Characterization of orientation correlation kinetics: chiral-mesophase domains in suspensions charged DNA-rods

Kyongok Kang
Forschungszentrum Jülich, Institute of Biological Information Processing IBI-4, Biomacromolecular Systems and Processes, Jülich, Germany
E-mail: k.kang@fz-juelich.de

Keywords: orientation correlation kinetics, chiral-mesophase domains, image-time correlations of fourier transformed images, averaged twist angles and domain sizes, suspensions of charged dna-rods, decouplings of orientation distributions

Supplementary material for this article is available online

Abstract
Bacteriophage DNA fd-rods are long and stiff rod-like particles which are known to exhibit a rich equilibrium phase behavior. Due to their helical molecular structure, they form the stable chiral nematic (N*) mesophases. Very little is known about the kinetics of forming various phases with orientations. The present study addresses the kinetics of chiral-mesophases and N*-phase, by using a novel image-time correlation technique. Instead of correlating time-lapsed real-space microscopy images, the corresponding Fourier images are shown for time-correlated averaged orientations. This allows to unambiguously distinguish to detect the temporal evolution of orientations on different length scales, such as domain sizes (depending on their relative orientations), and the chiral pitch within the domains. Kinetic features are qualitatively interpreted in terms of replica symmetry breaking of elastic deformations in the orthogonal directional axes of chiral-mesophase domains, as well by the average twist angle and the order parameter. This work can be interesting for characterizing other types of charged rods, mimicking super-cooled liquids and orientation glasses.

1. Introduction

Among many biological systems exhibiting enzymatic cleavages, the DNA replication is well played by an initiative role with gene protein functions in the change of shapes and forms. The relations between structural and functional filamentous bacteriophages (so called Ff coliphages) are extensively reviewed by Rasched and Oberer in 1986 [1], where the genetic and physical maps of DNA bacteriophage fd are depicted in great detail with 10 featuring gene proteins. In particular, the regulation of bacteriophage DNA fd is initiated by a gene 2 protein (g2p), a male-specific infection androphage for enzymatic activity and the duplex DNA replication with polymerase (holoenzyme) that converts the single-stranded super-coiled DNA to unwind fd-replicated forms [2]. This is mainly done by extracellular complex gene-5 protein (g5p) dimers forming helicase conformation [3], a key function allowing a weak interaction for the specific reversible binding affinity of the enzyme to its cleavage site. Nevertheless, there are few unclear functions and roles of gene proteins (for instance, gXP and the g4p-g1p morphogenesis towards the intergenetic region (IR) form). This ambiguity can be then interesting for a thermodynamic system with degrees of freedom in bulk motions of polar solvents. However, the gap of understanding single bacteriophage DNA strands towards collective behaviors of forming stable phases is still far challenging task to be exploited.

Up to now, most fibrous structures that may exist in the domains, consisting aligned fibers or irregular anisotropic particles, for para-crystals or amorphous morphologies [4], are probed by diffraction and scattering methods (introduced by A. Guinier). Protein mixtures are examples of natural systems that mimic rich phase behavior similar to colloids [5], as well fluctuations of orientational glass (found for a small protein levoglucose), which could be distinguished from the structural glass [6]: Typically, when the small domains are formed by

© 2022 The Author(s). Published by IOP Publishing Ltd
fluctuations, the spectral density of the structures starts to broaden with non-exponential relaxations in length scales. Thus, the elasticity of inner structures within the domains and enhanced anomalies near the phase transition are interesting phenomena for the kinetics of orientational motions in terms of order parameter and microscopic stresses [7].

In the case of charged rod glass, previously reported for suspensions of DNA fd-rods [8, 9], the invariant dynamics (for oriented textures and particles) is referred to the Wigner glass, and the time evolution can be also expressed in the polar presentation of orientations via Fourier space [10]. Dynamical arrest then leads to long-lived non-equilibrium states of matter such as glasses and gels [11], where slow time variables evolve in non-ergodic relaxations at different length scales either frozen-in or the intermediate states between the fast and slow processes.

Thus, the system of exhibiting hierarchical classes demands the kinetics of orientation orders with random degrees of freedoms in a measurable (continuous) time scale: As examples, the grain size of microdomains for cylindrical block copolymers are analyzed by Fourier filtering for the local order parameter with a relevant correlation length in the nematic state [12]. Also, the orientational orders of microdomains (2D striped patterns) consisting of PS-PI diblock polymers are discussed by the kinetic scaling law for the smectic layers of topological constraints, from the annihilation of disclinations, annealing temperatures and time. It gives the exponent power of an annealing time to be lower (0.25) than the case of 2D nematic system (0.5), due to the dynamic growth of domains [13]. The orientational dynamics have even shown that domains adapted to the planar devices with self-assembly of nanocolloidal rods, where the kinetics of long-ranged order in porous structures for thin metallic films (of anodic aluminum) of different growth rates (for a time exponent of 0.2) in plane orientations [14, 15].

Another indication of the metastable ordering in orientational glass is the ‘cluster reversion’, found in cyanoadamantane with the characteristic kinetics for the freezing of random strain field of a orientational cluster (see temperature-time diagram in figure 14 of [16]). The intrinsic twist viscosity of a nematic director has been captured in the reorientation kinetics against frictional forces among the neighboring domains as a small noise, in aramid solution via deuterium nuclear magnetic resonance (NMR) measurements [17].

