Light-responsive self-assembly towards supramolecular machines

Supaporn Kwangmettatham

LIGHT-RESPONSIVE SELF-ASSEMBLY TOWARDS SUPRAMOLECULAR MACHINES

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DISSERTATION

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the degree of doctor at the University of Twente,
on the authority of rector magnificus
prof. dr. T. T. M. Palstra,
on account of the decision of the graduation committee,
to be publicly defended
on Thursday June 7, 2018 at 12.45 h

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I'm basically an organic chemist...

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Chapter 1

General introduction

1.1 Introduction

“...Despite the fabulous advances in science and engineering over the past century, manifested most clearly by modern aircraft, we are nevertheless humbled by the realization that we still cannot synthesize a bird, a single cell of the bird or even one of its complex biological machines.” Ben L. Feringa (2017)¹

Molecular machines are systems in which a stimulus triggers the controlled motion of one molecular or submolecular component relative to another and potentially results in a net task (or work) being done.² In cells, biological molecular machines are key elements to maintain life,³ as they support a plethora of functions, ranging from cargo transportation, processive synthesis of proteins, to the generation of directional forces that drive mechanics of cell. The perspective of generating forces in artificial molecular systems opens new avenues of research to achieve a high degree of functionality, in a process that could be compared to what man-made materials have achieved with aircrafts that mimics flying birds, or with computers that mimic brain functions.^{4,5}

The sophistication reached by organic chemistry has now enabled the design and synthesis of many dynamic molecules that display controlled shape changes with an ever-increasing degree of sophistication,⁶⁻⁸ including molecular pincers⁹, ratchets,¹⁰ rotors,¹¹ pumps¹² and switches¹³ or even molecular assembly lines capable of synthesizing peptides.^{14,15} However, as movement of molecular machines has been mastered primarily in solution, harnessing their mechanically-relevant motion to produce work remains a major challenge. This is mostly because in the absence of any

cooperative behavior, the operation modes of molecular machines remain localized to the nanoscale. How to integrate synthetic molecular machines into hierarchical assemblies so that they can generate directional forces thus remains a major contemporary challenge. In this manuscript, we contribute to this challenge by drawing inspiration from cellular microtubules.¹⁶⁻¹⁸

Cellular microtubules are self-assembled tubes that can generate pushing or pulling forces by assembly or disassembly. They are self-assembled out of tubulin proteins, and therefore they can be considered as biological supramolecular machines.¹⁹ In this regard, we propose to move up in length scales, moving from synthetic molecular machines to “synthetic supramolecular machines”, in which light-responsive units are integrated into finite supramolecular entities. We anticipate that noncovalent synthesis will potentially facilitate transferring and amplifying the generated forces across increasing length scales, and eventually facilitate the production of mechanical work.²⁰

1.2 Aim and outline

The aim of this research is to create light-controlled supramolecular machines that can generate force, and eventually mechanical work. Drawing inspiration from cellular microtubules, the work involves a special focus on supramolecular tubular systems. The strategy is based on integrating molecular photo-switches (molecules that can be reversibly shifted between two or more stable states), such as azobenzenes (Chapter 3-4) and spiropyrans (Chapter 5-6), into amphiphilic molecules that are self-assembled in water. Upon irradiation with light, the molecular switch converts into its photo-isomer, which has a different geometry and possibly also different properties, resulting in macroscopic changes in the supramolecular structures.

In **Chapter 2**, a literature review is presented on the design and synthesis of stimuli-responsive tubular systems, with a special focus on analogies with the working principles of cellular microtubules.

Chapter 3 demonstrates the integration of azobenzene molecular switches into self-assembled tubular structures that convert light into tubular strain. The accumulation of strain leads to disassembly of tubules, along a complex mechanism that is reminiscent of the mechanism by which cellular microtubules pull chromosomes apart, during the cell division process.

In order to convert the force produced by these wholly artificial tubules into useful mechanical work, **Chapter 4** presents a strategy to interface these supramolecular machines with surfaces, by modifying their external surface. This modification of the tubular shell is achieved by co-assembling the original building blocks (the tubules from chapter 3) with new building blocks that contain a biotin linker. Further, the biotin-streptavidin interaction allows interfacing the tubules with a surface, while preserving their original dynamic properties.

Spiropyran photo-switches are used as a key components to control self-assembled morphologies in chapter 5 and 6. **Chapter 5**, focusses on the use of light to induce the transient expansion of vesicles. These vesicles are self-assembled out of spiropyran amphiphiles and, upon converting the merocyanine open form into the spiropyran closed form, the vesicle expands, reversibly, following an original mechanism based on self-limiting growth.

Chapter 6 deals with mimicking the out-of-equilibrium self-assembly of cellular microtubules. The design involves the use of spiropyran photoswitches that accommodate changes of amphiphilicity of the building blocks. Transient generation of the self-assembling isomer is anticipated to facilitate out-of-equilibrium self-assembly upon irradiation with light.

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Chapter 2

Artificial tubules towards supramolecular machines

Molecular machines lie at the heart of biological processes, and thus they have inspired chemists for decades. While extensive research on synthetic molecular machines has been performed, harnessing the work they generate into complex mechanical functions remains an open challenge, that will require integrating these machines, motors and switches, into complex systems where function can be emerge. Among other functions, the generation of measurable and directional forces holds special interest, such as those cellular microtubules generate when pulling chromosomes apart during the cellular division, or when pushing through plasma membrane in damaged cells. The versatility of microtubular operation in the cell sets the stage for the potential their artificial counterparts would hold. Building microtubule-like function in synthetic systems would open up the possibility to produce directional measurable forces that can transfer across multiple length scales and produce useful work. In this introductory chapter, we provide a literature overview of recent advances on designing artificial supramolecular tubules that respond to external stimuli.

2.1 Introduction

The cell is the basic structural and functional unit of living organisms. Enormous activities in cell keep running endlessly as long as life exist, at out-of-equilibrium, by the key components “*molecular machines*” (e.g. swimming motion of bacteria by bacterial flagellar motor, translation and synthesis of proteins by ribosomes, transporting of cargoes by kinesin motor proteins, moving of cells and generating of forces by microtubules, etc.).¹⁻⁷ Now the structures, functions of individual components, and even entire biochemical pathways have been elucidated.⁸⁻¹¹ The versatility of these dynamic systems and the large number of functions supported by biological molecular machines have inspired chemist for decades,^{2,12-16} and the versatility, usefulness, precision of the tasks performed by biological molecular machines set the stage for wholly artificial, complex, dynamic and out of equilibrium systems that would be driven by molecular motors, switches and rotors. Incorporating such dynamic molecules into synthetic systems with a high degree of functionality would set the stage for synthetic supramolecular systems to reach an unprecedented level of complexity, by achieving dynamic, directional function in far from equilibrium conditions, and thus entering length scales at which cellular machineries operate typically, a realm that no man-made technology has reached before.

Recently, synthetic molecular machines have been reported, that display a similar unidirectional rotation as motor proteins,¹⁷⁻¹⁹ a directional movement similar to myosin,²⁰⁻²⁴ and a sequence-specific synthesis of peptide chains, as ribosomes do.²⁵⁻²⁷ On the other hand, creating systems that display microtubule-like function remains an ongoing challenge (Figure 2.1). Cellular microtubules generate forces by either polymerization, or depolymerization of their supramolecular tubular architecture.²⁸⁻³⁰ Thus to achieve similar functions and mechanisms in synthetic systems, non-covalent synthesis appears to be a promising strategy. The key challenge, in this context, is to couple the self-assembly process to the externally triggered molecular reactivity.

Figure 2.1 Mimicking biological molecular machines functions by synthetic systems. (A) From the bacterial flagellar motor to an overcrowded-alkene based artificial molecular motor. Adapted with permission from ref [10] and ref [17]. Copyright 2014 Pearson and 2017 Royal Society of Chemistry. (B) From the ribosome to a synthetic molecular peptide synthesizer. Adapted with permission from ref [11] and ref [16]. Copyright 2017 Elsevier and 2015 American Chemical Society. (C) From kinesin to a molecular walker. Adapted with permission from ref [7] and ref [24]. Copyright 2004 Springer Nature and 2017 American Chemical Society. (D) The design and synthesis of wholly artificial molecular machines that are endowed with microtubule-like functions remains an ongoing challenge. Adapted with permission from ref [6]. Copyright 2009 Springer Nature.

In this introductory chapter, we describe how cellular microtubules generate directional forces in cells, with a special focus on which specificities of the tubular architecture mediate these complex mechanisms, and we show how this structure and associated mechanisms have inspired chemists in the design and synthesis of artificial tubules. Further, we will discuss selected examples of stimuli-responsive tubules, and lay out how these approaches could lead to achieving microtubule-like functions in the future.

2.2 Cellular microtubules

Microtubules are vital elements of the cytoskeleton, that are involved in mitosis, cell motility, intracellular transport, secretion, the maintenance of cell shape and cell polarization.⁶ These microtubules are polarized structures of non-covalent, tubular biopolymers composed of $\alpha\beta$ -tubulin heterodimer subunits.

In mammalian cells, a single microtubule is usually comprised of 13 protofilaments that associate laterally to form a 24 nm wide hollow cylinder (Figure 2.2A).³¹⁻³⁴ Because the $\alpha\beta$ heterodimers associate head-to-tail, a microtubule is polarized: in each protofilament, the $\alpha\beta$ heterodimers are oriented with their β -tubulin monomer pointing towards the faster-growing end (plus end) and their α -tubulin monomer exposed at the slower-growing end (minus end). As a consequence of this structure, different polymerization rates are observed at each of the two ends of the microtubule. Besides α -tubulin and β -tubulin, a third tubulin isoform is involved, γ -tubulin, that functions as a template for the correct assembly of the microtubules.^{35,36}

Figure 2.2 Schematic representation of cellular microtubules and the mechanism by which they generate forces. (A) Structure of a microtubule consist of two proteins, α -tubulin and β -tubulin. (B) The polymerization and depolymerization of microtubules are controlled by guanosine-5'-triphosphate (GTP). Adapted with permission from ref [6]. Copyright 2009 Springer Nature. (C) Force production by polymerization (growth) of a microtubule against a boundary, e.g. breaking through the plasma membrane, deformation of nucleus, and positioning of organelle. Insertion of new $\alpha\beta$ -tubulin subunits at the end exerts a pushing force on the boundary (brown). (D) Force production by depolymerization. One possible mechanism for the conformational wave model of microtubule depolymerization exerting a pulling force to separate chromosomes (purple) during mitosis. (E) An alternative mechanism to (D) known as the biased diffusion model.³⁰ These mechanisms are still under discussion, and we anticipate that building a wholly artificial system from the bottom up is likely to contribute to an improved understanding. Adapted with permission from ref [30]. Copyright 2016 Wiley-VCH.

