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Orientation Kinetics of Chiral-Nematic N* Domains in Suspensions of Charged DNA Rods

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Abstract

Many biological systems exhibit natural regulation mechanisms as necessary functions. Proteins, peptides, charged receptors, RNAs, and DNAs clearly demonstrate such varieties of structural dynamics in their biocomplexity. To understand the proper structural functionality of biomolecules, this requires integrated information from initiation, regulation, transfer and transport to desirable sustainable processes. Here, we demonstrate the orientation kinetics of stable chiral-nematic (N^*) domains that are consistently formed in the suspensions of charged DNA rods at low ionic strengths. This is enhanced by large access of released dissociated condensed ions and the mobile diffusive ions of DNA rods, acting onto the perpendicular motions of the DNA rods in bulk. The quantification of collective orientations for these interacting charged DNA rods is extracted by image-time correlation (ITC) performed in Fourier transforms (FTs) for overall spectral density distributions. Three distinguishable length scales are clearly shown corresponding to the relevant motion of the N^* domain in parallel, perpendicular and the optical pitch within the domains as well as the kinetics of local distributions in FTs. Particularly strong concentration transitions are confirmed by replica symmetry breaking of elastic deformations in N^* domains in terms of the average twist angle and the order parameter. This work can be interesting for sufficient cooling of the given concentration of charged DNA rods, at low ionic strengths (below the critical value), mimicking super-cooled liquid and orientation glass in other biomacromolecules.

PACS numbers:

Introduction

The structures of crystals as disordered and amorphous morphologies can be characterized by diffraction and scattering methods, as introduced by A. Guinier, including the fibrous structures as para-crystals consisting of domains (with aligned fibers) or irregular anisotropic particles [1]. Protein mixtures are natural systems mimicking colloidal behaviors [2] and the structural fluctuation of orientational glass is also found for a small protein (levoglucose) away from the structural glass that has initial caging, with non-exponential relaxations in broader length scales [3]. Typically, spectral density starts to broaden when observable structures are formed via fluctuations of domains. It is therefore interesting to see how the elasticity of inner structures are arranged within the domains. Anomalies are essential in elasticity so as to be enhanced near the orientation phase transition in such a way that the kinetics of orientational motions slow down in terms of order parameter and microscopic stresses [4]. In the case of charged rod glasses, the invariant of rotor dynamics is often referred to as the Wigner distribution function (for a finite system), where the time evolution can be deformed conveniently in the polar presentation of orientations or in the Fourier space [5]. Dynamical arrests can exist in glasses, where a slow time variable evolves in non-ergodic relaxations at different length scales either as the frozen or the intermediate states between fast and slow processes [6]. Thus, tracing the system with proper time scales is essential for the hierarchical classes, preserving unknown degrees of freedoms. Such commonly known examples are the orientation orders for the block copolymers: the grain size of the microdomains of cylindrical block copolymers are analyzed by Fourier filtering, depicting the local order parameter for the relevant correlation length in the nematic state [7]. Furthermore, the orientational orders of the microdomains of 2D striped patterns consisting of PS-PI diblock polymers are investigated using the kinetic scaling law for the smectic layers of topological constraints that originated from the annihilation of disclinations, annealing temperatures and time. The exponent power of the annealing time is found to be lower than 0.25, as was observed in the 2D nematic system (0.5), due to the dynamic growth of domains [8]. Furthermore, the self-assembly of nanocolloidal rods is considered to be useful for employing the orientational dynamics and performing efficient diffraction of the planar devices for the spacing of layers with controlled orientations [9]. The kinetics of long-ranged ordering are also shown for thin metallic films (of anodic aluminum), forming porous

structures during anodization, which leads to domains of different growth rates (for a time exponent of 0.2) in plane orientations [10]. Irrespective of the above findings, the “cluster reversion” is interestingly found to be the metastable ordering in the orientational glass of cyanoadamantane with the characteristic feature of relevant kinetics (see temperature–time diagram in Fig. 14 in Ref. [11]). The observation is interpreted as the occurrence of orientational glass, accompanied by the freezing of random strain field of a cluster coupled to the orientational order parameter. The reorientation kinetics of the nematic director are then measured by a molecular level of deuterium nuclear magnetic resonance (NMR) in aramid solution, showing a small noise of the intrinsic twist viscosity for frictional forces among the neighboring domains [13].

