiral pitch appeared towards larger FT-space lobes in the right of figure 2(a). The central intensity peaks are due to the chiral-nematic ($N^*$) domain size and the outer 4 intensity lobes are due to the chiral pitch length. The results strongly support that the visualization of distribution of averaged orientations for chiral-nematic ($N^*$) domains in figure 2(b), as well the local symmetry breaking at the concentrations (of $N^*$-X pattern transition line). The reason for such breaking symmetry is originated by the existence of collective self-assembly of slight axial asymmetry in the molecular structures of charged DNA rods (in figure 2(b). The collective arrangement of DNA rods are shown in the optical morphology under cross polarization for the chiral-nematic $N^*$ phase by rotating the samples. Also, the actual Fourier transforms of orientation distribution in figure 2(b), reveal the partial symmetry breaking in the reflection (thick green line) and the blue and red lines indicate the global orientations as the primary (blue lines) and secondary axes (red lines). The normalized angular distributions are shown in figure 2(c), where the primary peaks show a rather monotonic increase with an increase of the rotational angle, while the secondary peaks are not only broadly distributed, but also the half peaks can be seen for the symmetry breaking in the reflection of the primary half.

2.2. Image-time correlations

To analyze the direct orientation texture dynamics in equilibrium, various DNA rod concentrations were measured at different ionic strengths by image-time (or video) correlation functions, $C_r(t)$. A time series of images was recorded using a CCD camera, where images are typically taken for a larger time variable, every 1030 minutes, over a time span of about 415 weeks. The orientation textures of a long waiting time (40 hours to 3 months) for equilibrium states at a given ionic strength and various concentration are captured under the polarized light. The sample is loaded in the planar round cuvette with a 1 mm thickness and imaged with a long-collimated telescopic lens to ensure a larger field of view using a high-resolution CCD camera. Each single image in a time trace is used to construct an image correlation function from the region of interest, which is typically $300 \times 300$ (see figure 2 in [12]). Special optics for imaging such broader ROI views (18mm) are achieved using a telescopic lens under the white light with two crossed polarizer sheets. Details of the principles for image-time correlation spectroscopy can be found in [27]. From the recorded time-lapsed movie data, image-time correlations are used for the smaller ROIs (of 7 mm) to obtain here the decay times of bulk orientation textures. The correlation functions at different waiting times are then shown for various 7d concentrations at the lowest ionic strength (0.032 mM salt), also corresponding to the supplementary data, Movies 2–4, in figure 6 available online at stacks.iop.org/JPCO/5/065001/mmedia. Here, the focus is on quantifying a new type of glass, known as chiral glass, that is appeared in the current system and distinguished from the structural glass of an initial caging. The evidence of a chiral glass is discussed in terms of the divergence of relaxation times and the optical pitch variances for the slowly rotating fields.

3. Results

3.1. Salt-dependent equilibrium phase behaviors of DNA-virus rod suspensions

A bacteriophage fd is a rod DNA strand consisting of 50 amino acids fd coat proteins (see table 1 in [28]). The single molecular structure of filamentous bacteriophage fd is a long (L = 880 nm) and thin (bare diameter of $D = 6.6$ nm) rod DNA strand with a left-handed chiral core [29, 30]. The persistence length of an fd virus particle is about 2200–2800nm and the surface is surrounded with 2700 fd coat proteins that are the twisted
helical structure along the filament axis [31], with total charges of an fd virus as 8800 and 7500 elementary charges [32]. The orientations and collective alignments of charged DNA virus fd rods are shown in the equilibrium phase diagram (figure 1), with their effective interactions in the plane of DNA rod concentration and the ionic strength plane. The structural glass is observed to be confined within the helical domains with phase boundaries at a higher fd concentration for a particularly low ionic strength of 0.16–0.8 mM. By reducing the DNA virus fd concentration, both the chiral-nematic N\(^*\) phase and X-pattern are extended to the broader ranges of DNA rod concentrations. The most pronounced chiral phases are observed at the lowest ionic strength (0.032mM) with corresponding polarized optical morphologies of the N\(^*\) phase and helical domains and their existing slow dynamics. The phase behaviors close to the critical ionic strength of 1–2 mM clearly deviate from the stable chiral-nematic N\(^*\) phase (below 0.8 mM salt), while the planar nematic state is present above the isotopicnematic coexistence phase and above the critical ionic strength. In addition, dynamic pathways of RSB are depicted as PATHS I, II, and III in the phase diagram demonstrating the main features with different orientations of chiral-mesophases (in figure 2).

The application of accessible mobile ions is well demonstrated in the low-frequency electric field-induced dynamical states for the concentration of an isotopicnematic coexistence in [33–35]. The governing equations of motions for the effective twist interaction of charged rods can be expressed by the Smoluchowski equation for the probability density function (PDF) of density and orientations in the overdamped limit. The forces and torques due to rodrod interactions are then incorporated through the total potential energy. The orientation dependence of the particle is described by both \(D_t\) and \(D_r\) for the orientationally averaged translational diffusion coefficient and the rotational diffusion coefficient of interacting rods, respectively. Furthermore, the gradient...
The operator of the rotating field is in relation to the Cartesian coordinates as the cavity loop, $C_p$. This is due to randomized orientations of charged fd rods, with long-ranged electrostatic interactions in the bulk elastic medium. By increasing the concentration, RSB occurs at an intermediate concentration of charged DNA fd rods in the X-pattern, while the replica of helical domains is formed at higher concentrations at half the size of $N^*$ domains close to the concentration of structural glass.
3.2. Rotating field cavity loops and the pitch variances at the X-pattern