Microtubules are dynamic supramolecular polymers that assemble spontaneously in the presence of a guanosine-5'-triphosphate (GTP) (Figure 2.2B). They continuously undergo cycles of polymerization and depolymerization known as dynamic instability.^{31,37} GTP is required to be bound to β -tubulin for individual dimers to be added. Polymerization occurs at the (+) end of microtubule when new GTP- $\alpha\beta$ -tubulin dimers from the cytoplasmic pool are added. These new tubulin

subunits form a GTP-tubulin ‘cap’ at the (+) end that stabilizes the microtubule.^{31,38} After polymerization, the tubulin subunits hydrolyze the GTP that is bound to them into GDP, and thus the GTP cannot be exchanged anymore. Thus the rest of the microtubule is mostly composed of GDP-tubulin subunits. If the GTP cap is lost by hydrolysis, the microtubule enters a rapid state of depolymerization from the (+) end, that is referred to as a “catastrophic” event in view of its abruptness. Both polymerization and depolymerization mechanisms can generate forces in the living cell and can provide energy to perform mechanical work as described below.

Polymerization Forces. A microtubule growing against a barrier can exert a pushing force (Figure 2.2C).^{28,39-42} Such forces are involved in subcellular processes, such as the positioning of organelles and the deformation of cellular structures.

Depolymerization Forces While the polymerization of microtubules can generate pushing forces, their depolymerization can also be harnessed to generate pulling forces, provided that a link is maintained between an object and the depolymerizing tip of the microtubule (Figure 2.2D-E).^{28,30,43-46} The separation of chromosomes during mitosis is a major cellular process, in which the forces generated by microtubule depolymerization play a key role.*

* Here and in the whole manuscript, I use the term “pulling force” as convention to describe the force that is applied by disassembling cellular microtubules. However, I note that this process can also be considered as a pushing process, but in the opposite direction compared to the direction of the force generated when microtubules grow.

2.3 Bio-inspired strategies towards supramolecular tubular machineries

The motivation to work with tubular systems lies in the versatility displayed by cellular microtubules that operate in the cell. The propensity to produce forces efficiently and in the right temperature regime, with control over directionality, is thus inherently encoded into their tubular structure: they are in essence supramolecular cylinders that are hollow and stiff, and that are assembled from molecules that undergo chemically fueled conformational switching.^{37,47}

At a scale that is intermediate between molecules and macroscopic materials, i.e. for dimensions that are relevant both to cellular architectures and to supramolecular chemistry, investigations of dynamic mechanical properties have been limited to flexible supramolecular polymers so far.⁴⁸⁻⁵⁰ The fast re-equilibration that characterizes molecular fibers is a major hurdle for transmitting the dynamic behaviour of supramolecular fibers into directed mechanical action – if a supramolecular architecture re-equilibrates by adjusting its size against a load/wall continuously, then it will not be able to push or pull.

Recent investigations have shown that upon drying, the self-assembly of dipeptide fibers mediates chemo-mechanical transduction when the water droplets dry out – the microscopic crystallization of the dipeptides can pierce the interface between water and oil.⁵¹ This process is not reversible and remains a microscopic effect. Further, pioneering research on the dynamics of supramolecular fibers has revealed that the building blocks exchange rapidly between the assembled and the free state,⁵² which precludes a controlled access to out-of-equilibrium states. Moreover, the persistence length of classical supramolecular polymer fibers is insufficient to produce a force, i.e. a force can be transmitted only if the fiber does not bend easily, and fibers are too flexible.

Tubular architectures display vastly higher bending stiffness than fibers, because of their large diameter. Tubules are also expected to exhibit a much slower exchange rate between free molecules and molecules in the tube, because the exchanging molecules need to overcome both longitudinal forces, as in fibers (along the length of the tube), but also they need to overcome lateral forces (around the tube). Because of the slower exchange rates between molecules in and outside the tube, tubular structures have higher propensity to reside out-of-equilibrium, which is a prerequisite to achieve transduction of forces effectively.

2.4 Stimuli-responsive supramolecular tubules

Several approaches are available to build synthetic tubular structures based on non-covalent synthesis (Figure 2.3). Typical strategies include rolling or sealing at opposing edges of a two-dimensional sheet-like material, assembling of stave or rod-like subunits into barrel or bundle-shaped frameworks, coiling into hollow or helical conformations of linear precursors, and stacking of macrocycle or disc or sector-shaped subunits.⁵³ A lot of examples have been shown in the intensive reviews on synthetic organic nanotubes and supramolecular nanotubes based on amphiphilic molecules.⁵³⁻⁵⁵ However, the vast majority of examples report non-responsive systems, and this lack of dynamic character limits their potential for nanotechnologies.

Pre-programming a dynamic supramolecular behaviour thus requires re-engineering of the building blocks. Incorporation of the functional groups that alter the properties such as geometry, ionic property, polarity, etc., upon exposing to the external stimuli lead to change of non-covalent interaction and reorganization of macroscopic supramolecular structures. Possible stimuli range from salt ions, small organic molecules, pH, temperature, redox activation, and light. Hereafter, we describe recent advances in the design and synthesis of stimuli-responsive tubular architectures, categorized by stimuli.

Figure 2.3 General strategies to build supramolecular tubules. (A) Folding of a flat sheet. (B) Coiling of linear precursors to form helical or hollow structures. (C) Rod-like molecules can be assembled in a barrel-like fashion to form molecular bundles. (D) Stacking of macrocycles yields continuous tubes. (E) Sector or wedge-shaped molecules can assemble into discs that subsequently stack to form continuous cylinders, similar to macrocycles. Adapted with permission from ref [53] and ref [54]. Copyright 2001 Wiley-VCH and Copyright 2005 American Chemical Society.

2.4.1 Chemo-responsive tubules

In situ chemical transformation of the components in a system is a straightforward way to render responsiveness of a supramolecular system by weakening, breaking, or disturbing the non-covalent interactions in self-assembled structures.

Tubular structures can dissociate into toroids, when triggered by a silver salt (Figure 2.4A).⁵⁶ In the work by Myongsoo Lee *et al.*, the nanotube is self-assembled from amphiphile **1**, which contains a rigid V-shaped aromatic segment, and an oligo ether dendron. This amphiphile self-assembles in water under the combined action of hydrophobic effects, π - π stacking interactions, and shape recognition, in order to form hexameric macrocycles that stack on top of each other to form tubes. Upon addition of a silver salt, Ag^+ binds with nitrile group and triggers the dissociation of the tubules into discrete stacks of toroids. Further investigations by this group have involved the substitution of the terminal hydrophobic moiety of the bent-shaped core in amphiphile **1**, into other substituents, such as a pyridine (Figure 2.4B). The resulting amphiphile **2** self-assembles into flat sheets consisting of a zigzag

conformation through π - π stacking interactions.⁵⁷ The sheets reversibly transform into helical tubules at higher concentration and into discrete dimeric macrocycles at a lower concentration in response to Ag^+ ions through reversible coordination interactions between the pyridine units of the aromatic segments on one hand, and the Ag^+ ions on the other hand.

Figure 2.4 Chemo-responsive tubules based on bent-shaped amphiphiles. (A) Adapted with permission from ref [56]. Copyright 2010 Wiley-VCH. (B) Adapted with permission from ref [57]. Copyright 2013 American Chemical Society. (C) Adapted with permission from ref [58]. Copyright 2014 American Chemical Society.

Further, the same group of Myngsoo Lee has also demonstrated thin nanofibers that can inflate to form nanotubes upon guest encapsulation (Figure 2.4C).⁵⁸ Amphiphiles **4** features a bent-shaped aromatic segment connected by a *m*-pyridine unit and aldehyde group at the end. The aromatic segments self-assemble into paired dimers which stack on top of each other to form thin nanofibers. The nanofibers

inflate into helical tubules through the formation of hollow cavities triggered by *p*-phenylphenol, a H-bonding guest. This transformation can be reversed upon addition of ammonium hydroxide that causes deprotonation of the *p*-phenylphenol to its phenolate form, followed by the liberation of the guests from the cavity. The reversible inflation of the nanofibers arises from the packing rearrangements in the aromatic cores from transoid dimers to cisoid macrocycles, driven by the reversible H-bonding interactions between the pyridine units of the aromatic cores and the *p*-phenylphenol guest molecules.

Self-assembly of peptide-based amphiphiles is a popular strategy to realise molecular architectures with various morphologies depending on the amount and type of amino acid subunits.⁵⁹ Therefore it is not surprising that the peptide-based amphiphiles have also been used to design and synthesize responsive tubular systems. Ulijn's group has reported enzyme-triggered self-assembly of peptide nanotubes by subtilisin (a hydrolytic enzyme from *Bacillus licheniformis* which hydrolyses the methyl ester).^{59,60} Nanotubular networks of 9-fluorenylmethoxycarbonyl-tri-leucine (**Fmoc-L₃**) were produced *via* enzyme-triggered self-assembly (Figure 2.5A) whereby the non-assembling precursor, **Fmoc-L₃-OMe**, was converted into the **Fmoc-L₃** upon subtilisin-catalysed hydrolysis of the methyl ester, leading to hydrogel formation. In this system, the rate of the catalytic self-assembly could be tuned by changing the amount of enzyme that was present in the system.⁶¹ The increasing amount of enzyme facilitates transfer of chiral information from the molecular scale to the supramolecular structures. In the presence of a high enzyme loading, the amplification of the chirality of the system was remarkably enhanced.

Figure 2.5 Chemo-responsive tubules. (A) Peptide amphiphiles. Adapted with permission from ref [60]. Copyright 2010 Royal Society of Chemistry. (B) DNAs. Adapted with permission from ref [62]. Copyright 2010 Springer Nature. (C) Tetrameric proteins. Adapted with permission from ref [63]. Copyright 2016 American Chemical Society.