The mechanism of a glass-forming system is described theoretically by a general network theory for various systems of metallic alloys, silicates, and proteins [16]. In particular, the protein HIV protease is simulated by a “pebble game” or a bead-spring model [17, 18]. The model simplifies the rigid region of a mechanical network away from the soft one as a dynamic mode of proteins approached by the classical Lagrangian methods. The question arises regarding how many particles are engaged for active sites as well as the shape consisting in the concentration or the volume fractions. To date, there have been a lack of experiments on biological systems demonstrating orientational glasses in bulk. In addition, most orientation order glasses are studied in the thermotropic system or multi-component alloys near the transition point. Here, the suspensions of charged DNA rods in equilibrium phase behaviors at low ionic strengths are introduced as a good lyotropic system, where the orientation kinetics are analyzed and characterized by means of an advanced method of image-time correlations (ITCs) in the Fourier transforms (FTs). The equilibrium phase behaviors are explored systematically by varying the concentration of DNA rods at a given low ionic strength: the chiral-nematic N^* phase and the hierarchical chiral mesophases, X-pattern and helical domain (HD) phase are observed above the isotropic–nematic coexistence concentrations [14]. In particular, the intermediate concentration of DNA rods, the X-pattern is formed as a new type of glass (forming the cavity loops, a chiral glass) where replica symmetry breaking (RSB) occurs, in contrast to the structural glass [19]. The results of experimental findings clearly demonstrate differences in the averaged domains and orientation angles in both time and concentrations.

The key questions addressed in this paper are as follows: (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 in terms of determining the critical concentration (of RSB) and hierarchical chiral mesophases? (IV) What are the characteristics of N^* domains, in the lyotropic orientational system? (V) What are the “effective” reduced parameters in the orientation kinetics of N^* domains? (VI) Are there any possible relations to the thermotropic system resembling the thermodynamic system? If so, then how and why is the case?

Results

The quantification of collective orientations for charged DNA rods provides the time-dependent averaged orientations of chiral nematic N^* domains, effectively performed by image-time correlations (ITCs) in FTs. The characteristic features are then obtained from the results of data analyses as more direct evidence of replica symmetry breaking of N^* domains for the ensemble averaging of orientations. The particular aim here is to perform ITCs in FTs by varying the concentrations and waiting times developed by the in-house automated program that is rigorously used for the conversion of real images in morphology to FTs, followed by the calculations of ITCs for spectral intensity distributions in FTs. Further details of data analyses are described in the later section on methods.

Concentration-dependent orientations of twisted chiral nematic N^* -domains

The polarized optical morphologies are collected from the birefringence of alignments to investigate orientations of charged DNA rods under the crossed polarizers, resulting in the degree of optical anisotropy. Then, the order parameter can typically be estimated or calculated for proper average orientations with statistical tools for long waiting times. It is therefore a rather demanding task to acquire the data to capture the whole slow dynamics leading to the equilibrium phase behaviors simultaneously in real space and the corresponding spectral intensity distribution in FTs. The corresponding FTs of polarized optical morphologies of chiral nematic (N^*) domains are shown in Fig. 2 for varying concentrations of charged DNA rods (Fig. 1) at the given lowest buffer solution (0.032 mM

FIG. 1: Polarized optical morphologies of chiral nematic N^* domains for different concentrations of charged DNA rods (in 0.032 mM Tris/HCl buffer), indicated as unique phases, in the real space. Left: raw data, right: enhanced images taken after a long time at 135 hours. The individual N^* domains contain shorter length scales of optical pitch ($100-200 \mu m$). The field of view is $7 \times 7 mm^2$.

