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LIQUID CRYSTAL TEMPLATING

SPATIAL AND DYNAMIC CONTROL OF FUNCTIONAL SOFT MATERIALS

PROEFSCHRIJT
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Radboud University
Is a man not entitled to the sweat of his brow?

- ANDREW RYAN
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INTRODUCTION
1 INTRODUCTION

Nature has the fascinating ability to precisely create an incredible range of complex structures on multiple length scales from a range of molecular building blocks such as DNA, RNA, proteins, sugars and lipids. These essential bricks and the assembled hierarchical structures are able to perform numerous functions such as providing (electrical) communication, structural integrity, nutritional transport from a molecular to a cellular level and up to the organism level itself. Life in a macroscopic sense can only function if the structural organization of these molecular building blocks is precisely controlled in space and time.

Nowadays, scientists are trying to apply Nature’s tricks to the playground of the lab. Soft matter, such as 1D self-assembling supramolecular and polymeric materials are examples where individual building blocks are brought together by delicate covalent and non-covalent interactions, forming larger hierarchical assemblies. These individual building blocks can be chemically tuned to introduce a range of specific characteristics to the assembly (mechanical stability, electro-optic response), allowing ultimately for designing desired functions into the assembled structure. As a consequence, the macroscopic organization and their coupled functionality allows these soft materials to be used for a wide array of applications, ranging from sensing devices (which are able to pick up tiny amounts of specific biomolecules in solution), the creation of artificial tissue up to materials printed on plastics which are able to convert solar light into electricity. Due to their ease of processability and versatility in introducing specific properties and functions, these types of soft materials have the potential to provide a cost-effective alternative to many current-day inorganic solid state devices.

Despite the advantages that polymeric and supramolecular materials offer, a key aspect that holds back their application is the difficulty in controlling the spatial organization of these assemblies across multiple length scales. Although the order within the assembly (nanometer scale) is extremely high, when dissolved most supramolecular materials form macroscopic assemblies. Examples where spatial control at larger length scale is highly beneficial or even crucial for device performance are in opto-electronic devices, as well as in tissue engineering. In the latter for instance, a macroscopically organized polymer scaffold is necessary for the growth of highly aligned tissue, such as muscle fibers and neural tissue.

ORGANIZING SOFT MATTER 1.1

To spatially control the hierarchical organization of these soft materials, many strategies have been employed. As a classical top-down technique, photolithography is able to create well-defined features with a nanometer resolution on a high throughput basis, but clean rooms are needed and application of high UV doses to various biomaterials is a concern. Soft lithography is a flexible cost-effective alternative, as it is compatible with fragile biomaterials although creating complex patterned devices remains challenging due to limited in-plane registration during multiple stamping steps.

Using electric fields can be very useful since complex patterned electrodes might be used, where each individual pixel can be locally manipulated (analogous to LCD technology). Unfortunately, many soft materials possess low susceptibilities to electric fields, which require the use of very high/unpractical fields. Furthermore in (ion-rich) aqueous solutions, especially when using such electric fields, parasitic heating effects (Joule/dielectric heating) may disrupt molecular self-assembly and electrochemical degradation can even destroy the material.

Magnetic fields are better suited since they do not suffer from such disruptive (temperature) effects. Other advantages are that it is a contact free technique, it works even in large volumes (while surface-oriented techniques only have a finite working distance) and off-plane alignment is easily possible. One major drawback is that it is impossible to make complex patterns on multiple length scales of soft matter with this technique. Secondly, many soft materials have low susceptibilities to magnetic fields (similar to electric fields), resulting in the need for very high and power-consuming fields to align these materials. Consequently, examples of alignment of soft matter in aqueous and non-aqueous solutions using electric fields and magnetic fields (Figure 1) are relatively limited.
Lastly, shear flow is a very practical technique used to align soft matter although often the compound needs to possess liquid crystalline characteristics. Unfortunately, similar to magnetic fields and electrospinning, it is impossible to make smaller complex patterns on micron-sized length scales.

All techniques discussed have specific advantages and disadvantages which make them very useful only for specific situations. The general alignment technique should have the combined advantages of:

1. being compatible with a wide range of materials (especially fragile biomaterials);
2. having the ability to create complex patterns on multiple length scales;
3. facile operation in any basic laboratory.

One alignment technique which potentially does have these combined advantages, is liquid crystalline (LC) templating. This relatively new strategy has the intrinsic advantages that the (LC) template can be readily addressed by many different alignment techniques and that it can template the organization of a guest inside the LC host. This chapter introduces LC templating. The subsequent chapters in this thesis explore different aspects of this technique. First, liquid crystallinity is introduced.

Electrospinning is a very useful technique since it is able to align materials processed from a wide range of solvents into high aspect ratio fibers. Furthermore it is able to integrate patterned electric and magnetic field setups which locally align the electrospun fibers into basic in-plane patterns, although creating more complex patterns on micron length scales is impossible.

The liquid crystalline phase is a state of matter in between the solid and liquid phase; it has both solid-like ordering and fluid properties of a liquid. LCs can be classified by their physical properties. First of all, so-called thermotropic LCs (TLCs) consist of anisotropic molecules (e.g. rod-shaped or discotic) which self-organize in a range of LC phases at specific temperatures without any additional solvent present. In the most basic phase, the nematic phase, the LC molecules (also called mesogens), only have orientational order. In micron-sized domains, all molecules align in one direction, defined by the director. A chiral or cholesteric phase is formed by chiral LCs or by the addition of a chiral dopant which introduces a twist in the position of the molecules perpendicular to the director. Besides orientational order, mesogens can also have positional...
order, where the molecules have translational symmetry, resulting in a range of so-called smectic phases (1D positional order) or columnar phases (2D positional order).

When increasing the temperature for all these LC phases, they undergo one or more phase transitions, for example from smectic to nematic and eventually to the isotropic phase where all positional and orientational order is lost. Due to the long-range interactions between LC mesogens, spanning many micrometers, the multitude of mesophases can be distinguished under a polarized optical microscope. In a polarized optical microscope, the different refractive indexes parallel and perpendicular to the long axis of the LC molecules (also called birefringence) creates interference patterns of light in the visible range, where the observed color results from a phase shift, which depends on thickness of the LC layer and its birefringence (related to the molecular structure and the specific LC phase it is organized in). The phase of the LC can be derived by looking at the defect texture, which results from this interference color in combination with the spatial organization of the LCs around defects (which give rise to patterns that are unique for every mesophase). One example is displayed in Figure 4 where a so-called Schlieren texture (which is characteristic for the nematic mesophase) is visible under a polarized optical microscope.

Another class of LCs are so-called lyotropic LCs (LLCs). In contrast with TLCs, mesophase formation in LLCs depends on both temperature and the concentration of the compound in a solvent. As a sub-class of LLCs, amphiphilic LLCs are characterized by molecules composed of separate hydrophilic and hydrophobic sections. At specific concentrations (above their so-called critical micellar concentration or CMC) these molecules form rod-shaped or disk-shaped micelles, where the organization is governed by the polarity of the solvent; in polar solvents, the hydrophilic section and in non-polar solvents the hydrophobic part is in contact with the solution. At increasing concentrations these micelles self-organize in hierarchical liquid crystalline structures (Figure 5), for instance hexagonal and lamellar arrangements. Such amphiphilic LLCs can be found in countless examples all around us, ranging from soap solutions (in which micelles are able to encapsulate non-polar compounds in water) up to the formation of higher ordered bilayer membranes in living cells from such amphiphilic molecules.

**Figure 4** Polarized optical microscopy image showing a characteristic nematic schlieren texture for a liquid crystal. Reproduced from Ref. 35.
1.2 Liquid Crystals

An interesting class of LLCs are so-called chromonics or lyotropic chromonic LCs (LCLCs). LCLCs are rigid board-like molecules consisting of aromatic cores with water-solubilizing groups on the periphery. They stack face-to-face in columnar aggregates in water due to π-π stacking and hydrophobic interactions (FIGURE 6). The spacing between the individual stacked mesogens for these materials is about 3.4 Å, which is roughly the Van der Waals distance between aromatic rings. At certain concentrations and temperatures these stacks form specific LC phases such as nematic, smectic and cholesteric-like phases, where the columnar stacks contain tens to hundreds of LCLC units. Due their often rigid aromatic cores, many of them are highly absorbing in the visible range and in fact many commercial organic dyes have also shown LCLC behavior. Another example includes both DNA and RNA which can be considered as polymers with side-chain chromonic LC pendants.

One of the first molecules to be studied in detail was disodium cromoglycate (DSCG), which was earlier discovered to be an effective anti-asthmatic drug. It forms a nematic phase at room temperature at about 12 wt% in water and despite its aromatic structure it is optically transparent in the visible range. The second most widely studied chromonic is Sunset Yellow (SSY), a brightly orange colored compound that is also commercially used as a food dye. Both DSCG and SSY have very similarly shaped phase diagrams, although SSY forms room temperature nematic mesophases at much higher concentrations (30 wt% in water). On the other side of the spectrum, some chromonics are able to form LCLC phases at far lower concentrations, such as benzopurpurin 4B (0.4 wt%) and IR-806 (0.5 wt%) and pinacyanol acetate (0.75 wt%).

The columnar LCLC aggregates can be aligned in-plane and homeotropically through various surface treatments, although controlling the surface anchoring of these materials remains a challenge since the anchoring energy of these phases on conventional surfaces is very weak compared to conventional thermotropic LCs (1 to 2 orders of magnitude weaker). The induced alignment is often not stable over time.
1.2 LIQUID CRYSTALS

Lastly, anisotropically shaped minerals can form LC phases when dispersed at sufficient volume fractions in a solvent. Examples of such colloidal or mineral liquid crystals are goethite and beidellite which both form liquid crystalline phases at specific volume fractions in water at room temperature. Due to their high susceptibilities they can be easily addressed by both low magnetic and electric fields. Their sizes are much bigger than other conventional molecular LCs. In contrast to conventional LCs, temperature has a marginal effect on the stability of these phases, since Brownian motion plays an insignificant role for these relatively large particles. Due to their sizes and high densities, these board-shaped nanoparticles slowly sediment to the bottom of a solution.

1.2.1 LC APPLICATIONS

LCs have the unique ability to form domains whose orientations can be directed and extended to much larger length scales by applying external fields (electric, magnetic, shear) or directing surfaces (such as rubbed polyimide or photosensitive command layers), resulting in a uniformly aligned mono-domain at macroscopic length scales. The intrinsic anisotropy and ease of manipulation of their alignment forms, together with their optical properties, the basis of a wide range of applications. One key example is the development of liquid crystal displays (LCD) TV screens. In LCDs, thermotropic LCs are uniformly aligned by a directing surface (for example rubbed polyimide) and its local orientation is switched by individual electric fields on a patterned electrode setup. As a result, the orientation of LCs in each a compartment (or pixel) can be individually addressed, resulting in an on-off switch for local light transmission.

The ability to manipulate the organization of LCs in time and space can also be used to create stimuli-responsive materials manipulated by heat or light, such as in LC polymer networks. Using photosensitive anisotropic surfaces, these liquid crystalline-functionalized networks can be patterned across multiple length scales and by applying external stimuli (such as heating to the isotropic phase or by excitation of an incorporated photosensitive moiety) the local LC director is altered, resulting in a 3D macroscopic deformation of the network. Because a range of external stimuli can be transferred into programmable mechanical motion, these materials are very promising as low-cost mechanical components within many microtechnology applications.

Besides actuation, LC polymers also find application in optical sensing. By introducing chirality into the LC polymer, circular polarized light (corresponding to the pitch length of the elastomer) can be selectively reflected by these materials. A change in the environment (by a temperature change, specific light illumination and/or presence of analytes), induces an alteration of the pitch length which results in a macroscopic color change, making these smart materials ideal for low-cost sensing devices.

In addition to the aforementioned applications, TLCs have also been used in biomedical engineering and sensing devices within biological environments. The group of Nicholas Abbott has pioneered this field with their work on TLCs that detect a wide range of biological entities such as bacteria, viruses, cells, DNA, proteins, endotoxin and lipids within aqueous solutions. In general, when a biological interaction takes place along the interface between a TLC and the aqueous solution, the local orientation of the TLC molecules is altered, resulting in an LC reorientation over much larger length scales where it can be easily picked up by observing the change in texture under a polarized optical microscope.
The fluidic but anisotropic properties of LCs are suitable for their applications as an organizational template for other functional non-LC materials. A so-called LC template, which acts as an anisotropic solvent, is able to direct the organization of dispersed or dissolved materials (which do not possess liquid crystalline character) within its matrix through elastic-mediated interactions and reorientational shear forces (Figure 9). The LC template itself can be manipulated by conventional external stimuli such as directing surfaces and shear flow. Also due to its long range cooperative interactions and anisotropic polarizability, an LC template can also be addressed by relatively low electric and magnetic fields, which allows for controlling the organization of dispersed materials which normally can not be addressed by these external fields (due to their inherent low susceptibilities). The resulting macroscopic alignment of the dispersed functional materials arising from these templated alignment techniques in turn improves their optical, electrical and/or mechanical properties.

Unfortunately, many TLCs and amphiphilic LLCs (acting as detergents) are toxic to living matter [73,74], making in-situ application of bulk LCs with these fragile biomaterials challenging. However in the last few years, there has been an increased interest in using chromonic lyotropic LCs (LCLCs) for these purposes. Since these materials do not form micelles but rather elongated stacks and as such do not insert in the bilayer membranes of living cells, these materials are compatible in a wide range of biological conditions [69,74,75], even allowing for manipulation of living materials [76-78] and the detection of biological events [79].

Besides these examples there are many emerging applications in the LC field. Particularly exciting examples are the creation of LC nanostructured materials for electric storage and water-filtration applications [80-82] and LC colloidal assembly for a wide range of photonic applications [83]. For information on other LC-related topics and applications, the 2012 review of Lagerwall and Scalia is highly recommended [35].

Besides improving the properties of the templated materials, LC templating has additional benefits over other techniques. Since this approach does not depend on specific interactions with the template, it can be applied to organize a wide range of (non-liquid crystalline) materials. Furthermore, LCs can be locally manipu-
1.3 LC ALIGNMENT TECHNIQUES

The first step in LC templating is to control the organization and the alignment of the liquid crystalline template (which is also often crucial for LC applications in general). The most-used approaches are briefly discussed below.

1.3.1.1 RUBBED POLYIMIDE

For the last decades, rubbed polyimide has been the prime alignment technique for LCs and templates alike, since it is very effective and its application (spin-coating, curing and rubbing) is straightforward. Rubbing has been used for many years in the mass-manufacturing LC display industry. Spincoated polyimide gives rise to random planar alignment. By unidirectionally rubbing polyimide with a piece of velvet or an abrasive pad, grooves are created and the top polymer layer is reoriented due to the applied shear force. These anisotropic (molecular) topographies cause the mesogens to anchor in-plane unidirectionally on such surfaces and as a result the surface ordering is extended into the bulk solution up to roughly 100 microns distance.

1.3.1.2 SELF-ASSEMBLING MONOLAYERS

For homeotropic alignment on a substrate, it is convenient to employ self-assembling monolayers (or SAMs). These molecules are often built up from molecules composed of a long aliphatic tail and a functional head group (such as thiol or silane) that covalently binds to a substrate. The long alkyl tail provides homeotropic anchoring conditions for many liquid crystals. Shorter tails give planar alignment, but one needs topological structures to induce unidirectionality.

Despite the ease of using rubbed polyimide surfaces for LC alignment, complex patterning over multiple length scales with this technique is practically impossible, since it is mechanically extremely difficult and very time-consuming to locally rub polyimide precisely on a micron-scale. One very promising alternative is to use photoalignment on so-called photo-addressable command layers (CLs).

In general, CLs are polymers functionalized with photosensitive groups such as cinnamates, coumarines and azobenzenes that can be spincoated on a substrate. Cinnamate- and coumarine-functionalized polymers can undergo a [2+2] cycloaddition crosslinking reaction when subjected to light. When linearly polarized light is used, these command layers are crosslinked in a specific orientation with respect to the polarization of the light, resulting in an anisotropic molecular topography of the command layer. In the case of azobenzene-functionalized polymers, these chromophores undergo a light-mediated reversible cis-trans isomerization. When embedded within a spincoated polymer matrix and when subjected to linearly polarized light, these moieties undergo subsequent reversible cis-trans isomerizations until they are oriented with their long axis perpendicular to the plane of the linearly polarized light. By using multiple illumination steps and photomasks, CLs can be locally aligned in-plane in any pattern with domain sizes ranging from microns to centimeters. Using this technique, CLs can be spincoated over a wide range of surfaces, including patterned electrodes substrates, curved substrates and even in confined geometries, where traditional alignment techniques form a challenge.

CLs have been used to control the organization of thermotropic LCs, as well as LCLCs such as DSCG. In the latter case a surfactant is needed which anchors to the hydrophobic CL and couples the in-plane organization of the CL to the hydrophilic LCLC. One important step forward showing the high-throughput mass market potential of CLs was when Sharp implemented this non-contact LC alignment technique in 2009 in favor of mechanical rubbing of polyimide for the mass-production of their next-generation LCD TVs.
Besides directing surfaces, both electric and magnetic fields can be used to align LC templates, which in turn align the dispersed materials by reorientational shear forces and elastic interactions. In electric fields, LCs can align due their own permanent electric dipole moment. Even if LC molecules don’t possess a permanent dipole moment, the application of an electric field induces a small dipole moment, which allows for alignment perpendicular or parallel to the field. Similar to electric fields, magnetic fields can also align LCs due to their intrinsic magnetic dipole moment or induced magnetic dipole moment by the application of a field.

The advantage of using an electric field is that by using complex patterned setups or in combination with directing surfaces, LCs can be switched between several orientations, in-plane and homeotropic, which is the main technological feature behind LC display technology. An additional advantage arises when electric fields are applied to an LC template. In this situation, the dispersed functional material within the aligned template can physically bridge the electrodes, after which this material can act as a transducer in a biosensor or as a transistor in molecular electronic devices.

Using magnetic fields for LC template alignment has the advantages that it is a contact free method; no specific surfaces are needed within a container. Secondly, magnetic fields are effective in the bulk of a sample, therefore it can be used to align far larger volumes of LC templates (and dispersed functional materials) in comparison to polyimide rubbed cells, where surfaces in general can only direct LCs up to 100 micron. Furthermore, off-axis alignment is straightforward by tilting the sample in any orientation with respect to the field. Also homogeneous and inhomogeneous magnetic fields can be altered in space and/or time (by controlling the rotation of the sample and/or by changing frequency of the field), allowing even more complex LC template organizations.
A beautiful example was demonstrated by the group of Akagi who used a chiral LC to template the polymerization of helical polyacetylenes (Figure 11). Besides the creation of hierarchical helical structures over multiple length scales, the polyacetylene films showed high electrical conductivities due to its templated organization. Looking at other semiconducting n-conjugated polymeric materials, poly(3-hexylthiophene), a key component in organic solar cells (where spatial distribution of donor and acceptor materials is essential) was incorporated in a block copolymer. When templated by a nematic LC on a rubbed polyimide surface, highly anisotropic absorption and polarized photoluminescence emission emerged, due to induced macroscopic alignment of poly(3-hexylthiophene) fibrils.

LC templates can be subjected to several external directing fields at the same time, allowing for dynamic manipulation of the LCs and its dispersed material in time and space. As an example, many applications of carbon nanotubes (CNTs), which have very promising electronic properties, require both macroscopic alignment and a reorientational response to a stimulus. By using an LC template in combination with rubbed polyimide and an external switchable electric field, the orientation of both single-wall and multi-wall CNTs was switched by the application of an electric field, resulting in far higher conductivities when the CNTs are oriented along the field (due to their high anisotropic conductivities along the tubes).

Vice versa, polymers are also known to influence LCs, such as using polymers to stabilize the formation of so-called blue phases. This related class of polymer-liquid crystal composites has great potential as an electro-optical material, but these types of composite materials will not be discussed in this review.

Aligned functional soft materials are very well suited as amplifying sensor components. If these materials are employed in aqueous environments, they can probe both biological and chemical events, potentially leading to the development of a wide range of biomedical and opto-electronic devices. In order to apply LC templating to these aqueous solutions, water-soluble mesogens are required. Despite numerous application of thermotropic LC templating in non-aqueous solvents, in water-based systems, there are only sparse examples where unidirectional macroscopic alignment of aqueous-processable soft matter is realized. A reason for the limited success in aqueous systems is that conventional water-based amphiphilic lyotropic LCs are notoriously difficult to align in-plane with common surface treatments such as rubbed polyimide. Furthermore, ion-rich lyotropic templates are often incompatible with electric fields. Moreover, an amphiphilic lyotropic LC can readily function as a surfactant which interferes with the desired self-assembly process of a supramolecular material. There have been several examples where a range of both soft and harder materials (including 1D nanowires and nanoparticles) have been synthesized in amphiphilic LC templates, although the lack of long range in-plane alignment techniques of these lyotropic LC phases holds back its application of creating functional 1D nanomaterials on macroscopic device length scales. In only one example, Lagerwall et al. have shown that CNTs can be dispersed in an amphiphilic LLC matrix, which is uniaxially aligned by applying a magnetic field, resulting in a macroscopic unidirectional orientation of water-dispersed single-walled carbon nanotubes (Figure 12)

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**Figure 11** Scanning electron microscopy images of chiral LC templated helical polyacetylene at different magnifications. Adapted from Ref. 102.

**Figure 12**
1.3 Liquid Crystal Templating

Additionally, Abbott et al. found that motile bacteria formed linear multi-cellular assemblies (Figure 13), due to an interplay between the elastic-mediated attractive forces in the LCLC and flagella-derived dissociative forces. This group also studied bacteria motion in the nematic-isotropic interfaces of an LCLC, where controlled spindle-like domains or tactoids can be formed of nematic regions within an isotropic medium or vice versa. At the cusps of these domains, two topological defects are located, which are known to attract other micron-sized particles in order to reduce the elastic penalties at these locations. In this particular work, once the bacterium encountered such a tactoid, it followed the curved interface until it left the tactoid at the position of the topological defect. Furthermore, by tuning the size of these tactoids (controlled by temperature), the capture and release of the bacteria could be controlled.

As the use of LCLCs slowly expands into the biological domain, we can expect many more both creative applications and methodological studies emerging from these tunable anisotropic biocompatible solutions in conjunction with a wide range of other biomaterials.
1.3.2.3  COMPLEX PATTERNED SOFT MATTER

Although LC templates in theory can be manipulated locally (e.g. by applying local electric fields), in literature almost all examples have focused on basic unidirectional alignment. Fortunately, new possibilities have emerged to achieve full spatial control of functional materials due to the development of photosensitive command layers for LC alignment. Besides adoption by the industry as an non-contact alternative to mechanical rubbing of LC displays, this technique has also been applied to various functional materials, although liquid crystalline behavior is required. Examples include organic stimuli-responsive polymeric LC networks, 1D LC organic semiconducting polymers and oligomers, aligned crystallization of poly(di-n-hexylsilane), which forms a columnar LC phase at higher temperatures. LC functionalization (in order to increase the compatibility with command layers) is often undesired since laborious synthesis steps are necessary which might negatively impact the eventual functionality of the material. As expected, examples of patterned non-LC materials are very sparse; only pentacene was successfully aligned using this approach. The LC templating approach might be a powerful route to fully control the spatial organization of a wide range of materials. The LC solvent can be easily patterned on an organized command layer, where in turn it is able to orient various functional materials, ranging from organic semiconducting polymers, aqueous supramolecular materials up to even cells and bacteria. Despite the obvious potential behind this technique, so far only few examples are present this technique has been applied to amphiphilic and chromonic LLC-silica hybrids. To date, a huge amount of opportunities left in this field are waiting to be explored.

**Figure 14** LCLC-silica nanohybrid patterned on photopatterned command layer (PPLC). Reproduced from Ref. 88.
1.4 OUTLINE OF THIS THESIS

In this thesis, the concept of liquid crystal templating is taken a step forward in different directions; we apply liquid crystal templates to aqueous solutions and combine it with different external stimuli in order to control the spatial organization of dispersed functional materials and in some cases to form complex structures.

In chapter 2, full spatial control over the organization of water-processable supramolecular materials was achieved by using photosensitive command layers and LCLC templates which direct the organization of these materials in complex in-plane micrometer to centimeter patterns. After alignment, photopolymerization was used in order to form (locally) aligned arrays of optically active π-conjugated polydiacetylenes, which show strong linear polarized absorption characteristics. Additionally, after photopolymerization, the template could be removed by a simple washing step, leaving the crosslinked structures intact on the surface.

Chapter 3 describes unidirectional alignment and complex structure formation of peptide amphiphiles in magnetic fields by using the same LCLC template described in chapter 2. First of all, macroscopic alignment of these materials was achieved in 2 Tesla magnetic fields, which is a tenfold lower than without the aid of a template. Furthermore, photopolymerization of the self-assembled amphiphiles (similar to chapter 2) was used in order to form aligned arrays optically active π-conjugated polydiacetylenes. Additionally, 20 Tesla magnetic fields were used to form higher ordered structures of these materials by exploiting the opposite diamagnetic anisotropies of the template and the self-assembled amphiphilic materials.

In chapter 4, an LC templating strategy was employed to control the organization of water-processable supramolecular materials using electric fields. The template was changed to a mineral LC template (based on colloidal goethite) as it is much more compatible with electric fields. With this goethite template, dispersed peptide amphiphiles were aligned and positioned by using electric fields, which is the first time synthetic supramolecular/polymeric materials can be manipulated in aqueous solutions. Besides alignment and positioning, depletion induced peptide amphiphile bundle formation was studied over time in relation to different concentrations of the mineral LC template.

The application of LCLCs in sensing and optical devices heavily relies on homogeneous and stable alignment on anisotropic surfaces; a challenge which, so far, has not been solved adequately. In chapter 5, unparalleled alignment homogeneity and stability of LCLCs on rubbed polyimide was achieved by the addition of a small amount of a non-ionic surfactant to the LCLC solutions. We apply this concept to both closed LCLC cells and dried-in solid LCLC films. We demonstrate how to obtain high quality alignment by controlling the concentration and the nature of the surfactant, in particular its hydrophilic/lipophilic balance (HLB value) and discuss other critical parameters.

Integrating patterned liquid crystal templates with external electric fields is a very promising route to dynamically manipulate the full spatial organization of aligned functional soft matter. In chapter 6, dye-functionalized polysicyano-peptides dispersed in a thermotropic LC template (showing strong absorption and linear dichroism) were spatially patterned using photosensitive command layers. The orientation of these polymeric materials was reversibly switched using AC electric fields, where the electric field strength is directly related to the extent of the polymer rotation, which gives another level of dimensional control over these dispersed soft materials.

1.5 REFERENCES
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2 PATTERNING OF SOFT MATTER ACROSS MULTIPLE LENGTH SCALES

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ABSTRACT

Controlling the organization of functional supramolecular materials at both short and long length scales as well as creating hierarchical patterns is essential for many biological and electro-optical applications. It remains however an extremely challenging objective to date, particularly in water-based systems. In this work, we demonstrate that we can organize water-processable self-assembling materials from micrometers to centimeters in any direction using liquid crystal templating in combination with photo-addressable command layers. The structural assemblies that we prepare are readily transformed into optically active π-conjugated polymers after photopolymerization. After this step, the template may be removed to leave the functional material well-organized on the substrate. The advantage of this approach is that it lacks specific molecular interactions and uses simple techniques such as spincoating and photo-illumination and it may therefore be applied to a wide range of (aqueous) materials.

2.1 INTRODUCTION

Self-assembling supramolecular structures are excellent building blocks for the development of a wide range of functional molecular biomedical and optoelectronic devices. These soft materials have the potential to provide a bottom-up, low-cost, flexible alternative to many inorganic solid state devices. Water-processable functional supramolecular materials are particularly suited to probe biological processes in an aqueous environment. Lack of spatial control at larger length scales is one of the key aspects that holds back their application at the moment. While the order within an assembly (nanometer scale) is extremely high, when dispersed, most supramolecular materials form macroscopically isotropic assemblies. Moreover spatial control at device dimensions is challenging. Examples where such control is highly beneficial or even crucial for device performance are in opto-electronic devices and in tissue engineering, where a macroscopically organized polymer scaffold is necessary for the growth of highly aligned tissue, such as muscle fibers and neural tissue.