Other biological building blocks can also be used as building blocks for responsive tubules. DNA nanotubes were reported, that displayed selective loading and releasing of cargo.⁶² These triangular-shaped DNA nanotubes were formed by the combined self-assembly of small DNA triangles (7 nm, molecule 5) and large DNA triangles (14 nm, molecule 6), connected by linking strands (Figure 2.5B). These tubules could, size-selectively, encapsulate citrate-coated gold nanoparticles in the larger triangular areas. The release of this cargo was triggered by adding a DNA eraser strand (ES1') in the mixture, that was able to remove the linking strands, leading to the transformation of nanotubes 7 into their single stranded form 8, that released the particle cargo spontaneously.

The large biological building blocks such as protein has also been used as building blocks to form a microtubule-like structures with a control of the self-assembly and disassembly processes by supramolecular competition (Figure 2.5C).⁶³ The protein soybean agglutinin tetramer (SBA) has sugar-binding sites which specifically bind with *N*-acetyl- α -D-galactosamine (GalNAc) or α -D-galactopyranoside (Gal) sugars moiety in the ligands. Then the π - π stacking interaction of Rhodamine B (RhB) in the ligands induces SBA to form tubular structures. The driving forces for self-assembly are molecular recognition between SBA and GalNAc/Gal, and π - π stacking interaction of RhB. Addition of β -cyclodextrin (β -CD) causes dissociation of microtubes by hosting incorporating RhB in the ligands. A subsequent addition of a competitive guest 1-adamantane hydrochloride (Ada), which binds β -CD more strongly than RhB, induced the reformation of the protein microtubes.

2.4.2 pH-responsive tubules

While chemo-responsive systems, as discussed above, always require specific chemicals and chemical processes, pH-responsive systems are generally simpler to design and the response comes from the environment. The pH is readily controllable by buffer solution, and its biocompatibility could be beneficial for *in vivo* applications.

The pH changes can trigger helicity switching of virus-like nanostructures (Figure 2.6).⁶⁴ The shell of virus-like nanostructures are formed by co-assembly of pyridine-based molecule **9** and pyridinium-based cationic molecule **10** which creates positively charged pore. A double-stranded DNA molecule is encapsulated inside the pore by electrostatic interactions between positively charged pyridinium cations and negatively charged phosphate anions of DNA. At pH = 7.4, the coated molecules wrap around native DNA to form filamentous virus-like nanostructures with right-handed helical conformation. When lowering the pH down to 5.5, the DNA changes from right- to left-handed helical structures and induces simultaneous helicity switching of its coat assembly, accompanied by a contraction in the diameter of the coat assembly.

Figure 2.6 pH-responsive tubules. (A) Structures of pyridine- and pyridinium-based molecules. (B) Schematic representation of the DNA-coat assembly at pH 7.4. (C) Schematic representation of the molecular rearrangement of the coat molecules on the reversible switching of the DNA-coat assembly. (D) Collective motion in the helicity inversion of DNA and the synthetic coat assembly triggered by a pH change. Adapted with permission from ref [64]. Copyright 2017 Springer Nature.

2.4.3 Thermo-responsive tubules

Thermo-responsiveness is one of the fundamental and the most accessible property of supramolecular systems, as non-covalent interactions are inherently susceptible to a thermal activation, and temperature is relatively easy to control.

The thermo-responsive nanotubes with helicity inversion have shown in Figure 2.7A.⁶⁵ This system is based on bent-shaped aromatic amphiphiles **13** self-assemble into hexameric macrocycles in aqueous solution, forming chiral tubules by spontaneous one-dimensional stacking with a mutual rotation in the same direction. The adjacent aromatic segments within the hexameric macrocycles reversibly slide along one another in response to external temperature triggers that weaken the H-bond between water and pyridine, resulting in pulsating motions of the tubules accompanied by a chiral inversion. The aromatic interior of the self-assembled tubules can encapsulate hydrophobic guests such as C₆₀ (fullerene). Using a thermal trigger, C₆₀ could be released through the pulsating motion of the tubules.

Another example of “breathable” thermo-responsive tubules was thereafter reported by the same group (Figure 2.7B).⁶⁶ In this work, the tubular walls consist of laterally associated primary fibrils stacked from disc-shaped molecules **14** in which the discs readily tilt by means of thermally regulated dehydration of the oligoether chains placed on the wall surfaces. Upon heating, the ethylene oxide chains on the tubular walls are dehydrated, resulting in the switching between open and closed states. The pore switching can mediate a controlled water-pumping catalytic action for the dehydrative cyclization of adenosine monophosphate (AMP) to produce metabolically active cyclic AMP (cAMP). The close state of tubular pore confines the space which considerably restrict rotational degrees of freedom during the cyclization of AMP, which lowers the activation energy of the ring-closure reaction. Moreover, the closing of tubular pore can prevent by addition of molecule **15** as inhibitor. Intercalation of **15** in tubular structures can prevent tilting of disc planes and pore closing, upon heating.

Figure 2.7 Thermo-responsive tubules based on different building blocks. (A) Bent-shaped amphiphiles. Adapted with permission from ref [65]. Copyright 2012 American Association for the Advancement of Science. (B) Ring-shape amphiphiles. Adapted with permission from ref [66]. Copyright 2015 Springer Nature.

2.4.4 Redox-responsive tubules

Redox reactions are chemical reaction associated with a loss or gain of one or more electrons, by an applied electrical potential or chemical agents. Redox activation can be considered as a clean stimulus when using electrochemical methods to trigger the redox reaction.

An example of redox-responsive metal-organic nanotubes, consisting of ferrocene-based tetratopic pyridyl ligands mixed with AgBF_4 , are shown in Figure 2.8.⁶⁷ The Ag^+ ion binds with pyridine, in FcL_1 or FcL_2 , and resulting in a doubly bridge metal-organic macrocycles which eventually stacks to form the hollow nanotubes. Through oxidation of the ferrocene groups by cyclic voltammetry, the

tubes could be cut into stable, large rings and then reversibly reassembled into nanotubes by reduction of the ferrocene groups.

Figure 2.8 Redox-responsive tubules based on ferrocene derivatives. Adapted with permission from ref [67]. Copyright 2014 American Association for the Advancement of Science.

2.4.5 Light-responsive tubules

While those previous stimuli have been successfully used for realizing responsive tubules, these stimuli cannot be continuously supplied to an isolated system, which is where light appears as an ideal alternative. Light has more advantages because it can deliver to the closed system, leaves no chemical waste, the specific wavelength can be selected, and can achieve spatio-temporal control. To use light as a stimulus, the light-responsive units (such as molecular switches which their isomer has different geometry or properties, or molecular motors which has unidirectional rotation) are

required. In this section, light-responsive tubular structures in the recent years will be introduced.

Figure 2.9 Various types of light-responsive tubules. (A) Azobenzene peptide amphiphiles. Adapted with permission from ref [68]. Copyright 2015 Wiley-VCH. (B) Overcrowded alkene amphiphiles. Adapted with permission from ref [69]. Copyright 2011 Springer Nature. (C) Load of vesicles into nanotubes by osmotic pressure. Adapted with permission from ref [70]. Copyright 2015 Wiley-VCH.

Azobenzenes are one of the most simple and versatile photoswitches that have shown strong potential for constructing light-responsive tubules. An example of tubule forming peptide-based amphiphilic building blocks (MGlyAzo) is shown in Figure 2.9A.⁷¹ The glycine moiety acts as a H-bonding unit to construct self-

assembled nanotubes and the azobenzene moiety provides the *trans-cis* photoisomerization. Upon UV light irradiation, the diameter of nanotubules shrinks from 20 nm to 15 nm. Unfortunately, the small nanotubes are not relax back to the original tubules shapes by visible light, it changes the morphologies to helical nanotapes. The morphologies of self-assembly are controlled by different arrangement of molecules in supramolecular structures. Later, they have reported the additional morphologies transformation when the hydrophilic glycine is extended to di- and tri-glycine units.⁶⁸ The diglycine (**DGlyAzo**) shows the imperfection nanoring upon UV irradiation. When it relaxes with visible light, it transforms to the intermediate nanoring and then assembles to the nanotubes which has larger diameter than the initial nanotubes (25 nm vs 7 nm). For the triglycine moieties (**TGlyAzo**), the intermolecular H-bonding is too strong to permit the changes of morphology.

Next to azobenzenes, overcrowded alkenes can also be used to control disassembly of vesicle-capped nanotubes, upon light irradiation (Figure 2.9B).⁶⁹ The structure of amphiphile **16** combines the overcrowded alkene unit with hydrophilic oligoethylene glycol head groups and hydrophobic alkyl tails. This molecule forms bilayer by means of interdigitation of the opposing amphiphilic molecules. Under aqueous condition, it self-assemble into nanotubes. Addition of phospholipid 1,2-dioleoyl-*sn*-glycero-3-phosphate (DOPC), which improves solubility of amphiphiles, resulted in micrometre-long nanotube structures and also led to the formation of nanotubes capped with DOPC vesicles. Treatment of vesicle-capped nanotubes with detergent (Triton X-100) dissolves the phospholipid capping vesicle without affecting the nanotube. Subsequent removal of detergent with Biobeads followed by freeze-thaw cycles regenerates the vesicle-capped nanotubes. Irradiation with UV light leads to cyclization within the bilayer and eventually resulting in tube disassembly process. Moreover, with inducing the osmotic pressure by adding NaCl or evaporation of water during the ageing process, the vesicle cap can loaded into the nanotubes (Figure 2.9C).^{70,72} Introducing chirality into overcrowded alkene amphiphiles could also use

to control the morphology of self-assembled structures, which possibly by the increasing of hydrophobic volume that distorts the long range packing of amphiphiles and lead to shorter tubes.⁷³

Reversible reorganization between two different self-assembled morphologies, nanotubes and vesicles, has been realized by light-responsive amphiphilic molecular motors (Figure 2.10A).⁷⁴ Both molecules, **18** and **19**, are initially form tubular structure when co-assembly with DOPC in a 1:1 ratio. Upon UV light irradiation, the tubules change to vesicles and the reversed transformation from vesicles to nanotubes was achieved by heating followed by freeze-thaw. The mechanism underlying these changes in morphology is based on the fact that each photoisomer is characterized by a different packing parameter, which is a combination of volume and length of the hydrophobic half and the surface area of the hydrophilic part of an amphiphile.