As it seen in figure 2(b), the averaged orientations for collective DNA rod arrangements in the chiral-nematic N* phase is presented well in the FTs for depicting the projection of bulk polarization states. The main feature of the global orientations of the N* phase is as follows: The secondary Fourier orientation peaks are shown (in region 3) at the angle of $\pi/2$, among equal distributed peaks, with 8-fold symmetry, $\pi/4$ in the FT image observed in the orientations. The half region 4 is most pronounced for the broken axial symmetries in the primary axes (see 1–5 and 2–6), the reflection symmetries of regions 4–7, and regions 3–8. The axial symmetries exist in the primary axes (regions 1–5 and 2–6), while the reflection symmetries are broken with the FT image of regions 4–7 and 3–8. More interestingly, the orthogonal secondary axes (3–7 and 4–8) show broken symmetries for both the axial and reflection directions for regions 3–8 and 4–7 as well nearby regions 3–4 and 7–8. This suggests that the symmetry breaking of a chiral-nematic N* phase is originated by either the collective or cooperative effect of the twist elasticity or the intrinsic molecular axial symmetry of individual charged DNA rods (5-fold, not 6-fold).

To further discuss the replica and RSB presented in the equilibrium phases, simple illustrations of self-similarity with cavity loops are provided in the phase diagram of figure 3 as a means of illustrating the bulk depolarized optical morphologies of the chiral mesophases. The replica method is shown, with an increase of the DNA rod concentration, as the chiral-nematic N* phase and a hierarchical chiral mesophase of helical domains above the nematic-isotropic coexistence concentration. The local helicity axes are indicated as $h_n$, in the top left of figure 3(a), and the cavity loop is $C_h$ in the depolarized axes of analyzer A and polarizer P. It is worth noting the X-pattern, between the chiral-nematic N* phase and the helical domains, that occurs by keeping the local orientations in such a way preventing the collective neighboring of charged DNA rods in the middle. The X-pattern is located with a wide range of low ionic strengths in the intermediate DNA rod concentration, below the critical ionic strength (1–2 mM) in the phase diagram. Even more noticeable is the fact that RSB occurs at the hierarchical chiral mesophase of the X-pattern, bounded within the lower and upper phase boundaries, with two interesting characteristics: (i) the mechanical kink observed at the lower phase boundary of the X-pattern, from the N* phase, and (ii) the undulating upper phase boundary of the X-pattern, toward the helical domains and the structural glass, at higher DNA rod concentrations. This upper phase boundary appears to be different with the lower boundary line broadly parabolic at low ionic strengths. The reason for such differences indicates the RSB is driven by the orientational order (at lower phase boundaries) and the particle density (at upper phase boundaries). Although the size of a cavity loop is similar to the size of N*-phase domains, the local helicity axes (in the planar alignment) are excluded from the cavity loop due to being decoupled in the X-pattern (see left panel of figure 3(a)), and bounded for the formation of cavity loops via opposite directions in the rotating field, as shown in figures 3(a) and (b). As the DNA rod concentration increases, the helical domains are formed as the half size of N*, close to the concentration of structural glass. Due to an increase of the DNA rod concentration, or the long waiting time, the most possible local orientations for two neighboring charged DNA rods is the X-pattern, where the cavity loop is bounded within the planar nematics. This can be understood as the replica symmetry breaking (RSB), which occurs in the X-pattern, as being indicative of a chiral glass consisting of cavity loops.

More characterizations of collective orientations of dynamic self-organization for interacting charged DNA viruses are shown for the real-space and Fourier transforms As shown before, the self-similarity of the bulk depolarized optical morphology of the N* phase and helical domains again appears at a decreased length scale with an increase of the DNA rod concentration in the equilibrium orientation textures at a given low ionic strength (0.032 mM salt in PATH I). Such self-similarity occurring in the polarized ‘fingerprint’ textures of the chiral-nematic N* phase also appears in the helical domains at shorter length scales. Figure 4 presents the different helical domains with their distributions of orientations by varying the rotational angles. The cavity loops of helical domains $H_d$ also appear in the real space. The collective alignments of chiral charged DNA rods occur in both bulk orientations and the projection of orientations as the sum of two independent orthogonal polarizations. The various optical morphologies of helical domains feature different sizes of cavity loops appearing in the helicity axes, shown as the FT and real space in figure 4. This indicates that the bulk twist elastic deformations also occur due to the local orientations for the higher concentration of tightly packed helical domains $H_d$. The asymmetric and nonlinear distributions of orientations in the helical domains can be directly observed in the Fourier space (see FT images 1 and 6 in figure 4), indicating that the local symmetry is also related. Irrespective of the similarity of the twisted nematic and cholesteric fingerprint textures, these helical domains include combined topological defects and the continuous change of local helicity within dark disclinations as well the cavity loops that are shown in the current system of charged rods for the X-pattern (see Movie 1 in figure 3) and in the $H_d$ in figure 4(b), which, however, is not in the cholesteric liquid crystal materials for thin double layers. What has been varied in our phase diagram is the concentration of the rods and the ionic strength. The ionic strength changes not only the effective aspect ratio, but also changes the softness of the interaction potential between the rods, which in turn affects the twist–contribution to the interaction potential. The latter induces a transition between nematic and chiral nematic. In addition, the number of ions that
dissociate/associate from/to the rods is affected by both the rod concentration and ionic strength. Experiments on uncharged rods with a chiral structure of their core would be desirable to disentangle the effects of concentration and ionic strength. A further concern of the relation between the aspect ratio and the helical twist power of rods for chiral-mesopahses will be discussed separately.