Tris/HCl). By varying the concentration of DNA rods, the center zone of the FT represents the orientational distributions of N^* domains as the parallel and perpendicular component, slightly tilted in the horizontal and vertical axes in FTs, respectively (see Fig. 2 and Fig. 3). The tilt from two orthogonal axes in the FT peaks is due to the existing local orientations of N^* domains collectively averaged from the concentration-dependent orientations in Fig. 2, which can be seen clearly in the supplementary movie data in Fig. 3: Below the $N-N^*$ transition concentration, only the center peak is shown in FT, while the various FT spectral distributions occur at higher concentrations. Here, three distinguishable FT spacings are obtained for length scales corresponding to the spectral distances of chiral nematic N^* domains, decomposed as two orthogonal axes in the center zone of FTs for the parallel $q_{D,\parallel}$ and perpendicular $q_{D,\perp}$ components, and the optical pitch inside the N^* domains as q_P . Moreover, the approach towards an equilibrium in the slowly varying time is shown by

FIG. 2: The Fourier transforms (FTs) of real images corresponding to polarized optical morphologies (shown in Fig. 1) and the supplementary movie data of slow dynamics for different concentrations of charged DNA rods. The center zone of the FT represents the orientational distributions of N^* domains, $q_{D,\parallel}$ as the parallel, and $q_{D,\perp}$ the perpendicular component, surrounded by the outer regime q_P for the pitch length. Both orientation distributions of N^* domains and optical pitch vary in time, which is also presented in Fig. 3. The scale bars (mostly on the right) in the FT space are shown as $132 \mu m^{-1}$, $176 \mu m^{-1}$, and $264 \mu m^{-1}$, for concentrations of DNA rods of $3.8 mg/ml$, $5.4 mg/ml$ and $14 mg/ml$, respectively. The FT peaks are mostly tilted from two orthogonal axes due to the existing local orientation N^* domains.

the 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 (Fig. 2 and Fig. 3). In particular, the most drastic changes in FTs are seen in the middle concentrations of the X-pattern occurring as a critical concentration at low ionic strengths [19]. Also, the local orientational

intensity distribution profiles show that both axes contribute to unique behaviors between these two orthogonal directions of N^* domains by varying the concentration of DNA rods (see supplementary data movies, Movies F–I in Fig. 2). Temporal changes of the orientation distributions of the N^* domains are shown in the FTs in Fig. 3, where the N^* domains with optical pitch vary very slowly over time (135–240 hours). By further increasing the concentration of DNA rods in the X-pattern approaching the X–HD transition (see comparison on 10.5 mg/ml (Movie K) and 14 mg/ml (Movie L)), half-sized domains appear, resulting in an FT spacing that is twice as large (Fig. 4). Further comparison of the critical concentrations (near the X-pattern) are later shown at even longer waiting times, t_W , between the N^* –X transition (at 4.7 mg/ml) and the X-pattern (5.4 mg/ml) (see Fig. 6).

Kinetics of orientation distributions of N^* -domains, image-time correlations in FTs

Image-time correlation (ITC) spectroscopy is used to extract more characteristic features in the FT images. Compared to the ITCs in real space [19], the ITCs in FTs turn out to be a rather direct way of determining the average orientational motions of N^* domains from the changes of spectral intensity distributions. The main features of equilibrium phases at a low ionic strength below the critical value (at 1.2 mM Tris/HCl buffer) are as follows: N^* phase is stabilized by oriented N^* domains that appear orthogonally together with the N^* optical pitch (stripes in the N^* domains) inside. The corresponding FT of the N^* phase (2.6 mg/ml) is shown in Fig. 3(a) for the averages of distributions in the orientations of overall N^* domains in the center FTs, compared to a more pronounced stable N^* phase (3.8 mg/ml) in Fig. 3(b). However, above the N^* phase (5.4 mg/ml), a unique phase of X-pattern occurs such that the N^* –X pattern transition has noticeably different intensity distributions; the reflection symmetry is broken in the axes of the N^* domain perpendicular. $q_{D,\perp}$ is also independently shown as the diverging intensity profiles in FTs (see Fig. 3(c)), while the parallel component $q_{D,\parallel}$ appears to localized spherical distributions.

The average size of the spectral domains, $\langle q_D \rangle$, also increases with an increase of the concentration of DNA rods due to the smaller sizes of the domains that are seen in real space. Furthermore, in Fig. 3(d), for the X-pattern at a higher concentration, twice as large spacing is observed in FTs compared to Fig. 3(a) as well as more visible differences between $q_{D,\parallel}$ and $q_{D,\perp}$. The system is even more clearly presented in the orientations, such that

FIG. 3: 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 an ionic strength of 0.032 mM.