Despite the various underlying mechanisms of glass-forming systems in the metallic alloys, silicates, and proteins, theoretical description is rather simplified by a network theory [18]. For instance, the protein HIV protease is simulated by a ‘pebble game’ or a bead-spring model [19, 20], where the dynamic mode of proteins is simplified by a rigid region of a mechanical network, kept away from the soft one. The question arises then how many particles (in number density) are engaged for active sites as well the shape consisting in the concentration or volume fractions. To approach more realistic theoretical frameworks, experiments on mesoscopic scale of biological systems are also useful. Besides, the most orientation order glasses are studied so far in the thermotropic system or multi-component alloys near the transition point. The purpose of a present paper is to use this novel image-time correlation technique to investigate the concentration-dependent (lyotropic) system in the glass, where the orientations of chiral-mesophase domains are important, in the suspensions of charged DNA-rods at low ionic strengths in equilibrium, for the characterization of such orientation kinetics.

We use here a novel experimental method of an image-time correlation (ITC) in the Fourier transforms (FTs) for the data analysis. The equilibrium phase behaviors of charged DNA-rods are explored systematically by varying the concentration at a given low ionic strength (0.052 mM Tris/HCl buffer); the chiral-nematic N*-phase and the hierarchical chiral mesophases, X-pattern and helical domain (HD) phase are observed (notified as N-phase), above the isotropic-nematic (I-N) coexistence concentrations [21]. A special interest is the intermediate concentration of DNA rods, as the X-pattern is formed a new type of glass (forming the cavity loops for a chiral glass), with a replica symmetry breaking (RSB), distinguished from the structural glass in real-space averaged domains in time and concentrations [22]. More generally, the formation of chiral phases in various types of systems is not yet fully understood, and is currently active field of research [23–25], as well with the self-assembly and the phase behavior of DNA duplexes has been investigated [26]. In this paper, the quantification of collective orientations for charged DNA rods are further characterized in details of time-dependent averaged orientations of chiral-nematic N*-domains, performed by image-time correlations (ITCs) in Fourier-space. The results of characterization are then directly evaluated for the replica symmetry breaking of N*-domains by the ensemble averaging of orientations. The details of data analyses are described throughout the paper with following leading questions: (I) To what extent the twist angles of collective orientations in chiral-nematic N*-domains occur by varying the concentration and waiting time? (II) What are the orientation kinetics and order parameters approaching the equilibrium time? (III) Are there any differences in the results of ITCs in real space and FTs, for determining the critical concentration (of RSB) and hierarchical chiral-mesophases? (IV) What are the characteristics of N*-domains and the ‘effective’ parameters in the lyotropic orientational system? The novel image-time correlation technique based on time-lapsed Fourier transformed microscopy images introduced in this paper has the advance over previous work based on real-space images, that a clear distinction
can be made between the growth kinetics on several length scales, thus distinguishing, for example, the growth of domain size and the temporal development of the chiral pitch.

2. Concentration-dependent orientations of twisted chiral-nematic N°-domains

The optical morphologies of collective orientations for aligned charged DNA rods are collected from the birefringence under crossed polarizers, resulting in the degree of optical anisotropy. The order parameter is then estimated (or calculated) by the average orientations in long waiting times (typically as 135–240 h). In practice, acquiring the whole data of ‘randomly’ slow dynamics leading to the equilibrium is quite demanding in both real- and the corresponding spectral intensity in Fourier-space. For the characterization, polarized optical morphologies are obtained, for varying the concentration of DNA-rods, shown in figure 1, at a longer waiting time of 135 hours and more. In an increase of the concentration, the isotropic-nematic coexistent N-phase, a chiral-nematic N°, and an X-pattern, and the helical domain (HD) phase are formed. The corresponding FTs of polarized optical morphologies are then obtained (by a program) from converting systematically fast Fourier transform (FFT) images in time, which is shown in figure 2, at a given low buffer solution (0.032 mM Tris/HCl).

By varying the concentration of DNA rods, the center zone of the FT represents orientational distributions of N°-domains that are distinguished by the parallel and perpendicular component, $q_{D,\parallel}$ and $q_{D,\perp}$, slightly tilted in the horizontal and vertical axes in FTs, respectively (see figures 2 and 3). As it can be seen in overall FTs, the slight tilt of two orthogonal axes in the FT peaks is originated by existing local orientations of N°-domains, and collectively averaged for the concentration-dependent orientations (see the supplementary movie data in figure 2 available online at stacks.iop.org/JPCO/6/015001/mmedia): Below the N-N° transition concentration (1.8 mg/ml), only the center peak is shown in FT, without chiral-nematic N°-domains. However, at higher concentrations, more profound changes of orientations occur in FT spectral distributions in time: figure 3(a) shows the example of intensity distribution profiles in FTs: for the concentration of DNA rods (10.5 mg/ml) as the X-HD transition at a waiting time of $t_w \sim 30$ h, while the comparison of FTs for two high concentrations (10.5 mg/ml and 14 mg/ml) is shown in figure 3(b) at a longer waiting time ($t_w \sim 100$ h). Here, three distinguishable FT spacings are obtained in length scales corresponding to the spectral distances of chiral-nematic N°-domains; two orthogonal axes in the center zone of FTs are decomposed as the parallel $q_{D,\parallel}$ and perpendicular $q_{D,\perp}$ components, as well as the optical pitch inside the N°-domains as $q_P$. Moreover, visible differences of intensity distributions in FT lobes appearing between the $q_{D,\parallel}$ and $q_{D,\perp}$ for all the concentrations, above the N-N° transition (figures 2 and 4), towards the equilibrium.