So far, different strategies toward macroscopic alignment of polymeric and supramolecular materials have been developed, including photolithography, soft lithography, electrospinning, electric and magnetic field alignment as well as shear flow alignment. These techniques all have demonstrated their benefits, but also strong limitations such as incompatibility with (aqueous) soft matter, low susceptibilities and/or poor spatial control across multiple length scales.

In this chapter, we use liquid crystal (LC) templating with patternable substrates to obtain full spatial control in our self-assembled materials. This approach has numerous advantages: (i) it does not depend on specific interactions between the assembly and the template and thus it can be applied to a wide range of materials; (ii) any desired (hierarchical) structure can be imprinted on the substrate and reproduced in the assembly; (iii) the desired product can be (chemically) modified after organization (in our case to generate optically active π-conjugated polymers) and (iv) the template can be removed which only leaves the functional material on the substrate. In non-aqueous solvents (bulk thermotropic liquid crystals), the concept of LC templating was demonstrated successfully but in aqueous solutions unidirectional alignment at large length scales is rarely realized, let alone locally controlled. The limited success in water is related to the amphiphilic lyotropic LCs that are notoriously difficult to align on commonly used substrates such as rubbed polyimide. In addition, these lyotropic LCs are incompatible with electric fields (because of dielectric and Joule heating as well electrochemical degradation) and they can interfere with the desired self-assembly process of a supramolecular material. To overcome these disadvantages, we use a template of a lyotropic chromonic LC (LCLC) which is a rigid plank-like molecule that, when dissolved in water, stacks face-to-face in columnar aggregates. At sufficient concentrations these aggregates form liquid crystalline phases. In-plane and out-of-plane alignment of these phases has been reported although controlling the surface anchoring of these materials remains a challenge. Currently, templating by LCLCs has been restricted to controlling the unidirectional organization of water-compatible materials such...
as carbon nanotubes\textsuperscript{39} and motile bacteria\textsuperscript{40-42}, as well as the patterning of an inorganic LC-silica hybrid\textsuperscript{43}.

Here, we present a generic method to organize soft materials in a complex pattern across multiple length scales using LCLC templating. With two different well-studied amphiphilic supramolecular materials, we demonstrate the concepts of our approach in sequential steps. In the first step, we achieve centimeter-scale unidirectionally aligned assemblies by using the LCLC template on conventionally rubbed polyimide surfaces. Next, we show that we have full control over the spatial organization, from micron to macroscale, by combining LCLC templating with tunable photopatterned substrates. Lastly, we show that post-modification of the pre-organized assemblies yields highly stable, aligned and optically active 2D materials in any desired shape and size.

2.2 MATERIALS AND METHODS

We organized two functional amphiphiles 1 and 2, (\text{\textbf{FIGURE 1}}) with a different self-assembly motive. Both classes of amphiphiles have both promising biomedical and sensing applications (especially 2 and its wide range of analogues)\textsuperscript{11-14}, but also contain a diacetylene group that can be polymerized after assembly, which offers opportunities to develop their electro-optic properties.

\textbf{FIGURE 1} Materials used for LCLC templating. Molecular structures (a) of bolaamphiphile UD\textsubscript{12}U\textsuperscript{1a}, UD\textsubscript{10}U\textsuperscript{1b}, peptide amphiphile 2, DSCG, PMAz and Triton X-100. Cryo-transmission electron microscopy (Cryo-TEM) images (b,c) of \textit{1a} and \textit{1b} (0.14 wt% in water) respectively. TEM image (d) of a single fiber of 2 assembled in milli-Q. The UV-vis spectra (e) of \textit{1a}, \textit{1b} and 2, show strong light absorption of the amphiphilic polydiacetylenes after photopolymerization. Images (b,c,e) are adapted from Ref.\textsuperscript{44} by permission of The Royal Society of Chemistry.

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The non-ionic bolaamphiphiles we studied, \textit{1A} and \textit{1B} (\text{\textbf{FIGURE 1}}) consist of two oligo(ethyleneoxide) outer segments and a hydrophobic interior featuring two urea groups and alkyl-spacers. In water, both \textit{1A} and \textit{1B} form 1D rodlike aggregates, which are isotropically distributed over the sample (\text{\textbf{FIGURE 1B, 1C}}). Additionally, peptide amphiphile 2 was used, which consists of a 25 carbon hydrophobic tail and a hydrophilic GAGAK oligopeptide sequence and was previously studied in our group\textsuperscript{45,46}. In aqueous solutions, 2 self-assembles through hydrophobic shielding and hydrogen bonding interactions, forming micron to millimeter long twisted beta-sheet fibers (\text{\textbf{FIGURE 1D}}). Peptide amphiphiles are promising materials for a range of biomedical applications including tissue engineering and bone mineralization\textsuperscript{11-14,47}. The work of Stupp and others underline the necessity for controlling the spatial organization of assemblies over multiple length scales in order to engineer highly aligned tissues\textsuperscript{13} and for integrating these materials within biosensing and electronic devices\textsuperscript{1}.

Similar to the bolaamphiphiles \textit{1a} and \textit{1b}, peptide amphiphiles such as 2 also form isotropic assemblies in water (\text{\textbf{FIGURE S1A,B,C}}), but large scale organization has been reported\textsuperscript{14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38}. Compounds \textit{1A}, \textit{1B} and 2 are equipped with diacetylene moieties, which can be crosslinked by topological photopolymerization, which induces strong light absorption in the visible range (\text{\textbf{FIGURE 1E}}). The difference in the sharpness and position of the peaks between the compounds can be attributed to subtle changes in the packing of the side chains of the polydiacetylenes\textsuperscript{44}. The resulting n-conjugated polymer backbone introduces dimensional stability of the self-assembled structures, which allows us to study them in detail and in addition gives these materials strong anisotropic chromatic properties\textsuperscript{44}. The combination of spatial control, dimensional stability through crosslinking and the molecular optical properties makes these self-assembled structures ideal candidates for biomedical sensing and engineering applications\textsuperscript{44,45,46,47,48,49,50,51,52,53}. 

\begin{figure}[h] 
\includegraphics[width=\textwidth]{figure1.png} 
\caption{Materials used for LCLC templating. Molecular structures (a) of bolaamphiphile UD\textsubscript{12}U\textsuperscript{1a}, UD\textsubscript{10}U\textsuperscript{1b}, peptide amphiphile 2, DSCG, PMAz and Triton X-100. Cryo-transmission electron microscopy (Cryo-TEM) images (b,c) of \textit{1a} and \textit{1b} (0.14 wt% in water) respectively. TEM image (d) of a single fiber of 2 assembled in milli-Q. The UV-vis spectra (e) of \textit{1a}, \textit{1b} and 2, show strong light absorption of the amphiphilic polydiacetylenes after photopolymerization. Images (b,c,e) are adapted from Ref.\textsuperscript{44} by permission of The Royal Society of Chemistry.}
\end{figure}
2.3 RESULTS

2.3.1 UNIDIRECTIONAL MACROSCOPIC ALIGNMENT

To align both types of amphiphiles on a macroscopic scale, we used an LCLC template based on disodium cromoglycate (DSCG, FIGURE 1A) which at 14 wt% in water forms a nematic liquid crystal phase at room temperature. Using conventional glass cells coated with rubbed polyimide layers, DSCG was aligned macroscopically and templated the organization of the amphiphiles dispersed in the solvent. After full alignment and subsequent photopolymerization of the amphiphiles, we studied the organization of the assemblies in detail using (polarized) optical microscopy. For scanning electron microscopy (SEM) imaging, the polyimide-coated cells were opened and the DSCG template was washed from the substrates. The improved mechanical properties as a result of the photopolymerization process prevented the disassembly of the amphiphiles during the washing process.

FIGURE 2 shows polarized optical microscopy (POM) and SEM images of unidirectionally aligned samples of 1A, 1B and 2 in DSCG between two parallel rubbed polyimide-coated glass substrates. For all three samples between crossed polarizers, we observe one single color (FIGURE 2A, D AND G), indicating unidirectional alignment of the LCLC. The variation in colors between the cells relates to a small difference in cell thicknesses. The LCLC stacks are aligned along the rubbing direction (vertical), as confirmed by analyzing the optical appearance after inserting a quarter wave plate between analyzer and sample in the POM stage (FIGURE S3).

After removing the polarizers, (FIGURE 2B, E AND H) one can clearly see the assembled amphiphiles that are almost perfectly aligned in the direction of the DSCG stacks. The structures are larger than the nanometer-wide structures one obtains from assembly in plain water (FIGURE S1C). This is also confirmed by the SEM images that show bundles of thin assemblies, which we attribute to depletion effects and elastic forces induced by the LCLC. Once assembled, stray light or irradiation with UV light polymerizes the diacetylene moieties, as visible by a distinct color change of the amphiphiles in both the LCLC cells (FIGURE 2) and in bulk nematic DSCG solutions (FIGURE S4). The macroscopic alignment...
of the n-conjugated backbones of these amphiphilic polydiacetylenes induces strong linear polarized absorption characteristics (FIGURE 3). Furthermore, the polymerized assemblies are very stable in demanding conditions, even at temperatures as high as 90 °C (FIGURE S5). When we apply the same temperature sweep to non-polymerized assemblies (FIGURE S6), we observe that 2 completely dissolves while 1A partially dissolved due to its low solubility in the LCLC. Microscopy images of control experiments where solutions of 1A and 2 are dissolved in milli-Q in the absence of DSCG or in the isotropic phase of DSCG (5.1 wt%) show no macroscopic alignment of self-assembled 1A (FIGURE S7) and 2 (FIGURE S1) on rubbed polyimide surfaces.

2.3.2 FULL SPATIAL CONTROL

So far, we created homogeneously aligned assemblies, which can also be prepared by other techniques, for instance flow alignment and magnetic fields. For truly spatial control however, one wants to locally direct this organization in any desired direction. To this end we used a photopatternable substrate or command layer (CL) to pattern our LCLC template. CLs are frequently used to control the organization of thermotropic LCs and (to a lesser extent) LCLCs such as DSCG. In the latter case, a surfactant is needed which anchors to the hydrophobic CL and couples the in-plane organization of the CL to the hydrophilic LCLC. Beyond thermotropic liquid crystals and their elastomers, command layers have been used to organize few other materials such as liquid crystalline silica and pentacene.

In our work we employ a photopatternable CL based on a azobenzene polymethacrylate (PMAz, FIGURE 1A), which allows for micron scale pattern formation using simple procedures based on multiple polarized light illumination steps with photomasks. PMAz combined with DSCG requires the use of a surfactant to align the LCLC. In the early stages of the project, we were unable to reproduce the results by Fujiwara and Ichimura who studied DSCG alignment on PMAz command layer. This relatively simple procedure solves the outstanding challenge in the literature to obtain truly local orientational control in aqueous materials, without the need to resort to external stimuli such as electric fields.

Whilst 1A (vide supra) and 1B (FIGURE S9) follow the patterned DSCG template to generate complex structures, the directional organization of peptide amphiphile 2 was not compatible under these conditions, likely due to interference of the surfactant with the self-assembly process. Also at other concentrations and with other (mixtures of) surfactants, we observed no long-scale fibrous assembly of peptide amphiphiles and/or no patterned alignment of the LCLC with respect to the CL (FIGURE S10).

To prepare the spatially organized bolaamphiphilic assemblies, we patterned a PMAz layer in three consecutive irradiation steps (with three different polarization directions) using a photomask designed after the logo of our IMM institute (for details see SUPPORTING INFORMATION, FIGURE S11). After the CL patterning procedure, an aqueous mixture of 1A, DSCG and Triton X-100 at 50 °C (in the isotropic phase) was allowed to flow in the cell by capillary forces and the sample was cooled to allow the formation of the nematic phase of DSCG and the assemblies of 1A. The procedure, schematically shown in FIGURE 4, uses simple techniques and equipment that is available in virtually any chemistry lab.
2.3 Results

The POM images of the sample clearly show the pattern of the in-plane aligned LCLC template (Figure 5), where the DSCG stacks are oriented perpendicular to the vector of the linear polarized light (Figure S12). In addition, we observe bundled aligned fibers which follow the locally aligned LCLC template. The dimensions of these assemblies are in line with the aligned structures of 1a within a polyimide rubbed LCLC template (Figure 2), as observed by optical microscopy (Figure S13) and SEM (Figure 6). Control experiments in the absence or at low concentrations of DSCG (Figure S14) do not show any amphiphile alignment with respect to the photopatterned CL. In samples without a surfactant present, DSCG is unable to follow the organization of the underlying CL (Figure S15). Under the experimental conditions of the prepared sample depicted in Figure 5, we observe some tactoids in the nematic LCLC. These are small isotropic droplets in the nematic matrix that are related to the type of surfactant, its concentration and the exact temperature. They can be removed (or induced) by changing the surfactant (type or concentration) or by changing the stability of the nematic phase (by altering the DSCG concentration or temperature).

Figure 5 Spatially organized amphiphiles on multiple length scales in a LCLC template on a photopatterned CL. POM images of a mixture of 1a (0.086 wt%), DSCG (14.2 wt%) and Triton X-100 (0.26 wt%), after capillary insertion in a patterned PMAz ITO/glass cell, in which bundles of fibers are aligned locally in each patterned LCLC domain (the direction is indicated by the single white double-sided arrows). A 137nm quarter waveplate is inserted between sample and analyzer (depicted by the white dashed arrow). The cell thickness is not entirely uniform, yielding a color gradient over the sample. POM image (a), which was composed of multiple smaller POM images, shows an overview of the in-plane patterned LCLC domains grouped in three distinct orientations (indicated by the yellow double-sided arrows). Panels (b-d) are close-ups of areas with multiple orientations. The white dashed letters “Inst” in image (d) are inserted to show that the patterned resolution of the LCLC is not sufficiently high enough to make these micrometer-sized letters (photopatterned in the CL) clearly legible.

Figure 6 SEM images of aligned bundles (a) of 1a on a photopatterned CL after the cell has been opened and the LCLC template has been washed away. Image (b) shows bundles of 1a at higher magnification.
2.4 DISCUSSION

We believe that elastic and shear forces generated by the LCLC template are responsible for the local macroscopic alignment of the amphiphiles. As the sample is cooled from the isotropic phase, we initially observe that the multiple LCLC domains present, slowly reorient to the underlying PMAz command layer (FIGURE S16A). During mass reorientation of the LCLC domains into larger domains, the non-polymerized amphiphilic fibers and bundles of 1A undergo shear and elastic forces and consequently follow the orientation of the template. As a result of depletion and elastic forces, these amphiphiles form anisotropic micron-sized bundles that are visible with optical microscopy (FIGURE S16B) and polymerize over time by exposure to stray light. The presence of the amphiphiles reduces the attainable resolution. Whereas a 35 μm resolution is feasible for DSCG in the absence of 1A (FIGURE S17A,B), the ~50 μm wide lettering below the Institute’s logo becomes illegible with 1A present (FIGURE 5D). The small features are better reproduced when the alignment angle between adjacent areas decreases (FIGURE S17C,D). We tentatively attribute the decrease in resolution to the high stiffness of the assemblies that is further increased by the bundling process and to the competition of DSCG interactions between the CL and the assemblies. Assembly stiffness, depletion interactions, anchoring energies and device geometry should be optimized in order to obtain the highest possible resolution using the technique presented here.

2.5 CONCLUSION

Overall, we have demonstrated for the first time that it is possible to fully control the spatial organization of water-processable supramolecular materials across multiple length scales (from microns to centimeters). The combination of LCLC templating with photo-addressable command layers allows for structure formation in any shape and size. When applying this approach to amphiphilic supramolecular materials, the introduction of diacetylene moieties directly transforms these assemblies into π-conjugating optically active materials. After assembly and fixation, the template can be removed by a simple washing step, leaving the organized functional structures on the substrate.

One of the major advantages of the technique we present here that it can be applied to spatially organize a wide range of soft matter since the alignment procedure relies solely on orientational shear and elastic forces from the LC template instead of delicate short range electrostatic or Van der Waals interactions. From an application perspective, we envisage a plethora of biomedical and molecular opto-electronic applications. For example in biosensing, we expect a greatly increased electrical transduction efficiency along unidirectionally aligned polymer backbones (functionalized with sensing elements). Alternatively, spatially patterned bioscaffolds can be used to create cell-seeding islands as well as forming highly aligned tissue.

Furthermore, this technology can be readily used to generate complex architectures, for instance on curved surfaces or confined geometries, such as microfluidics [7]. Hierarchically ordered in-plane geometries [61,63,64] and off-axis alignment [69] allow the organization to extend even in the third dimension. Since the technology mainly relies on conventional spin coating and photo-illumination and due to the implementation of CLs by Sharp in 2009 for the production of their next-generation LCD TVs [72], we strongly believe commercial application and mass-manufacturing of such CL-based devices is within reach.

ACKNOWLEDGEMENTS 2.6

We would like to acknowledge Dr. Laura Cattaneo and Tonnie Toonen for their help with the photoalignment setup and the TechnoCentrum for the fabrication of the cell construction apparatus. We would also like to thank Dr. Laurens de Haan for helpful discussions.
the patterned CL. Most likely a surfactant with a lower HLB-value is necessary (with a larger lipophilic
along the patterned CL. Therefore, we turned to a different type of surfactant, Triton X-100, with a
Unfortunately, the addition of this surfactant to nematic DSCG did not facilitate in-plane alignment
Ichimura and coworkers used a poly(oxyethylene) laurel ether surfactant, Emulgen 108 with a HLB-value (which indicates the ratio of hydrophilic and lipophilic sections of a surfactant) of 12.1 at 0.1 and 0.3 wt% concentrations to align DISC. [9] We used a similar molecular type of surfactant (Brij 12) supplied by Sigma Aldrich which has a higher HLB-value of 16.9. Unfortunately, the addition of this surfactant to nematic DISC did not facilitate in-plane alignment along the patterned CL. Therefore, we turned to a different type of surfactant, Triton X-100, with a lower HLB-value, which when mixed with DISC, did facilitate alignment of the nematic DISC along the patterned CL. Most likely a surfactant with a lower HLB-value is necessary (with a larger lipophilic content) to provide a stronger anchoring to the lipophilic CL, dominated by the anisotropy of the monolayers.
Furthermore, Ichimura and co-workers used DSCG at much lower concentrations to form a nematic phase. In our work, these concentrations form a biphasic nematic/isotropic mesophase. Secondly, they state the addition of surfactant is necessary to generate the nematic phase, although it is commonly known that DSCG forms a nematic phase at room temperature by dissolution in water at a range of concentrations. Thirdly, we spin coated PMAz on cleaned ITO/glass substrates rather than on cleaned glass substrates since the polymer solution did not adhere properly to the glass, causing ruptures in the film when the cells were filled with an aqueous solution. Spin coating PMAz on top of a layer of (non-rubbed) polyimide also helped to circumvent this issue.

2.8 SUPPORTING INFORMATION

MATERIALS

DSCG and Triton X-100 were purchased from Sigma Aldrich and used as such. Details concerning the synthesis of 1A, 1B, 2 and PMAz can be found below.

POLYIMIDE & PMAZ CELL PREPARATION

For polyimide cell fabrication, microscope glass slides (VWR) were cut into pieces 20 x 20 mm and 24 x 24 mm. ITO/glass plates (CECiooP, PGO) were used for PMAz cell fabrication and cut in similar dimensions. After scrubbing the plates with a toothbrush and soap, they were rinsed with demineralized water and sonicated in ethanol (99.8%) for 30 minutes. Subsequently, the glass plates were dried under nitrogen flow and additionally cleaned for 30 minutes using a Novoscan PSD UV Surface Decontamination System. The glass plates were spin coated (SPINi50 Spin Processor) with polyimide (Pyralin PI 2555, HD Microsystems in N-methyl-2-pyrrolidone (2 wt%) for 107 s at 2000 rpm, after which they were baked at 180 °C for 2 hours and rubbed with a home-built rubbing machine. The ITO/glass plates were spin coated with PMAz in p-xylene (0.6 wt%) for 107 s at 2000 rpm. Polyimide and PMAz cells were fabricated by sandwiching two plates with a 23 μm mylar spacer, after which the plates were glued on two opposing sides with two-component epoxy glue and dried for at least one hour before use.

COMMAND LAYER PHOTOALIGNMENT PROCEDURE

A setup (FIGURE 7) was used consisting of a Newport Oriel Apex illuminator, a converging lens, a BG39 Bandpass filter, polarizer sheet and a sample stage in which preconstructed PMAz cells were clamped. The lamp was turned on for 10 minutes before starting the photoalignment procedure. In-plane patterned CLs were created by irradiating the PMAz cells for 3 minutes after which the polarizer was rotated and a part of the cell was covered with a photomask (designed after the logo of the IMM institute). Another 3 minute irradiation step aligned the uncovered part of the CL with respect to the orientation of the polarizer sheet, resulting in an in-plane patterned CL. For creating in-plane patterned CLs with more than 2 in-plane angles, additional irradiation steps were performed with additional photomasks and different orientations for the polarizer.

FIGURE 7 Schematic overview of the photoalignment setup.

SAMPLE PREPARATION

1A was dissolved (assisted by multiple sonication and vortexing steps) in a milli-Q stock solution (2 wt%) in an eppendorf tube. 50 μL of the solution was taken and added up to 500 μL volume with milli-Q. 500 μL of this solution was added to 500 μL of a Triton X-100 in milli-Q solution (0.6 wt%). The combined mixture was homogenized thoroughly on a vortex mixer and a specific amount was added to a vial containing solid DSCG to reach the desired concentration (14.2 wt%). After addition, the mixture was heated gently with a heatgun to fully dissolve DSCG into the aqueous solution. For samples without Triton X-100, the preparation was analogous to the previous example, except that 1A (0.1 wt%) was prepared directly from the corresponding stock solution (2 wt%). For preparing solutions containing 2, a mixture of 2 in milli-Q (1 mL, 0.03 wt%) was prepared in an eppendorf tube which was sealed with parafilm. For samples containing Triton X-100, a specific amount of 2 was weighed in an eppendorf tube after which a solution of Triton X-100 in milli-Q (0.3 wt%) was prepared in an eppendorf tube which was sealed with parafilm. For samples containing Triton X-100, a specific amount of 2 was weighed in an eppendorf tube after which a solution of Triton X-100 in milli-Q (0.3 wt%) was added. Aluminum foil was wrapped around the tube in order to prevent polymerization of assembled 2 by stray light before sample preparation and wrapped in parafilm to fixate the aluminum foil. The solution was heated in a 50 °C water bath for 30 minutes after which the solution was sonicated at 50 °C for 15 minutes. After sonication, the mixture was heated to 90 °C for 1 minute, after which the mixture was cooled down to room temperature outside of the water bath. Using a 1 mL syringe, the solution was added on a weighing balance to solid DSCG with a HPLC filter (0.2 μm membrane size) in order to remove any large prepolymerized aggregates of 2 present. The resulting suspension was heated to 90 °C to fully dissolve DSCG in the aqueous solution and completely disassemble 2 to allow temperature
induced assembly within the LCLC template during cooling the solution to room temperature.

**SOLUTION INSERTION IN CELLS**

Solutions of 1A, 1B or 2 in milli-Q with DSCG were inserted in pre-constructed parallel rubbed polyimide/glass cell at room temperature in the nematic phase or alternatively at 50 °C in the isotropic phase through capillary force. Solutions of 1A or 2 in milli-Q with DSCG and Triton X-100 were inserted in the isotropic phase by capillary force in pre-constructed PMAz coated ITO/glass cells, which were heated for 5 minutes at 50 °C and maintained at that temperature in a Linkam TMS 92 temperature controlled stage during filling. After filling, the cells were sealed with epoxy glue and dried for at least 1 hour after which the samples were investigated with optical microscopy. During drying, the glue might expand a few microns, resulting in different thicknesses of the DSCG layer between cells, which explains the variation of the interference colors visible between samples in **Figure 2** and **Figure 5**.

**PHOTOPOLYMERIZATION OF AMPHIPHILES & CELL OPENING**

After the amphiphilic nanostructures were aligned within the LCLC template, they were photopolymerized by keeping them for a few hours uncovered on a lab table, exposed to sunlight and/or conventional lighting. Alternatively, 5 minutes of illumination (10 cm distance from the source) with a conventional UV light source was sufficient to polymerize the amphiphiles. The bundled nanostructures turned blue due to the crosslinking of the diacetylene-functionalized backbones. The glass cells were opened by soaking them in dichloromethane for 30 to 45 minutes. After this period, the softened epoxy glue was peeled off the glass by using a scalpel. The glass cells were pried open and the LCLC was removed by adding several drops of milli-Q on the glass plates, which were removed after 15 minutes by tilting the glass plate on a piece of KimWipe.

**SYNTHESIS OF 1A AND 1B**

The synthetic procedure towards 1A and 1B was followed according to a previous literature procedure¹, besides that the 11-aminoundecanoyl-(poly(ethylene glycol)-monomethylether)-ester was synthesized according to another protocol⁴. Within this protocol an enzymatic coupling was performed (described below) for the synthesis of N-(tert-butyloxy carbonyl)-11-aminoundecanoyl-(poly(ethylene glycol)-monomethylether)-ester.

N-(tert-butyloxy carbonyl)-11-aminoundecanoic acid (3.05 g, 9.95 mmol), poly(ethylene glycol)-monomethyl ether (M<sub>n</sub> ca. 350) (4.23 g, 11.94 mmol) and Novozym 435 (2.12 g, 30 wt%) in 40 mL CHCl₃ were placed in a round bottom flask and the reaction mixture was stirred at 47 °C and 750 mbar in a rotary evaporator for 5 hours. To the resultant solution, molecular sieves were added and the mixture was allowed to react overnight. The suspension was filtered and the filtrate evaporated to dryness. The product was purified using flash column chromatography (silica gel, DCM/Ethanol 23:2 v/v) yielding 1a (4.29 g, 6.75 mmol, 78%) as a viscous transparent oil.

¹H-NMR (400 MHz, CDCl₃, T=295K): δ = 4.55 (bs, 1H, NH), 4.17 (t, 2H, J(H,H) = 4 Hz, CH₂OCO), 3.7-3.5 (m, 30H, OCH₂), 3.33 (s, 3H, OCH₃), 3.05 (q, 2H, J(H,H) = 6.7 Hz, CH₂N), 2.27 (t, 2H, J(H,H) = 8 Hz, CH₂CO), 1.60-1.53 (m, 2H, CH₂CH₂CO), 1.39 (s, 9H, C(CH₃)₃), 1.23-1.20 (m, 14H, CH₂).

¹C-NMR (400 MHz, CDCl₃, T=295K): δ = 173.79, 156.02 78.94, 71.99, 70.68, 70.56, 69.25, 63.39, 50.09, 40.67, 34.23, 30.11, 29.50, 29.38, 29.29, 29.25, 29.13, 28.49, 26.82, 24.93.

FT-IR (cm⁻¹): 3364, 2925, 2857, 1734, 1712, 1520, 1455, 1390, 1365, 1350, 1248, 1170, 1102, 1041, 950, 854, 732, 554.


GPC (CHCl₃; PS standards): M<sub>n</sub> = 1092 g/mol, PDI = 1.04.

**SYNTHESIS OF 2**

2 was synthesized according to a previous report⁵.
**PMAZ SYNTHESIS**

4-Methacyrloyloxyazobenzene (MAz) and the homopolymer PMAz were synthesized according to a previous report and characterized with IR, UV-vis and GPC (Figure S18, S19 and S20).

**POLARIZED OPTICAL MICROSCOPY**

(P)OM images in Figure 2, 3, 5S, 5T, 5V, 5S, 5T, 5U, 5V, 5W, 5X, 5Y, 5Z, 5AA and 5AB were taken with a Leica DM-RX polarized optical microscope and a Evolution VF camera. The OM images of bundles of 1A and 2 within LCLC templates in these figures were recorded as usual and afterwards a background image was subtracted to remove any out of focus textures on the image due to dust or grease present on some parts of the microscope (e.g. on lenses, microscope slides). Furthermore for Figure 2B,E,H, 3, 5S, 5T, 5U, 5V, 5W, 5X, 5Y, 5Z and 5AA the Levels tool in Photoshop CS5 was used to increase the contrast of the assemblies of 1A, 1B and 2 with respect to the background in order to improve the visibility of the aligned structures. (P)OM images in Figure 5, 5S, 5T, 5U, 5V, 5W, 5X, 5Y and 5Z were taken with a Olympus BX60 polarized optical microscope and a CoolSNAP-Pro camera. For determining the orientation of the LCLC stacks, a U-TP137 137nm retardation plate was used to determine the respective LCLC orientation within the sample cells. (P)OM images in Figure S1A,B,D,E,S6,5S and S1A with a Carl Zeiss Jenaval polarized optical microscope and an AmScope MD900E camera.

