Molecular machines for life-like adaptive matter

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Chapter 1

General introduction

1.1 Introduction

Life is sustained by the operation of bio-molecular machines that drive organisms away from equilibrium and support motile behavior, intracellular transport, transcription of genetic information and the synthesis of essential metabolites and proteins.¹

Organic chemists have long taken onto the challenge to create artificial molecular machineries that would be operating as many complex functionalities as their natural counterparts. This grand challenge has been recognized by the award of the 2016 Nobel prize for chemistry for the design and synthesis of molecular machines.^{2,3} Arguably, achieving control over molecular motion is a great achievement of man-made molecular machines. However, while the general chemical principles that rule this motion have been explored to a formidable extent,⁴ mechanisms to amplify the motion of molecules into the purposeful movement of materials have yet to be fully elucidated.^{5,6} Early attempts to harness molecular operations into macroscopic motion have led to shape-changing materials that switch between steady states. In stark contrast, living matter is capable to adapt and perform purposeful continuous motion by synchronizing the operation of

molecular machines in time and space, a feature which is unmatched in artificial systems.

In this manuscript I address this challenge by exploring the role that asymmetry and chirality play in amplifying molecular motion in high-order anisotropic media.

1.2 Aim and outline

The aim of this research is to develop materials with life-like properties that have the propensity to adapt their macroscopic functions to changes in the environment. Here, the strategy to engineer life-like matter draws inspiration from biological systems, where the operation of molecular machines is amplified from the molecular up to the macroscopic level, in highly ordered soft matter.

In Chapter 2 I present an overview on life-like motion from artificial molecular machines and discuss some general physico-chemical mechanisms that govern motile systems driven by molecular machines, with a special focus on systems that are fueled by light. The chapter reviews simple shape transformations all the way to complex molecular systems regulated by photo-mechano-chemical feedback loops that work continuously and remain away from equilibrium, for as long as they receive energy. The structure of this manuscript mirrors the general guidelines and roadmap provided in this review chapter.

Chapter 3 takes onto the challenge to design and create polymers that mimic the complex mechanical properties of muscles. These materials are capable to perform measurable work, either softening or stiffening in response to environmental cues, and they respond to stress with a non-linear deformation. Significantly, these bioinspired soft materials use mechanically active molecules to drive their life-like mechano-adaption.

Chapter 4 features a new design for overcrowded-alkene based molecular motors, that allows amplifying their chirality in anisotropic fluids effectively. The design of the molecular motors allows to address three separate chiral conformations stable in time and that twist liquid crystals. The range of chirality control over the cholesteric liquid crystal and the stability of the chiral structures formed is unmatched by molecular switches.

In **Chapter 5** a molecular system close to a structural transition is driven away from equilibrium by the winding action of molecular motors. Upon illumination with laser light the motor mediates the formation of a vortex that rotates continuously, regulated by a photo-mechano-chemical feedback loop, for as long as energy is provided in the form of light.

Chapter 6 shows that inanimate matter can be combined with artificial molecular motors to approximate the sophisticated and purposeful behavior of bacteria in water. Specifically, chiral microcompartments akin to protocells are shown to display helical motility, and this motile behavior is modified by the operation of the motors. The key role of chirality in this motion allows controlling the re-direction dynamics of these compartments.

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Chapter 2

Life-like motion from artificial molecular machines

Essentially all motion in living organisms emerges from the collective action of molecular machines transforming chemical energy, harvested ultimately from light, into ordered activity. Inspired by nature's biomolecular machines, chemists have moved from building static molecular structures to achieve dynamic control creating artificial molecular machines that display controlled and sometimes even directional motion in response to light. But to be practically valuable, the motion of these light-fueled molecular machines will have to be coupled to the rest of the world. Drawing inspiration from the complex functional movement seen in the plant and in the animal world, chemists have succeeded in harnessing molecular dynamics to do useful work at the macroscopic level. We review this recent progress here, and show how inspiration from living matter has driven research away from the engineering of switchable materials, by embracing the full complexity of the molecular worlds and moving towards autonomous and sometimes adaptive molecular systems that work continuously under the effect of illumination. We thus find evidence that molecular motion can be engineered into highly sophisticated movements resembling those developed by evolution-optimized processes in the natural world.

2.1 Introduction

Life is intimately connected to motion at all length scales – from the taxis of a bacterium to the regular oscillation of a beating heart, all the way to the biomechanics of the human body: the dynamics of life are born at the molecular level, and they follow the rules of molecular motion. Molecules consume fuel, and the energy is channeled into specific modes of molecular movement rather than being dispersed. These molecular-scale dynamics are then amplified by ingenious coupling with their environment, and eventually support shape-transformation in a broad range of functions, from adaptive stiffness (e.g. by the elongation of muscle fibers)¹, to autonomous motion (e.g. by the deformation of cilia and flagella)², color changes (e.g. by the swelling of cells in chameleon skin)³, topological transformation (e.g. by the gastrulation of embryos)⁴ and eventually life (e.g. the repetitive beat of a heart). Re-engineering the biological strategies that support motion will thus provide valuable insights into strategies to outperform our current materials in terms of energy management, actuation, and sensing.

The maturity reached by synthetic organic chemistry has enabled the design of a plethora of dynamic molecules that display controlled shape changes with an ever-increasing degree of sophistication. These achievements have been highlighted in recent reviews^{5,6,7,8,9,10,11}, in opinion papers^{12,13} and in comments following the attribution of the 2016 Nobel Prize for Chemistry to the design and synthesis of molecular machines^{14,15}. While the movement of molecular machines has been mastered in solution, harnessing their

mechanically-relevant motion to produce work remains a key challenge because i) liquids are isotropic media and in the absence of any cooperative behavior, the operation modes of molecular machines remain localized to the nanoscale, and ii) fluids are not endowed with any shape that would eventually mediate function. Integrating synthetic molecular machines into hierarchical assemblies so that they can generate macroscopic motion and ultimately function, thus remains a contemporary challenge, as the operation in solvents precludes the creation of mechanized morphologies and thus any production of work.

By contrast, the work performed by bio-molecular machines is harnessed cooperatively in space and time through hierarchical self-assembly across increasing length-scales, to yield macroscopic motion. This macroscopic motion that is the hallmark of living systems is also regulated by feedback loops, which allow organisms to sense and adapt to external stimuli, and give thus purpose to their action.^{16,17} For molecular machines to support life-like motion at all scales, we argue that four mechanisms are essential: i) the transduction of energy into relevant shape changes at the molecular level, ii) the harnessing of molecular changes in a cooperative fashion, iii) their amplification, and finally iv) the regulation of macroscopic motion in space and time by feedback loops.

In this contribution, we review how these mechanisms have been re-engineered by using light-fueled artificial molecular machines in wholly synthetic systems and materials. Our choice to focus on light-driven motion

is motivated by the fact that photochemical processes are not constrained by microscopic reversibility. While the dynamics of chemical processes are invariant under time reversal, i.e. a reaction can occur reversibly according to the same mechanism independently of where the equilibrium is shifted, processes that are driven by light do not have to be time symmetric. With photochemical reactions, the large energy input allows reactions to occur on higher potential energy surfaces (excited singlet or triplet states), where molecules have higher degrees of freedom to undergo transformations (configurational changes, dissociation, association, etc...) which would be impossible in chemically-driven systems. In the specific case of light-driven molecular machines, the transition from the higher potential energy surfaces to the ground state is accompanied by dramatic structural changes capable to generate forces on their surroundings.^{18,19} In addition, with adequate molecular design, light-driven chemical reactions can also lead to the formation of high energy conformational states, that are capable of producing a power stroke by release of strain.

Major achievements in the recent decade only hint at the sophistication of the macroscopic shape-transformations that can be achieved in living matter, both in terms of complexity and precision of movement, as well as in the accuracy of motion control. The challenge ahead is to reach beyond one-dimensional transformations of mechanized matter, e.g. bending movement or translation, towards truly mechanized materials that work away from

equilibrium, grow and eventually evolve by adapting to their environment actively.

This review chapter is divided into four main sections that feature recent progress in increasing order of movement complexity, with a final outlook into continuous processive operations orchestrated in time (Scheme 1). In the first section we address how molecular operations can be transduced into simple macroscopic shape changes, with special emphasis on incorporating molecular machines into topologically and hierarchically structured molecular environments. The second section shows how motility can emerge from these simple macroscopic changes, provided that they are coupled with chirality, and thus promote asymmetric interactions with the environment. The third section reviews recent progress in harnessing the continuous operation of molecular rotary motors in gels. The fourth and final section will shed light on recent approaches towards autonomous oscillating systems, *i.e.* systems that integrate a regulatory feedback loop in their design.

Scheme 1. Transduction of molecular motion across increasing length scales to macroscopic movement.

2.2 Macroscopic shape changes from molecular operations

Early attempts to set photo-chemistry into motion have focused on amplifying molecular operations into the macroscopic shape transformation of materials. Depending on the range of target macroscopic motion, whether a simple bending or a complex helical motion, molecular systems can be engineered so that molecular forces are transferred to larger molecular structures efficiently. Such an amplification is enhanced in higher order materials e.g. crystals, liquid crystals, and gels (Box1).

Box 1. Effect of order changes on the macroscopic deformation of soft materials.

2.2.1 Molecular crystals

Single crystals arguably constitute a straightforward system to program the amplification of movement.^{20,21,22,23} The precise organization of the molecules

in the crystal lattice is determined by molecular symmetry and therefore they obey known mathematical rules. Moreover, crystal structures can be characterized at the molecular and supramolecular level by X-ray analysis, allowing for a clear structural picture of the responsive materials.

Single crystals of diarylethenes are light-responsive (Figure 1a) and, typically, they have been long known to bend away from a light source (Figure 1b)^{24,25}. Being the closed form of the molecular switch thermally stable, it is possible to lock the UV generated shape. When exposed to visible light, the diarylethene switch opens back and the initial shape can be recovered (Figure 1c). To rationalize the bending motion of diarylethene single crystals it is useful to approximate the molecular shape of diarylethene to a triangular object (this simplification is just to describe a general phenomenon and by no means can explain many of the photoresponsive single crystals systems). By considering the top part of the fluorinated cyclopentene ring as one of vertex of the triangle, and the two extremes of the phenyl rings as the other two, we can argue that the open form is represented by a triangle with wider base and shorter height than the closed form. By increasing the amount of closed diarylethene switch upon UV irradiation through the thickness of the crystal, we have a formation of gradient of closed form (due to a filter effect). The difference in geometrical shape of the molecules and their gradient distribution through the thickness create strain which is accommodated by a curvature of the crystal, resulting in the characteristic bending away from the light source (Figure 1b). It is

worth noting that this system makes use of the general principle of anisotropic molecular shape change to drive motion (see Box 1). This is of general relevance and can be found in many natural and artificial responsive materials.

The speed of shape change of the diarylethene single crystals is comparable to that of piezoelectric crystals (about 25 μ s), and the photocyclization of diarylethenes in the crystal state can happen as fast as 10 ps. While the timescale of the actuation is unmatched by light responsive polymer systems, single crystals have two major drawbacks: first of all, from purely a mechanical standpoint they are extremely brittle, and second their dimensions are limited to hundreds of microns in length. However, in spite of these challenges, the brittleness of single crystals has been harnessed to engineer photosalient single crystals that can convert light energy into mechanical strain which is accumulated over time and then released in a short kinetic energy burst.

Plants can accumulate energy and release it abruptly to support a mechanically relevant function, such as seed dispersal.^{26,27} Uchida et al. have reproduced such a strategy to engineer photosalient responses in hollow crystals (Figure 1d).²⁸ These hollow crystals contain a mixture of two conformers of the molecule (Figure 1e), which can both undergo photocyclization upon UV irradiation, causing the a- and b- axes to expand about 0.40 % and the c-axis to contract about 0.48% (Figure 1f). This shape change of the crystal faces leads the hollow crystals to bend convexly during

irradiation, and, being the four corners of the hollow structure fixed, the strain accumulated is released by an abrupt burst of the crystals (Figure 1f).

The photosalient effect works only for hollow crystals in the case of diarylethene, because of the relatively small anisotropic deformation of the molecule and consequent modest translation to the all crystal. However explosive release of energy has been observed also in bulk crystals when more dramatic molecular shape change occurs. Naumov et al.²⁹ reported on bursting crystals driven by a [2+2] photocycloaddition that leads to the formation of dimers in the crystal state (Figure 1g). Upon the fast dimerization of the organometallic complex the crystal becomes highly heterogenic. The heterogeneity of the crystal is followed by and expansion of the unit cell volume, however the time available to release stress accumulated in the crystal is too short, making the bursting of the crystal the only possible pathway to release energy (Figure 1h).

Despite displaying fast actuations and offering the possibility to harvest energy, highly ordered systems such as molecular crystals have their limitations. The difficulty in pre-programming the macroscopic properties (i.e. elastic modulus, shapes, flexibility) of single crystals makes the development of adaptive motile materials based on them a challenge. Reducing the degree of order of the material by combining the orientational order of liquid crystals (see Box1) with the mechanics of polymers, allows overcoming these limitations partially.

Figure 1. Light-induced strain in single crystal systems. **a**, Reversible photochemical cyclization of a diarylethene. **b**, Schematic representation of the strain induced in the single crystal due to the molecular shape change from the open form (blue) to the closed form (red). The formation of a gradient of closed form makes the crystal bend away from the light source. **c**, Image of photo-induced bending and unbending of a diarylethene crystal (Adapted with permission from Ref. 25, Nature). **d**, Reversible photochemical cyclization of a diarylethene. **e**, Packing of the two open diarylethene conformers in a unit cell. **f**, Schematic representation of the hollow crystals formed by the compound in **d** and its shape change induced by irradiation at 313 nm. The insets show the irreversible photo-salient effect of the hollow crystal imaged by SEM. **g**, Photochemical [2+2] cycloaddition of two complexes of $[\text{Zn}_2(\text{benzoate})_4(2'\text{-fluoro-4-styrylpyridine})_2]$. **h**, Popping of a $[\text{Zn}_2(\text{benzoate})_4(2'\text{-fluoro-4-styrylpyridine})_2]$ single crystal under UV exposure. Part **e** and **f** are adapted with permission from Ref. 28, Wiley. Part **h** is adapted with permission from Ref. 29, Wiley).

2.2.2 Liquid crystal networks

Arguably, approaches based on liquid crystals have shown to be particularly promising in bringing the motion of molecular machines to the macroscale. Mesoscopic materials are indeed sufficiently organized to amplify the nanoscale operation of artificial molecular machines with a sense of directionality and along multiple length scales, yet they retain a fluid character that accounts for their high responsiveness to small changes in molecular structure or composition, or in the environment.^{30,31} The long-range orientational order of liquid crystals also enhances the efficiency of chiral transmission from the molecular scale upwards.^{32,33} However, their fluid character remains a limitation in the production of macroscopic work. It is possible to retain the liquid crystal order by using cross-polymerization, to form liquid crystal polymer networks^{34,35}. Molecular machines can organize similarly to the liquid crystal molecules and their shape changes can induce anisotropic shape transformation either by inducing disorder or by applying molecular forces to the polymer network (Scheme 1, Box 1).

The dynamic molecules used to activate liquid crystal networks are typically azobenzenes, although other switches, such as hydrazones, have been used recently³⁶. Azobenzenes are a class of photoswitches that reversibly undergo tremendous light-induced shape change, from rod-like (*trans* form) to bent-shaped state (*cis* form) and vice-versa (Figure 2a).^{37,38} By populating the *cis*-state the internal order of the LC is reduced, contrary to the rod-like *trans* state that is compatible with the liquid crystal order.^{39,40} For the purpose of

this review we are going to discuss only liquid crystals formed by rod-like molecules. The organization of these nematic liquid crystals is characterized by the average orientation of the long molecular axes, referred as the director $\mathbf{n}$ (Box 1).

Azobenzene can be incorporated in three ways in a liquid crystal network: doping, attachment to the polymer network as a side chain, and covalent embedment into the network, as a cross-linker.^{41,42,43,44} The latter approach allows for more efficient propagation and amplification of molecular shape changes to the network.⁴⁵ Together with the reduction of order upon photoisomerization, when incorporated as cross-linker unit in a liquid crystal network, azobenzene exercises pulling forces on the polymer chains resulting in the anisotropic shrinkage of the network. In some cases, azobenzene is not used as a photomechanical modulator but rather as a dye, which after absorbing UV light, dissipates energy increasing the temperature, reducing the liquid crystal order.^{46,47} We refer to this phenomenon as photothermal effect.

Figure 2a schematically shows how the shape change of azobenzene cross-linker in LC network with unidirectional molecular alignment contracts the material along the director and expands it in the orthogonal direction (Box1). This phenomenon underlies the general operation of shape-shifting LC materials capable to convert light energy in useful mechanical work.

This anisotropic deformation driven by molecular switches can be further amplified to the macroscopic scale in a variety of complex deformation

modes such as: bending, curling, twisting, winding, etc. which can be pre-programmed at the molecular level either by choosing the spatial organization of the director, or by engineering illumination conditions. Usually this strategy is implemented by freezing the LC configuration during polymerization^{35,43}, however there are examples of reconfigurable LC networks, for which the configuration can be modified after polymerization⁴⁸. We will consider how different orientations of the liquid crystals pre-define the macroscopic shape change upon light irradiation.

The simplest organizations for the liquid crystal monomers are the planar and homeotropic/vertical alignments, where the molecules are all aligned in the same direction (see Figure 2b). Side irradiation of the film with planar alignment leads to bending of the film towards the light source, accompanied by positive photogenerated stress (pulling forces, Figure 2b, left). Bending occurs due to the high optical density of the film in the spectral range of the light source used (filter effect). Most of the light is absorbed at the exposed side, which generates a gradient of the strain, reflecting the population of *cis* form of azobenzene, across the thickness causing out-of-plane deformation. On the contrary, due to expansion in orthogonal direction to molecular orientation generating pushing forces (negative photogenerated stress), film with homeotropic LC alignment bends away from the light source (Figure 2b, right). These deformation modes have been used in many responsive liquid crystal networks.⁴³

Complex helical geometries in plants result from the interplay of two orthogonally oriented anisotropic layers of cellulose and their mechanical movement has been studied for hundreds of years.⁴⁹ Botanical strategies for achieving motion often rely on generating helical systems such as in coiling plant tendrils,⁵⁰ spasmoneme springs⁵¹ and the opening of seedpods.^{52,53} While a number of artificial systems can mimic these biological devices, molecular based systems capable of driving such deformations are in their infancy. Katsonis and coworkers have pioneered the design of active helical geometries by using the twist configuration to mimic the orthogonal deformation modes of cellulose fibers in plants.⁵⁴ Additionally, they have originally demonstrated that mimicking the coiling strategy of plant tendrils allows smart materials to perform useful work. In the twist configuration, liquid crystals are oriented perpendicularly at the top, and at the bottom of the thin film, so that they twist across the thickness of the film (Figure 2c). The helical shape is determined by the combination of gradient in cross-linking density and orientation of the liquid crystal director in the mid-plane. The mid-plane orientation changes depending on the angle at which the ribbon forming helix is cut out of the film. This method offers the possibility to encode a variety of shapes in one single polymer film (Figure 2d). Upon illumination, the biomimetic springs wind or unwind depending on their handedness. These artificial tendrils can perform mechanical work and have been used to lift objects dozens of times their weight (Figure 2e). Moreover, they show non-linear mechanical properties under tension.⁵⁵

Many plants rely on high power burst of kinetic energy to propagate seeds. We mimicked the mechanism by which the seedpods of an orchid tree produce work to propagate seeds.⁵⁶ Each artificial valve is composed of a thin film of liquid crystal elastomer. Micro patterning within these films allows fabrication of two series of 'bars', one of which is shape-persistent to function like cellulose fibers in plant tissues, while the other bars are photo-active and change shape under stimulus, like the functional organic matrix of plants that swells to induce motion. We note that in order to design the desired function here, we needed to establish methods to photo-generate chiral shapes, using only non-chiral molecules. Molecular action amplified efficiently by the accumulation of small strain and its brutal release.

In the splay alignment, the liquid crystal molecules on one side are oriented planarly and homeotropically on the opposite side (Figure 2f). After cross-polymerization, this order is encoded in the polymer network. The amplitude of bending is greatly enhanced, in these materials, because there is a synergy between the pushing forces and the pulling forces generated at opposite sides of the film. Notably the bending occurs so that the side with homeotropic orientation always remains outside the curvature.

Materials with splay molecular configuration are used in many devices with bio-inspired design and functionality. In bacteria and other microorganisms, cilia are essential to locomotion², and their operation is characterized by an asymmetric shape transformation. Such an asymmetric change in the body shape is a key requirement for microorganisms to swim

in water, a physical rule that was coined as the scallop theorem by Edward Purcell in his talk on life at low Reynolds number.⁵⁷ The asymmetric flapping of cilia could be re-engineered artificially (Figure 2f).⁵⁸ Using two different azobenzenes, it became possible to selectively address the bending of each part of the sample, allowing for control of four different shape states by irradiation with spectral composition of the light. By inject printing technique, the authors created artificial cilia that by design of illumination conditions can move in a non-reciprocal way, mimicking their natural counterpart.

Other plant-inspired functional systems were later developed by the group by Priimagi and co-workers, including a night flower⁵⁹, and an artificial flytrap⁶⁰. When an object enters the optical field (488 nm laser light coming from integrated optical fiber), it produces an optical feedback by scattering light to the surface of the film which bends quickly capturing the object (Figure 2g). This design allowed for the system to receive signal from the environment and respond autonomously to perform a functional operation, such as grasping. Responding to environmental changes autonomously is a key characteristic of living systems, where the response is coupled to function aimed at preservation.

The planar, splay and twist liquid crystal organizations are most commonly used to amplify molecular shape transformation into macroscopic motion. They can also be combined in a single material, by using micropatterning strategies, e.g. photoalignment techniques⁶¹. Other strategies to encode

complex deformation include patterning of liquid crystalline order^{62,34} and cross-linking density of polymer network. We envision future developments by combining these with 3D printing, or with microfluidic strategies that will lead to shape-shifting unit cells.

Figure 2. Versatility of shapes and actuation modes from liquid crystal networks with integrated molecular photo-switches. **a**, A unidirectionally aligned liquid crystal network undergoes anisotropic shape transformation as a result of the isomerization of the azobenzene being incorporated in the network covalently. **b**, Influence of alignment on the deformation modes of liquid crystal networks. A film with planar alignment bends towards the source of light and thus generates mechanical stress, while homeotropic alignment facilitates onwads bending and thus the materials bends in the opposite way. **c**, Twist configuration of liquid crystal molecules. **d**, The springs display versatility in shapes and actuation modes, depending on their shape and chiral aspect. **e**, A soft spring lifts a weight upon irradiation with light, thus producing mechanical work. Figures **d**, **e** were adapted

with permission from Ref. 54, Nature. **f**, Splay alignment, when one of the substrates promotes planar alignment whether the opposite one forces the molecules to orient perpendicularly. Operation of an artificial cilium made of liquid crystal network with splay alignment. A cilium is composed from two parts loaded with two different azobenzene compounds absorbing in different spectral ranges. Four positions of cilium can be addressed selectively by color composition of light producing a motion that mimics that of cilia. Adapted with permission from Ref. 58, Nature. **g**, An artificial fly trap. An azobenzene-containing liquid crystal film in splay alignment is coupled to an optical fiber. The light coming from the fiber scatters from the trapping object initiating the bending of the ribbon, and thus closing of the fly trap. Adapted with permission from Ref. 60, Nature.