FIG. 4: An example of the intensity distribution profiles in FTs: (a) for the intensity profiles in enhanced FT images, and the black and white image, 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) and a longer waiting time ($t_W \sim 100 h$). The corresponding length scales in the FT are shown in the left panel and the actual intensity profiles on the right, extracted from the reading pixels. Here, 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 emerging in the direction of $q_{D||}$, while the other diverges in the axis of $q_{D\perp}$ of the N* domains due to the elastic N* domains. The outermost regime is shown for the relevant chiral pitch q_P .

the decomposition of the Fourier component is carried out to depict the corresponding N* domain as parallel and perpendicular in the center zone of intensity lobes. Such features are illustrated in Fig. 4 (see also Movie K and Movie L) for a comparison of two high concentrations for the X-pattern (10.5 mg/ml) and X–HD transition (14 mg/ml), respectively. Intriguingly, the overall sum of the intensity distributions interacts well between the N* domains and the chiral pitch within the domains. This therefore supports the notion that

the orientations of N^* domains are affected by the elastic deformations, leading to rather sharp transitions near the N^* -X pattern and X-HD concentration, found as the RSB for symmetry breaking [19]. A more detailed description of the elastic properties of N^* domains near the X pattern is given in the following subsection.

To quantify the characteristic features of morphological changes in the time-lapsed images, the two-dimensional image matrix is considered to be converted as the numbers of all 2D array intensity values for each pixel [25]. The image-time correlation function is defined as follows: $I(t)$ is the instantaneous transmitted intensity detected by a given pixel of the CCD camera. For the time traces recorded for all these pixels, the image-time correlation function $C_V(t)$ is defined as:

$$C_V(t) = \frac{\langle (I(t) - \langle I(t) \rangle) (I(0) - \langle I(0) \rangle) \rangle}{\langle (I(0) - \langle I(0) \rangle)^2 \rangle} \quad (1)$$

where V indicates the “video”, or time-lapsed images, and the brackets $\langle \dots \rangle$ denote the averaging of the whole field of views in the CCD camera pixels at 2D (i, j) matrix indices. Each individual image in a time trace is used to construct an image correlation function that is variable for the region of interest in the square (e.g, 512×512) pixels and int time, depending on the application. The image-time correlation is also applied to various other systems to extract different features of dynamical images [25]-[28]. Here, the application of ITCs in FTs is focused on obtaining the information of collective orientation degrees of freedom for charged DNA rods observed in slow times and the effective order parameter.

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

The mechanical behavior of the (network) glasses can be physically demonstrated by the existing rigidity of the molecules in variations of the soft phonon mode and the discrete glassy percolation [22], which often occur through the optical contrast by the mechanical anisotropy of crystallinity and molecular orientations. In practice, the optical birefringence and elastic moduli are then realized by the anisotropic shape of rod-like molecules due to their rigidity in the core, embedded in an elastic medium [23, 24]. In addition, a computational algorithm of generic rigidity in 2D percolation (called a “pebble game”) is used to predict the order parameter for first- and second-order transitions with few critical exponent powers.

Calculations of central force rigidity percolations are then mapped to the heat capacity, distinguishing the free energy distributions of bond and site percolation as the result of the “floppy” model in the glassy system. However, they are still restricted to 2D and not yet available in 3D [23, 24]. Therefore, it is worthwhile to validate the RSBs in the current lyotropic system, which is uniquely observed in the X-pattern, as a chiral glass resembling 3D orientation glass [19].

The results of systemic quantification for overall changes are shown in Fig. 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 where q_D , θ_{tw} , and τ indicate the N^* domain size, twist angle, and the lag (or delay) time of waiting, respectively. The performance of the ITC function in FTs is shown in Fig. 5(a) with different waiting times of $t_W \sim 0\text{ h}, 62\text{ h}, 80\text{ h}$ and 100 h , and 100 h and a delay time (or a lag time), $\tau = t - t_W$ for the comparable equilibrium time, $t_{eq} \sim 86\text{ h}$ in previous observations [20, 21].