Besides numerous achievements in controlling morphological changes, first steps have been taken towards demonstrating abilities of light-responsive tubules to generate nanoscale forces. We have developed light-powered tubules that convert light into tubular strain (Figure 2.10B).⁷⁵ Our tubular architectures are incorporating azobenzenes as light-responsive units. Upon UV irradiation, the geometrical changes associated with the *trans*-to-*cis* isomerization weaken the π - π stacking interactions and disrupt the organization of the tubules. As the strain accumulates within the tubules, eventually they break up. This mechanism is reminiscent the generation of forces by disassembly of cellular microtubules

Figure 2.10 Light-responsive supramolecular tubules. (A) Molecular motor-based amphiphiles. Adapted with permission from ref [74]. Copyright 2016 American Chemical Society. (B) Azobenzene bent-shaped amphiphiles.

Artificial muscle-like function performed by a supramolecular assembly has been reported recently. (Figure 2.11).⁷⁶ In this work by Feringa *et al.*, amphiphilic molecular motors are incorporated non-covalently into hierarchically organized nanofibers. Upon irradiation with UV light, the motor undergoes a conformational change, and the rotary motion powers the photo-actuation of the fiber which bends towards the light source in both aqueous solution and in air. The photo-induced bending of the fiber also mediates the lifting of weight. Overall, this work demonstrates macroscopic mechanical motion caused by supramolecular assembly of a small rotary motor molecules.

Figure 2.11 Motor-based tubules displaying light-driven muscle-like function. Adapted with permission from ref [76]. Copyright 2017 Springer Nature.

Besides wholly synthetic systems, molecular switches can be combined with barrel-shaped proteins and further assemble into light-responsive nanotubes. Aida and co-workers have shown the formation of supramolecular nanotubes by mutant chaperonin GroEL_{Cys} with spiropyrans (Figure 2.12A).^{77,78} In their work, Cys amino acids in mutant GroEL were allowed to react with spiropyrans (SPMI) to form GroEL_{SP}. The self-assembly into nanotubes was then triggered in the presence of divalent ions such as Mg²⁺, Ca²⁺, Mn²⁺, Co²⁺, and Zn²⁺ which stabilized by an ion bridges between each MC units. The formation of ion bridge spontaneously occurs when SP thermally isomerizes into the MC form, this conversion can be accelerated by exposure to UV light. The dissociation of chaperonin nanotubes was induced by adding EDTA, as this strong metal chelator removes the bridging ions, or by illumination with visible light, as closing the merocyanine into a spiropyran destroys the ion bridges.

Alternatively, the addition of adenosine-5'-triphosphate (ATP) can induce conformational changes in the chaperonin units, which generates a mechanical force that lead to the disassembly of the nanotubes by breaking multivalent GroEL_{MC} connections (Figure 2.12C).⁷⁹ The post modification of the GroEL surface, with boronic ester motif, allows cellular uptake of the nanotubes. With an encapsulated drug inside its cavity, it can be considered as a robotic nanotubular carrier. This system exhibits targeted guest release depending on the ATP concentration and shows a preferential accumulation of the nanocarriers in a tumour tissue. This tubular nanocarrier is a promising system for drug delivery as it is pharmacokinetically better than spherical structures.⁸⁰

The length of these nanotubes can be controlled by the ratio between GroEL_{Cys} and a molecularly engineered protein end-capper (SR_{MC}), a single ring version of GroEL_{Cys} (Figure 2.12D).^{81,82} As the length of these tubular structures is significantly affect the cellular uptake and circulation behaviour, the nanotubes that longer than 100 nm are less likely to be taken up into cells.

Furthermore, the modified GroEL is a versatility system that experimentally proof 1D array of superparamagnetic iron oxide nanoparticles (SNPs) for the first time (Figure 2.12E).⁸³ GroEL can act as a protein jacket and encapsulated SNPs in its cavity (GroEL_{MC}⊃SNP) to show the magneto-induced lateral assembly and form bundles of tubules. Upon turn on and off the magnetic field, this tubules show a repeatedly bundled and unbundles without denaturation. The hierarchical assembly of long 1D objects is suggested for the development of conceptually new soft materials. Further, this concept is reminiscent of the biological role of Lamin protein that structurally supports the nucleus by forming mechanically tough filaments through longitudinal growth and subsequent lateral attraction.⁸⁴

Figure 2.12 Light-responsive tubules based on mutant chaperonin proteins. Adapted with permission from ref [82]. Copyright 2017 American Chemical Society.

2.5 Conclusions and perspectives

Building responsive supramolecular tubules could open up the possibility towards artificial supramolecular machineries which can generate measurable forces at the nanoscale and beyond, and ultimately establish operational principles for chemo-mechanical transduction in supramolecular systems.

While a number of synthetic and responsive tubules have been reported in the last decade,^{53-55,85-87} only a very limited number of these systems have demonstrated the generation macroscopic work.^{88,89} As most of them operate in solution, the main challenge to transfer work from molecular to macroscopic levels lies in overcoming viscous forces and the Brownian storm.^{16,17}

Possible approaches to harness, combine and transfer small work across increasing length scales include the integration of dynamic molecules into polymer networks,⁹⁰ liquid crystals,^{91,92} supramolecular systems,^{88,89} and the interfacing of these small molecules with surfaces.⁹³ Prolonging systems out of equilibrium continuously require maintaining dynamic changes of molecular states, while avoiding accumulation of chemical waste products. Typical examples include the use of oscillating chemical reactions or light.⁹⁴⁻⁹⁶

Future advances are likely to contribute to engineering all-synthetic mimics for cellular microtubules, as part of a rudimentary model for the cytoskeleton. This bottom-up approach complements the top-down approach towards the artificial cell, that has been initiated by synthetic biologists.⁸ Its added value lies in the possibility to adjust and tune the properties of each molecular component individually, and eventually to mediate a better understanding of each specific contribution into the mechanics of the cell. By engineering non-equilibrium states of self-assembled systems, these systems can be pre-programmed to perform specific and unprecedented mechanical functions in synthetic systems.⁹⁴

Further, incorporating such dynamic molecules into synthetic systems with a high degree of functionality would set the stage for synthetic supramolecular systems to reach an unprecedented level of complexity, by achieving dynamic, directional function in far from equilibrium conditions, and thus entering length scales at which cellular machineries operate typically, with man-made nanotechnologies.

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Chapter 3

Molecular photoswitches mediating the strain-driven disassembly of supramolecular tubules

Chemists have created molecular machines and switches with specific mechanical responses that were typically demonstrated in solution, where mechanically relevant motion is dissipated in the Brownian storm. The next challenge consists of designing specific mechanisms through which the action of individual molecules is transmitted to a supramolecular architecture, with a sense of directionality. Cellular microtubules are capable of meeting such a challenge. While their capacity to generate pushing forces by ratcheting growth is well known, conversely these versatile machines can also pull microscopic objects apart through a burst of their rigid tubular structure. One essential feature of this disassembling mechanism is the accumulation of strain in the tubules. We envision a strategy toward supramolecular machines generating directional pulling forces by harnessing the mechanically purposeful motion of molecular switches in supramolecular tubules. Here, we report on wholly synthetic, water-soluble, and chiral tubules that incorporate photoswitchable building blocks in their supramolecular architecture. Under illumination, these tubules display a nonlinear operation mode, by which light is transformed into units of strain by the shape changes of individual switches, until a threshold is reached and the tubules unleash the strain energy. Our findings pave the way toward supramolecular machines that would photogenerate pulling forces, at the nanoscale and beyond.

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3.1 Introduction

Essentially all motion in living organisms emerges from the collective action of molecular machines transforming chemical energy into ordered activity. Inspired by nature's machinery, chemists have moved from building static molecular structures to designing and synthesizing molecules that display mechanically relevant motion,^{1,2} such as molecular switches,^{3,4} pincers,⁵ motors,⁶⁻⁸ pumps, shuttles,^{9,10} muscles,¹¹ walkers,¹² robotic arms,¹³ artificial peptide synthesizers,¹⁴ and self-propelled molecular cars exhibiting directional motion.¹⁵ The operation of these small molecules has been studied in solution primarily, where any mechanical action is overwhelmed by random Brownian motion.

Remarkably, nature's molecular machines also operate in a liquid environment, where despite the Brownian storm and the constant flux of building blocks they generate strong directional forces¹⁶ and synthesize essential molecules.¹⁷ Two characteristics underpin evolutionary designs: first, nonequilibrium operation of supramolecular machines is maintained by constant influx of chemical fuels and second, these machines are integrated into even larger supramolecular assemblies such as filaments, membranes, or tissues, to guide and coordinate the overall operation against the Brownian storm.

Reaching this remarkable level of functionality in artificial systems requires strategies where mechanically purposeful molecular motion can be transmitted effectively into motion at the supramolecular level—a challenge that has thus far proven elusive.

Cellular microtubules are versatile supramolecular machines that are capable of producing two types of directional forces under continuous influx of energy: they pull chromosomes apart through catastrophic disassembly, and shape-shift cells as they grow using chemical energy.¹⁸ This capacity to produce forces efficiently is inherently encoded into their supramolecular architecture: microtubules are in essence stiff cylinders that are self-assembled from molecules that undergo chemically fueled

conformational switching between assembling and nonassembling forms.¹⁹ Although the functional disassembly of cellular microtubules is still not understood fully, it appears that conformational changes in the tubules' building blocks induce a strain that builds up and releases abruptly to produce a directional mechanical force upon disassembly,²⁰ a process that has been described theoretically by the conformational wave model.^{21,22}

Here, we report the creation of synthetic supramolecular tubules in which the structural change at the level of individual building blocks is controlled by photochromic switching, which contributes to pushing the system gradually toward higher energy states, and eventually induces its catastrophic disassembly (Figure 3.1A). Light constitutes an ideal source of energy that can be supplied continuously to an isolated system to obtain structural switching—as opposed to previously employed external environmental triggers that do not modify the structure of the building blocks [i.e., salinity²³ and temperature²⁴]. Notwithstanding the tubular shape, a number of pioneering reports on supramolecular fibers^{25,26} have demonstrated strategies to achieve out-of-equilibrium operation.^{27–30} The mechanism of assembly of supramolecular polymers, mostly fiber-like objects, has attracted much attention,^{31,32} but much less is known about the mechanism by which these fibers disassemble.²⁹ In addition to the presence of a hollow cavity core that is intrinsic to a tubule,³³ a major difference between supramolecular tubules and fibers lies in the rigidity of the tubules versus the flexibility of the fibers. Consequently, from a dynamic point of view, synthetic tubules can be expected to exhibit a much slower exchange rate between building blocks in solution and building blocks in the tubule, which also substantially impacts their disassembly mechanism. Herein we describe how these molecular specificities can be encoded into a complex supramolecular system that operates in water and demonstrate that this strategy results in a nonlinear, three-step mechanism that mimics the conformational wave disassembly of cellular microtubules.²²

Figure 3.1 Concept of strain buildup disassembly of synthetic supramolecular tubules and molecular design of the building blocks. (A) Disassembly of synthetic tubules by light. Hydrophobic interaction, π - π stacking, and shape recognition drives the spontaneous assembly of the stable building blocks into supramolecular tubules in water. Light-triggered *trans*-to-*cis* isomerization within the hydrophobic part causes shape changes and eventually disassembly of the tubular structure. (B) Chiral building block (1) incorporating (*S*)-stereocenters in its hydrophilic moiety and its achiral counterpart (2). Notably, the chirality of building blocks has been proven to influence equilibrium dynamics of supramolecular assemblies dramatically.³⁴ Upon irradiation with UV light ($\lambda = 365$ nm), the building block undergoes *trans*-to-*cis* isomerization. The *trans*/*cis* ratio at photostationary state is $\sim 30/70$ (Figure 3.14). The reverse *cis*-to-*trans* switching can be realized thermally (with a half-life of *ca.* 12 h at 20 °C), or by irradiation with visible light (Figure 3.5).