**ELECTRON MICROSCOPY**

For SEM imaging, samples were coated with gold/palladium using a Cressington 208HR sputter coater at 20 mA for 10 seconds. SEM images were taken on a JEOL 6330 Cryo Field Emission Scanning Electron Microscope at 3 keV. TEM images were taken with a JEOL 1010.

**IR SPECTROOSCOPY**

IR spectrum of PMAz (Figure S18) was taken with a Bruker Tensor-27.

**UV-VIS SPECTROSCOPY**

UV-vis spectrum of 2 (Figure 1E) and PMAz (Figure S19) were taken with a Jasco J-815 CD spectrometer.

**GEL PERMEATION CHROMATOGRAPHY (GPC)**

GPC spectrum of PMAz (Figure S20) was taken with a Shimadzu GPC LC20AT.

**SUPPORTING FIGURES**

**Figure S1** Control experiments of 2 on rubbed polyimide. Microscopy images of 2 (0.1 wt%) assembled (a,b,c) in the absence of DSCG and (d,e,f) of 2 (0.05 wt%) in isotropic DSCG (5.1 wt%). In both situations OM images (a,d) show single bundled aggregates without any macroscopic alignment. The horizontal stripes in the background in image (d) are present due to interference of the lab lighting with the camera of the OM. POM images (b,e) show no birefringence of the medium, even at higher exposure times for the microscope camera. SEM images (c,f) show thin fibrous structures organized isotropically. The rubbing in all samples is in the vertical in-plane direction. The concentration of 2 in these control samples is higher than those in Figure 2G,H, 3, 5S, 5T, 5U, 5V, 5W, 5X, 5Y, 5Z and 5AA, since the control experiments were done with an older batch of 2 from which a significant part of material had been prepolymerized (by stray light or unknown aging effects), even when stored in an aluminum foil wrapped vial and stored in the freezer. The experimental conditions were optimized when using a fresh batch of 2, and a significant lower concentration was needed to obtain the same density macroscopically aligned bundles in a DSCG template (determined visually by POM and OM), since a much smaller percentage was prepolymerized over time before sample preparation.
Supporting Information

2.8 Supporting Information

Figure S2: Michel Levy chart. The chart was used to determine which of the interference colors present (when the aligned nematic phase of DSCG was inspected with POM and a 137nm quarter waveplate) can be attributed to a higher or lower phase shift. Obtained from: [http://www.olympusmicro.com/primer/techniques/polarized/michel.html](http://www.olympusmicro.com/primer/techniques/polarized/michel.html).

Figure S3: Determination of LC alignment direction on rubbed polyimide. POM image of a mixture of 1a (0.086 wt%), DSCG (14.2 wt%) and Triton X-100 (0.26 wt%), showing a unidirectionally aligned DSCG domain in the direction of a rubbed polyimide surface without (a) and with a (b,c,d) quarter waveplate inserted between analyzer and sample. The central white double-sided arrows indicate the rubbing direction. DSCG is negatively birefringent as it forms elongated aggregates due to π-π stacking of individual stacked plank shaped molecules. The stacked aggregates in the domains which show a higher order (green) interference color (c) are aligned with their long axis parallel to the slow axis of the quarter waveplate. When rotating the sample 90° (d), the interference color changes to (lower order) red, indicating the LCLC stacks are aligned parallel to the fast axis of the quarter waveplate and thus parallel to the rubbing direction.

Figure S4: Bulk solutions of amphiphiles in nematic DSCG before and after photopolymerization. Vial of 2 in nematic DSCG/water before (a) and after (b) photopolymerization, when the solution turns blue. The red particles in the solution in (a) are a few prepolymerized aggregates (which are colored red due to the temperature sweep applied during sample preparation). These aggregates passed through the HPLC filter (0.2 μm membrane size) during sample preparation (see supporting information). Image (c) shows vials of solutions of 1a and 1b in nematic DSCG/water before (left) and after (right) photopolymerization, when both solutions visibly change in color.

Figure S5: Effect of temperature on photopolymerized 1a and 2 in LCLC template. OM images of (a,b,c) 1a (0.086 wt%) and DSCG (14.2 wt%); at room temperature (a), during heating to the isotropic phase (b), and at 90° Celsius (c). During melting, the isotropic islands of DSCG grow in size and at one point the interface layer comes into contact with bundles of 1a. At that point the long-range attractive interactions force the bundles of 1a to the interface layer between nematic domains and the isotropic islands, which reduces the volume of LC distortions and the resulting total free energy of the system. Since the islands grow over time during melting and consequently displaces the interface layer, the bundles are partially transported in this moving interface layer, resulting in a slight disordered alignment after heating to the
complete isotropic phase. OM images of (d,e,f) $2$ (0.026 wt%) and DSCG (14.2 wt%), at room temperature (d), during heating to the isotropic phase (e) and at $90^\circ$ C (f). The cells were kept at $90^\circ$ C for at least 10 minutes, during which the overall appearance of the fibrous assemblies of $1a$ and $2$ did not change (besides that bundles of $2$ become more reddish in color).

Figure S7: Control experiments of $1a$ on rubbed polyimide. Microscopy images of $1a$ (0.1 wt%) assembled (a,b,c) in the absence of DSCG and (d,e,f) $1a$ (0.095 wt%) in isotropic DSCG (5.1 wt%) on polyimide. The rubbing of the polyimide in all samples is in the vertical in-plane direction. OM image (d) shows bundles of $1a$ organized unidirectionally in random in-plane orientations which are slightly birefringent as visible in the respective POM image (e) at higher exposure times for the microscope camera. Despite assembled in an isotropic DSCG medium (d,e,f), bundles of $1a$ still form due to depletion induced phase separation. Assemblies of $1a$ don't show such micron-sized bundles (a,b,c).

Figure S6: Effect of temperature on non-photopolymerized $1a$ and $2$ in an LCLC template in rubbed polyimide cells (vertical direction). OM images of (a,b) $1a$ (0.086 wt%) and DSCG (14.2 wt%), at room temperature (a) and at $90^\circ$ Celsius (b). OM images of (c,d) $2$ (0.026 wt%) and DSCG (14.2 wt%), at room temperature (c) and at $85^\circ$ Celsius (d). Non-polymerized assemblies of $1a$ do not dissolve (due to its low solubility in water), while $2$ completely dissolves. The horizontal stripes in the background in images (a,b,d) are present due to interference of the lab lighting with the camera of the OM.

Figure S8: Anisotropic absorption characteristics of unidirectionally aligned photopolymerized amphiphiles. OM images depicting the strong linear polarized absorption characteristics of unidirectionally aligned $1a$ (a,b) and $2$ (c,d) when the aligned bundles are aligned parallel or perpendicular to the linear
polarization of the light, indicated by the double-sided white arrows. OM images are taken of mixtures of (a,b) 1a (0.086 wt%) and (c,d) 2 (0.026 wt%) in nematic DSCG (14.2 wt%) on a vertically rubbed polyimide glass cell and at the exact same spot for each individual cell.

**Figure S1** Spatially organized 1b on multiple length scales in a locally aligned LCLC template on a photopatterned CL. POM image of a mixture of 1b (0.086 wt%), DSCG (13.8 wt%) and Triton X-100 (0.26 wt%), after capillary insertion in a patterned PMAz ITO/glass cell. A 137 nm quarter waveplate is inserted between sample and analyzer (depicted by the white dashed arrow). The double-sided white arrows indicate the orientation of the LCLC stacks as well as micron-wide bundles of 1b in each domain.

**Figure S2** LCLC template of 2 on photopatterned CL. POM images (a,b) and OM image (c) of 2 (0.003 wt%), DSCG (14.2 wt%) and Triton X-100 (0.26 wt%) on a photopatterned command layer. Blue arrows indicate local in-plane orientation of DSCG. Image (c) shows localized red 1b clusters on the interface between isotropic droplet and nematic DSCG. No aligned 1b bundles can be observed in the entire cell.

**Figure S3** Schematic view showing CL pattern used in Figure 5. The CL depicted was patterned in three orientations (indicated by black, grey and red colored domains). Lines within these domains indicate the orientation of the polarizer during photoalignment.
Microscopy images (d,e,f) show 1A (0.095 wt%) and Triton X-100 (0.28 wt%) in isotropic DSCG (5.1 wt%) on photoaligned PMMA. OM image (d) shows some bundles present close to the edge of the cell. In the center of the cell, no bundles can be observed. Most likely, these large bundles are only present at the edges since the concentration of DSCG at that location is high enough (because of water evaporation at the edges of the cell) to deplete bundles of 1A into dimensions which can be observed with optical microscopy. These bundles don’t follow the organization of the photoaligned CL, as can be seen in OM image (d) and SEM image (f).

Figure S15: POM image of DSCG (14.2 wt%) without addition of a surfactant and after capillary insertion in a photopatterned CL cell. The blue double-sided arrow indicates the direction of the photoaligned azobenzene-moieties of the CL. Due to the absence of surfactant (such as Triton X-100), the hydrophilic LCLC is unable to orient along the aligned hydrophobic CL, resulting in the formation of multiple domains of nematic DSCG.

Figure S16: Process of DSCG alignment and bundle formation of 1A after insertion in a patterned CL cell. POM images showing a mixture of 1A (0.086 wt%), Triton X-100 (0.26 wt%) and DSCG (14.2 wt%) in a photoaligned PMMA cell during cooling down from the isotropic phase to the nematic phase (a). The white circle in (a) depicts the round DSCG domain (from the letter “i”) which is slowly forming and is clearly visible in (b) after the nematic phase has fully formed. In (b), bundles of 1A can be seen oriented within the aligned nematic DSCG domains. The white double-sided arrows indicate the local DSCG orientation.
Supporting Information

2 Patterning of Soft Matter Across Multiple Length Scales

Figure S17: Attainable resolution of LCLC template on a photopatterned CL. POM images (a,b) of in-plane patterned DSCG (13.8 wt%) and Triton X-100 (0.26 wt%) on a photoaligned PMAz CL. White double-sided arrows indicate the LCLC orientation. A different photomask was used to imprint a much smaller pattern of the IMM institute compared to the larger pattern imprinted in Figure 5 and Figure S17C, S17D. In image (b), the smallest details visible in the logo are in the range of roughly 15 μm. POM image (c) of a mixture of 1a (0.086 wt%), Triton X-100 (0.26 wt%) and DSCG (4.2 wt%) shows the letters "Inst" (which is a exactly the same part of the photomask used for the pattern in Figure S17C) and the respective OM image (d) (after removing polarizer and analyzer from the POM stage). Note that the smallest detail is in the order of roughly 100 μm. In the presence of 1a, the resolution of the LCLC patterns is roughly 6 fold lower, since the bundles of 1a are not flexible enough to follow the reorienting forces induced by the torque which is generated by the large angular offset between the LCLC patterned domains which anchor to the PMAz CL (facilitated by the surfactant). Since the LCLC also anchors to the bundles of 1a, the competition between the long-range interactions of the LCLC with CL as well as the bundles, combined with the bulk elastic properties of the LCLC, causes the LCLC domains to deform, lowering the resolution of the patterns.

Figure S18: IR spectrum of PMAz. 1748 (vC=O), 1600 (aromatic), 1486 (aromatic), 1094 (vC-O).

Figure S19: UV-vis spectrum of PMAz in chloroform.
FIGURE S20 GPC graph of PMAz.

SUPPORTING INFORMATION REFERENCES

CHAPTER 3

DIRECTED PEPTIDE AMPHIPHILE ASSEMBLY USING AQUEOUS LIQUID CRYSTAL TEMPLATES IN MAGNETIC FIELDS
3 DIRECTED PEPTIDE AMPHIPHILE ASSEMBLY USING AQUEOUS LIQUID CRYSTAL TEMPLATES IN MAGNETIC FIELDS

This chapter has been adapted from the article: Pim van der Asdonk, Masoumeh Keshavarz, Peter C.M. Christianen, Paul H.J. Kouwer, *Soft Matter*, 2016, 12, 6518-6525.

**ABSTRACT**

An alignment technique based on the combination of magnetic fields and a liquid crystal (LC) template uses the advantages of both approaches: the magnetic fields offer non-contact methods that apply to all sample sizes and shapes, whilst the LC templates offer high susceptibilities. The combination introduces a route to control the spatial organization of materials with low intrinsic susceptibilities. We demonstrate that we can unidirectionally align one such material, peptide amphiphiles in water, on a centimeter scale at a tenfold lower magnetic field by using a lyotropic chromonic liquid crystal as a template. We can transform the aligned supramolecular assemblies into optically active π-conjugated polymers after photopolymerization. Lastly, by reducing the magnetic field strength needed for addressing these assemblies, we are able to create more complex structures by initiating self-assembly of our supramolecular materials under competing alignment forces between the magnetically induced alignment of the assemblies (with a positive diamagnetic anisotropy) and the elastic force dominated alignment of the template (with a negative diamagnetic anisotropy), which is directed orthogonally. Although the approach is still in its infancy and many critical parameters need optimization, we believe that it is a very promising technique to create tailor-made complex structures of (aqueous) functional soft matter.

3.1 INTRODUCTION

Self-assembly in aqueous solutions is an excellent approach to fabricate functional materials that can interface with biological and biochemical processes, for instance within sensing devices. Controlling the macroscopic organization (such as micropatterns and alignment) of such aqueous supramolecular materials across multiple length scales is essential for device performance, but remains a major challenge to date. Over the years, many techniques have been developed to control the spatial organization of aqueous self-assembling materials, including photolithography, soft lithography, electrospinning, electric fields, and magnetic fields. All these techniques have demonstrated their use in specific situations, but they also suffer from limitations that are often directly associated to the organization of aqueous soft materials. For instance, such materials frequently display a low susceptibility and incompatibility to strong electric fields. Also complex pattern formation of these structures over multiple length scales on a wide range of surfaces is very challenging.

We recently explored liquid crystal templating as a tool to direct the spatial organization of supramolecular materials in water. This approach offers a number of unique advantages: (i) LC templating does not depend on delicate molecular interactions and is therefore suitable to organize a wide range of soft materials; (ii) the liquid crystal (LC) template itself is highly susceptible to external stimuli and is readily manipulated to generate complex patterns; (iii) after alignment, organization and optional post-modification, the LC template can be removed, leaving only the aligned functional material on the substrate. LC templating has been applied successfully to align organic and, to a lesser extent, aqueous functional materials. Mostly, surface interactions were used to control the alignment of aqueous soft matter, although one example of carbon nanotube alignment in a magnetic field aligned lyotropic liquid crystal was published.

Here, we use the advantages of LC templating and combine them with the unique features of magnetic field alignment, which is an intrinsic contact-free alignment technique (no specific directing surfaces needed). We demonstrate that this approach allows for the formation of complex patterns by cleverly exploiting the differences in sign and strength of the diamagnetic anisotropies of the LC template and the dispersed soft material.

To demonstrate this concept, we aligned and patterned peptide amphiphiles (PA) in a lyotropic chromonic liquid crystal (LCLC) template in a magnetic field. In an aqueous LCLC template solution, PA self-assembles into nanometer-wide fibers and progressively bundles into hierarchical micron to millimeter-sized structures. We show that we need a tenfold lower magnetic field to align these amphiphilic bundles over a centimeter range in the presence of the magneti-
3.2 MATERIALS AND METHODS

![Molecular structures (a) of PA and DSCG. Transmission electron microscopy (b) images of PA assembled in water (top) and in nematic DSCG in water (bottom), showing in both cases a fibrous twisted beta-sheet structure.](image)

The peptide amphiphile that we use are self-assembling supramolecular materials which have shown a lot of promise in both biomedical engineering and sensing applications. Its synthesis and characterization has been reported before. The amphiphile consists of a 25 carbon hydrophobic tail and a GAGAK hydrophilic head section (Figure 1A). In both water and in nematic DSCG at room temperature, PA self-assembles due to hydrophobic-hydrophilic interactions, forming long 1D beta-sheet fibers (Figure 1B). In water, these beta-sheets form isotropic assemblies at larger length scales. Furthermore, PA was functionalized with a diacetylene-moiety, which allows us to crosslink these materials by a topological photopolymerization step, resulting in a greatly improved mechanical stability and a strong chromatic response due to the \( \pi \)-conjugated backbones. For device applications, such as in tissue engineering and molecular electro-optics, controlled long range ordering of these materials is essential. Macroscopic alignment was realized with shear flow, electrospinning and a high magnetic field approach, whereas multi-length scale control was accomplished using soft lithography and recently with an LCLC template on photopatterned substrates. The high magnetic field experiments showed that, at sufficient field strengths, PA assemblies align with their long axis parallel to the magnetic fields (with the hydrogen bonds parallel and the alkyl chains perpendicular to the field). The magnetic alignment of such materials depends on the anisotropy of the diamagnetic susceptibility and the strength of the applied field. We calculated a value of \( \Delta \chi = \chi_{\parallel} - \chi_{\perp} \approx -61 \times 10^{-12} \text{m}^3 \text{mol}^{-1} \) for the molar diamagnetic susceptibility for PA (see Supporting Information). The number is negative (i.e. PA will align with the molecular axis perpendicular to the field, but 1D assemblies will align parallel to the field) and small, which confirms that even for large assemblies high magnetic fields are necessary to obtain alignment.

We chose disodium cromoglycate (DSCG) as a lyotropic chromonic liquid crystal (LCLC) template to direct the organization of PA in magnetic fields. DSCG is a rigid plank-like molecule that consists of an aromatic core with water-solubilizing groups on the periphery. In water, the molecules stack face-to-face in columnar aggregates due to \( \pi \)-\( \pi \) stacking and hydrophobic interactions, and at certain concentrations and temperatures, these aggregates form nematic and smectic phases. Recently, nematic DSCG solutions have been used to template the organization of motile bacteria and the alignment of carbon nanotubes and peptide amphiphiles.

The molar diamagnetic susceptibility of DSCG is positive and much larger. We calculated a value of \( \Delta \chi = \chi_{\parallel} - \chi_{\perp} \approx 1226 \times 10^{-12} \text{m}^3 \text{mol}^{-1} \). Now the molecules will order with their long axis parallel to the field and the 1D assemblies will order in a plane perpendicular to it. As the concentration PA is much higher than the concentration PA, one expects that DSCG can be aligned at much lower field strengths. Indeed, experimentally was found that magnetic fields as low as 0.7 T are sufficient to align a nematic solution of DSCG. By placing DSCG solutions in a confined space (such as in a glass cell of a several microns spacing), the stacks are forced to align in-plane and perpendicular to the magnetic field.
3.3 RESULTS AND DISCUSSION

3.3.1 UNIDIRECTIONAL AlIGNED ARRAYS OF \( \pi \)-CONJUGATED PePTIDE AMPHIPHILES

To create unidirectionally aligned arrays of dichromic \( \text{PA} \), we filled glass cells (23 micron spacing) with a mixture of \( \text{PA} \) (0.026 wt\%) and DSCG (13.7 wt\%) in milli-Q. The glass cells were sealed with epoxy glue to prevent water evaporation and after 1 hour of drying, the cells were loaded in the magnetic field setup. The temperature was increased to 80 °C after which a magnetic field (\( B = 2 \text{ T} \)) was turned on. With the magnetic field on, the sample was cooled to room temperature over a period of 40–60 minutes. Subsequently, the magnet field was switched off and the sample was removed. Polarized optical microscopy (POM) was used to investigate the assembly and alignment of \( \text{PA} \) and DSCG.

**Figure 2** shows microscopy images of \( \text{PA} \) and DSCG after cooling to room temperature in the presence of a 2 T magnetic field. DSCG forms a unidirectionally aligned monodomain over centimeter dimensions (**Figure 2A**). The DSCG stacks are aligned in-plane and perpendicular to the direction of the magnetic field, as confirmed by analyzing the optical appearance after inserting a quarter wave plate between analyzer and sample in the POM stage (**Figure S1**). Over the same centimeter length scales, OM reveals extended fibrous bundles aligned (**Figure 2B**), which are macroscopically aligned self-assembled \( \text{PA} \) fibers, oriented parallel to the DSCG template and thus perpendicular to the magnetic field direction. Furthermore, in many locations, spindle-like structures are observed (**Figure 2C**) from which aligned fibrous bundles emerge. We believe that these spindles are nucleation sites of the initial \( \text{PA} \) fibers formation from which (aligned) fiber growth was initiated later in the cooling process.

After assembly in the magnetic field, a photopolymerization step (initiated by leaving the glass cells uncovered on a lab table for several hours or by a 10 minute illumination with a conventional UV-lamp) crosslinked the aligned \( \text{PA} \) bundles, which consequently became bright blue in color due to the \( \pi \)-conjugated backbone structure (**Figure 2B,C**). At this stage, the cells were opened and DSCG was washed away, leaving mechanically stable \( \text{PA} \) bundles (as a result of the photopolymerization process) organized unidirectionally on the substrate. Scanning electron microscopy (SEM, **Figure 2D**) shows massive arrays of fibrous \( \text{PA} \) bundles unidirectionally aligned perpendicular to the magnetic field. Control experiments demonstrated the role of the liquid crystalline template. Without DSCG present, we found no macroscopic fiber alignment for either the samples grown in the absence (**Figure S2**) or in the presence (**Figure S1**) of the magnetic field.

Mixtures of \( \text{PA} \) in DSCG in the absence of a magnetic field showed random in-plane LCLC alignment, resulting the formation of disordered fibers (**Figure S4**). We found that the applied magnetic field has no observable influence on the observed \( \text{PA} \) bundle morphology; the microscopy images of mixtures of \( \text{PA} \) and DSCG assembled in the absence of a magnetic field (**Figure S5**) show similar \( \text{PA} \) assembly morphologies, compared to when \( \text{PA} \) is assembled in a DSCG template with a magnetic field applied (**Figure 2**).
Results and Discussion

Directed Peptide Amphiphile Assembly Using Aqueous Liquid Crystal Templates in Magnetic Fields

Due to the macroscopic unidirectional alignment of the n-conjugated backbones of these amphiphilic polydiacetylenes, strong linear dichroism is induced (Figure 3). Linearly polarized light parallel to the backbone of the PA assemblies is strongly absorbed (Figure 3A), whereas the absorption of linearly polarized light oriented perpendicular to the assemblies is drastically reduced (Figure 3B). Additionally, the polymerized assemblies are very stable in demanding conditions (Figure S6), even at temperatures as high as 90 °C.

Figure 4 schematically displays the templating mechanism responsible for macroscopic PA alignment. At high temperatures (> 80 °C) both PA (grey, Figure S7) and DSCG* (yellow) are (mostly) molecularly dissolved (Figure 4A). Cooling to 50 °C initiates self-assembly of PA fibers, which are not responsive to the 2 T magnetic field thus orient isotropically (Figure 4B). Simultaneously during cooling, the self-assembled DSCG stacks have grown long enough to form nematic LCLC domains wherein the stacks align perpendicular to the magnetic field (Figure 4C) as a result of their negative diamagnetic anisotropy. The presence of PA bundles may facilitate LCLC formation (Figure S8) since we observed a slight increase (from 32.7 to 33.7 °C) in the clearing temperature of DSCG (13.4 wt%) with PA present. Whilst the LC domains expand into a single monodomain, reorienting shear forces and elastic mediated forces direct the alignment of PA nanofibers parallel to the LCLC template (Figure 4D). Meanwhile during the cooling process, depletion interactions with the LCLC solvent forces these nanometer-wide fibers to form much thicker bundles (Figure 4E, Figure S9). After photopolymerization, the PA assemblies are very stable due to the crosslinked diacetylene cores which are oriented parallel to the fiber’s long axis (indicated by the vertical dark blue line in the inset in Figure 4F). Due to the increased stability, the template can be readily removed. In addition, the PA bundles show strong linear polarized absorption characteristics because of the presence of a transition dipole moment parallel to the n-conjugated polydiacetylene PA backbones.

Figure 5 Microscopy images of PA (0.026 wt%) in DSCG (13.7 wt% in milli-Q) after alignment in a 2 T magnetic field, showing a strong dichroic response. The temperature of the sample was raised to 65 °C (isotropic phase of DSCG) to remove dichroic contribution from the DSCG birefringence. The double-sided white arrows in the top right corner indicate the orientation of the polarizer in the OM stage. When the polarizer is aligned along the backbone of the PA assemblies (a) light is strongly absorbed due to the aligned π-conjugated backbones. When the polarizer is oriented perpendicular to the backbones of the PA, light absorption is reduced (b). Due to the increased temperature, the PA bundles undergo a color change from blue (Figure 2) to red.

Figure 4 Cartoon of the mechanism of LCLC (yellow) templated PA (grey) alignment in low (2 T) magnetic fields. Cooling the sample from a molecularly dissolved PA and DSCG solution at high temperatures (panel a) first leads to PA assembly which are not affected by the small magnetic field (panel b). At slightly lower temperatures, we find the DSCG transition into the nematic-isotropic biphasic regime. The nematic droplet (in an isotropic continuum) are aligned by the 2 T field and the PA in it will be reoriented by the liquid crystalline matrix (panel c). Further cooling increases the fraction of (aligned) nematic DSCG solution until at room temperature a continuous nematic phase is formed. At the same time, the PA monomers continue to assemble into increasingly long fibers. As their environment now is homogeneously aligned (panel d), we find full efficient templating to the PA assemblies. Keeping the sample at room temperature results in a further (lateral) assembly process of the PA fibers (panel e).
Hierarchically Patterned π-Conjugated Peptide Amphiphiles

Besides utilizing a template to realize macroscopic unidirectional peptide amphiphile orientation, we used the orthogonal diamagnetic anisotropies of DSCG and PA to create complex hierarchical PA structures in high magnetic fields. At these high fields, we anticipated a competition between the two PA alignment forces: the LCLC templates that directs the orientation perpendicular to the magnetic field and the (high) magnetic field itself, which forces the PA stacks to organize parallel to the field.

Sealed glass cells containing PA (0.026 wt%) and DSCG (13.7 wt% in milli-Q) were placed in a high magnetic field setup. The glass cells were heated to 80 °C and a 20 T magnetic field was applied (in plane of the sample). After cooling the cells to room temperature, the magnetic field was switched off and the sample was studied with (polarized) optical microscopy (Figure 5).

Surprisingly, we observed complex LC director fields (Figure 5A) as well as areas of large single domains in the range of several millimeters (Figure 5B). In contrast, when DSCG was aligned in the absence of PA (Figure S10), in a high magnetic field, a perfectly aligned monodomain was obtained. Upon closer inspection with optical microscopy (Figure 5C), we observed two distinct organizations of fibrous aggregates present everywhere in the sample: (i) fibers aligned parallel to the direction of the applied magnetic field, sometimes in conjunction with spindle-like structures (aligned perpendicular to the field); and (ii) fibers aligned perpendicular to the field in both bundle- and spindle-like formations, identical to the assemblies found after applying a 2 T magnetic field sweep (Figure 2).

After a photopolymerization step, the orthogonally aligned PA assemblies turned blue in color (Figure SC), analogous to the assemblies in Figure 2. After the cross-linking step, the glass cells were opened and the LCLC was washed away. We used SEM (Figure 6) to investigate the hierarchical structures with the two distinct organizations at higher magnifications in order to unravel its morphology. Figure 6A,B and C shows aligned fibrous bundles oriented parallel to the field, in conjunction with perpendicularly aligned spindle-like aggregates. In some locations (Figure 6D), only spindle-like aggregates were found (oriented perpendicular to the magnetic field) without any conjoined bundles that were aligned parallel to the field. These structures are also visible with optical microscopy (Figure 5C, top right corner) and are identical to the assemblies found after a 2 T magnetic field sweep (Figure 2).
3.3 RESULTS AND DISCUSSION

3.3.1 Directed Peptide Amphiphile Assembly Using Aqueous Liquid Crystal Templates in Magnetic Fields

Directed peptide amphiphile (PA) assembly with fibers oriented both perpendicular and parallel to the magnetic field. Image (c) shows unraveled fibers (perpendicular aligned to the field) from the thicker unidirectionally aligned bundle (parallel to the field). Image (d) shows a unidirectionally aligned spindle similar to a 2 T aligned PA bundle (Figure 2C).