The most drastic changes in FTs are captured in the middle concentrations of the X-pattern (see figure 4(c) at 5.4 mg/ml in times), occurred as a critical concentration at low ionic strengths [22]. Also, the local orientational intensity distribution profiles show that both axes contribute to unique behaviors between these two orthogonal directions of N°-domains, $q_{D,\parallel}$ and $q_{D,\perp}$, by varying the concentration of DNA rods (see the supplementary data movies, Movies F-I in figure 2). Temporal changes of the orientation distributions of the N°-domains in the FTs of figure 4, show that the N°-domains with optical pitch vary slowly over time (135–240 h). By further increasing
the concentration of DNA rods in the X-pattern approaching the X-HD transition (in the comparison of 10.5 mg/ml (Movie K) and 14 mg/ml (Movie L)), half-sized reduced domains appear, resulting in an FT spacing that becomes twice as large (figure 4). Further different orientation distributions of the critical concentrations (near the X-pattern) are provided for longer waiting times, $t_W = 200–220$ h, between the N$^*$-X transition (at 4.7 mg/ml) and the X-pattern (5.4 mg/ml) (see figure 7).

3. Kinetics of orientation distribution of N$^*$-domain, image-time correlation in FT

The particular interest is focused to demonstrate the long-time equilibrated orientation distributions via image-time correlation (ITC) in FTs, performed in-house automated program that is rigorously employed for the conversion of real images in morphology to FTs, followed by the calculations of ITCs for spectral intensity distributions. Image-time correlation (ITC) spectroscopy is then used to extract characteristics in the FT images: Compared to the ITC in real space [22], the ITC in FT turns out to be a rather direct way of visualizing the average orientational motions of N$^*$-domains from the temporal changes of spectral intensity distributions. As previously found at a low ionic strength, the equilibrium phases below the critical value (at 1.2mM Tris/HCl buffer) [21] carry out the following features: (i) N$^*$-phase is stabilized by oriented chiral-nematic N$^*$-domains that appear orthogonally together with the N$^*$ optical pitch (stripes in the N$^*$ domains) inside domains. The corresponding FT of the N$^*$ phase (2.6 mg/ml) is shown in figure 4(a) for the averages distributions in orientations of overall N$^*$-domains, in the center FTs, compared to a more pronounced stable N$^*$ phase at a higher concentration (3.8 mg/ml) in figure 4(b). (ii) However, above the N$^*$ phase (5.4 mg/ml), in an increase of the concentration, a unique phase of X-pattern occurs such that the N$^*$-X pattern transition has notably different intensity distribution; the reflection symmetry is broken in the axes of N$^*$-domain perpendicular, $q_{D, \perp}$, shown independently as the diverging intensity profiles in FTs (see figure 4(c)). On the contrary, the parallel
component $q_{D\parallel}$ appears to be localized as the spherical distribution. (iii) The average size of the spectral domains, $\langle q_D \rangle$, increases with an increase of the concentration of DNA rods due to the smaller sizes of domains seen in real space. Also, the X-pattern (in figure 4(d)) at a higher concentration, has shown twice larger spacing in FTs, compared to a chiral-neatnic N*-phase (in figure 4(a)) with visible differences between $q_{D\parallel}$ and $q_{D\perp}$. (iv) Furthermore, a clear decomposition of Fourier component is carried out in the orientations, corresponding to the N*-domains as parallel and perpendicular in the center zone of intensity lobes. Such features are illustrated in figure 3(b) (see the Movie K and Movie L in figure 2), for a comparison of two high concentrations of the X-pattern (10.5 mg/ml) and X-HD transition (14 mg/ml), respectively. Rather broad bright intensity rings (at low frequency) are formed at the central core of the FT. Notable differences between the two orthogonal axes are shown in the center zone; one is the emerging in the direction of $q_D$, and the other diverges in the axis of $q_D$ of the N*-domains, while the outermost regime is for the relevant optical pitch $q_P$. The FT space of domains and optical pitch are 260 $\mu$m$^{-1}$, and 380 $\mu$m$^{-1}$, respectively.

Figure 3. An example of the intensity distribution profiles in FTs: (a) for the intensity profiles in enhanced FT images, and the black/white image with intensity profiles, for the concentration of DNA rods (10.5 mg/ml) as the X-HD transition at a waiting time of $t_{w} \sim 30$ h, (b) The comparison of FTs is shown for two high concentrations (10.5 mg/ml and 14 mg/ml) at a longer waiting time ($t_{w} \sim 100$ h). Rather broad bright intensity rings (at low frequency) are formed at the central core of the FT. Notable differences between the two orthogonal axes are shown in the center zone; one is the emerging in the direction of $q_D$, and the other diverges in the axis of $q_D$ of the N*-domains, while the outermost regime is for the relevant optical pitch $q_P$. The FT space of domains and optical pitch are 260 $\mu$m$^{-1}$, and 380 $\mu$m$^{-1}$, respectively.

The quantification of morphological changes in time-lapsed images are done as follows: the two-dimensional image matrix is converted to the numbers of all 2D array intensity values for each pixel [27]. The image-time correlation function is then defined by the instantaneous transmitted intensity, $I(t)$ detected by a given pixel of the CCD camera. For the time traces recorded for all these pixels, the image-time correlation function CV ($t$) is defined as, $C_V(t) = \frac{\langle I_i(t_i) - \langle I_i \rangle \rangle (I_j(t_j) - \langle I_j \rangle)}{\langle I_i \rangle \langle I_j \rangle}$, where $V$ indicates the ‘video’, or time-lapsed images, and the brackets $\langle \cdots \rangle$ denote the averaging of whole field of views in the CCD camera pixels at 2D ($i, j$) matrix indices. Each individual image at a time trace is used to construct an image correlation function, depending on the application, such that the region of interest in the square (e.g, 512 $\times$ 512) pixels, performed for various other systems [27–29]. Here, the application of ITCs in FTs is particularly aimed to obtain the collective orientation degrees of freedom for charged DNA rods observed in slow times and the effective concentration-dependent order parameter in bulk.
The consistent measurements of orientations over time, $C_0(q_D, \theta_{nm}, \tau)$, are depicted by the ITC function in FTs, shown in Figures 5 and 6, where the orientations of N*-domains are averaged within the given FT regions for the Fourier spectral intensity distributions. Depending on the measurement time, or the delay (lag) time, $\tau$, the