2.2.3 Molecular self-assemblies

Muscles are the quintessential paradigm of a hierarchical organisation capable of producing work, and they are formed by self-assembly. However, in fully artificial molecular systems, harnessing cooperative molecular motion remains often hampered by the instability of supramolecular structures during molecular shape transformation, commonly leading to disassembly⁶³, and the poor mechanical resistance of supramolecular assemblies. However, Feringa and co-workers have recently succeeded in encoding muscle-like behavior in anisotropic hydrogels⁶⁴ driven by molecular rotary motors^{65,66,67,68} displaying unidirectional rotation around C=C bond as depicted in Figure 3a (description of unidirectional rotation will be given in the third paragraph below). Organizing amphiphilic molecular motors over multiple length-scales into long supramolecular polymers allowed for the formation of nanofibers with 5-6 nm diameter. When the nanofibers containing solution was drawn into CaCl₂ aqueous solution cross-

linking between the carboxylates and calcium cations yielded a string of supramolecular hydrogel (95 wt.%) with unidirectional aligned bundles of fibers. X-ray measurements showed that light increases the diameter of the nanofiber (Figure 3b). The light-induced rotation of molecular motors in the fiber bundle initiates the contraction of the nanofibers along the long axis, while their diameter expands to preserve the total volume in an isovolumetric shape transformation. Collectively this action results in the bending of the string towards the light source (Figure 3c,d) similarly as described for LC networks with unidirectional planar alignment. Annealing of the string at 50°C promotes thermal helix inversion of the motors with relaxation to the initial state allowing the string to recover the native shape.

While providing inspiring proof for the potential of this research, the shape-changing materials reported so far and described in this section are mostly bending and inducing translation; essentially the transformations are one-dimensional. These materials are also characterised by a moderate versatility – one set of building blocks typically yields one functional system only, with a limited number of simple morphing modes. The state of the art is that each new functional shape change demands a new strategy to be developed from bottom-up, including the design of the dynamic molecular element, its coupling to the functional environment, aspects related to chirality etc.; to date, general design guidelines have not been established. The mechanized materials reported so far are still far from reaching the functional sophistication of nature's shape-shifting systems, both in the complexity of

the shape change as well as in the resulting movement and in the versatility of their operation modes.

Figure 3. Light-driven molecular motors setting self-assembled molecular materials in motion. **a**, Photoisomerization of an overcrowded alkene-based molecular motor. **b**, Upon photoisomerization of the motor, the self-assembled nano-fiber in which the motor is embedded, expands. **c**, The scheme of bending of bundle of aligned fibers upon illumination. The mechanism involves contraction of the fibers along their long axis. **d**, A supramolecular string of bundles in water actuates towards the light source (at the left). Unbending occurs when thermal helix inversion of the molecular motors occurs as a result of annealing. Figures **a-d** were adapted with permission from Ref. 64, Nature.

2.3 From molecular motion to motility

Motility, intended as the capability of a body to transduce energy into motion of its center of mass, is an essential characteristic of living organisms enabling to sustain functions such as feeding, multiplying and eventually evolving. Transduction of simple shape changes into motility is not trivial due to the need for asymmetry of interactions with the surroundings. This asymmetry can be achieved either by sophisticated autonomous motion which is non-invariant under time reversal⁵⁷ or by engineering symmetry breaking in the interactions between the body and the environment. In this section we will deal with the latter case, since the former is still in its infancy, and we will consider the following types of motility driven by molecular action: swimming, rolling, inching.

Palfy-Muhoray and co-workers reported on the light induced motility of a liquid crystal elastomer swimming in water.⁶⁹ The film with uniaxial planar orientation was doped with an azo-dye. When the film was placed on the water surface and the center was irradiated with a laser beam, the photothermal effect induced a rapid bending of the sample, yielding a saddle-like deformation. The momentum from the deformation is transferred to the fluid, pushing the elastomer away from the light. This general physical mechanism can be observed for many swimming creatures (e.g. flatfish).

A similar mechanism of swimming by creation of undulatory waves propagating along the body of micro-swimmer was developed by Fischer

and co-workers⁷⁰. The authors fabricated a cylindrical object from a liquid crystal elastomer incorporating an azobenzene, with the liquid crystal being aligned unidirectionally (Figure 4b). Spatio-temporally modulated light was used to achieve non-reciprocal deformation of a microrobot's body allowing directional locomotion (Figure 4b). By scanning the cylindrical body of the microrobot with light modulated in intensity, travelling waves of protrusions pushed the body in opposite direction at a speed of up to 2-3 $\mu\text{m/s}$.

Rolling based locomotion was achieved by designing ribbon of LC polymer network containing azobenzene cross-linker with twist alignment which formed helical shape upon illumination.⁷¹ Longer illumination times led to the rolling of the spring (Figure 4c). The mechanism of the movement was explained by momentum generated from unwinding of the spring upon inhomogeneous exposure (since the sample was irradiated from top) and friction with the surface. The momentum initiates the shift of the center mass of the spring in a rolling way accompanied by the change of irradiation profile which allows regenerating the forces and sustaining photomotility. Moreover, it was shown that the directionality of such movements is partially controlled by angle between the helix axis and the edges of the ribbon.

Inching is another widely used biological strategy for locomotion of invertebrate and it has inspired researchers in engineering caterpillar-like soft microrobots.⁷² By fabrication of alternately patterned uniaxial molecular

alignment in LC elastomer film doped with azo-dye, it was possible to mimic the rectilinear body deformation of caterpillars.⁷³ Upon stimuli induced reduction of order by either temperature or light, the film displayed a periodical undulation along the body. This deformation was induced by slowly scanning a laser beam along the sample, and resulted in the appearance of travelling deformation waves that shifted the body forward (Figure 4d, left). This approach allowed these artificial robots to execute various tasks like inching up a slope (Figure 4d, right), pushing objects, etc.

This inching motion was designed by introducing asymmetry in the initial state of the soft robot, simplifying the control over the external stimulus. As proved in azo-dye doped liquid crystal network,⁷⁴ with alternating splay alignment having an asymmetric accordion shape, performs inching motion upon temporally (non-spatial control) modulated laser light (Figure 4e). The directionality of the movement was enhanced by using surfaces with anisotropy in the friction coefficient.

A common strategy in these examples is to engineer non-reciprocal motion by spatio-temporal control of light irradiation. However, this control must be coupled with fast relaxation of the material on a time scale which so far has been proved possible only by using photothermal effect. Even though the role of the molecular switch is limited to the latter effect, this paragraph shows the importance of asymmetry in transducing motion across length-scales, also in inanimate matter.

Figure 4. Harnessing molecular motion for motility. **a**, A thin film of liquid crystal elastomer doped with azobenzene floats on a liquid. The film first deforms and then swims away from UV irradiation. The movement is caused by the transfer of energy. The black arrow shows the bending and dashed white curve displays the nematic director. (Adapted with permission from Ref. 69, Nature) **b**, Locomotion of a micro-robot based on travelling-wave deformation modes. The microscope objective projects the dynamic light pattern onto the soft robot, which deforms in a selective fashion. On the right, the deformation of cylindrical micro-robot upon exposure with modulated light pattern travelling from left to right, which results in translation in opposite direction. The body of the robot is ~ 1 mm long. (Adapted with permission from Ref. 70, Nature) **c**, Locomotion of a liquid crystal polymer spring incorporating an azobenzene in its design. Illumination increases the twist in the spiral which is hindered against the slip by friction with the surface of the paper on which it moves. Friction acts tangentially to the sample creating unbalanced forces pushing the center of mass. As the spiral rolls, the irradiation profile shifts accordingly, regenerating the forces and sustaining locomotion for as long as the system received light. (Adapted with permission from Ref. 71, Nature) **d**, The soft robot made of liquid crystal elastomer film with patterned molecular orientation is able to climb a

slope. The laser beam is scanned along the robot body and induces temperature increase. Exceeding the phase transition temperature (T_{pt}) the film contracts along the nematic director causing the shape change. The scanning laser beam creates the wave of shape shifting which results in caterpillar-like motion in scanning direction. (Adapted with permission from Ref. 73, Wiley) **e**, Liquid crystal azobenzene-doped elastomer film with alternated splay alignment pattern demonstrate light-fueled inching locomotion. Alternated light actuation leads to the extension and bending of the sample laying on the surface with blazed grating profile (see inset) which causes directional caterpillar-like movement. (Adapted with permission from Ref. 74, Wiley).

2.4 Harnessing the continuous operation of molecular machines in soft adaptive materials

Switchable materials are typically powered by molecular switches (diarylethenes, azobenzenes, hydrazones, etc...), and the materials usually reflect the bistability of these switches (see Box 2). Early attempts to reach beyond switchable materials, towards truly mechanized matter that performs work out of equilibrium, have thus made use of molecular machines that move continuously, in order to convert light into continuous motion.^{75,76,77} These examples have evidenced that amplifying the continuous operation of molecular machines is far from being trivial, because photochemical processes irretrievably leads to steady states (photostationary states) which are stable under continuous illumination. Arguably, the systems that have revealed the most perspective so far have involved overcrowded alkene-based molecular motors.^{78,79}

Light-driven overcrowded alkene-based molecular motors feature a rotor and a stator (Figure 5a), with a central double bond around which light-

induced isomerization occurs (Figure 5a). Contrary to the *trans*-to-*cis* isomerization of stilbenes or azobenzenes, that are characterized by stochastic changes in the molecular shape, overcrowded-alkene based motors display a unidirectional rotation which is caused by the asymmetry of the free energy landscape due to intramolecular steric hindrance. Complete cycle of molecular rotation includes a thermal helix inversion step allowing to lock different rotational states. The unidirectionality of molecular rotation together with the possibility to tune the thermal helix inversion process by chemical design or molecular environment provides a versatile tool for development novel functionalities and operational mechanisms.

Topology plays a very important role in harnessing the rotary motion of molecular motors across increasing length scales. Giuseppone et al. recently reported on the macroscopic contraction of an organogel formed by swelling polymer containing molecular motors.⁷⁸ A polymer network of molecular motors was formed by using a click-reaction to couple the rotor to the stator (Figure 5b). Swelling the polymer network in an organic solvent resulted in a soft material in which motor rotation resulted in an entanglement and collapse of the diluted polymer network (Figure 5c). The organogel shrunk isotropically in response to illumination, and eventually down to 20% of its initial volume (Figure 5c). The overall process took about 2 hours, until eventually the material underwent abrupt failure by oxidation of the axle of the molecular motor (Figure 5a). The original volume of the material could

not be recovered because the polymer network is mechanically locked by the unidirectional rotation. The material needs a pathway to release the strain accumulated by the entanglement of the polymer network, in order to feature reversibility in its expansion. The reversibility of the macroscopic contraction of the gel was demonstrated by incorporating diarylethene into the network as a modulator unit (Figure 5e, f).⁷⁹ The mechanism goes as follows: with UV light, the molecular switch converts into its closed form, allowing the molecular motor to wind and entangle the polymer network until there is not enough free volume for further shrinkage. In contrast, when exposed to visible light, the diarylethene molecules open and the thiophene ring is “free” to rotate around the single bond connecting it to the perfluorinated cyclopentene (Figure 5e, right), and release the stress accumulated. The contraction upon UV exposure occurs in 140 minutes, while when irradiated with visible light the gel regains its original volume in 300 minutes (Figure 5g), i.e. the response remains very slow.

In contrast to the hierarchically structured materials described above, these molecular motors-based gels represent a class of isotropic materials, which without inherent anisotropy (gradients, patterning, etc...) perform shape deformation (see Box 1). These systems exemplify the role of topology in the design of active machine-driven polymers that operate out-of-equilibrium.

Figure 5. Harnessing out equilibrium rotary motion of molecular motors for energy storing organogels. **a**, Rotary cycle for a overcrowded alkene-based light-driven molecular motor. The rotor part is represented in blue while the stator part is in red. Illumination results in the isomerization of the central double bond to form a conformation that is less stable energetically, and thus relaxes thermally by

undergoing a helix inversion. **b**, Structure of a molecular motor that can be incorporated in an organogel-forming polymer network, through four connection points. **c**, Time evolution of the relative volume of the organogel upon exposure with UV light. **d**, Images of the organogel at different illumination times. The organogel shrinks considerably before breaking up. **e**, Motor and modulator forming the polymer network swollen to an organogel. The modulator allows strain to be released, because free rotation is allowed around the single bond, in the open form of the switch. **f**, Schematic representation of a polymer network formed by the motor and modulator unit. **g**, Relative volume dependence on the irradiation times. Panels **b**, **c**, and **d** are adapted with permission from Ref. 78, Nature. Panels **e**, **f**, and **g** are adapted with permission from Ref. 79, Nature).

2.5 From switchable matter to oscillating systems and materials

Molecular materials capable of continuous and processive operation upon constant energy input would bridge the gap between active materials, inherently limited by the stability of the molecular switches used in the system, and living matter, that can produce work continuously thanks to regulatory mechanisms. Introducing feedback loops as regulatory system^{80,81,82} for active materials will be central to the sophisticated behavior of artificial systems (see Box 2). While engineering of oscillating chemical reactions has been a challenge undertaken in the last two decades, these periodic changes in concentration have been rarely coupled with mechanically relevant actions^{83,84,85,86} and are based purely on regulatory feedback loops acting through variation of concentration of active species.

Box 2. From switchable materials to life-like processive systems.

In contrast to chemical feedback loops regulating many biological processes, Broer and co-workers engineered a continuous propagating wave-like motion in light-responsive liquid crystal polymer networks, by using a “physical” negative feedback loop in azobenzene-containing liquid crystal networks in a splay configuration (Figure 6a).⁸⁷ When the film is constrained at both ends and the high intense (510 mW cm^{-2}) illumination occurs at an angle, waves start propagating continuously. These waves can propagate in both directions depending on the orientation of the liquid crystal with respect to the light source (Figure 6b). This oscillating behavior resulted from a combination of deformation caused by photothermal effect and the self-shadowing⁸⁸, which sustains a negative feedback loop. The mechanical wave-like deformation has been also successfully theoretically modelled correlating the strain developed and released with the liquid crystal order during the oscillations (Figure 6c).

This achiral responsive material relies on stimulus which can break the symmetry of the system enabling continuous out-of-equilibrium operation. In the liquid crystal polymer network above, the symmetry breaking comes from the specific illumination conditions. However, symmetry breaking elements can be introduced in the system by means of molecular chirality, which allows for spontaneous symmetry breaking upon continuous energy input. Light-driven rotary motors possess remarkable molecular helicity, which can be transduced across length-scales in a liquid crystal.^{89,90} This functionality coupled with their light-fueled molecular shape-changes make them ideal candidates for the design of out of equilibrium molecular systems. Recently, two elegant systems were reported, in which the molecular motors work in synergy with liquid crystals to induce macroscopic motion in either non-continuous or continuous fashion (Box 2).

A pioneering system features a thin film of helix-based liquid crystal, prepared by doping a nematic liquid crystal with molecular motors.^{91,92,93} The helical shape of the motors is transmitted to the liquid crystals by chiral elastic forces, and thus the molecules organize into a helix. Upon illumination, the molecular motors convert into an isomer with opposite molecular helicity. This inversion is successfully transmitted to the liquid crystal and as a result, the screw sense of the cholesteric helix also inverts with light.^{89,94} Cholesteric film under 'hybrid' confinement conditions have one side in planar alignment, and the other side in perpendicular alignment, typically at the interface with air. These films are well-known to display a

fingerprint texture at the interface with air (the pattern in Figure 6d is a typical example of fingerprint texture). Upon photo-inversion of the screw sense, the fingerprint pattern starts rotating clockwise, passing through a state where the helices unwind, and the fingerprints disappear. Eventually the helices rewind with opposite handedness, still rotating clockwise, until the photo-stationary state is eventually reached. When a micrometer size glass rod is placed on top of the fingerprint, the object also rotates (Figure 6d).⁹¹ The possibility to transduce the operation of nanometer sized molecular machines to a microscopic object is a remarkable achievement, albeit occurring transiently only, while the system converts from one stable state to another (Box 2).

We recently have overcome this limitation in the continuous rotation of a light-driven vortex.⁹⁵ For this system to work, it is essential that the illumination occurs locally (here with a laser), because it forces any twisted structures that are formed to remain spatially confined. In this illuminated area, structural transitions are engineered at the molecular level by using a co-doping strategy, where a passive chiral dopant and active molecular motors are combined to induce a pitch that is typically larger than the thickness of the cell (Figure 6e). In this geometrical confinement, the twist is suppressed, and this situation where the chirality of the system cannot be expressed and is referred as a 'frustrated' state. When the sample is locally illuminated with a laser, the molecular motors start winding the cholesteric helix reducing its pitch until the latter is short enough to cause a localized

transition from a frustrated achiral state to a chiral state with a supramolecular knot, having a diameter that is about twice the waist of the laser beam (Figure 6f). When this knot is illuminated with double laser power, a knot-to-vortex transition takes place, and the newly formed vortex starts rotating immediately. The chirality of the vortex is encoded by chirality of the knot: in other words, a left-handed knot always evolves into a left-handed vortex (Figure 6g). The direction of vortex rotation is predetermined by the handedness of the defect formed, *i.e.* left-handed vortices rotate clockwise (Figure 6h), while right handed vortices rotate counterclockwise. The rotation persists under continuous illumination, with no sign of fatigue for dozens of illumination hours.

The mechanism elucidates the sustained vortex rotation as a feedback loop between the differential diffusion of activated and non-activated forms of the chiral motors and their twisting action: as the axial symmetry of the system is broken upon formation of the vortex, because of the presence of a kink, the diffusion of the active motor becomes angle dependent and consequently the strength of the twisting operated by the motors becomes angle dependent as well. A new chiral landscape arises where: i) the tightness of the helix determines the differential angular diffusion of the motors, and, ii) in return, the motors twist the vortex more efficiently in this new spatial distribution, and iii) the twist rearrangement of the rotating vortex will influence the differential angular diffusion of the motors, and so on. This continuous reciprocal effect between the motor and its chiral

environment constitutes a photo mechanochemical feedback loop that sustains a continuous rotation, for as long as the system is illuminated.

Figure 6. Light fueled processive systems in anisotropic molecular materials. **a**, Azobenzene molecules used in liquid crystal polymer networks. **b**, Wave-like deformation of a liquid crystal elastomer with splay configuration, that is clamped at both ends. The red arrows show the propagating direction of the wave. The top scheme represents the irradiation set-up, while the bottom scheme is for the case of irradiation of the side with homeotropic anchoring conditions. **c**, Simulation and movie of a wave-like deformation due to self-shadowing in a liquid crystal elastomer (scale bar 5 mm). **d**, Molecular motor rotating a microrod laying on the surface of a

cholesteric liquid crystal film. The rotation of texture from the initial state to the photostationary state drives the rotation of the microscopic object on the surface. The cholesteric helix is right-handed initially, and undergoes helix inversion to reach left handedness at photo-stationary state (scale bar 50 μm). **e**, Co-dopant strategy for fine pitch tuning and light-driven inversion of chiral nematic handedness. Molecular motors are used as photo-active dopant, (R = phenyl or methyl). A bridged binol derivative is used as a co-dopant that is not light-responsive. Photo-isomerization ($\mathbf{m} \rightarrow \mathbf{m}^*$) and thermal relaxation ($\mathbf{m}^* \rightarrow \mathbf{m}$) are associated with helix inversion. **f**, Motor-doped liquid crystal film in a sandwich-type glass cell promoting perpendicular orientation of the liquid crystal. Local illumination with a Gaussian laser beam ($\lambda = 375 \text{ nm}$) triggers winding of the cholesteric helix. **g**, Knot formed by winding of the cholesteric helix at laser power of $\sim 1.25P$ min (left) and vortex formed upon symmetry breaking due to increase in irradiation power ($\sim 2P$ min, right). **h**, A left-handed vortex rotates clockwise for as long as irradiation lasts (scale bar 20 μm). **b** and **c** are reproduced with permission from Ref 87, Nature. **d** is adapted from Ref. 91 and 93 (Nature and American chemical society respectively). **e**, **f**, and **g** are adapted with permission from Ref. 95, Nature.

2.6 Conclusions and outlook

Analysis of the recent literature allows drawing the outlines of a general roadmap towards the design of systems and materials with life-like adaptive functions (Scheme 1). The design starts with the choice of mechanically relevant operation, that acts as transducer of light energy. Molecular machines that can be used as transducers are molecular rotary motors, hydrazones, diarelethenes, azobenzene, etc. These molecular machines feature molecular shape changes upon light illumination that can be harnessed if these active molecules are incorporated in high order molecular materials such as crystals, liquid crystals, molecular self-assemblies and polymer networks. The higher-level order allows converting the effects of

molecular shape changes into disorganization. In ordered matter, this disorder can be introduced by heating, or in a more controlled manner, by the emergence of new isomeric structures which disrupt the organization of its surrounding, resulting in anisotropic shape changes. In addition, molecular shape changes apply forces on polymer networks and this tension gives rise to stress. Overall, both disorder and mechanical forces contribute to building up mechanical strain, which is responsible for motion at larger length scales. Typically, this motion allows transitioning between two macroscopic states. These switchable materials, while paving the way towards a new materials era, do not yet adapt to their environment and typically do not display autonomous continuous operation. Considering materials under a new paradigm where the material is both the machine performing the task rhythmically and the physical object on which the task is performed, we can take lessons from living matter in order to imagine the next generation of functional molecular materials that will be driven by molecular machines and controlled by regulatory feedback loops. We anticipate that sustaining continuous oscillatory behavior in artificial systems will require fine kinetic tuning of the interplay between positive and negative feedback loops (Scheme 1).

We thus envision that in order to reach beyond current levels of functionality, adaptive materials will need to incorporate mechano-chemical feedback loops in their design. As the rules of molecular motion and its transduction across increasing length-scales gradually come to light, the

effect of external mechanical forces on the operation of molecular machines and chemical reactions has still to be fully explored and reveal all its opportunities^{96,97,98,99}. Harnessing these two phenomena synergistically will reveal a scenario where the forces generated by molecular machines modify the materials and, reciprocally, the material regulates the operation of the active molecular components. Future systems and materials controlled by such regulatory mechano-chemical feedback loops will support their autonomous continuous operation away from equilibrium.

