Chapter 3  Nematic DNA thermotropic liquid crystals with photo-responsive mechanical properties

Abstract

Over the last decades, water-based lyotropic liquid crystals (LLCs) of nucleic acids have been extensively investigated because of their important role in biology. Alongside, solvent-free thermotropic liquid crystals (TLCs) from DNA are gaining great interest, owing to their relevance to DNA-inspired optoelectronic applications. Up to now, however, only the smectic phase of DNA TLCs has been reported. The development of new mesophases including nematic, hexagonal, and cubic structures for DNA TLCs remains a significant challenge, which thus limits their technological applications considerably. In this work, we demonstrate a new type of DNA TLC that is formed by electrostatic complexation of anionic oligonucleotides and cationic surfactants containing an azobenzene (AZO) moiety. DNA-AZO complexes form a stable nematic mesophase over a temperature range from -7 to 110 °C and retain double-stranded DNA structure at ambient temperature. Photoisomerization of the AZO moieties from the E- to the Z-form alters the stiffness of the DNA-AZO hybrid materials opening a pathway towards the development of DNA TLCs as stimuli-responsive biomaterials.
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Introduction

Lyotropic liquid crystals (LLCs) of DNA obtained by supramolecular self-assembly have attracted considerable attention as these condensed DNA mesophases have broad biological and biotechnological significance in an aqueous environment.1-7 A variety of LLC structures ranging from nematic phases, over smectic and hexagonal states to cubic phases based on pristine DNA8-15 and DNA-surfactant complexes16-28 have been obtained by tuning DNA concentration, length, and surfactant type. These seminal findings enabled the development of powerful tools for drug delivery and gene therapy applications.29-32 Although most investigations of DNA LCs are currently limited to aqueous solutions, research examining anhydrous DNA thermotropic liquid crystals (TLCs) is gaining momentum33 owing to its relevance in DNA-based optoelectronic applications.34-37 Recently, for example, a new class of smectic DNA TLCs has been generated by electrostatic complexation of single stranded (ss) oligonucleotides with surfactants containing two flexible alkyl chains.38,39 Based on this type of DNA TLCs, an electrochromic device that exhibits a clock function and a ceiling temperature indicator was fabricated.40 Despite these advances it is imperative to enrich ordered mesophases of DNA TLC materials with a greater degree of tunability and control over their electrical, optical, and mechanical properties as well as to retain the double-stranded (ds) conformation of DNA in DNA-TLCs.

Light can be dosed with spatial, temporal, and energetic control and hence can be regarded as the optimal physicochemical stimulus to manipulate the properties of TLCs.41,42 Consequently, there has been widespread and persistent interest in the development of photo-responsive TLC polymers exploiting the characteristics of photoisomerization and photoalignment.43,44 For example, with the introduction of photochromic azobenzene units into TLCs, molecular switching events can be transmitted and amplified from the molecular to the macroscopic level in the form of mechanical work.45 Hence, this opens an attractive route to realize actuator,46,47 plastic motor,45,48 shape memory,49,50 and solar-energy harvesting systems.51 To implement these attractive features of TLC polymers into a biological context, there is considerable demand for the development of TLC systems based on biomacromolecular architectures with photo-responsive mechanical properties52-54 for the fabrication of smart soft biomaterials utilized as artificial skins and muscles.

Here we report a new class of DNA TLC materials exhibiting nematic mesophases whose mechanical behaviors can be controlled conveniently by irradiation with light. Since DNA TLCs exhibit remarkable mechanical properties,38 we reasoned that modulation of these characteristics by the means of light-irradiation would add an unprecedented level of control over this type of material.
Nematic DNA thermotropic liquid crystals with photo-responsive mechanical properties

Nematic DNA TLCs were prepared by electrostatic complexation of double- and single-stranded oligonucleotides with cationic surfactants containing two aliphatic chains and one aromatic azobenzene moiety (AZO) followed by dehydration. The light-induced \( E \)- to \( Z \)-isomerization proceeds smoothly in these solvent-free DNA-AZO complexes and grants access to photocontrol over their mechanical performance in case of double stranded DNA.

**Results and discussion**

Initially, we synthesized a new cationic surfactant containing a quaternary ammonium group and two hydrophobic alkyl chains that are separated by a benzoic acid unit and an azobenzene moiety (AZO) through a four-step route (Scheme 1, experimental details and characterization are presented in the Supporting Information). In brief, the hydroxy group in position 4 of the \( \beta \)-resorcylic acid derivative 1 was reacted with bromobutane to yield the corresponding ether 2. Subsequently, the second hydroxyl group in position 2 was also converted into the corresponding ether by employing 1,4-dibromobutane to yield compound 3. The quaternary ammonium compound 4 was obtained by employing trimethylamine. In a transesterification reaction, 4-((4-octylphenyl)diazenyl)phenol was introduced to furnish the azobenzene containing surfactant 5 (AZO) exhibiting branched alkyl chains.

\[ \text{Scheme 1. The synthesis route of the cationic surfactant (AZO) containing a quaternary ammonium group, two hydrophobic alkyl chains and two aromatic units including an azobenzene moiety.} \]

Afterwards, the photoisomerization behavior of the AZO surfactant was investigated employing UV-vis absorption spectroscopy during irradiation with UV-light (Figure 1A). Before UV-
irradiation, the azobenzene exists predominantly in the $E$-form characteristically indicated by the $\pi$-$\pi^*$ transition at ca. 330 nm.$^{41,55}$ We attribute the absorption band with a maximum at about 260 nm to the $\pi$-conjugated benzene rings present in both isomers. Upon UV-irradiation at 365 nm, the absorption intensity of the $\pi$-$\pi^*$ transition at around 330 nm decreased and the n-$\pi^*$ absorption peak at ca. 434 nm increased indicating successful $E$- to $Z$-photoisomerization of the AZO surfactant. Based on the time-dependent UV-vis absorption spectra, an $E$- to $Z$-isomerization efficiency of at least 84% was determined in the AZO surfactant, which is in agreement with the analysis of proton nuclear magnetic resonance spectroscopy ($^1$H-NMR) (Figure S7). After the exposure to visible light (450 nm,), the azobenzene could not be transformed back fully to the original $E$-form (Figure S8). Due to the absence of an isosbestic point over the course of the irradiation, we attribute this effect to unspecific photodegradation of the AZO surfactant due to prolonged light exposure under aerated conditions.