3.2 Results and discussion

3.2.1 Molecular Design

The use of water as a solvent requires the engineering of photoresponsive building blocks that spontaneously self-assemble in water. Our design involves a V-shaped aromatic core in which two azobenzene photoswitches are incorporated as shape-changing activators (Figure 3.1B). The V-shaped core is connected to branched hydrophilic oligoether chains that help solubilize the molecules in water and imbues them with an amphiphilic character. In the absence of UV light, the planar *trans* form is present, and the self-assembly of the building blocks into tubes is thus expected to be driven by the combination of hydrophobic effects, π - π interactions and shape recognition, as has been reported for other shape-persistent building blocks.^{23,24,35} In contrast, the *cis* form disrupts both planarity and the V shape of the hydrophobic part and our design thus builds on the concept that *trans*-*cis* isomerization should promote switching between the assembling and nonassembling forms of the building blocks (Figure 3.1).

3.2.2 Self-Assembly of Synthetic Tubules in Water

In water, both building blocks **1** and **2** form noncovalent hexameric macrocycles that stack on top of each other to form tubules (Figure 3.1A), as manifested in the elongated architectures observed in the transmission electron micrographs (Figures 3.2A for **1**, and 3.15C for **2**). Atomic force microscopy (AFM) also confirms these findings (Figure 3.2B). Cryo-TEM images reveal the internal aromatic part of the tubules with the uniform size along its whole length (Figure 3.2C). The measured diameter ($d \approx 5$ nm) corresponds to the expected diameter of the hexamer (Figure 3.2D). The periodicity of bundled tubules indicates the external diameter of the tubule is *ca.* 11 nm, in agreement with the value estimated from molecular models (Figures 3.2D and 3.2E). TEM micrographs indicate an external diameter of *ca.* 7 nm for both **1** and **2** (Figures 3.15), which we attribute to a difference in sample

preparation; when out of solution the hydrophilic chains interdigitate partially upon drying.

Figure 3.2 Synthetic tubules spontaneously form in water. (A) TEM micrographs of the tubules formed by building block 1 (83 μM). (B) AFM phase image of the synthetic tubules self-assembled from the achiral building block 2 in water and deposited on mica. (C) Cryo-TEM micrograph of the tubules formed by building block 2 (1.3 mM). (D) Model of the tentative hexameric non-covalent macrocycle composing the tubules with the characteristic diameters (Chem3D[®]). (E) Measurements of the external diameter from the periodicity of the bundled tubes 2 (1.33 mM) observed by Cryo-TEM. External diameter of the tubules was measured at 3 different areas and subsequently averaged.

The structural information provided by microscopy is complemented by spectroscopic data. Upon self-assembly of **1** in water, a CD signal appears with a zero-crossing point that corresponds to the λ_{max} of the azobenzene chromophores. The intensity of the CD signal increases with the concentration of **1**, indicating that **1** assembles into tubules with a preferred handedness (Figure 3.3A). The shape of the CD spectrum remains constant between 1 μM and 83 μM , which indicates

that the supramolecular structures are robust and uniform. A CD signal is not observed over the same concentration range when **1** is dissolved in acetonitrile, which indicates that molecular tubes do not form in acetonitrile. Achiral building block **2** does not yield a CD signal in water, which we attribute to the formation of tubules without preferential handedness.

Figure 3.3 (A) CD spectra at incrementally increased concentrations of **1** between 1 μM (absence of the CD signal) to 83 μM (the highest CD intensity). (B-C) Emission spectra of Nile Red solution (0.94 μM) in water with different concentration of (B) chiral azobenzene **1** and (C) achiral azobenzene **2**. (D) Determination of the critical aggregation concentration for building blocks **1** and **2** using the fluorescent probe Nile red (0.94 μM). The ordinate shows the ratio of intensities of the fluorescence bands (612 and 650 nm for **1**; 621 and 647 nm for **2**) (red dots, **1** and black square, **2**). Nile red present in the hydrophobic environment of the tubules shows increased fluorescence intensity and an emission maximum at $\lambda_{\text{max}} \sim 620$ nm compared with $\lambda_{\text{max}} \sim 650$ nm in water.

Effective control over the dynamics of the synthetic tubules was achieved close to the critical aggregation concentration, where small changes in concentration can drive substantial changes at the supramolecular level. The critical aggregation concentration was determined by plotting changes in the fluorescence of a probe (Nile red) that favors hydrophobic cavities,³⁶ for increasing concentrations of building blocks, and was found to be $\sim 1\ \mu\text{M}$ (Figure 3.3B-D).

Synthetic supramolecular architectures are dynamic in nature and, to date, their design has been such that they readily adapt to changing boundary conditions. As soon as the conditions change, the rapid exchange between the assembled building blocks and the isolated building blocks secures a fast re-equilibration process. In contrast, we anticipated that if the exchange between the assembled building blocks and those in solution is slowed, then the architectures will display a tendency to linger out of equilibrium, in a higher energy state. Nature's supramolecular machines have evolved in such a way that they can operate out of equilibrium and employ this higher energy state to perform useful tasks. To examine the exchange rate between building blocks forming the tubules and those in solution, we mixed two populations of the individually preformed tubules formed by chiral **1** and achiral **2**. Upon mixing an aqueous solution of the chiral tubules **1** with another aqueous solution containing tubules **2** the CD signal remained constant for 24 h (Figure 3.4A). In contrast, upon mixing the same tubules in water/acetonitrile (95/5) the CD signal increased and revealed amplification of chirality, an effect known as the sergeants and soldiers principle.³⁷ These data indicate that in water the exchange of molecules between the tubules and the solution is slow. In contrast, this exchange is fast in the water/acetonitrile mixture, likely because of the higher solubility of all building blocks in acetonitrile.

Figure 3.4 The preformed supramolecular tubules remain in an out-of-equilibrium kinetic trap. (A) We monitor the evolution of the CD signal after mixing a 1:1 ratio of preformed chiral tubules from **1** (2.5 μM) and preformed achiral tubules from **2** (2.5 μM) and compare the dynamic behavior in either pure water (black line) or in water/acetonitrile 95/5 (red line). Over 24 h, chiral amplification is apparent in water/acetonitrile and is translated into a gradual increase of CD signal, which indicates that the chiral and achiral building blocks are mixing to form a larger number of chiral tubes (i.e., the system displays a sergeants and soldiers effect). In stark contrast, chiral amplification is not observed in water. The absence of chiral amplification indicates that in water exchange between the building blocks, which the tubules are composed of, does not occur. These data indicate that in water and over the timescale of the measurements, the structure of the tubules is kinetically trapped at room temperature. (B) CD signal of **1** recorded at $\lambda = 390 \text{ nm}$ in water (2.5 μM), for varying temperatures.

Further insight into the equilibrium dynamics of the tubules formed by **1** can be extracted from the temperature dependence of the CD spectra. Overall, the intensity of the CD signal recorded at $\lambda = 390 \text{ nm}$ decreases with an increasing temperature, until it completely disappears at 55 °C (Figure 3.4B). The disappearance of the CD signal most likely originates from the greater thermal energy that decreases the average length of the tubules until they become soluble. While at temperatures between 40 °C and 55 °C the CD signal decreases linearly, below 40 °C the system starts exhibiting a nonlinear behavior. Between 25 °C and 35 °C the intensity of the CD signal decreases by less than by 10%, whereas between 35 °C and 40 °C the CD signal abruptly reduces by 50%. This shows that the assembled tubules are kinetically

trapped around room temperature within the timescale of the measurements, whereas at elevated temperatures the structure dynamically adapts to temperature changes. Although the response of the self-assembled tubules to temperature cannot be directly compared with the situation when the system responds to structural changes of the building blocks, it gives a valuable insight into the dynamics of the molecular exchange between the assembled and free state. Overall, we conclude that at ambient temperature the system of tubules formed in a purely aqueous environment has the tendency to linger out of equilibrium after it has been disrupted.

3.2.3 Conversion of Light into Supramolecular Strain

Next, we studied the dynamics of the synthetic tubules under a continuous influx of energy, through irradiation with light. The azobenzene units in the aromatic core of the building blocks provide the light-responsive trigger for the disassembly. In acetonitrile, where tubules are not formed, we verified that the azobenzene unit displays the expected photoswitching behavior, in both building blocks **1** and **2** (Figure 3.5). Upon irradiation of the *trans* form with UV light ($\lambda = 365$ nm), *trans*-to-*cis* isomerization occurs. These large geometrical changes are altering planarity and conjugation, and therefore they are manifested in a decrease of the π - π^* absorption band at $\lambda_{\text{max}} = 367$ nm and a less pronounced increase of the n - π^* absorption around 450 nm.

Illumination of the tubules (**2**, 1.33 mM) for less than 20 min did not yield a significant change in either their length (Figure 3.6A-B) or diameter (increased by ~ 1 nm, Figure 3.17). The marginal increase in length falls within the experimental uncertainty. Further, there are always 1 μM of nonassembled building blocks in solution (as indicated by the value of the critical aggregation concentration before irradiation), but their low number cannot account for this marginal length increase at a concentration of 1.33 mM.