The local helical domains are shown with various depolarized morphologies of different defect lines of bulk twisted disclination lines. In addition, for a fixed low ionic strength of 0.032 mM salt, and an increase of the DNA rod concentration, a very slow RSB process occurs to form a cavity loop, which leads to an X-pattern between the chiral-nematic N* phase and the helical domains $H_d$ (in PATH I). Optical pitch variance is depicted in figure 5:

Figure 3. Replica and RSB presented in the equilibrium phase diagram of charged chiral DNA rods: (a) Simple illustrations of self-similarity with cavity loops in bulk depolarized optical morphology of N* phase and helical domains in the phase diagram of DNA rod concentration and ionic strength. In the top left, local helicity axes are indicated as $h_i$, and the cavity loop is $C_j$ in the depolarized axes of analyzer $A$, and polarizer $P$. (b) Representative orientation textures of chiral mesopahses show a chiral-nematic, X-pattern, and chiral glass, with an increase in the concentration of DNA rods for a low ionic strength (0.032 mM). The red arrows indicate the local helicity and cavity loops formed in different microscopic length scales in the equilibrium orientation textures. See the supplementary data Movie 1 for the chiral glass.
At a fixed low ionic strength, not only does the optical pitch decrease, but also the twisting of the neighboring domains occurs with increasing DNA rod concentration. Figure 5(a) shows the entire field of view for measuring the optical 2π pitch and one of the sites is illustrated in the raw intensity profiles of figure 5(b). Different regions of many fingerprint orientation textures are selected for the field of view (see the yellow bars for optical pitches in the pixels). The optical 2π pitch variances are provided in figure 5(c), with the different chiral mesophases indicated above the IN coexistence as a chiral-nematic N*, X-pattern, and helical domain, respectively, with increasing DNA rod concentration. The red line appears to be the ‘true’ pitch, taken as the largest value in variances for a given DNA concentration. The average pitch is indicated in figure 5(c) as the blue line, which intriguingly modulates in the N* phase and the X-pattern, but again coincides with the true pitch of the helical domains at a higher concentration. A possible explanation for this is not only the finite characteristic length scales of the helical twist power, but also the orientation textures occurring in the finite system with an internal stress release allowed in many interacting ‘twisted’ boundaries as a function of DNA rod concentration. The characteristic length of pitch variance is found to be a function of rod concentration.
where \( L_{\text{max}} \approx 515 \mu m \) is the largest length for a given concentration, and \( \tilde{c} \) is the characteristic concentration. The \( C_{N^*} \approx 1.7 \text{ mg ml}^{-1} \) is the upper nematic binodal concentration. The averaged pitch length is also distinguished by the concentration as

\[
\langle L_0 \rangle \sim L_0 \exp \left\{ \frac{\tilde{c}^*}{c - C_{N^*}} \right\} \quad \text{for } C_{N^*} < c < C_0,
\]

\[
\sim L_0 \exp \left\{ \frac{-\tilde{c}^*}{c - C_{N^*}} \right\} \quad \text{for } c > C_0.
\]

Figure 5. Optical pitch variances: (a) The real view of pitch samplings and (b) an example of the pitch measurements (after 24 hours) at a low ionic strength of 0.032 mM salt. (c) The optical \( 2\pi \) pitch variance as a function of DNA rod concentration. Time-lapsed images are collected for different fd concentrations for a low ionic strength (0.032 mM salt). The red line represents the true pitch of the largest value; the blue for the average value of the measured pitch. A divergence of pitch is shown in the \( N'N \) phase transition at lower rod concentration as well the saturation of pitch (60 \( \mu m \)) at higher DNA rod concentrations.

\[
L_p \sim L_{\text{max}} \times \exp \left\{ \frac{\tilde{c}}{c - C_{N^*}} \right\},
\]