We postulate that these two distinct PA fiber orientations actually are the result of competition in alignment introduced by the positive diamagnetic susceptibility of PA and perpendicular alignment along the LCLC template induced by its negative diamagnetic susceptibility.

FIGURE 7 schematically shows the prime processes cooling from the molecularly dissolved state at 80 °C (Figure 7A) in the presence of a high (20 T) magnetic field. Again at approximately 50 °C, PA fiber (grey) formation commences which now is directed by the strong magnetic field (Figure 7B), yielding bundles parallel to the field. Due to the time and temperature-dependent depletion interactions and the ongoing PA assembly, these fibers continue to grow in dimensions (Figure 7C). Simultaneously during cooling, small nematic DSCG domains form and grow to eventually form large nematic domains throughout the sample. Newly formed and still dissolved PA fibers may now follow the elastic forces induced by the nematic DSCG phase and orient perpendicular to the fibers that are already present before. They may give rise to new bundles that are entirely aligned perpendicular to the field (Figure 6A) or associate tangentially to the existing parallel bundles (Figure 6A, B, C and 7D).

The elastic forces are not high enough to reorient large PA bundles, which maintain their parallel orientation. One should consider, however, that phase formation in DSCG sets in at a lower temperature than PA assembly. The fact that we see significant amounts of perpendicularly oriented PA fibers suggests that the elastic forces are stronger than the magnetic forces (at 20 T). This is supported by the observation of tiny fibers emerging from the large parallel aligned bundles that unravel and predominantly bend in the direction of the nematic LCLC template (Figure 6C). These assemblies then act as a nucleation point for further PA assembly and bundling until room temperature is reached (Figure 7E).
2 and Figure 6d (perpendicular to the magnetic field direction and parallel to the DSCG stacks) were observed (Figure S11A,B). We found similar PA aggregates (again perpendicular to the field) when PA was assembled at lower concentrations (0.013 wt%) in a DSCG template at 20 T (Figure S11C). Apparently, at these lower concentrations the assembly of PA is dominated by the LCLC template despite the presence of the high magnetic field. On the other hand, at increased PA concentrations (0.039 wt%), the orthogonal structures similar to the ones in Figure 5C and Figure 6A,B were observed (Figure S11D). The higher PA concentrations result in the formation of larger assemblies which in turn have an increased diamagnetic susceptibility and hence an increased responsiveness to the magnetic field. This is further supported by control experiments which show that 0.1 wt% PA (without DSCG present) assembled at 20 T form macroscopic aligned bundles along the magnetic field direction, while at 0.015 wt% PA (without DSCG present) at 20 T no macroscopic alignment is observed (Figure S12).

Overall, the structure formation of these supramolecular materials in LCLC templates and magnetic fields depends on the relative strengths of the two opposing contributions, which both can be tuned independently. The shear and elastic forces of the template can be tailored by the particular LCLC used, as well as the temperature and concentration of the template. The diamagnetic susceptibility of PA can be tuned by its concentration and also the temperature. In addition, the magnetic field itself is an important parameter as is the (temperature, time and concentration dependent) depletion effect. A more detailed understanding of all of these factors will be the starting point to control and utilize the complex structures that are available through these methods.

### 3.4 CONCLUSION

In this work, we show that we can create unidirectional aligned arrays of functional supramolecular materials on a centimeter scale by combining LCLC templates with 2 T magnetic fields. After photopolymerization, these materials show strong linear polarized absorption characteristics due to the macroscopic aligned π-conjugated backbones. Their increased mechanical strength also allows for the removal of the LCLC template by a simple washing step, leaving the optically active amphiphiles unidirectionally aligned on the substrate. With this approach it is possible to align these materials at 10 times lower magnetic field strengths than previously reported.

This approach has the great advantage that it can be applied to macroscopically align a wide range of soft matter, since the alignment procedure solely relies on orientational shear and elastic forces and lacks the requirement for delicate molecular interactions that require fine tuning. Since we are also able to drastically lower the requirements for magnetic field alignment with this approach, we envisage the use of small commercially available permanent magnets, for instance based on neodymium, integrated within tabletop setups for applying this technique to a huge number of aqueous functional soft materials.

Additionally, the reduction of the field required for alignment opens up the opportunity to create more complex structures at much higher fields. We created orthogonally aligned PA assemblies by exploiting the opposite diamagnetic anisotropies of our LC template and self-assembled amphiphilic materials. Such self-assembled off-equilibrium structures are impossible to create on such a small length scale using other conventional alignment techniques. We believe, therefore, that this approach is very promising to create tailor-made complex structures of (aqueous) functional soft matter highly beneficial for device applications in many diverse areas. A better control of the critical parameters that we set out in the chapter will be necessary to achieve this.

**ACKNOWLEDGEMENTS 3.5**

We would like to thank both Dr. Britta Ramakers and Dr. Dennis Löwik for the synthesis of PA and the TechnoCentrum for the fabrication of the cell construction apparatus.
3.6 REFERENCES


We noticed that a part of the PA aggregates bundles are not completely aligned parallel or perpendicular to the magnet field direction (figure 5c, figure 5a-b), which we can attribute to the orthogonal alignment forces acting on the organization of bundled PA fibers (positive diamagnetic susceptibility versus GLC template shear and elastic forces) which are in the same order of magnitude. A similar effect was described before in Ref. 34.
3.7 SUPPORTING INFORMATION

MATERIALS

**PA** was synthesized according to a previous report\(^1\). DSCG was purchased from Sigma Aldrich and used as such.

The energy related to the magnetic moment of anisotropic molecules induced by an external magnetic field depends on the orientation of the molecules with respect to the magnetic field direction. The magnetic moment of a molecule induced by an applied magnetic field depends on the molar magnetic susceptibility, \(\tilde{\chi}\),

\[
\tilde{\chi} = \begin{pmatrix}
 x\chi'x' & 0 & 0 \\
 0 & y\chi'y' & 0 \\
 0 & 0 & z\chi'z'
\end{pmatrix}
\]

**Scheme 1** Schematic representation of a molecule in a magnetic field. The \(x'\), \(y'\) and \(z'\) are the principle molecular axes and \(x\), \(y\), \(z\) are the lab axes.

where \(x'\), \(y'\) and \(z'\) are the principle axes of the molecule (scheme above). The diamagnetic susceptibility of an organic molecule is given by the sum of all contributions of the different chemical groups\(^2\). The susceptibility of most of the chemical groups is known and can be found in literature\(^1\). Based on this reference, we calculated the molar magnetic susceptibility of DSCG and found that it will align with their long axis in the plane parallel to the magnetic field since its susceptibility along the \(x\)-axis is higher than all the other directions (\(\Delta \chi = \chi_{xx} - \chi_L = 1266 \times 10^{-12} \text{ m}^3 \text{ mol}^{-1}\) with \(\chi_{xx} = \chi_{yy}\) and \(\chi_L = \chi_{zz}\)).

The same calculations were carried out for **PA** monomers. The results show that **PA** monomers will be aligned perpendicular to the magnetic field direction because its susceptibility along the \(z\)-axis is higher than all the other directions (\(\Delta \chi = \chi_{z} - \chi_L = -611 \times 10^{-12} \text{ m}^3 \text{ mol}^{-1}\)).

**Glass Cell Preparation**

Microscope glass slides (VWR) were cut into pieces 14 x 20 mm and 18 x 24 mm. After scrubbing the plates with a toothbrush and soap, they were rinsed with demiwater and sonicated in ethanol (99.8%) for 30 minutes. Subsequently, the glass plates were dried under nitrogen flow and additionally cleaned for 30 minutes using a Novascan PSD UV Surface Decontamination System. Cells were fabricated by sandwiching two plates with a 23 μm mylar spacer, after which the plates were glued on two opposing sides with two-component epoxy glue and dried for at least one hour before use.

**Sample Preparation**

A solution of **PA** in milli-Q (0.03 wt%) was weighed in an empty eppendorf tube. The solution was homogenized with a vortex mixer and sealed with parafilm, after which the tube was wrapped in aluminum foil (to prevent premature photopolymerization of the **PA** by stray light) and fixated with parafilm. The solution was heated in a 50 °C water bath for 30 minutes after which the solution was sonicated at 50 °C for 15 minutes. After sonication, the mixture was heated to 90 °C for 1 minute, after which the mixture was cooled down to room temperature outside of the water bath. Using a 1 mL syringe, the solution was added on a weighing balance to solid DSCG with a HPLC filter (0.2 μm membrane size) in order to remove any large prepolymerized aggregates of **PA** present. The resulting suspension was heated to 90 °C to fully dissolve DSCG in the aqueous solution and completely disassemble **PA** to allow temperature induced assembly within the LCLC template during cooling the solution to room temperature.

**Magnetic Field Application**

After preparing the solution, the glass cells were filled. **PA** solutions in milli-Q and DSCG were inserted in the isotropic phase by capillary force in pre-constructed glass cells, which were preheated for 5 minutes at 50 °C and maintained at that temperature in a Linkam TMS 92 temperature controlled stage during filling. After filling, the cells were sealed with epoxy glue and dried for at least 1 hour after which the samples were placed in the magnetic field setup. After placing the glass cells in the magnetic field setup, the temperature was increased to 80 °C.
and maintained for 10 minutes. The magnetic field was applied and slowly over a period of 30 - 50 minutes, the sample was cooled down to room temperature. The sample was removed from the setup and investigated using optical microscopy.

**PHOTOPOLYMERIZATION**

After the amphiphilic nanostructures were aligned within the LCLC template, they were photopolymerized by keeping them for a few hours uncovered on a lab table, exposed to sunlight and/or conventional lighting. Alternatively, 5 minutes of illumination (10 cm distance from the source) with a conventional UV light source was sufficient to polymerize PA. The bundled nanostructures turned blue due to the crosslinking of the diacetylene-functionalized backbones.

**CELL OPENING & SEM IMAGING**

The glass cells were opened by a 30-45 minute soaking procedure in dichloromethane. After this period, the softened epoxy glue was peeled of the glass by using a scalpel. The glass cells were pried open and the LCLC was removed by adding several drops of milli-Q on the glass plates, which were removed after 15 minutes by tilting the glass plate on a piece of KimWipe. Due to the presence of external (capillary) forces during the opening of the cells and washing/drying of the plates, the cells were opened only after a few days when polymerized PA bundles had grown several microns wide (due to time-dependent depletion forces of PA bundles in the DSCG template). Sub-micron thick PA bundles (with less interconnected physical crosslinks) might not be strong enough for the capillary forces present during the cell opening and washing procedure. For SEM imaging, the plates were dried overnight and coated with gold/palladium using a Cressington 208HR sputter coater at 20mA for 10 seconds. SEM images were taken on a JEOL 6330 Cryo Field Emission Scanning Electron Microscope at 3 keV. TEM image was taken with a JEOL 1010.

OM images in **figure 2B, C, 3, S6** were taken with a Leica DM-RX polarized optical microscope and a Leica DMC2900 camera. After taking the image, a background image was subtracted to remove any out of focus textures on the image due to dust or grease present on some parts of the microscope (e.g. on lenses, microscope slides).

POM images in **figure 2A, S1 and S12A** were taken with an Olympus BX60 polarized optical microscope and a CoolSNAP-Pro camera. For determining the orientation of the LCLC stacks, a U-TP137 137nm retardation plate was used to determine the respective LCLC orientation within the sample cells. (P)OM images in **figures 5, S2, S3, S4, S5, S8, S9, S10, S11 and S12B** are taken with a Carl Zeiss Jenaval polarized optical microscope and an AmScope MD900E camera. Photoshop CS5 (Levels tool) was used to enhance the contrast of the OM images in **figure 2B, C, 3, S2, S5, S6, S9, S11d and S12B** in order to improve the visibility of the PA fibers.

**SUPPORTING FIGURES**

**FIGURE S1** Determination of LCLC alignment direction after 2 Tesla magnetic field application. POM image of a mixture of PA (0.026 wt%), DSCG (15.7 wt%), showing a unidirectionally aligned DSCG.
Supporting Information

Figure S4: POM (a) and OM (b) image of a mixture of PA (0.026 wt%), DSCG (13.7 wt%) in the absence of a magnetic field. POM image (a) shows random in-plane orientation of DSCG. When removing the polarizer and analyzer (b) only tiny PA aggregates can be observed organized isotropically. The horizontal stripes in the background in image (b) are present due to interference of the light with the camera of the OM.

Figure S5: OM image of DSCG (13.4 wt%) and PA (0.015 wt%) at room temperature after cooling down from 80 °C (0.6 °C/min) and photopolymerization. No magnetic field was applied during the cooling down process. The OM image shows a photopolymerized spindle-like assembly of PA fibers similar in morphology to the assemblies annealed in a 2 T magnetic field (Figure 2).

Figure S6: Effect of temperature on photopolymerized PA (0.026 wt%) and DSCG (13.7 wt%) in a 2 T magnetic field aligned LCLC template. OM image shows aligned photopolymerized bundles at room temperature (a), during heating to the isotropic phase (b) and at 65 °C (c). The cells were also heated at 90 °C for at least 10 minutes, during which the overall appearance of the fibrous assemblies of PA hardly changed. The darker out-of-focus textures are dust and/or grease spots on the outside of the glass cell.
Supporting Information

3 Directed Peptide Amphiphile Assembly Using Aqueous Liquid Crystal Templates in Magnetic Fields

Figure S7: Dynamic light scattering (DLS) plot of PA (0.1 wt% in water) during cooling down procedure. When the sample is cooled down, at roughly 50 °C, the overall scattering starts to increase, indicating the onset of PA assembly formation. DLS investigations of PA in DSCG was impossible due to the large scattering of nematic DSCG and its high concentration relative to PA.

Figure S8: POM images of (a) DSCG (13.4 wt%) and (b) DSCG (13.4 wt%) and PA (0.026 wt%) during an annealing procedure from 80 °C (0.6 °C/min) in the absence of a magnetic field. Both POM images show the onset of birefringence (lightly colored structures emerging from the dark background) arising from nematic domain formation of the LCLC. The presence of PA (b) may facilitate the onset of nematic DSCG formation due to the slightly higher isotropic to nematic transition temperature (33.7 °C compared to 32.7 °C).

Figure S9: OM images of DSCG (13.4 wt%) and PA (0.026 wt%) during an annealing procedure from 80 °C (0.6 °C/min) in the absence of a magnetic field. At 33.4 °C (a) a few PA bundles can be observed, which during cooling down (b) have grown substantially, while other bundles have emerged which are large enough to be observed with the OM. The contrast was enhanced in this image using Photoshop (Levels tool) and the OM image was converted to greyscale in order to increase the visibility of the bundles. The horizontal stripes in the background in both images are a result of interference of the light with the camera of the OM.

Figure S10: POM image DSCG (13.7 wt%) after 20 T magnetic field alignment. Double white-sided arrow indicates magnetic field direction. The horizontal stripes in the background in image (b) are present due to interference of the light with the camera of the OM. The variation in color relates to a small difference in local thicknesses of the cell.

Figure S11: (P)OM images of PA assembled in nematic DSCG (13.7 wt%) after magnetic field alignment. POM image (a) shows a DSCG monodomain after the sample was cooled at 15 T. OM image (b) shows...
the presence of similar spindle-like aggregates as in the 2 T experiment (figure 2). OM images (c) shows spindle-like PA (0.031 wt%) aggregate after cooling down in nematic DSCG (13.7 wt%) in a 20 T magnetic field aligned. When the PA concentration is increased to 0.039 wt% (d), similar structures (assembled in nematic DSCG (13.7 wt%) and in a 20 T magnetic field) emerge compared to the ones in figure 5 and 6.

**FIGURE S12** OM images of (a) PA (0.1 wt% in water) and (b) PA (0.015 wt%) after cooling down to room temperature in a 20 T magnetic field (horizontal direction) and subsequent photopolymerization. At a high PA concentration of 0.1 wt% (a), macroscopic fiber bundle alignment can be observed while at a lower concentration (0.015 wt%) only isotropic aggregates are present which do not align along the magnetic field.

**SUPPORTING INFORMATION REFERENCES**


DIRECTING SOFT MATTER IN WATER USING ELECTRIC FIELDS
4 DIRECTING SOFT MATTER IN WATER USING ELECTRIC FIELDS

This chapter has been adapted from the article: Pim van der Asdonk, Stijn Kragt, Paul H.J. Kouwer, ACS Appl. Mater. Interfaces, 2016, 8, 16303–16309.

ABSTRACT

Directing the spatial organization of functional supramolecular and polymeric materials at larger length scales is essential for many biological and molecular opto-electronic applications. Although the application of electrical fields is one of the most powerful approaches to induce spatial control, it is rarely applied experimentally in aqueous solutions, since the low susceptibility of soft and biological materials requires the use of high fields, which leads to parasitic heating and electrochemical degradation. In this work, we demonstrate that we can apply electric fields when we use a mineral liquid crystal as a responsive template. Besides aligning and positioning functional soft matter, we show that the concentration of the liquid crystal template controls the morphology of the assembly. As our setup is very easy to operate and our approach lacks specific molecular interactions, we believe it will be applicable for a wide range of (aqueous) materials.

4.1 INTRODUCTION

1D supramolecular and polymeric materials assembled in aqueous solutions are excellent materials for a wide range of opto-electronic applications, especially in situations where biological environments need to be probed. From a bottom-up perspective, these building blocks can be chemically tuned to introduce a range of functional characteristics into the assembly (e.g. mechanical stability, electro-optic response, detection of specific biological events by sensing elements). In solution however, most 1D supramolecular and polymeric materials form isotropic assemblies on a macroscopic scale (despite their high definition on the nanoscale) which greatly limits their device applicability. Over the past decades, spatial control (alignment and positioning) of these water-processable materials has proven extremely difficult to achieve, particularly at larger length scales.

Borrowing the concepts of LCD technology, the use of (locally applied) electric fields may be one of the most generic and powerful approaches to induce spatial control. Although examples of soft matter manipulation in organic solvents have been reported, its applicability in water has rarely been successful. One can identify two major challenges. First, the inherently low susceptibility of soft matter to electric fields requires the use of high fields. Second, the parasitic heating effects (Joule heating, dielectric heating) and electrochemical degradation that are associated with high electric fields in (ion-rich) aqueous media can prevent the assembly process or even destroy the structures. Any technique that addresses these drawbacks will meet popular demand in a wide variety of research fields.

We used liquid crystals (LCs) to overcome these challenges. As a result of their collective behavior, LCs are very susceptible to electric fields and, even at low fields, high degrees of order can be obtained. As such, the LC can become an anisotropic solvent that templates the organization of other functional materials, either dissolved or dispersed in the material. In addition, the absence of delicate interactions between the dispersant and the matrix renders the technique generally applicable. LC templating has found good use for functional materials in organic (bulk liquid crystal) materials, although most often substrates rather than electric fields are used to induce macroscopic order.

LC templating in aqueous environments requires water-soluble liquid crystals (lyotropic LCs). The difficulty to transfer order from a substrate to the aqueous bulk makes examples of surface-directed alignment sparse. We recently proposed a new method, based on chromonic liquid crystals, to organize supramolecular and polymeric materials across multiple length scales. We are however not aware of any templating examples of electric-field driven alignment in aqueous anisotropic media.

In this chapter, we demonstrate electric field-controlled alignment in an aqueous environment using an athermal lyotropic mineral liquid crystal (LMLC). To illustrate the concept, we align and position peptide amphiphiles that show great promise in the biomedical field, in particular for macroscopically aligned samples, and in opto-electronics.
4.2 MATERIALS AND METHODS

As an anisotropic electric field responsive template, we used colloidal goethite. Goethite are board-shaped iron-oxide nanoparticles (α-FeOOH). At volume fractions larger than ~8.5% (dependent on the aspect ratio of the sample) they form a nematic liquid crystalline phase in water. Because of their much larger dimensions (FIGURE 1A) compared to molecular thermotropic and lyotropic LCs, mesophase formation is virtually temperature independent. At lower volume fractions, goethite is so-called paranematic; no long-range orientational order exists, but nematic order is readily introduced by applying small external stimuli, such as magnetic or electric fields. The high susceptibility and the athermal behavior makes goethite an excellent candidate for an aqueous liquid crystal template. Goethite samples were synthesized following the literature procedure as detailed in the SUPPORTING INFORMATION.

The peptide amphiphile (PA, FIGURE 1B) that we align and position in this study consists of a pentapeptide (GAGAK amino acid sequence) equipped with a 25 carbon tail which, in that contains two triple bonds. In water, the peptide amphiphile self-assembles into twisted beta-sheet like nanofibers (FIGURE 1C) through a hydrophobic collapse of their alkyl tails and internal hydrogen bonding. The resulting twisted ribbons have cross sections of nanometer dimensions (~8 x 15 nm), a twist periodicity of ~90 nm and a length of the order of hundreds of microns or even longer. At larger length scales, this material forms isotropic assemblies. In the past, a range of techniques has been used to control its unidirectional organization across multiple length scales since for many biomedical and opto-electronic applications, macroscopic alignment of these functional supramolecular materials is crucial for its application. The aliphatic tail of PA is functionalized with a diacetylene moiety, which can be crosslinked by photopolymerization, after the materials is fully assembled. The induced polymerization greatly increases the mechanical stability and of the self-assembled amphiphiles. In addition, it introduces strong chromatic properties due to its π-conjugated backbone. The synthesis and characterization of PA have been described earlier. Our studies do not indicate that PA assembly in the presence of goethite is significantly different than in its absence; the PA fibers photopolymerize as usual. Later in the chapter, we do show that the goethite concentration determines the extent of lateral peptide amphiphile aggregation.

4.3 RESULTS AND DISCUSSION

ALIGNMENT OF GOETHITE DISPERSIONS 4.3.1

First, we tested the response of an isotropic (1.9 v/v%) and a nematic dispersion (12.5 v/v%) of goethite in water under the application of an AC electric field (1 MHz). Rectangular capillaries (0.05 mm x 0.50 mm x 5 cm) filled with the dispersions were placed in the electric field setup and were studied using polarized light microscopy (FIGURE 2A). The results showed a significant alignment of the goethite particles in the electric field direction. The extent of alignment was found to increase with the goethite concentration and the electric field strength. These findings are consistent with previous studies on the alignment of LCs in electric fields.

In order to apply an electric field in the aqueous solutions, we used the electric field capillary setup (FIGURE 2B) that Dozov et al. developed to study the electric field induced alignment of LMLC beidellite particles in water. This setup has some great advantages when applied in aqueous solutions: (i) the electrodes are not in contact with the solution, which prevents electrochemical degradation of the solution; (ii) at high frequencies, electrokinetic effects such as convection and flow alignment of the particles are prevented, although some Joule heating is still present; and (iii) the electrodes are not physically attached to the capillary wall, which gives necessary experimental flexibility. Detailed information about the electric field setup can be found in the SUPPORTING INFORMATION.
4.3 Results and Discussion

4.3.2 Alignment and Positioning of PA Fibers

After determining the optimum electric field strength for goethite alignment, we prepared solutions containing PA (0.15 mg mL\(^{-1}\)) and goethite (6.5 v/v\%) in water (intermediate concentration, 6.5 v/v\% in the paranematic phase) in water (see Supporting Information for the detailed procedure). We placed the capillary containing a freshly prepared sample in our electric field setup under a (P)OM stage. The electrodes were positioned at a slight oblique angle, which results in a non-uniform electric field strength. The assembly and bundling of peptide amphiphiles is a gradual process and we expected that the exact times when an electric field is switched on (and switched off) plays a critical role in the assembly process and thus in the resulting macroscopic organization of PA. Factors to consider are the PA self-assembly time, the alignment time for self-assembled PA fibers in the (reorienting) goethite dispersion and the bundling time of PA fibers.

When an AC electric field (0.4 V μm\(^{-1}\), 1 MHz) was applied to the PA/goethite dispersion, 15 minutes after sample preparation, we observed the formation of small assemblies after approximately 80 minutes, which align along the direction of the electric field (Figure 3). Furthermore, we noticed that these bundles slowly moved toward the strongest electric field (Movie S1), vide infra. In a control experiment without the electric field present (thus PA assembly in a paranematic 6.5 v/v\% goethite solution), we observed isotropically organized bundles (Figure S2). In a second control experiment with the electric field applied, but without the goethite template present (attempted electric field alignment of PA assembled in water) we did not observe assemblies at all (Figure S3). Under these conditions, the peptide amphiphiles will assemble into (isotropically distributed) fibers, but they will not organize into bundles\(^{44}\), which leaves them too small to be visualized with an optical microscope.

**Figure 2** Isotropic and nematic goethite alignment induced by AC electric field. Paranematic goethite (1.9 v/v\%) at 0 V (a), 0.49 V μm\(^{-1}\), 1 MHz under crossed polarizers parallel (b) and at 45° (c) with respect to electric field direction. Nematic goethite (12.5 v/v\%) at 0 V (d), 0.38 V μm\(^{-1}\), 1 MHz under crossed polarizers parallel (e) and at 45° (f) with respect to electric field direction. (g) Birefringence intensity for different goethite solutions under crossed polarizers (45° with respect to electric field direction) at different electric field intensities. The values were calculated by converting the colored POM images to greyscale images and analyzing the greyscale intensity on the black to white scale in a range from 0 to 256. The difference between the intensities of the birefringence of the two concentrations can not be compared directly, since slightly different microscope settings (lamp intensity, exposure time) were used for each series of POM images.

**Figure 3** Electric field alignment and positioning of PA bundles in a goethite template. The image shows aligned PA bundles (0.15 mg mL\(^{-1}\)) in goethite (6.5 v/v\% ) in a 153 V, 1 MHz AC electric field. The black

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**Note:** The images and figures mentioned in the text are not explicitly described here, but they are expected to accompany the text for visual understanding.
time dependency of electric field application

The time when the electric field is applied and removed during the hierarchical PA assembly process greatly influences the overall morphology and characteristics of the formed bundle network. Overall, we distinguish three time intervals.

Time interval #1: Interfering with nucleation: When the AC electric field was switched on immediately after sample preparation, many times we did not observe any bundle formation, even after the field was switched off again and after waiting for several hours. The underlying cause for the inhibition of bundle formation is currently unknown. Possibly, the PA did assemble into nanofibers (which are too small to detect with optical microscopy) and the electric field prevented further aggregation to form larger bundles.

Time interval #2: Window for manipulation: In the intermediate interval, we have a time window that we can use to manipulate the assembly. We studied two different scenarios. In the first, a continuous field was applied to the sample somewhere between 15 minutes and 2.5 hours after sample preparation. The field was then maintained for several hours. After about 4 hours after applying the field, the assembly and bundling process of the PA was almost finished and the unidirectionally aligned bundled network did not increase significantly in size anymore. This scenario was followed to produce (FIGURE 3). As a result, the highly anisotropically bundled PA network itself does not change anymore when the field is switched off and the goethite template undergoes the transition from the paranematic to the isotropic phase (FIGURE S4).

FIGURE 4 shows the same sample depicted in FIGURE 3, but at a much later stage in the alignment process (24 hours after the field was switched off and the electrodes were removed from the capillary). The orientation of the highly aligned bundled network which formed at high field strength in between the two electrodes was not affected by the removal of the field (bottom FIGURE 4D). We observed, however that after field removal some additional PA bundling continued (now isotropically) at that location (top FIGURE 4D). Also bundle formation in the areas covered by the aluminum electrodes is visible. Going from the outside of the electrode inwards, the organization of the PA bundles becomes progressively more anisotropic. This result is in line with the electric field strength penetration in the setup as modelled by Dozov and coworkers.

Additionally, we observed that in between the (slightly oblique) electrodes, most bundles appear at or move to the point of the highest electric field intensity (FIGURES 3 and 4), despite the small difference in the local field strength (approximately 0.05 V μm⁻¹) across the sample (MOVIE S1). This effect is commonly observed in dielectrophoresis studies. As a result, the density of bundles becomes very high and their entanglement is efficient, which locks the formed anisotropic structure quickly in place and renders it stable to changes in growth conditions, for instance when the field is removed and the goethite template loses its anisotropy. In more advanced electrode setups, this effect can be readily exploited to tailor the local density, organization and orientation of PA-based or other soft polymer scaffold materials.
foil electrodes placed around the capillary. After switching on the field (b), these bundles immediately realign in the direction of the electric field (indicated by the double sided white arrow). After switching off the field again the bundles relax (c) and realign again (d) when the field is switched on. Out of focus spots are dust particles on the outside of the capillary.