Figure 3.5 UV-vis spectra of the isolated building blocks **1** and **2** in acetonitrile. (A) After UV irradiation and relaxation under ambient light for the chiral compound **1**. (B) Cycles of UV irradiation ($\lambda = 365$ nm, 15 s) and relaxation with the ambient light for the compound **1**. (C) UV-vis spectra of thermal relaxation of the chiral azobenzene **1** and (D) achiral azobenzene **2** under dark.

After 60 min of irradiation, cryo-TEM images show that the tubules become shorter (Figure 3.6C) and that their number increases (Figure 3.18). The distribution in length shows the presence of shorter tubules after 60 min of UV irradiation with the disappearance of long tubules (>300 nm) compared with the distribution before irradiation or after irradiation for 20 min (Figure 3.6D–F). Combined, these observations indicate that the tubules break into shorter segments, tentatively at positions where the azobenzene groups are switched to the *cis* form. Dynamic light scattering (DLS) data obtained in situ, at lower concentrations, support this conclusion further (100 μ M, Figure 3.7). At concentrations of ~ 1.33 mM used in the cryo-TEM measurements, complete disassembly of the tubules was not observed, as the remaining concentration of the *trans* form at the photostationary state is considerably greater than the critical aggregation concentration.

Figure 3.6 Structural evolution of the synthetic tubules formed by **2** upon irradiation with light. (A) Cryo-TEM picture before UV irradiation, (B) after 20 min of irradiation, (C) and after 60 min of irradiation in water ($\lambda = 365$ nm, $c = 1.33$ mM). The darker spherical objects are artifacts from the cryogenic medium. (D) Length distribution before UV irradiation, (E) after 20 min of UV irradiation, and (F) after 60 min of UV irradiation.

Figure 3.7 Dynamic light scattering measurements of the size of the tubules formed by compound **2** in water before (black line) and after 100 min of UV irradiation (red line, $c = 100$ μ M).

At lower concentrations of **1** in water (2.5 μM), full disassembly is achieved (Figure 3.8A). Parallel monitoring of the disassembly process by CD and by UV-visible (UV-vis) absorption spectroscopy shows that the complete disassembly is achieved after 35 min of irradiation, that is, before a photostationary state is reached (Figure 3.8B), with the *trans/cis* ratio reaching 83/17 only (Figure 3.19).

Figure 3.8 Conversion of light into molecular and supramolecular strain. (A) Evolution of the CD spectra of tubules formed by **1** (2.5 μM solution of **1** in water) as a function of the time upon irradiation (measured at 0, 1, 2, 5, 10, 20, 35, 50, and 60 min) with UV light ($\lambda = 365$ nm). The disappearance of the CD signal after 35 min indicates that the tubules have disappeared. (B) Absorption spectral changes induced by irradiation with UV light.

The photo-triggered decrease in the CD signal does not follow a monoexponential behavior (Figures 3.8A and 3.9A). Instead, 80% of the CD signal is lost abruptly within the first minutes of irradiation, when only 7% of the *transform* has switched into the *cis* form. We attribute this first phase to a reduction of the helical twist, which is accompanied by a red shift of the wavelength of maximum absorbance λ_{max} (Figures 3.8B, 3.9B and 3.20). Such a red shift indicates a change toward another molecular organization (J-aggregates),³⁸ leading us to conclude that the building blocks slide with respect to each other, within the tubules. After 5 min, when the *trans/cis* ratio is estimated at 93/7, the λ_{max} reaches its maximum value and remains constant for a further 30 min of irradiation (Figures 3.8B and 3.9B). During this time, the CD signal continues to decrease, albeit at a slower rate. After 35 min

of irradiation, the λ_{max} starts to shift toward shorter wavelengths, while the CD signal approaches zero. Once irradiation stops, the building blocks undergo thermal *cis*-to-*trans* switching (Figure 3.5); however, full reversibility of the supramolecular system is not achieved in these experimental conditions, likely because constant recovery of the *trans* form does not mediate the same cooperative effects as in a situation that involves the *trans* form exclusively.

When Nile red (0.94 μM) is encapsulated in the tubules formed by **1** in water (2.5 μM), the changes in UV-vis absorbance and CD spectra upon irradiation are the same as in the absence of the fluorescent probe (Figure 3.21), which indicates that the encapsulation of the Nile red does not alter the photo-triggered disassembly pathway. Release of Nile red expected to occur alongside the disassembly of the tubules reflects the complex disassembly process. The release of Nile red is manifested in changes in fluorescence. During the first minutes of irradiation the intensity of the fluorescent signal increases (Figures 3.9C and 3.21), which indicates an uptake of additional Nile red. A blue shift of the maximum emission wavelength (Figure 3.21) corresponds to a reorganization of the hydrophobic cavity, which is consistent with the red shift observed by UV-vis spectroscopy. After the initial increase of intensity, the fluorescent signal starts steadily decreasing (Figures 3.9C and 3.21), which indicates that Nile red is released and the tubules are disassembling.

3.2.4 Insights into the Mechanical Operation of the Tubules

Overall, irradiation of the self-assembled tubules pushes them out of equilibrium and toward a complex stepwise disassembly (Figure 3.9). First, when irradiation begins, the *trans* form of the azobenzene switches to the *cis* form at numbers that remain sufficiently low to be stabilized within the tubules, without forcing them to disassemble. The strain induced by the nonplanar and bended *cis* form is balanced by the free energy that would be required to solubilize the *cis* form in water.

Figure 3.9 Proposed mechanism for the light-triggered disassembly of synthetic tubules in water. (A) CD intensity at $\lambda = 400$ nm and (B) position of λ_{max} during the light-triggered disassembly in water ($2.5 \mu\text{M}$ of **1** in water). (C) Fluorescence intensity of Nile red ($0.94 \mu\text{M}$) at λ_{max} during the light-triggered disassembly of tubules formed by **1** in water ($2.5 \mu\text{M}$). (D) Scheme showing the stepwise mechanism of disassembly in water. Strain energy accumulates until it induces explosive breakage of the tubules.

As irradiation proceeds, the increasing amount of *cis* form gradually builds up further strain. Next, a critical amount of *cis* form within the tubules is exceeded and they start to break up, most likely at places where the concentration of the *cis* form is the highest in the tubules. The breaking of the tubules decreases the effective length of the hydrophobic cavity where the fluorescent probe can be encapsulated, which results in its gradual release and decrease of fluorescence (Figure 3.9C). Finally, when the tubules become shorter than their critical nucleation size they disappear completely. This final phase of the disassembly comes with a clear blue shift of the λ_{max} , as a sizeable fraction of the molecules are in the *cis* form and consequently start dispersing from the assembled state into individually solubilized molecules. The absence of isodichroic point is in agreement with switching from a CD-active assembly to a CD-inactive solution of molecules.

Our results also show that the exchange rate of the building blocks has a significant impact on the dynamics of the system. Irradiation of the tubules in a water/acetonitrile (95/5) solution is not accompanied by a shift of the absorption band. The CD signal consistently shows a simple behavior (Figure 3.10), which indicates that in the presence of an organic solvent the disassembly loses its complexity and follows a simple process, where the initial lag phase and buildup of strain are typically absent.

Alternative mechanisms for the dynamic molecular behavior on which we report can be excluded on the basis that they fail to support experimental evidence, including (i) the possibility of slow dissolution of the *cis* form and subsequent reequilibration of the assemblies that would be dictated by a lower concentration of the *trans* form in the system and (ii) the hypothesis that *trans*-to-*cis* isomerization would occur at a higher rate at the edge of the tubules, and induce dissolution of the *cis* form. In both cases, the tubules would undoubtedly shorten rather than break and, moreover, fast re-equilibration within the timescale of minutes would be required, which clearly does not happen in the current system (Figure 3.4). Furthermore, these alternative

mechanisms would not account for the observed spectral shifts, nor would the change of solvent alter the observed behavior.

Figure 3.10 Comparison of the initial state of the disassembly process in pure water and water/acetonitrile mixture (95/5). (A) UV-vis and (B) CD spectra of a solution of the tubules formed by **1** at 2.5 μM in water/acetonitrile mixture (95/5) upon UV irradiation. (C) UV-vis and (D) CD spectra of a solution of tubules formed by **1** at 2.5 μM in water upon UV irradiation. (E) CD signal at 390 nm and (F) UV-vis $\lambda_{\max}$ shifts *vs* irradiation time in water (black dots) and in water/acetonitrile mixture (red dots).

3.2.5 Strain Buildup and Tubule Destabilization Captured by All-Atom Simulations

Atomistic modeling provides further insight into the strain buildup in the tubules. Atomistic models for **1** and for the corresponding equilibrated tubule are shown in Figure 3.11. Tubule **1** was equilibrated and found very stable during 150 ns of an all-atom molecular dynamics (AA-MD) simulation (Figure 3.12). As used recently in the study of molecular transitions in supramolecular polymers,³⁹ starting from equilibrated tubule **1** (Figure 3.11A) we used well-tempered metadynamics⁴⁰ to calculate the minimum energy that is necessary for *trans*-to-*cis* isomerization in a monomer within the tubule (Figure 3.13). We used this information to set up out-of-equilibrium AA-MD simulations where *trans*-to-*cis* isomerization could be observed iteratively. The C-N=N-C dihedral angle potential of the assembled monomers was modified to disfavor the *trans* form, and as a result switching to the *cis* form was induced in the course of the simulation. This condition is consistent with all monomers being uniformly irradiated by light, while each transition depends on the crowding around individual monomers.

During simulations, the azobenzenes were seen to undergo *trans*-to-*cis* isomerization, which allows monitoring of the energy ΔE absorbed by the assembly, as a function of the increasing percentage of *cis* form in the tubule (Figure 3.11B). ΔE correlates to the strain energy that builds up in the system. The results demonstrate that the accumulation of strain in a tubule is nonlinear. In particular, while below $\sim 10\%$ of *trans*-*cis* transition we observe an initial phase where the supramolecular structure can keep the ΔE constant ($\Delta E \sim 2 \text{ kcal}\cdot\text{mol}^{-1}$, average value per-monomer in the system), above $\sim 15\%$ the ΔE rises considerably. Analysis of how much the monomer arrangement in the assembly deviates from the ordered one in the original tubule (Figure 3.11C, $\Delta\Phi$) shows that structural deformations in the tube are also nonlinear. Globally, the system shows a “stop-and-go” behavior, where the tubule accumulates energy, which is then released as plastic deformations of the structure. In fact, above $\sim 15\%$ of *trans*-to-*cis* isomerization we clearly observe the

appearance of holes in the tubule (Figure 3.11D), which constitutes further evidence of structural collapse.

