Self-Assembly of Aqueous Soft Matter Patterned by Liquid Crystal Polymer Networks for Controlling the Dynamics of Bacteria

Netra Prasad Dhakal

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SELF-ASSEMBLY OF AQUEOUS SOFT MATTER PATTERNED BY LIQUID CRYSTAL POLYMER NETWORKS FOR CONTROLLING THE DYNAMICS OF BACTERIA

By
Netra Prasad Dhakal

A Thesis
Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science

The University of Memphis
August 2020
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Netra Prasad Dhakal
June 16, 2020
Memphis, TN, US
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**List of Abbreviations:**

<table>
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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>LC</td>
<td>Liquid Crystal</td>
</tr>
<tr>
<td>LCLC</td>
<td>Lyotropic Chromonic Liquid Crystal</td>
</tr>
<tr>
<td>DSCG</td>
<td>Disodium Cromoglycate</td>
</tr>
<tr>
<td>POM</td>
<td>Polarizing Optical Microscope</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribo Nucleic Acid</td>
</tr>
<tr>
<td>SD1</td>
<td>Azo dye</td>
</tr>
<tr>
<td>RM257</td>
<td>2-Methyl-1,4-phenylene bis (4-(3-(acryloyloxy) propoxy) benzoate</td>
</tr>
<tr>
<td>CCD</td>
<td>Charged Coupled Device</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of Interest</td>
</tr>
<tr>
<td>LCN</td>
<td>Liquid Crystal Polymer Network</td>
</tr>
<tr>
<td>DMF</td>
<td>N-N Dimethylformamide</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning Electron Microscopy</td>
</tr>
<tr>
<td>nm</td>
<td>nanometer</td>
</tr>
<tr>
<td>µm</td>
<td>micrometer</td>
</tr>
<tr>
<td>JXLC2000</td>
<td>mixture of liquid crystals</td>
</tr>
</tbody>
</table>
List of Symbols:

n  director field
S  order parameter
D  dimension

ne  refractive index of extraordinary ray
no  refractive index of ordinary ray

I0  Intensity of incident light
I  Intensity of light

P  Polarizer
A  Analyzer

φ  rotation angle
p  probability

m  topological charge
Chapter 1

Introduction

1.1 Introduction to Liquid Crystals

The commonly known states of matters are solid, liquid, gas etc. The most important property of solid is its long range orientational order of molecules or atoms. Whereas the most important property of liquids is it can flow. The liquid crystal (LC) is a state of matter which has the property of both that of liquid and crystal. LCs can flow like liquid and has long range orientational order like crystal\(^1\). Due to this versatile property, LCs are interesting material and used in many applications like display, composite material, bioengineering and many more.

1.2 Classification of Liquid Crystals

Liquid crystals can be classified into two classes: Thermotropic and Lyotropic

![Classification of Liquid Crystals](image)

Fig 1. 1 Classification of Liquid Crystals
1.2.1 Thermotropic Liquid Crystals

The property and phase of liquid crystals depends on temperature. At a high temperature the molecules lost the orientational order and called isotropic phase, below the isotropic phase the molecules have some orientational order called nematic phase. Due to the temperature regime and orientational order, the thermotropic liquid crystals can further be divided into calamitic (rod like), Discotic (disc like) and Sanidic (lath like). The spatial and temporal average of long axis of molecules is called a director which is denoted by $n$.

To describe the liquid crystal a scalar order parameter, $S$, is defined as

$$S = \langle P(\cos \theta) \rangle \left( \frac{3\cos^2 \theta - 1}{2} \right)$$

(1)

Where $P$ is the second Legendre polynomial and $\theta$ is the angle between the local orientation of molecule and director field ($n$). For perfect alignment of molecules, $S=1$, and for random alignment $S=0$. The value of $S$ lies between 0.3 to 0.8 for different phases of thermotropic liquid crystals.

Fig 1. 2 (a) Nematic phase (b) Isotropic phase
As the temperature of the substance increases, the order parameter decreases to zero for critical temperature. These rod/disc shape molecules have elastic behavior with the external stimuli like electromagnetic field and hence show birefringence, which is useful to make display of many electrical devices like computer, mobile etc.

1.2.2 Lyotropic Liquid Crystal

Lyotropic liquid crystals are formed by dissolving amphiphilic mesogens in a solvent. The amphiphilic mesogens are compound having both hydrophilic and hydrophobic parts in the structure. The property of Lyotropic liquid crystals depend on temperature and concentration of the materials. As we increase the concentration of amphiphilic mesogens in solvent, the mesogens first dispersed randomly, after reaching critical micelle concentration (cmc), they start to aggregates to form micelle. These micelles further formed ordered structure like hexagonal, cubic, lamellar, disc etc. the common examples of lyotropic liquid crystals are soap, detergent, surfactants, lipids, proteins, etc.
Another way to form lyotropic liquid crystal is by the spontaneous self-organization of anisotropic nanoparticles from isotropic to nematic phase transition. According to Onsagar, the orientational order is achieved above critical concentration and hence LC phase is formed. Lyotropic Chromonic liquid crystals (LCLC) are different from amphiphilic due to their special character. They are typically plank-like molecules with the aromatic central core and hydrophilic ionic (hydrogen bonding) groups at the periphery which are dissolved in water (solvent) and self-assemble into column-type aggregates by $\pi-\pi$ stacking. The resulting aggregates self-organize into a nematic liquid-crystal phase when a certain concentration or temperature is reached. Compared to other lyotropic system, chromonic do not have critical micelle concentration (cmc). LCLC are formed by one dimensional molecular self-assembly and are sometimes also called “living polymers” and “wormlike micelle”. Some examples of LCLC are DNA, Proteins, Myelin from proteins and lipids etc. Fig 1. 4 shows a typical structure of most commonly and widely studies Lyotropic chromonic Liquid Crystal Disodium cromoglycate (DSCG), which is also known as anti-asthmatic drug.