Further characterization of the pristine surfactant was conducted by polarized optical microscopy (POM) analysis revealing that the lyophilized AZO surfactant is birefringent (Figure 1B), highly viscous, and has a TLC phase at room temperature. Differential scanning calorimetry (DSC) unraveled two endothermic peaks at -5 and 50 °C corresponding to the crystalline-LC and LC-isotropic transitions, respectively (Figure 1C). The ordered structural features of the AZO surfactant were analyzed by small-angle X-ray scattering (SAXS). In the AZO mesophase, the sharp first-order reflection peaks of $E$- and $Z$-isomers and their following harmonics are characteristic of long-range ordered lamellar structures (Figure 1D, black curve). Based on the analysis of the two sharp first order reflection peaks at $q = 1.64$ nm$^{-1}$ and $q =1.83$ nm$^{-1}$ ($d = 2\pi/q_1$), the smectic layer spacing of the two isomers can be determined to 3.82 nm for the $E$- and 3.43 nm for the $Z$-isomer, respectively, approximately corresponding to the molecular dimensions of the AZO isomers. We note that the layer spacing difference of ca. 0.39 nm of the two isomers also corresponds to the length change of about 0.35 nm from $E$- to $Z$-azobenzene.$^{56,57}$ Moreover, after irradiation with UV-light overnight, the sample was remeasured by SAXS at room temperature. We found that the intensity of the first-order diffraction of $E$-AZO decreased significantly and the diffraction peak of $Z$-AZO was increased (Figure 1D, red curve) clearly indicating that the $E$- to $Z$-isomerization of azobenzene can be performed successfully in the liquid crystalline phase. It should be noted, however, that unspecific photodegradation of the AZO molecule cannot be excluded fully because of UV exposure overnight. Conversely, after irradiation with 450 nm light for 24 h, the $Z$- to $E$-isomerization could not be observed in the AZO LC material. Packing effects may be responsible
for this irreversible photoisomerization behavior in the AZO LC phase as the isomerization process is accompanied by a considerable geometrical rearrangement.58

Figure 1. Characterization of the pristine AZO surfactant. (A) UV-vis absorption spectra of the AZO surfactant in aqueous solution over the course of irradiation with UV-light accompanied by E- to Z-isomerization (concentration 65 μM). (B) POM image of the birefringent AZO surfactant at room temperature. Scale bar is 100 μm. (C) DSC traces with phase transition temperatures of the AZO surfactant (at a heating/cooling rate of 5 °C·min⁻¹). Two endothermic peaks at -5 and 50 °C indicate the crystalline-LC and LC-isotropic transitions of the AZO surfactant. (D) SAXS profiles of the AZO surfactant recorded before and after the application of UV irradiation. The sharp first-order reflection peaks of E- and Z-isomers and their following harmonics are characteristic of long-range ordered lamellar structures of the AZO surfactant. The inset represents the molecular packing model of the surfactant. Isomerization of the AZO samples was performed employing a UV-lamp (0.5 mW·cm⁻²) at λexc = 365 nm

Subsequently, a 22mer single stranded oligonucleotide and its complementary sequence were synthesized by conventional solid-phase synthesis.59 The purity and molecular weight were confirmed by polyacrylamide gel electrophoresis and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) respectively (Figure S9). Mixing of an aqueous solution of 22mer dsDNA with cationic surfactants (AZO) results in precipitation of the dsDNA-AZO complex, which is then obtained in pure form after centrifugation and lyophilization
Quantitative component determination of the dsDNA-AZO complex can be carried out by NMR (Figure S10) revealing the stoichiometry of the 22mer dsDNA and the AZO surfactants to be 1:52 (i.e. ca. 1.2 AZO surfactant molecules per phosphate of the oligonucleotide). This indicates that a small number of extra surfactant molecules are present in the complex which we attribute to the π-π and alkyl chain interactions among the AZO surfactants. Thermogravimetric analysis (TGA) of the dsDNA-AZO sample revealed a water content of less than 5 wt% and also demonstrated the thermal integrity of the complex up to 200 °C. Above this temperature, decomposition starts (Figure S11). At room temperature, the dsDNA-AZO material is soft and can be pressed. Birefringence with typical schlieren textures is observed (Figure 2B) indicating the formation of a nematic TLC phase in the dsDNA-AZO complex. Temperature-dependent POM analysis showed that the birefringent nematic textures melt away completely above 110 °C leaving only transparent isotropic liquid (Figure S12). Differential scanning calorimetry (DSC) results showed one broad endothermic peak from 45 to 120 °C in the virgin heating cycle, which may correspond to thermal de-hybridization of dsDNA in the TLC material (Figure S13). Additionally, a phase transition from crystal to LC was observed at around -7 °C.