Figure 3.11 Strain build-up and tubule destabilization captured by all-atom simulations. (A) Atomistic models of building block **1** and of the unperturbed original tubule. (B) Energy absorbed by the tubule (ΔE , values per-monomer) as a function of the percentage of *cis* form. (C) Deviation from the ordered arrangement of the monomers in unperturbed **1** ($\Delta\Phi$, in percentage), as a function of the percentage of *cis* form. Data are averaged data from three replica simulations. (D) Snapshots of tubule from **1** at the start (0%) and once *trans*-to-*cis* isomerization has reached 20%. Aromatic units are colored gray, internal water molecules are colored red while external water molecules are blue (PEG groups are not shown for clarity; they appear as voids). Above 20% of *trans*-to-*cis* isomerization, water diffuses in and out of holes in the structure (black circle).

These simulations are representative of a reduced portion of an infinite supramolecular tubule and are limited to a 100-ns timescale. However, while not allowing us to observe full disassembly of the tubules into monomers during the runs, the model provides evidence of the structural and energetic impairment introduced into the assembly by increasing levels of *trans*-to-*cis* isomerization, which are the key molecular factors underpinning tubule disassembly by exposure to light.

Figure 3.12 AA-MD equilibration of tubule 1. (A) Energy of tubule 1 as a function of AA-MD simulation time. (B) Root mean square deviation (Rmsd) of the atoms of tubule 1 as a function of AA-MD simulation time. (C) Deviation (in percentage) in the order parameter ($\Delta\Phi$) as a function of simulation time calculated respect to the ordered assembly of the monomers in equilibrated tubule 1. All data show that tubule 1 reaches stable equilibration along the AA-MD run.

Figure 3.13 Azobenzene *trans*-*cis* transition in monomer 1. (A) Free-energy profiles for *trans*-*cis* transition as captured by WT-MetaD simulations in disassembled in monomer 1 (black) or in monomer 1 as assembled in the tubule (red). (B) Dihedral potential term for the azobenzene *trans*-*cis* transition in monomer 1. Black: original non-modified dihedral (GAFF force field, Eq. 1). Red: modified dihedral (see Eq. 2) used in the out-of-equilibrium AA-MD simulations.

3.3 Conclusions

A complex, multistep, light-driven disassembly of aqueous supramolecular tubular assemblies has been demonstrated. The initial conversion of light into tubular strain, which is stored and accumulated before the system breaks apart, is reminiscent of the strain-driven disassembly of cellular microtubules, by which these biological machines generate forces that pull chromosomes apart—another challenge is to combine this operation mode with their complementary operation mechanism, where they push objects by ratcheting upon growing. Here, the strain energy that accumulates during the initial phase is related to the number of azobenzene units present in the *cis* form and the difference between the free energy gained by insertion of either the *trans* form (negative) or the *cis* form (positive) to the tubular structure. Ultimately, this system shows potential toward transducing the mechanical action of photoswitches across length scales, as in the process of vision, where large geometrical changes associated with double-bond isomerization are phototransduced by supramolecular assemblies, to yield macroscopic phenomena. We envision that in the future such synthetic molecular self-assembled architectures will be capable of generating directional forces at the nanoscale (e.g., by deformation of vesicular walls).

3.4 Acknowledgments

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3.5 Materials and methods

3.5.1 General

Materials. Reagents and solvents were purchased from commercial sources and used without further purification unless stated otherwise. ^1H - and ^{13}C -NMR were recorded at 400 and 100.6 MHz, respectively. 2D COSY and HMQC experiments were used to assist on NMR peak assignments. Chemical shifts are reported in δ = units (ppm) relative to the residual protonated solvent signals of CDCl_3 (^1H -NMR: δ = 7.26 ppm) and CD_3CN (^1H -NMR: δ = 1.94 ppm). Thin-layer chromatography (TLC) was carried out using Merck silica gel 60 on aluminum sheet, with visualization by UV light and by charring with a potassium permanganate solution (2%) and sodium hydrogen carbonate (4%) in water. Column chromatography was carried out using Merck silica gel 60 (230–400 Mesh). ESI-MS spectra were obtained for samples dissolved in DCM-MeOH at low μM concentrations.

UV-visible and circular dichroism (CD) spectroscopy. The solutions were prepared with concentrations in the micromolar range, at least 18 hours before the irradiation experiments, from a concentrated stock solution in water. All solutions were kept in the dark at all times. UV-visible spectra were recorded at room temperature with a Perkin Elmer Lambda 850 UV-visible spectrometer in 1-cm quartz cells. CD spectra were recorded with a Jasco J-1500 spectrometer. For experiments in water/acetonitrile, acetonitrile was added to a solution of the molecules in water, before irradiation.

Determination of critical aggregation concentrations. Critical aggregation concentrations were determined following a reported procedure.³⁶ Briefly, Nile Red at a concentration of 0.94 μM in milliQ water was used as a fluorescent probe. Dilutions of **1** and **2** were prepared in dye solution and incubated at room temperature for at least 15 min. Fluorescence emission spectra between 580–720 nm were recorded

using a fluorescence spectrophotometer (Perkin Elmer LS55) using an excitation at $\lambda = 550$ nm.

Dynamic Light Scattering. The samples were prepared a day prior to the acquisition. UV irradiated samples were measured immediately after the irradiation has stopped. DLS measurements were carried out using a Nanotracs (Anaspec) particle analyzer. For each condition, five measurements of 120 s were performed, with a set-zero time of 180 s. Data was analyzed assuming a refractivity index of 1.45. Measurements were carried out at a concentration 80 μ M. Particle size was determined following the intensity distribution.

Cryo-TEM. The solutions were prepared by solubilizing the compounds in deionized water at 1.33 mM, one day before the acquisition of micrographs. These solutions were used directly after irradiation. The cryo-TEM samples were prepared by depositing a few microliters of these solutions on carbon-coated grids (Quantifoil 3.5/1, Quantifoil Micro Tools, Jena, Germany). After blotting the excess liquid, the grids were vitrified in liquid ethane (Vitrobot, FEI, Eindhoven, The Netherlands) and transferred to a Philips CM 120 microscope equipped with a Gatan model 626 cryo-stage operating at 120 kV. Micrographs were recorded under low-dose conditions with a slow-scan CCD camera. The highest density is obtained from the electron dense aromatic part of the building blocks.

Transmission electron microscopy (TEM). The samples were prepared by drop-casting 80 μ M water solutions on carbon grids (Formvar/Carbon 200 mesh, Copper). Prior to measurements the samples were stained by Uranyl acetate. TEM micrographs were recorded using a Philips CM300ST - FEG microscope.

Atomic force microscopy (AFM). The samples were prepared by drop casting a solution of tubules in water, on freshly cleaved mica. After blotting the excess liquid, the samples were dried in air several hours and characterized by in tapping mode (Nanoscope IV). The AFM images were analyzed with the LA 1730 software.

Measurements of the diameter and length of the tubules. The Image J 1.46 software was used to analyze AFM images and (cryo-)TEM micrographs. Manual analysis led to determination of the length and diameter of the tubules. Longer tubules are underrepresented as their length was measured only within the size of the micrographs at the suitable magnification.

Irradiation experiments. The samples were irradiated by using a bluepoint LED Hoenle Technology lamp (300 mW.cm^{-2} , $\lambda = 365 \text{ nm}$).

3.5.2 Synthesis and characterization

Compounds **1** and **2** were synthesized according to a **Scheme 1**. Compounds **3**,⁴¹ **6**,⁴² and **7**⁴³ were synthesized according to reported procedures.

Scheme 1. Synthetic route towards **1** and **2**.

(*E*)-4'-((4-iodophenyl)diazenyl)-[1,1'-biphenyl]-4-carbonitrile (4).

4'-Aminobiphenyl-4-carbonitrile (87 mg, 0.45 mmol, 1 eq.) and **3** (105 mg, 0.45 mmol) were dissolved in acetic acid-EtOAc 1:1 (4 mL). The reaction mixture was stirred at 40 °C and an orange solid was formed after 20 min. The reaction was stirred for 5 h. The precipitated compound was filtered and washed with water, then re-dissolved in dichloromethane, dried over Na₂SO₄, and concentrated to give **4**. Yield: 140 mg (70%). ¹H-NMR (400 MHz, CDCl₃) δ 8.03 (d, *J* = 8.6 Hz, 2H), 7.89 (d, *J* = 8.7 Hz, 2H), 7.77–7.73 (m, 6H), 7.68 (d, *J* = 8.7 Hz, 2H). ¹³C-NMR (100.3 MHz, CDCl₃) δ 152.5, 152.1, 144.7, 141.9, 138.6, 132.9, 128.2, 128.0, 124.7, 123.9, 118.9, 111.7, 98.3. ESI-MS (*m/z*) calculated for [C₁₉H₁₂IN₃H]⁺ 410.02, found: 410.29.

(*E*)-4'-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)diazenyl)-[1,1'-biphenyl]-4-carbonitrile (5).

A solution of **4** (150 mg, 0.12 mmol), KOAc (35.3 mg, 0.36 mmol, 3 eq.), bis(pinacolato)diboron (138 mg, 0.18 mmol, 1.5 eq.) and a catalytic amount of Pd(dppf)Cl₂ (3.5 mg) in dry and degassed dioxane (3 mL) was stirred at 80 °C overnight. The solution was cooled down, diluted with dichloromethane and washed with water, then dried over MgSO₄, filtered and concentrated. The residue was purified by chromatography in toluene-hexane 9:1→toluene-MeOH 99:1 to give **5** as an orange solid. Yield: 75 mg (50%). ¹H-NMR (400 MHz, CDCl₃) δ 8.04 (d, *J* = 8.6 Hz, 2H), 7.98 (d, *J* = 8.4 Hz, 2H), 7.92 (d, *J* = 8.4 Hz, 2H), 7.76 (m, 6H), 1.38 (s, 12H). ¹³C-NMR (100.3 MHz, CDCl₃) δ 154.5, 152.7, 144.7, 141.7, 135.8, 132.9, 128.2, 128.0, 123.9, 122.2, 118.9, 111.6, 84.3, 25.1. ESI-MS (*m/z*) calculated for [C₂₅H₂₄BN₃O₂H]⁺ 410.20, found: 410.08.

