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## RESEARCH ARTICLE OPEN ACCESS

# Shear-Induced Supramolecular Organization of Calf-Thymus DNA in Solution: Insights from SAXS Coupled with Rheology

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## ABSTRACT

The investigation of DNA's dynamic and flow properties in solution is crucial for understanding its biological roles. DNA double helix strands interact and, at high concentrations, they form liquid crystalline phases, which are influenced by molecular weight and ionic strength. At rest, Small Angle X-ray Scattering (SAXS) shows that DNA double helix strands exhibit an electrostatic correlation peak whose intensity decreases in the presence of external salt. In this study, shear-induced liquid crystalline textures were observed in high molar mass calf-thymus DNA solutions (CT-DNA) at concentrations higher than 7 mg/mL using birefringence visualizations and rheology measurements. Then, SAXS, combined with rheology, was used to examine this supramolecular organization under flow, revealing orientations of CT-DNA with preferential inter-chain distances. Upon relaxation, a transition from anisotropic to isotropic behavior was observed in those DNA solutions. To summarize, it is shown that CT-DNA's supramolecular organization at rest is progressively modified under flow and significantly influenced by DNA concentration, shear rate, and ionic strength.

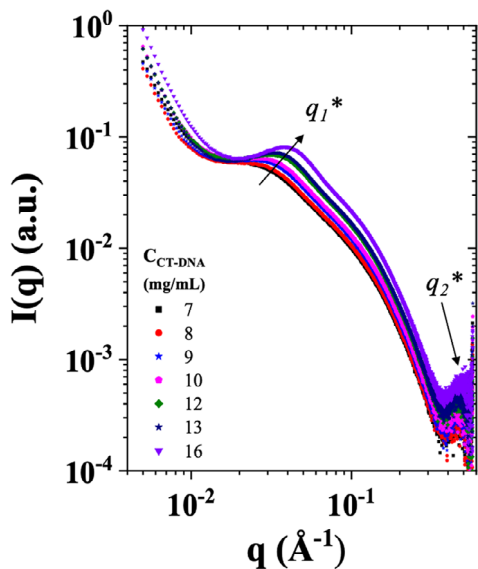
## 1 | Introduction

The organization and dynamics of DNA in solution are key to its biological functions, influencing processes like gene regulation, replication and transcription [1]. At low DNA concentrations ( $C_{DNA}$ ), DNA behaves as a semiflexible polymer chain [2, 3]; however, as  $C_{DNA}$  increases, DNA double helix strands begin to interact, leading to entanglement, which influences its viscoelastic properties [4]. Beyond a critical concentration, these interactions drive a phase transition to liquid crystalline states,

where DNA molecules align into nematic or cholesteric phases depending on factors such as ionic strength and molecular weight (MW) [5]. In moderately concentrated solutions ( $C_{DNA} > 7$  mg/mL), Calf thymus-DNA (CT-DNA) strands in the isotropic phase gradually align under flow, forming birefringent textures [4, 6]. In this work, we used Small Angle X-ray Scattering (SAXS) coupled with rheology to study CT-DNA's supramolecular structure along the flow curve and during relaxation after an imposed shear rate [7]. We examined the anisotropy of CT-DNA's supramolecular organization in water and buffer solutions. The

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**FIGURE 1** | SAXS intensity  $I(q)$  plotted against the scattering vector  $q$  for CT-DNA solutions in TE buffer at concentrations between 7 and 16 mg/mL.

impact of  $C_{\text{DNA}}$  and ionic concentration on CT-DNA solution organization under flow and during relaxation was analyzed by studying the azimuthal variation of the correlation peak  $q^*$  obtained by SAXS. After the apparent stationary state of the stress was reached for a given shear rate, the 2D X-ray images taken during relaxation showed a structural transition from anisotropic to isotropic behavior in the CT-DNA solution