![Fig 1. 4 Structure formula of DSCG](image-url)
Controlling the superstructures of water-based soft matter is a thriving area of contemporary research and development crossing boundaries of physics, chemistry, and biology. It provides tools to build functional materials for various biological and optical applications. In this endeavor, controlled molecular self-assembly of liquid crystalline aqueous soft materials, that is, lyotropic chromonic liquid crystals (LCLCs), has been yielding remarkable applications such as polarizers, electro-optical devices, and templates for the self-assembly of peptide amphiphiles. Because of its nontoxicity to biological cells, Lyotropic chromonic liquid crystal (LCLC) has been used for bio detection and controlling the dynamic behavior of bacteria.

1.3 Polarizing optical microscopy (POM)

Liquid crystals are optically active, anisotropic materials. they have two refractive indices; one is parallel ($n_\parallel$) to the optic axis and another is perpendicular ($n_\perp$) to the optic axis. Light passing through liquid crystals usually decomposes into two rays: ordinary and extraordinary, whose polarizations are perpendicular to each other. They have different velocities and refractive indices and appear to have a phase difference $\delta$.

$$\delta = \frac{2\pi}{\lambda}(n_e - n_o)d$$ \hspace{2cm} (2)

where, $\lambda$ is the wavelength of light, $n_o$ and $n_e$ refractive index for ordinary and extraordinary ray respectively. Let $\varphi$ be the angle between the optic axis and the direction of light propagation and liquid crystal material is placed between two crossed polarizers, then the transmitted intensity of monochromatic light is given by

$$I = I_0 \sin^2(2\varphi) \sin^2(\delta/2)$$ \hspace{2cm} (3)

where $I_0$ is the incident light intensity, $\delta$ is the phase difference and $\varphi$ is the azimuthal angle, i.e. the angle between the analyzer and the projection of the optic axis onto the sample plane. shows the polarized optical microscope (POM) configuration. Rotation of the birefringent/anisotropic
sample will induce an intensity change due to the change of azimuthal angle $\varphi$ if $\sin(\delta/2)$ is not zero. Thus, POM images of liquid crystal textures provide information about the alignment and director. Fig 1.5 shows the Polarizing Optical Microscope.

Fig 1.5 polarizing optical microscope (POM) (downloaded from Nikon website)

1.4 Defects in LC

The liquid crystals molecular interaction is very weak, they can be deformed by mechanical force, electromagnetic (electric, magnetic) field, and interfacial forces. As compared to uniform alignment of director field, the free energy increases in the deformed form. The basic deformations are splay, twist and bend as shown in Fig 1.6.
Splay and bend distortion can be achieved by placing liquid crystal inside the two substrates having wedge shape substrate. Twist can be achieved by placing liquid crystals inside uniform substrate and twisting them. The local optical properties of liquid crystals are determined by director field. Inside the defect, the director field changes abruptly, become inhomogeneous and show singularity. Due to the inhomogeneous director field, the defect appears dark when observed through polarizing optical microscope (POM). The study of defect is equally important in nanoscience, biology, and cosmology. The cosmological defects have similar properties as that of topological defect of liquid crystal except they have different scale. For the LC the orientation of molecules is represented by the director field \( \hat{n} \), the director varies from place to place so \( \hat{n} \) can be represent as a function of position \( \hat{n} = \hat{n}(\vec{r}) \). Mainly there are two types of defects point and line defect.

The strength (s) of defect is a number with sign which is obtained by moving around a closed path, the director rotates by s multiples of \( 2\pi \). Mathematically the director field along XY plane is defined by the angle \( \theta \), by \( \hat{n}(x,y) = (\cos \theta, \sin \theta, 0) \), where \( \theta(x,y) = m \tan^{-1} \frac{y}{x} + \theta_0 \) as shown in Fig 1. 7.
Fig 1. 7 Schematic illustration of the commonly observed Schlieren defects of strength and sign (a) $s = +1$ (b) $s = -1$ (c) $s = +1/2$ (d) $s = -1/2$

1.5 Outlines

The main goal of this thesis is to enhance the use the photopatterning technique to control self-assembly of water-based soft matter by using a various type of liquid crystals and polymers. The first chapter contains a brief introduction of liquid crystals, their classifications, polarizing optical microscope, defects formation and director field.
Second chapter consists of preparation of liquid crystalline phase of DNA, photopatterning of DNA, characterization of DNA alignment by measuring transmitted intensity of DNA, patterning of DNA with topological defect and arbitrary pattern and their possible applications.

Third chapter consists of fabrication of liquid crystal polymer network (LCN), their characterization, patterning of LCLC on LCN and application of pattern LCLC to control the dynamics of bacteria.

Chapter four consists of further applications of liquid crystal polymer network (LCN), to control the growth and proliferation of neuron cells and neuron tumor cells.

1.6 List of publications

The results presented in this thesis are a part of the following publications\textsuperscript{10-12}


DNA is the building blocks for most of the living organisms. The double helix structure of DNA was first discovered by Watson and Crick in 1953\textsuperscript{13}. Nature can precisely control molecular self-assembly of DNA to form complex structures on multiple length scales\textsuperscript{14}.

The macroscopic organization of DNA has several potential applications such as biosensing devices, tissue engineering, inorganic solid-state and opto-electronic devices. In order to get potential applications, the DNA should be well ordered organized which can control the spatial organization of these assemblies across multiple length scales. Several methods are proposed to create large-scale, well-ordered, and oriented DNA structures by using evaporation\textsuperscript{15,16}, magnetic field\textsuperscript{17}, mechanical shearing\textsuperscript{18}, molecular combing\textsuperscript{19-22} and topographic control\textsuperscript{23-25}.

Yoon et. al. studied the molecular self-assembly of DNA by using shearing method. They studied the alignment of col phase of crude DNA and showed well aligned regular stripe pattern of DNA by simple mechanical shearing\textsuperscript{18}.

Woang et.al investigated the ring like DNA drops which form a periodic zigzag structure during drying process. They explain this phenomenon is due to the liquid crystal elasticity of DNA\textsuperscript{15}.