where \( L_{\text{max}} \approx 214 \mu m \) is the limiting value of the pitch length at the concentration of \( C_{N^*} \), and decreases to 60 \( \mu m \) at the highest concentration. The spread of length decreases with an increase fo the DNA rod concentration. Note that there is a visible modulation of the averaged pitch length (\( L_0 \)) below the critical concentration of \( C_0 \), while the above is shown with the coincident decay with the true pitch. As it can be seen in figure 5(c), a critical DNA rod concentration is occurred at \( C_{N^*} < C_0 < C_{\text{RSB}} \), with the reduced pitch distributions from 110 – 200 \( \mu m \) at the critical concentration of \( C_0 \approx 4 – 7 \text{ mg ml}^{-1} \). The characteristic decays of averages pitch variances are different forms in the concentration, expressed qualitatively as the \( \tilde{c}^* \).
Here, the $\tilde{c}$ measures the characteristic decay of the pitch variances in the concentration. The main features of the pitch variances are found to be as follows: (i) There is a divergence of the pitch at a lower concentration above the upper binodal concentration of the IN coexistence phase, $C_{L-N}$, appearing on the chiral-nematic N* phase, $C_{N*}$. The broader distribution of pitch is also seen at such a concentration and at 100 to 500 $\mu$m, is most pronounced at the lowest ionic strength of 0.032 mM salt. Similar pitch divergence behaviors are observed at another low ionic strength (0.16 mM salt), but with a slightly shorter pitch. In addition, the pitch boundary is frozen at a higher ionic strength (0.8 mM salt) at the LTKA23, facing the stable nematic state, close to the critical ionic strength. (ii) A modulation of the average optical pitch is seen with a maximum value of 280 $\mu$m and a minimum value of 110 $\mu$m for the chiral-nematic N* phase and X-pattern, respectively. This suggests that larger accesses of rotational freedoms exist among charged DNA rods in the lower concentration of the N* phase and the X-pattern, below the higher packing of the helical domains, $H_f$ for such a low ionic strength. (iii) A saturated value of the optical pitch is obtained at 60 $\mu$m for the helical domains with increasing DNA rod concentration, as indicated by the red arrow bar in figure 5(c). This might be due to the increase of interactions for crowded DNA rods as well as the enhanced twist elasticity with a critical rod density for a given Debye screening length. Whether or not this twist elasticity can be described by the equation of motions relating to the cavity loop, particularly below the critical ionic strength, is open to discussion.

### 3.3. Divergence of the exponent power in Relaxations via Image-time Correlation

As mentioned previously, the distinction between the chiral glass and the spin glass is characterized by the decoupling scenario of chirality and spins, similar to weakly anisotropic Heisenberg-like spin glasses [18, 36] in 3D space. Of particular interest for a chiral glass is the divergence of correlation length that occurs slightly earlier than that of spin glasses at the critical temperature, just below the freezing of a glass state. For the mixed chirality state, the correlation length exponent of the disordered 3D Heisenberg model is found to be close to the value of 1$^*$ [37]. There, the chirality appears to be a continuous transition with a circulating (electric) current that generates the magnetic moment, which evidently is a different feature compared to the structural glass for the initial freezing in time.

To quantify the relevance of RSB, the characteristic time and length scales of the change of orientation textures of the chiral-nematic N* phase are measured by means of image-time correlations at the lowest ionic strength (0.032 mM salt). Time-lapsed images (and movie data) are provided in figure 6 for different DNA rod concentrations (2.6 mg ml$^{-1}$, 4.7 mg ml$^{-1}$, and 10.5 mg ml$^{-1}$) and waiting times. Image-time correlation is used as an experimental method for different waiting times as well as for obtaining the optical pitch variance in figure 5, with corresponding depolarized optical morphologies in figure 6. Intriguingly, a notable difference between the true and averaged pitch length for both the chiral-nematic N* phase and the X-pattern are seen up to the helical domains at higher concentrations. In addition, the monotonic decrease of the pitch length at helical concentrations for longer waiting times. The quantification of the pitch of reaching the equilibrium time is performed by image-time correlations. The characteristic time and length scales of the change of orientation textures of the chiral-nematic N* phase and the X-pattern are seen up to the helical domains at higher concentrations. In addition, the monotonic decrease of the pitch length at helical domains is found to coincide with the true pitch. The undulation of the averaged pitch indicates that a finite length scale for given charged rod concentrations is affected by the existing orientation degree of freedoms.

The image-time correlation function is defined as

$$C_V(t) = \frac{\langle (I(t) - \langle I(t) \rangle)(I(0) - \langle I(0) \rangle) \rangle}{\langle (I(0) - \langle I(0) \rangle)^2 \rangle},$$

(3)

where the brackets $\langle \cdots \rangle$ denote averaging over all CCD-camera pixels. Each single image in a time trace is used to construct an image correlation function from the region of interest (300 $\times$ 300 pixels). Imaging of a broader ROI view (18 mm) is performed using a special telescopic lens under the white light with two crossed polarizer sheets. Details of the principles of image-time correlation spectroscopy can be found in [27].

From the recorded time-lapsed movie data, image-time correlations are used to obtain the decay times of bulk orientation textures. The correlation functions for different waiting times are shown for 6 fd concentrations at the lowest ionic strength of 0.032 mM salt. The characteristic length of optical pitches decreases at higher concentrations for longer waiting times. The quantification of reaching the equilibrium time is performed by obtaining the time when there is no further change to the decay rates of correlation functions (as indicated by arrows in figure 6(b)). At the lowest ionic strength (0.032 mM salt), image-time correlations are obtained at different fd concentrations for the orientation textures of the N* phase, where equilibration also occurs earlier (about 30 hours in total) than at the ionic strength of 0.16 mM (86 hours). Slightly increased pitch lengths are observed at a lower ionic strength of 0.032 mM as well as the broadening of the N* phase (see PATH I in the phase diagram of figures 1 and 3). This is a result of the increased ion condensation, which leads to a longer ranged electrostatic repulsion that effectively screens the chiral core-core interactions for the I-N coexistence concentration, revealed by a weak AC electric field [38].