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Chapter 3

Muscle-like mechanical adaptability in light-responsive polymers

The cooperative operation of artificial molecular motors and switches has recently been amplified by polymer-based strategies that have led to versatile motion at the macroscale. As shape-shifting polymers incorporating molecular switches have become ever more sophisticated in their morphing capabilities, a major remaining challenge is to encode muscle-like mechanical adaptability during their operation and to explore its molecular origin. Here, we describe light-responsive soft actuators in which molecular switches encode changeable stiffness by controlling interfacial tension, in a phase heterogeneous design that combines free liquid crystal molecules with a liquid crystal polymer network. Significantly, in the presence of a large fraction of free molecules, light triggers an unprecedented and reversible photo-stiffening, analogous to the mechanical behaviour of myosin-powered muscle fibers. Our bio-inspired polymer nanocomposites shape-shift and adjust their stiffness to the task they have to perform, also while they move, which might result in the creation of compliant robots that can move, shape-shift and modulate stiffness.

3.1 Introduction

Recent developments in synthetic organic chemistry have supported the design and synthesis of molecular motors^{1,2}, rotors^{3,4}, switches^{5,6,7} and other dynamic molecules that display mechanically relevant motion in response to external stimuli^{8,9,10,11}, with unprecedented sophistication. Strategies to make these molecules work together, in space and time, have allowed amplifying their nanoscale operation modes into purposeful shape transformation at the macroscopic level.^{12,13,14,15,16,17,18} Notably, approaches involving liquid crystals have turned out to be particularly successful, because the long-range order of these systems couples to molecular motion efficiently. Examples range from chiral liquid crystals^{19,20} to liquid crystal elastomers²¹ and other liquid crystal networks.²² Inspired by the mechanics without muscles of biological systems,²³ liquid crystal networks incorporating light-responsive molecules have been developed to mimic cilia movement,²⁴ tendrils,^{12,25} opening of seedpods,²⁶ flytraps,^{27,28} and, continuous wave propagation.²⁹ These macroscopic shape transformations, as complex as they are, have so far not been combined with controllable changes in mechanical properties,³⁰ and this lack of mechanical adaptability limits the interactions that active elements of soft robotics would establish with unpredictable environments. In most cases, the photo-activation of the molecular switches irremediably leads to a softening of the polymer by ways of fluidization^{31,32}. In contrast, biological tissues are archetypes of mechanically adaptive materials that can modulate their stiffness whilst they perform work. In muscles, strain stiffening typically occurs during mechanical loading. The operation of

myosin motor proteins is amplified over increasing length scales to yield active generation of work by the muscle fibers, and these muscle fibers display non-linear strain-stiffening, which provides mechanical protection against rupture.³³ Recent work has endeavored to program stiffness control in man-made artificial materials,^{34,35,36,37}. These mechanically responsive systems are, however, inherently passive and thus not capable of combining shape-shifting properties with an active modulation of stiffness.

Here, we show wholly artificial shape-shifting materials that also display complex mechanical adaptability, including light-controlled softening and stiffening as well as non-linear responses to stress. Our strategy involves incorporating phase heterogeneity in liquid crystal polymer networks, by adding a free liquid crystal that does not take part in the network. When large fractions of free liquid crystal are embedded in the network, the photo-induced modification on the polarity of the polymer network induces large changes in the miscibility between the phase segregated free liquid crystal on one hand, and the polymer network on the other hand. The interfacial tension between these two phases is thus strongly enhanced, which eventually mediates an unprecedented photo-stiffening behaviour. These mechanically adaptive materials can efficiently extract the work produced by molecular switches by generating stresses in the MPa order. Moreover, in combination with helix-based shape geometries, they display stimuli-responsive enhancement of their nonlinear response to stress, which is a salient characteristic of myosin-activated muscle fibers.

3.2 Results and discussion

3.2.1 Bio-inspired design of mechanical adaptability

The design of our system combines long range order and nano-phase heterogeneity with the stimuli-responsive properties of azobenzenes. Azobenzenes are artificial molecular switches that convert from a rod-like *trans*-form to a bent *cis*-form under irradiation with UV light, and this transformation is reversible once the light is turned off. Such an azobenzene switch was covalently incorporated in a liquid crystal polymer network as a crosslinker, to ensure that the light-induced shape transformation of the switch can induce large changes in the polarity and in the order of the polymer network. The liquid crystal polymer network was combined with a nematic liquid crystal that is based on cyanobiphenyls, and is known to be partially miscible with the liquid crystal polymer network (composition shown in Supplementary Figure 1).

The phase heterogeneous design draws inspiration from mutable collagenous tissues, that provide a supportive framework for animals such as sea cucumbers and starfish, by giving them the ability to alter the stiffness of their skin rapidly and reversibly. In these echinoderms, the interplay between collagen fibers and a viscoelastic matrix results in a non-linear mechanical response that is combined with stiffening under mechanical stress.^{38,39} Bio-inspired approaches combining soft polymers with rigid cellulose nanofibers has yielded wholly artificial chemo-responsive materials that become soften upon exposure to water,⁴⁰ or stiffen upon

changes in temperature⁴¹. Here, we introduce phase heterogeneity by forming a photo-responsive liquid crystal network *in-situ*, within a free liquid crystal that does not take part into the network. Upon formation of the polymer network by cross-linking, phase separation spontaneously occurs between the polymer network and the free liquid crystal, and yields a large interfacial area. In the resulting nanocomposite material, both the polarity and the morphology of the polymer network are modified by photo-activation of the azobenzene (Figure 1), thereby diminishing the miscibility of the two components, and below we show that these photo-induced modifications mediated by the azobenzene have a major impact on the stiffness of the soft actuator.

Figure 1. Design and composition of the mechanically adaptive polymers. The zoom shows the molecular components of the network and the free fraction of liquid crystal which phase separate. The photo-induced isomerization of azobenzene increases the polarity of the fibers and thus affects the orientation (anchoring) of the free liquid crystal molecules, and their miscibility to the network.

The proportion of free liquid crystal was allowed to vary in the nanocomposite materials (for compositions see Supplementary Table 1). Their morphology was characterized by atomic force microscopy (AFM), and revealed a nanocomposite material (Figure 2). After washing the free liquid crystal away from the polymer network, the characteristic stretching band of the nitrile group around 2200 cm^{-1} disappeared from the infrared spectrum (Supplementary Figure 2), which confirmed the presence of phase heterogeneity in the original material. The morphology of the washed materials reveals the liquid crystal network, with distinct differences in pore sizes between the upper side and the bottom side of the material (Figure 2). This difference in morphology originates from the asymmetric irradiation

conditions, that create a gradient in cross-linking density through the thickness of the film. This strategy was previously exploited to generate gradient to control the shape-shifting of polymer springs.¹²

Figure 2. Morphological investigations. **a**, Atomic force microscopy image of the mechanically adaptive polymer composites. **b**, Image of the side exposed to light during photopolymerization, after the free liquid crystal is washed away with acetonitrile, the morphology of the polymer network is revealed. **c**, Opposite side of the film washed with acetonitrile. Scale bar is 1 μm . The AFM images are shown in phase mode.

3.2.2 Light-responsive modulation of stiffness

The stiffness was investigated in cut strips of thin films. When cut out parallel to the liquid crystal director these ribbons remained flat. The stiffness of the ribbons was measured by their continuous stretching either under ambient light that does not yield significant photo-isomerization of the azobenzene, or under irradiation with UV light. The relative extension of the composite, e.g. the strain, was recorded against the stress applied by the pulling forces during the elongation process (Figure 3a shows a schematic of the experimental setup), to yield a stress-strain curve (Figure 3). The slope of this curve equals the Young modulus, which is a measure of how much the

materials resist to deformation - with materials becoming softer when the Young modulus decreases, and stiffer when it increases. Importantly, the ribbons were always stretched along the liquid crystal director, thereby excluding any substantial contribution from soft elasticity.⁴² In *ex-situ* experiments, two different ribbons were used to estimate the stiffness; one in the absence of any irradiation, and another one was measured in the fully switched photostationary state (Figure 3b,d). *Ex-situ* measurements allow the modulus to be precisely determined, with no artefacts arising from pre-stretching. *In-situ* experiments were carried out with a ribbon that was extended, first under ambient light conditions, and further under constant UV illumination (Figure 3c,e). In the absence of irradiation, stress-strain curves show a linear response to pulling, indicating that the deformation is completely elastic and therefore reversible, *i.e.* no plastic deformation is observed until the ribbons break (Figure 3b,d black curves).

Upon irradiation with UV light, the polymer nanocomposite shows drastically different mechanical properties, depending on the proportion of free liquid crystal that is present in the polymer network. With a low proportion of free liquid crystal, photo-induced softening was consistently and exclusively observed for both *in-situ* and *ex-situ* irradiated samples (Figure 2b,c). Such a photo-induced softening is in line with earlier reports on the behaviour of liquid crystal and amorphous azobenzene-containing polymers, in the absence of any phase heterogeneity. Shimamura *et al.*³¹ have reported that irradiation of cross-linked azobenzene-containing liquid-

crystalline polymer films leads to a decrease of Young's modulus, and Kumar *et al.* have also documented the photo-fluidization of similar materials³². In these homogeneous materials, the rod-like shape of the *trans*-azobenzene is compatible with the liquid crystal order, whereas the crescent shaped *cis*-azobenzene that is formed by light disrupts the liquid crystal organization. *I.e.*, the *trans*-to-*cis* photo-isomerization of the switches disrupts the organization of the polymer, and the network undergoes light-induced softening by fluidization. By contrast, and rather counter-intuitively, we found that networks containing a high percentage of free liquid crystal stiffen upon irradiation with light, and both *ex-situ* and *in-situ* photo-tensile experiments evidence a large photo-induced increase in Young modulus (Figure 3d,e).

The proportion of the free fraction thus appears to be a critical factor in the mechanical photo-response of the nanocomposite polymer material. In the absence of light, the Young modulus decreases linearly when the proportion of free liquid crystal increases, from an average 220 MPa to 60 MPa (Figure 3f, where R is the molar ratio between the free liquid crystal and the azobenzene). Such a softening is not surprising, as small molecules are known to behave as plasticizers when incorporated into polymeric materials⁴³. Under UV illumination however, the decrease of the modulus with increasing proportion of free fraction becomes less pronounced, and therefore, at values beyond a given proportion of free fraction ($R \approx 3.9$, Figure 3f), the Young modulus of the irradiated materials overtakes that of the

materials in the ground state. This threshold composition corresponds to the transition between the photo-softening and the large and counter-intuitive photo-stiffening response that was not observed in liquid crystal polymer networks where photo-softening has been reported consistently^{31,32}.

In order to quantify the light-induced change of stiffness in the ribbons, the photo-induced variation of modulus $\Delta E \% = (E_{PSS} - E_0)/E_0 \times 100$ (where E_{PSS} is the Young modulus at the photostationary state and E_0 is the Young modulus in ambient conditions) was plotted as a function of the proportion of free liquid crystal in the material. Arbitrarily, we attribute negative values to photo-softening, and positive values to photo-stiffening. We observe that the variation in stiffness increases with the proportion of free liquid crystal in the nanocomposite and changes sign at $R \approx 3.9$ (Figure 3g). The nanocomposites exhibit considerable mechanical contrast under irradiation with light, with softening by up to 40 % or stiffening by up to 50 % of their initial modulus, depending on the proportion of free liquid crystal in the material.

We surmise that the unprecedented photo-stiffening that we observe for larger fractions of free liquid crystals, originates from a decrease in the solubility of the free liquid crystal within the polymer network. Such an enhanced incompatibility creates elasto-capillary forces that oppose the formation of interfacial area and thus tends to resist against deformation – which is observed as a stiffening. This mechanistic explanation is supported by recent work according to which microscopic liquid inclusions can

increase the stiffness of soft solids when interfacial tension opposes elastic deformation – provided that these inclusions are sufficiently small.⁴⁴ Here, we propose that light enhances the interfacial tension, because compatibility between the free liquid crystal and the liquid crystal polymer network is decreased, as the azobenzene-doped polymer fibers become more polar, and the *cis*-rich disordered polymer network becomes less compatible with the liquid crystalline organization of the free liquid crystal. Indeed, there is recent literature indicating that polymer networks can be miscible with nonreactive liquid crystals only up to a certain threshold, and that the miscibility decreases once the azobenzene switches to the *cis*-form.^{45,46} Ryabchun *et al.* have shown how a liquid crystal gel with a low polymer content expels a free liquid crystal doped with azobenzenes upon irradiation with light.⁴⁵ Broer *et al.* have reported on the light-driven expulsion of free liquid crystal molecules from azobenzene-activated gels of smectic liquid crystals that behave as photo-sponges. Significantly, for concentrations such as reported here, where the free liquid crystal fraction remains below 50% of the total weight, expulsion of the free fraction does not occur.⁴⁶ Essentially, the mechanical adaptability that we put forward thus originates in the operation of the molecular switches, provided that there is enough free liquid crystal to create a minimum of interface. The switch-driven stiffness change is fully reversible and the material returns to its original function either upon thermal relaxation or by back photo-isomerization under visible light irradiation.

Figure 3. Active stiffness control with light. **a**, Scheme of experimental set up for photo-tensile experiments. The polymer is stretched parallel to the molecular alignment (indicated with a black arrow). The sample can be activated by UV-light ($\lambda = 365\text{nm}$) to actively control the stiffness of the material. **b**, *Ex-situ* stress-strain curves for material with $R = 1$ in room-light condition (black squares) and under irradiation (blue circles). We define R as the molar ratio between the free liquid crystal and the azobenzene. **c**, *In-situ* stress-strain curve for material with $R = 1$. The film is initially strained in ambient condition (black squares) and then exposed to UV light (blue circles). **d**, *Ex-situ* stress-strain curves for material with $R = 4.7$ in room-light condition (black squares) and under irradiation (red circles). **e**, *In-situ* stress-strain curve for the same material. The polymer is first strained (black squares) and then exposed to UV light (red circles). **f**, Average Young modulus before (squares) and after irradiation (circles) irradiation for different values of R . **g**, Photo-induced variation of the Young modulus, for different proportions of free liquid crystal in the composite ($\Delta E = \frac{E_{PSS} - E_0}{E_0} \times 100$).

3.2.3 Mechanical forces from shape-changing polymer composites

It is well-known that liquid crystal polymer networks can change shape in response to light, provided that they incorporate light-responsive molecular switches covalently in their design^{12,25,26}. These shape-shifting properties are preserved in the nanocomposite materials, and thus also occur whilst the material adapts its stiffness. Upon irradiation with UV light on one side of the material, the photoisomerization of the azobenzene from the rod-like *trans*-state to the bent *cis*-state disrupts the liquid crystalline alignment sufficiently to deform the material (Supplementary Figure 3). These shape-shifting properties can be used to perform work¹². We have consequently investigated how much stress our materials can impart at varying intensities of irradiation. For this, a ribbon was clamped between a force sensor and a motorized stage, and pre-strained at +0.1% its original length, to prevent shape changes by illumination and thus prevent artefacts in the stress measurements. By forcing the strain to remain constant, *i.e.* by keeping the elongation of the material constant under illumination, the force sensor records the stress that is applied by the azobenzene-activated polymer composite. Two regimes are clearly distinguishable for the photo-generation of stress, depending on the intensity of light (Figure 4a). At low intensities (17 mW/cm²), the generation of stress follows the kinetics of the azobenzene, and eventually reaches a plateau corresponding to the photostationary state. In this regime, the stress is exclusively generated by the action of the molecular switch. Upon irradiation with higher intensities of light (above 38

mW/cm²) both the generation and the release of stress are instantaneous, which is typical for temperature-driven actuators that quickly dissipate heat after exposure. An infrared camera confirms that for high illumination powers, the samples can reach a maximum temperature of 85 °C (Figure 4b). All the experiment are reported for low UV light intensities (17 mW/cm²), unless otherwise stated. At such low intensities there is no detectable increase in temperature, which means that the photo-thermal effects are avoided and that the molecular switches are determining the dynamic stiffness.⁴⁷ Controlling the light intensity allows to tune the force generated by both photo-softening and photo-stiffening polymer composites (Figure 4c). Overall photo-stiffening composites generate larger stresses (in the MPa order) than their photosoftening counterparts with increasing differences for higher light intensities.

Figure 4. Possibility to generate stress under illumination. **a**, Stress generated by a photo-stiffening polymer ($R = 4.7$), for increasing intensities of light ($\lambda = 365$ nm): 1 (17 mW/cm²), 2 (38 mW/cm²), 3 (118 mW/cm²), 4 (307 mW/cm²), and 5 (381 mW/cm²). At low intensities, the response of the polymer is regulated by the molecular switch. At higher intensities (2-5), the temperature intervenes. **b**, Infrared images of a photo-stiffening polymer under different intensities of light irradiation. **c**, Photo-generated stress of photo-stiffening (red squares) and photo-softening (blue circles, $R = 1$) polymers as a function of light intensity.

3.2.4 Muscle-like mechanical response in springs of the polymer composites

Muscle-like tunable stiffness is a quintessential property for future soft actuators. At rest, passive muscle fibers elongate in a nonlinear fashion (Figure 5a). Such an adaptation of stiffness to stretching is an in-built mechanical property that protects the muscle fibers from breaking.³³ In active muscle fibers, the same nonlinear response to stretching is observed, but the steeper slope of the force curve highlights that this nonlinear behavior occurs within a range of larger stiffness, which is a prerequisite to muscle

performance. Such a complex behavior can be encoded in the materials that we put forward, to yield a unique system that does not only mimic the static mechanical responses of relevant bio-active materials but it is also active and mimics these processes while at work.

For this, we prepared helical springs out of the photo-stiffening polymers described above, by adding a small amount of chiral dopant and confining the material in a glass cell that promotes a twist across the thickness of the sample (Figure 5b). After polymerization, ribbons cut out from the film are helically shaped as a result of the chiral organization of the molecules combined with a gradient in cross-linking density.^{12,25} These springs respond to light with shape transformations such as winding or unwinding. They lift more than ten times their weight, thereby performing measurable work (Supplementary Figure 6). When stretched, the springs elongate, and their characteristic geometrical parameters such as diameter and pitch are modified (Figure 5c), and they display a nonlinear strain-stiffening response (Figure 5d), like all soft springs do⁴⁸. The elongation of the spring shows negligible hysteresis upon cycling due to the fact that most of the energy goes into geometrical changes and the pulling thus has little impact on the molecular structure of the polymer (Figure 5d). Only once the springs are unwound are the pulling forces applied to the covalent network itself. The transition from a non-stressed to a stretched network is marked by a dramatic decrease in the elongation of the polymer springs, and is responsible for their overall non-linear behavior.

Combining this nonlinear response, that is inherent to the geometrical design of the spring, with the active stiffness control provided by phase heterogeneity allows encoding muscle-like mechanical adaptation artificially (Figure 5e). When the spring is stretched in room-light condition, a typical strain-stiffening behavior is observed whereas under irradiation with light, the curve shifts significantly towards lower strains (Figure 5e, red). This shift originates in the photo-stiffening of the nanocomposite polymer and endows the spring with complex, biomimetic mechanical adaptation properties. For comparison, springs designed to undergo photo-softening were mechanically tested both when inactive and when activated by light. Once the springs are photo-activated, the force-displacement curve shifts towards higher strain (Supplementary Figure 7), opposite to what is observed with their photo-stiffening counterparts.

Figure 5. Muscle-like mechanical behavior from photo-responsive springs. **a**, Nonlinear extension of passive and active muscle fibers in response to a pulling force. Reproduced from R. Shadmehr & M. A. Arbib, *Biol. Cybern.* 66, 463-477 (1992). **b**, Twist configuration of liquid crystals in a glass cell. **c**, Response of a liquid crystal polymer spring to increasing pulling forces. **d**, Loading (increasing pulling force) and unloading (decreasing pulling force) of the springs, displaying a reversible non-linear strain-stiffening behavior, where hysteresis is negligible. **e**, Muscle-like behavior of ground state (black) and light-activated (red) polymer springs ($R = 4.7$), as shown from force-extension curves.

3.3 Conclusion

This mechanical adaptability studies add to the mechanical intelligence of soft actuating materials, by showing how the dynamic mechanical properties of shape-shifting polymers can be engineered by means of interfacial tension in a phase heterogeneous design. In particular, we show that either light-

induced softening, or an unprecedented light-induced stiffening of these elastomers can be programmed by rational design of their composition. In combination with the molecular action of the switches, the liquid crystalline character mediates shape-shifting, while the dynamic phase heterogeneity provides mechanical adaptability. These experiments constitute an original approach to translate the nanoscale action of molecular switches into both a macroscopic shape transformation, and into dynamic mechanical properties as mediated by their light-induced change in polarity. Based on this, we were able to create polymer springs that mimic the mechanical response of muscles by combining the nonlinear response to strain that is inherently encoded in the spring-like geometry, with the light-tunable stiffness of phase-heterogeneous liquid crystal polymer networks. Our results show that bio-inspired approaches to compliant soft materials offer the potential for creating a broad repertoire of dynamic materials that mimic the mutable mechanical properties of biological tissues, and support the development of artificial muscles and active compliant mechanisms for soft robotics.

3.4 Materials and methods

Materials. Monoacrylate 4-methoxybenzoic acid 4-(6-acyloyloxyhexyloxy)phenyl ester, 97% (Synthon Chemicals), monoacrylate 4[4(6-acyloxyhex-1-yl)oxyphenyl]carboxy-benzonitril, 97% (Synthon Chemicals), diacrylate 1,4-bis[4-(6-acyloyloxyhexyloxy)benzoyloxy], 97% (Synthon Chemicals), E7 liquid crystal (Merck Millipore), S-811 chiral dopant: S-

octan-2-yl 4-((4-(hexyloxy)benzoyl)oxy)benzoate (Merck Millipore), photo-initiator: phenylbis(2,4,6-trimethylbenzoyl)phosphine oxide, 97.0% (Irgacure 819; Sigma-Aldrich). Dichloromethane HPLC grade was purchased from Sigma-Aldrich and ethanol (99.9%) was purchased from VWR. Description for the synthesis of the azobenzene switch can be found in the supplementary data section.

Sample preparation. Cut edges glass slides (Thermo Fischer Scientific) were cut in 25x25 mm square with the help of a diamond tip pen. The glass squares were immersed in ethanol and sonicated 30 min at room temperature. The slides were air-dried and spin-coated with a Sunever 150 alignment layer polyimide (Nissan Chemical Industries) using a Laurell WS-650 spin coater. The slides were heated on a hot plate at 180°C for an hour. Alignment was applied by rubbing the slides with a velvet cloth. Spacers of 50 µm were placed on both edges of the slides and the cell was assembled with epoxy glue. For tensile experiments cells with unidirectional planar alignment were fabricated, while biomimetic springs were obtained with cells with twist configuration (perpendicular alignment of the two glass slides).

The monomers and the photo-initiator were weighted separately, dissolved in dichloromethane and mixed in a glass vial. Dichloromethane was evaporated by heating the open vial in an oven, in the dark, at 60 °C overnight. The mixture was then heated at 80 °C for 30 min.