Moreover, the ordered nematic features of the dsDNA-AZO TLC were analyzed by SAXS revealing a broad diffraction peak corresponding to the d spacing of 4.95 nm (Figure 2C, black curve). Besides the use of ds DNA, nematic TLCs from ss DNA were fabricated (Figure S14 and S15) and SAXS data indicate a nematic mesophase with a diffraction spacing of 4.5 nm for this LC complex. We attribute these values to the average diameter of the DNA-surfactant complexes that are composed of DNA units of 1-2 nm thickness and interdigitated AZO surfactant molecules of 3-4 nm thickness. It should be noted that this average diameter of the ssDNA-AZO complex was only 0.55 nm smaller than the one of the mesophase formed by dsDNA-AZO, which is less than the diameter difference (ca. 1 nm) of ssDNA and dsDNA.60,61 We ascribe this difference to partial denaturation of the double stranded DNA in the solvent-free dsDNA-AZO mesophase. Additionally, the DNA-AZO TLC materials were characterized by SAXS after UV-irradiation (Figure 2C, red curve; Figure S14B, red curve). We found that the diffraction spacing decreased hinting towards a successful E- to Z-isomerization in the TLC materials. However, unspecific photodegradation of the AZO molecule may contribute to this decrease because of overnight UV exposure. The most important result of the structural analysis is that the electrostatic assembly of anionic DNA and cationic AZO induces rearrangement of the AZO molecules from an originally lamellar phase in the pristine AZO surfactant (Figure 1) to a nematic mesophase in the DNA-AZO complexes.
**Figure 2.** Preparation and characterization of the dsDNA-AZO nematic liquid crystals. (A) The dsDNA-AZO nematic TLC material is formed by electrostatic complexation of double-stranded 22mer oligonucleotides and AZO surfactants. (B) POM image of the dsDNA-AZO complex at room temperature showing typical schlieren textures characteristic of a nematic mesophase. Scale bar is 100 μm. (C) SAXS profiles of the dsDNA-AZO complex (25 °C) recorded before and after the application of UV-irradiation. Two broad diffraction peaks corresponding to the d spacing of 4.95 and 4.65 nm indicate the E- to Z-isomerization of the azobenzene moiety in the nematic TLC material. Isomerization of the dsDNA-AZO samples was performed employing a UV-lamp (0.5 mW·cm−2) at λexc = 365 nm.

In order to study the mechanical properties of the TLC materials, we subjected them to dynamic mechanical analysis employing a shear rheometer. We determined storage moduli (\(G'\)) representing the elastic portion and loss moduli (\(G''\)) as a measure for the viscous portion at an applied strain of 0.5. Expectedly, the pristine AZO surfactant behaves liquid-like as evidenced by the larger loss moduli (\(G''\)) compared to the storage moduli (\(G'\)) over the measured frequency range (0.1-20 Hz) (Figure 3A, black curve). When the nematic DNA-AZO samples were measured, typical LC viscoelasticity was observed by the emergence of a \(G'\) to \(G''\) crossover at 11 Hz (Figure 3A, blue curve and red curve). We attribute the increased elastic moduli of the DNA-AZO mesophases, as
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cmpared to the pristine AZO surfactant, to the additionally introduced DNA backbone. Notably, when backbone rigidity increased from ssDNA to helical dsDNA the stiffness of the TLC materials was enhanced and viscosity increased significantly (Figure 3B). The mechanical properties of the complexes comprising Z-isomer could not be investigated employing shear rheometry as most likely penetration depth of UV-light into the film is not sufficient to change the bulk mechanical properties of the sample sufficiently to be detectable beyond the error of measurement.

![Figure 3. Dynamic mechanical analysis of the TLC materials employing a shear rheometer. (A) Storage (G') and loss (G'') moduli as function of shear frequency of the AZO surfactant (black curve), the dsDNA-AZO (pink curve), and the ssDNA-AZO (blue curve) complexes (ε = 50%, T = 25 °C) and (B) corresponding viscosity measurement at f = 1 Hz (ε = 50%, T = 25 °C).](image)

Therefore, we investigated the photo-responsive mechanical properties of the TLC materials employing AFM-based nanoindentation. Three thin films including pristine AZO surfactant, dsDNA-AZO, and ssDNA-AZO were prepared by drop-casting and deposited on Si substrates. Firstly, the pristine film of AZO surfactant was investigated. Upon UV-irradiation (λ_{exc} = 365 nm, 3.6 mW·cm^{-2} for 15 minutes) alteration of the surface topography was observed by AFM imaging (Figure 4). Concomittantly, the stiffness of the material decreased significantly as expressed through the spring constant k. Notably, no heating of the film sample was noticed while irradiating our setup. The observed effect thus indicates that the E- to Z-isomerization of the azobenzene moiety can alter the stiffness of the surfactant in the film.
Figure 4. AFM-based nanoindentation on a thin film of the AZO surfactant. (A) Surface topography of the film before UV-irradiation with a roughness of around 13 nm (color scale 15 nm). AFM image was taken in peak force tapping mode using SNL-A cantilever. (B) Superposition of F-D curves taken from the surface in panel A, at a tip velocity of 1 µm·s⁻¹. (C) Histogram of calculated spring constant of the AZO thin film from the F-D curves in panel B, considering \( K_{\text{cantilever}} = 0.35 \) N·m⁻¹, \( K_{AZO} = 0.241 \pm 0.031 \) N·m⁻¹. (D) Surface topography of the film after irradiation with UV-light (\( \lambda_{exc} = 365 \) nm, 3.6 mW·cm⁻², 15 min). The roughness decreased to around 2 nm (color scale 3 nm). (E) Superposition of F-D curves taken from the surface in panel D. (F) Histogram of calculated spring constant of the AZO thin film from the F-D curves in E, \( K_{AZO} = 0.145 \pm 0.0 \) N·m⁻¹.