Fig. 5(b) also shows the image-time correlation functions in FTs by varying the 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) as well as the delay time ($\tau \sim 120\text{ h}, 60\text{ h}, 40\text{ h}$ and 20 h). Below the lower N^* domains in the concentrations of 1.8 mg/ml and 2.6 mg/ml, an increase in correlations is obtained due to the presence of nematic N domains compared to the chiral nematic N^* domains. This can be partly explained by the instability of thermal fluctuations near the N – N^* transitions. In contrast, ITCs in FTs decrease at higher concentrations, with slight indications of local oscillation peaks. These local oscillatory behaviors of image-time correlations are originated by the slow fluctuation in time of variation intensity due to the elastic motions of largely fluctuating N^* domains in the collective orientation distributions. Most ITCs in FTs can be fitted by a single exponential decay function except for the local oscillatory behaviors at longer times and low concentrations for large intensity fluctuations. The intensity correlations in FTs are related to the elastic motions of N^* domains. When the time is $t > t_{eq}$, there are no visible changes in orientations, while when the time is short ($t < t_{eq}$), large variations of correlations can still be seen in FTs, as can be seen in the data analysis (see also Fig. 7(a)).

More direct evidence of physical observations for the replica symmetry breaking (RSB) appears in the middle concentrations (of the X-pattern), occasionally in the fast time scale with mechanical kinks [14]. Without any loss of information, maintaining reliable continu-

FIG. 5: Image-time correlation function in the FT, $C_\theta(q_D, \theta_{tw}, \tau)$, for the quantification of random orientations of N^* domains: (a) as a function of the concentration of charged DNA rods for different waiting times of t_W : 0 h, 62 h, 80 h, and 100 h. (b) Image-time correlation functions in the FT for 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. Most ITCs in FTs perform a single exponential decay function, except for the local oscillatory behaviors and low concentrations for large intensity fluctuations. The intensity correlations in FTs are related to the elastic motions of N^* domains, and the characteristic parameters of ITCs are extracted from the results of fittings corresponding to the N^* domain size, q_D , twist angle, θ_{tw} , and delay time, τ .

ous measurements over a long period of time is important here to capture such occasional stochastic processes. Then, rather sharp kinetic changes of critical concentrations are obtained (see Fig. 6 (a) and (b)) for the comparison of 4.7 mg/ml (N^* phase) and 5.4 mg/ml (N^* -X pattern) at even longer waiting times (up to $t_W = 220$ h). Sensitive changes of cor-

FIG. 6: The kinetic changes of critical concentrations for the comparison of 4.7 mg/ml (N^* phase) and 5.4 mg/ml (N^* -X pattern) at even longer waiting times (up to 220 hours): (a) The N^* domains in real space, (b) the corresponding FTs, and (c) the image-time correlation functions in FTs for different waiting times. Rather rapid changes of correlation functions are seen in the concretion of DNA rods in 4.7 mg/ml, where the N^* domains become unstable towards the X-pattern compared to the N^* -X pattern transition concentration (5.4 mg/ml). The FT spacing is $\sim 176 \mu m^{-1}$. Clearly diverging edge parts of FTs are shown at the bottom left of (b) in the X-pattern, which is not discernible in the image of (a) in real space.

relation functions are also shown in Fig. 6(C) for different longer waiting times in both concentrations of DNA rods in 4.7 mg/ml, where the N^* domains become unstable towards the X-pattern, and the 5.4 mg/ml at the N^* -X pattern transition concentration. The finding confirms that more precise determinations are greatly affected by the decoupling of orientation N^* domains between the two orthogonal axes in the direction of $q_{D\perp}$ and $q_{D\parallel}$. In particular, there is a divergence of length scales in the component of $q_{D\perp}$ and the relevant chiral pitch q_P in the X-pattern, where the effective decoupling of $q_{D\perp}$ and $q_{D\parallel}$ then leads to RSB. The further realization of RSB is accurately observed in the ITCs of FTs at a concentration of DNA rods of 5.4 mg/ml, which is the concentration where the mechanical kink randomly appears in the real space in a short period of time before reaching an equilibrium [14, 19, 27]. This can be understood as the density of the system being compensated by the orientation orders of chiral nematic N^* domains at the N^* -X transition line, with the