The glass cells were pre-warmed at 60 °C and the mixture was introduced into the cell by capillary forces, using a pre-warmed metallic tip. Once the

cell was filled, it was covered by aluminum foil and heated at 80 °C for a few minutes to prevent crystallization. After cooling back the cell, a cut-off filter ($\lambda > 425$ nm, Edmund Optics) was placed on top of it and photopolymerization was performed with an Edmund Optics MI-150 high-intensity illuminator over 3 h (the polymerization temperature was adjusted for each mixture). The cells were finally placed in an oven at 60 °C overnight for post-curing.

3.5 Supplementary data

Supplementary Figure 1. Chemical composition of the free liquid crystal fraction

Supplementary Figure 2. ATR-IR spectra of film before (bottom) and after (top) washing with acetonitrile (ACN). On the right is a particular of the spectra on the absorption of nitrile.

Supplementary Figure 3. Bending motion of a shape-shifting polymer upon illumination. The alignment of the liquid crystal is parallel to the long axis of the ribbon. The bending motion is mediated by a build-up in strain through the thickness of the polymeric ribbon.

Compound	R=1	R=1.9	R=2.9	R=3.8	R=4.7	R=5.6
C6BPN	12.4 mol% 10.8 mg	12.3 mol% 10.8 mg	12.1 mol% 10.8 mg	12 mol% 10.8 mg	11.8 mol% 10.8 mg	11.7 mol% 10.8 mg
C6BP	60.1 mol% 53 mg	56.6 mol% 50.5mg	53.1 mol% 48 mg	49.7 mol% 45.5 mg	46.4 mol% 43 mg	43.2 mol% 40.5 mg
C6M	18.1 mol% 27.7 mg	17.8 mol% 27.7 mg	17.6 mol% 27.7 mg	17.4 mol% 27.7 mg	17.2 mol% 27.7 mg	17 mol% 27.7 mg
Azobenzene	4.2 mol% 5mg	4.2 mol% 5mg	4.1 mol% 5mg	4 mol% 5mg	4 mol% 5mg	3.98 mol% 5mg
E7	4.1 mol% 2.5mg	8 mol% 5mg	12 mol% 7.5 mg	15.7 mg 10 mg	19.5 mol% 12.5 mg	23.1 mol% 15 mg
Irgacure 819	1 mol% 1mg	1 mol% 1mg	1 mol% 1mg	1 mol% 1mg	1 mol% 1mg	1 mol% 1mg

Supplementary Table 1. Chemical composition of the liquid crystal mixtures.

Supplementary Figure 4. Glass transition temperature (T_g) for mixtures with different R .

Supplementary Figure 5. In situ stress-strain curves for LCPN with 4 (left) and 20 mol % E7 content (right). The films are irradiated with UV light of 17 and 300 mW/cm² intensities.

Supplementary Figure 6. Winding polymer spring lifting an object ca. 10.5 times its weight (11.67 mg mass of the object, 1.1 mg mass of the polymer spring). The work performed by the spring is ca. 0.64 μJ (6.1 J/mol of Azo).

Supplementary Figure 7. Photoinduced softening of a liquid crystal polymer spring ($R = 1$). Nonlinear mechanical response (strain stiffening) of a liquid crystal polymer spring. The curve in black shows the force-displacement curve of the spring. The curve in blue shows the photo-induced softening upon UV irradiation.

Supplementary Figure 8. Infrared spectra of components on the left and of LC mixture before (blue) and after polymerization (red) on the right.

Synthesis

Supplementary Scheme 1. Synthetic route for the synthesis of the azobenzene diacrylate.

Compound 1. Compound 1 was synthesized according to previously reported procedure.⁴⁹

Compound 2. Compound 2 was synthesized according to previously reported procedure.⁵⁰

Azobenzene diacrylate (Azo). Compound 2 (100 mg, 0.185 mmol), potassium acrylate (81.5 mg, 0.74 mmol), and potassium iodide (24.5 mg, 0.148 mmol) in anhydrous DMSO (5 mL) were stirred at 100 °C under N₂ atmosphere for 72 hours. After cooling down to room temperature, the reaction mixture was poured into water. The solution was filtered under vacuum and washed twice with water. The yellow solid was dissolved in dichloromethane/acetone and dried over Na₂SO₄. After filtration and evaporation of the organic solvent the azobenzene was isolated as a yellow solid (90 mg, 95%). ¹H-NMR (400 MHz, CDCl₃) δ 7.87 (d, *J* = 9.0 Hz, 2H), 6.98 (d, *J* = 9.0 Hz, 2H), 6.40 (dd, *J* = 17.3, 1.5 Hz, 1H), 6.12 (dd, *J* = 17.3, 10.4 Hz, 1H), 5.82 (dd, *J* = 10.4, 1.5 Hz, 1H), 4.18 (t, *J* = 6.6 Hz, 2H), 4.03 (t, *J* = 6.4 Hz, 2H), 1.92 – 1.78 (m, 2H), 1.77 – 1.67 (m, 2H), 1.58 – 1.41 (m, 4H).

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Chapter 4

Engineering multistate cholesteric helices with light-driven molecular motors

Unravelling the rules of molecular motion at all scales will help us understand how living matter could have ever emerged from inanimate matter, and this understanding will also support the development of soft materials with dynamic optical functions. Here we present a novel design for overcrowded-alkene based molecular motors that feature a rich conformational chiral landscape, including three distinguishable chiral states. Amplification of their chirality-derived operation in a liquid crystal yields multistate cholesteric helices with a versatile dynamic behavior that can be controlled with light, precisely and reversibly. These highly dynamic and photo-inverting helices associated to our molecular design are likely to support unprecedented developments in the field of tunable reflectors and diffraction gratings, and for the recording of optical information.

4.1 Introduction

Harnessing the motion of molecular machines into macroscopic function is a contemporary endeavor that is likely to bring responsive soft matter closer to the sophistication of biological systems and materials^{1,2,3,4,5}. As in biological systems, successful approaches to make molecular machines work at larger scales have involved self-organized soft matter, ranging from hydrogels^{6,7}, to self-assembled monolayers⁸, artificial muscles⁹ and other polymer systems¹⁰. Arguably, approaches based on liquid crystals have shown to be particularly promising in bringing the motion of molecular machines to the macroscale. Mesoscopic materials are indeed sufficiently organized to amplify the nanoscale operation of artificial molecular machines with a sense of directionality and along multiple length scales, yet they retain a fluid character that accounts for their high responsiveness to small changes in molecular structure or composition, or in the environment.¹¹ The long-range orientational order of liquid crystals also enhances the efficiency of chiral transmission from the molecular scale upwards. Twisted liquid crystals thus constitute an ideal amplification medium for chiroptical switches^{12,13,14}, and specifically for overcrowded alkene-based light-driven molecular motors, whose helical chirality inverts in each switching step^{15,16}. In chiral nematic liquid crystals incorporating molecular motors, self-organized helices indeed respond to light not only with large changes in their twist and orientation, but also with inversion of their screw sense¹⁷. These dynamic helices have mediated the development of rotating surfaces^{18,19}, revolving vortices,²⁰ reconfigurable chiral droplets²¹, and adaptive optical materials^{22,23}.

However, the molecular motors that have been used in liquid crystals so far, go through two different chiral states during their rotation, and therefore the dynamic helices in which they are incorporated mostly switch from a stable state to a transient state under irradiation with light. Having multi-stable dynamic helices that are also capable of inverting their screw sense thus remains a challenge in terms of molecular design of molecular motors.

Here, we present a novel design for overcrowded-alkene based molecular motors that features a rich conformational chiral landscape, including three different chiral states (Figure 1a). This molecular design allows taking full advantage of the multistate switching that characterizes the operation of the motors, in pre-programming the formation of a range of helices with both screw senses, combined with the possibility to fine tune the helical twist with light (Figure 1b). Besides presenting multi-stable and versatile optically responsive soft materials, this work contributes to a better understanding of the mechanisms by which molecular machines transfer chirality across length scales in soft matter.²⁴

Figure 1. Molecular motor design and operation. **a**, Rotary cycle of a first-generation molecular motor upon light and heat (one enantiomer is shown only). **b**, Schematic representation of a cholesteric supramolecular helix undergoing helix inversion in response to an external stimulus.

4.2 Results and discussion

4.2.1 Design of molecular motors and their action

Our design is based on a symmetric overcrowded-alkene, with identical bottom and upper halves. Upon light-induced rotation, these motors undergo large chiral shape transformations. Their molecular anisometry is modified by varying the position of substituents at the motor core, to optimize their compatibility with liquid crystals, allowing chirality to be transduced across length-scales more efficiently (Figure 1). In motors **1-3**, hexyl chains are attached to the motor core with or without a phenyl spacer.

The synthesis of motor **1-3** is described in the supporting information (Scheme S1 and Supplementay Figure 1-12). The absolute configurations of all the enantiomers of **1-3** are determined according to our previous report²⁵ and detailed in the supporting information as well.

The unidirectional rotary cycle starts with the (*R,P*)-*trans*-stable isomer (Figure 1a). Upon UV illumination ($\lambda = 312$ nm) the central double bond undergoes a *trans-cis* photoisomerization step and results in the formation of an energetically less-favorable (*R,M*)-*cis*-unstable isomer that adopts inverted (*M*) helicity.¹⁵ The photoisomerization process is reversible if 365 nm irradiation is applied to *cis*-unstable isomer. Meanwhile, the obtained (*R,M*)-*cis*-unstable isomer is also able to go through an energetic downhill thermal helix inversion step to yield (*R,P*)-*cis*-stable isomer. This thermal step is irreversible and therefore ensures the unidirectional rotation of the motor. Note that the helicity of the molecule is reversed again from *M* to *P* during the thermal isomerization step from *cis*-unstable isomer to *cis*-stable isomer. If *cis*-stable isomer is exposed to 312 nm UV light then the further rotation to the *cis*-unstable-state has been observed. Such rotation occurs through the intermediate formation of *trans*-isomer as it is shown in Figure 1a.

Figure 2. Partial ^1H -NMR spectra of **2** (CD_2Cl_2 , 20 °C). **a**, stable *trans*-**2**, before irradiation ($\lambda = 312$ nm). **b**, after irradiation; and **c**, after standing at 50 °C in the dark overnight. One enantiomer is shown only.

^1H -NMR spectroscopy was employed to study the motor cycle of molecules **1-3**. Figure 2a presents the partial ^1H -NMR spectrum of *trans*-**2** in CD_2Cl_2 . According to our previous studies of similar symmetric motors, in which the upper and bottom halves are identical^{26,27}, the multiplet at 3.1 ppm, double doublet at 2.8 ppm and doublet at 2.4 ppm can be assigned to the aliphatic protons H_a , H_b and H_c of the five membered ring, respectively. The sample was irradiated ($\lambda = 312$ nm) at 20 °C and downfield shifts of all absorption were observed, which is indicative of the formation of a new isomer i.e. unstable *cis*-**2** (Figure 2b). Notably, H_c shifts from 2.4 ppm (doublet) to 2.7 ppm (double doublet). The new absorption at 3.3 ppm can be identified as

H_b, and the multiplet at 3.7 ppm can be assigned as H_a in the unstable *cis* isomer. Photostationary state was reached upon extended irradiation, and integration of the proton H_a signals in the *trans* and unstable *cis* isomer indicated a photo-conversion ratio of 87/13 (unstable *cis*-2/*trans*-2). The sample was kept in the dark at 50 °C overnight. The occurrence of thermal helix inversion from unstable *cis*-2 to stable *cis*-2 is indicated by up-field shifts of all absorptions (Figure 2c). The ratio of stable *cis*-2/*trans*-2 is equivalent to the ratio of unstable *cis*-2/*trans*-2. It indicates the absence of the thermal *E*-*Z* isomerization and therefore ensures the unidirectionality of the thermal isomerization of unstable *cis*-2. Motors **1** and **3** also show similar changes in spectra and PSS ratios are determined to be 72/28 and 77/23 (*cis*-unstable /*trans*), respectively (Supplementary Figure 13-14). In addition, the rotation cycle of the motor was followed by UV/Vis absorption spectroscopy. UV/Vis absorption spectra of the (*R,P*)-motors **1-3** show that the band corresponding to the *trans*-state is the most blue-shifted, whereas the *cis*-stable-state is characterized by a modest red-shift (Figure 3). The *cis*-unstable-state shows the main absorbance band substantially at a longer-wavelength region ($\lambda > 355$ nm). The absorption spectra of all three forms of **3** are shifted bathochromically compared to the other motors, likely because the alkyloxy-substituents in *para*-position increase the conjugation between the two halves of the.

These motors are also fluorescent (Figure 3a-c), with emission from the *trans*-state being more intense than from both *cis*-states. Motors **2** and **3** have very

similar behavior depending on the state of the motor, except for the fact that, the emission of motor **3** is shifted bathchromically similar to the change in absorption spectra going from **2** to **3**. The Stokes shift for *cis*-unstable-isomer is significantly smaller than for *trans*- and *cis*-stable-isomers. The emission of motor **1** is characterized by larger Stokes shifts, especially for the *cis*-unstable-isomer.

Circular dichroism (CD) spectroscopic studies of molecular motors **1-3** (Figure 3) have shown that *trans*- and *cis*-stable-states have the same chirality as is evident from the negative absorptions around 320 nm (black and blue lines). Meanwhile, exposure of any of these forms to UV light ($\lambda = 312$ nm) leads to an inversion of chirality as reflected by the positive absorptions around 340 nm (red lines), which is associated with the formation of the *cis*-unstable-state of the motors. Based on these data, we can expect photoinduced supramolecular helix inversion of cholesteric liquid crystals doped with these motors, although the transfer of chirality from molecular to supramolecular level is far from trivial.²⁸

Figure 3. Structure, absorbance, emission and circular dichroism spectra of motors. (*R,P*)-1 (a), (*R,P*)-2 (b) and (*R,P*)-3 (c). The spectra were recorded in DMSO at room temperature. The *cis*-unstable state was obtained by irradiating the *trans*-state with UV light ($\lambda = 312$ nm), until a photo-stationary state (PSS) was reached. For the emission spectra, the excitation wavelengths were $\lambda = 300$ nm for motor 1 and 2, and $\lambda = 320$ nm for motor 3.

4.2.2 Molecular motors in nematic liquid crystals

The orientation of the molecular motors in the liquid crystal host determines the optical properties of the cholesteric helix, and its propensity to amplify molecular motion along length scales. We have thus investigated the alignment of the motor in the liquid crystal by using racemic mixtures, in order to prevent the formation of a twist in the liquid crystal, and thus

facilitate the study of the motor's alignment with respect to the host. Linear dichroism spectroscopy allows to estimate the orientation of the electronic transition moment in the molecule and, consequently, of the molecular motor itself in respect to the orientation of liquid crystal molecules (Figure 3). Moreover, it allows calculating the dichroism D , that indicates absence of any order for $D = 0$, and perfect order for $D = 1$, respectively (see equation 3 in Methods). These values are shown Figure 4.

Interestingly the long molecular axis of the *trans*-state of motor **1** has a preferable orientation along liquid crystal alignment with a notable degree of ordering (Figure 4a). At the same time, *cis*-states are randomly distributed (in terms of transition moment directionality) in the oriented medium, which we associate with the very low anisotropy of the molecular shape. Motor **2** shows a nontrivial behavior (Figure 4b), namely, the *trans*-state is oriented along the orientation of the liquid crystals, while both *cis*-forms of **2** have an orthogonal orientation facilitating its biphenyl fragments to be co-directed with liquid crystal molecules (Supplementary Figure 15). Motor **3** in the *trans*-state displays the highest degree of orientation within the series ($D \sim 0.76$) along the orientation of the liquid crystal, which corresponds to the best examples of dichroic dyes in liquid crystalline matrices.²⁹ The high value of the dichroism is related to the long rod-like and rigid shape of the molecule. Both *cis*-states of motor **3** also have a predominant orientation as shown in Figure 4c but with significantly lower dichroism likely due to their bent-shape.³⁰ If we consider the values of dichroism of each particular state for all

motors, we can single out that the degree of ordering of the *trans*-, *cis*-stable- and the *cis*-unstable-states increases in the series of motor **1**, **2** and **3**, respectively, which is associated with a more pronounced rod-like shape of the motor molecules facilitating their alignment in liquid crystal medium.

Figure 4. Alignment of the motors with respect to the liquid crystal host. In order to estimate the alignment of the motors, racemic mixtures (1 wt%) were embedded in ZLI-1695 as a liquid crystal host medium that does not absorb in the UV region, and thus does not interfere with UV-visible spectroscopy. Home-made quartz cells with a nominal gap of 10 μm and rubbed PVA alignment layers were used, to promote unidirectional molecular alignment. On the structures of motor **1** (a), motor **2** (b), and motor **3** (c), the calculated momentum of the electron transition ($S_0 \rightarrow S_1$) is indicated by purple arrows (details for the calculation are described in the methods part). The molecular motors are represented with respect to the molecular alignment

of nematic host molecules (represented in the background), accordingly to the experimental data from linear polarized light absorbance, that is plotted on the polar plots (right panel). These polar plots show an angular dependence for the maximum of absorbance, *i.e.* they indicate that the motors indeed adopt a preferred orientation within the anisotropic molecular matrix. Dichroism values are provided in the figure and reflect the degree of alignment of the motor molecules, where 0 is disorder and 1 perfect alignment. Positive and negative values correspond to the orientation of the motor's momentum of electron transition, respectively, along and perpendicularly to the liquid crystal orientation.

4.2.3 Molecular motors twisting liquid crystals

Next the behavior of these enantiomerically pure motors as chiral dopants in liquid crystal medium were studied. A small amount of a chiral dopant embedded into a liquid crystalline nematic medium induces the formation of a helix, with a twist that is characterized by a phenomenological parameter called the helical twisting power (HTP, see Equation 1):

$$\text{HTP} = P^{-1}/Cee \quad (1)$$

where P is the cholesteric pitch, C is the concentration of the chiral molecules, and ee is the enantiomeric excess. By convention, the HTP is positive when the cholesteric helix is right-handed and negative when it is left-handed.¹¹ Its absolute value is the sum of the individual contributions of all enantiomers present in the system, and is determined by the geometry of the chiral moieties and the nature of the molecular interactions between the components of the liquid crystal.¹⁷

In this work we have used a mixture of cyanophenyl-cyclohexyl derivatives (detailed structures are shown in SI) that is liquid crystalline at room temperature and, importantly, does not absorb at $\lambda = 312$ nm. The helical twisting powers of all enantiopure (*R,P*)-enantiomers, determined by the Grand-Jean Cano method (see methods part),³¹ are shown in Table 1, and indicate that (*R,P*) motor **1** induces a left-handed cholesteric helix in both *trans*- and *cis*-unstable-state, however with a moderate strength. (*R,P*) motor **2** is characterized by higher twisting powers, comparable to those of second-generation molecular motors,³² and also shows inversion of handedness of the cholesteric helices (Figure 1b). (*R,P*)-motor **3** in *trans*- and *cis*-stable-state induces a left-handed cholesteric helix, moreover PSS mixture upon irradiation of 312 nm enriched with *cis*-unstable-state of **3** possesses the same left-handed helical structure. However, given the 77/23 isomeric composition (*trans/unstable-cis*) at photostationary state, the calculated helical twisting power of *cis*-unstable-state **3** is low (HTP = +4.8), and indicates that helix inversion occurs upon UV illumination as well.

Analyzing how molecular chirality is transduced to the supramolecular level of cholesteric helices it should be noted that, from the data reported, there is do not directly correspond to any theoretical models of chirality transduction, unlike in binols or binaphthyls.³³ A close look into molecular structures of the motors in the three distinct states (*trans*, *cis*-unstable, *cis*-stable) suggests these molecular motors embed two axial chiral moieties. One of them is formed by two benzyl rings forming the stiff stilbene unit,

which is extended in the case of motor **2** and **3**, analogous to cisoidal/transoidal conformation of binols. The other is accommodated by the C=C bond and two carbons atoms at the stereogenic allylic position in the cyclopentene rings. When individual contributions to the twisting power have the same sign, they enhance each other and yield a high value for the resulting HTP – in the opposite case they compensate each and yield low HTP values. It is of key importance to define the axis along which the transduction of molecular chirality occurs in the most efficient way. In some sense such explanation goes along with disk-/rod-like shape anisotropy approach proposed previously.³⁴ All data presented in Table 1 can be explained by the interplay of the two different axial chiralities mentioned above. A detailed description of the motor structures is provided in supporting information (Supplementary Figure 16-18).

It is worth mentioning that due to the extension of the molecular shape of the motor **2** in the position 3 and 3' (see Figure 1a) the HTP of both *cis*-states was enhanced more than two times compared to motor **1**. On the other hand, such structural changes of motor **2** in the *trans*-state results in inversion of the HTP' sign likely due to the selective enhancement of the axial chirality formed by the stilbene moiety. The extension of the core of the motor in the position 4 and 4' (see Figure 1a) leads to about a twofold increase in the HTP of *trans*-state of motor **3**, while both *cis*-forms have lower HTPs compared to the motor **1** (additional data on HTP values on motors in different liquid

crystal media and those for opposite enantiomers can be found in Supplementary Table 1 and 2, respectively).

Based on the experimental results on the transduction of chirality reported here, it is evident that straightforward chemical modification of the first-generation motor core yields materials with different properties in terms of their chiral amplification in liquid crystals. However, the photochemical reactions yielding unidirectional rotary motions of all three motors can induce an inversion of the HTP sign, which allow us to control the type of supramolecular helical structure under the action of an external stimulus (light, heat), as schematically shown in Figure 4b and discussed further on.

Motor	<i>trans</i>	312 nm PSS ^a	<i>cis-unstable</i> ^b	<i>cis-stable</i>
1	-26,4	-42,3 / -44,9	-48,6	+25,3
2	+4,8	-77,6 / -87,4	-88,9	+75,5
3	-49,9	-7,8 / -9,7	+4,8	-17,4

Table 1. Light-induced helix inversion . Values of helical twisting powers (HTP_{wt.%} in μm^{-1}) for each (*R,P*)-enantiomer of the considered motors, in all three stable states that can be reached during the motor cycle. *Cis-unstable* forms were generated *in-situ*, by starting either from the *trans*-state or from the *cis-stable*-state, and irradiating with light at $\lambda = 312$ nm. Positive and negative HTP values correspond to right- and left-handed helices, respectively. ^a: 312 nm PSS was reached by conversion of *trans* isomer. ^b: HTP value calculated based on measured composition of 312 nm PSS.

The data given above describe static cases of the application of motors as chiral dopants in liquid crystals. Let us now consider their dynamic behavior, in particular how the twisting powers of change under external stimuli. Figure 5 shows the full cycle of rotation of first-generation molecular motors presented here and their change in HTP in each state. The notation $[\Delta\text{HTP}]$ indicates that the HTP changes without helix inversion. Positive and negative values correspond to increase and decrease in HTP, respectively. The notation $\{\Delta\text{HTP}\}$ indicates that the changes in helical twisting power are also associated with dynamic helix inversion. Figure 5 shows that all three motors allow adjusting the helical twist within a wide range. For all motors (**1-3**) the cholesteric handedness can be selectively addressed by irradiation at $\lambda = 312$ nm, or at $\lambda = 365$ nm, or by heat.