Next, the AFM measurements on the dsDNA-AZO sample were carried out and revealed notable changes in the mechanical characteristics over the course of irradiation with UV light. Before UV exposure, the sample surface was heterogeneous with an elastic constant varying...
Figure 5. AFM-based nanoindentation on a thin film of the dsDNA-AZO complex. (A) Surface topography of the film before irradiation with UV-light, with a roughness of around 65 nm (color scale 86 nm). (B) Superposition of F-D curves taken from the surface in panel A, at a tip velocity of 1 μm·s⁻¹. (C) Histogram of calculated spring constant of the dsDNA-AZO thin film from the F-D curves in panel B with $k_{	ext{cantilever}} = 0.35$ N·m⁻¹. The calculated spring constant is highly variable from 1.4 to 12.0 N·m⁻¹. (D) Surface topography of the film after the application of UV-light ($λ_{	ext{exc}} = 365$ nm, 3.6 mW·cm⁻², 15 min). The roughness is around 65 nm. (E) Superposition of F-D curves taken from the surface in panel D. (F) Histogram of calculated spring constant of the dsDNA-AZO film from the F-D curves in E, $K_{\text{dsDNA-AZO}} = 1.6±0.5$ N·m⁻¹.

between 1.4 and 12.0 N·m⁻¹ (Figure 5). However, after the samples were irradiated with UV-light, a surprisingly homogeneous stiffness of the same surface with an elastic constant of 1.6±0.5 N·m⁻¹ (mean ± sd) was measured. The film also became more soft as evidenced by the absent of breakthrough events in the force-distance curve. Control experiments involving the ssDNA-AZO film
indicate no apparent difference in stiffness before and after UV-irradiation for these films (Figure S16). These results suggest that photoswitching of the azobenzene moiety in the dsDNA-AZO complex might induce a structural change large enough to be reflected in the macroscopic properties of the material. Interestingly while the pristine films of AZO surfactant show a large change in surface roughness after UV irradiation, this change is absent in the dsDNA-AZO and ssDNA-AZO TLC materials.

Conclusions

A structurally novel azobenzene surfactant containing a quaternary ammonium group and two alkyl chains was successfully synthesized. The pristine surfactant material forms a broad mesophase with a layered structure. Once it is complexed with DNA, photo-responsive nucleic acid-based thermotropic liquid crystals (TLCs) are obtained. In stark contrast to the pristine surfactant, a nematic mesophase over a broad temperature range from -7 to 110 °C was detected. The hybridization state of double stranded DNA is preserved in the TLCs at room temperature. The viscoelastic properties (elastic moduli and viscosity) of the DNA-AZO complexes correlate with the structure of DNA. In comparison to previous reports involving smectic DNA TLCs containing another type of surfactant, which lacks an aromatic moiety, we conclude that the introduction of an appropriate surfactant is important to modulate the mesophase structure and mechanical performance of the resulting complexes. Remarkably, upon irradiation with UV-light, the E- to Z-isomerization of the azobenzene moiety was successfully realized in the solvent-free DNA-AZO TLC materials. Concomitantly, photo-responsive mechanical manipulation could be achieved, whereby the stiffness of the TLC materials is in general smaller after the treatment with UV light. The characteristics of DNA fluidity and stimuli-responsive mechanical behavior might allow the development of DNA-based smart materials by further exploiting their recognition or self-healing properties. Furthermore, it should be mentioned that sunspecific photodegradation of the AZO moiety exists in the TLC systems. Thus, optimization of the electronic properties of the AZO molecules to improve their photostability will play an important role to expand multifunctionality and practical applications of DNA-AZO TLC materials.

Experimental section

Materials: 4-octylanilne, phenol, 2,4-dihydroxybenzoic acid, 1-bromobutane, 1,4-dibromobutane, N,N'-dicyclohexylcarbodiimide (DCC, 99%), 4-dimethylaminopyridine (DMAP, 99%), and trimethylamine solution (4.2 M) were obtained from Sigma-Aldrich. All the starting compounds for
the synthesis of AZO surfactant were used without further purification. All solvents and reagents for oligonucleotide synthesis were purchased from Sigma-Aldrich and Novabiochem (UK). Solid supports (Primer SupportTM, 200 µmol∙g⁻¹) from GE Healthcare were used for the synthesis of DNA. 3-hydroxypicolinic acid was used as matrix during mass spectrometry. Ultrapure water with a resistivity of ca. 18.2 MΩ·cm was used for all experiments. Other solvents used in the work were analytical grade.

**Characterization:** UV-Vis spectra were measured on a JASCO V-630. FT-IR spectra were recorded by a Bruker IFS88 instrument. NMR spectra were measured on a Varian Mercury NMR spectrometer at 25 °C (400 MHz for ¹H-NMR; 100MHz for ¹³C-NMR). Liquid chromatography-mass spectrometry (LC-MS) analysis were performed on a Waters Xevo G2 UPLC/TOF. DNA sequences were purified by high-performance liquid chromatography (HPLC, ÄKTA DNA explorer, GE Healthcare). Mass spectrometric analysis was performed using a 4800 MALDI-TOF/TOF Analyzer. Polarized optical microscopy (POM) was conducted on a Zeiss Axiophot. Thermogravimetric analysis (TGA) was carried out using a TA Instruments Q1000 system in a nitrogen atmosphere and with a heating/cooling rate of 10 °C/min. Small-angle X-ray scattering (SAXS) was performed by employing a conventional X-ray source with radiation wavelength of λ=1.54 Å and a Bruker Nano/microstar machine was used to obtain small angle scattering profiles, where the sample-to-detector distance was 24 cm. The sample holder is a metal plate with a small hole (diameter ca. 0.25 cm, thickness ca. 0.15 cm), where the X-ray beam passes through. The scattering vector \( q \) is defined as \( q = 4\pi\sin\theta/\lambda \) with \( 2\theta \) being the scattering angle. Rheology was investigated by a shear strain controlled Bohlin VOR rheometer (Bohlin Reologi AB) with two stainless steel fixtures.