Morii et. al. prepared homogeneous oriented molecular chain of DNA by drying semidiluted solution in horizontal magnetic field. They also claim that DNA chains are aligned not only by a magnetic field but also by the interfacial effect that induced the chains to fit along the air-liquid interface\textsuperscript{17}.

Bensimon et. al. found that DNA molecules attached at one end of a solid surface can be extended and aligned by a receding air-water interface by the process molecular combing. They also claim
that this process can extend chromosomal Escherichia coil DNA fragment and detect minute quality of DNA. Yoon et al. demonstrated a shear induced flow and micro posts can be used to create microstructure of DNA arrays. They used patterning application to characterize anisotropic DNA.

However, these approaches control only the local domains instead of larger structures. Control of DNA molecular self-assembled structures with predetermined architecture and functionality through multiple length scales is highly desirable and is one of the major goals of biological engineering and materials science. The ordering structures of DNA molecules can be based on its liquid-crystal phase with spontaneous orientational order. If the concentration of an aqueous DNA solution reaches about 50 mg/mL, DNA molecules will self-assemble into a nematic phase. If the concentration is high enough, up to 200 mg/mL, the DNA solution will display a columnar phase. The nematic ordering of DNA structures can be controlled by using the surface patterning technique.

2.2 Experimental methods and Materials

We follow the recently proposed approach for surface photoalignment that is based on plasmonic photomasks used to irradiate bounding plates with light of a controlled polarization pattern, as described in detail in Refs 27,28. The photosensitive material used here is an azo dye, SD1 [Fig. 2.1], from DIC, Inc. It is mixed with N, N dimethylformamide (from Sigma, Inc.) at a concentration of 0.2 wt% and spin-coated onto the cleaned glass substrates at 3000 rpm for 30 s. After baking at 120°C for 15 min, the SD1-coated substrates are exposed in the photopatterning setup shown in Fig. 2.2 for 5 min. In the photopatterning setup, we use an X-Cite 120 metal halide lamp as a source of nonpolarized light to illuminate the plasmonic metamasks; the lamp output is
the strongest in the spectral range between 350 and 450 nm. The plasmonic photomask is made of a quartz substrate coated with aluminum films of 150 nm thick. The aluminum film is perforated by means of electron beam lithography and reactive ion etching with rectangular nanoapertures, each of 220 nm in length and 100 nm wide. An unpolarized light beam goes through the photomask and becomes linearly polarized, and the polarization is perpendicular to the long side of the nanoaperture. By designing the orientations of the nanoapertures in space, the light polarization can also be manipulated to vary from point to point. Patterns with spatially varying linear polarizations are projected and imprinted on the azo-dye SD1 layer.  

![Molecular structure of (a) liquid-crystalline monomer reactive mesogen RM257 (b) photosensitive azo-dye SD1](image-url)

Fig. 2. Molecular structure of (a) liquid-crystalline monomer reactive mesogen RM257 (b) photosensitive azo-dye SD1
The light is emitted from the illumination lamp and patterns of linearly polarized light distribution are created after the plasmonic photomask, which are projected on the sample coated with azo-dye SD1 through two objective lenses. The predesigned patterns will be imprinted on to the SD1 layer as shown in Fig. 2.

**2.3 Result and discussion**

Sodium salt of DNA from salmon testes (from Sigma Inc.) with 2000 base pairs is used in this work. This DNA is considered to be a semiflexible polymer with a persistence length of 50 nm and nematic liquid-crystal phase will form at concentrations from 50 to 200 mg/ml. DNA in the nematic phase with a concentration of 100 mg/ml is used, which has an isotropic phase at 95°C, and an isotropic-nematic phase transition at 90°C. All experiments are conducted at 25°C in the nematic phase.

**2.3.1 Planar alignment of DNA structures**
Aqueous DNA solution with a concentration of 100 mg/ml is prepared and one drop of this solution is placed on one patterned substrate with an aligned liquid-crystalline polymer layer. The other cleaned glass substrate will cover this DNA drop. The gap between the two substrates are set by 5-μm glass spacers. This sample is promptly sealed by 5 min epoxy glue to avoid water evaporation. Then the sample is heated up to 95°C to remove all flow alignment and other undesirable effects. After the sample is cooled to room temperature, the sample is imaged by using

![Fig. 2](image-url)

Fig. 2. 3(a) Aligned DNA is placed at 45° between the crossed polarizers (b) engaged λ-plate POM (c) Aligned DNA is placed at 135° between the crossed polarizers, (d) engaged the λ-plate POM a polarizing optical microscope (POM). We use a 50X-1000X advanced upright polarized light
microscope from Amscope with both 10×Plan, NA=0.25 objective, and 20×Plan, NA=0.40 objective, lenses. Optical microscopic images are captured by using a 20MP USB3.0 BSI C-mount microscope camera from Amscope (resolution 5440×3648 pixels). Fig. 2. 3 (a) shows that after the nematic DNA solution contacts the liquid-crystalline polymer layer with a planar alignment, DNA chains will be aligned uniformly following this planar fashion. The alignment of DNA structures can be directly determined by using POM with a retardation λ plate. The λ plate has a slow axis along 45° and alignment of DNA parallel to this axis will show yellowish domains Fig. 2. 3 (a) and Fig. 2. 3 (b), and perpendicular to this axis will show blueish domains, Fig. 2. 3 (c) and Fig. 2. 3 (d).

![Graph](image)

**Fig. 2. 4** Intensity measurement by rotating the planar-aligned DNA sample from 0° to 180°; I is the normalized intensity and φ is the rotation angle.
We rotate the DNA sample with planar alignment at room temperature at steps of 5° and record POM images at every step. After that, for each image, a 1×1mm² area is selected as the region of interest (ROI) and average intensity in the ROI is measured and normalized by using Image J software. The plot of intensity at each step versus rotational angle is obtained and fitted by using I \( \propto \sin^2(2\varphi) \), where I is the intensity and \( \varphi \) is the rotation angle, as shown in Fig. 2.411.