## 2 | Results and Discussion

### 2.1 | DNA Organization in TE Buffer by Static SAXS Measurements

Figure 1 shows the SAXS intensity  $I(q)$  for CT-DNA solutions with  $C_{\text{DNA}}$  up to 16 mg/mL, illustrating the impact of  $C_{\text{DNA}}$  on the first correlation peak,  $q_1^*$ . This peak arises from long-range electrostatic repulsions between DNA chains and is strongly dependent on polymer concentration, i.e.,  $C_{\text{DNA}}$ . As  $C_{\text{DNA}}$  increases,  $q_1^*$  shifts

to higher  $q$  values and becomes sharper, indicating enhanced intermolecular interactions and reduced intermolecular spacing. The evolution of  $q_1^*$  with  $C_{\text{DNA}}$  follows the expected scaling law  $q^* \sim C_p^{1/2}$ , consistent with previous studies [7]. At  $q$  values greater than  $0.3 \text{ \AA}^{-1}$ , a second peak emerged,  $q_2^*$ , attributed to the DNA form factor, which reflects the intrinsic structural features of the individual DNA chains [7].

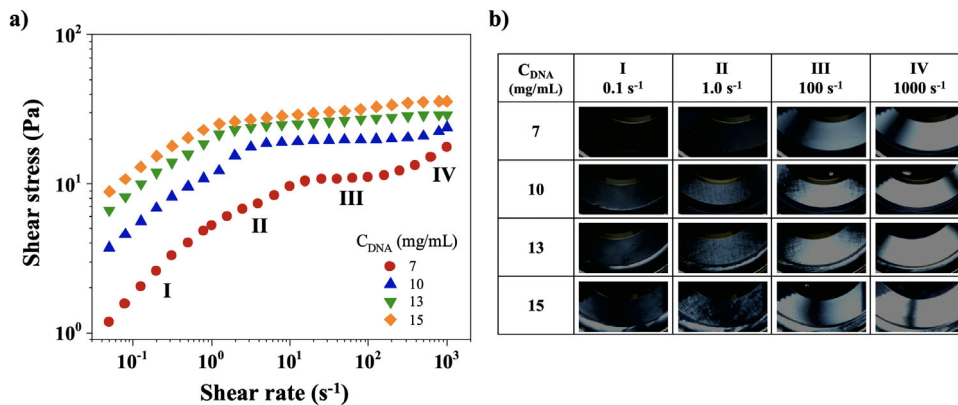
### 2.2 | Shear Flow Properties and Birefringence of CT-DNA Solutions

The CT-DNA rheological behavior under flow was studied for solutions with  $C_{\text{DNA}}$  up to 16 mg/mL (Figure 2a). Visual observations between crossed polarizers revealed shear-induced birefringence at characteristic shear rates, depending on  $C_{\text{DNA}}$  (Figure 2b).

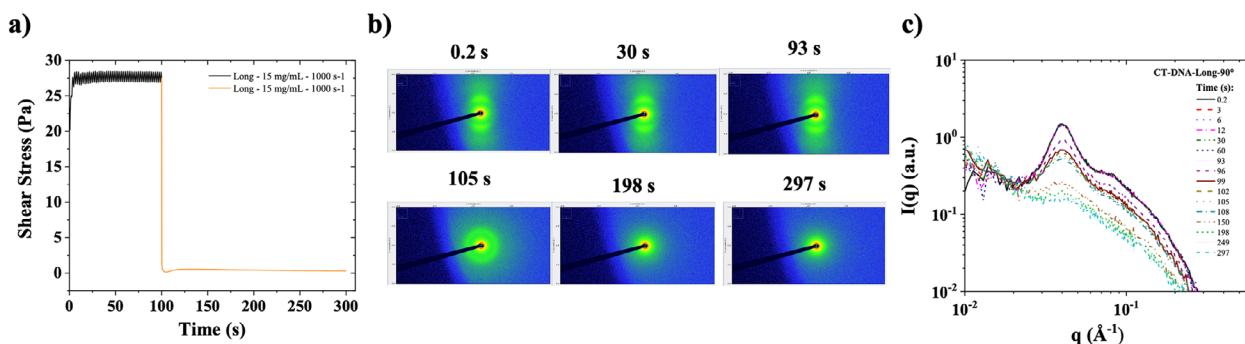
In shear stress curves (Figure 2a), a plateau is observed at concentrations of CT-DNA solutions of 7 mg/L and above, consistent with previous reports [4]. This plateau corresponds to the formation of liquid crystalline textures, where DNA strands begin to align under flow. In the flow curves for each concentration, we identified two shear-thinning regions (I, II), followed by the shear stress plateau (III), which is associated with the formation of liquid crystalline textures. The final stage results in a uniform anisotropic monodomain (IV).