These data also show unprecedented opportunities to dynamically regulate the cholesteric pitch of a LC film for instance motor **2** allowing ΔHTP as high as $160\ \mu\text{m}^{-1}$. It should be emphasized that *trans*- and *cis*-stable-states of **1-3** are thermally stable and the *cis*-unstable-forms are relatively stable at room temperature characterized by half-lives exceeding 100 hours, these new motor designs are, to the best of our knowledge, the first three-stable-state single-molecule system controlled by light. More importantly, besides the possibility to switch the systems driven by these motors between three states, in fact, any intermediate state can be kinetically trapped purposefully by controlling the irradiation time.

Figure 5. Cyclic operation of the molecular motors. The scheme shows the photochemical transformations that an *(R,P)*-enantiomer undergoes during the rotation cycle, and how these transformations are associated with large changes in the helical twisting power (HTP). The notation $[\Delta\text{HTP}]$ indicates that the HTP changes without helix inversion. The notation $\{\Delta\text{HTP}\}$ indicates that the changes in helical twisting power are associated with helix inversion.

To estimate the stability of *cis*-unstable forms of motors in LC, 1 wt% of *trans*-motors were used as dopants. *Cis*-unstable-states were generated in the liquid crystal with $\lambda = 312$ nm light, the relaxation of *cis*-unstable- to *cis*-stable-state in the dark was followed by spectral changes at room temperature (Figure 6). We found that the *cis*-unstable-state of motor **1** is less stable than **2** and **3**; with a half-life time of ≈ 113 h (Figure 6). *Cis*-unstable-stables of motors **2** and **3** in the liquid crystals are characterized by

substantially higher stabilities, with half-life times of 824 h and 955 h, respectively (full spectra are provided in Supplementary Figure 19). The stability of the photo-generated *cis*-unstable isomer is affected not only by molecular structure but also by the environment.^{35,36} Overall the *cis*-unstable isomer of motors **1-3** are characterized by considerably longer half-life times than in isotropic solution (Supplementary Figure 20). In addition to study the stability of the *cis*-unstable-isomer, we have also addressed how the HTP values change during the relaxation, and the data are shown in Supplementary Figure 21. The kinetic curves differ from those shown in Figure 6, because the Δ HTP depends on the initial (*cis*-unstable) and final (*cis*-stable) values of HTP (see Table 1).¹⁷ The half-life time of the *cis*-unstable-state ranges from several days to tens of days in liquid crystals.

Figure 6. Relaxation kinetics and fluorescence properties of the motors in the liquid crystal. Kinetics of relaxation from the *cis*-unstable-state to the *cis*-stable-state of motors **1-3** in a liquid crystalline environment. The thermal isomerization was followed by UV-vis spectroscopy at room temperature in the dark. The *cis*-unstable state was generated *in-situ* by irradiating the *trans*-isomer at $\lambda = 312$ nm. The home-made quartz cells have a nominal cell gap of 10 μm , and were filled with the nematic liquid crystal doped with 1wt% of the (*R,P*)-**2** enantiomer.

In order to demonstrate the capabilities of molecular motors in LC film for optical applications, we developed a material consisting of nematic matrix ZLI-1132 doped by 4 wt% of *cis*-stable-isomer of motor 2. The planar oriented layer of this mixture reflects green light. Figure 7a shows how irradiation at $\lambda = 300$ nm affects the position of the selective light reflection band. The initial state has a right-handed helical structure with selective light reflection in the green spectral region ($\lambda_{\text{max}} = 550$ nm) according to Equation 2. Importantly the supramolecular helical structure of a certain handedness reflects circularly polarized light with the same handedness selectively. Exposure to $\lambda = 300$ nm leads first to the unwinding of the helix with the corresponding shift of the reflection band to the infrared region, followed by inversion of chirality and subsequent left-handed twist of the helical structure resulting in the hypsochromic shift of the reflection due to the rotation of the motor from *cis*-stable- to the *cis*-unstable-isomeric form. Two states with opposite handedness of cholesteric structure are separated by a region with an unwound helical structure since molecular motors with opposite signs of HTP compensate each other in this area (according to Equation 2).

$$\lambda_{\text{max}} = nP \quad (2)$$

where n is the average refractive index, and P is the helix pitch. Overall, the cholesteric material we have developed allows adjusting the optical properties in a wide spectral range and to invert the sign of helix in controlled way under the action of UV light. If the irradiation is stopped at a certain time (certain dose of exposure), the color of the sample can also be

fixed because the isomeric composition of the motor is stable in time. Controlling the dynamic helices can also be performed locally, with spatial resolution (Figures 7b,c). Figure 7b shows the image of a tulip observed under natural (non-polarized), left-circular polarized (LCP) and right-circular polarized (RCP) light. Area (1) is non-irradiated and reflects green RCP light as shown in transmission spectra (Figure 7b). The area corresponding to the flower was exposed to UV light to reach helical structures that reflect green and red colors. The tulip selectively reflects the LCP light, while the area around reflects RCP light. Where the areas with opposite chirality touch each other, we observe a thin black border that corresponds to areas where the helix is completely unwound, and thus light is not reflected. Other patterns can be designed as well (Figure 7c). As the images are formed in liquid layers, their stability is limited to only one hour, because the motor diffuses in lateral directions. However, diffusion does not constitute a fundamental limitation as it is possible to take advantage of polymer to stabilized liquid crystals³⁷ by using cross-polymerizable liquid crystalline compounds or side-chain liquid crystal polymers, instead of low-molar-mass liquid crystals in further applications.

Figure 7. Photo-patterning multiple reflection colors in a single material. **a**, Upon exposure to UV light ($\lambda = 300$ nm), the change in period of the helix is associated with a shift of the selective light reflection peak, whereas the inversion of handedness is associated with an inversion of circular polarization. The thin film was formed in a planar cell, with a liquid crystal doped with motor 3 in the *cis*-stable state (4 wt%). The notations RH and LH indicate right- and left-handed geometries, respectively. **b**, **c**, The motor design we put forward allows photo-patterning of multiple, stable colors in a thin film of liquid crystal. The thin films of motor-doped liquid crystals were visualized under natural (non-polarized) light and, left-handed or right-handed circular polarized light. Samples were irradiated at $\lambda = 300$ nm, through a mask, for varying times, until helix inversion was achieved. The reflection from the cholesteric helices can be visualized selectively by using circular polarizers, while irradiating with natural (non-polarized) light allows visualizing both RH and LH reflection colors. The transmission spectra corresponding to the different colours are provided in the right panel. **d**, Absorbance and fluorescence spectra of LC film doped with motor (R,P)-2. The inset shows a fluorescence image for a sample in the *cis*-stable-state, irradiated through a mask with $\lambda = 365$ nm light (the excitation wavelength was $\lambda = 300$ nm). The flower pattern consequently corresponds to an area where the motor is in the *trans*-state, while the rest is in the *cis*-stable state, and these two areas are distinguishable by having different fluorescence properties. The dimension of the sample is 2×2 cm.

It is important to address at which extent the molecular motors influence the liquid crystalline environment and *vice-versa*. We highlight how the emission properties of the motors are heavily dependent on the interaction with soft matter. In the liquid crystal phase, the fluorescence of each of the three stable states is modified substantially (Figure 7d for motor **2**, Supplementary Figure 22 for motors **1** and **3**). Their emission features a long wavelength emission band for the *cis*-forms, in particular for the *cis*-stable form.

Finally, the motor design we put forward, and the associated multi-state helices, also allow photo-controlling fluorescence in soft matter. Similarly to optical image recording in Figure 7b, fluorescent patterns can be encoded in the material due to the emitting properties of different isomers. The spatial distribution of isomeric forms of the motor can be seen by the naked eye (Figure 7d). On an image displaying different fluorescent colors, the *trans*-isomer of motor **2** fluoresces in the ultra-violet (inside of the tulip), while the rest of the sample display emission in the visible spectral range inherent to initial *cis*-stable-state of motor **2**. As the polarization of the emitted light is defined by the chirality of the helix,^{38,39} it becomes possible to envision switching the handedness of the emitted light, reversibly.

4.3 Conclusion

Integrating light-driven molecular motors into liquid crystals allows transducing their operation at the macroscopic level. Asymmetry is key to

this mechanism, and therefore a molecular design featuring a rich chiral landscape, such as the one we put forward, allows utilizing the full potential of multistate switching. Encoding multistate switching in a higher order soft material allows targeting more than one final optical state with one stimulus, thus paving the way for smart multifunctional materials. In the quest for dynamic liquid crystal based optical materials, our new motor design allows inverting stable helices with light, *i.e.* light transforms a dynamic helix into its mirror image. This property translates into complex optical image recording for which it becomes possible to encode, in the same sample, areas of similar pitch but opposite handedness, *i.e.* handedness can be encoded in a smart optical material, and its color as well, independently. We anticipate that the highly dynamic and photo-inverting helices associated to our molecular design will bring dynamic optical materials to the next level, with unprecedented developments in the field of tunable reflectors and diffraction gratings, and in optical information recording. Overall, the work also adds to our understanding of the rules governing chiral amplification in functional soft matter.

4.4 Acknowledgements

We acknowledge Dr. Jiawen Chen for the synthesis of molecular motors used in this work. We acknowledge Dr. Dimitri Morozov for performing the DFT calculations. We acknowledge Prof. Ben Feringa for the scientific discussion of the data.

4.5 Materials and methods

Sample preparation. The nematic hosts ZLI1132 and ZLI1695 were purchased from Merck and used as received. The motor-doped cholesteric liquid crystals were prepared by dissolving the necessary amounts of motors and nematic hosts in dichloromethane, followed by solvent evaporation and drying in vacuum. Next, the motor-doped cholesteric liquid crystals were introduced by capillarity into sandwich-like quartz cells or wedged-like cells. Optical properties of the motors in liquid crystalline environment were investigated in home-made quartz cells of 10 μm thickness. Unidirectional orientation of liquid crystal molecules was achieved by alignment coatings (rubbed polyvinylalcohol layers on quartz substrates).

Measurements. The polarized optical microscopy investigations have been performed using a microscope BX51 (Olympus). The absorption and transmittance spectra were measured using a spectrometer HR2000+ (Ocean Optics). For the polarized light absorbance measurements, spectrometer was equipped with a rotatable polarizer (Glan-Taylor prism). Dichroism (D) has been calculated according to the Equation 3:

$$D = (A_{\perp} - A_{\parallel}) / (A_{\perp} + A_{\parallel}) \quad (3)$$

where $A_{\parallel}$ and $A_{\perp}$ are the absorbance of light polarized parallel and perpendicular to the liquid crystal alignment direction.

Fluorescence measurements were performed using a LS55 (Perkin Elmer) spectrometer. As light sources for photooptical studies, LEDs (Thorlabs)

with wavelengths 300 nm ($I \sim 2.7 \text{ mW/cm}^2$) and 365 nm ($I \sim 10 \text{ mW/cm}^2$) and a mercury lamp (Spectroline) with a 312 nm ($I \sim 2.5 \text{ mW/cm}^2$) filter. The intensity of the light has been measured using a power meter PM-100D (Thorlabs).

HTP values have been determined by the Cano method. For practical guidelines see ref. 20. The sign of cholesteric helical structure was determined exploiting rotatable analyzer as previously reported.⁴⁰ The wedge cells were purchased from E.H.C. Co. (Japan).

Calculations. The structures of motors **1**, **2** and **3** have been optimized in *trans*-, *cis*-stable-, and *cis*-unstable conformations, using density functional theory with PBE0 functional⁴¹ and cc-pVDZ basis set. A conductor-like polarizable continuum model (C-PCM)⁴² has been used to model DMSO solvent with $\epsilon = 46.7$. Vertical excitation spectra calculation has been performed at the optimized geometries with time-dependent density functional theory (TD-DFT) method using Tamm-Dancoff approximation,⁴³ PBE0 functional and cc-pVDZ basis set. The same C-PCM solvent model of DMSO has also been used in spectra calculations.⁴⁴ Vertical excitation energies, orientations of molecular dipole moments, as well as $S_0 \rightarrow S_1$ transition dipole moments have been extracted from those calculations. The GAMESS(US) quantum chemistry package⁴⁵ has been used to perform optimizations and spectra calculations.

4.5 Supplementary data

Synthesis and characterization. Chemicals were purchased from Acros, Aldrich, Fluka or Merck and were used as received. Solvents for extraction and chromatography were technical grade. All solvents used in reactions were freshly distilled from appropriate drying agents before use. All reactions were performed under inert atmosphere (Ar). Analytical TLC was performed with Merck silica gel 60 F254 plates and visualization was accomplished by UV light. Flash chromatography was carried out using Merck silica gel 60 (230-400 mesh ASTM). NMR spectra were recorded on Varian AMX400 (^1H : 400 MHz, ^{13}C : 100 MHz) and Varian Unity Plus (^1H : 500 MHz, ^{13}C : 125 MHz) spectrometers. The deuterated solvents (CD_2Cl_2 and CDCl_3) were treated with Na_2CO_3 , molecular sieves (4 Å) and degassed by argon prior to use. Chemical shifts are denoted in parts per million (ppm) relative to the residual solvent peak (CD_2Cl_2 : ^1H δ = 5.32 ppm, ^{13}C δ = 53.84 ppm; CDCl_3 : ^1H δ = 7.26 ppm, ^{13}C δ = 77.0 ppm). The splitting parameters are designated as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets. High-resolution mass spectrometry (ESIMS) was performed on a LTQ Orbitrap XL spectrometer with ESI ionization. Enantiomeric excess values were determined by chiral HPLC using a Shimadzu LC-10ADVP HPLC equipped with a Shimadzu SPD-M10AVP diode array detector.

Supplementary Scheme 1. Synthetic route towards the light-driven molecular motors **1**, **2** and **3**.

Enantiomeric pure compounds **S1**, **S2**, and **S3** were prepared according to procedures described in the literature. (Neubauer, T. M.; van Leeuwen, T.; Zhao, D.; Lubbe, A. S.; Kistemaker, J. C. M.; Feringa, B. L. *Org. Lett.* **2014**, *16*, 4220-4223).

Motor 1. A mixture of enantiomeric pure *trans*-**S1** or *cis*-**S1** (348 mg, 1.0 mmol), potassium carbonate (690 mg, 5.0 mmol) and 1-bromohexane (495 mg, 3.0 mmol) in acetonitrile (25 mL) was heated at reflux for 16 h. After

cooling, ethyl acetate (50 mL) and water (50 mL) were added to the mixture. The water layer was extracted with ethyl acetate (50 mL) and the combined organic layers were washed with brine and dried over Na₂SO₄. The solvent was evaporated and the residue was purified by flash column (SiO₂, pentane:dichloromethane = 3:1) to yield **1** (362 mg, 70%) as a colorless oil. *Trans-1*: ¹H NMR (400 MHz, CD₂Cl₂) δ 6.56 (s, 2H), 4.10 – 3.85 (m, 4H), 2.94 – 2.80 (m, 2H), 2.57 (dd, *J* = 14.2, 5.7 Hz, 2H), 2.29 (s, 6H), 2.23 – 2.14 (m, 7H), 1.89 – 1.75 (m, 4H), 1.54 (s, 6H), 1.45 – 1.33 (m, 7H), 1.09 (d, *J* = 6.4 Hz, 6H), 1.01 – 0.89 (m, 6H). Enantiomers were analyzed by chiral HPLC, Chiralpak AD-H (99.2% *n*-heptane/ 0.8% *i*-PrOH), 40 °C, 0.5 mL/min, retention times (min) 10.9 (R) and 17.1 (S). *Cis-1*: ¹H NMR (400 MHz, DMSO-*d*₆) δ 6.34 (s, 2H), 3.51 (t, *J* = 6.7 Hz, 4H), 3.18 (t, *J* = 6.6 Hz, 2H), 2.93 (dd, *J* = 15.0, 6.4 Hz, 2H), 2.33 (d, *J* = 15.2 Hz, 2H), 1.99 (s, 6H), 1.81 – 1.73 (m, 4H), 1.38 (d, *J* = 14.9 Hz, 8H), 1.25 (dd, *J* = 7.6, 3.9 Hz, 8H), 0.96 (d, *J* = 6.6 Hz, 8H), 0.87 – 0.80 (m, 6H). Enantiomer were analyzed by chiral HPLC, Chiralpak AD-H (99% *n*-heptane/ 1% *i*-PrOH), 40 °C, 0.5 mL/min, retention times (min) 10.4 (R) and 15.5 (S). ¹³C NMR (126 MHz, CDCl₃) δ 171.0, 159.0, 158.5, 145.0, 144.7, 144.3, 143.6, 138.5, 136.4, 133.8, 132.9, 125.2, 123.1, 114.4, 113.5, 71.3, 71.0, 44.8, 44.5, 41.0, 40.7, 34.3, 34.2, 32.2, 32.2, 28.6, 28.5, 25.3, 25.3, 23.1, 21.9, 21.5, 21.3, 18.9, 16.9, 16.7, 16.7. HRMS (ESI-TOF) *m/z*: Calcd for C₃₆H₅₃O₂ [M + H]⁺ 517.40401; Found 517.40327.

Motor 2. A mixture of enantiomeric pure *trans*-**S2** or *cis*-**S2** (474 mg, 1.0 mmol), Pd₂(dba)₃ (37.5 mg, 0.04 mmol), S-Phos (38 mg, 0.10 mmol), K₃PO₄ (636 mg, 3 mmol) and (4-(hexyloxy)phenyl)boronic acid (424 mg, 2.05 mmol) in toluene (15 mL) was heated at reflux for 16 h. After filtration, the solvent was evaporated and the residue was purified by flash column (SiO₂, pentane:dichloromethane = 6:1) to yield **2** (482 mg, 72%) as a white solid.

Trans-2: ¹H NMR (400 MHz, CDCl₃) δ 7.33 (d, *J* = 8.4 Hz, 4H), 6.96 (d, *J* = 8.0 Hz, 6H), 4.01 (t, *J* = 6.5 Hz, 4H), 3.03 (t, *J* = 6.2 Hz, 2H), 2.76 (dd, *J* = 14.5, 5.6 Hz, 2H), 2.27 (d, *J* = 28.6 Hz, 14H), 1.89 – 1.77 (m, 4H), 1.55 – 1.45 (m, 4H), 1.36 (q, *J* = 3.6 Hz, 8H), 1.13 (d, *J* = 6.4 Hz, 6H), 0.92 (s, 6H). Enantiomers were analyzed by chiral HPLC, Chiralpak AD-H (99.2% *n*-heptane/ 0.8% *i*-PrOH), 40 °C, 0.5 mL/min, retention times (min) 10.9 (R) and 17.1 (S).

Cis-2: ¹H NMR (400 MHz, CDCl₃) δ 7.32 – 7.16 (m, 4H), 6.98 – 6.82 (m, 6H), 4.00 (t, *J* = 6.6 Hz, 4H), 3.41 (t, *J* = 6.6 Hz, 2H), 3.13 (dd, *J* = 15.0, 6.3 Hz, 2H), 2.49 (d, *J* = 15.0 Hz, 2H), 2.27 (s, 6H), 1.89 – 1.72 (m, 4H), 1.48 (s, 10H), 1.37 (d, *J* = 3.8 Hz, 8H), 1.14 (d, *J* = 6.7 Hz, 6H), 0.92 (m, 6H). Enantiomer can be analyzed by chiral HPLC, Chiralpak AD-H (99% *n*-heptane/ 1% *i*-PrOH), 40 °C, 0.5 mL/min, retention times (min) 12.5 (R) and 20.5 (S). ¹³C NMR (126 MHz, CDCl₃) δ 160.5, 145.7, 144.5, 144.0, 142.5, 137.7, 133.4, 133.0, 132.8, 132.34, 116.5, 70.7, 44.4, 41.3, 34.3, 32.0, 28.5, 28.5, 25.3, 23.2, 22.3, 21.0, 21.0, 16.7. HRMS (ESI-TOF) *m/z*: Calcd for C₄₈H₆₁O₂ [M + H]⁺ 669.46661; Found 669.46627.

Motor 3. A mixture of enantiomeric pure *trans*-**S3** or *cis*-**S3** (474 mg, 1.0 mmol), Pd₂(dba)₃ (37.5 mg, 0.04 mmol), S-Phos (38 mg, 0.10 mmol), K₃PO₄

(636 mg, 3 mmol) and (4-(hexyloxy)phenyl)boronic acid (424 mg, 2.05 mmol) in toluene (15 mL) was heated at reflux for 16 h. After filtration, the solvent was evaporated and the residue was purified by flash column (SiO₂, pentane:dichloromethane = 6:1) to yield **3** (473 mg, 70%) as a white solid. Trans-**3**: ¹H NMR (400 MHz, CDCl₃) δ 7.27 (d, *J* = 8.1 Hz, 8H), 6.93 (d, *J* = 8.6 Hz, 5H), 6.84 (s, 1H), 4.00 (t, *J* = 6.6 Hz, 4H), 3.39 (t, *J* = 6.6 Hz, 2H), 3.15 (dd, *J* = 15.0, 6.3 Hz, 2H), 2.49 (d, *J* = 15.2 Hz, 3H), 2.19 (s, 6H), 1.92 – 1.75 (m, 5H), 1.56 (d, *J* = 24.4 Hz, 16H), 1.43 – 1.29 (m, 9H), 1.14 (d, *J* = 6.8 Hz, 6H), 0.92 (d, *J* = 2.3 Hz, 8H). Enantiomers can be analyzed by chiral HPLC, Chiralpak AD-H (99.2% *n*-heptane/ 0.8% *i*-PrOH), 40 °C, 0.5 mL/min, retention times (min) 17.3 (R) and 20.7 (S). Cis-**3**: ¹H NMR (400 MHz, CDCl₃) δ 7.29 (d, *J* = 8.3 Hz, 4H), 7.02 – 6.87 (m, 7H), 4.01 (t, *J* = 6.5 Hz, 5H), 2.98 (t, *J* = 6.2 Hz, 2H), 2.77 (dd, *J* = 14.5, 5.6 Hz, 2H), 2.47 (s, 6H), 2.30 (d, *J* = 14.3 Hz, 2H), 2.13 (s, 6H), 1.82 (d, *J* = 7.4 Hz, 5H), 1.52 (d, *J* = 22.6 Hz, 13H), 1.40 – 1.31 (m, 9H), 1.15 (d, *J* = 6.4 Hz, 6H), 1.04 – 0.79 (m, 11H). Enantiomer can be analyzed by chiral HPLC, Chiralpak AD-H (99% *n*-heptane/ 1% *i*-PrOH), 40 °C, 0.5 mL/min, retention times (min) 16.7 (R) and 20.1 (S). ¹³C NMR (126 MHz, CDCl₃) δ 160.5, 147.8, 143.1, 142.8, 142.3, 137.2, 135.5, 133.1, 132.4, 130.5, 116.6, 116.5, 70.7, 44.3, 42.1, 34.3, 32.0, 28.4, 25.3, 24.6, 23.3, 23.3, 22.4, 19.1, 18.9, 16.7. HRMS (ESI-TOF) *m/z*: Calcd for C₄₈H₆₁O₂ [M + H]⁺ 669.46661; Found 669.46628.