2.3.2 Photopatterning DNA structures with topological defects

We first design the pattern with director field \( \hat{n} = (n_x, n_y, n_z) = (\cos\theta, \sin\theta, 0) \), where \( \theta(x,y) = m \tan^{-1}(y/x) + \theta_0 \), \( m \) is the topological charge; the phase \( \theta_0 \) sets the type of distortions. Fig. 2.5 (a) shows a pattern of director field with \( m=1/2 \) and \( \theta_0 = \pi/2 \). The director field can be simulated to gain direct visualization of the textures between cross polarizers. For every pixel, the local intensity through the crossed polarizers can be calculated as \( I \propto \sin^2[2\theta(x,y)] \). The obtained intensity map will be normalized by dividing the intensity of every pixel by the maximum intensity of all pixels. Thus, the image graphic of the simulated texture between crossed polarizers can be plotted in Mathematica. The simulated texture between crossed polarizers is shown in Fig. 2.5 (b). The POM image shows the texture of DNA alignment with two extinctions, Fig. 2.5 (c), which is in agreement with the simulation. The POM image with the engaged \( \lambda \) plate is another confirmation that orientation of DNA ordering follows the predesigned pattern, Fig. 2.5 (d). If the pattern is designed with \( m=1 \) and \( \theta_0 = \pi/2 \) [Fig. 2.5 (e) and Fig. 2.5 (f)], the patterned DNA structures adopt bent distortions, with four extinctions imaged by POM [Fig. 2.5 (g)], and alignment along the slow axis of the \( \lambda \) plate shows a blue color, Fig. 2.5 (h). Pattern of DNA structures with higher topological charge, such as \( m=3/2 \) [Fig. 2.5 (i) and Fig. 2.5 (j)], is also created. The POM image of this pattern shows six extinctions, Fig. 2.5 (k), as confirmed by inserting the \( \lambda \) plate, Fig. 2.5 (l)11.
Fig. 2. (a) Director field of a topological defect with $m=1/2$ and $\theta_0 = \pi/2$. (b) Simulated texture of $1/2$ defect in part (a) between crossed polarizers. POM images of orientations of DNA structures by this $1/2$ defect without (c) and with (d) the $\lambda$ plate. (e) Director field of a topological defect with $m=1$ and $\theta_0 = \pi/2$ adopting pure bent distortions. (f) Simulated texture of circular +1 defect in part (e). POM images of arrangements of DNA structures by this circular+1 defect without (g) and with (h) the $\lambda$ plate. (i) Director field of a defect with $m=3/2$. (j) Simulated texture of 3/2 defect in part (i). POM images of DNA alignment by this+3/2 defect without (k) and with (l) the $\lambda$ plate. The scale bar is 50 μm.
2.3.3 Photopatterning DNA structures with 2D lattice of topological defects

To demonstrate the capability of this photo-patterning technique, DNA patterns with more complex structures are created. An array of (+1/2, -1/2) defects is designed with

$$\theta(x, y) = \sum_i m_i \tan^{-1} \frac{y}{x - ix_0},$$

where $m_i = 1/2$, $m_{i+1} = -1/2$, $x_0 = 50 \mu m$ is the distance between two adjacent half defects.

Fig. 2.6 Photo-patterning DNA structures with 2D array of topological defects. (a) Director field of a 2D lattice of defects (+1/2, -1/2); (b) Simulated texture of director field in part (a) between crossed polarizers; (c)-(d) POM images of DNA alignment by the pattern without (c) and with $\lambda$-plate (d), respectively; (e) Director field of a 2D defect array of (+1, -1); (f) Simulated texture of director field in part (e); (g)-(h) POM images of DNA pattern by the (+1, -1) defect array without (g) and with $\lambda$-plate (h), respectively; (i) Director field of a 2D defect array of (+3/2, -3/2); (j) Simulated texture of director field in part (i); (k)-(l) POM images of DNA alignment by the (+3/2,
-3/2) defect array without (k) and with $\lambda$-plate (l), respectively; P and A represent polarizer and analyzer; Scale bar is 50 $\mu$m.

Then we put these two rows of defect array with a distance $y_0 = 100\mu$m to form a 2D (+1/2, -1/2) defect array, Fig. 2. 6 a-b. DNA chains follow the pre-designed pattern and POM textures are shown in Fig. 2. 6 c-d. A 2D lattice of alternating (+1, -1) defects are created with

$$\theta(x, y) = \sum_{i,j} m_{i,j} \tan^{-1} \frac{y - jy_0}{x - ix_0},$$

where $m_{i,j} = 1$, $m_{i,j+1} = -1$ and $x_0 = y_0 = 100\mu$m is the distance between the defects, Fig. 2. 6 e-f. The POM images in Fig. 2. 6 g-h show the alignment of DNA structures by the predesigned pattern in Fig. 4f. A more complex pattern of DNA ordering with 2D lattice of (+3/2, -3/2) defects are shown in Fig. 2. 6 i-l.

### 2.3.4 Photo-patterning DNA structures with scale-up arbitrary geometries

Scale-up arbitrary shapes of DNA structures can be created by programming the patterns. For example, letters of “U” and “M” with alignment along 135° are designed, Fig. 2. 7 a and the patterns of DNA structures are imaged by POM in Fig. 2. 7 b-c. The domains with “UM” align DNA chains along 135° showing bright texture, Fig. 2. 7 b and the alignment of DNA structures is confirmed with $\lambda$-plate inserted, Fig. 2. 7 c.

Fig. 2. 7 (a) Designed alignment with 135° with respect to the horizontal direction; (b)-(c) POM images of patterns of DNA chains in the form of letters “UM” without (b) and with (c) $\lambda$-plate;
Other scale-up arbitrary patterns such as *Mona Lisa* and *The Starry Night* paintings are also created, Fig. 2. 8 a-b. Fig. 2. 8 a shows the mask of *Mona Lisa* painting with white pixels transmitting light with linear polarization along 45° and black pixels blocking light. POM image shows that DNA chains are patterned following the design with bright domains aligned 45° with respect to the polarizer,

Fig. 2. 8 (a) Mask with pattern of *Mona Lisa* painting with white pixels transmitting linearly polarized light along 45° and black pixels blocking light; (b) POM image of the pattern with bright pixels that DNA chains are aligned 45° following the design.