Both the decay time and the decay rate are obtained from the fits of image-time correlations. The fitting function is chosen as the single-stretched exponential function,
where $B$, $A$, $\beta$, and $\tau$ are the fitting parameters for the background, amplitude, stretching exponent, and decay time, respectively. The time constant $\tau$ is a measure of the decay time where the orientation texture changes and the inverse of the decay time is the decay rate ($\Gamma = 1/\tau$). Equilibration occurs with a typical waiting time of tens of hours (shown in figure 7(a)) and the decay time, $\tau$, increases with longer waiting time, $t_w$. The inverse of decay time is the decay rate, $\Gamma$, which measures the change of orientations. To obtain further valuable information on

$$C_{\beta\Gamma}(t) = B + A \exp \{ -(t/\tau)^\beta \},$$  

Figure 6. Results of image-time correlations: (a) Time-lapsed images and movie data (Movies 13) of a chiral-nematic N* phase being formed at the fd concentrations (top: 2.6 mg ml$^{-1}$) and the hierarchical chiral mesophases (middle: 4.7 mg ml$^{-1}$, bottom: 10.5 mg ml$^{-1}$) at the lowest ionic strength (0.032 mM salt). (b) Image-time correlation functions, $C_{\beta\Gamma}(t)$, performed for the chosen field of images for different fd-concentrations (2.6 mg ml$^{-1}$, 4.7 mg ml$^{-1}$, 5.4 mg ml$^{-1}$, 7.6 mg ml$^{-1}$, 10.5 mg ml$^{-1}$, and 14.0 mg ml$^{-1}$) with different waiting times. The supplementary movies are provided as Movies 2, 3 and 4. The arrow indicates the increase in waiting time.
the slow relaxations, the reduced time is introduced as $t_R = (t - t_L)/t_L$, where the long waiting time required to reach the metastable state is obtained as $t_L \sim 40 - 50$ hours. An exponential decay rate is observed as a function of $t_R$, which is measured for different DNA rod concentrations, as shown in figure 7(b).

The characteristic decay rate and relaxation time are plotted as a function of DNA concentration in figure 8. A simple illustration of the concentration-dependent chiral mesophases is shown in figure 8 as a chiral nematic $N^*$ phase, X-pattern, and helical domains, $H_d$, with increasing DNA rod concentration, as well as the cavity loops $C_c$ and the helicity $h_i$ depicting the chiral glass that appeared in the X-pattern concentration. The decay rate is fitted as a function of the reduced time $t_R$ by

$$
\Gamma = \Gamma_c \exp \left( \frac{t_R}{\tau_c} \right) + \Gamma_0,
$$

where $\Gamma_c$ and $\tau_c$ represent the concentration-dependent characteristic decay rate and relaxations, and $\Gamma_0$ depicts the intrinsic decay rate. If we consider the dimensionless time for the relaxation with a reduced time for a given concentration, then we observe the logarithmic slowing down in the decays as

$$
\ln \left[ \frac{\Gamma - \Gamma_c \exp \left( \frac{t_R}{\tau_c} \right)}{\Gamma_c} \right] \sim C_{RSB} = -\frac{t_R}{\tau_c}.
$$

Note here that the decay rates are concentration-dependent, and the RSB occurs when the concentration is reached as $c \rightarrow C_{RSB}$. Thus, if we fit the above independent observations in terms of $C_{RSB}$, the characteristic decays of the X-pattern decrease as a function of concentration,

$$
\ln \left( \Gamma_c \right) \sim -0.725(c - C_{RSB}),
\tau_c \sim \exp \left\{ -1.082(c - C_{RSB}) \right\} + 0.111,
\Gamma_0 \sim \exp \left\{ +1.20(c - C_{RSB}) \right\} + 0.00048,
$$

where the above observations are a function of concentration. $\Gamma_c$ is the amplitude of decay rate that contributed very small energy dispersion (several tens of $\mu$Hz) when entering the X-pattern (at $C_{N^*}$), which is noted by the slope where $\gamma = 0.725$ and which slows down approaching the finite length correlations of helical domains, $H_d$. The actual fitting of the above observations is performed in the concentration regime of $C_{N^*} < c < C_{RSB}$, bounded in the phase boundaries, before the helical domain $H_d$ phase. Although a slight increase of the rate occurs at a concentration of 8 mg ml$^{-1}$, a significant decrease of the decay rate is seen upon increasing the concentration to the upper-phase boundary in figure 8(b). A similar tendency is observed with the relaxations in figure 8(c), with a sudden decrease in the exponential slope upon an increase of the rod concentration. The fit is obtained with an amplitude of 25.6 and a background of 0.111. A steady residual decay rate of $\Gamma_0$ is found with an amplitude of 0.462 and a very low background of 0.0005 when varying the concentration.

When the DNA rod concentration reaches $C_{N^*} < C_0 \sim 4$ mg ml$^{-1} < C_{RSB}$ for the given low ionic strength (0.032 mM salt) at the lower-phase boundary of the X-pattern, the as $\tau_c$ is observed with a slope of $\gamma = 1.087$. Interestingly, for a residual decay rate of $\Gamma_0$, a concentration gap is obtained between the $N^*$ phase and the X-pattern, where the growth of the exponent slope is $\gamma = 1.20$, as shown in figure 8(d). Thus, the above exponent

Figure 7. The results of image-time correlations for orientation textures as a function of waiting time for different fd concentrations, measuring (a) decay time $\tau$ and (b) decay rate $\Gamma$ as a function of the reduced time of $t_R = (t - t_L)/t_L$, where the $t_L$ represents the long amount of time required to reach the metastable state (taken as 42 hours).