AFM based nanoindentation experiments were carried out using Bruker cantilever SNL-A. The force-distance (F-D) curves were recorded at a tip velocity of 1 µm/s. The spring constants of the film surface were calculated from the linear fraction of the F-D curves. The slope of the indentation curves provides the effective spring constant \( (K_{\text{effective}}) \) fitted to the equation \( 1/K_{\text{effective}} = 1/K_{\text{Cantilever}} + 1/K_{\text{film}} \), i.e. considering the film and the cantilever serve as two springs in series. The cantilever spring constant \( (K_{\text{Cantilever}}) \) is regarded as 0.35 N/m, which is the nominal value provided by the manufacturer (Bruker). Notably, all the experiments are comparative in nature, i.e. comparison of the same film before and after the application of UV light irradiation. The calculated spring constant is not the absolute value of the material, since it would be affected by the absolute spring constant of the cantilever, the thickness as well as the roughness of the film.
**Synthesis details and characterization of AZO surfactant:** the cationic surfactant containing a quaternary ammonium group and two hydrophobic alkyl chains that are separated by a benzoic acid unit and an azobenzene moiety (AZO), was synthesized in six steps from commercially available starting materials (Scheme S1). The synthesis route was inspired by procedures for similar azobenzene derivatives reported in the literature (Scheme S1). The synthesis of the azobenzene derivative I and the ethyl ester of β-resorcylic acid 1 were already described in the literature and were prepared accordingly.66,67

**Scheme S1. Synthesis scheme of AZO surfactant 5.**

Step 1: synthesis of 4-((4-octylphenyl)diazenyl)phenol (I).

4-octylaniline (2.5 g, 12.2 mmol) was dissolved in a mixture of diluted aqueous HCl (25 mL, 3M) and EtOH (25 mL) and stirred in an ice bath. To this solution, cold aq. NaNO2 sol. (0.894 g, 13 mmol dissolved in 10 mL H2O) was added dropwise and stirring was continued for 0.5 h. Then, the solution of the diazonium salt intermediate was added dropwise to an aqueous solution (20 mL) containing phenol (1.15 g, 12.2 mmol) and NaOH (0.96 g, 24.4 mmol) at 0 °C. After stirring overnight, the resulting solution was neutralized with HCl. The precipitate was filtered off and washed with water. After lyophilization overnight, product I was obtained in 88% yield. 1H-NMR (400 MHz, CD3OD): δ = 7.81-7.72 (m, 4 H), 7.33-7.28 (m, 2 H), 6.93-6.87 (m, 2 H), 2.70-2.64 (m,
2 H), 1.66-1.64 (m, 2 H), 1.36-1.27 (m, 10 H), 0.92-0.86 (m, 3 H). $^{13}$C-NMR (100 MHz, CDCl$_3$): $\delta$ = 158.4, 150.9, 147.1, 146.2, 129.2, 124.9, 122.7, 116.0, 36.0, 32.0, 31.4, 29.6, 29.4, 22.8, 14.3.

**Figure S1. NMR analysis of compound I.** a) 1H-NMR spectrum of I (25 °C, 400 MHz, CD3OD). b) 13C-NMR spectrum of I (25 °C, 100 MHz, CDCl$_3$).

**Step 2: synthesis of ethyl 2,4-dihydroxybenzoate (I).**

2,4-dihydroxybenzoic acid (25 g, 162.2 mmol) was dissolved in EtOH (125 mL) and then heated to 60 °C. To the solution, concentrated H$_2$SO$_4$ (9 mL) was added dropwise. Then, the reaction was refluxed overnight. After cooling to r.t., the pH of the solution was adjusted to 5-6 using a sat. aq. NaHCO$_3$ sol. Et$_2$O was used to extract the reaction solution and after the evaporation of the solvent
in vacuo, the crude product was purified through column chromatography on silica gel (EtOAc:hexane = 1:8). The yield was 72%. \(^1\)H-NMR (400 MHz, \((\text{CD}_3)_{2}\text{SO})\): \(\delta = 10.81\) (s, 1 H), 10.47 (brs, 1 H), 7.63 (d, J=8.8 Hz, 1 H), 6.37 (dd, J=8.8 Hz, 1 H), 6.29 (d, J=2.4 Hz, 1 H), 4.30 (q, J=7.2 Hz, 2 H), 1.30 (t, J=7.2 Hz, 3 H). \(^{13}\)C-NMR (100 MHz, \((\text{CD}_3)_{2}\text{SO})\): \(\delta = 169.5, 164.4, 163.1, 131.6, 108.4, 104.1, 102.6, 60.8, 14.1\).

\textbf{Figure S2.} NMR analysis of compound 1. \(a\) \(^1\)H-NMR spectrum of 1 (25 °C, 400 MHz, \((\text{CD}_3)_{2}\text{SO})\). \(b\) \(^{13}\)C-NMR spectrum of 1 (25 °C, 100 MHz, \((\text{CD}_3)_{2}\text{SO})\).

Step 3: synthesis of ethyl 4-(butoxy)-2-hydroxybenzoate (2).

1-bromobutane (1.4 mL, 13 mmol) was added dropwise to an acetone solution (30 mL) of compound 1 (2 g, 11 mmol) and K₂CO₃ (2.28 g, 16 mmol) and the mixed solution was heated at 70 °C for 24 h. When the solvent was evaporated, the crude product was re-dissolved in CHCl₃ and then filtered. After removing CHCl₃ \textit{in vacuo}, the residue was purified by column chromatography.
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(EtOAc:hexane = 1:15). The title compound was obtained in 70% yield. $^1$H-NMR (400 MHz, CDCl$_3$): $\delta = 11.05$ (s, 1 H), 7.72 (d, $J =$ 8.8 Hz, 1 H), 6.42-6.39 (m, 2 H), 4.35 (q, $J =$ 7.2 Hz, 2 H), 3.96 (t, $J =$ 6.4 Hz, 2 H), 1.77-1.72 (m, 2H), 1.52-1.43 (m, 2 H), 1.38 (t, $J =$ 7.2 Hz, 3 H), 0.96 (t, $J =$ 7.2 Hz, 3H). $^{13}$C-NMR (100 MHz, CDCl$_3$): $\delta = 170.1$, 165.2, 163.9, 131.2, 107.8, 105.5, 101.1, 68.0, 61.0, 31.1, 19.3, 14.3, 13.9. IR [cm$^{-1}$]: 3170-3000 ($\nu$ C-H, aromatic), 2970-2860 ($\nu$ C-H, CH$_2$, CH$_3$), 1726 ($\nu$ C=O), 1660-1450 ($\nu$ C=C, aromatic), 1450-1300 ($\delta$ C-H, CH$_2$, CH$_3$), 1250-970 ($\nu$ C-O, aromatic). LC-MS (ESI$^+$): 239.0996 (239.1205 calcd.) for [C$_{13}$H$_{18}$O$_4$+H]$^+$.