### 2.3 | Shear-Induced Transition in DNA Organization Followed by Rheo-SAXS Measurements

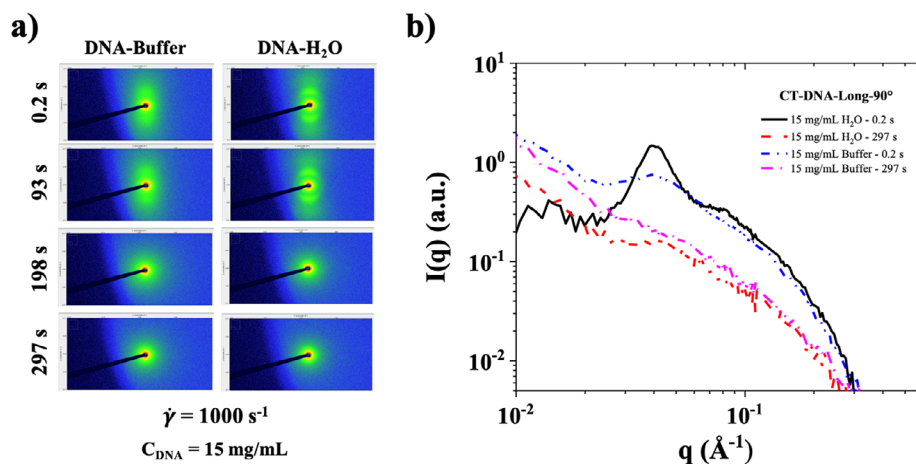
Figure 3a shows the evolution of shear stress over time for a CT-DNA solution in water at 15 mg/mL, subjected to a shear rate of  $1000 \text{ s}^{-1}$  for around 100 s, followed by relaxation of the sample up to 300 s. At this high shear rate, DNA domains align, exhibiting anisotropic behavior as shown in Figure 3b (up to 100 s). Upon cessation of shear, over 100 s, a phase transition from anisotropic to isotropic occurs (Figure 3b), indicating that DNA chains reorganize progressively into a random configuration. Figure 3c shows the time-dependent decrease in intensity for the



**FIGURE 2** | a) Shear stress as a function of shear rate for DNA solutions at 7, 10, 13, and 15 mg/mL in TE buffer at 20°C. b) Visualizations of each DNA solution under shear between radially oriented crossed polarizers at shear rates selected from the flow curve (I to IV). Each image represents a steady-state visualization at a specific imposed shear rate.



**FIGURE 3** | a) Shear stress evolution as a function of time after applying a shear rate of  $1000 \text{ s}^{-1}$  during 100 s. b) Time-resolved SAXS patterns during flow and relaxation obtained from a longitudinal configuration. c) SAXS azimuthal intensity evolution during stress application and subsequent relaxation at selected time points.  $C_{\text{DNA}} = 15 \text{ mg/mL}$  in  $\text{H}_2\text{O}$ .



**FIGURE 4** | a) Time-resolved SAXS patterns during shear ( $1000 \text{ s}^{-1}$  during 100 s) and relaxation in DNA solutions at  $15 \text{ mg/mL}$  in buffer and  $\text{H}_2\text{O}$ , obtained from an azimuthal configuration. b) SAXS intensity for CT-DNA solutions in buffer and  $\text{H}_2\text{O}$  at  $15 \text{ mg/mL}$ .

first-order peak ( $q_l^*$ ), which diminishes during the relaxation phase.