Figure 4. Temporal changes of FTs for orientational distribution N*-domains: (a) in the lower N* phase (2.6 mg/ml), (b) N* phase (3.8 mg/ml), and (c) N*-X pattern (5.4 mg/ml), and (d) X-pattern (10.5 mg/ml) for the ionic strength of 0.032 mM Tris/HCl buffer. The corresponding scale bars of FT-space are notified at the most right panels.
Figure 5. Image-time correlation function in the FT, $C(q, \Theta, \tau)$, for the quantification of random orientations of $N^*$-domains, for different waiting times of $t_W$: 0 h, 62 h, 80 h, and 100 h, by varying concentrations of DNA-rods: 1.8 mg/ml, 2.6 mg/ml, 3.5 mg/ml, 3.8 mg/ml, 5.3 mg/ml, 5.4 mg/ml, 10.5 mg/ml, and 14 mg/ml. Here, the delay time $\tau = t - t_W$ is 120 h, 60 h, 40 h, and 20 h. The characteristic parameters of ITCs, extracted from the result of fitting parameters corresponding to the $N^*$-domain size, $q_D$, twist angle, $\Theta_{tw}$, and delay time, $\tau$. 
kinetic fractions of orientational distribution of N\(^\ast\)-domains are extracted by the fits. The fitting function of the ITC in FTs is chosen here as a single mode decay function, as \( C_\theta \sim A e^{-\Gamma t} + B \), in terms of the three characteristic parameters, defined as the amplitude \( A \sim S \), the background \( B \sim q_D \), and the decay rate \( \Gamma \sim \theta_{tw} \) interpreted as the order parameter, N\(^\ast\)-domain size, and twist angle, respectively. Here, \( q_D \), \( \theta_{tw} \), and \( \tau \) indicate the N\(^\ast\)-domain size \( (q_D) \), twist angle \( (\theta_{tw}) \), and the lag (or delay) time \( (\tau) \) of waiting, respectively. The results of physical observation for overall changes are then shown in figure 5, with the average coherence for orientations analyzed by an image-time correlation (ITC) function, \( C_\theta(q_D, \theta_{tw}, \tau) \), obtained for a long measuring time: The performance of ITC function in FTs is shown by varying the concentrations of DNA rods \( (1.8 \, \text{mg/ml}, 2.6 \, \text{mg/ml}, 3.5 \, \text{mg/ml}, 3.8 \, \text{mg/ml}, 5.3 \, \text{mg/ml}, 5.4 \, \text{mg/ml}, 10.5 \, \text{mg/ml}, \text{and} \, 14 \, \text{mg/ml}) \), and the delay time \( (\tau \sim 120 \, \text{h}, 60 \, \text{h}, 40 \, \text{h}, \text{and} \, 20 \, \text{h}) \). The image-time correlation functions of Fourier transformed images are related to the elastic motions of N\(^\ast\)-domains. ITCs in FTs perform a single exponential decay function, except for the local oscillatory behaviors at longer times and low concentrations \((\text{at large intensity fluctuations})\). Based on the elastic orientations of N\(^\ast\)-domains, there are no visible changes in orientations at

Figure 6. Image-time correlation function in the FT, \( C_\theta(q_D, \theta_{tw}, \tau) \), for the quantification of random orientations of N\(^\ast\)-domains, as a function of concentration of charged DNA rods at different waiting times of \( t_w = 0 \, \text{h}, 62 \, \text{h}, 80 \, \text{h}, \text{and} \, 100 \, \text{h} \), from the left right panels. The arrow indicates the increase of concentrations of charged DNA fd-rods.
longer times, above the equilibrium time \((t > t_{eq})\), however larger variations of correlations exist in the earlier time \((t < t_{eq})\). Thus, this implies that reaching an equilibrium time is indeed important to determine the thermodynamics for a lyotropic system.

4. Elastic kink of \(N^*\)-domains near the X-pattern (a chiral glass)

When the (network) glasses consisting rigidity of the molecules in variations of the soft phonon mode and discrete glassy percolation \([30]\), the optical contrast can be revealed by the anisotropy of crystallinity and molecular orientations. In particular, the optical birefringence and elastic moduli are exhibited by the rod-like molecules of the rigid core, embedded in an elastic medium \([31, 32]\). However, when the core of particle is less rigid than the outer structure, its thermal fluctuations vary by some extent and deviate from the scaling laws. In addition, a computational algorithm of generic rigidity is limited to predict the order parameter, only in 2D percolation, mapping to the heat capacity, for the free energy distributions of bond and site percolation in the glassy system. There are no yet reliable theories in 3D bulk properties \([31, 32]\). Thus, it is worthwhile to evaluate the RSB that experimentally observed in X-pattern, as a chiral glass resembling 3D orientation glass \([22]\). The direct evidence of physical observation for the replica symmetry breaking (RSB) is shown by the mechanical kinks randomly occurred in the fast time scale, in the middle concentrations \((4–6 \text{ mg/ml})\), shown in figure 8, as the consequence of an effective decoupling between \(q_D\) and \(q_{D'}\), for varying the concentrations and different waiting times. The more vivid realization of