Figure S3. NMR analysis of compound 2. a) $^1$H-NMR spectrum of 2 (25 °C, 400 MHz, CDCl$_3$). b) $^{13}$C-NMR spectrum of 2 (25 °C, 100 MHz, CDCl$_3$).

Step 4: synthesis of ethyl 2-((4-bromobutyl)oxy)-4-(butoxy)benzoate (3).

The mixture of compound 2 (2g, 7.5 mmol), 1,4-dibromobutane (15 mmol), and K$_2$CO$_3$ (1.4 g, 10.2 mmol) was dissolved in acetone (30 mL). Then the solution was heated to 75 °C for 24 h. After that,
the hot solution was immediately filtered to remove inorganic salts and the residue washed thoroughly with hot acetone. The filtrate was collected and the acetone was removed \textit{in vacuo}. The crude product was purified by column chromatography on silica gel (EtOAc:hexane = 1:20). The yield of the reaction was 80% . $^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ = 7.82 (d, J = 8.4 Hz, 1 H), 6.46 (dd, J = 8.8 Hz, 2 H), 6.43 (d, J = 2.4 Hz, 1 H), 4.30 (q, J = 7.2 Hz, 2 H), 4.02 (t, J = 6.0 Hz, 2 H), 3.97 (t, J = 6.4 Hz, 2H), 3.50 (t, J = 6.4 Hz, 2 H), 2.16-2.09 (m, 2 H), 2.01-1.95 (m, 2 H), 1.79-1.72 (m, 2 H), 1.52-1.41 (m, 2 H), 1.35 (t, J = 7.2 Hz, 3H). 0.96 (t, J = 7.2 Hz, 3 H). $^{13}$C-NMR (100 MHz, CDCl$_3$): $\delta$ = 165.9, 163.7, 160.5, 133.9, 112.7, 105.3, 100.2, 68.0, 67.6, 60.4, 33.7, 31.2, 29.4, 27.8, 19.3, 14.5, 13.9. IR [cm$^{-1}$]: 2960-2870 (ʋ C-H, CH$_2$, CH$_3$), 1718 (ʋ C=O), 1610-1460 (ʋ C=C, aromatic), 1440-1290 (δ C-H, CH$_2$, CH$_3$), 1250-1020 (ʋ C-O, aromatic). LC-MS (ESI$^+$): 375.0582 (373.0936 calcd.) for [C$_{17}$H$_{25}$BrO$_4$+H]$^+$. 

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure_s4.png}
\caption{NMR analysis of compound 3. a) $^1$H-NMR spectrum of 3 (25 °C, 400 MHz, CDCl$_3$). b) $^{13}$C-NMR spectrum of 3 (25 °C, 100 MHz, CDCl$_3$).}
\end{figure}
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Step 5: synthesis of 4-(5-(butoxy)-2-(ethoxycarbonyl)phenoxy)-N,N,N-trimethylbutan-1-aminium bromide (4).

Compound 3 (2 g, 4.83 mmol) and Me₃NH (11.4 mL, 48 mmol) were dissolved in EtOH (35 mL). Then, the solution was heated to 80 °C for 24 h. The crude product was purified by column chromatography on silica gel (CHCl₃:MeOH = 10:1) in 71% yield. ¹H-NMR (400 MHz, CDCl₃): δ = 7.61 (d, J = 8.8 Hz, 1 H), 6.28-6.26 (m, 2 H), 4.03 (q, J = 7.2 Hz, 2 H), 3.89 (t, J = 5.6 Hz, 2 H), 3.81-3.73 (m, 4 H), 3.27 (s, 9 H), 1.95-1.88 (m, 2 H), 1.79-1.73 (m, 2 H), 1.59-1.52 (m, 2 H), 1.33-1.24 (m, 2 H), 1.14 (t, J = 6.8 Hz, 3 H), 0.77 (t, J = 7.2 Hz, 3 H). ¹³C-NMR (100 MHz, CDCl₃): δ = 164.6, 163.5, 160.1, 133.2, 111.4, 105.2, 99.6, 67.8, 67.6, 65.6, 59.8, 52.7, 30.7, 24.8, 20.1, 18.8, 14.0, 13.5. LC-MS (ESI⁺): 352.2482 (352.2093 calcd.) for [C₂₀H₃₄NO₄]⁺.

![Figure S5. NMR analysis of compound 4. a) ¹H-NMR spectrum of 4 (25 °C, 400 MHz, CDCl₃). b) ¹³C-NMR spectrum of 4 (25 °C, 100 MHz, CDCl₃).](image)
Step 6: synthesis of 4-(5-butoxy-2-((4-((4-octylphenyl)diazenyl)phenoxy)carbonyl)phenoxy-N,N,N-trimethylbutan-1-aminium bromide (5).

Compound 4 (2 g, 4.2 mmol) and sodium hydroxide pellets (0.5 g, 12.5 mmol) were dissolved in a mixture of methanol (30 mL) and water (3 mL). Then, the solution was heated to 70 °C for 4 h. After the reaction, the pH of the solution was adjusted to around 2 by adding concentrated HCl (12 M). The solvents were removed in vacuo and then the intermediate was re-dissolved in CHCl₃ and filtered. Next, a mixture of the acid intermediate (1.57 mmol), compound I (3.5 mmol), DCC (0.34 g, 1.72 mmol), and DMAP (96 mg, 0.79 mmol) were dissolved in anhydrous CH₂Cl₂ (35 mL) and the solution was stirred overnight at r.t. After the reaction, the solution was filtered and washed with CH₂Cl₂. The crude product was collected by evaporation of the solvent in vacuo.