The actual fitting of the above observations is performed in the concentration regime of $C_{N^*} < c < C_{RSB}$, bounded in the phase boundaries, before the helical domain $H_d$ phase. Although a slight increase of the rate occurs at a concentration of 8 mg ml$^{-1}$, a significant decrease of the decay rate is seen upon increasing the concentration to the upper-phase boundary in figure 8(b). A similar tendency is observed with the relaxations in figure 8(c), with a sudden decrease in the exponential slope upon an increase of the rod concentration. The fit is obtained with an amplitude of 25.6 and a background of 0.111. A steady residual decay rate of $\Gamma_0$ is found with an amplitude of 0.462 and a very low background of 0.0005 when varying the concentration.

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power \( \gamma \) carries qualitatively depending on the inverse of the charged DNA rod concentration for the limiting value of the decay rate above a threshold DNA rod concentration when entering the X-pattern. This agrees with the possibility of the dynamical scaling of chiral glass in terms of the overlap for the chirality, as the decay rate \( \Gamma \) decreases. Here, \( \Gamma \) also corresponds to a small energy dispersion (several tens of \( \mu \) Hz), which is \( 10^{-5} \) lower than the colloids and \( 10^{-8} \) smaller than the liquid crystalline phase. At higher concentrations, above a DNA rod \( \text{fd} \) concentration of \( C_{\text{RSB}} \sim 7.4 \times 10^{-8} \text{mg ml}^{-1} \), the exponent power saturates at a value of \( \gamma \sim 1.082 \) with the reduced time of \( t_R \). In contrast, no slope is found at lower concentrations, indicating that the probabilistic realization of orientation textures is rather ergodic (as a replica), which is seen in the optical pitch variations for both the \( N^* \) phase and the X-pattern below the \( H_d \). At this low ionic strength, the decay rate \( \Gamma \) is also far too slow at higher DNA rod concentrations. This is not the case at higher ionic strengths (above 1-2 mM salt). Thus, the system demonstrates the feasible realization of a chiral glass for charged DNA-virus rod suspensions by effectively approaching the phase transition to the glass state for lower ionic strengths.

Typically, the orientational glass occurs before the structural glass appears, as seen in the binary mixture of the anisotropic system. Similarly, in this system, the chiral glass occurs in the vicinity of a structural glass, appears with a finite size of cavity loops, and is bounded within the stable planar nematic state. Furthermore, the maximized rotating fields of the orientations for DNA rods can be coupled to the twist elasticity at higher concentrations, exhibiting the tightly packed density of the replica of helical domains \( H_d \) (as illustrated in figure 3). According to the replica method, the stress can be negligible, provided that the structure decay is small compared to a loading shear modulus in the \( N^* \) phase. However, when the RSB occurs, the local mechanical stress releases exist as the ‘kinks’, which are briefly observed at the lower phase boundary of the X-pattern, from the \( N^* \) to X-pattern transition in PATH III. Furthermore, at the upper boundary of the X-pattern, the undulation of the boundary lines is shown with the occasional appearance of small voids (i.e. air bubbles), which indicates that the frustrated time (or the effective temperature) may exist for such slow processes as a

Figure 8. Replica symmetry breaking (RSB) of chiral glass: (a) Simple illustration of concentration-dependent chiral mesophases, a chiral nematic \( N^* \) phase, X-pattern, and helical domain, \( H_d \), with increasing DNA rod concentration. Cavity loops \( C_j \) and helicity \( h_i \) are indicated with the chiral glass that appears in the X-pattern concentration. (b) Characteristic decay rate and (c) relaxation time are shown with (d) the background decay rate as a function of DNA rod concentration. Here, the X-pattern is denoted by the lower-phase (pink line) and upper-phase (green line) boundaries (see the slope \( \gamma \)), which is seen slowing down approaching the finite length correlations of helical domains, \( H_d \). The characteristic concentration for phase boundaries of X-pattern are indicated as arrows with \( C_{\text{NSB}} \) and \( C_D \) for lower- and upper-phase, respectively.
logarithmic in time). Therefore, the decay rate becomes a non-zero finite value in the infinitely long-time metastable thermodynamic glass state. This might also explain why the optical pitch variances are modulating for the average values for the N* phase and the X-pattern (figure 5(c)) below the $H_d$ where the overlap volumes are accessible in rotating fields to form the cavity loops.

The slowing down process (at a logarithmic in time) of charged DNA rods at low ionic strengths is caused by the thick electric double layers that form a larger Debye screening. Furthermore, the stable chiral mesophases are well maintained with the long-range electrostatic hydrodynamics and local ‘twist’ elasticity of charged DNA rods. RSB also occurs and is demonstrated by the change of configurations from the N* phase to the X-pattern, where the repelling forces are stronger for the given rod concentration (or the effective density). However, the replica occurs at a higher packing density (or rod concentration) in $H_d$ and the reduced length scale of the pitch and domains is again shown without any indication of cavity loops, which includes the frustrated time (or effective temperature) for the very slow process. Therefore, the decay rate of $H_d$ becomes a non-zero finite value in the infinitely long-time metastable thermodynamic state.

4. Summary and discussion

The RSBs are shown here by the dynamic pathways of PATHS I, II, and III in the equilibrated phase diagram for interacting charged DNA virus fd rods crossing various chiral phase boundaries at varying low ionic strengths. This is also driven by the salt-dependent microscopic dynamics of collective thermal fluctuations that are coupled to orientations. The correlation length is shown with the concentration-dependent optical pitch of the chiral-nematic N* phases, the X-pattern, and helical domains, and is measured by image-time correlations [12, 39]. Variations of orientation distributions are also presented by real-space and Fourier transforms Chiral pitch variances depend on the effective increase of released condensed ions, where the range of electrostatic repulsion is large compared to the screening of chiral interactions.