![Figure 7](image-url)
RSB is found at a middle concentration of DNA rods, 5.4 mg/ml, which is also the concentration where the mechanical kink appeared randomly in the real space, for a short period of time, before reaching to an equilibrium [9, 21, 22]. This is originated by the density being compensated by the orientations of chiral-nematic N*-domains, at the N*-X transition concentration, and balanced by two orthogonal orientation axes of chiral-mesophase domains. However, further increase of the density (at a higher rod-concentration), the replicas of smaller helical domains (formed in the X-HD transition) occur, similar to the optical morphology of larger scale N*-domains for lower concentration in an equilibrium phase [22], in which now the density of charged DNA rods overcomes against their orientations. According to above observations, the driving mechanisms of RSB are suggested as follows: A sudden reverse of cluster (or domains) may occur in the development of orientation orders, similarly forming a microscopic lattice at the (thermotropic) glass-like transitions (figure 13 of [16]). The intensity fluctuations of orientations are then a precursor to such transitions steady until the actual development of cluster occurs in a finite space (e.g. a sudden jump or mechanical kink), followed by a weak time dependence for a long period of time (likewise the behavior of effective temperature-time diagram in figure 14 of [16]). In addition, the reason for replica symmetry breaking (RSB) occurring in the middle concentration (between above the N*-X-pattern and below the X-HD transition), is resulted by a diminishing of N*-domains in an increase of DNA rod concentration. Whether this can be relevant with an unusual isotope effect, observed in a high-temperature superconductor [33] would be an open concern; for instance, whether the X-pattern is a coexistent state between the partially molten state of N*-domains against disordered (in isotropic) and the ordered as out of the plane (homeotropic-nematic) state or not. More details of the equilibrium phase diagram formed in stable phases of charged DNA rods are shown different at the higher concentration (14 mg/ml) for higher ionic

\[ S, \langle q^2 \rangle \times 0.53 \text{ mm}^{-1} \]

\[ @ t_W \sim 0 \text{ h} \]

\[ @ t_W \sim 0.032 \text{ mM Tris/HCl buffer} \]

\[ @ t_W \sim 62 \text{ h} \]

\[ @ t_W \sim 80 \text{ h} \]

\[ @ t_W \sim 100 \text{ h} \]

**Figure 8.** The order parameter, \( S \), as a result of image-time correlations in FTs, as a function of waiting time, \( t_W \), the concentration of DNA rods, and the delay time \( \tau \) of 60 h, 40 h, and 20 h. Note that there are sharp differences in the middle concentrations of DNA rods (in 4.7–6 mg/ml), depending on the characteristic time for \( t < t_{eq}, t \sim t_{eq}, \) and \( t > t_{eq} \), respectively.
strengths, which is discussed compared to below and near the critical ionic strength (of 1.2 mM) for the long-time kinetic arrests [21, 22].

5. Characteristics of N⁺-domains: Twist angle, domain size and order parameter in concentrations

As shown before, the kinetics of orientation distribution in the suspensions of charged DNA rods are shown by the effective parameters as long waiting times and varied concentration in the lyotropic system. The characteristic crossover in orientations occurs at a concentration of DNA rods for 4–6 mg/ml (see figure 8), by the amplitude of order parameter, S and the averaged background value that obtained from the inverse of N⁺-domain size, 〈qD〉. Also, the findings are in good agreement with the result in a real space, where the kinetics of orientation distribution of N⁺-domains are taken at a typical equilibrium time of teq ~ 80 h [22]. Therefore, when the duration time is similar or larger than the equilibrium time (see t ∼ teq or t > t eq in figure 8), rather significant changes are seen in the critical concentration regimes. However, for the shorter duration time, at t < t eq, the critical concentrations are mostly hindered by the apparent changes, at high concentrations. The resulting characteristics of featured parameters for ITC in FTs are plotted in figure 9 as a function of concentration. The simple illustrations of systematic increase in the concentration of charged DNA rods are shown in figure 9(a), at the lowest ionic strength (of 0.032 mM) exhibiting the characteristic changes of orientation distribution in FTs, for the N-N⁺ transition, N⁺ phase, N⁺-X pattern, X-pattern, and X-HD transition phase. For the low concentration of N-N⁺ transition, the N⁺-domains show dominant peaks in an increase of the concentration, while at a high concentration of X–HD transition, above the RSB, chiral-mesophases are being half-sized smaller in real-size, carrying out twice larger outer intensity lobes of pitches (see the left illustrations of figure 9(a)).

The decays of ITC function in FTs, Cd(q_D, θ_mon, τ), are presented by the twist angles in time correlations, θ_mon, in figure 9(b). The inverse of chiral-mesophase domains, 〈q_D〉, and the order parameter, S, are presented in Fig. 9(c), and figures 9(d), (e), respectively obtained from the fittings. Due to the finite Fourier component analysis, the background value is seen for the stationary value corresponding to the N⁺-domains as 〈q_D〉. In addition, the average twist angle, 〈θ_mon〉, is converted from the decay constant of ITC in FTs, as the multiplication of a complete 2 π turn (see figure 9(b)) for the sum of possible spherical intensities. The values of concentration-dependent averaged order parameter, 〈S〉, in figures 9(d) and (e), a.e. estimated separately from the effective Debye screening length and dissociation constants for the release of condensed ions at a given ionic strength. Thus, in the results of figure 9, clearly visible gaps are obtained from the above characteristic parameters, where the middle-concentration is identified as the RSB (notified as the pink region in figure 9). A special note is that the monotonically decreased reduced order parameter is found before the occurrence of RSB concentration, while above in the concentration of 10 mg/ml, relatively large spread of order parameters are observed, depending on both concentration and lag-times in figures 9(d) and (e). This hints that still local orientations of smaller helical domains may possibly to progress microscopically in a hierarchical chiral-mesophases.