4.3 Results and Discussion

4.3.4 Controlling Bundle Sizes

Besides alignment and positioning of PA bundles in the electric field-aligned goethite template, we also studied the formation of the bundles as a function of time. Over time we observed that their density increased, but also that the average thickness of these bundles increased, first rapidly and then leveling off. We postulated that this bundling process is induced by depletion interactions between the anisotropic goethite particles and the (also anisotropic) self-assembled PA fibers. Consequently, we expected that we could control the bundling process by tuning the concentration of goethite in the solution.

We prepared samples of PA (0.15 mg mL\textsuperscript{-1}) assembled in goethite templates of different concentrations, in the absence of an electric field. OM images were taken over time and imaging software was used to measure the bundle areas (see Supporting Information for the detailed procedure). Since we are limited by the resolution of the optical microscope, we can only observe bundles of PA composed of the approximately 20 nm wide nanofibers (Figure 1B) when they reach diameters of around 1 μm. Figure 6 shows, as a function of time, the total PA fiber areas for different goethite concentrations, averaged over multiple microscopy images. The goethite concentration dependence is clear, and after full statistical analysis (see Supporting Information) is also significant. Below a threshold goethite concentration of <6 v/v%, no bundle formation is observed within the first hours of the assembly process. After a full day, some PA bundles can be observed for the 4.3 and 1.9 v/v% samples (Figure S5). At goethite concentrations beyond the threshold, bundles can be observed much earlier and bundles become thicker at increasing template concentrations.

Additionally, we measured evolution of the bundle sizes for a mixture of PA (0.15 mg mL\textsuperscript{-1}) in goethite (6.3 v/v%) assembled in an electric field (the AC field of 0.4 V μm\textsuperscript{-1}, 1 MHz, was turned on 150 minutes after sample preparation). The bundle sizes of these fibers are lower than when assembled in the absence of an electric field at the same concentration. This difference may have an origin...
in sample temperature, where Joule heating as a result of the electric field can elevate the temperature in the solution\(^n\), which in turn changes the kinetics of PA self-assembly and bundling process. Secondly, both the increased sample temperature and electric field induced macroscopic alignment of PA and goethite may alter the depletion interactions, which influences the overall PA bundle formation. Alternatively, the electric field itself may change the assembly kinetics in a similar way as it influenced the PA assembly rates when applied immediately after sample preparation (as in Time interval \#1).

We believe that this approach unlocks the use of electric fields to manipulate functional materials in aqueous solutions. It can be applied to a wide range of materials in aqueous solutions since LC templates do not rely on specific interactions between the template and dispersed assembly. In addition, the setup is very easy to build and operate. We ultimately envisage that this approach is integrated in LC display technology, where complex patterned electrode setup can be used to actively control the organization (e.g. by aligning and switching) of aqueous functional materials on many levels.

\section*{ACKNOWLEDGEMENTS}

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\section*{CONCLUSION}

Overall, we have demonstrated for the first time that it is possible to use high frequency AC electric fields to align and position self-assembling supramolecular, polymeric materials in aqueous solutions. Our approach uses a mineral LC template that is able to transfer its orientation to growing peptide amphiphile nanofibers and bundles, yielding macroscopically aligned samples. As the template is highly susceptible to electric (and magnetic) fields, it is possible to locally control the orientation but also the bundle density. Furthermore, we showed that the concentration of the mineral LC template controls the size of the bundles formed. The template may be removed after stabilizing the assemblies by post-modification (such as photopolymerization) by opening the setup and immediately washing out the goethite particles.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure6.png}
\caption{Calculated bundle areas of PA (0.15 mg mL\(^{-1}\)) measured over time in different goethite solutions. Insets show images of PA bundles after Cellprofiler analysis at two different times in a 10.8 v/v\% goethite solution. The areas within the red outlines is used for bundle area determination. Non-fibrous structures (e.g. dust) which are also outlined in red by Cellprofiler are manually removed so that the areas of these particles are not used for the total PA bundle area calculation. Error bars indicate 90\% confidence intervals for the means.}
\end{figure}
4.6 REFERENCES

4.7 SUPPORTING INFORMATION

SAMPLE PREPARATION

From a (vigorously stirred) diluted stock solution of goethite (for preparation, see Supporting Information), a pre-determined amount was taken and centrifuged at 10000 rpm for 1 hour. After centrifugation, water was removed from the top layer in order to concentrate the solution to the desired volume fraction and the resulting suspension was homogenized with a vortex mixer. Afterwards, PA was weighed in an empty eppendorf tube and a specific amount of freshly prepared goethite solution was added to the tube. The PA concentration (in mg mL\(^{-1}\)) was determined as the PA weight in relation to the volume of water. The eppendorf tube was homogenized with a vortex mixer and sealed with parafilm, after which the tube was wrapped in aluminum foil (to prevent premature photopolymerization of the PA by stray light) and fixated with parafilm. The PA/goethite solution was heated in a 50 °C water bath for 30 minutes and sonicated at 50 °C for 15 minutes. After sonication, the mixture was heated to 90 °C for 1 minute to molecularly dissolve the peptide amphiphiles, after which a 5 cm long rectangular capillary (0.05 × 0.50 mm, 5005, VitroCom) was filled with the solution at room temperature by capillary action.

ELECTRIC FIELD SETUP

To apply an electric field to the goethite solutions, a previously described set-up was used (Figure 1d,e). The capillary (containing a freshly prepared goethite solution) was placed between two glass plates (25 × 25 mm) and glued on both ends with epoxy glue. Two aluminum foil strips (5 × 1 cm) acting as electrodes, were fixed with their long axis on a separate microscope slide using scotch tape, leaving a very small gap in between. This microscope slide was placed under the microscope and two cables were used to connect the aluminum electrodes to the amplifier (Krohn-Hite 7602M) and function generator (HAMEG HM8130). Under the microscope, the electrodes were pushed closer to each other (typically 300–500 μm) and the slide was fixed to the x-y stage with scotch tape. The capillary between two glass plates was carefully placed over the microscope slide in between the two aluminum foil strips, which were then tightly folded around the capillary in direct contact with the capillary outer wall. After positioning the capillary under the microscope, scotch tape was used to fixate the glass plates at the ends of the capillary on the x-y stage.

PA FIBER DIMENSIONS ANALYSIS

In order to determine the increase of the area of the PA fibers in the goethite solution over time (without the presence of an electric field), for each concentration, 3 capillaries were filled and placed on a microscope slide after which the edges were glued with epoxy glue to avoid water evaporation from the sample. A red marker was used to mark each individual spot for analysis (3 per capillary). Over time, optical microscopy images were taken of 9 spots in total. To quantify bundle formation, the images were processed using a pipeline written in CellProfiler (version 2.1.1). In this pipeline a number of steps were performed to improve fiber visibility prior to analysis. First of all, the images were corrected for unequal illumination (CorrectIlluminationCalculate), the grayscale of the images was inverted (InvertForPrinting), long and thin features (fibers) were enhanced twice (EnhanceOrSuppressFeatures), the difference between fore- and background was enhanced (ImageMath), a threshold was applied to remove possible strong features such as any presence of dirt within the solution (ApplyThreshold), the intensity was rescaled (RescaleIntensity) and background was removed as much as possible (ApplyThreshold). This threshold used an adaptive threshold strategy with a MoG (Mixture of Gaussian) method. Now, the images were ready for the actual analysis and the objects (fibers) were identified based on intensity (IdentifyPrimaryObjects). The identified objects were filtered on area, minor axis length and eccentricity to remove a significant part of objects that were not PA fibers (FilterObjects). Fragments of PA fibers that touched each other were now unified (ReassignObjectNumbers) after which the objects were filtered again on area, minor axis length and major axis length in order to remove a significant part of small objects found in the background (FilterObjects). An image with outlines of the remaining objects on top of the earlier rescaled image was saved together with an image displaying the object numbers (Overlay outlines, DisplayDataOnImage and SaveImage). The area of the objects were measured after conversion from pixels to μm (MeasureObjectSizeShape and Calculate Math) and the data was exported to a spreadsheet (ExportToSpreadsheet). The images were checked visually for objects which were not PA fibers and these were removed from the data by hand. Formation of PA bundles also occurred in
the z-direction. Images were taken in different focal planes and only the bundles which were optimally focussed were used for bundle area calculation.

**GOETHITE SYNTHESIS**

Goethite was synthesized using a previous described procedure. For the synthesis, Fe(NO₃)₃·9H₂O (Sigma Aldrich), TMAH (tetramethylammonium hydroxide, Sigma Aldrich, 25% w/w in water) and HNO₃ (65%, Merck) were used as received. Ferric nitrate nonahydrate (36.4 g, 90.2 mmol) was dissolved in distilled water (560 mL). The tetramethyl-ammonium hydroxide solution (200 mL) was added to the solution under stirring. After stirring (30 minutes), the solution was poured into a glass bottle and closed carefully with a screw cap. The bottle was placed in a pre-heated oven (T = 120 °C). After 2 hours, the color of the solution changed from very dark red-brownish to ochre indicating the formation of the goethite particles. After 18 hours in the oven, all goethite particles were precipitated at the bottom of the flask and a colorless supernatant remained. The supernatant was removed and replaced by milli-Q (760 mL). The solution was homogenized by vigorously shaking and divided equally over 18 centrifugation tubes. The tubes were then centrifuged (7500 rpm, 35 minutes). The supernatant was removed and replaced with milli-Q (42.2 mL in each tube). The solution was homogenized by using a vortex and centrifuged (7500 rpm, 40 minutes). The supernatant was removed and replaced by HNO₃ (3 M in milli-Q, 42.2 mL in each tube). Again the solution was homogenized by using a vortex and centrifuged (7500 rpm, 45 minutes). The supernatant was removed and replaced with milli-Q (42.2 mL in each tube) and the pH was checked using pH paper and found to be around 3.5. The solutions were poured together in 1 bottle and the w/w% of this stock solution was determined by weighing an aliquot, drying it and weighing it again. The v/v% was deduced from this using the intrinsic density of the goethite particles (ρ = 4.26 g mL⁻¹) and was found to be 0.26 v/v%. The particles were further analyzed by powder X-ray diffraction (FIGURE S6) and transmission electron microscopy (TEM). For TEM, the solution was diluted 3000 times. X-ray diffraction showed that the synthesized particles overlap well with a goethite reference. The TEM images were analyzed by hand using ImageJ to determine the average dimensions of the particles: \( <L> = 169.56\) nm (σL = 0.10 nm) and \( <W> = 33.50\) nm (σW = 0.17), which results in an aspect ratio of \( L/W = 5.06\).

Statistical analysis was performed in order to determine whether there was a significant difference in the calculated PA bundle areas between capillaries of the same PA/goethite concentration and between different PA/goethite concentrations. First of all, tests were conducted to see whether bundle areas between the 3 separate capillaries containing the same PA/goethite solution were similar in the same time intervals. The following two-tailed F-test was used in order determine whether the variances between two capillaries were homogeneous (we assumed the data was distributed normally):

\[
F_{cal} = \frac{s_p^2}{s_2^2}
\]

Here, \( s_1 \) is the variance of the capillary with the biggest variance over the 3 spots and \( s_2 \) the variance of the capillary with the smallest variance over the 3 spots. This calculated \( F \) value was compared to a tabulated value for a two-tailed F-test (\( \alpha = 0.05 \)) with degrees of freedom of \( n_1 - 1 \) and \( n_2 - 1 \), in which \( n_1 \) is the number of spots for the capillary with the biggest variance and \( n_2 \) the number of spots for the capillary with the smallest variance. All variances of all the capillaries were homogeneous according to this test. The following two-tailed T-test (\( \alpha = 0.05 \) and degrees of freedom equal to \( n_1 + n_2 - 2 \)) was used to see whether bundle areas between the 3 separate capillaries containing the same PA/goethite solution were similar at each time interval.

\[
t_{cal} = \frac{|x_1 - x_2|}{\sqrt{s_p^2 \left( \frac{1}{n_1} + \frac{1}{n_2} \right)}}
\]

Here, \( x_1 \) and \( x_2 \) are the mean values of two capillaries and \( s_p^2 \) a pooled variance of the homogeneous variances of the two capillaries calculated with the following formula:

\[
s_p^2 = \frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{(n_1 - 1) + (n_2 - 1)}
\]

The calculated t-value (\( t_{cal} \)) was found to be smaller than the tabulated value (\( t_{tab} \)). Thus, the difference between the means was considered to be not
significant, which was found for all experiments when comparing bundle areas between capillaries of equal mixtures. When comparing the difference in PA bundle areas between the 6.3 v/v% and 10.8 v/v% goethite solutions, the values for the bundle areas (taken from all capillaries of a single concentration in specific time intervals) were averaged (5 to 7 measurements per averaged mean). From the averaged means, the variances for both concentrations were compared with each other at each similar time interval using a two-tailed F-test ($\alpha = 0.05$). According to the F-test the variances were homogeneous in all cases. A two-tailed T-test ($\alpha = 0.05$) was used (using the similar equations displayed above) to compare whether the bundle area of PA in the 10.8 v/v% goethite solution was significantly larger than in the 6.3 v/v% solution. The value of $t_{cal}$ was found to be larger than $t_{tab}$ for each time interval where bundles were identified, indicating that bundle areas of PA in a 10.8 v/v% goethite solution are significantly larger than those in 6.3 v/v% goethite.

(POLARIZED) OPTICAL MICROSCOPY

The (P)OM images in figure 2, 3, 4, S1, S3 and S4 were taken with a Olympus BX60 polarized optical microscope and a CoolSNAP-Pro camera. (P)OM images in figure 5 and movies S1 and S2 were shot with a Carl Zeiss Jenaval polarized optical microscope and an AmScope MD900E camera. (P)OM images in figure 6 (for calculating the bundle sizes), S2 and S5 were taken with a Leica DM-RX polarized optical microscope and a Evolution VF camera.

TRANSMISSION ELECTRON MICROSCOPY

TEM images in figure 1 were taken with a JEOL 1010 TEM.

X-RAY POWDER DIFFRACTION

The X-ray powder spectrum in figure S6 was taken with a Bruker D8 Advance.

SUPPORTING FIGURES AND MOVIES

Movies S1 and S2 can be found here: [HTTP://TINYURL.COM/ZRF62FX].

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**FIGURE S1** Goethite alignment (6.4 v/v%) determination under an electric field using a 137 nm waveplate. Images (a,b) show a goethite solution (6.4 v/v%) aligned with an 0.41 V $\mu$m$^{-1}$, 1 MHz electric field. Image (b) shows that when direction of the field is perpendicular to the waveplate orientation (striped single arrow), the interference color is almost black. In this situation, the retardation is decreased, which indicates that the waveplate is perpendicular to the long axis of the aligned goethite. Therefore, the long axis of the goethite particles are aligned parallel to the applied field.

**FIGURE S2** Bundles of PA (0.15 mg mL$^{-1}$) in goethite (6.3 v/v%) organized in an isotropic network when no electric field is applied, 5 hours after sample preparation. Dark shadow on the left of the image is an out-of-focus dot used for marking the target spot on the capillary.

**FIGURE S3** PA (0.15 mg mL$^{-1}$) in water when an AC electric field is applied (0.62 V $\mu$m$^{-1}$, 3 MHz).
4.7 Supporting Information

**Supplementing Information References**


EXTREMELY STABLE AND HOMOGENEOUS LYOTROPIC LIQUID CRYSTAL ALIGNMENT ON ANISOTROPIC SURFACES
5 EXTREMELY STABLE AND HOMOGENEOUS LYOTROPIC LIQUID CRYSTAL ALIGNMENT ON ANISOTROPIC SURFACES

This chapter has been adapted from the article: Pim van der Asdonk, Peter J. Collings, Paul H.J. Kouwer, J. Am. Chem. Soc. 2016, submitted.

ABSTRACT

Lyotropic chromonic liquid crystals have great potential in both biosensing and optical devices due to their biocompatible nature and strong optical characteristics. These applications, however, demand a homogeneous and stable alignment on anisotropic surfaces; a challenge which, so far, has not been solved adequately. In this work, we show how to drastically increase the quality of in-plane alignment and stability of these liquid crystals on conventional rubbed polyimide substrates by the addition of a small amount of a non-ionic surfactant. Samples with surfactant show excellent alignment that is stable for months, whilst control samples without surfactant show much poorer alignment that further deteriorates in days. Also, well-aligned dry films of chromonics can be prepared following our approach. We demonstrate how to obtain high quality alignment by controlling the concentration and the nature of the surfactant, in particular its molecular structure and hydrophilic/lipophilic balance (HLB value) and discuss other critical parameters. We believe that this approach may very well be essential for advancing the applicability of these water-based, biocompatible, and often highly dichroic materials for a wide range of uses.

5.1 INTRODUCTION

In the liquid crystalline (LC) phase, molecules have the ability to flow like a liquid, but possess positional order similar to a solid. This intermediate state of matter gives rise to intrinsic anisotropy on the micron scale, but allows for long-range alignment when directed by external fields and/or strong anchoring interactions with substrates. This addressability, together with the anisotropic optical properties, forms the basis for a wide range of applications, including liquid crystal displays (LCDs). Whilst research on liquid crystals has strongly focused on LCD technology in the past decades, currently, many new and exciting applications are emerging that require novel materials properties and new engineering methods. The last decade has seen an increasing interest in synergizing unique LC characteristics with aqueous environments, for instance for the development of biological sensing applications\textsuperscript{3}, (biocompatible) templates which align and manipulate (living) anisotropic materials\textsuperscript{6-10} and highly dichroic LCs for facile fabrication of optical elements\textsuperscript{11-17}.

This particular field of application requires different, water compatible materials. Excellent candidates are so-called lyotropic chromonic liquid crystals (LCLCs), which are rigid, plank-like molecules consisting of aromatic cores with hydrophilic groups on the periphery. In water, these molecules spontaneously stack face-to-face in columnar assemblies due to π-π stacking and hydrophobic interactions. At certain concentrations and temperatures, these stacks form liquid crystalline phases\textsuperscript{13,14} such as nematic and columnar phases. Typical members of the class of LCLCs are highly conjugated dyes that are functionalized with water-soluble (often ionic) groups\textsuperscript{14}. The rigid, aromatic cores of LCLCs give these materials excellent optical properties, such as strong linear dichroism, high birefringence and in some cases high absorbance in the visible range.

Typically, LCLCs are applied either in closed cells where one benefits from the dynamics of the anisotropic solution or, alternatively, as a thin film after water evaporation from an anisotropic LCLC solution. In any application, however, high quality, homogeneous and stable macroscopic alignment is absolutely crucial for device performance. In practice, this requirement is not easily or adequately met. Earlier work to obtain large scale alignment follow substrate-based approaches developed for thermotropic LCs, including alignment on polyimide substrates\textsuperscript{18,19}, rubbed glass\textsuperscript{20,21}, nanopatterned polymer films\textsuperscript{22,23}, self-assembling monolayers\textsuperscript{24,25}, and photosensitive surfaces\textsuperscript{26-28}. In general, such substrates align thermotropics much better than LCLC solutions. Good alignment of specific LCLCs can be realized on tailored substrates\textsuperscript{29}, but overall, both in-plane\textsuperscript{30} and homeotropic\textsuperscript{31} alignment is plagued by defects and stability issues. This may not come as a large surprise, considering the hydrophobic/hydrophilic mismatch between some of these substrates and LCLCs: the directing surfaces are often hydrophobic while in-plane aligned LCLC assemblies (dissolved in water) are covered in hydrophilic groups on the outside. Even without this mismatch, for example when rubbed glass is used, the in-plane anchoring strength measured...
for two LCLCs is weak, ~0.3 μN/m\(^2\). The overall lack of understanding of LCLC anchoring on surfaces, together with poor alignment and the stability issues resulting from defect formation, currently hamper innovative LCLC-based applications from entering the market.

In this work, we show how to drastically increase the quality of the macroscopic in-plane alignment of various LCLCs on conventional rubbed polyimide substrates by the addition of a tiny amount of a non-ionic surfactant. Our strategy is suitable for both LCLC solutions in closed cells as well as applications using dried-down anisotropic films. Besides greatly improving the degree of alignment, we also find that the surfactants introduce unprecedented long-term stability in closed LCLC cells. Whilst the alignment in samples without surfactant deteriorates within hours or days, the addition of a surfactant stabilizes the patterns for over several months without any loss in alignment quality. In this manuscript, we discuss how the alignment quality can be tuned by the concentration and the nature of the surfactant.

### 5.2 MATERIALS AND METHODS

#### 5.2.1 LYOTROPIC CHROMONIC LIQUID CRYSTALS

In this work, we investigated two of the most carefully studied LCLCs ([FIGURE 1A](#)), disodium cromoglycate (DSCG) and Sunset Yellow FCF (SSY). When dissolved in water, both materials form stacks (their length is temperature dependent), which can form a nematic phase. At room temperature, DSCG forms the nematic phase between 12–16 wt% in water. Despite its aromatic structure, it is optically transparent in the visible range. DSCG has been applied as a biosensor\(^1\), an optical compensator for LCDs\(^16\) and as an LC template for several functional materials\(^11,12\), even including motile bacteria\(^8-10\). SSY is commercially used as a highly absorbing (food) dye. It forms a room temperature nematic phase between 29 and 39 wt% and although studied academically in detail\(^20,21,29,32-35\), SSY is found less in LCLC-based applications, probably due to its high absorbance in the visible range.

![Molecular structures of (a) lyotropic chromonic liquid crystals DSCG and Sunset Yellow FCF; (b) surfactants used in this study with their respective HLB (hydrophilic/lipophilic balance) values.](#)

#### 5.2.2 SURFACANTS

For the non-ionic surfactants, we selected a wide range of commercially available surfactants, including Triton X analogues, Span 20, Tween 60, Tween 85, Brij S20 and DOSS ([FIGURE 1B](#)). The hydrophilic-lipophilic balance (HLB value) of these surfactants is a measure of their relative hydrophilicity and is an important parameter for its surface-active properties. The surfactants in this study have HLB values ranging between 8.6 (poorly water-soluble) and 17.6 (very hydrophilic). To study solely the dependency of LCLC alignment versus the relative hydrophobicity of the surfactant, we selected the fully water-soluble Triton X family. These surfactants have the same hydrophobic head group, which is substituted with an ethylene glycol tail of different lengths, which gives rise to a broad range of HLB values. In recent work, we added Triton X-100 to properly align DSCG on a photo-patterned hydrophobic substrate\(^12\).
5.2.3 POLYIMIDE COATED CELLS

We perform our experiments on conventional and commercially available rubbed polyimide (PI) substrates, which are the default alignment substrates used in both academia and the (LCD) industry. Prior to rubbing, spin-coated PI induces random planar LC alignment. Unidirectional rubbing with a cloth, velvet or an abrasive pad introduces grooves and reorients the top polymer layer\textsuperscript{36,37}, which in turn provides unidirectional alignment of the adjacent liquid crystalline material. In this work, we used KPI-300 as a PI aligning layer in both commercial 20 μm spaced anti-parallel rubbed PI cells and single PI-coated substrates (both produced by Instec Inc.). Preliminary work on other commercially available PI substrates (PI-2555, HD Microsystems and PI LX5400, Hitachi) gave analogous results.

5.3 RESULTS

5.3.1 LCLC ALIGNMENT QUALITY AND STABILITY IN CLOSED CELLS

To study the surface alignment of DSCG and SSY on rubbed PI in closed cells, we prepared mixtures of DSCG (16 wt% in water) and SSY (33 wt% in water) with and without the non-ionic surfactant Triton X-100 (0.24 mM, 0.016 wt%). Anti-parallel rubbed PI cells were filled with the LCLC or LCLC/surfactant solution in the nematic phase by suction-filling and closed with epoxy glue to prevent water evaporation over time. On purpose, we choose the flow direction perpendicular to the rubbing direction to exclude flow alignment effects. The cells were then heated to the isotropic phase and cooled back to room temperature. After brief equilibration at room temperature, the alignment quality was monitored as a function of annealing time at room temperature using polarized optical microscopy (POM). We found that homogenous alignment in DSCG samples (3 hours) was reached much faster than in SSY samples (3-10 days).

Both DSCG and SSY with 0.24 mM Triton X-100 (\textbf{FIGURE 2A,B}) form a completely homogenous monodomain (with a few small air bubbles)\textsuperscript{38}, which is in stark contrast to DSCG and SSY without the addition of Triton X-100 (\textbf{FIGURE 2C,D}). POM investigations with a waveplate (\textbf{FIGURE S1}) confirm that in both DSCG samples (with and without surfactant), the LCLC stacks align parallel to the rubbing direction, although DSCG without surfactant locally forms large domains with an off-axis alignment with respect to the rubbing direction, as indicated by the elongated dark red domains in \textbf{FIGURE 2C}. Furthermore, in DSCG samples without surfactant, stripes are observed that run parallel to the LCLC flow direction (perpendicular to the rubbing direction), which are attributed to shear flow-induced alignment of DSCG stacks, originating from the cell filling process. Heating the cells to the isotropic phase reduces their occurrence, but does not fully erase the shear flow alignment effects in these cells. In the samples with Triton X-100 added, these features are not observed, most likely due to the formation of an interface layer of surfactant between the hydrophobic PI and aqueous DSCG solution.

\textbf{FIGURE 2} POM images showing homogeneous alignment of (a) DSCG (16 wt%) and (b) SSY (33 wt%) both containing Triton X-100 (0.24 mM). Both POM images show a uniform color, indicating a homogeneously in-plane aligned LCLC mono-domain. In the inset in image (b), the crossed polarizers are turned 45° with respect to the rubbing direction, which gives maximum transmission through the sample. (c) POM image of DSCG (16 wt%) without Triton X-100, which shows slight off-axis alignment of DSCG with respect to the PI rubbing direction and small streaks parallel to the flow direction and perpendicular to the rubbing direction. (d) POM image of SSY (33 wt%) without Triton X-100, which shows homeotropic alignment of SSY, confirmed when rotating the sample under the crossed polarizers (inset). The small bright spots in panels (a) and (c) are defects around small air bubbles in the sample.
SSY with Triton X-100 at the same concentration also forms a homogeneous in-plane aligned monodomain (Figure 2B) with the orientation of the LCLC stacks along the rubbing direction (Figure S2). Surprisingly, SSY without surfactant forms a homeotropically aligned monodomain (Figure 2D) on the unidirectionally rubbed PI surface, as confirmed when rotating the sample under crossed polarizers. Although rubbed PI surfaces in general align LCLCs along the rubbing direction, homeotropic alignment of SSY has also been observed on a different type of unrubbed PI.

In addition to addressing the alignment quality challenge, the surfactant also enormously improves the stability of the LCLC alignment. POM analysis (Figure 3A–C) shows that DSCG alignment of samples with Triton X-100 is perfectly stable over very long periods of time. Even after 4 months, the visual appearance of the macroscopic monodomain was indistinguishable from the monodomain formed 50 minutes after the annealing procedure. In contrast, after a few days, DSCG samples without the surfactant show a progressive in-plane realignment away from the rubbing direction, visible by the strong increase in transmission in the POM images (Figure 3E,F,G). Unstable alignment of LCLCs on rubbed PI\(^a\) and homeotropic alignment layers\(^{30,31}\) is common and has been reported before. The reorientation that we observe is directly traced back to flow-alignment effects; the POM image after 24 days (Figure 3G and Figure S3) show two domains with DSCG alignment nearly orthogonal to the rubbing direction. Analogous to the DSCG results, nematic solutions of SSY with the addition of Triton X-100 are extremely stable over several weeks (Figure 3D). SSY without Triton X-100, however transforms from the initial homeotropic alignment to a micron-sized grainy in-plane alignment over time (Figure 3H). It is remarkable that the annealing times for both the nematic DSCG and SSY solutions are very different. Whereas DSCG forms a complete monodomain 3 hours after the annealing treatment, monodomain formation of nematic SSY takes at least several days (with Triton X-100: 3 days at 0.48 mM and 10 days at 0.24 mM).