The main results are summarized as follows: (i) In terms of RSB just below the structural glass, both helical domains and the X-pattern are stable at high concentrations for low ionic strengths. The divergence of relaxation time and the exponent power of correlation length are supported by the phenomenological observation of relaxations for orientations via the image-time correlation. Extremely long-time kinetic arrests (LTKAs) are observed for both structural glass (for 300 hours) and the chiral glass (30010,000 hours) with the decay rates being quantified for long-time arrested orientation textures. (ii) The X-pattern, which relates to the dynamic pathway of PATH II in the equilibrium phase diagram, reveals a cavity loop that is directly observed as a chiral glass bounded within the planar nematic phase. The rotating fields of charged DNA fd rods are enhanced by the cooperative twist effect at low ionic strengths. (iii) The optically stable pitch variances and the divergence of a pitch and the exponent power are determined by varying the DNA fd rod concentration at the lowest ionic strength, which is obtained by image-time correlation. The release of condensed ions for charged DNA viruses (fd) was sufficiently large for the degree of accessible mobile ions, resulting in thicker electric double layers for such lower ionic strengths. (iv) Moreover, the occurrence of chiral-glass originates from an intrinsic orientational disorder—to order transition in chirality of lyotropic systems, contrary to the ‘structural’ glass where the morphology slowly ages over a few hundred hours. It turned out that the chiral glass releases internal stress at a critical concentration (around 4–8 mg ml$^{-1}$), as shown in figures 5(c) and 8(d), which is followed by ‘freezing’ in a helical state at longer waiting times for higher concentrations of DNA rods.

Near the structural glass, both helical domains and the X-pattern are stable at high concentrations via effective interactions. The relaxation times for the orientations are observed by the slowly varying rotating fields of charged DNA rods, which are further enhanced by their ‘twist’ effect at higher DNA rod concentrations and confirmed by the divergence in relaxation and pitch length. Here, one of the long-time kinetic arrests, LTKA2, is the metastable mixed state of RSB, an X-pattern, which represents the chiral glass. The main feature of a chiral glass appears to be the finite size of the cavity loops, bounded with the planar nematic state.

Several distinguishable equilibrium orientation textures exist in the equilibration time of 30010,000 hours before any indication of frustrations (or freezing) occurring. For a larger time window, below the structural glass, the high concentration merges with an X-pattern, revealing the decoupling of local helicity and the cavity loop. It can therefore be concluded that the X-pattern (at low ionic strengths) is the most probable state of a long-living metastable type of chiral glass. The splitting of cavity loops (C) and helicity axes ($h_i$) are then obtained, depending on the concentrations (or packing density) of charge chiral rods, and the configuration of a chiral glass is seen in the X-pattern for the replica symmetry breaking.

In the canonical ensemble, the microscopic states are closed as the thermodynamic properties of semiclassical gasses, driven by the number of particles for the given same internal energy of all microscopic states, as well for the Debye model of solids for the phonon collective modes at low temperatures. Near the critical point, density fluctuation becomes correlated over a large distances that can be mapped in a scaling law.
However, when the system is open as having varied number of particles and energy, the probability density is varied with the thermodynamic variables as the temperature and chemical potential. In the latter case, there is a discontinuity of the release in heat capacity at the critical temperature. Such experimental realizations are shown in the superfluid. The continuous phase transitions is well established by the Ginzburg-Landau theory relating to the order parameter. Typical behaviors of free energy for the continuous phase transitions from the isotropic to the nematic phase are illustrated for an increase in concentration at the top of figure 9. First-order transitions occur irrespective of the breaking symmetry above the isotropic-nematic coexistence concentration, where the chiral-nematic N* phase occurs.

If the effective volume fractions of a given low ionic strength is taken as \( f_{\text{eff}} \), the expectation value for the order parameter \( S \) is suggested in figure 10. In order to describe the dynamics of melting and the forming of nematic domains, an equation of motion should be derived for the orientational order parameter tensor \( S \). This tensor is defined as the ensemble average of the dyadic product of the unit vector \( \hat{u} \) that specifies the orientation of a rod, \( S(t) \equiv \langle \hat{u} \cdot \hat{u} \rangle(t) \).

As analogy of the superfluid, the gauge symmetry appears with a phase transformation, in such a way that all phases of wave functions are varied in the phase lag at the critical point. The phase factor of a slowly varying functional in concentration (likewise the pressure and temperature in the thermodynamic system) remains negligible. Spontaneous broken symmetry occurs at the X-pattern, indicating the existence of a gapless phase mode as a non-zero decay rate for the symmetry-restoring soft Goldstone mode (illustrated in figure 9 at the lower panels for non-zero decay rate). A conclusive review of the critical phenomena of quenched disorder is

**Figure 9.** A simple illustration of the free energy versus order parameter as a function of the concentration-dependent phases: isotropic, isotropic-nematic coexistence (above), and the chiral mesophases (below). The chiral nematic N* phase is shown with a shift of the minimal order parameter, the X-pattern with an increase in free energy, and the helical domain with an increase of the DNA rod concentration. There might be an increase of Gibbs free energy for the helical domains, with the occurrence of voids and specific heats at higher concentrations. Here, the concentration is indicated as \( C_{I-N}, C_{N^*}, C_{\text{RSB}}, \) and \( C_D \) for isotropic-nematic coexistence, chiral-nematic N*, RSB (in X-pattern), and the helical domains, respectively.
discussed in [42]. Here, in the current system, a finite discontinuity can be clearly seen across the critical concentration in the bottom right of figure 9 (likewise for ferroelectric or antiferroelectric materials). A simple illustration of the free energy versus an order parameter (see figure 9) as a function of the concentration-dependent phases: isotropic, isotropic-nematic coexistence (above), and chiral mesophases (below). The chiral-nematic N° phase is shown with a shift of the minimal order parameter, the X-pattern with an increase of the free energy, and the helical domains with an increase of the DNA rod concentration for a given low ionic strength.