6. Discussion and conclusion

We have shown here the collective changes of orientations approaching to the equilibrium phase diagram of charged DNA rods at low ionic strengths by the average orientational distribution of N⁺-domains over a long time shown in FTs, for various stable chiral-mesophases and glasses. One of the most unique observations is the X-pattern in FTs as the diverging domains near the RSB. The overall orientations of charged DNA rods are responded as an increase of DNA-rod concentration, by following reasons: (i) There is an asymmetric transition of a non-monotonic N-N⁺ line, in the phase diagram above the upper binodal line (I-N transition) and below the X-pattern. This asymmetry becomes symmetric, in the N⁺–X transition phase boundary line with a variation of ionic strength for two chiral-mesophases (in the X-pattern and helical domains, HDs). This is now explained and clarified by the decoupling mechanism of two independent degrees of freedom in the orientational axes of q_D, and q_D in FTs. (ii) Below the critical ionic strength, pronounced chirality expands as the concentration increases, such that the most pronounced stable chiral-nematic N⁺ phase at the N⁺–X transition. This is relevant with the intrinsic microscopic relaxations in the lower binodal that as far slower than the reorientation of an aligned planar nematic phase at higher ionic strengths [21, 22], (iii) The increase of FT spectral spacing corresponds rigorously to a decrease of the N⁺-domain size as the replica of half-sized twisted helical domains at higher concentrations (at the HD-phase) near the X-pattern, above the N⁺–phase boundary. Also near the critical ionic strength (confirmed independently as 1.2 mM), found in [21, 22], the long-time kinetic arrests (LTKA1 and LTKA2) in the equilibrium phase behaviors at low ionic strengths, have been observed smaller center peaks, due to the fact that larger scales of domains are accompanied by scattered intensity fluctuations of chiral-mesophases.
On the contrary, above the critical ionic strength, the coexistence of I-N is located at a narrow gap for different long-time kinetic arrest (LTKA3) of N-N* phase, which shows bright peaks in the center zone in FTs for two emerging phases of N-N* and I-N transitions in the equilibrium. Therefore, the current system demonstrates the macroscopic behavior of orientation dynamics resembling the slow dynamics of orientation glasses found in metallic alloys (see figure 5 in [18]) of rigid rod-shape.

Figure 9. (a) Simple illustrations for the orientation distribution, equilibrated in the actual FT intensities with FT space of domains, increased with an increase of DNA rod-concentration, from 132 μm⁻¹, 176 μm⁻¹, 264 μm⁻¹, to 380 μm⁻¹. (b)–(e) The characteristic fitting parameters for (b) the orientations of twist angles, \( \theta_{tw} \), (c) the average size of N*-domains, \( \langle q_D \rangle \), and (d) the preferred order parameter, \( S \), for different delay times. Larger view of the order parameter in the rectangular area of (d) is shown in (e), with an estimated value \( \langle S \rangle \). The pink region indicates the RSB occurs (in the X-pattern), in which overall orientations are different below and above the concentration in equilibrium.

[21]. (iv) On the contrary, above the critical ionic strength, the coexistence of I-N is located at a narrow gap for different long-time kinetic arrest (LTKA3) of N-N* phase, which shows bright peaks in the center zone in FTs for two emerging phases of N-N* and I-N transitions in the equilibrium.
molecules. Furthermore, there are two effective parameters for a lyotropic system: The first parameter of collective motions for these charged DNA rods is the concentration-dependent orientation distribution revealing the intermediate concentration of the X-pattern between N'-X and X-HD phase boundaries at a low ionic strength, as a unique replica symmetry breaking (RSB) as a chiral glass [22]. This has been evidently highlighted here by the Fourier transformed images, with the effective decoupling of orientation axes in $q_{D1}$ and $q_{D2}$. This is fundamentally responsible for the RSB of chiral-mesophase domains, for varying both concentrations and waiting times, by means of the image-time correlation in FTs. As the result, the characterization of domain formation are determined by the twist angles; overall orientations of N'-domains that enable to predict order parameter. Moreover, the decoupling and divergence of orientations for N'-domains in $q_{D1}$, distinct from the $q_{D2}$, are all carried out in featured parameters for the orientation distribution. More intriguingly, the replica of smaller helical domains (HDs) forms again, by upon increasing the concentration (after the RSB concentration), and continuous changes in orientation evolving to a local symmetry breaking within the direction of $q_{D3}$ in the concentration (14 mg/ml) of X-HD transition (see the Movie L in figures 2 and 9(a)).

The second effective parameter in realization of the current system is the time to reach an equilibrium state, for depicting the unique concentration of RSB, in a similar way to define the ‘critical isotherm’ (see figure 30 in [7]), discussed in thermodynamics for both the order parameter and the microscopic stress of elastic deformation. The microscopic orders are essential for orientational anomalies in the vicinity of transitions, occasionally accompanied by the elastic waves (resembling cavity loops observed slowly over time as a chiral glass in [22]). This suggests then the X-pattern (at given low ionic strengths) is formed possibly by a rotational diffusion and affected by the annihilation of twisted chiral-nematic N'-domains, which are reoriented at long waiting times in equilibrium, effectively by pronounced perpendicular motions of thick electric double layers, for low ionic strengths. This may be understood as the sufficiently slower cooling in the case of supercooled liquids, where most charged particles and ions exhibit often no crystallization by long-ranged repulsive interactions in the equilibrium thermodynamic system. On the contrary, at a higher ionic strength, above the critical value (1.2 mM), relatively fast diffusion along the spatial extent of the DNA rod direction for the overall kinetics. Thus, the ionic-strength-dependent electric double layers for charged DNA rods are underlying mechanism for given waiting times, characteristic reduced time, promoting enhanced contrast in such rich phase behaviors, including the RSB. Therefore, in conclusion, the results of image-time correlation (ITC) of Fourier transformed images, evidently facilitate the investigation of orientation kinetics of N'-domains, driven by the ambient dissociation/association from the condensed ions, cooperated by mobile diffusive ions surrounding interacting charged DNA rods in the equilibrium. Finally, the work provides not only the useful application of ITCs in FTs, but also a possible driving mechanism of RSB in the orientation of charged DNA rods, for the lyotropic system. The fundamental physics for the long time it takes for crystallization to occur, underlies most probably the same principles as for other systems before phase separation occurs, like other types of charged systems, supercooled liquids, and glasses.