FIG. 7: Results of image-time correlations in FTs:(a) the order parameter, S , and the average size of N^* domains, $\langle q_D \rangle$, as a function of waiting time, t_W , and the concentration of DNA rods, where the delay time τ is 60 h, 40 h, and 20 h, respectively. Interestingly, there are sharp differences in the middle concentrations of DNA rods (in 4.7–6 mg/ml), when performing the observation, depending on the characteristic time for $t < t_{eq}$, $t \sim t_{eq}$, and $t > t_{eq}$. (b) Simple illustrations of changes in the collective orientations with an increase of DNA rod concentration at a low ionic strength. For the low concentrations of the N– N^* transition, the N^* domains are shown as dominant peaks which increase with an increase of the concentration due to the domains being half a size smaller with the outer intensity lobes of pitches. A special observation of the X-pattern in FTs are the diverging domains near the RSB.

balance breaking in the X-pattern through RSB. However, when the density increases at a higher concentration of the X–HD transition, the replicas of smaller helical domains appear again, similar to the optical morphology of N^* domains in the equilibrium phase [19].

The RSB may then be relevant with a sudden reverse of cluster (or domains) in the development of orientation orders, forming a microscopic lattice at the (thermotropic) glass-like transitions (Fig. 13 of Ref.[11]). Then, the intensity fluctuations of orientations are a precursor to such transitions that are steady until the actual development of the cluster occurs in a limited space (e.g. a sudden jump or mechanical kink) and is followed by a weak time dependence for a long period of time (see effective temperature–time diagram in Fig. 14

of Ref.[11]). An unusual isotope effect is also observed in a high-temperature superconductor [12], which compromises an open question as to whether or not the X-pattern is a coexistent of the partially molten state of N* domains against more disordered (in isotropic) or ordered out of the plane (homeotropic nematic) states in bulk.

Characteristics of N* domains: Twist angle, domain size and order parameter

As the characteristics of the overall orientations of charged DNA rods in the lyotropic system, the effective parameters are the long waiting time and concentration. The characteristic crossover occurs at a concentration of DNA rods of 4–6 mg/ml, as shown in Fig. 7(a) for the amplitude of order parameter, S and the background as the average value of the inverse of the N* domain size, $\langle q_D \rangle$, which is in qualitative agreement with the start of the ITC in the real space. Figure 7(b) illustrates the simple characteristic features of FTs with an increase of the concentration depicted as stable phases. Therefore, the kinetics of the orientations of N* domains are reasonable when taking the typical equilibrium time as $t_W \sim 80 h$ [19]. When the duration time is similar or larger than the equilibrium time, as in Fig. 7(a), rather more significant changes are seen in the critical concentration regimes (see example of $t \sim t_{eq}$ or $t > t_{eq}$). In contrast, for the shorter duration time, $t < t_{eq}$, in Fig. 7(a), the critical concentrations are strongly hindered by the apparent changes at high concentrations.

The coherent measurements of orientations over time are depicted by the ITC function in FTs, $C_\theta(q_D, \theta_{tw}, \tau)$, for the Fourier spectral intensity distributions, where the orientations of N* domains are averaged within the given FT regions. Depending on the measurement time, or the delay (lag) time, τ , the kinetic fractions of the orientational distributions of N* domains can be extracted. The fitting function of the ITC in FTs is chosen here as a single mode decay function, as $C_\theta \sim A e^{-\Gamma t} + B$, where the three characteristic parameters are defined as $A \sim S$, $B \sim q_D$, and $\Gamma \sim \theta_{tw}$, interpreted as the order parameter, N* domain size, and twist angle, respectively.

The resulting characteristics of the featured parameters for ITCs in FTs are plotted in Fig. 8 as a function of concentration. The decay constant of $C_\theta(t)$ is also present in the twist angles in time correlations as well as the order parameter and the inverse of the N* domains that are obtained from the fittings, as shown in time (Fig. 7(a)) and concentration

FIG. 8: Results of the characteristic fitting parameters of image-time correlations in FTs: (a) the average orientations of twist angles, θ_{tw} , (b) the average size of N^* domains, $\langle q_D \rangle$, and (c) the preferred order parameter, S , for different delay times. A larger view of the order parameter is shown in (d). Here, the estimation of concentration-dependent order parameter, $\langle S \rangle$, is shown such that the dissociation constant (as $643 e^-$) and an effective diameter ($\sim 292 \text{ nm}$) are considered and inserted as a visual guide in (c) and (d). The pink regions indicate the concentration at which RSB occurs (in the X-pattern), where the ensemble average of the overall intensity distributions of orientations differs both below and above the concentration in equilibrium.