## 2.4 | Effect of Ionic Concentration on Shear-Induced Transition in DNA Organization

Figure 4 shows the SAXS relaxation patterns and SAXS intensity influenced by the ionic concentration (water is compared with buffer).

Figure 4a shows that the ordering phase relaxation time is shorter in the absence of external salt. Additionally, the intensity of the correlation peak ( $q_l^*$ ), as observed in water, decreases gradually in the presence of external salt (Figure 4b). This behavior reflects salt-induced screening of electrostatic interactions, reducing their effective range of interactions.

## 3 | Conclusions

Shear rate-induced transition from isotropic to anisotropic liquid crystalline textures was observed in CT-DNA solutions above  $7 \text{ mg/mL}$ . During relaxation, a transition from anisotropic to isotropic behavior was observed. The evolution of  $q_l^*$  correlation peak with  $C_{\text{DNA}}$  follows the scaling law  $q^* \sim C_p^{1/2}$ . The pres-

ence of external salt screens electrostatic interactions, reducing DNA relaxation time and the intensity of the correlation peak ordering during flow and relaxation. These results highlight the supramolecular organization of DNA, which is strongly modified under flow in dependence on the applied shear rate,  $C_{\text{DNA}}$  and ionic concentration.

## 4 | Experimental Section/Methods

### 4.1 | Materials and Solutions Preparation

CT-DNA solutions (MW =  $6\,560\,000 \text{ g/mol}$ ) were prepared in water (10 and  $15 \text{ mg/mL}$ ) and a buffer (7–16  $\text{mg/mL}$ ) with Tris-HCl (100 mM) and EDTA (10 mM) at pH 7.4 (TE buffer). The buffer was diluted 9:1 with HPLC water to prepare DNA solutions (ionic concentration  $\sim 10 \text{ mM}$ ). Solutions were stored at  $4^\circ\text{C}$  to avoid degradation.

### 4.2 | Rheological Measurements and Visual Observations under Crossed-Polarizers

The rheological behavior of CT-DNA TE buffer solutions (7–16  $\text{mg/mL}$ ) was studied at  $20^\circ\text{C}$  using an AR-G2 rheometer with a 40 mm,  $1^\circ 5' 2''$  steel cone. Steady-state and relaxation

measurements were conducted over a shear rate range of  $5 \times 10^{-4}$  to  $1000 \text{ s}^{-1}$ . Birefringence was examined with a Physica MCR 301 rheometer (Anton Paar) using a 43 mm glass plate-plate geometry (0.65 mm gap), placed between crossed polarizers, one below the fixed plate, and the other one above the geometry. Samples were illuminated with a white light source that was placed below the first polarizer, and birefringence intensity under shear was recorded at  $20 \text{ }^\circ\text{C}$  using a Logitech network camera and/or a coupled device camera (Sony DSLR-A580) with an adapted commercial zoom (Ex-Sigma Macro 105).

### 4.3 | SAXS Scattering Measurements Coupled to Rheology

Static SAXS experiments were performed on ID02 high brilliance beamline at European Synchrotron Radiation Facility (ESRF, Grenoble, France). Rheo-SAXS measurements were conducted on the SWING beamline at SOLEIL synchrotron (Gif-sur-Yvette, France). Sample shearing was synchronized with SAXS signal acquisition using an Anton Paar MCR 501 controlled-stress rheometer. CT-DNA samples were loaded into a Couette cell with a  $500 \text{ }\mu\text{m}$  gap, made of polycarbonate for optimal X-ray transmission. SAXS measurements were performed at  $12 \text{ keV}$  with a fixed sample-to-detector distance of  $1 \text{ m}$ . For each shear rate or strain value, 2D SAXS patterns (100 frames,  $200 \text{ ms}$  exposure) were recorded, with only longitudinal results presented here. Data were normalized, solvent contributions subtracted, and analyzed using Foxtrot 3.5 and SASView.

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#### Conflicts of Interest

The authors declare no conflicts of interest.

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