7. Experimental details

7.1. Sample preparation and optical measurements of birefringent charged DNA rods

Suspensions of charged DNA rods are prepared from the concentrated stock of DNA viruses (fd) at an ionic strength of 20 mM and dialyzed against the buffer solution of a lower ionic strength (0.032 mM Tris/HCl buffer) for 2 consecutive days using the Slide-A-Lyzer Dialysis Cassette Extra Strength, 10,000 MWCO, 0.5–3 ml capacity membrane cassette purchased from Thermo scientific Inc. (Lot No LL151432). The buffer is exchanged for a fresh new one after 24 hours for further purification of both the solvents and the sample. The concentrated suspension of DNA viruses (fd) is then prepared by a Donann equilibrium with a buffer solution. The concentrations of DNA viruses are measured by optical density (OD) at a wavelength of 269 nm to weigh the mass of $W_f$, for the very small amount (10 μl) using a UV/Vis spectrometer (Cary, 50 Bio, Win UV scan Application, Varian, Australia, Pty, Ltd). The fd concentration is then obtained by the relation

$$[fd] = \frac{OD}{W_f} \times 0.26042,$$

where the factor of 0.260 42 ~ 1/3.84 is considered for the extinction (absorption) coefficient of a single fd virus as 3.84. For lower ionic strengths, the same procedures are followed to measure the concentration of DNA suspensions by repeating the sampling process in 5 times. The dissociation constant and an effective diameter for the ionic strength of 0.032 mM Tris/HCl buffer are considered as 643 μm and ~292 nm, respectively.

The optical measurements are done by a commercially available Quartz transparent cylinder cuvette with a thickness of 1 mm and a diameter of 20 mm (120 QS 1 mm, Hellma Precision in Spectro-Optics) is used to contain an approximately 380 μl sample volume. The sample holder is placed between two crossed polarizer sheets to capture polarized images of the birefringent orientation texture. In addition, the large field of view is
captured by a long-distance telescopic (InfiniProbe, Infinity, Boulder, CO, USA) lens, placed in front of the CCD camera (AxioCam Color A12–312). The entire measurement is performed by an automatic save setting for the slow-motion sequences of images for a time interval of every 30 minutes recorded for 10–30 (90) days by the image software (Axivert, Carl Zeiss).

7.2. Orientation distributions and image-time correlation (ITC) in Fourier transformed (FT) images
The conversion of FFT transforms of the real images is performed by writing a short script to read the movie data (avi) files as stacks. The FFT is then applied to each image and saved as a new image stack using the image software available online (via online ImageJ/Fiji version ImageJ 2.1.0; Java 1.8.0. − 172). Thus, the rigorous conversions of all different concentrations for long measurement times are performed in the same way for the previous steps before calculating the actual image-time correlation (ITC) in FTs. The in-house-developed image-time correlator (program) is used to calculate the coherence of morphological changes in the images (BMP files). The original ITC program was invented by Prof. J.K. G. Dhont for the characterization of field-induced dynamical states of the current system of charged DNA rods [27]. Here, in this work, the images of FTs are additionally obtained from the corresponding images, captured in the real space for the long-time traces (120–240 hours) to extract the averaged overall orientational distributions in the intensity fluctuations. The in-house-developed ITC program is quite robust and effective. For instance, a single image–time correlation takes only a few seconds for the whole sequence of time-lapsed images (or the video data, typically for 512x512 pixels at a total of 500 time frames) via the user-friendly window setting (developed by Dr. H. Kriegs, IBI-4, FZJ). The quantification of orientation changes in the time-lapsed FT images here is performed by 2D array intensity values prepared from the reconstruction of image data to the (i, j) index intensity values for black/white 2D ASCII formatted data to calculate the image-time correlation function in the pixel–pixel intensity auto-correlation, which was first introduced in [27]. The reconstruction of image data is taken from the collected time-lapsed images in such a way that each image is subtracted from the overall averaged intensity value at a given time. Then, the image–time correlation function in the pixel–pixel intensity auto-correlation, in the black/white 2D ASCII formats, is kept for reading the instantaneous transmitted intensity taken by a given pixel of the ROI in the CCD camera. The image-time correlation function \( C_{ij}(t) \) is calculated for the time traces recorded for all these pixels, with the averaging of CCD camera pixels used to subtract from the individual pixel–pixel intensities. Each single image in a time trace is used to construct an image correlation function that has a flexible region of interest as the square pixels (e.g. 300 × 300 or 512 × 512). The initial time can be chosen as a good estimate for depicting the average of dynamical images in time sequences, followed by a normalization with the total sum of the pixel intensity correlation at an initial time frame. Here, the results of ITC in FTs, \( C_d(q_D, \theta_{in}, \tau) \), are fitted by a single decay function in time to characterize the three representative parameters, which are interpreted as \( A \sim S \), \( B \sim \langle q_D \rangle \), and \( \Gamma \sim \langle \theta_{tw} \rangle \), for the order parameter, average size of \( N^+ \)-domains, and twist angle, respectively.

Acknowledgments

Author KK thanks to Prof. Jan Dhont, who originally invented ITC, and Dr. H. Kriegs (IBI-4 in FZJ) for the script of converting from the series of movie data (avi) files to FFT images in stack so that the rigorous data analyses on ITC in FTs are available here for valuable discussions.

Data availability statement

All data that support the findings of this study are included within the article (and any supplementary files).