(Fig. 8(b)). Due to the finite Fourier component analysis, the background value here is seen as required for the stationary value corresponding to the N^* domains. In addition, the average twist angle, $\langle \theta_{tw} \rangle$ is converted from the decay constant of the ITC in FTs as the multiplication of a complete 2π turn (see Fig. 8(a)) so that the sum of possible spherical intensities can be considered. The values of the concentration-dependent averaged order

FIG. 9: The equilibrium phase diagram and corresponding FTs formed in phases of charged DNA rods as a function of low ionic strength, reproduced from Ref.[14, 19], are presented with the average orientation distributions of N^* domains over a long time. The changes in the orientations of N^* domains are shown with a comparison of the higher concentration (14 mg/ml) at higher ionic strengths below and near the critical ionic strength (of 1.2 mM) for 2 long-time kinetic arrests (LTKA1 and LTKA2) at 0.08 mM and 0.5 mM, respectively. Above the critical ionic strength, the middle concentration (of 7.4 mg/ml) is compared between the LTKA3 (at 2 mM) and the N^* -X pattern (at 0.032 mM). The equilibrium phase diagram is reproduced from Ref. [14].

parameter, $\langle S \rangle$, is included in Fig. 8(c) and 8(d), estimated from the effective Debye screening length and dissociation constants for the release of the condensed ions at a given ionic strength to clearly highlight the middle-concentration gap presented as the RSB (see pink range in Fig. 8).

Summary

The equilibrium phase diagram of charged DNA rods is provided in Fig. 9 as a function of low ionic strength for various stable chiral mesophases, with the average orientational distributions of N^* domains over a long time also shown in FTs. The systematic increase in the concentration of charged DNA rods at the lowest ionic strength (of 0.032 mM) exhibits the characteristic changes of collective orientations in FTs as the N - N^* transition, N^* phase, N^* -X pattern, X-pattern, and X-HD transition phase: (i) The asymmetric transition lines of N - N^* occur in the phase diagram above the upper binodal line of the I - N transition and below the X-pattern, observed as a non-monotonic increase. This asymmetry becomes symmetric again in the N^* -X transition phase boundary line with a variation in ionic strength for two chiral mesophases (of the X-pattern and helical domains, HDs). This can be explained by the decoupling of two independent degrees of freedom in the orientational axes of $q_{D\perp}$ and $q_{D\parallel}$. (ii) Below the critical ionic strength, pronounced chirality expands as the concentration increases for the most pronounced stable chiral nematic N^* phase before the N^* -X transition occurs. The trend is also seen in the lower binodal, relating to the intrinsic microscopic relaxations of charged DNA rods where the effective diffusion of the given concentration (or the crowding) is considered compared to the orientations. The reorientation of the aligned planar nematic phase at higher ionic strengths is shown to be much faster than the perpendicular diffusion [14, 19]. (iii) The increase of the FT spectral spacing corresponds 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. (iv) Close to the critical ionic strength (confirmed separately as 1.2 mM), two long-time kinetic arrests (LTKA1 and LTKA2) are also shown for different features at high concentrations of 14 mg/ml; larger scales of domains appear near the critical ionic strength, accompanied by intensity fluctuations of nematic domains. (v) In contrast, above the critical ionic strength, the coexistence of I - N is located with a narrow gap of the N - N^* phase, as seen in LTKA3, which contrasts significantly with the $N + N^*$ and I - N transition lines in the equilibrium diagram in Fig. 9 (see X-pattern below and LTKA3 on the right) with dominant peaks in the center zone in FTs. Further details on the equilibrium phase diagram of charged DNA rods at low ionic strengths can be found in Ref. [14, 19].