Fig. 2. 9 a. Mask with pattern of *Van Gogh’s The Starry Night* painting and POM image of the pattern of DNA chains are shown in Fig. 2. 9 a-b. Note that the patterns in these experiments are scaled up to several millimeters range. For higher magnification to obtain larger scale patterns, which will cause more image aberration during the patterning and the resolution will be compromised¹¹.
Fig. 2. 9 (a) Mask with pattern of Van Gogh’s The Starry Night painting (b) POM image of the pattern with bright pixels that DNA chains are aligned along 45° direction.

2.3.5 Result and discussion

The aligned DNA chains are widely used as a template for the self-assembly of bacteria, gold nanorods, thermotropic liquid-crystal molecules, etc. As a type of aqueous lyotropic liquid crystal, the ordered DNA structures shown here can potentially be designed to direct the assembly of peptide amphiphiles and other kinds of biomolecules. The concentration of DNA we use has anisotropic phase at a temperature of 95°C. At temperatures higher than this temperature, the DNA molecules are oriented randomly, but at lower temperatures, such as room temperature, the molecules recover the designed ordered structures. If DNA is used as a template material, this property will help to realize the reconfiguration of suspended biomaterials by simply changing the temperature. Due to the simplicity and versatility of our technique in this work, this programmable and biocompatible soft material, across multiple length scales, will find applications as templating tools for various guest functional materials to follow the orientations of ordered DNA chains. To summarize, space-varying DNA self-assembled structures, in a deterministically predesigned manner, can be created by using the photopatterning technique. Various configurations of DNA
orientations with a 2D lattice of topological defects are achieved by placing DNA molecules inside a cell coated with photopatterned liquid crystalline polymer. Additionally, this method can be finetuned to scale up and arbitrary patterns of DNA chains in the millimeter-scale range are created. The arbitrary patterns of DNA structures are not limited to the current work and can be programmed to different geometries due to the versatility of the designed patterns. The resulting programmable and predesigned DNA self-assembled structures across multiple length scales will open opportunities for designing dynamic functional bionanomaterials and developing electro-optical and biosensing devices.
Chapter 3: Self-assembly of Aqueous Soft Matter Patterned by Liquid Crystal Polymer Networks

3.1 Introductions

In this work, we propose to use liquid crystal polymer networks (LCN) to control the superstructures of one aqueous soft material called lyotropic chromonic liquid crystals (LCLCs) which shows spontaneous orientational order by stacking the plank-like molecules into elongated aggregates. We synthesize a layer of patterned LCN film by a nematic liquid crystal host in which the spatially varying molecular orientations are predesigned by plasmonic photo-patterning. LCLCs are typically plank-like molecules which are dissolved in water and self-assemble into column-type aggregates by $\pi-\pi$ stacking. The resulting aggregates self-organize into a nematic liquid crystal phase when a certain concentration or temperature is reached. Due to its non-toxicity to biological cells, LCLC has been used for bio-detection and controlling the dynamic behavior of bacteria. Among all the strategies to align LCLCs, buffed films of polyimide is the most used due to its simplicity. However, it is very difficult to produce spatially varying alignment of LCLC by using polyimide. Hence, two branches of techniques have been developed. One is to fabricate topographic features such as micro-channels by using lithography technique, and the other one is to use photo-patterned polymers. The microchannels will align the LCLC aggregates parallel to the channel alignment, whereas the photo-patterned polymers will make the LCLC aggregates aligned perpendicularly to the polymer alignment.

As a stimuli-responsive polymer, liquid crystal polymer networks (LCN) have been widely used in soft robotics, artificial muscles and actuators, etc. In this work, we will synthesize a layer of LCN film as the substrate to control the supramolecular self-assembly of LCLCs. This LCN film is composed of polymer filaments which are templated by a nematic liquid...
crystal host. The molecular orientations of this liquid crystal template are predesigned by plasmonic photo-patterning technique\textsuperscript{27}. These patterned polymer filaments form nanoscale spacing between them. When LCLC aggregates are interfacing with this templated LCN film, they are aligned parallel to the patterned polymer alignment, which is fundamentally different from the previous work\textsuperscript{36,37}. It indicates that the polymer filaments with nano-spacing behave similarly as the fabricated micro-channels to confine the LCLC aggregates parallel to the polymer alignment. Based on this finding, we also demonstrate that the dynamics of suspended bacteria in LCLC solution will be directed following the direction of patterned polymer filaments. The demonstrated spatially controlling of molecular assembly of aqueous soft matter by using stimuli sensitive LCN film opens opportunities in advanced functional materials and dynamic biomolecular devices.

3.2 Experimental Results

3.2.1 LCN film fabrication

We used the recently invented photo-patterning technique based on the plasmonic metamasks\textsuperscript{27,28}. The mask is made of a thin layer of aluminum film with spatially varying orientation of nanoslits. A nanoslit has aperture of a length 220 nm and width 100 nm. The light transmitted through the nanoslits get linear polarized perpendicular to the designed nanoslits\textsuperscript{43}. This light of polarization pattern will define the director pattern on the substrates with a layer of photosensitive material of azodye. The azodye SD1 (from \textit{DIC}) is mixed with N, N-dimethylformamide (DMF) solvent at the concentration of 0.2wt\%. The SD1 solution is spin coated on the cleaned substrates which have been treated by oxygen plasma for 1min. The coated substrates are then baked at 120\textdegree C for 15 min. Two SD1 substrates are assembled with 20\,\mu m glass spacers. Afterward, the sample is placed in the photo-patterning setup for 5min and the pattern of light polarization will be imprinted on the SD1 layer, Fig. 3. 1 a.
Fig. 3. 1 Fabrication of patterned liquid crystalline polymer networks (LCN) film. (a) Predesigned patterns are imprinted on the substrates coated with azodye layer; (b) Acrylate-based monomer mixed in nematic liquid crystal solution is filled in the patterned sample; The monomer will be templated by the patterned liquid crystal molecular orientations; (c) The liquid crystal host will be washed out after photo-polymerization; A thin layer of LCN film will be attached on one side of glass substrate