Supplementary Figure 3. ¹³C NMR spectrum (126 MHz, CDCl₃) of compound 1.

Supplementary Figure 4. ¹H NMR spectrum (400 MHz, CDCl₃) of *trans*-2.

Supplementary Figure 5. ¹H NMR spectrum (400 MHz, CDCl₃) of *cis*-2.

Supplementary Figure 6. ¹³C NMR spectrum (126 MHz, CDCl₃) of compound 2.

Supplementary Figure 7. ^1H NMR spectrum (400 MHz, CDCl_3) of *trans*-3.

Supplementary Figure 8. ^1H NMR spectrum (400 MHz, CDCl_3) of *cis*-3.

Supplementary Figure 9. ^{13}C NMR spectrum (126 MHz, CDCl_3) of compound **3**.

Supplementary Figure 10. High resolution mass spectrum of motor 1.

Supplementary Figure 11. High resolution mass spectrum of motor 2.

Supplementary Figure 12. High resolution mass spectrum of motor 3.

Supplementary Figure 13. Partial ^1H -NMR of **1** (CD_2Cl_2 , 20 $^\circ\text{C}$) **a**, stable *trans*-**1**, before irradiation ($\lambda = 312 \text{ nm}$); **b**, after irradiation; and **c**, after standing at 50 $^\circ\text{C}$ in the dark overnight.

Supplementary Figure 14. Partial ¹H-NMR of **3** (CD₂Cl₂, 20 °C) **a**, stable *trans*-**3**, before irradiation ($\lambda = 312$ nm); **b**, after irradiation; and **c**, after standing at 50 °C in the dark overnight.

Supplementary Figure 15. Polarized absorbance spectra of racemic mixtures of motor **1** (a), **2** (b), and **3** (c) embedded in unidirectional aligned liquid crystal medium. LC cells are made of quartz with rubbed PVA alignment coating; cell gap – 10 μm . The angle of light polarization is with respect to molecular orientation of liquid crystal molecules and is shown for each graph.

Transduction of molecular chirality. To explain the transduction of chirality from molecular motors to cholesteric helices, we propose to consider two different axial chirality formed by the stilbene moiety and from the stereogenic allylic methyl group. These axial chiralities are highlighted in yellow. Each axial chirality is characterized by an handedness (right or left), a pitch of propagation (P_a and P_p for the stilbene and the methyl respectively), defined by the relative distance of the rigid element which extend further from the central double bond along the helix propagation, and a radius (R_a and R_p for the stilbene and the methyl respectively). The larger the radius and pitch the larger the contribution to the chirality transduction. Different isomeric forms twist the liquid crystal molecules along a different axis as indicated by the direction of the pitch in the figure.¹ On the right-hand side the handedness of the resulting cholesteric helix are schematically shown. The geometrical parameters of pitch and radius were extracted from the optimized structures of motors obtained as described in the method part (see main text). The only limitation of this approach is the prediction of transduction of chirality where axial chiralities are not very well defined (i.e. *trans*-stable isomer of motor 3).

¹ di Matteo, A.; Todd, S. M.; Gottarelli, G.; Solladié, G.; Williams, V. E.; Lemieux, R. P.; Ferrarini, A.; Spada, G. P. Correlation between Molecular Structure and Helicity of Induced Chiral Nematics in Terms of Short-Range and Electrostatic-Induction Interactions. The Case of Chiral Biphenyls. *J. Am. Chem. Soc.* **2001**, 123 (32), 7842–7851.

Supplementary Figure 16. Transduction of molecular chirality to the supramolecular level for (*R,P*) motor **1**.

Supplementary Figure 17. Transduction of molecular chirality to the supramolecular level for (*R,P*) motor **2**.

Supplementary Figure 18. Transduction of molecular chirality to the supramolecular level for (R,P) motor 2.

Motor	LC host	<i>trans</i>	<i>cis-unstable</i>	<i>cis-stable</i>
1.	E7	-20,9	-26,7	+20,2
	ZLI 1132	-26,4	-42,3	+25,3
	ZLI 1695	-23,1	-30,5	+19,2
2.	E7	+8,4	-16,3	+74,6
	ZLI 1132	+4,8	-77,6	+75,5
3.	E7	-45,1	-28,7	-10,6
	ZLI 1132	-49,9	-7,8	-17,4

Supplementary Table 1. HTP_{wt.%} values of molecular motors (*R,P* enantiomers) in different LC matrices.

Motor	LC host	<i>trans</i>	<i>cis-unstable</i>	<i>cis-stable</i>
1.	E7	+18,6	+23,5	-24,1
	ZLI 1132	+28,4	+45,3	-42,2
2.	E7	-21,7	+29,1	-71,9
	ZLI 1132	-24,2	+76,3	-84,2
3.	E7	+52,1	+28,9	+2,5
	ZLI 1132	+41,3	+5,0	+16,1

Supplementary Table 2. HTP_{wt.%} values of molecular motors (*S,M* enantiomers) in different LC matrices.

Supplementary Figure 19. Absorption spectra of thermal isomerization of *cis*-unstable isomer to *cis*-stable isomer of motor **1** (a), **2** (b), and **3** (c) obtained after 312 nm exposure in LC cell. *R,P* enantiomers of the motors dissolved in ZLI1132 liquid crystal host. Quartz LC cells are made with rubbed PVA alignment coatings (cell gap – 10 μm .)

Supplementary Figure 20. Kinetic studies of thermal helix inversion of unstable-cis isomer to stable-cis isomer. The kinetics of thermal helix inversion of the *cis*-unstable isomer to *cis*-stable isomer of motor **1-3** were determined by UV-vis absorption spectroscopy. Using the Eyring equation, the Gibbs free energy and the corresponding half-lives of **1-3** at 20 °C were obtained. Motor **1** has a half-life of 18.6 h at r.t. while motor **2** and **3**, with phenyl spacers present, show longer half-lives (25.8 h for **2**, 43.3 h for **3**). The long half-life is essential for further modulation of motor to address distinct helical states by light and heat within the liquid crystals.

Eyring plot for the thermal helix inversion of **1(a)**, **2(b)**, **3(c)** in heptane. The linear fitting of $\ln(kh/k_0T)$ by $1/T$ using Eyring equation $\ln \frac{k}{T} = \frac{-\Delta H^\ddagger}{R} \cdot \frac{1}{T} + \ln \frac{k_B}{h} + \frac{\Delta S^\ddagger}{R}$. The rate constants of the first-order decay k were obtained from equation $A/A_0 = e^{-kt}$, at four different temperatures. Half-life of motor **1** is 18.6 h, motor **2** is 25.8 h and motor **3** is 43.4 h at 20 °C.

Supplementary Figure 21. Relative variation of motors' HTPs during thermal isomerization from *cis*-unstable state obtained by irradiation at 312 nm.

Supplementary Figure 22. Absorption and emission spectra of racemic mixture of motor **1** (a) and motor **3** (b) dissolved in ZLI1695 liquid crystal host. LC cells are made of quartz with rubbed PVA alignment coatings (cell gap – 10 μm). Excitation wavelengths are 300 nm and 320 nm for motor **1** and **3**, respectively.

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Chapter 5

Revolving supramolecular vortex fueled by light in motor-doped liquid crystals

Molecular machines operated by light have been recently shown to be able to produce oriented motion at the molecular scale as well as do macroscopic work when embedded in supramolecular structures. However, any macroscopic movement irretrievably ceases as soon as the concentration of the interconverting molecular motors or switches reaches a photo-stationary state. To circumvent this limitation, researchers have typically relied on establishing oscillating illumination conditions – either by modulating the source intensity or by using bespoke illumination arrangements. In contrast, here we report a supramolecular liquid crystalline based system in which the emergence of oscillating patterns is encoded at the molecular level. Our system comprises chiral liquid crystal vortices that revolve continuously when illuminated, under the action of embedded light-driven molecular motors. The vortex rotation is sustained by the diffusion of the motors away from a localized illumination area. The interplay between the twist of the supramolecular structure and the diffusion of the chiral molecular motors creates continuous, regular and unidirectional rotation of the vortex under non-equilibrium conditions.

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5.1 Introduction

Nature's molecular machinery drive processive motion over increasing length scales and with control over space and time, by taking advantage of the sharp structural changes that occur close to phase transitions¹ in an open system that exchanges molecules and energy continuously². Abrupt qualitative responses to small variations in environmental conditions are thus a key feature for designing dynamic phenomena such as oscillating reactions or pattern formation, and for out-of-equilibrium operation modes to emerge from reaction-diffusion mechanisms³.

By contrast, despite the ability to harness the mechanical operation of artificial molecular machines in molecular systems^{4,5}, and to produce work at the macroscopic scale^{6,7,8}, these systems driven by man-made molecular machines have yet to be engineered to perform continuous operations autonomously, especially at the micro- and macroscopic scale. The major challenge in the development of systems capable to perform continuous rhythmic motion is to overcome the limitation caused by steady state dynamics (in chemically driven systems) or photo-stationary states (in photochemical systems)^{9,10}. While these equilibrium states can be populated and depopulated on demand, by controlling externally the input of the stimulus that shifts the equilibria^{11,12,13,14,15,16}, such strategy is inherently devoid of any autonomous and adaptive response of the system. Therefore, the development of autonomous operating systems driven by molecular machines requires strategies to establish concerted feedback mechanisms

that can control the concentration of active and inactive species in space and time¹⁷.

Here, we couple the localized operation of molecular machines with diffusion in a liquid crystal that is close to structural phase transition, and demonstrate the sustained rotation of supramolecular vortices. Our strategy involves helix-based liquid crystals that incorporate a molecular motor in a situation of geometrical confinement that prohibits the formation of the supramolecular helix. The frustration is released by light-induced isomerization that promotes isothermal twisting of the helix, and eventually yields chiral topological structures. Above a given threshold in the illumination power, a symmetry-breaking topological transition takes place that directs the robust and continuous rotation of a vortex. Combining the operation of artificial molecular machines with reaction-diffusion¹⁷ across length scales adds to our understanding of chemical dynamics in soft matter,¹⁸ comes closer to evolutionary strategies^{19,17} and paves the way towards life-like mechanized matter²⁰.

5.2 Results and discussion

5.2.1 Design of frustrated photo-responsive chiral liquid crystal for vortex generation.

We use overcrowded alkenes Ph-**m** or Me-**m** as molecular motors (Figure 1a). Upon illumination, **m** transforms into its unstable isomer **m**^{*}, while the reverse transformation **m**^{*}→**m** occurs at room temperature spontaneously. Once these molecular motors are incorporated in an achiral liquid crystal, amplification of chirality yields a photo-responsive cholesteric liquid crystal. This helix-based liquid crystal is further confined between two glass slides, each of them promoting perpendicular orientation of the liquid crystal molecules. When the gap L of the cell is typically smaller than the helical pitch p of the cholesteric liquid crystal (the distance over which the average molecular orientation of the liquid crystal rotates by 2π , and whose sign defines the handedness of the cholesteric helix), the helix is suppressed²¹. This geometrical frustration can be released when energy kicks in locally, by the use of either electric fields²² or laser beams²³ and yield a variety of complex twisted topological structures²⁴ that are all characterized by a constant cholesteric pitch.

Here, the geometrical frustration is released by winding the helix mechanically, *i.e.* by the twisting action of the molecular motor, in a system where **m** is combined with a shape-persistent chiral co-dopant (Supplementary Figure 1). Co-doping is necessary because most photo-

responsive chiral dopants induce helical unwinding under illumination, and here releasing geometrical frustration requires photo-induced winding. By adjusting the relative proportions of motor and shape-persistent co-dopant, and their concentrations, we have prepared cholesteric liquid crystals that display opposite helical handedness at full photo-conversion, *e.g.* provided that the illumination is sufficiently strong (Supplementary Table 1). The initial helical pitch p_0 first increases under illumination, and while the motor continues to photo-convert, the cholesteric helix reverses its handedness and starts winding continuously in the opposite direction (Figure 1a), until it reaches its saturated photo-stationary value p_∞ . We use steady irradiation conditions provided by a focused laser beam at wavelength $\lambda = 375$ nm (Figure 1b), and observe the system under crossed linear polarizers (Supplementary Figure 2). In practice, the emergence of any three-dimensional liquid crystal structure is thus assessed by a two-dimensional pattern.

Figure 1. Motor-doped liquid crystals winding under irradiation, and associated chiral structures. **a**, Molecular motor used as photo-active dopant, with a substituent R on the stereogenic centre. Two derivatives were used: Ph- m (R = phenyl group), and Me- m (R = methyl group). A bridged binol BB was used as a passive co-dopant. When mixed with a nematic liquid crystal, the dopant and co-dopant generate a photo-responsive cholesteric liquid crystal by combined chirality transfer. Helix inversion is associated both with the photo-isomerization $m \rightarrow m^*$, and with its spontaneous reverse transformation $m^* \rightarrow m$. **b**, Motor-doped liquid crystal film with thickness $L = 10 \mu\text{m}$ confined between two glass slides promoting perpendicular orientation of the molecules and illuminated locally using a Gaussian laser beam with a beam waist $w = 5 \mu\text{m}$ at wavelength $\lambda = 375 \text{ nm}$. **c**, **d**, Axisymmetric chiral patterns with opposite handedness observed by polarized optical microscopy between crossed linear polarizers. Such patterns can be sustained for a minimal illumination power of $\sim 1.25 P_{\text{min}}$. The dashed twisted crosses are a guide for the eyes, to identify the broken chiral symmetry in the pattern. **e**, **f**, A secondary structural symmetry breaking takes place spontaneously as the power increases up to $\sim 2 P_{\text{min}}$, and leads to a vortex that features a kink. This vortex rotates continuously, and both its handedness and the direction of rotation are predetermined by the axisymmetric pattern from which it results (**c** or **d**). The dashed spirals emphasize the handedness of the vortex and the solid white arrow shows the direction of rotation. Scale bars = $20 \mu\text{m}$.

5.2.2 Light-driven generation of supramolecular vortex

Earlier work showed that the mechanical winding provided by the motors can generate several types of twisted structures, above a minimal power $P_{\min}$ ²⁵. Here we report on out-of-equilibrium dynamics that occur via a structural transition, when the illumination power is used as a control parameter. At power values $\sim 1.25P_{\min}$ we can stabilize a twisted liquid crystal structure associated to an axially symmetric and chiral pattern, and that can be either right-handed or left-handed (Figure 1c,d). Increasing the irradiation power further and up to $\sim 2P_{\min}$, we unveil a breaking in axial symmetry that defines a structural transition: the axial symmetry of the chiral pattern is broken and yields another, much larger chiral pattern (vortex) that exhibits a kink (Figure 1e,f), and the handedness of which is pre-determined by the handedness of the axisymmetric pattern (Figure 1c,d). Strikingly, the emergence of this vortex is accompanied by its continuous, regular, and unidirectional rotation, in a direction that is pre-determined by its handedness (Figure 2a,b). The fact that the diameter of the beam is three to five times smaller than the diameter of the pattern is a clear manifestation that $\mathbf{m}^*$ diffuses away from the illuminated zone. When the light is turned off, the pattern vanishes away. Constant and stable rotation is sustained under steady illumination conditions – up to forty hours of continuous rotation have been observed for both directions of rotation, without any signs of fatigue (Figure 2c). Plotting the correlation coefficient between an image observed at any time t and a reference image, quantifies the similarity

between two images over time (Figure 2c). The corresponding Fourier spectrum provides a quantitative analysis for the rotation rate (Figure 2d), and highlights that the revolving vortex is robust in its operation. Moreover, this rotation does not depend on the polarization of the laser light, and has been observed with both linearly and circularly polarized light.

Figure 2. Light-driven chiral vortices revolving unidirectionally. **a,b**, Snapshots of chiral patterns revolving counter-clockwise and clockwise over one period. The polar angle ψ of the structural kink (see white arrow) in the (x,y) plane is plotted against time on the right side. The line refers to the best linear fit of the data. **c**, Correlation coefficient X between any image and the reference image at $t = 0$. The inset evidences a period $T \sim 20$ min. **d**, Fourier spectrum of $X(t)$ plotted around the mean rotation frequency $f_0 = 1/T_0$. The red curve is a Gaussian fit. Scale bars = 20 μm .

5.2.3 Revolving supramolecular vortex

We have investigated how the rotation rate depends on the use of a given enantiomer of either Ph-**m** or Me-**m**. We conclude that there is a general character to the rotation, as seen in Figure 3a, where the period of rotation T_0 correlates with the diameter d of any revolving vortex (experimentally, a range of different diameters can be accessed by using different irradiation powers). Specifically, the results evidence an exponential dependence of the rotation period with the diameter of the vortex that is valid for both motors and independently of the direction of rotation. This trend is not compatible with a quadratic law $T_0 \sim d^2/D$ that can be inferred from a simple dimensional analysis. This exponential dependence thus suggests that, in addition to diffusion, another parameter plays a key role in establishing the rotation, and this is probably the long-range orientational order that is characteristic for liquid crystals. Further, the rotation rate is influenced by the bulkiness of the substituents at the stereogenic center; specifically, it has been shown that an increase of steric hindrance speeds up the rotary motion²⁶. As Me-**m*** relaxes faster than Ph-**m***, the former motor displays a tendency to generate smaller vortices (square labels) than the latter (circular labels), and smaller diameters are associated with faster vortex rotation. Both enantiomers can lead to clockwise and counter-clockwise rotation, which means that the direction of rotation cannot be predicted by choosing one enantiomer over the other, but is instead pre-programmed at the

supramolecular level, as illustrated in Figure 1c-f. Two enantiomers induce the same rotation rates.

Figure 3. Universal character of the revolving chiral structures. **a**, Rotation period T_0 as a function of the structure diameter d for molecular motors Ph-**m** and Me-**m**, and for both clockwise and counter-clockwise rotation. The diameter d is defined experimentally as the diameter of the largest bright ring of the pattern. The solid line refers to an exponential fit. Insets: polarizing microscopy snapshots of revolving chiral patterns obtained with Me-**m** (squares) and Ph-**m** (circles). Scale bars = 20 μm . **b**, Dependence of the structure diameter d as a function of the power of the incident ultraviolet laser beam, where $d_{\min}$ and $P_{\min}$ refer to the smallest stable and static structure. The experimental data are plotted against data extracted from simulations based on a simplified reaction–diffusion model ('with diffusion') and from a purely kinetic model ('without diffusion'), both neglecting the coupling with the twisted three-dimensional liquid crystal structure (see Methods). The diffusion constant is approximated to be the same for all the motors, and is denoted as D_0 .

The diameter of the chiral pattern d depends on the incident reduced power P (Figure 3b). As frustration is released when the pitch magnitude $|p|$ is below the critical value p_c , this means that in the areas where the pattern is

formed, $|p| < p_c$. We can thus estimate the diameter of the structure from the condition $|p(d/2)| = p_c$, and further show, thanks to a reaction diffusion model developed by our collaborators, that the diameters of the patterns that are observed experimentally correspond to diffusion coefficients of the molecular motors $D_0(\text{Ph-}\mathbf{m}) \sim 5.25 \pm 3.75 \cdot 10^{-11} \text{ m}^2/\text{s}$ and $D_0(\text{Me-}\mathbf{m}) \sim 2.35 \pm 1.55 \cdot 10^{-11} \text{ m}^2/\text{s}$. This range of values is in good agreement with literature values²⁷ and thus confirms that diffusion is key in the emergence of these structures.

5.2.4 Role of helix inversion in the stabilization of the vortex

We also demonstrate that the occurrence of helix inversion plays a key role in preserving the integrity of the light-fuelled rotor. For a cholesteric liquid crystal with a composition that does not allow helix inversion, the axisymmetric structure is destabilized into the outwards growth of fingerprints, and the transition to a rotational regime does not occur (Figure 4a,b). Moreover, the diameter of the structure in the case of helix inversion is larger than without, which further indicates that helix inversion promotes radial confinement of the liquid crystal reorientation, as supported by our model (Figure 4c). Indeed, the latter predicts a much steeper pitch increase along the radial coordinate in helix-inverting cholesteric helices, a feature which is associated with a larger elastic energy barrier, and thus makes it

difficult for the chiral pattern to grow. This underpins the role of elastic chiral gradients in the emergence of rotation.

Figure 4. Helix inversion promotes spatial confinement, and thus preserves the integrity of the revolving patterns. **a**, Transient dynamics leading to continuous rotation in the presence of helix inversion (147 s). Scale bars = 20 μm . **b**, Transient dynamics leading to fingerprint pattern formation without cholesteric helix inversion (~75 s). Scale bar = 20 μm . **c**, Simulated dependence of the helix pitch on the distance to the ultraviolet beam axis with d_{inv} and $d_{\text{no-inv}}$ the estimated size of the twisted structures with and without helix inversion, which is derived from the frustration condition $|p(d/2)| = p_c$. Simulation parameters: $p_0 = 16 \mu\text{m}$; $p_\infty = 1.6 \mu\text{m}$; diffusion coefficient, $D_0 = 3.5 \times 10^{-11} \text{ m}^2\text{s}^{-1}$; Gaussian beam power, 20 nW (with helix inversion, i.e. $p_\infty = -1.6 \mu\text{m}$) and 5 nW (without helix inversion, i.e. $p_\infty = +1.6 \mu\text{m}$); Gaussian beam waist, $w = 5 \mu\text{m}$.

The rotation of the vortex can be harnessed into the rotation of a particle-like liquid crystal structure, which behaves like an independent object, of diameter $\sim 30\ \mu\text{m}$ (Figure 5). Its trapping results from the complex elastic landscape that emerges as a response to the inhomogeneous liquid crystal orientation. In contrast to what was observed in other systems⁹, the rotation speed of the cargo particle equals that of the revolving chiral pattern, the latter being only marginally influenced by the presence of the cargo.

Figure 5. Orbital transport of cargo. Off-axis rotational transport of a cargo, here a particle-like liquid crystal structure, being subjected to an orbiting trajectory under the effect of the revolving pattern. The rotation period is ~ 23 min.

5.2.5 Proposed mechanism for vortex rotation

Our experimental findings suggest the establishment of a photo-mechanochemical feedback loop that control the rotation of the vortex. Localized illumination of motor-doped liquid crystal in confinement generates axisymmetric toroid-like structures. As long as the axisymmetry of the supramolecular structure is preserved (Figure 1c,d), the diffusion of chiral motors along gradients of concentrations remains invariant per rotation, and thus it never yields any rotation even when $\mathbf{m}$ and $\mathbf{m}^*$ have

different diffusion rates. In contrast, once axial symmetry is broken and the vortex is formed (Figure 1e,f), the twist of the medium and thus the propagation rate of the motors become angle-dependent. This angular dependence causes a twist gradient, as a result of the long-range orientational order characterizing liquid crystals, in which a local change of twist also affects neighbouring molecules. Reciprocally the new angular dependence also redefines the angular redistribution of the diffusing motors. This mutual dependence leads to the continuous rotation of the twist caused by the molecular motors, which we detect experimentally as a rotating structure, appearing as a rigid-body vortex. In addition, it is the chiral nature of the vortex's structure that provides the directional character to the liquid crystal reorganization.