Figure 10. A schematic of the order parameter for hierarchical chiral mesophases, in which PATH I is applicable in the equilibrium phase diagram in figures 1 and 3. Above: free energy versus an order parameter in the X-pattern, where RSB occurs below and above the C_{RSB} concentration. Below: illustrations of concentration-driven orientation texture as the chiral-nematic domains, cavity loop, and helical domains (from left to right) for an increased concentration of charged DNA rods, corresponding phases in the concentrations of C_{1-N} ∼ 1.5 mg ml^{-1}, C_{N°} ∼ 1.8 mg ml^{-1}, C_{RSB} ∼ 7.4 − 8 mg ml^{-1}, C_{D} ∼ 10.5 mg ml^{-1} for the lowest ionic strength (0.032 mM).

The RSB is then caused by different interactions meeting at C_{RSB} < C_{CRSB}: (I) At C_{N°} < C < C_{RSB}, the pronounced optical pitch lengths < L_0 are modulating in the concentration, while the order parameter S is maximized below the occurrence of RSB (see dashed green line in figure 10) and the chiral-nematic N° phase remains stable. (II) However, above C_{RSB}, the pitch length decreases monotonically while there is also a sharp decrease of the order parameter, leading to the formation of cavity loops in the X-pattern. Here, the cavity loop is created by the substantial amount of dissociated ions existing around DNA rods that drive to repel each other with collective rotational dynamics by increasing density (and concentration) in the long-range electrohydrodynamics. When density is increased, the reduced amplitude fluctuations are also less for a given
low ionic strength. Thus, the shorter rod rod interaction range becomes stronger, preventing the formation of cavity loops, and approaching the helical domains. Such concentration-driven orientation textures are illustrated in the lower panel of figure 10 for the chiral-nematic domains, cavity loops, and helical domains (from left to right), with an increase of the charged DNA rod concentration. Whether or not the above observations are related to any similarity with the superfluid hydrodynamics for propagating density (and sound) waves in the dispersion relation requires further investigation.

As a theoretical interest, the semi-empirical expression for the cavity loop can be expressed by the ‘twist’ interaction potential for a finite domain size with the rotating fields. The system has already shown the bifurcation diagram of the order parameter for isotropic-nematic binodal concentrations [35]. An equation of motion for the spinodal decomposition of a nematic state can be derived from the Smoluchowski equation through a Ginzburg-Landau expansion up to the fourth order in the orientational order parameter. Then, it depicts a preference for a non-parallel, twisted orientation of two rods, in the averaged manner, where the energetically unfavorable overlap of diffuse double layers in parallel orientation [43]. However, above the chiral-nematic N′ concentration, the hierarchical chiral mesophases have not yet been considered in equilibrium thermodynamics. This is the first realization of an enhanced ‘twist’ effect in chiral glass while maintaining the long-time stable cavity loops. RSB turns out to be originated by the finite size effect, appeared before the occurrence of helical domains as the replica, for increasing concentration at a low ionic strength. Further analyses of the concentration dependent kinetic in averaged orientation distributions of chiral-nematic domains are underway with the image-time correlations in Fourier transforms separately. By approaching the critical ionic strength (PATHS II or III in the phase diagram), the relative amount of dissociation ions of DNA rods decreases, while the rotational motions are hindered by the translations. Their microscopic dynamics therefore become faster again compared to the orientation dynamics, with the freezing of orientation textures demonstrated by the initial caging, as structural glass, at a high concentration (LITKA23 in phase diagram).

Intriguingly, the upper-phase boundary of the X-pattern undulates with occasional voids that indicate the release of stress, similar to the heat capacity for the temperature response, observed in the chiral glass for interacting charged DNA rods. Also, the results suggest that the semi-empirical expression for the ‘twist’ interaction potential may explain the cavity loops (very slow rotation of the local morphology), shown in X-pattern, between the chiral-nematic phase (N′) and helical domains. The occurrence of a very slow rotating field is many order of magnitude weaker (10^-5 – 10^-8) relaxations than the case of colloids and liquid crystals.

As a follow up work, determination of the weak twist elasticity can be described by the equation of motions for the density and order parameter, relating to the cavity loop, by varying the ionic strength up to the critical value. Then the kinetics of averaged orientations for chiral-mesophases are to be quantified via image-time correlations in Fourier space, for the order parameter and helical twit power (HTP). Whether the cavity loops can be relevant in the dynamical states by field-induced polarization or not would be another interest [35]. As a foundation for this challenge, it is useful to acknowledge the existence a chiral glass for charged DNA rods, in which the physics of this subject can be further explored to discuss charge effects in effective temperature of the thermodynamics.

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