In summary, the current system demonstrated well the orientations of charged DNA rods,

resembling the slow dynamics of orientation glasses that are often found in metallic alloys (see Fig. 5 in Ref. [16]) or the rigid rod-shape molecules. Furthermore, the intermediate concentration of the X-pattern between the N*-X and X-HD phase boundaries at a low ionic strength is found to be a unique RSB (as a chiral glass exhibiting cavity loops) [19], which has now also been confirmed by a significant gap in the concentration of charged DNA rods. A direct analysis of the overall orientations of N* domains enables the twist angles, domain size, and order parameter to be extracted for the determination of characteristic orientations by image-time correlation (ITC) in FTs. Moreover, the most interesting finding is that the decoupling and divergence of orientational motion N* domains in $q_{D\perp}$, away from the $q_{D\parallel}$, is more responsive to RSB. However, the replica forms again, consisting of small helical domains (HDs) with an increase of the concentration and the orientation kinetics that evolves to a further local symmetry breaking in the direction of $q_{D\parallel}$ (see FT of the X-HD transition in Fig. 9). Replica symmetry breaking (RSB) also occurs particularly strongly in the middle concentration, leading to a diminishing of N* domains both above the N*-X-pattern and below the X-HD transition of half-sized helical domains (HD) with an increase of DNA rod concentration. This indicates that the time to reach equilibrium state is an effective parameter for depicting the unique concentration of RSB, possibly in a similar way to defining the “critical isotherm” (see Fig. 30 in Ref. [4]), as discussed in thermodynamics for both the order parameter and the microscopic stress of elastic deformation. The orientation and elastic properties are therefore relevant for demonstrating the sensitive measure of orientational anomalies in the vicinity of the transition point, even by releasing the elastic waves (resembling cavity loops observed very slowly over time as a chiral glass in Ref.[19]). This then opens up the question as to whether or not the X-pattern (at low ionic strengths) can be expressed by an enhanced rotational diffusion for charged DNA rods as the chiral glass (cavity loops) and affected by the annihilation of twisted chiral nematic N* domains. Based on the averaged order parameters of charged DNA rods (at low ionic strengths) obtained directly by ITCs in FTs, it is highly possible for the N* domains to be reoriented at long waiting times in equilibrium by pronounced perpendicular motions within thick electric double layers for sufficiently slower cooling. In contrast, a faster diffusion process occurs for the parallel direction of DNA rods at a higher ionic strength above the critical value (1.2 mM), leading to an easier alignment along the charged DNA rods. The ionic strength-dependent electric double layers of charged DNA rods are therefore an

essential driving mechanism for demonstrating such rich phase behaviors observed in this system, including the RSB. The results of ITCs in FTs evidently provide the orientation kinetics of N^* domains, as affected by the presence of dissociation/association from both condensed ions and mobile diffusive ions surrounding the interacting charged DNA rods in the equilibrium. The findings in this work illustrate both the use of extensive applications of ITCs in FTs and strong evidence of RSB in the orientation kinetics of collective interactions for charged DNA rods as a good model system of lyotropic bulk orientation glasses, to be applicable for other charged biomacromolecules.

Methods

Sample preparation and optical measurements of 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 analyzed against the buffer solution of a lower ionic strength (0.032 mM Tris/HCl buffer) for 2 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 Donnan 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_g for the very small amount ($10\ \mu l$) 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 $[fd] = \frac{OD}{W_g} \times 0.26042$, where the factor of $0.26042 \sim 1/3.84$ 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 5 times. For the optical measurements, a commercially available Quartz transparent cylinder cuvette with a thickness of 1 mm and a diameter of 20 mm (120 QS 1mm, Hellma Precision in Spectro-Optics) is used to contain an approximately $380\ \mu l$ 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).

Orientation distributions and image-time correlation spectroscopy in FTs

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 as 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 characterization of field-induced dynamical states of this current system of charged DNA rods [25]. 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 fluctuations. The in-house-developed ITC program is very robust and effective. For instance, a single image-time correlation only takes 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 Ref. [25]. 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, 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 $C_V(t)$ 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 variable region of interest as the square pixels (e.g. 300×300 or 512×512). The initial time can then be a good estimate for depicting the average of the 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, $C_\theta(q_D, \theta_{tw}, \tau)$, are fitted by a single decay function in time to characterize the 3 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.

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