To evaluate the effect of the Triton X-100 concentration on the LCLC alignment quality, we prepared samples of DSCG or SSY with different surfactant concentrations and subjected them to a straightforward POM-based analysis. In short, after complete annealing, a POM image is taken between crossed polarizers with the analyzer parallel to the rubbing direction. A second image is taken between parallel polarizers, both perpendicular to the rubbing direction. For perfectly aligned samples, the crossed polarizer image is black, while the parallel polarizer image is very bright. In less perfectly aligned samples, the crossed polarizer image is black, while the parallel polarizer image is very bright. For quantification, the averaged intensities of both images were measured using Photoshop (masking the areas around air bubbles) and used to calculate the intensity ratio $I_\parallel/I_\perp$, which is a good measure for the quality of alignment. A ratio approaching zero indicates excellent alignment, whereas higher $I_\parallel/I_\perp$ values mark off-axis alignment. For each sample, nine spots (2.5 x 2.5 mm) were measured and the averaged ratios per sample were plotted versus the concentration of Triton X-100 (for details on the procedure, see the Supporting Information).
The quantitative analysis of the effect of Triton X-100 on the DSCG alignment (Figure 4) shows that at low surfactant concentration (0.08 mM) the alignment has improved considerably, but that the maximum affect is reached at concentrations of 0.24 mM and beyond. The alignment quality of SSY as a function of surfactant concentration shows a similar trend. Without the addition of Triton X-100, the SSY alignment is homeotropic. With 0.08 mM Triton X-100 added the LCLC aligns in-plane and with increasing larger concentrations (0.24 mM and 0.48 mM), the ratio $I_{\perp}/I_{\parallel}$ becomes much smaller, indicating that the homogeneity of the LCLC alignment is further increased.

![Figure 4](image)

**Figure 4**: Quantitative analysis of the alignment quality of DSCG (16 wt%) and SSY (33 wt%), expressed by the intensity ratio $I_{\perp}/I_{\parallel}$ (see main text) versus the concentration of Triton X-100. Note that a logarithmic y-axis has been used in order to visualize the differences at higher surfactant concentrations. The POM images used for analysis (Figure S4) were taken after complete annealing of the samples (DSCG: 3 h, SSY: 14 days).

When further increasing the surfactant concentration, we found that at Triton X-100 concentrations of 1.12 mM, small micron-sized isotropic tactoids emerge in the homogeneously aligned nematic DSCG (Figure S5). At this high concentration, the surfactant not only covers the hydrophobic substrate, but is also present in the bulk LCLC solution, where it alters the DSCG phase diagram and reduces the nematic/isotropic transition temperature. At much higher concentrations (4.81 mM), we also observe much larger isotropic regions (Figure S6), for which also phase separation of Triton X-100 rich areas from the nematic DSCG starts to play a role. Despite the presence of the large and/or small isotropic islands at excess Triton X-100 concentrations, the texture of nematic DSCG between the islands is visually identical in homogeneity to the samples containing 0.24 mM and 0.48 mM Triton X-100 (Figure S7).

### HYDROPHILICITY OF THE SURFACTANT

5.3.3

Surfactants are built up from hydrophilic and hydrophobic (lipophilic) moieties. Their relative sizes and thus the relative hydrophilicity is expressed by the surfactant's hydrophilic/lipophilic balance (HLB) value. The HLB value is given on an arbitrary range from 0–20, where a value >10 roughly marks water solubility of the surfactant. Using the family of Triton X surfactants (Figure 1B) with the same hydrophobic group and different size hydrophilic tails (and thus different HLB values), we studied the effect of surfactant hydrophilicity (Figure 5A). Quantitative analysis of the POM images (Figure 5B) showed that surfactants with lower HLB values (shorter hydrophilic tails) have a larger positive effect on the homogeneity of the DSCG alignment on rubbed PI. In samples with Triton X-100, the alignment is extremely homogeneous, while with progressively less hydrophobic surfactants (with higher HLB values) at the same concentration, the alignment quality decreases. Even for these samples, however, the alignment quality is markedly better than the samples lacking surfactants.

### NATURE OF THE SURFACTANT

5.3.4

Besides the concentration, the type of surfactant requires optimization for the combination of substrate and LCLC. One approach is to adjust the HLB value (previous section); another is to change the hydrophobic and hydrophilic moieties. We investigated the alignment quality of DSCG on polyimide with a range of commonly applied non-ionic surfactants with different molecular structures (Figure 1B) as well as the ionic surfactant DOSS. Quantitative analysis (Figure 5C) shows that all surfactants increase the alignment quality of DSCG, although the effective concentration varies from one surfactant to another. Triton X-100 clearly has the largest positive effect on the alignment quality, despite its higher HLB value (13.4) than Span 20 (8.6). At higher concentrations of Brij S20 and Tween 60, small isotropic islands emerge (indicated by the asterisk in Figure SC), which arise from either phase separation of surfactant rich regions and/or a reduction of the DSCG clearing temperature, analogous to what is observed at high Triton X-100 concentrations. Similar to Triton X-100, the alignment of DSCG induced by all surfactants in Figure 1B is extremely stable, even after several weeks (Figure S8).
5.3.5 SURFACTANT MIXTURES

It is common practice in surfactant engineering to mix surfactants of different HLB values to optimize the desired effect. Also, in particular applications (such as in biosensing), some surfactants might alter the structural integrity of fragile biomaterials, and thus should be avoided. To this end, we explored the possibility of mixing two surfactants, Triton X-100 and X-102, to obtain the desired homogeneous and stable LCLC alignment (FIGURE 5D). Triton X-100 is the best performing surfactant for the alignment of DSCG on the KPI-300 polyimide, but at 0.04 mM, the concentration is too low to reach the maximum effect. Addition of small amounts Triton X-102 shows that at intermediate concentrations (0.04 + 0.12 mM), optimum alignment can be realized. Analogous to the results with individual surfactants, the mixtures are also able to stabilize the in-plane aligned LCLC phase for long periods of time (FIGURE S8E). By employing a mixing strategy, even poorly soluble surfactants can also be used. Such surfactants (generally with a low HLB value) do greatly improve the LCLC alignment, but due to their poor solubility in water, micron-sized aggregates form which locally deform the bulk LCLC alignment. Addition of sufficient amounts of high HLB surfactants results in the formation of water-soluble co-micelles (FIGURE S9), which allows for diffusion to the hydrophobic interface. Besides the increased flexibility in surfactant usage, mixing surfactants is also a great tool to tune the alignment quality by carefully choosing the composition of the mixture: the type of surfactants and their concentrations.

5.3.6 LARGE SCALE HOMOGENEOUS ANISOTROPIC SOLID FILM FABRICATION

The cells that we studied were closed with epoxy and the formation of fully homogeneous textures was allowed to proceed for days when necessary. For the formation of solid films, however, single substrates are used and the annealing process competes with water evaporation. This often results in poor (large scale) alignment quality of the films. Here, we study how surfactants are able to improve the film homogeneity. In our experiment we deposited several drops of an aqueous isotropic solution of DSCG (7 wt%) and Triton X-100 (0.48 mM) on a single PI-coated substrate. Afterwards, the plate was tilted in order to allow the drops to flow over the entire substrate (not parallel to the rubbing direction) which forms a thin layer of several microns thick. The plate was placed in a custom built humidity chamber in order to reduce the water evaporation rate and allow annealing of the nematic DSCG layer that slowly emerged when a fraction of the water evaporated from the layer. After the LCLC had formed a defect-free homogeneously aligned nematic LCLC layer, the top plate of the humidity chamber was opened slightly which allowed the remaining water to evaporate at a higher rate in order to form the solid film.

FIGURE 6 shows POM images of a dried-in solid DSCG film on a rubbed PI substrate (FIGURE 6A-B). When rotating the crossed polarizers, the images turn from bright green to dark, indicating a homogeneously aligned monodomain. The stripes in the images are the result of annealing defects and can be removed by optimizing the preparation procedure*[^5]. Although the isotropic solution randomly spread over the substrate, we found that the orientation of the LCLC stacks is parallel to the rubbing direction (FIGURE S10). Averaged over various spots of the solid film (4 in total), we found for DSCG/Triton X-100 on rubbed PI a dichroic ratio $R_{\lambda=325nm} = A_{\parallel}/A_{\perp} = 3.0$, where $A$ is the absorbance parallel or perpendicular to the rubbing direction (more details can be found in the Supporting Information). In previous work on dried-in sheared DSCG films on glass,
5.4 DISCUSSION

5.4.1 OPTIMUM SURFACTANT AND SURFACTANT CONCENTRATIONS

We attribute the relatively poor alignment of DSCG on (rubbed) PI in the absence of a surfactant to shear flow alignment effects. As the sample is filled, DSCG molecules or short stacks anchor to the PI substrate in the flow direction. A heating and cooling cycle does not completely remove the adhered molecules from the surface and as a result they compete with the rubbed PI surface for bulk DSCG alignment, resulting in the formation of alignment defects. We found that gradually, the flow-induced alignment effects dominate, which results in a large in-plane reorientation of the LCLC domains. In our commercial cells, the flow direction was perpendicular to the rubbing direction, which made the effect dramatic, but even when parallel, the flow profile is not always perfectly in line with the PI alignment.

A small amount of non-ionic surfactant is expected to form a layer at the interface between the hydrophobic PI and the aqueous DSCG solution, which prevents the formation of the flow-induced DSCG adhesion to the surface, but is still able to couple the (molecular and/or topographical) anisotropy of the rubbed polyimide effectively to the bulk LCLC. The lowest effective concentration for Triton X-100, where the surface alignment homogeneity and stability of both DSCG and SSY are optimized, is between 0.24 mM and 0.48 mM (Figure 4). Assuming the formation of an interface layer, we can estimate the concentration necessary to completely cover the surface, i.e., the critical surface aggregation constant $c_{CSA}$:

$$c_{CSA} = \frac{x}{a \cdot h \cdot N_A}$$

where $x$ is the number of surfaces which need to covered ($x = 1$ in the case for a dried down solid film and $x = 2$ for an enclosed cell), $a$ is the hydrophobic area coverage per surfactant molecule, $h$ is the thickness of the LCLC layer in the cell or on the single substrate and $N_A$ is Avogadro’s number. In our experimental setup with $a = 0.55$ nm$^2$ for Triton X-100, we find $c_{CSA} = 0.30$ mM, which is very close to the lowest effective concentration of 0.24 mM. This indicates that a large fraction of the added surfactant anchors to the surface, instead of being dissolved molecularly or in micelles. At higher surfactant concentrations, the alignment quality of the LCLC does not improve any further since the PI surface is already fully covered; and at lower concentrations the alignment quality decreases, since the PI surface is covered only partially with the surfactant. Still, this situation gives much better results than the sample without surfactant.

Looking at the relative hydrophobicity of a surfactant (indicated by its HLB-value), we observe that within the Triton X-100 family, the surfactants with the lower HLB-value (with a larger relative hydrophobicity) give the highest alignment quality. In these samples, a larger fraction of the surfactant will assemble at the...
interface. High HLB surfactants will partition more in the aqueous solution and higher concentrations are needed to cover the substrate and realize the same level of alignment quality. The larger amounts of surfactants dissolved in the bulk LCLC, however, can easily result in shifts in the phase diagram and/or cause phase-separated surfactant rich areas as we found for Triton X-100; concentrations beyond 1.12 mM within closed cells (figure S5, figure S6), and in anisotropic solid films at a concentration of 0.72 mM (figure S11).

Surfactants with lower HLB values than Triton X-100 (13.4), such as Span 20 (8.6) and DOSS (10.9) do show great homogeneity and stability of the nematic DSCG phase, but the alignment quality of Triton X-100 is still superior. Clearly, the molecular structures of the surfactant and the polyimide play an important role. The molecular structure of KPI-300B has not been disclosed, but we know it contains, like many other PIs, aromatic rings in the structure. We hypothesize that the phenyl rings of the hydrophobic block of Triton X-100 may provide a strong interaction with the (aligned) PI molecules, causing the formation of an ordered and stable interfacial layer. In short, to determine the ultimate surfactant, one should consider the overlap in molecular structures of the surfactant and the PI as well as the HLB value of the surfactant, which should optimally fall in a range between 8 and 14. Using mixtures of surfactants gives an extra degree of flexibility in selecting the desired surfactant; even poorly soluble surfactants can be employed if they are paired with readily soluble surfactants.

5.4.2 MECHANISM OF ALIGNMENT

In general, there is still not much known about the role of surfactants in the mechanisms responsible for LCLC alignment on directing surfaces such as rubbed PI. Studies on PI have shown that rubbing both reorients the top polymer (sub-nanometer length scale) layer and forms microgrooves (nanometers in height and spaced tens of nanometers) [36,37]. Atomic force microscopy (AFM) analysis on the commercial substrates that we used gave results that are in line with grooves of 2-4 nm deep and spaced 20-40 nm apart (figure S14) [41,42]. For conventional thermotropic LCs, it is believed that both effects contribute to the LC alignment [43]. In the case of surfactant-mediated LCLC alignment on rubbed polyimide, we have reason to believe that both mechanisms are active as well.

For LCLCs, one should first consider the much larger length scales of the chromonic ‘mesogens’ compared to thermotropics. Due to the assembly process of tens to hundreds of DSCG molecules [43], the building blocks of its nematic phase are about 2 nm wide (roughly the height of the PI grooves) and 4 nm separated (several times smaller than the width of the grooves). For bulk LCLC alignment directional information needs to transfer through at least a monolayer of surfactant, which for Triton X-100 is about 4 nm.

Concerning the molecular anisotropy of the reoriented top polymer layer, directional information must be transferred through the surfactant layer, despite the mismatch in length scales and orientation between the aligned imide-units of the polymer (sub-nanometer) and the LCLC stacks (the stacks are aligned parallel to the PI backbones, but the DSCG molecules are perpendicularly oriented). Earlier work on DSCG alignment on photopatterable azobenzene-based command layers supports the presence of this mechanism. In this work, the command layer is spin coated on the (glass) substrate and aligned by irradiation with polarized light. This process exclusively gives a reoriented polymer later without a significant surface topology. On this surface, DSCG stacks orient parallel to the azobenzene-units of the command layer, resulting in high quality in-plane alignment [12].

A possible mechanism to transfer the alignment information from the command layer to the LCLC stacks may be related to the self-assembly of surfactant molecules into so-called hemi-micellar aggregates on the surface. Formation of such 1D aggregates has been reported for a wide range of non-ionic surfactants on various hydrophobic surfaces [44-47]. Although alignment of hemi-micellar aggregates along the crystal lattice of highly oriented graphite surfaces has been demonstrated, structure formation on rubbed polyimide or photosensitive command layers has not been reported. It is not unlikely that non-ionic surfactants form similar structures on these surfaces and, as such, provide a 1D groove-like structure with dimensions of nanometers (in height and spacing) that is responsible for the LCLC alignment. In the case of rubbed polyimide, this alignment mechanism works cooperatively with the rubbing induced anisotropic microgrooves (which are not present on photoaligned command layers).
5.6 ACKNOWLEDGEMENTS

We would like to thank Roel Hammink for the AFM measurements and Tonnie Toonen for help with the polarized absorption measurements setup.
5.7 REFERENCES

[38] The air bubbles are present in both samples are the result of the filling procedure of the cells (see SUPPORTING INFORMATION). These air bubbles deform the nematic director only locally and were masked in our subsequent quantitative analysis.
5.8 SUPPORTING INFORMATION

MATERIALS

DSCG, SSY and surfactants Triton X-100, Triton X-102, Triton X-165, Triton X-305, Span 20, Tween 60, Tween 85, Brij S20 and DOSS were purchased from Sigma Aldrich and were used as received, except for SSY, which was purified using a precipitation method.1

LC/LC/SURFACTANT SAMPLE PREPARATION

Surfactant was weighed in a glass vial and diluted with milli-Q water to make a 1.0 wt% stock solution. A small magnetic stirring bar was added and the vials were sealed with a cap and parafilm to prevent water evaporation. The stock solutions were stirred for at least one day before use. Before preparing the LC/LC/surfactant samples, the 1.0 wt% stock solution was vortexed for 10 seconds after which the solution was diluted with fresh milli-Q water to the desired surfactant concentration (in mM) and vortexed again. The concentration of surfactant in this manuscript is noted as the amount of surfactant (in mol) per volume of milli-Q (ignoring the contribution of the LC/LC to the total volume). Either DSCG or SSY was weighed in another vial and a specific amount of surfactant/milli-Q solution was added (in order to form the nematic phase). The solution was gently heated to ensure full dissolution of the solid LC/LC into the aqueous solution.

LC/LC CELL PREPARATION

A 1 mL syringe (NORM-JECT) was glued on one side with epoxy glue to one opening of the liquid crystal cell. After 1 hour of drying, the cell/syringe was rotated in order to glue the other edge. After 1 hour of drying, a Pasteur pipette was used in order to place a small drop on one opening of the cell (opposite from the glued syringe). After placing the droplet, the syringe was carefully pulled back in order to fill the cell completely with the nematic LC/LC solution. After completely filling the cell, the syringe could easily be broken off the edge of the cell and both openings were sealed with epoxy glue. After 1.5 hours of drying, the filled cell was placed in a preheated hot stage at 70 °C. Within 1 minute the nematic LC/LC had undergone a complete phase transition to the isotropic phase after which the cell was removed from the hot stage. After about 1.5 to 3 hours for DSCG and about 2-14 days for SSY (dependent on the surfactant concentration), the LC/LC had fully aligned to the underlying PI layer and polarized optical microscopy was used to investigate the alignment quality.

QUANTITATIVE ALIGNMENT QUALITY MEASUREMENTS

The cells were placed on the x-y stage of the polarized optical microscope. The cells were manually aligned in order to make sure the rubbing direction was exactly in the horizontal in-plane direction of the microscope. Two greyscale images at low magnifications (5x objective) were taken at each position in the cell (9 positions in total) in different analyzer/polarizer configurations. The first configuration (for convenience named perpendicular or ⊥) was with crossed analyzer and polarizer and with the analyzer parallel to the rubbing direction. The second configuration (named parallel or ||) was with both analyzer and polarizer perpendicular to the rubbing direction. The settings of the microscope (both hardware and camera software) were calibrated in order to maximize the “parallel” image without over-exposing the camera. After taking an image in this configuration, a second image was recorded in the “perpendicular” configuration after increasing the exposure time to a fixed value (which was used for all POM analysis experiments). When a sample is nearly perfectly aligned along the rubbing direction, I⊥ approaches zero. When a sample was not homogeneously aligned along the rubbing direction, any off-axis alignment of the director was picked up by the analyzer, resulting in an increased I⊥ (and, to a lesser extent, a decreased I||).

After recording 9 sets of images per cell (2.5 mm x 2.5 mm area per image), in Photoshop CS5 the operation Filter - Blur - Average was used order to determine the average grey value of the recorded images. The calculated I⊥ was divided by I|| (between 0-256) and this value was plotted in FIGURES 4 and 5.

DICHROIC RATIO MEASUREMENTS

A deuterium-halogen light source was connected through a fiber optics cable to an AvaSpec-2048 Fiber Optic Spectrometer, with in between a polarizer and the sample stage, on which the LC/LC coated PI/glass plate was placed. For each polarizer setting (parallel or perpendicular to the rubbing direction), a dark
Supporting Information

5.8 SUPPORTING INFORMATION

Supporting Information

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the phase shift responsible for the tactoids in FIGURE S5. Furthermore, larger phase separated isotropic regions are visible, which might be surfactant-rich areas phase separated from the nematic DSCG due to the high concentration of the surfactant or isotropic DSCG islands present due to a shift in the phase diagram.

**FIGURE S7** Alignment quality of DSCG (16 wt%) with 0.24 mM (a) and 4.81 mM (b) Triton X-100 added. In between the isotropic areas and outside the air bubble areas, the texture is visually identical for both samples.
Supporting Information

Figure S9: POM images of DSCG (16 wt%) with Tween 85 (0.08 mM) in a rubbed PI cell under parallel polarizers perpendicular to the rubbing direction (a) and crossed polarizers (b). The presence of insoluble surfactant aggregates, visible as tiny black spots in image (a), greatly reduces the alignment homogeneity of DSCG, visible by the numerous white spots in image (b). When Tween 60 (0.36 mM) is added (c,d), the alignment homogeneity is greatly increased due to co-micelle formation (which removes the insoluble Tween 85 aggregates). The large elongated black spots in images (a,c) are small air bubbles in the sample, which entered the sample during sample preparation. Around these small air bubbles, bright defects can be observed in the corresponding images under crossed polarizers (b,d).

Figure S10: POM images of a solid dried-in anisotropic DSCG film on rubbed polyimide with the addition of Triton X-100 (0.72 mM) under crossed polarizers and with a 137 nm waveplate inserted. The stacked assemblies in the domains that show a higher order (green/blue) interference color (a) are aligned with their long axis perpendicular to the fast axis of the quarter-wave plate. When rotating the sample 90° (b), the interference color changes to (lower order) yellow, indicating the LCLC stacks are aligned parallel to the fast axis of the quarter-wave plate and thus parallel to the rubbing direction.

Figure S11: POM images of a solid dried-in anisotropic DSCG film on rubbed polyimide with the addition of Triton X-100 (0.48 mM) under crossed polarizers, showing a homogeneously aligned solid film. The elongated isotropic islands present in the film (c) can be attributed to excess surfactant present in the bulk that has phase separated from the LCLC bulk during the drying process.
**Supporting Information References**


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**Figure S12** POM images of a solid dried-in anisotropic DSCG film on rubbed polyimide with the addition of Triton X-100 (0.04 mM) and Triton X-102 (0.12 mM) under crossed polarizers, showing a homogeneously aligned solid film. The non-uniform color difference in image (a) is attributed to a slight gradient in film thickness.

**Figure S13** POM image of a solid dried-in anisotropic DSCG film on glass with the addition of Triton X-100 (0.48 mM) under crossed polarizers, showing random LCLC domain formation.

**Figure S14** Atomic force microscopy (AFM) characterization of the PI surface topology. The 2D overview (left) shows that rubbing generates unidirectionally ordered grooves on the surface. Cross-sections perpendicular to the rubbing direction (right) show the typical length scales of the surface topology: grooves of ~1-4 nm deep with a spacing of ~20-40 nm.
CHAPTER 6

SPATIAL AND TEMPORAL PATTERNING OF POLYMERS IN ELECTRIC FIELD RESPONSIVE LC TEMPLATES
6 SPATIAL AND TEMPORAL PATTERNING OF POLYMERS IN ELECTRIC FIELD RESPONSIVE LC TEMPLATES

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ABSTRACT

Controlling the spatial and temporal organization of functional polymers is essential for the development of switchable soft-matter based electro-optical devices. By using a combination of a liquid crystal template, a photopatterning substrate (for spatial control) and electric fields (for dynamically switching) we show that we are able to dynamically control the spatial organization of polymers across multiple length scales (conveyed through the patterned liquid crystal template). The polymer that we organize is an azobenzene-functionalized polyisocyanide, whose stiff polymeric helix and laterally attached pendant azobenzene units induce tangential anchoring to the liquid crystal host. Due to the donor-acceptor functionalized azobenzene units, the polymeric material is strongly absorbing in the visible range for characterization purposes. We find that polymers align locally to the liquid crystal director field and reversibly change its orientation by the application of an electric field. Since this hybrid technique can be easily applied to other functional polymeric materials and relies on simple techniques, such as spincoating, photoalignment and electric fields, we believe it has great potential for the development of a wide range of switchable electro-optical devices.

6.1 INTRODUCTION

Polymeric materials form the basis of a wide range of technological applications. Their properties can be tailored by changing the molecular structure and backbone architecture. In addition, they are commonly easy to process and often cheap. Despite containing a well-defined (macro)molecular structure, at larger length scales polymeric materials are isotropic. For many applications this is no problem, but other applications strongly benefit from high structural definition at multiple length scales. To boost device performance, a number of techniques have been developed that address macroscopic length scales, including photo and soft lithography, electrospinning and the application of electric, magnetic or mechanical fields. Many of these techniques however, are not suited for both designing complex patterns, nor can they dynamically manipulate the polymer orientation after alignment/pattern formation. Such functionalities are critical for the development of switchable soft-matter based optical and electric applications.

In this chapter, we use liquid crystal templating (LCT) to generate complex and dynamic structures of polymer materials. LCT has been successfully applied to organize functional polymers in bulk liquid crystals and, to a lesser extent, in aqueous lyotropic or chromonic templates. The major advantages of LCT are its versatility, its high dimensional control and its dynamic addressability. The versatility stems from the absence of distinct molecular interactions between the template and the polymer. Dimensional control is given by the substrate; micrometer patterning techniques are already commercially applied in the LCD industry. Lastly, the liquid crystal template is readily manipulated using electric fields, again analogous to LCD technology, which introduces spatially controlled dynamics to the dispersed polymers. The promises of LCT has only partly been realized: by far the majority of studies are focused on plain long-range unidirectional alignment and only rare examples use complex structured substrates or electric fields to take full advantage of this approach.

In this paper, we use an approach to spatially and temporally control the organization of functional polymers, by applying a combination of a liquid crystal template, a photopatterning substrate and electric fields. This is to our knowledge the first example where both complex patterned in-plane alignment of functional polymers is obtained, combined with controlled reversible homeotropic switching of these materials.

In order to benefit from the anisotropic environment that the liquid crystalline matrix offers, one would like to use rigid or semi-flexible polymers with a persistence length larger or of the same order as the contour length. Polyisocyanides are such class of polymers through the formation of a rigid helix which is the result of the polymerization mechanism. The helical backbone conformation, supported by a β-sheet-like hydrogen-bond pattern at the periphery yields stiff polymer
6.2 MATERIALS AND METHODS

Polyisocyanides (PICs) are a class of long, helical and rigid polymers. The approximate \( \alpha \)-helical backbone conformation, which is the result of the polymerization mechanism, is stabilized through \( \beta \)-sheet like hydrogen bonding between adjacent peptide groups\(^6\). Earlier work in our group used the polymer as a rigid scaffold to precisely control the architecture of substituted chromophores, including D–π–A-based azobenzenes, which showed to have tremendous dipole moments\(^6\). Its intrinsic structural stiffness and molecular anisotropy makes PICs very responsive to elastic force alignment via LCT. To induce planar alignment of the surrounding liquid crystal host, we introduced mesogen-like substituents that were laterally grafted through a short spacer. To separate the absorbance of the \textbf{AzoPIC} (low concentration) from the 5CB liquid crystal (high concentration), we further introduced donor and acceptor groups on the chromophore. The structure and a sketch of the helical conformation are displayed in \textbf{FIGURE 1}.

The synthesis of \textbf{AzoPIC} is outlined in \textbf{SCHEME 1}. Details of the synthesis and characterization are given in the Supporting Information. Boc-protected alanine was equipped with a short spacer through a standard EDC coupling reaction. The hydroxyl-functionalized push-pull azobenzene \(^2\) was first deprotonated with t-BuOK and then added to bromide 1 to give the functionalized boc-protected alanine 3. Deprotection with EtOAc•HCl, formylation and dehydration using Burgess’ reagent yielded the isocyanide monomer 5 in an 18% overall yield. The polymerization of monomer 5 was initiated with a Ni\(^{2+}\) salt in the presence of a small amount of alcohol. The reaction was carried out in chloroform (which is common solvent for isocyanide polymerization reactions) or in 4-cyano-4’-pentyl-1,1’-biphenyl (5CB). In 5CB, the monomer concentration studied was relatively low (\( c = 0.035 \text{ wt\%} \)), due to the limited solubility of 5 in this liquid crystal (0.064 wt%).

\( ^6 \) AzoPIC is a functionalized polyisocyanide which can be expressed using the following formula: \( \text{AzoPIC} = \text{Boc-protected alanine} \rightarrow \text{isocyanide monomer} \rightarrow \text{polymer} \). The synthesis steps include deprotonation, addition, and subsequent reactions.

\( ^2 \) Azobenzene functionality is added to improve solubility and introduce programable optical properties.

\( ^3 \) Deprotection is achieved using a strong base, typically t-BuOK.

\( ^4 \) Formylation involves the conversion of an alcohol to an aldehyde.

\( ^5 \) Dehydration is achieved using Burgess’ reagent, an effective method for converting alcohols to isocyanides.

\( ^6 \) Polymerization is initiated by a metal ion, typically a Ni\(^{2+}\) salt, in the presence of a solvent.