A nematic liquid crystal JXLC2000 (from Grandinchem) is mixed with 5 wt% of acrylate-based reactive monomer RM257 (from Wilshire Technology INC.) and 0.05wt% of photoinitiator Irgacure 651 (from Ciba) on a hot plate with a temperature of 90°C for 5mins. This mixture is vortexed for 2 mins and then heated again for 30 mins to ensure a homogeneous mixture. Afterwards, this mixture is filled into the photo-patterned sample at the temperature of 90°C in isotropic phase, Fig. 3. 1 b. After the sample is cooled to room temperature, the monomers follow the liquid crystal host which is aligned by the director patterns on the SD1 layer. The sample is then polymerized by an unpolarized UV light (UVL-26 Handheld UV lamp, Analytikjena LLC) with the intensity 1.4 mW/cm² and wavelength 365 nm, for 1 hour. This sample is then soaked in Hexane (from Sigma) overnight to ensure the complete removal of LC host from the sample. After it is dried, the sample is placed in the liquid nitrogen tank for 1 min and carefully split by a razor
blade. One thin film of liquid crystalline polymer networks that replicates the director pattern of the LC host is attached to one side of the glass substrate, Fig. 3. 1c.

Fig. 3. 2 show the uniformly aligned polymer chains in the LCN film observed by polarizing optical microscope (POM). It indicates that the LCN film can be fabricated in a predesigned deterministic manner.

Fig. 3. 2 (a) Polarizing optical microscope (POM) image of uniformly aligned LCN film 45° with respect to the polarizer; (b) POM image of uniformly aligned LCN film rotated at 45° with red plate engaged. P and A represent polarizer and analyzer.

In order to show the capability of this technique, LCN film with different director patterns are created. As an example, the circular director field is designed as,

\[ \hat{n} = (n_x, n_y, n_z) = (\cos \theta, \sin \theta, 0), \]

where \( \theta(x, y) = m \tan^{-1} \frac{y}{x} + \theta_0 \), \( m = +1 \) is an integer topological charge and the phase \( \theta_0 = \pi / 2 \) sets the distortion with pure bend, Fig. 3. 3a. The fabricated LCN film with polymer chains in circular geometry is shown in Fig. 3. 3b.
A LCN film with a more complex pattern can also be created with a 2D lattice of alternating (+1, -1) defects as

\[ \theta(x, y) = \sum_{i,j} m_{i,j} \tan^{-1} \frac{y - jy_0}{x - ix_0}, \]

where \( m_{i,j} = 1 \), \( m_{i,j+1} = -1 \) and \( x_0 = y_0 = 100\mu m \) is the distance between the defects, Fig. 3. 4 a. The micrograph of LCN film is shown in Fig. 3. 4 b and the polymer chains follow the designed pattern.
3.2.2 Characterization of LCN film

The patterned LCN film is first characterized by Scanning Electron Microscope (SEM). The LCN film attached on the glass substrate is sputter coated with 60/40 Au/Pd with thickness of 5 nm. SEM imaging is performed by Pheonon Prox (Nanoscience Instruments). Fig. 3. 5 a shows that polymers form the filament structures following the designed pattern shown in Fig. 3. 1 a. The polymer filaments in the LCN film can also be directed into the circular pattern, Fig. 3. 5 b. Another orientational pattern of polymer configuration is shown in Fig. 3. 5 c with the director field \( \hat{n} = (n_x, n_y) = (\cos \theta, \sin \theta) \) with \( \theta(x, y) = m \tan^{-1} \frac{y}{x} + \theta_0 \), where \( m = -1 \) and \( \theta_0 = \pi/2 \). Note that there are several factors such as monomer type, concentration of monomer, polymerization temperature and UV dosage for polymerization influencing the morphology of the polymer filaments\(^{44,45}\). The liquid crystalline monomers used in this work are preferable to form polymer filaments with sizes of about 1 micrometer at the given concentration, temperature and UV dosage during the polymerization process.

Fig. 3. 5 (a) Uniform alignment of polymer filaments in LCN film; Red double-arrow is the uniform alignment direction; (b) Circular aligned polymer structures by the pattern shown in Fig. (c) Patterned LCN film with \( m = -1 \) and \( \theta_0 = \pi/2 \).
The roughness of the LCN film is characterized by a high-resolution 3D Profilometer (Filmetrics Inc.) using white light interferometry. The LCN sample is prepared for 3D profilometry by sputter coating a 10nm layer of AuPd. The 3D profile image of the LCN film is shown in Fig. 3.6 b. The roughness profile is measured across the top surface of the LCN film. It is shown that the height variation of polymer filaments in the LCN film is less than 1μm, Fig. 3.6 a-c, which is less than 5% compared to the LCN film thickness.

Fig. 3.6 (a) 3D profile image of LCN film with circular pattern measured by 3D Profilometer; (a) 2D mapping of the top surface of LCN film in part; (c) Measured roughness profile across the surface of the LCN film indicated by the red line shown in part.