ORCID iDs

Kyongok Kang @ https://orcid.org/0000-0002-8790-9350

References

[1] Rasched I and Oberer E 1986 Ff Coliphages: Structural and functional relationships Microbiological Reviews 50 401–27
[2] Meyer T F and Geider K 1982 Enzymatic synthesis of bacteriophage fd viral DNA Nature 296 826–32
[3] Brayer G D and McPherson A 1985 A model for intracellular complexation between gene-5 protein and bacteriophage fd DNA Eur. J. Biochem. 150 287–96
[4] Guinier A 1994 X-ray Diffraction in Crystals, imperfect Crystals, and Amorphous Bodies (New York: Dover Publications Inc) pp 314–7
[5] Loeb J 1922 The Explanation of The Colloidal Behaviors of Proteins LVI 731
[6] Tombari E and Johari G P 2013 Structural fluctuation and orientational glass of levoglucosan-High stability against ordering and absence of structural glass J. Chem. Phys. 142 104501
[7] Raikher Y L and Shliomis M I 1994 The effective field method in the orientational kinetics of magnetic fluids and liquid crystals Advances in Chemical Physics Series ed W Coeffey vol LXXXVII (New York: Wiley) pp 732–7
[8] Kang K and Dhont J K G 2013 Glass transition in suspensions of charged rods: structural arrest and texture dynamics Phys. Rev. Lett. 110 015901
[9] Kang K and Dhont J K G 2013 Structural arrest and texture dynamics in suspensions of charged colloidal rods Soft Matter. 9 4401
[10] Atakishiyev N M, Chumakov S M and Wolf K B 1998 Wigner distribution function for finite systems J. Math. Phys. 39 6247
[11] Palmer R G, Stein D L, Abrahams E and Anderson P W 1984 Models of hierarchical constricted dynamics for glassy realxaiton Phys. Rev. Lett. 53 958
[12] Harrison C et al 2000 Reducing substrate pining of block copolymer microdominas with a buffer layer of polymer brushes Macromolecules 33 857
[13] Harrison C, Adamson D H, Cheng Z, Sebastian J M, Sethuraman S, Huse D A, Register R A and Chaikin P M 2000 Mechanisms of ordering in striped patterns Science 290 1558
[14] Baker J L, W-Cooper A, Toney M F, Geissler P L and Alivisatos A P 2010 Device-scale perpendicular alignment of colloidal nanorods Nano. Lett. 10 195
[15] Napoltskii K S, Roslyakov I V, Eliseev A A, Byelov D V, Petukhov A V, Grigoryeva N A, Bouwman W G, Lukashin A V, Chumkaov A P and Grigoriev S V 2011 The kinetics and mechanism of long-range pore ordering in anodic films on aluminum J. Phys. Chem. C 115 23726
[16] Descamps M and Caucheteux C 1987 The orientational glassy state and glass transitions in cyanoadamantane: kinetics of metastable ordering and cluster reversion J. Phys. C: Solid State Phys. 20 5073
[17] Fan S M, Luckhurst G R and Picken S J 1994 A deuterium nuclear magnetic resonance investigation of orientational order and director kinetics in aramid solutions J. Chem. Phys. 101 3255
[18] Boolchand P, Lucovky G, Phillips J C. and Thorpe M F 2005 Self-organization and the physics of glassy networks Philosophical Magazine. 85 3823
[19] Jacobs D J and Thorpe M F 1995 Generic rigidity percolation: The pebble game Phys. Rev. Lett. 75 4051
[20] Jacobs D J and Thorpe M F 1995 Generic rigidity percolation in two dimensions Phys. Rev. E 53 3682
[21] Kang K 2021 Equilibrium phase diagram and thermal responses of charged DNA-virus rod-suspensions at low ionic strengths Sci. Repo. 11 3472
[22] Kang K 2021 Chiral glass of charged DNA rods, cavity loops J. Phys. Commun. 5 065001
[23] Malotke F, Succone M, Wölper C, Dong R Y, Michal C A and Giese M 2020 Chiral mesophases of hydrogen-bonded liquid crystals Mol. Syst. Des. Eng. 5 1299
[24] Wang- L, Urbas A M and Li Q 2020 nature-inspired emerging chiral liquid crystal nanostructures: From molecular self-assembly to dna mesophase and nanocolloids Adv. Mater. 32 1801335
[25] Bisoi- H K, Singh G, Fisch M R, Agar-Kooijman D M, Li Q and Kumar S 2019 Chiral and orientationally ordered fluid mesophases formed by oxadiazole bisaniline based achiral bent mesogens Liq. Cryst. 46 1373–82
[26] Gvozden K, Ratajczak S N, Orelana A G, Kentzinger E, Rückler U, Dhont J K G, De Michele C and Stiakakis E 2021 Self-Assembly of All-DNA rods with controlled patchiness Small 2(045)10
[27] Kang K 2011 Image time-correlation, dynamic light scattering, and birefringence for the study of the response of anisometric colloids to external fields Rev. of Sci. Instrum. 82 033903
[28] Kang K, Piao S H and Choi H J 2016 Synchronized oscillations of dimers in biphasic charged fd-virus suspensions Phys. Rev. E 94 020602(R)
[29] Kang K, Lee D and Seo J W 2021 Frequency-responsive cooperativity of graphene oxide complexes under a low AC bulk electric field J. Mol. Liq. 335 116151
[30] Phillips J C 2005 Topological theory of electron-phonon interactions in high-temperature superconductors Phys. Rev. B 71 184505
[31] Ward I M 1962 Optical and mechanical anistoropy inc crystalline polymers Proc. Phys. Soc. 80 1176
[32] Crawford S M and Kolsky H 1951 Stress birefringence in polyethylene Proc. Phys. Soc. B 64 119
[33] Gweon G H, Sasagawa T, Zhou S Y, Graf J, Takagi H, Lee D H and Lanzara A 2004 An unusual isotope effect in a high-transition-temperature superconductor Nature 430 187