\( ^7 \) Solubility limits the concentration of monomer in the liquid crystal.
RESULTS AND DISCUSSION 6.3

FULL SPATIAL CONTROL 6.3.1

For the isocyanide polymerization reaction, we prepared a solution of isocyanide monomer $5$ (0.035 wt% in 5CB) which was stirred for 4 days to ensure complete dissolution of the monomer. A part of this solution was set aside and used as a control sample. An amount of the solution was mixed with the nickel(II) perchlorate catalyst solution, such that the catalyst/monomer ratio was 1:500. This polymerizing mixture was introduced into a number of liquid crystal cells (see below) to follow the progress of the reaction with (polarized) optical microscopy. The remainder was polymerized in a reaction tube and followed with spectroscopic tools.

Polymer formation is best followed with circular dichroism (CD). Whilst the isocyanide monomer $5$ is CD silent (like many chromophore-substituted isocyanides), the polymer shows a Cotton effect right at the absorption wavelength of the azobenzene side groups (Figure 2). This is a clear indication that in the AzoPIC polymer, the chromophores are stacked in a helical configuration along the polymer backbone. The reaction proceeds slowly due to the low concentration of monomer and catalyst. The yield of the isocyanide polymerization reaction, often measured by disappearance of the characteristic isocyanide stretch in IR spectroscopy was not determined as the large number of cyano groups of the liquid crystal template obstructed quantification. The very small amounts of polymer (approximately 0.5 ng per cell) impeded molecular weight analysis. Following the formation of AzoPIC in chloroform did show the disappearance of the isocyanide stretch (Figure S1). Both $5$ and AzoPIC have a slight influence on the thermal properties of the liquid crystalline matrix; the presence of these compounds in both situations lowers the nematic-isotropic transition temperature $T_{NI}$ of 5CB by 0.3 °C.
For the microscopy studies, we introduced the polymerizing mixtures in two types of cells: standard parallel-rubbed polyimide (PI)-coated glass cells and custom-made photopatterned cells (detailed information about the sample and cell preparation can be found in the **Supporting Information**). The latter were prepared by spin coating a thin layer of PMAz on an electrode (indium tin oxide, ITO) covered glass substrate and patterning the PMAz layers in following three consecutive irradiations steps (with three different polarization directions) using a photomask designed after the logo of our IMM institute (**Figure S2**). After filling the cells, we observed that the LC locally aligned with respect to the patterned command layer (**Figure 2A**), where the direction of the 5CB mesogens is parallel to the photoaligned azobenzene moieties of PMAz (**Figure S3**). After a few hours, we observed the formation of micron-sized red colored bundles aligned parallel to the local LC director (**Figure 2B, C**). Control samples with 5 in 5CB without catalyst present in rubbed polyimide (**Figure S4**) or photopatterned PMAz (**Figure S5**) cells did not show any sign of the formation of (aligned) red bundles. After several days, a parallel-rubbed polyimide cell with 5/5CB/catalyst solution was carefully opened, the LC was washed away and scanning electron microscopy (SEM) showed small bundles organized on the substrate in the rubbing direction (**Figure 3D**).

**FIGURE 2** Circular dichroism (top) and UV-vis (bottom) spectra of 5 in 5CB before (blue) and after (red) adding catalyst solution. The solutions were diluted 6-fold with p-xylene to remove signal scattering induced by the nematic 5CB and to reduce the overall strong absorption of solution.

**FIGURE 3** Directed orientation of AzoPIC bundles by a 5CB template in a photopatterned PMAz cell (20 μm spacing). Polarized optical microscopy (POM) image (a,b) of a mixture of isocyanide monomer 5 (0.035 wt%), 5CB and catalyst solution, a few hours after capillary insertion in a photopatterned PMAz ITO/glass cell. Photopatterning yielded domains with three different orientations, as indicated by the light blue double-sided arrows. Panel (b) is a zoom from panel (a). The corresponding optical microscopy (OM) image (c) shows bundles of AzoPIC aligned to the local nematic director, parallel to the polarization direction. The thin black line that indicates the boundary between the photopatterned domains was added for visual clarity. The OM image was taken after removing the analyzer from the POM stage, whilst leaving the polarizer in the orientation given by the white double-sided arrow. The polarizer enhances the contrast of the bundled structures with the background (due to its strong dichroic properties), thus improving the general visibility of the bundles. After cell opening and removal of the template, SEM (d) shows bundles (assembled in a 5CB template) on rubbed polyimide aligned in the same direction as the rubbing direction (vertical).

The approx. 10 μm long structures are obviously longer and wider than single polyisocyanide chains and we expect them to be bundles of chains. These bundles are formed as a result of depletion interactions (which depend on time,
temperature and the AzoPIC length and concentration) between the anisotropic LC solvent and 1D AzoPIC. Figure 3C shows that the 5CB background however, is also still colored. This red shade originates from AzoPIC that is molecularly dissolved or in very small (microscopically invisible) bundles, or may come from unreacted isocyanide monomer as a result of the incomplete polymerization reaction. Further microscopy studies with polarized light showed that the red color is, in fact, polarization dependent and thus also aligns with the 5CB matrix (see backgrounds in Figure 4).

The AzoPIC bundles, which are locally aligned to the template show strong linear polarized absorption characteristics. In Figure 4, the polarization of the light source is rotated 360° in four 90° steps. When the polarizer is directed parallel to the anisotropic AzoPIC bundles, the bundles appear dark (Figure 4A,B), caused by the strong absorbance of the D–π–A azobenzene dyes grafted on the polymer backbone. While rotating the polarizer perpendicular to the bundles, they become almost indistinguishable from their background as the absorbance is minimal (Figure 4C,D). The azobenzene’s main absorption band (at λ\text{max} = 497 nm) is attributed to the n–n* transition. The corresponding dipole moment (along the long axis of the molecule) thus is oriented parallel to the director of the 5CB LCT. This also means that the attached azobenzene moieties are oriented largely parallel to the PIC backbone, as is schematically represented in Figure 1.

The orientation of monomer, dissolved polymer and or small bundles follows the same trend and here the dyes are also oriented parallel to the director (see for instance in the backgrounds of the top areas of Figure 4, panels a and c or the bottoms of panels b and d.

![Figure 4](image)

**Figure 4** Anisotropic absorption characteristics of locally unidirectionally aligned AzoPIC bundles on a photopatterned PMAz cell (20 μm spacing). AzoPIC bundles clearly show strong absorption characteristics when the polarizer is oriented parallel to the bundle direction (indicated by the white double-sided arrow). Note when the polarizer is oriented perpendicular to the bundle direction that the AzoPIC bundles are almost invisible. For visual clarity, the thin black line indicates the boundary between the photopatterned domains.

### Electric Field Switching

6.3.2

After micro-structuring the AzoPIC (assemblies) on the photopatterned PMAz command layer, we applied a homeotropic AC electric field (20 V, 1 V μm⁻¹, 1 MHz) to the PMAz cells to dynamically switch the polymers. Figure 5 and Movie S1 both show the reversible reorientation of the locally organized AzoPIC bundles in the 5CB LCT when a homeotropic electric field is applied. Figure 5A shows two domains with the nematic directors parallel (top) and oriented 45° (bottom) with respect to the polarizer (white double-sided arrow), which are separated by a thin black line. Before applying the field, the AzoPIC bundles are aligned in-plane within each corresponding domain. Directly after applying the field, 5CB immediately reorients homeotropically due to the electric field induced Fredericksz transition (Figure S6)\textsuperscript{a}, where the patterned domains are
now clearly separated by a defect line\(^{30}\). The AzOPIc bundles also undergo an in-plane to homeotropic reorientation (Figure 5B), but much slower; after several seconds the bundles are fully rotated in the homeotropic direction (Figure 5C). When switching off the field, the bundles slowly reorient due to the in-plane realigned bulk LCT (Figure 5D). Again, after several seconds the bundles are completely realigned in the planar orientation within their respective 5CB domain (Figure 5E). We believe elastic forces induced by the LC to be responsible for the realignment of these bundles when the bulk LCT changes its orientation with respect to the electric field, analogous to earlier examples where LCT and electric fields were applied to both dispersed carbon nanotubes\(^{6}\) and polymeric nanowires\(^{9}\).

Through POM analysis, we quantitatively determined the switching times of the AzOPIc bundles in relation to their bundle length (Figure 5F) by measuring the response rates of a number of individual bundles of different sizes. As could be expected based on their inertia, for smaller bundles the switching times are shorter while longer bundles respond slower. For the longest bundles, we anticipate that the bundles interact with the substrate, which can contribute to the observed slower in-plane to homeotropic transition. The response rate of the bundles is of the order of seconds, much slower than the polymer PFO nanowires in prepared the nematic host E7, for which 100 ms response times were recorded\(^{9}\). The latter one-dimensional structures were smaller (2 μm) and were switched at a slightly higher electric field (0.6 V μm\(^{-1}\)).

From the POM analysis, we also were able to measure the change in orientation of an AzOPIc bundle as a function of the field strength (Figure 6). After a threshold field of approximately 0.05 V μm\(^{-1}\), the 14 μm long AzOPIc bundle rotates from an in-plane orientation into a complete homeotropic orientation at 0.43 V μm\(^{-1}\). At intermediate electric field strengths (between 0.1 and 0.43 V μm\(^{-1}\)), the bundle adopts a stable partially rotated alignment where the degree of reorientation is related to the applied field.
6.4 CONCLUSIONS

In this paper, we have demonstrated a generic approach to spatially and temporally control the organization of functional polymers, by using a combination of a liquid crystal template, a photopatternable substrate and electric fields. The stiff helical backbone of the polyisocyanide we employ are functionalized with strongly absorbing D–π–A-azobenzenes, which induces both tangential anchoring to the LCT and also introduces strong dichroic absorption characteristics when dispersed in 5CB. We find that the functionalized polymers are aligned within domains formed by the locally photopatterned liquid crystal template. When applying an electric field, the orientation of these polymers can be reversibly switched. The electric field strength is also directly related to the extent of the polymer rotation, which gives another level of dimensional control over these dispersed soft materials.

Since in general, delicate molecular interactions between the LCT and the dispersed materials are absent, this hybrid approach can be applied to a wide range of other functionalized materials. Both the unique ability to dynamically switch the orientation of complex patterns of in-plane aligned materials and the adoption of photosensitive surfaces by the industry, should pave the way for the development and mass-manufacturing of soft matter based switchable optical and electric applications.

6.5 ACKNOWLEDGEMENTS

We would like to thank Merck for kindly providing 5CB and both Dr. Laura Cattaneo and Tonnie Toonen for their help with the photoalignment setup. We thank the TechnoCentrum for the fabrication of the cell construction apparatus and Onno van den Boomen for the design of the 3D AzoPIC model.
6.6 REFERENCES


We did not measure the delay in response time of 5CB resulting from the presence of AzoPIC (or the corresponding monomer 5). The response time of our matrix is still orders of magnitude faster than that of the AzoPIC bundles.

The presence of the defect line between the domains is attributed to the interfacial region between domains of strongly anchored in-plane aligned 5CB mesogens very close to the surface.
6.7 SUPPORTING INFORMATION

SYNTHESIS OF AZOPIC

INSTRUMENTATION

All chemicals were used as obtained, unless otherwise stated. Silica gel (0.040-0.060 mm) from Merck was used for column chromatography and silica gel 60 F254 coated glass plates (Merck) were used for thin layer chromatography. 1H NMR and 13C NMR spectra were recorded on an Inova 400 MHz or a Bruker Avance III 500 MHz spectrometer at room temperature. Chemical shifts are reported in ppm relative to tetramethylsilane (δ = 0.00 ppm). The following measurements were recorded at room temperature unless stated otherwise. UV-VIS spectra was performed on a Varian Cary 50 spectrometer, the CD spectra on a Jasco J800 spectrometer equipped with a temperature control unit, infrared spectra were recorded on the Bruker Tensor 27 FT-IR and the Thermo LCQ advantage max, ESI, equipped with an autosampler was used for mass spectrometry measurements.

Chemicals. All chemicals were used as received, unless noted otherwise. Azobenzene and PMAz were kindly provided by Merck.

COMPOUND 1

N-tert-Butoxycarbonyl-d-alanine (4.27 g, 22.6 mmol) and 3-bromo-1-amino-propane hydrobromide (2.83 g, 20.5 mmol) were dissolved in CH2Cl2 (150 mL). To this solution N,N-diisopropylethylamine (DIPEA, 2.91 g, 22.6 mmol), N-hydroxybenzotriazole (HOBT, 4.03 g, 23.6 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, 3.50 g, 22.6 mmol) were added. After stirring for 24 hours, the solution was washed consecutively with an aqueous 10% (w/w) citric acid solution (2 x 100 mL), H2O (100 mL), aqueous saturated sodium carbonate (2 x 100 mL), H2O (100 mL) and brine (100 mL). The organic layer was dried over Na2SO4, concentrated and subjected to column chromatography (EtOAc/CH2Cl2 1:4), yielding 1 as an off-white solid (3.69 g, 58%). Analysis: 1H NMR (400 MHz, CDCl3): δ 8.28 (d, J = 9.1 Hz, 2H, CH aromatic); 7.84 (d, J = 9.1 Hz, 2H, CH aromatic); 7.80 (d, J = 9.4 Hz, 1H, CH aromatic); 6.60 (s, 1H, NHboc); 6.34 (dd, J = 9.4, 2.6 Hz, 1H, CH aromatic); 6.18 (d, J = 2.6 Hz, CH aromatic); 4.84 (s, 1H, NH-alkyl); 4.24 (t, J = 6.9 Hz, 2H, CHO); 4.00 (q, J = 7.1 Hz, 2H, CH2); 3.55 (m, 2H, CH2OH); 3.46 (m, 2H, NH); 2.10 (quin, J = 7.1 Hz, 4H, CH2N); 1.36 (d, J = 9.1 Hz, 2H, CH2 spacer); 1.25 (t, J = 7.1 Hz, 6H, CH3 azobenzene); 1.20 (d, J = 7.1 Hz, 3H, CH1 alanine). MS-ESI m/z = 543 [M+H].

COMPOUND 3

A solution of 3 (1.30 g, 4.20 mmol) in DMF (20 mL) was cooled to 0 °C and slowly potassium tert-butoxide (0.47 g, 4.20 mmol) was added. The colour of the reaction mixture changed from dark purple to a dark blue. Bromide 1 (1.41 g, 4.48 mol) was slowly introduced in the reaction mixture, the ice bath was removed and the mixture was allowed to reach room temperature. To complete the reaction, the mixture was stirred at 100 °C overnight. After cooling, the reaction mixture was washed with dilute aqueous HCl (0.1 M, 100 mL), saturated sodium bicarbonate (100 mL) and water (200 mL). The organic layer was dried over Na2SO4 concentrated and subjected to column chromatography (EtOAc/CH2Cl2 1:4), yielding 3 as a red solid (1.06 g, 46%). Analysis: 1H NMR (400 MHz, CDCl3): δ 8.28 (d, J = 9.1 Hz, 2H, CH aromatic); 7.84 (d, J = 9.1 Hz, 2H, CH aromatic); 7.80 (d, J = 9.4 Hz, 1H, CH aromatic); 6.60 (s, 1H, NHboc); 6.34 (dd, J = 9.4, 2.6 Hz, 1H, CH aromatic); 6.18 (d, J = 2.6 Hz, CH aromatic); 4.84 (s, 1H, NH-alkyl); 4.24 (t, J = 6.9 Hz, 2H, CHO); 4.00 (q, J = 7.1 Hz, 2H, CH2); 3.55 (m, 2H, CH2OH); 3.46 (m, 2H, NH); 2.10 (quin, J = 7.1 Hz, 4H, CH2N); 1.36 (d, J = 9.1 Hz, 2H, CH2 spacer); 1.25 (t, J = 7.1 Hz, 6H, CH3 azobenzene); 1.20 (d, J = 7.1 Hz, 3H, CH1 alanine). MS-ESI m/z = 310 [M+H].

COMPOUND 4

The boc protecting group was removed by dissolving 3 (990 mg, 1.82 mmol) in a solution of HCl in ethyl acetate (2.3 M, 15 mL). The color of the reaction mixture changed from dark red to pale orange. After 5 hours stirring at room temperature, the reaction was quenched by the addition of aqueous NaOH (0.1 M, 100 mL). The organic phase was separated, washed with water (100 mL), dried over Na2SO4 and concentrated. The free amine was directed converted to the formamide by the addition of sodium formate (1.12 g, 16.5 mmol) and ethyl formate (30 mL) and heating the reaction mixture to reflux for 48 h. After cooling and removal of the solvent by evaporation, the crude product was subjected to column chromatography (EtOAc/CH2Cl2 1:4) to yield 4 as a red solid (789 mg, 80 %). Analysis: 1H NMR (400 MHz, CDCl3): δ 8.33 (d, J = 9.1 Hz, 2H, CH aromatic);
8.01 (s, 1H, CHO); 7.84 (d, J = 9.1 Hz, 2H, CH aromatic); 7.81 (d, J = 9.4 Hz, 1H, CH aromatic); 6.31 (dd, J = 9.4, 2.6 Hz, 1H, CH aromatic); 6.19 (d, J = 2.6 Hz, CH aromatic); 6.10 (s, 1H, NH formamide); 4.84 (s, 1H, NH-alkyl); 4.28 (t, J = 6.9 Hz, 2H, CH₂O); 4.20 (q, J = 7.1 Hz, 1H, CH alanine); 3.95 (m, 2H, CH₂NH); 3.48 (q, J = 7.1 Hz, 4H, CH₂N); 2.16 (quin, J = 6.9 Hz, 2H, CH₂ spacer); 1.27 (t, J = 7.1 Hz, 6H, CH₃ azobenzene); 1.15 (d, J = 7.0 Hz, 3H, CH₃ alanine). MS-ESI m/z = 471 [M+H].

**COMPOUND 5**

A mixture of 4 (750 mg, 1.59 mmol) and methyl N-(triethylammoniumsulfonyl) carbamate (Burgess’ reagent, 0.70 mg, 2.90 mmol) in CH₂Cl₂ was refluxed for 5 hours. After cooling, the reaction mixture was concentrated and the crude product subjected to column chromatography (acetone/CH₂Cl₂ 4:96) to give the isocyanide monomer 5 as a red solid (675 mg, 82 %) that was stored until polymerization reaction at –20 °C. Analysis: ‘H NMR (500 MHz, CDCl₃) δ 8.32 (d, J = 7.9 Hz, 2H, CH aromatic); 7.86 (d, J = 8.0 Hz, 2H, CH aromatic); 7.83 (d, J = 9.4 Hz, 1H, CH aromatic); 6.38 (dd, J = 9.4, 1.5 Hz, 1H, CH aromatic); 6.22 (d, J = 1.6 Hz, CH aromatic); 4.31 (t, J = 6.3 Hz, 2H, CH₂O); 4.05 (q, J = 7.0 Hz, 1H, CH alanine); 3.64 (m, 2H, CH₂NH); 3.52 (q, J = 7.0 Hz, 4H, CH₂N); 2.17 (quin, J = 6.7 Hz, 2H, CH₂ spacer); 1.51 (d, J = 7.0 Hz, 3H, CH₃ alanine); 1.27 (t, J = 7.0 Hz, 6H, CH₃ azobenzene). MS-ESI m/z = 471 [M+H]. FT-IR (cm⁻¹, ATR): 3296, 3100 (NH); 2141 (C≡N); 1666 (C=O); 1513, 1369 (NO₂); 1214 (tert. amine). UV-vis (EtOH): 𝜆_{max} = 500 nm.

The maximum solubility of 5 in 5CB was determined by sonicating an excess of 5 in 5CB for 10 minutes and filtering the suspension through a microfilter (0.2 μm pores). The filtrate was diluted 1000 fold with chloroform and the concentration of 5 in the solution was determined spectrophotometrically. The maximum solubility of 5 in 5CB is 0.064 wt-%, but to ensure full solubility of the monomer, default reactions were carried out at a slightly lower concentration of 0.035 wt-%.

**POLYMERIZATION OF 5 IN CHLOROFORM**

A round bottom flask (10 mL) was charged with isocyanide monomer 5 (14.0 mg) and distilled chloroform (1.0 mL). The initiator/catalyst solution (200 μL) was added and the progress of the reaction was followed by TLC and IR, UV-vis and CD spectroscopy. After full consumption of 5 (monitored by IR spectroscopy), disopropyl ether (10 mL) was added and the polymer was centrifuged (3000 rpm, 5 min). The clear solvent was decanted and the solids were washed twice more with disopropyl ether (10 mL) and with intermediate centrifugation steps. After purification, CH₂Cl₂ (20 mL) was added to prevent the polymer from drying in.

**PATTERNED AZOPIC ALIGNMENT, SWITCHING AND ANALYSIS**

**POLYMERIZATION OF 5 IN 5CB**

Monomer 5 was dissolved in 5CB and stirred for a few days to ensure complete dissolution. Nickel(ii)perchlorate was dissolved in ethanol (1 mL) and afterwards dry toluene (49 mL) was added. Using a micropipette, a small amount was added to the 5/5CB solution (1:500 catalyst/5 ratio). After swirling for a short time, rubbed polyimide and photoaligned PMAz cells were filled with a drop of the solution at room temperature through capillary force. (Polarized) optical microscopy was used to investigate the polymerization of 5 and subsequent alignment in the anisotropic cells. The remainder of the bulk solution was used for the spectroscopic analysis (UV-vis, CD).

**POLYIMIDE & PMAZ CELL FABRICATION**

The fabrication of rubbed polyimide and photoaligned PMAz cells (including the photoalignment process) has been described in previous work except that the cells were not completely sealed with epoxy glue.

**ELECTRIC FIELD APPLICATION**

Two electrodes were connected to the PMAz cell, which was placed in a custom designed cell holder on the x-y stage of the (P)OM. A function generator (HAMEG HM8130) was used to apply the electric field (continuous wave, 20 V, 1 V μm⁻¹, 1 MHz) to the LC template.
Supporting Information

**CELL OPENING**

The glass cells were opened by soaking them in dichloromethane for 30 to 45 minutes. After this period, the softened epoxy glue was peeled of the glass by using a scalpel. The glass cells were pried open and the LC was removed by adding several drops of diisopropylether on the glass plates, which were removed after 15 minutes by tilting the glass plate on a piece of KimWipe.

**(POLARIZED) OPTICAL MICROSCOPY**

(P)OM images in **Figure 3, 4, 55 and 56** were taken with a Leica DM-RX polarized optical microscope and a Leica DMC2900.

(P)OM images in **Figure 5** and 53 were taken with a Olympus BX60 polarized optical microscope and a CoolSNAP-Pro camera. For determining the orientation of the 5CB mesogens, a 530 nm retardation plate was used.

(P)OM images in **Figure 6** and 54 were taken with a Carl Zeiss Jenaval polarized optical microscope and an AmScope MD900E camera.

**ELECTRON MICROSCOPY**

For SEM imaging, samples were coated with gold/paladium using a Cressington 208HR sputter coater at 20mA for 10 seconds. SEM images were taken on a JEOL 6330 Cryo Field Emission Scanning Electron Microscope at 3 keV.

**UV-VIS & CD SPECTROSCOPY**

The UV-vis spectra of 5 and Azopic (**Figure 2**) were taken with a Jasco V-630 and the CD spectra were taken with a Jasco J-815 spectrometer.

**POLARIZED OPTICAL MICROSCOPY**

Leica DM-RX polarized optical microscope and a Leica DMC2900. Olympus BX60 polarized optical microscope and a CoolSNAP-Pro camera.

Carl Zeiss Jenaval polarized optical microscope and an AmScope MD900E camera.

**SUPPORTING MOVIES AND FIGURES**

Movie S1 can be found here: [HTTP://TINYURL.COM/HBSETV8](HTTP://TINYURL.COM/HBSETV8)

**FIGURE S1** Infrared spectra of a polymerization reaction of 5 in chloroform (1 wt%). Prior to adding nickel-catalyst (1:5000), the isocyanide peak at 2141 cm⁻¹ is prominently present (blue spectrum) but after addition over a couple of days it has completely disappeared (red spectrum), indicating a full conversion of 5 into AZOPIC.

**FIGURE S2** Schematic view showing how the command layer depicted in Figure 5 was patterned in three orientations (indicated by black, grey and red colored domains). Lines within these domains indicate the orientation of the polarizer during photoalignment.
**FIGURE S4** Determination of 5CB alignment with respect to PMAz. POM images with a 530 nm waveplate show two domains of locally aligned 5CB (with S present), where the orientation of the linear polarized light used for the photoalignment is given by the light-blue arrows. Some pressure was applied to one side of the cell which increased the spacing of the glass plates on one side, which caused a liquid front to move inside the cell. On the bottom right (a,b) and bottom left (c,d) part of images, there is a tiny layer of 5CB covering the insides of the PMAz coated plates, while the inside of the cell above the liquid front is fully filled with the 5CB solution. The layer thickness of the in-plane aligned bulk 5CB can also be drastically reduced bulk by applying a homeotropic electric field (b,d). The bulk LC reorient while a thin layer anchored strongly to the surface does not change its orientation. The thickness of the 5CB layer is related to the phase shift of the interference light. Since the layers below the liquid front are much thinner (also after applying an electric field in the domains above the liquid front), the phase shift is low enough to not be able to observe the shift in light color when a 530 nm wave plate is added. The phase shift decreases when the vector of the linear polarized of the photoalignment is parallel to the wave plate orientation (a,b) and increases when it is perpendicular to the vector of the light (c,d). This indicates that the azobenzene-moieties of the PMAz (which align perpendicular to the vector of the light) are parallel to the long axis of the 5CB mesogens.
**SUPPORTING INFORMATION REFERENCES**


**FIGURE S5** Microscopy images of 5CB (0.035 wt%) in a photoaligned PMAz cell. No aligned red microscopic bundles of Azopic can be observed in the cell. Dissolved 5 (responsible for the red background) shows absorption characteristics when the polarizer (indicated by the double-sided white arrow) is oriented parallel to the photoaligned azobenzene mesogens of PMAz within one of the two domains. For visual clarity, the thin black line indicates the boundary between the photopatterned domains.

**FIGURE S6** Polarized optical microscopy (POM) images of Azopic in 5CB (0.035 wt%) in a photoaligned PMAz cell before (a) and during (b) application of electric field (20 V, 1 V μm⁻¹, 1 MHz). POM image (b) shows a purple color which is present due to a smaller phase shift of the light, indicating that the bulk LC has oriented homeotropically due to the electric field. A thin layer of 5CB has remained anchored to the PMAz surface, which is responsible for the observed birefringence. The thick stripes represent defect lines which appear after the application of the electric field, similar to those in Figure 5.
7 OUTLOOK

7.1 KEY ASPECTS OF LIQUID CRYSTAL TEMPLATING

From the previous chapters, it has become clear that using liquid crystal (LC) templating as an alignment tool for device fabrication has many unique characteristics which allow it to complement (or distinguish itself from) other, more conventional techniques such as photolithography, soft lithography and electrospinning in many aspects. In this outlook, these unique aspects are summarized and put in perspective.

The first key aspect is the sheer range of potential materials which can be organized using LC templates. This thesis shows that LC templating can be expanded to the aqueous regime, by demonstrating full control over the spatial organization of water-processable supramolecular and polymeric materials across multiple length scales (Chapter 2). Since functionality can be easily introduced in these classes of soft materials (by monomeric design or through post-modification after assembly), the amount of applications which can emerge from these spatially organized functional materials possibilities is potentially huge. One of the main features of LC templating, both in water and organic bulk liquid crystals, is that the alignment of dispersed materials solely relies on the elastic and orientational shear interactions with the LC template, instead of delicate short range electrostatic or Van der Waals interactions. Specifically this aspect makes the LC templating approach suitable to organize and direct a large number of water-processable materials that are not easily addressed by other conventional alignment techniques. As it already has been demonstrated already that LCLCs such as DSCG are compatible with living matter, one can expect that other highly innovative biological applications will emerge in the near future.

The second aspect is that this technique in general allows for the creation of patterns of functional soft matter from micrometers to potentially meters which by itself is extremely difficult or practically impossible to achieve for (some) other conventional alignment techniques. As it already has been demonstrated already that LC-LCs such as DSCG are compatible with living matter, one can expect that other highly innovative biological applications will emerge in the near future.