### 3.2.3 Patterning aqueous LCLC

We use LCLC material disodium cromoglycate (DSCG) purchased from Alfa Aesar without further purification. DSCG is dissolved in deionized water at 14 wt% for nematic phase. One drop of DSCG solution is placed on the LCN film and it is covered by another oxygen plasma treated clean substrate\textsuperscript{11}. The gap between these substrates is set by 5μm glass spacers. Then the sample is sealed promptly by a 5min epoxy glue. The sealed sample is heated up to 40°C in the isotropic phase to remove all the flow alignment effect. Then it is cooled to room temperature in nematic phase for further study.
Fig. 3. 7 a-b are the POM images of the DSCG solution interfacing with uniformly aligned LCN film. When DSCG is in the biphasic temperature range, tactoid droplets will form on the surface of the LCN film\textsuperscript{46}. Fig. 3. 7 a show that the tactoids are aligned parallel to the LCN film which is aligned $45^\circ$ with respect to the polarizer. The cusps of spindle-shaped tactoids are tilted $45^\circ$ due to this alignment. As the temperature reaches the nematic phase, the DSCG becomes homogeneously aligned by the LCN film with polymer structures shown in the background within half an hour, Fig. 3.7 b. The column of disks with a double arrow indicates the alignment of DSCG aggregates following the polymer orientation at $45^\circ$\textsuperscript{12}.

![POM image](image1.png)

Fig. 3. 7 (a) As DSCG is in the biphasic temperature range, the spindle-shape tactoid droplets are aligned parallel to the uniform alignment of LCN film; (b) At room temperature, DSCG in nematic phase is homogeneously aligned by the LCN

The spindle-shape tactoids are also aligned by the LCN film with circular director pattern, Fig. 3. 8 a, The POM images of DSCG with the circular director field is shown in Fig. 3. 8 b.
Fig. 3. 8 (a) DSCG tactoids in biphasic temperature range (b) DSCG solution in nematic phase are aligned by the LCN film with a circular director field.

Another 2D lattice of (+1, -1) defects and the POM images of DSCG alignment are shown in Fig. 3. 9 a and Fig. 3. 9 b respectively.

Fig. 3. 9 (a) DSCG tactoids in biphasic temperature range (b) DSCG solution in nematic phase are aligned by the LCN film with 2D lattice of (+1,-1) defects.
3.2.4 Application of patterned aqueous LCLC

A direct application of the patterned LCLC is to interface with biological cells such as bacteria due to its biocompatibility. Bacteria strain 23857 of *Bacillus Subtillis* (from ATCC) with rod-shape of 5-7 μm long and 0.7 μm in diameter is used. They are cultured in a Terrific Broth (from Sigma-Aldrich) liquid medium in a shaking incubator at 35 °C for 12 hours. The bacteria are extracted from the growth medium as completely as possible by centrifugation. DSCG is dissolved in Terrific Broth at the concentration of 14%. Then this solution is added to the concentrated bacteria. The resulting mixture is then vortexed at 3,000 rpm to reach a homogenous dispersion. One drop of this mixture is then placed on the LCN film, which is then covered by another clean substrate. The sample is then sealed, heated to isotropic and cooled to nematic phase for further study.

When the bacteria are suspended in the DSCG patterned by uniformly aligned LCN film, they swim parallel to the uniform alignment of LCN film as shown in Fig. 3.10.

![Fig. 3. 10 Time sequence of images of two bacteria swimming in opposite directions parallel to the uniform alignment of LCN film scale bar 10μm.](image)

These two bacteria are circled by red lines and move in opposite direction indicated by white and red arrows respectively.
Fig. 3. 11 (a) Trajectories of swimming bacteria following the uniform alignment of LCN film; Different color codes indicate different bacterium individuals; (b) Measured probability of swimming direction of bacteria with respect to the alignment orientation of LCN film scale bar 100µm

The direction of bacteria motion is within 20° range compared to the orientation of LCN film, Fig. 3. 11 b. This is another evidence that the LCLC aggregates are aligned parallel to the polymer orientations in LCN film.

The trajectories of the bacteria can also be controlled by a patterned DSCG in the LCN film with a circular director field, Fig. 3. 12 a-c. The bacteria swim parallel to the circular polymer orientations in a bi-directional manner, Fig. 3. 12 c.

Fig. 3. 12 (a-b) Trajectories of swimming bacteria in the DSCG patterned by circular aligned LCN film; (c) Probability of azimuthal velocity $v_{\phi}$ of the swimming bacteria.
3.3 Results and Discussion

In the prior work ref. 36,37, the LCLC aggregates are aligned perpendicularly to the patterned polymers. However, in this work, the superstructures of LCLC molecules are aligned parallel to the patterned polymer filament structures in the LCN film. It has been demonstrated that due to the confinement effect, the LCLC aggregates are parallel to the microchannels fabricated by lithography technique 34,35. This indicates that the nanoscale spacing between the polymer filaments in the LCN film behave similarly as the fabricated microchannels to confine the LCLC aggregates parallel to the alignment of LCN film. However, the differences between these two techniques are also quite remarkable. The microfabricated channels are usually made with micron size periodicity, but there is no regular periodicity in the polymer network, as shown in Fig. 3. 6 a-c. Besides, the polymer chains inside the LCN film are very dense with nanometer scale spacing which is comparable to the chromonic LC molecular size. Hence, we attribute the alignment of chromonic LC aggregates by the LCN film to the confinement effect between the nanoscale spacing in the polymer chains. Based on this property, different director fields of LCLC aggregates patterned by the LCN film are demonstrated. This opens opportunities to more general biological applications involving living biological units. To this end, we demonstrate that by suspending bacteria in the patterned LCLC solution by LCN film, the dynamics of the bacteria can be directed by fine tuning the director field of LCN film.
Chapter 4: More Application of patterned LCN

When neural tumor cells were plated onto the templated LCN films, the cell alignment, migration, and proliferation were directed in both linear and curvilinear fashions following the pattern of the aligned polymer chains.

4.1 Controlled dynamics of neural tumor cells by uniformly aligned LCN

In order to test the directed dynamics of cells on the linear pattern aligned LCN film, human glioblastoma A172 cells due to their high motility. These cells were permanently transfected with mCherry red fluorescent protein to enable to observe them by fluorescence microscopy. A172 cells plated on the LCN were viable and proliferated as shown by the finding that cells plated at low density rapidly reached confluence. When confluence was reached, time-lapse live cell imaging was performed for 24 hours with a laser scanning confocal fluorescence microscope to monitor the behavior of the mCherry transformed cells.