Then, the crude product was re-dissolved in CH₂Cl₂ whereupon white precipitate formed. The precipitate was filtered and the filtrate was collected. This process was repeated until there was no white precipitate appearing anymore. Finally, the product was purified by column chromatography on silica gel (CHCl₃:MeOH = 10:1) in 69% yield. ^1H NMR (400 MHz, CD₃OD): δ = 8.12 (d, J = 8.0 Hz, 1 H), 8.01 (d, J = 8.0 Hz, 2 H), 7.87 (d, J = 7.6 Hz, 2 H), 7.41-7.38 (m, 4 H), 6.71-6.68 (m, 2 H), 4.22-4.11 (m, 4 H), 3.58-3.54 (m, 2 H), 3.06 (s, 9 H), 2.74 (t, J = 7.6 Hz, 2 H), 2.12-2.06 (m, 2 H), 1.99-1.92 (m, 2 H), 1.87-1.79 (m, 2 H), 1.73-1.67 (m, 2 H), 1.61-1.51 (m, 2 H), 1.38-1.32 (m, 10 H), 1.06-1.02 (m, 3 H), 0.93-0.91 (m, 3 H). ^13C NMR (100 MHz, CD₃OD): δ = 166.6, 165.0, 163.1, 154.5, 152.2, 151.7, 148.3, 135.5, 130.3, 124.9, 124.0, 123.9, 111.2, 107.2, 101.2, 69.3, 67.5, 53.4, 36.8, 33.0, 32.5, 32.3, 30.6, 30.4, 26.7, 23.7, 21.4, 20.3, 14.4, 14.1. IR [cm⁻¹]: 2960-2850 (ν C-H, CH₂, CH₃), 1731 (ν C=O), 1610-1460 (ν C=C, aromatic), 1440-1260 (δ C-H, CH₂, CH₃), 1200-1010 (ν C-O, aromatic). LC-MS (ESI⁺): 616.4109 (616.3333 calcd.) for [C₃₈H₅₄N₃O₄]⁺.
Figure S6. NMR analysis of compound 5. a) $^1$H-NMR spectrum of 5 (25 °C, 400 MHz, CD$_3$OD). b) $^{13}$C-NMR spectrum of 5 (25 °C, 100 MHz, CD$_3$OD).
Investigation of E- to Z-photoisomerization of the AZO surfactant by $^1$H-NMR spectroscopy. Aromatic region of $^1$H-NMR spectra of the AZO surfactant in CD$_3$OD ($\approx 7$ mM, 25 °C) were recorded before and after the application of UV irradiation at 365 nm (0.5 mW·cm$^{-2}$). The photoswitching behavior was determined in $^1$H-NMR spectra by integration of the respective NMR peaks, as E- and Z-isomers display well-separated and distinguishable resonances for aromatic protons. The proton at position $e$ was used as internal standard. Table 1 shows that there is 8.9% of Z-isomer before UV exposure. After UV irradiation, $\approx 94.0\%$ Z-isomer was obtained.

Table 1. $^1$H-NMR assignments for the E- and Z-isomers of AZO surfactant, and the composition of Z-isomer obtained using integration of respective NMR peaks.
UV-vis absorption spectra of the AZO surfactant in aqueous solution (ca. 30 μM) over the course of irradiation with UV-light and visible light. After UV exposure for 5 min, obvious E- to Z-isomerization was observed. But with the consecutive irradiation of visible light (450 nm, 30 μW·cm^-2), only a fraction of Z-isomer of the AZO could be transformed back to the E-isomer (ca. 50%) in this system.

**Synthesis of oligonucleotides:** 22mer oligonucleotide with a sequence 5’-CCTCGCTCTGCTAATCCTGTTA-3’ and its complementary sequence 5’-TAACAGGATTAGCAGAGCGAGG-3’ were synthesized using standard automated solid-phase phosphoramidite coupling methods on an ÄKTA oligopilot plus (GE Healthcare) DNA synthesizer.

**Figure S8.** UV-vis absorption spectra of the AZO surfactant in aqueous solution (ca. 30 μM) over the course of irradiation with UV-light and visible light. After UV exposure for 5 min, obvious E- to Z-isomerization was observed. But with the consecutive irradiation of visible light (450 nm, 30 μW·cm^-2), only a fraction of Z-isomer of the AZO could be transformed back to the E-isomer (ca. 50%) in this system.

**Figure S9.** Characterization of 22mer DNA by polyacrylamide gel electrophoresis (PAGE) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). (A) PAGE analysis. M: Ladder, Lane 1: 22mer DNA, Lane 2: complementary 22mer DNA, Lane 3: 22bp double-stranded DNA after hybridization. (B, C) MALDI-TOF MS of the 22mer and its complementary sequence. The measured and expected masses are in good agreement.
Preparation of DNA-AZO complexes: first, DNA hybridization was carried out in an aqueous buffer solution (10 mM MgCl₂, 50 mM NaCl, and 10 mM Tris-HCl, pH = 7.5) with a DNA concentration of 2.4 mM. The hybridized DNA solution was diluted by adding Milli-Q water. Thus, an aqueous solution of 22mer dsDNA with a concentration of ca. 300 μM was obtained. In a second solution made from mixed water and ethanol (v/v = 5:3), the concentration of AZO surfactant was adjusted to 2-3 mM at room temperature. Both solutions were combined in a ratio so that approximately 2 mol of surfactants equal 1 mol of phosphate group within DNA. After mixing aqueous solution of 22mer dsDNA with cationic AZO surfactant, a precipitate occurred. After centrifugation and lyophilization, the 22mer dsDNA-AZO complex was collected for further characterization. The 22mer ssDNA-AZO complex was prepared following the same procedures.