Overall, we propose that the emergence of rotation is based on a non-local feedback loop that involves the local concentrations of $\mathbf{m}$ and $\mathbf{m}^*$, and their spatial gradients, as well as twist gradients that occur across the liquid crystal structure. Indeed, the diffusion of a chiral species is influenced by the chirality of the liquid crystal in which they diffuse, and vice versa^{28,29}. Therefore, while the spatial distribution of the molecular motors defines the distribution of the pitch, in return, the elastic relaxation and the differential diffusion rates of $\mathbf{m}$ and $\mathbf{m}^*$ define a new chiral landscape (this interplay is illustrated in Supplementary Scheme 1).

5.3 Conclusions

Chirality is key to the emergence of rotation, and appears to be a universal design principle³⁰, as observed in the directional reorientation of passive liquid crystalline media employing unidirectional transfer of optical angular momentum carried by chiral light to achiral liquid crystals^{31,32} or unidirectional Marangoni flows driven by absorption of light in chiral liquid crystals³³. However, the latter systems lack any active molecular component, and therefore they also involve light intensity levels that are at least five orders of magnitude higher than the $\sim 50 \text{ mW/cm}^2$ used here, which is compatible with sunlight illumination. Another classic example of macroscopic dynamics in chiral liquid crystals is the so-called Lehmann rotation where chiral liquid crystals are driven by a gradient of temperature along the helical axis³⁴, and which remains an intriguing phenomenon to date³⁵. The same mechanism yields molecular precession of a chiral liquid-crystalline monolayer spread on a glycerol surface, however this precession remains short-lived³⁶.

By engineering reaction-diffusion processes in a medium characterized by long-range orientational order and taking advantage of large chiral gradients, we demonstrate the emergence of revolving vortices, in a phenomenon that appears as a supramolecular and chiral counterpart of the Belousov-Zhabotinsky reaction. These vortices maintain non-equilibrium conditions at length scales which are typically 10 thousand times larger than

their individual components. Even though we do not know whether the rotation involves fluid flow or if the rotation is a manifestation of the traveling front of the twist of the liquid crystal molecules, these results contribute to unravel the role of chirality in the developing of autonomous molecular systems that are capable of transforming light into work continuously.

5.4 Acknowledgements

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5.5 Materials and methods

5.5.1 General

Materials. Liquid crystal E7 was purchased from Merck. All reagents for the synthesis of Ph-**m**, Me-**m**, and **BB** were purchased from Sigma-Aldrich with the highest degree of purity. **BB** was synthesized reported according to previously reported procedures³⁷.

Characterization of the motors and shape-persistent dopants. Helical twisting powers (HTP) were measured in the nematic liquid crystal E7

(Merck), by using the Grandjean-Cano wedge method. For each motor enantiomer this yielded the following values: (*S,P*)-Ph-**m** $\text{HTP}_{\text{wt}\%} = +92 \mu\text{m}^{-1}$, (*R,M*)-Ph-**m** $\text{HTP}_{\text{wt}\%} = -92 \mu\text{m}^{-1}$, (*R,P*)-Me-**m** $\text{HTP}_{\text{wt}\%} = +46.3 \mu\text{m}^{-1}$, (*S,M*)-Me-**m** $\text{HTP}_{\text{wt}\%} = -65.1 \mu\text{m}^{-1}$. The HTPs of the shape-persistent dopants were (*R*)-**BB** $\text{HTP}_{\text{wt}\%} = -60.2 \mu\text{m}^{-1}$ and (*S*)-**BB** $\text{HTP}_{\text{wt}\%} = +60.3 \mu\text{m}^{-1}$. These values were used in order to prepare cholesteric liquid crystals with adequate photo-responsive behaviour.

Preparation of the photo-responsive cholesteric liquid crystals. One given enantiomer of molecular motor **m** and the **BB** enantiomer with opposite helical twisting power were dissolved in a commercially available liquid crystal E7 (Merck Japan), in proportions that are determined by using the equation $p = \sum_i (\text{HTP}_i c_i \text{ee}_i)^{-1}$, where c_i is the concentration of the i -th dopant, and ee_i its enantiomeric purity (this equation is valid only at low concentrations). By using this dependence of the helical pitch to the concentration of dopants, it is possible to pre-program the handedness of the cholesteric helix under irradiation (Table 1).

For each photo-responsive cholesteric liquid crystal, the value of the pitch was measured before illumination and at the photo-stationary state under uniform illumination with sufficiently high intensity by using the Cano-Grandjean wedge method, with a wedge angle α defined by $\tan(\alpha) = 0.0269$. The handedness of the cholesteric helix was determined by using a known procedure³⁸.

Further, the liquid crystal was placed into a glass cell of thickness $L = 10\ \mu\text{m}$ that consists of two parallel substrates providing perpendicular orientation for the liquid crystal molecules (E.H.C. Tokyo). The relaxation time of the cholesteric helix from p_∞ to p_0 was determined by monitoring the sample between crossed linear polarizers.

5.5.2 Synthesis and characterization

Ph-**m** and Me-**m** were synthesized by Supitchaya Iamsaard following Scheme 1 and Scheme 2 respectively. ^1H - and ^{13}C -NMR (measured for Ph-**m** and Me-**m**) were recorded at 400 and 101 MHz respectively. Chemical shifts are reported as δ (in ppm) in respect to the residual protonated solvent CDCl_3 (^1H -NMR: $\delta = 7.26\ \text{ppm}$, ^{13}C -NMR: $\delta = 77.2\ \text{ppm}$).

Scheme 1. Synthesis of Ph-**m**

Scheme 2. Synthesis of Me-m

2,3-dihydro-1H-cyclopenta[a]naphthalen-1-one (2). Under inert atmosphere 3-chloropropionyl chloride (7.5 ml, 78 mmol) pre-dissolved in dichloromethane (25 ml) was added dropwise to a suspension of aluminum chloride (13 g, 94 mmol) in dichloromethane (100 ml). The mixture was cooled to 0 °C and naphthalene (1) was added (10 g, 78 mmol). After going back to room temperature the mixture was stirred overnight. The mixture was concentrated in vacuo and 50 mL of sulfuric acid was added (96 %). The mixture was heated at 90 °C for 45 min. After cooling to room temperature, the mixture was poured onto 200 g of crushed ice and extracted with diethyl ether and ethyl acetate. The organic fractions were washed with water, sodium bicarbonate (saturated) and brine, dried over sodium sulfate, and filtered. The solution was concentrated under reduced pressure and the compound (2) was purified by column chromatography (hexane/ethyl acetate = 90/10). (Yield 70%).

2-phenyl-2,3-dihydro-1H-cyclopenta[a]naphthalen-1-one (3). Palladium acetate (220 mg, 1 mmol), 2-Dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl (920 mg, 2 mmol) and sodium *tert*-butoxide (60 mg, 5.9 mmol) were dissolved in 15 ml of dried toluene. **2** (520 mg, 3 mmol) and iodobenzene (400 mg, 2 mmol) were dissolved in dried toluene (10 ml) and added into the reaction flask. The mixture was heated under inert atmosphere at 120 °C overnight. After cooling down to room temperature, 100 ml of water was added and the mixture was extracted 3 times with diethyl ether, washed with water, dried over sodium sulfate, filtered and concentrated. **3** was obtained after purification by column chromatography (hexane/ethyl acetate = 95/5) (Yield 70%).

(2-phenyl-2,3-dihydro-1H-cyclopenta[a]naphthalen-1-ylidene)hydrazine (4). **3** (0.290 g, 1.12 mmol) and hydrazine monohydrate (1.8 ml) were dissolved in 1.8 ml of ethanol. The mixture was heated to reflux for 72 h in inert atmosphere. After cooling down to room temperature, 100 ml of water were added and the mixture was extracted 3 times with ethyl acetate, washed with water, dried over sodium sulfate, filtered and concentrated. **4** was purified by column chromatography (hexane/ethyl acetate = 90/10). (Yield 90%).

1-diazo-2-phenyl-2,3-dihydro-1H-cyclopenta[a]naphthalene (7). Under inert atmosphere compound **4** (0.272 g, 1.00 mmol) was dissolved in 10 ml of DMF. After cooling at -30 °C, [Bis(trifluoroacetoxy)iodo]benzene (0.435 g, 1.00 mmol) was added to the solution.

9H-fluorene-9-thione (6). 9-fluorenone (**5**) (0.218 g, 1.21 mmol) and phosphorus pentasulfide (0.490 g, 1.21 mmol) were added in toluene (10 ml) under inert atmosphere and the mixture was heated at 80 °C for 2h. After cooling at room temperature the solution was concentrated under reduced pressure and the residue was purified by column chromatography (hexane/dichloromethane = 9/1).

2-phenyl-2,3-dihydrodispiro[cyclopenta[a]naphthalene-1,2'-thiirane-3',9''-fluorene] (8). **6** was dissolved in DMF (10 ml) and added to the stirring solution of **7** at -30 °C. The solution was cooled to room temperature and concentrated under reduced pressure. The resulting oil was washed with a saturated aqueous solution of ammonium chloride and extracted with ether. The organic phase was washed with water, dried over magnesium sulfate, filtered and concentrated under reduced pressure.

9-(2-phenyl-2,3-dihydro-1H-cyclopenta[a]naphthalen-1-ylidene)-9H-fluorene (9) (Ph-m). The crude compound **8** was dissolved in xylene (20 ml) and heated to reflux overnight with triphenylphosphine (0.529 g, 2.02). The resulting mixture was cooled to room temperature, and iodomethane (0.793 g, 3.03 mmol) was added to remove the excess of PPh₃. After cooling at room temperature, the suspension was removed by filtration, and the solution was concentrated. The residue was purified by column chromatography (hexane/ethyl acetate = 19/1) to give 118 mg of compound **9**. The enantiomers were separated by chiral HPLC on a CHIRALPAK AD-H column using a methanol/ethanol (1:1) mobile phase. The (*S,P*)-Ph-**m** enantiomer was eluted

5 min after start (purity > 99%, enantiomeric excess > 99%), while (*R,M*)-Ph-**m** was eluted after 8 min (purity > 99%, enantiomeric excess > 99%). ¹H NMR (400 MHz, CDCl₃): δ 3.24 (d, ²J_{HH} = 12.0 Hz, 1H, CH₂), 4.00 (dd, ²J_{HH} = 12.0 Hz, ³J_{HH} = 4 Hz, 1H, CH₂), 5.38 (d, ³J_{HH} = 4 Hz, 1H, CH), 6.81-6.89 (m, 2H), 7.11-7.28 (m, 7H), 7.32 (t, ³J_{HH} = 8 Hz, 1H), 7.41-7.54 (m, 4H), 7.78 (d, ³J_{HH} = 8 Hz, 1H), 7.81 (d, ³J_{HH} = 8 Hz, 1H), 7.88 (d, ³J_{HH} = 8 Hz, 1H), 7.96 (d, ³J_{HH} = 8 Hz, 1H), 8.02 (d, ³J_{HH} = 8 Hz, 1H). ¹³C NMR (75 MHz, CDCl₃) δ 146.54, 146.32, 144.10, 140.21, 140.00, 139.65, 137.75, 137.19, 133.18, 132.90, 131.30, 129.17, 129.01, 128.94, 127.72, 127.39, 127.37, 127.36, 127.16, 126.55, 126.28, 125.97, 125.63, 124.55, 123.83, 119.65, 119.21, 56.72, 44.24.

Methyl 2-methyl-3-(naphthalen-2-yl)propanoate (11). LDA was generated by dropwise addition of n-BuLi (2.5 M, 4.15 ml, 10.38 mmol) to a solution of DIPA (1.82 ml, 12.98 mmol) in THF (50 ml) at 78 °C under inert atmosphere. Methyl propionate **10** (1 ml, 10.38 mmol) was added gradually into the solution. The solution was stirred for 1 h at 78 °C. Solution of 2-(bromomethyl)naphthalene (2.75 g, 12.45 mmol) in THF (15 ml) was then added to the flask at -78 °C. The reaction was stirred for about 15 mins before warming up to room temperature and stirring overnight. The reaction was quenched with saturated NH₄Cl (aq). Crude product was extracted with dichloromethane (3 times). The combined organic phase was washed with water, dried over magnesium sulfate and concentrated under reduced pressure. The product **11** was purified by column chromatography (hexane/ethylacetate = 1/49) yielding **11** (0.7446g, 31.41% yield).

2-methyl-3-(naphthalen-2-yl)propanoic acid (12). **11** (0.745 g, 3.262 mmol) was heated to reflux overnight with KOH (2.56 g, 45.66 mmol) in water (13 ml) and EtOH (13 ml) solution. After cooling to room temperature, a solution of 30% HCl (aq.) was added to neutralize the reaction. The resulting mixture was extracted with dichloromethane (3 times). Combined organic phases were then washed with water, dried over anhydrous magnesium sulfate and concentrated under reduced pressure. **12** was purified by column chromatography (hexane/ethyl acetate = 19/1). (76% yield).

hypochlorous 2-methyl-3-(naphthalen-2-yl)propanoic anhydride (13). SOCl₂ (1.93 ml, 26.41 mmol) was added into the solution of acid **12** (0.826 g, 3.855 mmol) in dichloromethane (40 ml) DMF (few drops). The resulting mixture was heated to reflux for 1 h. After cooling the solution at room temperature, the solvent was evaporated. Product **13** was used without further purification.

2-methyl-2,3-dihydro-1H-cyclopenta[a]naphthalen-1-one (14). Aluminum chloride (1.028 g, 7.71 mmol) was added into a solution of acid chloride **13** (3.86 mmol) in dichloromethane (150 ml) at 0 °C. The mixture was stirred for 45 mins. The reaction was quenched with saturated solution of NaHCO₃ at 0 °C. The mixture was extracted by dichloromethane (three times), dried over MgSO₄ and concentrated under reduced pressure. The product was purified by column chromatography (hexane/ethyl acetate = 49/1) to yield **14** (0.571 g, 76 %yield).

(2-methyl-2,3-dihydro-1H-cyclopenta[a]naphthalen-1-ylidene)hydrazine (15). A mixture of **14** (0.5769 g, 2.939 mmol) and hydrazine monohydrate (14 ml) was heated to reflux for 3 days. To the reaction mixture which was cooled down to room temperature, water was added. The resulting mixture was extracted with ethylacetate (three times). The combined organic phases were washed with water, dried over MgSO₄ and concentrated under reduced pressure. **15** was further dried under reduced pressure and directly used in next step.

1-diazo-2,3-dihydro-1H-cyclopenta[a]naphthalene (16). To a stirring solution of hypervalent iodine (5.49 g, 12.77 mmol) in DMF (35 ml) at -30 °C, **15** (12.77 mmol) in DMF (40 ml) was added.

2-methyl-2,3-dihydrodispiro[cyclopenta[a]naphthalene-1,2'-thiirane-3',9''-fluorene] (17). thio-ketone **6** (~12.77 mmol) solution in DMF (20 ml) was added to stirring solution of previous synthetic step (**15**). The reaction was stirred for about 30 mins before allowing to room temperature. To the reaction mixture at room temperature, saturated solution of NH₄Cl (aq) was added. The resulting mixture was extracted with ethyl acetate (three times). The combined organic layers were washed with water, dried over MgSO₄ and concentrated under reduced pressure. Product **17** was dried under reduced pressure and used in the next step without further purification.

9-(2-methyl-2,3-dihydro-1H-cyclopenta[a]naphthalene-1-ylidene)-9H-fluorene (18) (Me-m). A mixture of triphenyl phosphine (6.698 g, 25.54

mmol) and crude product **17** (12.77 mmol) was refluxed in xylene (100 ml) for 3 h. Iodomethane (2.85 ml) was added to the reaction mixture at room temperature and stirred for 1 h. The precipitate was filtered off and washed with hexane. The filtered solution was then mixed with water and extract with dichloromethane (3 times). The combined organic layers were then washed with water, dried over MgSO_4 and concentrated under reduced pressure. After that crude mixture **18** was purified by column chromatography (hexane/ dichloromethane=49/1) yielding **18** (0.573 g, 13.02 % yield). The enantiomers were separated by chiral HPLC on a CHIRALPAK AD-H column using a methanol/ethanol (1:1) mobile phase. (*R,P*)-Me-**m** was eluted 7 min from the start (purity = 89.9%, enantiomeric excess > 99%) and (*S,M*)-Me-**m** was eluted at 12 min (purity = 96.5%, enantiomeric excess > 99%). ^1H NMR (400 MHz, CDCl_3) δ 1.40 (d, $^3J_{\text{HH}} = 8$ Hz, 3H, CH_3), 2.80 (d, $^2J_{\text{HH}} = 16.0$ Hz, 1H, CH_2), 3.58 (dd, $^2J_{\text{HH}} = 16.0$ Hz, $^3J_{\text{HH}} = 4$ Hz, 1H, CH_2), 4.37 (m, 1H, CH), 6.74 (d, $^3J_{\text{HH}} = 8$ Hz, 1H), 6.82 (t, $^3J_{\text{HH}} = 8$ Hz, 1H), 7.23 (t, $^3J_{\text{HH}} = 8$ Hz, 1H), 7.33 (t, $^3J_{\text{HH}} = 8$ Hz, 1H), 7.39-7.43 (m, 2H), 7.48 (t, $^3J_{\text{HH}} = 8$ Hz, 1H), 7.77- 7.79 (m, 2H), 7.85-7.89 (m, 1H), 7.95 (t, $^3J_{\text{HH}} = 8$ Hz, 2H), 7.98-8.03 (m, 1H). ^{13}C NMR (101 MHz, CDCl_3) δ 151.30, 147.58, 140.22, 139.95, 139.68, 137.26, 136.51, 132.77, 131.01, 130.57, 129.97, 128.80, 127.65, 127.07, 127.05, 127.00, 126.72, 126.10, 125.94, 125.45, 124.22, 124.16, 119.81, 119.08, 45.67, 42.14, 19.47.

5.6 Supplementary data

Supplementary Figure 1. Chiral molecules used as helicity-inducing dopants. Enantiomeric pairs of molecular motor Ph-m (a,b) and Me-m (c,d), and enantiomeric pair of photo-persistent bridged binol derivative BB (e,f). The helical twisting powers (HTP) are measured for a single species of dopant in the nematic host E7 and reported in percentage of molar weight. In all cases, motors of the upper (respectively, lower) panel where mixed with the photo-persistent enantiomer from the upper (respectively, lower) panel too.

Supplementary Figure 2. Experimental setup. BS: beam splitter. The lens L is used to focus the laser beam on the frustrated slab of cholesteric liquid crystal. The sample is constituted by frustrated liquid crystal in the glass cell. P_{in} and P_{out} : crossed linear polarizers; Camera: Thorlabs DCC1645C CMOS camera.

Sample	Concentration of dopants	Pitch values		Pitch relaxation time (min)
		p_0 (μm)	p_∞ (μm)	
A*	(S,P)-Ph- m 0.83 % (R)- BB 0.99 %	+16.0	-1.7	20
B*	(S,P)-Ph- m 0.83 % (R)- BB 0.99 %	+16.0	-1.7	20
C	(S,P)-Ph- m 0.83 % (R)- BB 0.99 %	+16.0	-1.7	20
D	(S,P)-Ph- m 0.55 % (R)- BB 0.73 %	+11.4	-2.0	16
E	(R,M)-Ph- m 0.55 % (S)- BB 0.73 %	-12.4	+1.7	16
F	(S,P)-Ph- m 0.41 % (R)- BB 0.55 %	+15.7	-1.4	15
G	(R,P)-Me- m 1.05 % (R)- BB 0.71 %	+22.1	-1.2	9
H	(S,M)-Me- m 0.61 % (S)- BB 0.51 %	-15.6	+1.5	5
I	(R,P)-Me- m 1.44 % (R)- BB 0.82 %	+10.8	-1.7	6
J	(S,M)-Me- m 0.57 % (S)- BB 0.53 %	< -40	+2.9	8
K	(S,M)-Me- m 1.10 % (S)- BB 1.11 %	+15.0	+1.5	20

Supplementary Table 1. Description of all photo-responsive cholesteric liquid crystals designed for the study. Sample name, preparation date and molecular composition, pitch values before UV exposure (p_0) and after photo-stationary state is reached (p_∞), and the relaxation time of the cholesteric helix from p_∞ to p_0 when irradiation stops. * Samples A and B refer to two distinct cells prepared from the same mix. Samples A to J present helix inversion between the initial and the photo-stationary state ($p_0 p_\infty < 0$) while sample K is a non-inverting cholesteric liquid crystal ($p_0 p_\infty > 0$). In the main text, Figure 1, Figure 2 and Figure 5 show data from sample A. Figure 4 shows data from sample A and from sample K.

Supplementary Figure 3. Light-induced modifications in the spatial distribution of the molecular motors. Calculated radial profiles for the normalized concentrations of $\mathbf{m}$ and $\mathbf{m}^*$, namely c/c_0 and c^*/c_0 , from the simplified reaction-diffusion model in the case of Gaussian beam with power $P \sim 2.5P_{min}$, which corresponds to $\tilde{I}(r=0) \sim 1700$. Two situations are shown: with diffusion (solid lines) and without diffusion (dash-dotted lines).

Supplementary Scheme 1. Schematic representation of the interplay between physical and chemical parameters. Quantitative models to describe this phenomenon would involve two coupled partial differential equations describing the reaction-diffusion processes for the concentrations $c(\mathbf{r}, t)$ and $c^*(\mathbf{r}, t)$ of $\mathbf{m}$ and $\mathbf{m}^*$ respectively, with $\mathbf{r}$ the position and t the time. Namely, $\partial_t c = \nabla \cdot (D \nabla c) + R(c, c^*)$ and $\partial_t c^* = \nabla \cdot (D^* \nabla c^*) + R^*(c, c^*)$. In these equations, $D(\mathbf{r}, t)$ and $D^*(\mathbf{r}, t)$ refer to the diffusion constant of the motors, that depends on their position, and R and R^* are the local reaction terms that respectively account for the effect on $\mathbf{m}$ and $\mathbf{m}^*$, of the photo-excitation of $\mathbf{m}$ into $\mathbf{m}^*$, and on the thermal relaxation of $\mathbf{m}^*$ into $\mathbf{m}$. These equations would further be coupled to the liquid crystal. Under local illumination, the rotation involves inhomogeneous reaction and diffusion processes in the liquid crystal, that is characterised by long range orientational order. At the molecular level, the concentration c and c^* of the motors $\mathbf{m}$ and $\mathbf{m}^*$ are driven by the local

illumination, and by the specifics of the thermal relaxation. The resulting director field n (orientational degree of freedom) and flow field v (positional degree of freedom) at the microscopic level couple in a nonlocal manner to the concentrations fields via inhomogeneous differential diffusion rates D and D^* of the motors that depend on the liquid crystal pattern in which they diffuse.