Fig. 4. 1 Dynamics of the A172 neural tumor cells on the uniformly aligned LCN film. (a) Laser scanning fluorescence confocal microscope image of subconfluent A172 cells aligned following
the uniform alignment of LCN film; (b) Higher magnification fluorescent image of aligned A172 cells; (c) Trajectories of motion of A172 cells directed by the uniform alignment of LCN as seen during a 24 hours time lapse; (d) SEM image of the A172 cell alignment attached on the LCN film; (e) SEM image of one A172 cell attached to the uniformly aligned polymer networks; (f) Probability distribution (p) of the angle between cell migration and the alignment of LCN. Note that the white double arrow in all images indicates the direction of alignment of LCN.

The results showed that the cells were aligned uniformly along the direction of the aligned polymer chains, Fig. 4. 1 a-b. Cell motility was analyzed with Image J on time-lapse. The analyzed trajectories in Fig. 4. 1 c show that the cell migration was directed by the linear pattern of the LCN film. The cells moved at a speed of 3-10 μm/h. The angles between the cell motion and the alignment of polymer chains are in the range of less than 20°, Fig. 4. 1 f. The collective alignment of the cells was also visualized by SEM, which clearly showed the alignment of the cells was directed by the uniformly aligned LCN, Fig. 4. 1 d. Fig. 4. 1 e shows an individual cell body following the local alignment of the polymer network.

### 4.2 Curvilinear cell dynamics directed by circularly aligned LCN film

A172 cells plated on circularly aligned polymer networks did align curvilinearly following the circular LCN pattern, Fig. 4. 2 a. Since A172 cells are of neural origin, they have the capability to grow cytoplasmic extensions, similar to those found in differentiated neurons and astrocytes. Fig. 4. 2 a also shows that A172 cells can extend long cytoplasmic extensions and that these are directed curvilinearly by the circular alignment of the polymer network. The live imaging of the cell trajectories on the LCN film with a circular pattern were recorded and analyzed, Fig. 4. 2 b. The results show that the cells migrate along the circular tracks formed by the circularly aligned polymer chains. The azimuthal velocities of the cells show equal probability for them to move...
clockwise or counterclockwise, Fig. 4. 2 d. SEM image of the cells provided further evidence that the cell alignment or organization was manipulated by the templated pattern of the LCN film, Fig. 4. 2 c.

Fig. 4. 2. (a) Laser scanning fluorescence confocal microscopy image of curvilinear alignment of A172 cells on the LCN film with a director pattern of circular +1 defect; the fluorescence signal of the cells is overlayed with the circular LCN pattern; (b) curvilinear trajectories of directed A172 cells on circularly aligned LCN as determined during 24 hours; also see Supplementary Movie 4; (c) SEM image showing that cells plated on an LCN film with a circular pattern organize themselves in a circular manner; (d) probability distribution ($p$) of azimuthal velocity ($v_\phi$) of A172 cells migrating on the LCN film.
To summarize, space-varying DNA self-assembly structures in a deterministically predesigned manner can be created by using photo-patterning technique. Various configurations of DNA orientations with 2D lattice of topological defects are achieved by placing DNA molecules inside a cell coated with photo-patterned liquid crystalline polymer. Besides, this method can be fine-tuned to scale up and arbitrary patterns of DNA chains in the scale of millimeter range are created. The arbitrary patterns of DNA structures are not limited to the current work and can be programmed to other different geometries due to versatility of designed patterns. The resulting programmable and predesigned DNA self-assembly structures across multiple length scales will open opportunities for designing new dynamic functional bionanomaterials and developing electro-optical and biosensing devices.

we propose to pattern the aqueous soft matter, namely LCLC, through a photopatterned thin film of liquid crystalline polymer networks (LCN). We demonstrate that the LCLC aggregates are oriented parallel to the polymer filaments in the LCN film. The molecular orientation of the LCN film can be templated by a nematic liquid crystal host with topological defects and 2D defect lattice structures. The demonstrated capability shows immediate application of controlling the dynamics of biological units suspended in the patterned LCLC solution. The demonstrated spatially controlling of molecular assembly of aqueous soft matter integrated with its biocompatibility by using a stimulus sensitive LCN film opens new opportunities in advanced dynamic functional materials and their bioengineering applications.

The patterned polymer chains in the LCN film can direct the alignment of the long axis of cells and the trajectory of migration for both interphase cells and daughter cells immediately after cell division in both linear and curvilinear manners. The LCN films can also promote population
of cells to follow the polymer alignment in patterns resembling cell organization in tissues. Only recently, Sano et al.\textsuperscript{49} and Ladoux et al.\textsuperscript{50} observed that neural progenitor cells and epithelial cells can spontaneously form the nematic liquid crystal phase with topological defects, respectively. These randomly formed topological defects can orient cell migration and growth. Hence, the demonstrated capability in this work opens opportunities to imprint the topological defects in LCN films as a scaffold to provoke the organization of cell population in a controlled manner resembling that found in different biological tissues. Besides, due to the LCN’s sensitivity to external stimuli such as temperature and optical field \textsuperscript{51}, the cell dynamics and growth can also be regulated by programming the responsiveness of LCN. Such efforts are in progress.

The glioblastoma cell line used in this work extends cytoplasmic processes similar to those extended by neurons. We also show that these cytoplasmic extensions follow the directional cues imparted by the LCN pattern. The finding of the directional growth of cell extensions is a steppingstone for future studies on the use of templated LCN films to direct neuronal cell adhesion and the directionality of axons. This also suggests that patterned polymer networks can serve as scaffolds providing structural support and directional information for neuronal cell growth and axons, which are properties that can find application for nerve regeneration after injury.

These templated LCN films have predesigned polymer network patterns which can serve as pathways to control the direction of cell alignment, migration and orientation after mitosis. The capabilities demonstrated in this work open opportunities to employ synthesized polymer networks with versatile designed patterns to influence and promote the functionality and organization of cell populations in a manner that may lead to new advanced materials for nerve repair, tissue engineering and regenerative medicine applications.
References