The third aspect is that the characteristic aspects of LCs can be combined with templated functional materials for additional functionality. For instance, LCs can add specific optical characteristics to a device such as its inherent ability to alter the polarization of light as well as absorbing a specific part of the light (especially in the case of highly absorbing LCLCs used as laser-dyes). Also, since liquid crystals can deform locally around sub-micron objects (through anchoring interactions) and create much larger deformations (through long-range elastic deformations) at dimensions which can be detected under a polarized optical microscope, unique LC sensing capabilities can be integrated in applications where the spatial organization of functional materials needs to be controlled but also detected in time and space. As an example, Crystal Diagnostics is a company which has used this sensing feature to detect the presence of pathogens which together with antibodies are able to cluster microspheres in an LC medium, where the resulting deformation of the bulk LC can be visually detected under a microscope.

The fourth aspect is that LC templates can be easily integrated with other alignment techniques such as electric and magnetic fields which introduces dynamics and responsiveness to the system. In this regard, electric fields have the unique ability to manipulate the orientation of the dispersed low-susceptible functional materials (by addressing the highly susceptible LC template) and using existing LCD technology in order to create complex electric field-driven switchable setups. As shown in Chapter 4, it is now possible to align and manipulate supramolecular and polymeric materials in water using electric fields by using an athermal mineral LC template. With this approach a wide range of other materials can be manipulated, perhaps even living biomaterials in the future. In Chapter 6, instead of mineral LCs, we have applied electric fields to an organic non-ionic LC template on a photopatterned substrate in order to reversibly switch the orientation of polymeric assemblies from a patterned in-plane to a unidirectional homeotropic orientation. For aqueous systems, switching the orientation of dispersed functional materials in LC templates in complex patterns has not been demonstrated yet. So far, there are no reports where the required mineral LCs have been aligned on e.g. photopatterned command layers. Perhaps a similar surfactant strategy as employed in Chapter 2 can facilitate patterned alignment of the mineral LC on a photoaligned substrate.

Besides applying LC templates to directing surfaces and/or electric fields, templating dispersed soft materials within LCs in magnetic fields also has many
unique benefits. First of all, no directing surfaces are needed, out-of-plane is easily possible and the strength and direction of the magnetic field can be readily changed over time. In Chapter 3 we demonstrated that peptide amphiphiles can be aligned at ten times lower magnetic field strengths than previously reported, by using an LCLC template. This paves the way for high-throughput alignment of functional soft materials in LC templates (in both aqueous and organic solutions) on tabletop setups with for example permanent neodymium magnets. Also by lowering the required magnetic field strength for alignment, we can use strong magnetic fields to create complex assembled structures. We showed that we can obtain orthogonally structured assemblies in a magnetic field aligned LCLC, because of a competition between the orthogonal diamagnetic anisotropies of the peptide amphiphiles and LCLC template. The formed structures are also influenced by a range of interactions such as elastic and time-dependent depletion forces, magnetic field strength and temperature. However, in order to precisely control the formation of such structures, we need to better understand and control these interactions. Decoupling one or more of these interactions should help in this regard. One option is to remove the temperature influence on the formation of the dispersed material, for example by using preformed polymer dispersions in which the covalent polymeric structure is stable against the increased temperatures during the cooling down process in the magnetic field (compared to supramolecular materials where self-assembly is initiated at specific temperatures). A second option is to use athermal goethite templates (whose LC phase is not influenced by temperature) instead of temperature-sensitive LCLCs such as DSCG.

The fifth aspect is that photopatterned LC templates can be used to fabricate many diverse types of devices (from a hardware engineering perspective). Because it is based on spin coating and photoillumination, this technique can be readily applied to a wide range of different substrates (glass, plastics, conducting indium tin oxide etc.) and even on curved surfaces. These polymer-based photosensitive layers can also be applied to confined geometries, such as microfluidics. Using advanced photoalignment techniques frequently employed in the development of stimuli-responsive LC polymer networks, even hierarchically ordered in-plane geometries can be created. The use of photopatterned substrates with large pre-tilt angles might allow the organization to extent in the third dimension.

The sixth aspect is the ease of application of spatially patterned LC templates and the potential for integration within mass manufacturing. From an engineering perspective, the hybrid technology of photopatterning and LC templates can be readily applied in laboratories and in the manufacturing industry. Since the technology mainly relies on conventional spin coating and photo-illumination and due to the implementation of command layers by Sharp in 2009 for the production of their next-generation LCD TVs, commercial application and mass-manufacturing of such command layer-based devices should be within reach.

7.2 APPLICATION CRITERIA

There are several important criteria in LC templating which need to be considered in order to apply this technology for the development of devices. First of all, the concentration of the dispersed materials within the LC solvent can be a limiting factor for specific applications, for example, when a high density of aligned arrays of functional materials on a substrate is desired for specific applications, such as optical retarders or (wavelength-selective) polarizers. The solubility can be improved by carefully matching the LC and the dispersed functional material by considering their specific chemical composition. Furthermore, in the case of supramolecular and polymeric materials, the building blocks can be modified through organic chemistry to improve the solubility without radically impacting the self-assembly mechanism and elastic interactions with LC templates. On the other hand, for compounds which can be dispersed at high concentrations, the LC template might not be able to effectively direct the organization of the dispersed material, resulting in much lower degrees of alignment.

Secondly, depletion interactions with LCs force the polymers or assemblies into larger aggregates. This can give rise to unique organizations of the dispersed materials within the matrix, which may work well for specific applications, such as tissue engineering, where an improved mechanical strength of polymeric materials is desired and/or where cell-signaling receptors on the backbones of these materials need to be clustered. Controlling the depletion interactions would give rise to a better control over the macroscopically organized assembly. Besides the molecular design and compatibility between materials and host, temperature and time can also be used as parameters to control the diffusion rate.
Another criterion for applications derived from LC templating is the presence of the LC itself, which, therefore, should be carefully chosen. It can be beneficial as an additional responsive (sensing) agent, which can also add specific optical characteristics to the device (as explained above). If undesired, the template can be removed from the organized functional material on the substrate, provided that the mechanical stability of the organized soft material is strong enough to withstand the shear forces in the washing process. This required mechanical stability can arise, for example, from depletion induced bundling (see above), photopolymerization of internal crosslinkable moieties (such as internal diae-tylene-groups in peptide amphiphiles in chapters 2, 3 and 4) or improved non-covalent adhesion to the specific directing substrates (such as rubbed polyimide or photopatterned command layers).

A fourth criterion is the intended life-time of the LCLC-based device. In contrast to thermotropic LCs (with very high boiling points), LCLC phases are formed in water, which evaporates from the device over time. For devices designed for long-term stability, this should be avoided at all times, since it would greatly impact the organization of the LCLC (and the dispersed materials) and thus would be detrimental for the functionality of the device. Engineering completely sealed-off environments for such long life-time designed devices is essential.

Finally, in some cases the addition of a surfactant to the LCLC can be extremely beneficial as it greatly increases the alignment homogeneity and stability of LCLCs on rubbed polyimide (chapter 5) and photopatterned substrates (chapter 2). Although these surface-active agents greatly assist in the alignment of LCLCs on hydrophobic surfaces, specific surfactants at (too) high concentrations can negatively influence the functionality of the dispersed materials. As an example, they can prevent the self-assembly of supramolecular materials such as peptide amphiphiles (chapter 2), or analogously, alter the membrane integrity of bacteria and cells or simply introduce undesired isotropic micelles in the liquid crystal template. Choosing the specific surfactant (mixture) and concentration (e.g. below the critical micellar concentration) or perhaps developing new photosensitive command layers with side-chain surfactant moieties (replacing altogether the need for a surfactant additive) should address these challenges. Additionally, the molecular design of the functional soft materials can be tuned such that it inhibits dissolution of the material by incorporation within surfactant aggregates, for example by preventing similar types and sizes of hydrophilic/hydrophobic domains.

On a more general note, the amount of potential applications which can be derived from LC templating are truly staggering, looking at the vast catalogue of materials (with different functionalities) that can be organized as well as well as the multiple device configurations possible using conventional industrial manufacturing processes. A few potential applications are listed below. For instance, aqueous LC templating can be used for the development of biosensors based on hydrophilic polymer backbones (functionalized with sensing elements) patterned on substrates in between electrodes, which can detect specific (bio)molecules where the macroscopic alignment greatly increases the electrical transduction efficiency. Alternatively, spatially patterned bioscaffolds prepared by LC templating can be used to create cell-seeding islands, but also guide tissue growth (such as neuronal cells) along patterned hydrogels on 2D substrates. In a completely different field, switchable high performance photonic devices might be fabricated from optically active polymeric nanowires dispersed at large concentrations in spatially patterned LC templates with integrated electric fields. As mentioned above, potential applications of liquid crystal templating in aqueous or organic environments are found in very different fields of materials science. A more detailed technology assessment is required in order to identify the main areas where the technologies described in this thesis can lead to commercial application. Some of the key questions stated below can be used as a starting point for such an assessment.

What kind of applications can be developed with the techniques described in this thesis, even in the foreseeable future? Which companies might be interested? Does the researcher, supervisor(s) have such potentially interested companies within their network; which might be interested and can be easily approached? From an application standpoint, which specific requirements does the applications need to have? What are the currently best performing materials in a company’s portfolio which can potentially greatly benefit from LC templating? Is it feasible to start a small pilot with this technology? Can it be scaled up to the desired level (long term)? Is any future mass-market application held back by IP protection? Answering these questions should help to provide a basis on which a thorough technology assessment can be conducted in order develop routes to commercialization of liquid crystal templating.
SUMMARIES
8 SUMMARIES

8.1 ENGLISH SUMMARY

Nature’s craftsmanship is truly inspiring in the way it is able to form complex structures based on a range of building blocks (proteins, DNA, sugars, lipids and more). The formation of larger structures from these biomaterials and the ability to position and dynamically control the organization of these structures across many length scales has been fundamental for life to develop function and ultimately to flourish. As scientists we are intrigued by Nature’s nanotechnological toolkit and we are trying to apply similar techniques in order to create new functional materials for a wide range of purposes, ranging from developing nanocapsules which are able to deliver drugs to a specific point in the body of a patient, to creating large-scale devices which can efficiently transform the abundant solar power into clean electricity. Scientists are able to employ organic chemistry to build a huge array of complex, tailor-made molecules (as chemical building blocks) and allow these materials to form larger nanometer-sized functional structures. It remains a challenge however to control the spatial organization of these materials across multiple larger length scales.

In this thesis, the concept of liquid crystal templating as a technique to control the spatial organization of nanomaterials is taken a step forward. We apply this technique to aqueous solutions and combine it with various external stimuli, in some cases to create complex structures.

In **CHAPTER 1** describes the current state-of-the-art to realize multi-length scale organization in various types of soft matter.

In **CHAPTER 2**, we developed a technique based on photosensitive surfaces and liquid crystal templates which allows us to control the in-plane organization of water-processable 1D supramolecular materials in any programmable pattern from micron to centimeter length scales. After alignment, these amphiphilic materials can be photopolymerized which induces highly stable optically active \( \pi \)-conjugated polydiacetylenes, which show strong linearly polarized absorption characteristics. Due to the increased mechanical stability of the photopolymerized polydiacetylenes, a washing step can remove the liquid crystal template which leaves the micron-sized amphiphiles intact and organized on the surface.

Instead of applying directing surfaces, in **CHAPTER 3**, we used non-contact external magnetic fields in order to indirectly control the structure formation of 1D supramolecular materials in water. By applying a 2 T magnetic field, the liquid crystal template aligns parallel to the field. The dissolved amphiphilic building blocks in turn self-assemble into fibrous structures (which can only be aligned by very high fields above 20 T) and reorient in this matrix due to anisotropic elastic interactions. Through this template-approach, we managed to align these supramolecular materials at tenfold lower fields than previously reported. After alignment, photopolymerization transforms the aligned supramolecular materials into highly stable and optically active polydiacetylenes, which show linear polarized absorption characteristics. Additionally, the template can be removed by a washing step because of the increased mechanical stability. We also used this approach in tandem with a 20 T magnetic field, where we observe the formation of complex 2D structures due to competing alignment processes resulting from the opposite diamagnetic anisotropic of the template and the self-assembled amphiphiles.

Inspired by the technology behind LCD screens, electric fields have been used in the past to control the one-dimensional structure formation of polymeric materials. Unfortunately, application of electric fields in (ion-rich) aqueous solutions is troublesome due the presence of parasitic heating effects. In **CHAPTER 4** we demonstrate an approach which relies on a mineral-type liquid crystal template in water, which under the application of an electric field is able to align and position dispersed supramolecular materials. Besides showing for the first time that supramolecular and polymeric materials can be aligned in water using an electric field through this approach, we also show that the bundle sizes of the supramolecular assemblies can be tuned with the concentration of the employed mineral liquid crystal template.

Liquid crystal-based applications are heavily reliant on strong interactions between directing surfaces (often based on anisotropically treated polymeric coatings) and various liquid crystals, where the substrate provides stable and homogeneous alignment of the liquid crystalline bulk. Despite the development
of a range of very effective approaches for creating strong alignment for conventional thermotropic liquid crystals, applying these techniques to water-based liquid crystals on directing surfaces has been very challenging so far. In **CHAPTER 5**, we show that with a surfactant-based approach we are able to create extremely stable (over several months) and highly homogeneous alignment of lyotropic chromonic liquid crystals on standard rubbed polyimide surfaces. By mixing lyotropic chromonic liquid crystals with a tiny amount of non-ionic surfactant, the formation of an interface layer enhances the anisotropic interactions between the hydrophobic polyimide and the anisotropic hydrophilic liquid crystal medium. We apply our approach to both closed cells and dried-down chromonic films and we show how we can obtain such unprecedented high alignment quality by varying the molecular structure and concentration of the applied surfactant.

In **CHAPTER 6**, we combine photosensitive patterned surfaces and electric fields with a liquid crystal template to control the spatial organization of stiff organic polyisocyanopeptide polymers. We find that in-situ polymerization of the corresponding monomers yield bundles of polyisocyanopeptides, which follow the photoimprinted pattern on the substrate and show strong absorbance and linear dichroism. These dye-functionalized polymeric materials can be switched from an in-plane to homeotropic alignment using an electric field. The response time of the bundles (which is much slower than that of the liquid crystal template) strongly depends on the dimensions of the bundle. We also find that the strength of the applied electric field is directly related to the extent of the bundle reorientation, which enables another level of dimensional control over these aligned polymeric materials.

Finally, in **CHAPTER 7**, the unique aspects of liquid crystal templating are summarized and put in perspective. Furthermore, its potential in application development is discussed.
Het is werkelijk fascinerend hoe de natuur in staat is om complexe structuren te creëren uit verschillende bouwstenen (eiwitten, DNA, suikers, lipiden en meer). De formatie van nog grotere structuren uit deze biomaterialen en de mogelijkheid om deze te positioneren en dynamisch te controleren in tijd en ruimte op verschillende lengteschalen is een essentiële voorwaarde voor het functioneren van leven. Als wetenschappers zijn we geïntereard door het ware vakmanschap waarmee de natuur zulke complexe structuren creëert en ordent. Wij proberen soortgelijke technieken toe te passen om nieuwe materialen te ontwikkelen voor verschillende doeleinden. Te denken valt bijvoorbeeld aan de fabricering van nanocapsules die op een precieze locatie in het lichaam van een patiënt een medicijn kunnen afleveren, of de ontwikkeling van materialen die effectief het overschot aan zonlicht kunnen omzetten in duurzame energie. Wetenschappers zijn in staat om door middel van organische chemie een gigantische hoeveelheid complexe moleculen (chemische bouwstenen) te creëren en van deze componenten nanometer-grote functionele structuren te construeren. Het blijft echter een grote uitdaging om de ruimtelijke organisatie van deze nanomaterialen op verschillende lengteschalen te controleren.

Een manier om nanomaterialen ruimtelijk te organiseren is het gebruik van zogenaamde vloeibaar kristalline matrices. In dit proefschrift is het concept van deze techniek verder doorontwikkeld. We passen deze techniek toe in waterige oplossingen en we combineren het met externe stimuli om gecontroleerde complexe structuren te vormen.

In **HOOFDSTUK 1** is beschreven welke technieken momenteel toegepast worden om de ruimtelijke organisatie van nanomaterialen te controleren.

In **HOOFDSTUK 2** beschrijven we de ontwikkeling van een techniek, gebaseerd op foto-actieve coatings en vloeibaar kristalline matrices, waarmee het mogelijk is om de in-plane organisatie van 1D watergedragen supramoleculaire materialen in elk programmeerbaar patroon (van de micrometer- tot centimeter-lengteschaal) te controleren. Nadat we deze amfifiele materialen uitgelijnd hebben, kunnen we de structuren fotopolymeriseren waardoor ze mechanisch zeer stabiel zijn en door hun macroscopische uitlijning lineair dichroïsme vertonen. Vanwege de sterk verbeterde mechanische eigenschappen van deze n-geconjugeerde polydiacetylenen kunnen we het vloeibaar kristallijne matrix verwijderen door middel van een wasstap, waardoor vervolgens de uitlijndige micrometer-grote structuren intact en georganiseerd op het substraat blijven liggen.

In plaats van gebruik te maken van sturende coatings, hebben we in **HOOFDSTUK 3** externe magnetische velden gebruikt om indirect de structuurformatie van 1D watergedragen supramoleculaire materialen te controleren. Bij de toepassing van een 2 Tesla magneetveld wordt de vloeibaar kristallijne matrix uitgelijnd in de richting van het magneetveld. De opgeloste amfifiele bouwstenen in deze template organiseren zich in vezelachtige structuren (die normaliter alleen uit te lijnen zijn bij magneetvelden boven de 20 Tesla), die zich volgens heroriënten in de uitgelijnde matrix door middel van anisotrope elastische interacties. Via deze techniek zijn we in staat om de structuurformatie van deze materialen op meerdere lengteschalen te controleren bij tien keer lagere magnetische velden. Na uitlijning passen we een fotopolymerisatie stap toe om deze materialen te transformeren in optisch actieve en mechanisch zeer stabiele n-geconjugeerde polydiacetylenen, waarna we door middel van een wasstap de vloeibaar kristalline matrix kunnen verwijderen. We hebben deze techniek ook toegepast met 20 Tesla magneetvelden, waardoor complexe 2D structuren ontstaan. Deze zijn gevormd vanwege de onderlinge competitie in uitlijningskrachten tussen de tegenovergestelde diamagnetische susceptibiliteiten van de matrix en zelf-assembleerende supramoleculaire amfifiele.

Geïnspireerd door de technologie achter LCD-schermen hebben wetenschappers in het verleden veelvuldig elektrische velden gebruikt om de structuurformatie van een-dimensionale polymeren te controleren. Helaas is deze techniek niet toepasbaar in waterige (ion-rijke) oplossingen vanwege de aanwezigheid van parasitaire opwarmingsverschijnselen. In **HOOFDSTUK 4** hebben we een methode ontwikkeld die gebaseerd is op een mineraal-houdende flocuibre Een manier om nanomaterialen ruimtelijk te organiseren is het gebruik van zogenaamde vloeibaar kristalline matrices. In dit proefschrift is het concept van deze techniek verder doorontwikkeld. We passen deze techniek toe in waterige oplossingen en we combineren het met externe stimuli om gecontroleerde complexe structuren te vormen.

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Toepassingen van vloeibare kristallen zijn erg afhankelijk van sterke interacties tussen sturende oppervlaktes (vaak gebaseerd op anisotroop-behandelde polymeersubstraten) en verscheidene vloeibare kristallen. Ondanks dat er zeer effectieve technieken zijn ontwikkeld om sterke uitleiding te verzorgen voor thermotrope (niet-waterige) vloeibare kristallen, is het in de praktijk een grote uitdaging om deze technieken toe te passen op vloeibaar kristallen in waterige oplossingen in combinatie met deze sturende polymeersubstraten. In **HOOFTSTUK 5** laten we zien dat we door middel van het gebruik van oppervlakte-actieve stoffen, extreem stabiele (aantal maanden lang) en zeer homogene uitleiding kunnen verkrijgen voor waterige vloeibare kristallen op anisotroop-behandelde polyimideoppervlaktes. Door het toevoegen van een zeer kleine hoeveelheid oppervlakte-actieve stof aan het waterige vloeibaar kristal wordt er een tussenlaag gevormd dat sterke anisotrope interactie faciliteert tussen het hydrofiele vloeibaar kristal en het hydrofobe polymeersubstraat. We passen deze methode toe in afgesloten cellen en in dunne lagen van ingedroogd vloeibaar kristal. Verder laten we zien hoe men door middel van de keuze van het type en concentratie van oppervlakte-actieve stof zulke extreem stabiele en zeer homogene uitleiding kan creëren.

In **HOOFTSTUK 6** combineren we foto-actieve coatings met elektrische velden en vloeibaar kristallijn matrices (ditmaal in een niet-waterige oplossing) om de organisatie te controleren van stijve organische polyisocyanopeptide-polymeren. Door middel van polymerisatie van de opgeloste monomeren in de matrix worden micrometer-grote bundels van polyisocyanopeptides gevormd, die zich lokaal ordenen in patronen op het foto-uitgelijnde substraat en sterke lichtabsorptie en lineair dichroïsme vertonen. Door het gebruik van een elektrisch veld kunnen deze optisch actieve materialen vervolgens geschakeld worden vanuit de initiële orientatie naar een nieuwe orientatie, dus van in het vlak van het substraat naar loodrecht op het substraat respectievelijk. De schakeltijd van de bundels, die vele malen trager is dan de heroriëntatie van het vloeibaar kristallijn matrix, is sterk afhankelijk van de dimensie van de bundel. Verder is de sterkte van het elektrisch veld ook gecorreleerd aan de mate van heroriëntatie van de bundel, wat een extra mogelijkheid geeft om de structuurformatie van polymeren te controleren in tijd en ruimte.

Als laatste in **HOOFTSTUK 7**, vatten we de unieke aspecten van vloeibaar kristallijn matrix samen en analyseren we de potentie van deze technologie voor de mogelijke ontwikkeling van innovatieve applicaties.
ACKNOWLEDGEMENTS, CURRICULUM VITAE, PUBLICATION LIST & NOTES
9 ACKNOWLEDGEMENTS, CURRICULUM VITAE, PUBLICATION LIST & NOTES

9.1 ACKNOWLEDGEMENTS

It was never my intention to do a PhD in the first place. I had the feeling doing a PhD meant working solely by yourself in your own enclosed scientific area. During the period when I was about to finish my Master’s degree, I was pursuing other job opportunities. Around this time, there was however one particular fine evening where I had a beer with a good friend of mine who told me I should absolutely consider talking to his old supervisor from his Master’s internship. A few days later, I contacted a certain assistant-professor and asked him if I could talk to him about my prejudices concerning doing a PhD. I expected a short chit-chat, but after I gave a presentation to the Molecular Materials group (where only a handful were present due to the consequences of a specific PhD defense party) and a long but very pleasant conversation, my mind was set on working in this research group.

First of all I’d like to dearly thank this assistant-professor I mentioned earlier, Paul Kouwer. Ever since the start of my PhD it has been an absolute pleasure working with you. You always gave me a lot of space in allowing me to develop myself as a scientist while somehow you still managed to give me a nudge in the right direction whenever I seemed to have become stuck. From both a professional and personal view, I really enjoyed our meetings, discussing my latest results, future plans but also other non-work-related matters. I am also very indebted to you for all your help during the frantic end of my PhD. Without your timely and critical feedback on my chapters I would never have been able to finish my thesis before the end of my contract.

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I will try to do my utmost best to thank all the super nice people I have met during my time at the Faculty of Science in Nijmegen. Because of the sheer amount of people who came and went, it might be that I will miss out a few in the list below. My sincerest apologies in that case. First of all I would like to thank my lunch buddies Egle, Jialiang, Giorgio, Alexandra, Riccardo, Johnny, Miriam, Pat, Shaji, Seda and all other people from my department, in particular Jon, Roel Hamming, Maarten, Joan, Emilia, Joris, Sophie, Onno, Daniel, Marcel, Bob, Mario, Laurens, Rajat, Petri, Qi-Wei, Chenggong and the new additions Paula, Max, Bao, Ying and Hugbong (best of luck with your PhDs). Of course I can not forget the Wing 8 crew and all the cheerful lunches, fun BBQs, board game evenings, beers and strange fermented fish adventures we had: Loai, Marlies, Elena, Fei, Yingfeng, Yongjuan, Alaa, and the many students who have been part of this “isolated” part of the sub-faculty such as Sarah, Noël, Jordi, Shauny, Vincent, Alain, Martin, Tom, Ashish, Roy, Peer, Danny, Jelle, Josje, Corali and Sema. Outside of my own department and wing, I have met so many friendly people along my PhD journey such as Dave W., Roger, Marcel, Lise, Lianne, Joep v.d. W., Ruud, Mathijs, Mark, Nanda, Morten, Sanne, Albert, Maike, Stephanie, David F., Emilien, Ilya, Joost, Sjoerd, Venkat, Julian, Dani, Danny, Jos, Abbas, Rens, Ivan, René, Alejandra, Paul W., Marta, Paul T., Mireille and many others I forgot to mention. Personally, I had a blast organizing the annual Nolte-Meijer Cup, which were yearly football and volleyball matches between the chemistry departments of Eindhoven and Nijmegen. Gijs van Pruissen, thank you for my favorite non-scientific collaboration and my sincerest apologies to Roeland Nolte for not managing to take the Football cup back to Nijmegen (where we both know it belongs). Over the last four years I have visited many conferences, meetings and summer schools in various places in Europe and the United States and I had the pleasure to meet so many kind people in the same field. Therefore a shout-out to my liquid crystal buddies: Michael, Uroš, Luka, Gregor, Daniel and Lily.

Of course, I can not forget many of my friends who (outside of my work) greatly contributed to my mental well-being and as a result played indirectly a very important role in my PhD. Many, many thanks to Thijs and Roel Helmes (my awesome paranymphs), Lotte, Bram, Carolien, Nan, Neeltje, Floor, Bernard, Henk, Andrès, Sanne, Roel vd H., Anja, Koen, Nienieke, Isabel, Ramon, Luuk, Roel S., Marijn, Ine, Rein, Peter, Rebecca, Arno and Joep B. Here’s to many more drinks, skiing trips, festivals, board game evenings and great BBQs together in the future. Ans, Henk, Carolien, Jeroen, Luc, Laura, it has been a blast getting to know you (as my sort of backup family). I am looking forward to our next dinner parties where besides plenty of hilarious conversations you can amaze me again at the sheer amount of delicious food you are able to eat as a group.

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And finally my dear Vera, also as my PhD partner-in-crime. these last few years have been truly amazing, such as our wonderful trips around the world, many insightful discussions about our PhDs and in general all the good times/vibes together. I am looking forward to many more adventures with you.
9.2 CURRICULUM VITAE

Pim van der Asdonk was born on a very hot summer’s day on the 21st of June 1986 in Tegelen, the Netherlands. After finishing his secondary education at College Den Hulster in Venlo, where he obtained his VWO degree, he moved to Nijmegen to start his Chemistry education. After a short stint in the culinary domain where onions got the best of him, he pressed forward and obtained his Master’s degree in Chemistry in 2012. Shortly thereafter, he started as a PhD student in the group of Molecular Materials (funded by NanoNextNL), under the supervision of Dr. Paul Kouwer and Prof. dr. Alan Rowan, where he earned his doctorate’s degree in 2016.
9.3 PUBLICATION LIST


How can we manipulate incredibly small one-dimensional nano- and micrometer-sized objects? Can we align and position these structures in any desired pattern on a range of different surfaces? Is it possible to dynamically switch the orientation of these tiny objects?

In order to develop innovative applications using these molecular building blocks, it is essential to tackle such challenges. **Liquid crystal templating:** spatial and dynamic control of functional soft materials describes the development of a versatile approach which addresses these questions. By using anisotropic liquid crystalline solvents in combination with directing surfaces (rubbed polymer coatings or photosensitive command layers), electric and/or magnetic fields, the spatial organization of various polymeric and supramolecular materials can be controlled across multiple length scales. Furthermore, this technique can even be applied to (biocompatible) aqueous solutions. Finally, because of its integration with external electric fields, it is also possible to dynamically switch the orientation of these patterned one-dimensional nanomaterials.