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Chapter 6

Re-directional motile behavior in spiral droplets

Converting chemical energy into motion is essential to all forms of life. In unicellular organisms, it is the emergence of biomolecular machines that has arguably supported the evolution towards autonomous and purposeful motion. However, how uniquely directional motility can emerge from inanimate matter remains unclear. Here, we demonstrate cell-sized chiral droplets that propagate in water persistently, and trace the threads of a screw. Under illumination, these droplets re-orient in a direction that is determined by their chiral shape, and, significantly, the re-direction is guided by the chiral operation of artificial molecular motors. This motile behavior shows analogy with the helical motion and chirality-derived orientation behavior that aqueous micro-organisms have evolved. Our findings demonstrate that dynamic control over chirality and flow can be operated by molecular machines, and they add to a general understanding of how asymmetric molecular operations can be transferred across length scales to regulate sophisticated motion in living systems.

6.1 Introduction

Evolving active and purposeful motion has likely been essential to the emergence of life because it allows overcoming the inherent limitations of diffusive motion with respect to feeding, growing and multiplying. In swimming cells and aquatic micro-organisms, active and directional motility is supported by biomolecular machines, for example it is the flagellar motor of the *E.coli* bacterium that steers its motion, with chirality a salient feature in its operation.^{1,2} Chemists have succeeded in designing and engineering many classes of molecular machines and have harnessed asymmetry and chirality to drive their operation from the molecular level upwards.^{3,4,5,6,7,8} Recently, synthetic molecular machines were interfaced with soft matter to achieve complex macroscopic motion such as twisting springs,⁹ rotating patterns,¹⁰ and a pulsing vortex¹¹. However, in these examples the motion driven by molecular machines sees the center of mass of the object fixed, or in other words the molecular motion is not converted into macroscopic motility.

Simple shape transformations will not overcome the challenge of moving a microscopic object forward because movement at small scales requires a shape transformation of the body that breaks a time-reversal invariance.¹² Chirality thus plays a major role in motility below the microscale, and indeed, unicellular organisms typically rely on dynamic helix-based geometrical transformations in order to move autonomously. *E. coli* uses rotating helical flagella bundles to swim,¹ while the helix-shaped body of *H.*

pylori and *C. jejuni* mediate their efficient helical trajectories in challenging media¹³. Besides relying on chiral body shape transformations, the movement of most aquatic microorganisms and swimming cells demonstrate helical motion, including zooplankton, ciliates and bacteria. The evolutionary advantages for helical motion remain unclear, however this motile behavior is associated with a higher degree of directionality, in other words the directionality of helical trajectories is less sensitive to extracellular and intracellular random variations.¹⁴ Moreover swimming in a screw-like fashion offers opportunity for efficient re-orientation behaviors set to follow chemical and physical gradients.¹⁵ These chirality-derived re-orientation mechanisms can be based on biased random walk, such as the well-known ‘run-and-tumble’ of *E.coli*.¹⁶ However, less known is that deterministic directional changes are also frequent. In the ‘run, reverse and flick’ behavior of *Vibrio alginolyticus*, a marine bacterium that also moves along helical pathways, the flick angle is pre-programmed, *i.e.* the re-direction is deterministic, instead of occurring randomly.^{17,18} Another example is helical klinotaxis, that allows cells to sense their three-dimensional environment efficiently.¹⁹

A large number of different micro-objects have been synthesized that display chemotactic^{20,21} and phototactic behavior²² when moving in water, however the motile behavior of these synthetic ‘micro-swimmers’ has yet to demonstrate the sophistication of living systems, and in particular, these systems lack any active re-directional mechanism.²³ In many examples, the

catalytic decomposition of hydrogen peroxide leads to erratic motion,²⁴ with a notable lack of control over the rotational degree of freedom. Recent work reported that helical trajectories are obtained for droplets of chiral liquid crystals through the coupling between the internal chirality of the droplet and a gradient in surface tension at the interface with water, which generates a chiral flow that pinches the droplet forward in a screw-like movement.²⁵ However the correlation between the chirality of the droplets and their helical motion was unclear, and overall, the rules of artificial motile behavior in fluids remain elusive.

Here, we show that light-driven molecular motors can be incorporated in liquid crystal droplets, in order to endow these rudimentary micro-compartments with a helical motion and photo-responsive orientational behavior. Key to the mechanism that translates the operation of the molecular motors is the possibility to control the chiral flows generated by the interplay between the chiral shape of the droplets, and gradients in their surface tension—these gradients occur because micelles of surfactant in water solubilize small quantities of the liquid crystal in the droplet. The transfer of chirality involves a cascade of bottom-up events: i) the helical aspect of the molecular motors is converted into the helical organization of a chiral liquid crystal, ii) upon confinement in the form of a droplet, the liquid crystal adopts a spiral organization, and iii) as the liquid crystal is solubilized in the environment, gradients in surface tension are created, a chiral flow of fluid

matter emerges at the droplet/water interface, and this chiral Marangoni effect moves the droplet forward, along a helical path (Figure 1).

Figure 1. Chirality transfer in droplets of motor-doped chiral liquid crystals. The helicity of the molecular motors is amplified across increasing length scales, into the spiral organization of a liquid crystal droplet in water. The handedness of the spiral organization mirrors that of the liquid crystal phase, which is determined by the molecular motor. Upon the micelles of surfactant solubilizing some of the liquid crystal from the droplet, an instability is formed, and the droplet is pushed forward by a chiral Marangoni flow. The movement of the droplet traces the threads of a screw, with a handedness opposite to that of the spiral droplet. Using light, it is possible to control the chiral shape of the molecular motor, thus the chiral shape of the spiral droplet, and eventually the helical motion. Scale bar = 50 μm .

6.2 Results and discussion

6.2.1 Chirality-derived motility in spiral droplets

Overcrowded alkenes were used as light-driven molecular rotary motors (Me-**m** and Ph-**m**, Figure 2). Molecular motors are unique photo-responsive dopants for liquid crystals because their light-induced operation is known to induce a large-scale, precise, and reversible winding or unwinding of the cholesteric helix,²⁶ but they also combine these large pitch changes with the possibility of inverting the helical handedness.^{27,28} The asymmetric operation of the motor is amplified in nematic liquid crystals, thereby turning it into a cholesteric liquid crystal, with a helix-based organization (Supplementary Figure 1). The molecular motors twist the liquid crystal host molecules with a certain efficiency, known as Helical Twisting Power (HTP). The helical pitch, is defined as twice the geometrical period of the helix, and by the helical handedness (Figure 1).

Droplets of photo-responsive cholesteric liquid crystals were prepared with a typical diameter of 20 μm , by injection in an aqueous environment enriched in surfactant (tetradecyl-trimethylammonium bromide, TTAB). The excess surfactant is known to solubilize the liquid crystal, which is taken up by the surfactant in the form of filled micelles.²⁹ In order to prevent floating or sinking of the droplets, we used a mixture of water and D₂O as the aqueous phase to match the density of the liquid crystal.

Once the droplet is formed, it is pushed forward by the flow. The movement lasts 7-8 minutes and covers a total distance of several millimeters. The mechanism is understood as follows: the solubilization of the liquid crystal as mediated by the surfactant creates an instability, and the gradients in surface tension that are associated with this instability initiate a flow around the droplet, that is known as a Marangoni flow. This flow effectively pinches the droplet forward, and triggers its motion.^{25,29} In previous work, we demonstrated that when the liquid crystal molecules orient perpendicularly at the interface with water, droplets of cholesteric liquid crystals organize into a spiral (Figure 1).³⁰ Here we show that the spiral shape of these droplets mediates a non-equilibrium cross-coupling between a gradient of interfacial tension and a chiral flow inside the droplet, that results in helical motion (Figure 2a,b). The handedness of the motion is constant over time, and is always opposite to that of the liquid crystal helix (Figure 1). Chirality thus plays an essential role in controlling and regulating flows in and around fluid droplets, and in defining the geometry of their motile behavior.

Specifically, the period of the liquid crystal helix defines the geometry of the helical motion. Droplets of cholesteric liquid crystals doped with Me-**m** so as to induce a pitch of 4.3 μm , initially move in an erratic manner (Figure 2c). Over time, they transition into helical motion. This evolution is ascribed to the fact that Me-**m** is not taken up by the surfactant micelles, so over time its concentration in the shrinking droplet increases, consequently the pitch decreases, and the spiral gets tighter. The shrinking of the droplets thus

competes with the spiral tightening that comes from the motor increasing its concentration over time. The chirality confinement ratio $N = 2d/p$, where d is the diameter of the liquid crystal droplet and p is the pitch of the cholesteric helix, was used earlier by theoretical³¹ and experimental studies,³² and is a measure of the tightness of the spiral in the droplet, corresponding to the number of spiral turns. Consistently, in all experiments, we found that helical motion occurs only above a threshold in the $12 < N < 14$ range (Figure 2d). Below that threshold, the droplets lose their spiral character, and their motile behavior transitions to ballistic, or even erratic. Above that threshold, the chirality is successfully transmitted as torque on the droplet.

We used a non-photoactive chiral dopant to induce the spiral droplets (CB15, Figure 2c), in order to further investigate the influence of confinement on the helicity of the autonomic motion. With droplets of pitch ca 1.4 μm ($N = 22$), in contrast to what was observed with Me-**m**, the droplet starts moving in a helical fashion as soon as it is produced, and without any initial erratic movement (Figure 2d). Over time, the droplet shrinks in size and the number of spiral turns decreases until movement starts along an overall straight trajectory, with no control over the rotational motion. We explain this rectilinear motion by considering that CB15, is taken up by the micelles at the same rate as the liquid crystal is, because its structure is similar to that of the liquid crystal. As a consequence, the concentration of chiral dopant CB15 in the droplet remains constant over time, the pitch also remains constant, and therefore, the confinement ratio N decreases over time due to the

progressive reduction in droplet size. In this case, the threshold value of N below which there is no helical motility was found to be $N = 13$, *i.e.* it is consistent with the threshold value obtained with the previously described experiments involving Me-**m**. We conclude that this threshold in chiral aspect is general to all systems we have investigated.

A final confirmation for this chirality-derived mechanism is provided by spiral droplets of a liquid crystal that incorporates Ph-**m**. As discussed above for Me-**m**, the structure of the molecular motor Ph-**m** is such, that it is not leaking away from the droplet, while the liquid crystal is. As Ph-**m** winds the cholesteric liquid crystal tighter than Me-**m**, it is possible to form droplets that move helically right after they are produced, *i.e.* there is no need to wait for the dopant to concentrate in the spiral droplet in order to induce a transition from rectilinear to helical motion. As the concentration of Ph-**m** in the spiral droplet increases over time, the droplet rapidly becomes isotropic at room temperature, and the movement becomes erratic (Figure 2d).

We were also able to prove that the spiral pattern of the droplet determines the period and the radius of the motion (Figure 2b). The period and radius of the helical trajectories evolve differently with the confinement ratio N (which also evolves over time, as noted above). The pitch of the helical trajectory increases when the confinement ratio in the droplets increases, until it reaches a plateau at $N \approx 18$. The radius of the helical trajectory increases linearly with N . Increasing the chirality of the system thus causes

an increase in the torque within the droplet, and the trajectory of the droplet follows a helix with a larger radius (Figure 2e).

Along a helical trajectory, the average speed of a droplet of 20 μm in diameter is ca 18 $\mu\text{m/s}$. In comparison, *E. coli* with a cell body of typically 2-3 μm swims at a speed of 20-40 $\mu\text{m/s}$. As mentioned above, however, the spiral droplet is shrinking while it moves, and therefore the speed of the droplet is not constant over time. Specifically, we observe that the speed of a given droplet decreases exponentially over time (Figure 2f). Having plotted the speed of the droplets with respect to their size, we conclude that large droplets move faster (Figure 2g). An increase in speed with droplet size was reported earlier, albeit in isotropic oil droplets and only in specific conditions of surfactant concentration.³³

Figure 2. Chirality-derived movement in spiral droplets. **a**, Two dimensional trajectory of droplet of 5CB doped with Ph-**m** demonstrating active helical motion with time-lapse of the droplet along the trajectory. The spiral organization is visible as a pattern on the droplet. **b**, Schematic representation of a left-handed spiral droplet moving with a right-handed helical trajectory. The geometry of the motion is characterized by its radius (R , in red) and period (P , in blue). **c**, Structure of the

chiral molecular dopants used to induce the cholesteric phase in the liquid crystal droplets. CB15 is a non-active chiral dopant, hence the cholesteric helix remains unchanged by illumination. Me-**m** and Ph-**m** are both photo-active chiral dopants, with the capacity to invert the handedness of the liquid crystal phase upon illumination. Ph-**m** induces a larger twist in the liquid crystalline structure (Ph-**m** has a larger HTP). **d**, Evolution of the chirality confinement ratio N in time, for droplets of liquid crystal doped with CB15 (green square, initial pitch = 1.5 mm), Me-**m** (cyan circles, initial pitch = 4.3) and Ph-**m** (blue triangle, initial pitch = 1.6 mm). For Me-**m** and Ph-**m** after a certain time the concentration of dopant increases and the liquid crystal becomes isotropic. The solid lines are a guide for the eye. A typical evolution of N in time is shown for each dopant. **e**, Dependence of radius (R , red continuous line) and period (P , blue dashed line) from the chirality confinement N . The colored area represents the standard deviation. The motion was investigated for droplets doped with CB15. **f**, Time evolution of the speed for a cholesteric droplet doped with CB15 with initial diameter of ca. 19 μm . **g**, Speed of motile droplets as a function of their diameter, acquired both before and during motion.

6.2.2 Re-directional motile behavior

We have shown in earlier work that the operation of molecular motors inverts the cholesteric helix and therefore the spiral organization of the droplet also inverts actively under illumination.³⁰ As discussed above, the spiral droplets move along a helical path with a handedness that is opposite to that of the spiral. Remarkably, when the handedness of the spiral was allowed to invert rapidly (ca. 1 s), the droplets immediately initiated a significant re-direction in three dimensional space (Figure 3a). This photo-induced reorientation accompanied by the inversion of handedness of the trajectory is reminiscent of the motion of *C. reinhardtii*, a single-cell algae that is known to perform photo-taxis by helical klinotaxis for which the directional change is determined by the light intensity.¹⁵

Here, the change in the directionality of the spiral droplets was studied by tracking the run and tumbling of the droplets in three dimensions.. Measuring the angle θ between the direction before irradiation, and that after irradiation (Figure 3b,c) allows concluding that the sharp change in the direction of motion occurs within a cone of probability (Figure 3d). Upon analysis of trajectories performed by Me-**m** and Ph-**m** doped droplets (Supplementary Figure 2,3) it appeared that the angle of redirection was defined by the experimental conditions under which the spiral droplets were undergoing helix inversion, *i.e.* this helix inversion would always be triggered by us when the spiral droplets had similar values of confinement ratio $N \approx 32$ (Supplementary Figure 4). Correlating the value of this confinement ratio, at the moment of the chiral inversion, with the angle θ , allows confirming that this deterministic behavior is determined by the chiral shape of the spiral droplet. Specifically, larger values of N result in a larger photo-stimulated redirection. Realizing that the angle between the droplet axis, and the direction of the helical motion, is fixed during the motion provides additional insight into this re-directional behavior.³⁴ Upon inversion of chirality, the south-north axis of the droplet reconfigures differently, and, since its angle with the helical trajectory is fixed, the new direction is inherently dictated by this reconfiguration. Furthermore, the angle between the droplet axis and the trajectory is dependent on the period and radius of the motion, which is itself controlled by the chirality confinement as shown in Figure 2d (Supplementary Figure 5). The interplay

between these relations defines the angle of the photo-induced re-orientation.

Figure 3. Re-directional motile behavior of the photo-responsive spiral droplets. **a**, Three dimensional motion of a droplet incorporating Me-**m** motor. Upon rapid helix inversion, the spiral droplet operates a sharp change in the direction of movement. **b**, A sharp directional change is operated by the spiral droplets upon helix inversion. The re-direction occurs within a cone. **c**, Two dimensional trajectory of a spiral droplet that undergoes directional change upon abrupt helix inversion. The angle θ is defined as the angle between the helical axis of motion before illumination (black arrow) and after illumination (red arrow). **d**, Number of events of directional change associated with a given value of θ , for an average value $N \sim 32$.

The mean experimental value of θ is ca. 60 degrees. **e**, Dependence of θ from the chirality confinement ratio N . The angle of photo-stimulated re-direction increases with N (the number of twists in the spiral droplet).

We have also investigated the specifics for the re-orientation of the spiral droplets when the chirality inversion occurs slowly, *i.e.* when a lower intensity of irradiation is used. When the irradiation conditions are such that helix inversion occurs in ~ 8 seconds, the liquid crystal is allowed to transition in the pseudo-nematic state. During this time the droplet loses its spiral aspect, and the helical trajectory is thus allowed to collapse on its axis. We observe that this achiral organization is associated with a straight, rectilinear motion (Figure 4). The average value of the angle between the helical trajectory and the rectilinear trajectory was found to be 21 degrees, thus consistently smaller than the redirection angle that is associated with a sharp helix inversion (Figure 4). As the chiral information is lost, directionality is also lost, and the motile behavior loses its deterministic re-directional character.

Figure 4. Influence of the chiral shape of the droplet on the geometry of their motion. Three dimensional motion of a droplet under the operation of molecular motor Me-m. When the light power is sufficiently low, the helical trajectory unwinds slowly, becomes rectilinear, and then rewinds with helix inversion. The insets show the shape of the droplet during motion, and evidence that rectilinear motion is associated with the disappearance of the spiral feature. The direction of propagation is not modified significantly upon transition from helical to rectilinear motion.

6.3 Conclusions

We have demonstrated unprecedented sophistication in the motile behavior of synthetic micro-compartments in water. In our system, molecular motors are at the core of a mechanism that steers the helical movement, and guides a light-induced re-orientation. The mechanism of reorientation involves transmission of chirality towards higher levels: the chirality of the molecular motors is converted into the spiral organization of droplets, which is in turn transformed into helical motion that responds to photo-stimulated changes in period and handedness. This chirality-derived mechanism shows similarities with the rules that govern the motile behavior of unicellular

organisms¹⁴ such as bacteria and the malaria parasite,³⁵ as well as chiral neuronal motility.³⁶ The system constitutes a generalizable example where controlling chirality artificially results in directionality, and thus unveils the contribution of chirality to the rules of autonomic movement.³⁷ In a context where accumulating evidence suggests that cells are intrinsically chiral and that the cytoskeleton and motor proteins steer their chiral motility,³⁸ our work contributes to a chemo-physical understanding of how molecular torques can amplify chiral morphologies into chiral motility, and ultimately into the functional lateralization of cellular assemblies at higher levels. Future motile micro-compartments and protocells will gain directionality by taking advantage of chirality-derived mechanisms such as the one we describe and, in the longer term, coupling the operation of molecular machines with the re-directional motility of micro-compartments will provide new opportunities for sophisticated motile sensing.

6.4 Acknowledgements

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6.5 Materials and methods

Materials. The nematic liquid crystal 5CB and the shape-persistent chiral dopant CB15 were purchased from Merck and used as received. TTAB (tetradecyl-trimethylammonium bromide) was purchased from Wako. The light-driven molecular motors Ph-**m** and Me-**m** were synthesized *in-house*.¹¹

Preparation of liquid crystal mixtures and characterization. Chiral dopants (CB15, Me-**m** or Ph-**m**) were weighed in glass vials and then the desired volume of 5CB was added to the dopant. The content of the vial was heated above the isotropization temperature, above which the opaque material becomes fully transparent. The isotropic mixture was stirred at 40 °C and was let to cool to room temperature. The cholesteric liquid crystal was then introduced in a glass wedge cell (E.H.C. Co.) and its pitch was measured using the Grandjean Cano method, with a polarized optical microscope (Olympus BX51). The pitch of light-responsive liquid crystals doped with Me-**m** and Ph-**m** was measured in the dark, and subsequently at the photostationary state, by using $\lambda = 365$ nm collimated light from an LED (Thorlabs), until no further change in texture was observed.

Cholesteric droplets production and propulsion experiments. The cholesteric liquid crystal was injected with a microinjector (Femtojet, Eppendorf), using commercially available needles (Femtotips, Eppendorf) in an aqueous (25 wt % D₂O) surfactant solution (13 wt %, TTAB). The chamber containing the aqueous solution was prepared by applying an adhesive spacer (≈ 1 mm in thickness) onto a microscope slide. After injection, the

chamber was closed with a glass coverslip. The size of the droplets was tuned by changing the injection pressure and injection time of the Femtojet.

The motion of the spiral droplets was imaged with an inverted bright field microscope (DMI6000B, Leica). The time-lapse images were acquired using a CMOS camera (HXG40, Baumer, 10 fps). During motion, the focus on the droplet was adjusted manually. The XY coordinates of the XY stage (TANGO Desktop, Märzhäuser Wetzlar) and the Z coordinate of the motorized Z drive of objective lens (x20, Leica) were recorded using Labview. The light-responsive spiral droplets were allowed to move over at least 2 periods of the helical trajectory, then they were irradiated using high intensity (300 mWcm^{-2}) or low intensity (50 mWcm^{-2}) collimated UV light (365 nm). The intensity of the light was measured using a power meter PM-100D (Thorlabs).

Video analysis. The videos were analyzed using a custom written program in python.²⁵

6.6 Supplementary data

Supplementary Figure 1. Characterization of liquid crystal mixtures used for droplets production and propulsion. **a**, Structure of nematic liquid crystal 5CB used as host in all the mixtures. **b**, **c**, **d**, Polarized optical microscopy images of wedge cells containing liquid crystal mixtures doped with CB15 (10 wt %), Ph-**m** (0.68 wt %) and Me-**m** (0.38 wt %) respectively. The Grandjean-Cano lines are visible and were used to measure the pitch of the cholesteric phase. For **c** and **d** the image on the right corresponds to the liquid crystal at the photo-stationary state (inverted handedness), for which the pitch is also measured.

Supplementary Figure 2. 3D trajectories of the propulsion with abrupt helix inversion and directional change for droplets doped with Me-m. The red asterisks mark the switching event.

Supplementary Figure 3. 3D trajectories of the propulsion with abrupt helix inversion and directional change for droplets doped with Ph-m. The red asterisks mark the switching event.

Supplementary Figure 4. Probability plots. **a**, Normal probability plot for the angle θ in radian. **b**, Normal probability plot for the chiral confinement ratio N at the moment of irradiation with high intensity of UV light.

Supplementary Figure 5. Dependence of θ , period and radius of propulsion on the chirality confinement. The relative radius (R/d , where d is the diameter of the droplet) and the angle θ increase with the increase in chirality confinement ratio N . The relative pitch (P/d , where d is diameter of the droplet) decreases with an increase of N .

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