Additionally, a thin film of the 22mer dsDNA-AZO complex was fabricated by drop-casting for AFM based nanoindentation experiments. The lyophilized sample (1-2 mg) was dissolved in 30 μL CHCl₃. Then the solution was transferred to the smooth surface of a Si substrate (0.5×0.5 cm) by a pipette. After drying at ambient conditions for 48 hours, the film of the 22mer dsDNA-AZO complex was formed. Thin films of the 22mer ssDNA-AZO complex and pristine AZO surfactant were prepared following the same procedures.
Chapter 3

**Characterization of DNA-AZO complexes**

*Figure S10.* Analysis of the stoichiometry of the dsDNA-AZO complex by $^1$H-NMR (400 MHz). The signals of terminal methyl (marked by a, l) and aliphatic groups (marked by b-k) in AZO and methyl group of thymine in DNA (marked by i) were utilized to estimate the molecular ratio of dsDNA and AZO surfactant. The terminal methyl groups in AZO surfactant were used as an internal standard. The binding stoichiometry can be roughly calculated as the integration of protons difference (at chemical shift between 1.2-2.0) between the AZO and AZO-DNA complex. Assuming that one DNA molecule could combine with n AZO molecules (DNA:nAZO), then after complexation, the total number of protons at chemical shift between 1.2-2.0 can be expressed as: $(\text{DNA(T}_{11})) \times 3 + (\text{AZO(-CH}_2\text{-})_{10}) \times n$. According to the integration of the protons of AZO surfactant and dsDNA-AZO in their $^1$H-NMR as shown above, we have:

\[
11 \times 3 + 19.95 \times n = 20.58 \times n
\]

\[
n = 52
\]

As a result, the stoichiometric ratio of AZO and dsDNA is roughly 52:1.
**Figure S11.** Thermogravimetric analysis (TGA) of the 22mer dsDNA-AZO and 22mer ssDNA-AZO complexes. TGA analysis showed that the DNA-AZO materials exhibited water contents less than 5% (w/w) (black curve, dsDNA-AZO; red curve, ssDNA-AZO).

**Figure S12.** POM investigation of the dependence of temperature on the phase transition of the 22mer dsDNA-AZO complex. The birefringent nematic textures melt completely above 110 °C, leaving only transparent isotropic liquid (in D, insertion of a quarter wave plate). Scale bar is 100 μm.
Figure S13. DSC traces (at a heating/cooling rate of $5 \, ^{\circ}\text{C} \cdot \text{min}^{-1}$) of the 22mer dsDNA-AZO complex. In the 1st heating there is one broad endothermic peak from 45 to 120 $^\circ$C, which may suggest that thermal de-hybridization of dsDNA took place in the solvent-free LC material. Upon cooling, no exothermic peaks were observed, possibly due to a slow crystallization process. The DSC curve of the 2nd heating gave a phase transition from crystal to LC at around -7 $^\circ$C.
Figure S14. Preparation and characterization of the 22mer ssDNA-AZO nematic liquid crystals. (A) The ssDNA-AZO nematic TLC material is formed by electrostatic complexation of single-stranded 22mer oligonucleotides and AZO surfactants. (B) SAXS profiles of the ssDNA-AZO complex (25 °C) recorded before and after the application of UV irradiation. One broad diffraction peak corresponding to the d spacing of 4.55 nm was observed before UV irradiation, indicating a nematic phase. The average diameter of 4.55 nm can be explained by a ssDNA unit of ca. 1 nm thickness and interdigitated AZO surfactants of ca. 3.55 nm thickness protruding from the DNA backbone. After the application of UV light, the d spacing decreased to 4.39 nm indicating the E- to Z- photoisomerization of the azobenzene moiety in the material. Note, light treatment of the samples was performed by a UV lamp (0.5 mW·cm⁻²) at 365 nm. (C) POM image of the birefringence of the ssDNA-AZO complex showing a nematic mesophase. Scale bar is 100 μm.
Figure S15. Stoichiometry analysis of the ssDNA-AZO complex by $^1$H-NMR (400 MHz). Similar method as shown in Figure S10 was used to estimate the molecular ratio of ssDNA and AZO surfactant. The integrals protons of terminal methyl (l, a) and methylene groups (e, d, c, f) in AZO and ssDNA-AZO were considered for the determination. The total protons of ssDNA-AZO can be expressed as follows:

$$8\times 3 + 7.59\times n = 8.67\times n$$

$$n=22$$

As a result, the stoichiometric ratio of AZO and ssDNA is around 22.
Figure S16. AFM-based nanoindentation on a thin film of the ssDNA-AZO complex. (A) Surface topography of the film before the application of UV light, with a roughness of around 2 nm (color scale 2.7 nm). (B) Superposition of F-D curves taken from the film surface in panel A, at a tip velocity of 1 µm·s⁻¹. (C) Histogram of calculated spring constant of the ssDNA-AZO thin film from the F-D curves in panel B, considering $K_{\text{cantilever}} = 0.35$ N m⁻¹. The average value of the calculated spring constant is $K_{ssDNA-AZO} = 2.2 ± 0.3$ N m⁻¹. (D) Surface topography of the film after the application of UV light (365 nm, 3.6 mW·cm⁻², 15 min). The roughness is around 2 nm (color scale 2.9 nm). (E) Superposition of F-D curves taken from the surface in panel D. (F) Histogram of calculated spring constant of the ssDNA-AZO film from the F-D curves in E, $K_{ssDNA-AZO} = 2.2 ± 0.3$ N m⁻¹.
Reference

Nematic DNA thermotropic liquid crystals with photo-responsive mechanical properties