Compound 1 and 2. A solution of **5** (60 mg, 0.15 mmol, 3 eq.), **6** (or **7**) (54 mg, 0.05 mmol), Pd(PPh₃)₄ (6 mg) and aqueous Na₂CO₃ (2 M, 5 mL) in THF (8 mL) was degassed and stirred under N₂ atmosphere at 80 °C for 48 h. The layers were separated and the aqueous phase was extracted with EtOAc. The combined organic layers were washed with water, dried (MgSO₄), filtered and concentrated. The residue was purified by chromatography in EtOAc-MeOH 24:1→23:2 to yield compound **1** (or **2**) as an orange solid. Yield: 56 mg (74%) for **1** and 47 mg (62%) for **2**.

(**1**) ¹H-NMR (400 MHz, CD₃CN) δ 7.96 (dd, *J* = 8.6, 1.8 Hz, 8H), 7.90 – 7.76 (m, 16H), 7.55 (s, 1H), 7.25 (d, *J* = 1.4 Hz, 2H), 4.19 (d, *J* = 5.7 Hz, 2H), 3.60 – 3.27 (m, 64H), 3.24 (s, 12H), 2.36 (dt, *J* = 11.7, 5.9 Hz, 1H), 1.00 (d, *J* = 6.3 Hz, 12H). ¹³C-NMR (100.3 MHz, CD₃CN) δ 161.1, 153.3, 152.8, 145.0, 144.3, 142.7, 142.3, 133.8, 129.1, 129.0, 128.7, 124.3, 124.3, 114.0, 112.2, 75.6, 75.5, 72.6, 71.4, 71.1, 71.0, 70.2, 69.9, 68.0, 58.9, 42.0, 17.4. HR-MS (*m/z*) calculated for [C₈₈H₁₁₆N₆O₁₉H]⁺ 1560.8295, found 1560.8214.

(**2**) ¹H-NMR (400 MHz, CD₃CN) δ 8.03 (dd, *J* = 8.6, 1.6 Hz, 8H), 7.94 (d, *J* = 8.6 Hz, 4H), 7.90 – 7.83 (m, 12H), 7.63 (t, *J* = 1.4 Hz, 1H), 7.32 (d, *J* = 1.4 Hz, 2H), 4.23 (d, *J* = 5.6 Hz, 2H), 3.58 – 3.54 (m, 4H), 3.51 – 3.40 (m, 64H), 3.25 (s, 12H), 2.38 (dt, *J* = 11.9, 5.9 Hz, 1H), 2.06 (dt, *J* = 11.8, 6.1 Hz, 2H). ¹³C-NMR (100.3 MHz, CD₃CN) δ 161.1, 153.3, 152.8, 144.9, 144.2, 142.7, 142.2, 133.7, 129.1, 129.0, 128.7, 124.3, 119.6, 119.2, 114.0, 112.2, 72.5, 71.3, 71.1, 71.0, 70.9, 70.1, 69.8, 67.3, 58.8, 41.2, 40.9. ESI-MS (*m/z*) calculated for [C₈₄H₁₀₈N₆O₁₉H]⁺ 1505.77, found: 1505.80.

3.5.3 Supplementary information

Figure 3.14 Determination of the photo-stationary state in acetonitrile by ^1H -NMR. (A) Azobenzenes compound states. Upon UV irradiation the initial *trans-trans* azobenzene switches to the *cis-trans* and *cis-cis* forms. (B) ^1H -NMR (400 MHz in CD_3CN) spectra of chiral compound 1 and (C) achiral compound 2 at the initial state (red spectra) and after UV light irradiation ($\lambda = 365$ nm, green spectra). (D) Ratio of the ^1H -NMR of the integrated peaks (highlighted by the arrows) corresponding to the *trans/cis* signals of compounds 1 and 2. The smaller proportion of the *cis* isomer in the case of compound 1 is probably due to the fact that the photostationary state was not reached at that moment. Irreversible photo-degradation became more prominent and have not allowed us to analysis the NMR spectra unambiguously.

Figure 3.15 Formation of synthetic tubules in water. (A) TEM of the tubules with the chiral building block **1** (83 μM). (B) Grey value profile of an area of TEM picture A. (C) TEM of the tubules with the achiral building block **2** (80 μM). (D) Grey value profile of an area of the TEM picture C. (E) AFM phase image of the synthetic tubules self-assembled from the achiral building block **2** in water and deposited on mica. (F) Height profile from the AFM image.

Figure 3.16 Linear fitting of the thermal relaxation under dark for (A) chiral azobenzenes **1** and, (B) achiral azobenzene **2**. The law rate is $\nu = k_1[\text{cis}] - k_2[\text{trans}]$. A_{∞} is the absorption at the initial state at 360 nm and A_t the absorbance at the time t at 360 nm.

Figure 3.17 Modification of the diameters of the tubules 2 (1.33 mM) before and after 20 minutes of UV light irradiation. The measurements have been done by using the difference of the contrast on pictures at different areas of bundled tubules. Values are given in nm.

Figure 3.18 Comparison of the number of the tubules **2** (1.33 mM) before and after 60 minutes of UV light irradiation.

Figure 3.19 Estimation of the *trans/cis* ratio during the UV irradiation process at 35 min. From the calibration curve of the absorption at 360 nm (30520 L.mol⁻¹.cm⁻¹, *cis* form has negligible absorption at this wavelength) at changing concentration of the compound **1** in water, the amount of the *trans* form was estimated to be 83 %.

Figure 3.20 Observation of the 3 step disassembly process of the tubules by UV-vis for the achiral building block 2 in water. (A) UV-vis spectra upon UV irradiation. (B) λ_{max} versus irradiation time and the 3 observed steps.

Figure 3.21 Photo-triggered disassembly of tubules formed by compound 1 (2.5 μM) in water with encapsulated Nile Red (0.94 μM). (A) UV-vis, (B) CD and (C) fluorescent spectra upon UV irradiation.

3.5.4 Extended computational methods

Construction of the atomistic models and equilibration AA-MD simulations. The atomistic (AA) models for monomer **1** and supramolecular tubule **1** were built and parametrized according the procedure recently adopted for similar self-assembling motifs.^{39,44–47} In particular, the AA model of monomer **1** was parametrized based on the General Amber Force Field, GAFF (*gaff.dat*).⁴⁸ The initial structure of tubule **1** was built starting from eleven hexagonal macrocycles, each containing six monomers **1** (Figure 3.1A), which have been stacked on the top of each other along *z*-direction (initial stacking distance of 4.4 Å) with a mutual tilting angle of 16.4°. Such a starting configuration for tubule is consistent with recent literature report on a similar tubule.²³ This tubule portion (composed of 66 monomers) was inserted into a simulation box (initial *X* × *Y* × *Z* dimensions of 12 × 12 × 4.84 nm) grazing the tube in *z*-direction and filled of explicit TIP3P⁴⁹ water molecules. In this way, replicated along *z* via periodic boundary conditions, this system effectively modeled a portion of the bulk of an infinite (helical) tubule **1**.

All simulations were conducted with the GROMACS 5.1.2 software.⁵⁰ After initial minimization, tubule **1** was equilibrated for 150 ns of AA-MD in periodic boundary NPT (constant N: number of atoms, P: pressure and T: temperature) using a timestep of 2 fs and a 10 Å cutoff. This simulation time was sufficient for the tubule to reach stable structural and energetic equilibration in the AA-MD regime (Figure 3.19). All AA-MD runs were conducted at 300 K (27°C) using the v-rescale⁵¹ thermostat (coupling constant of 2.0 ps) and 1 atm of pressure using semi-isotropic pressure scaling (compatibly with the directional nature of the tubule) with coupling constant of 2 ps. The particle mesh Ewald (PME)⁵² approach was used to treat long-range electrostatics. The LINCS algorithm was used to constrain all bonds involving hydrogens.⁵³

Well-tempered metadynamics simulations. The WT-MetaD⁴⁰ simulations have been conducted using the GROMACS 5.1.2 software⁵⁰ and the PLUMED 2 plugin.⁵⁴ We used the C-N=N-C dihedral angle in the azobenzene tail of monomer **1** as the collective variable (CV) describing/biasing the *trans-cis* transition. In the WT-MetaD runs, we used a HILLS height of 0.28 kcal mol⁻¹ and Gaussian SIGMA of 0.35 rad. A bias factor of 10 or 40 was used for the WT-MetaD simulations of the *trans-cis* transition in monomer **1** as respectively disassembled (in water) or assembled in the tubule. The other simulation parameters are the same reported above. From the WT-MetaD simulations we obtained the free-energy profiles for the *trans-cis* transition of the azobenzene groups in monomer **1** as disassembled in water or assembled in the tubule (Figure 3.20A: black and red respectively). The transition requires more energy in the assembled state compared to the disassembled one due to the crowding present in the assembly (at least ~20 kcal mol⁻¹ for an assembled monomer *vs.* ~9 kcal mol⁻¹ for a disassembled one). In the experiments, light irradiation provides the energy necessary to this transition. We used this information to set up out-of-equilibrium AA-MD simulations where *trans-cis* transitions in the tubule could be monitored iteratively.

Out-of-equilibrium AA-MD simulations and analysis of strain build-up. We modified the native dihedral angle potential of the azobenzene groups in monomer **1** to observe spontaneous *trans-cis* transitions during an AA-MD run (Figure 3.20B). This is equivalent to adding a static bias to the azobenzene dihedral potential of the monomers in tubule **1**. In the equilibrium AA-MD, the C-N=N-C dihedral potential of the native azobenzene groups in monomer **1** was defined by the sum of two terms as in Eq. 1 (standard for this group in the GAFF force field):

$$V_{d,native}(\varphi) = k_1(1 + \cos(n_1\varphi - \varphi_{1,s})) + k_2(1 + \cos(n_2\varphi - \varphi_{2,s})) \quad (1)$$

where $k_1 = 3$ kcal mol⁻¹, $n_1 = 2$, $\varphi_{1,s} = 180^\circ$ and $k_2 = 2.8$ kcal mol⁻¹, $n_2 = 1$, $\varphi_{2,s} = 0^\circ$ (Figure 3.20B: black curve). In the out-of-equilibrium AA-MD runs, such dihedral potential was modified as in Eq. 2 for all monomers in tubule **1**:

$$V_{d,out-of-equil}(\varphi) = k_3(1 + \cos(n_3\varphi - \varphi_{3,s})) \quad (2)$$

where $k_3 = 6.2 \text{ kcal mol}^{-1}$, $n_3 = 1$, $\varphi_{3,s} = 180^\circ$ (Figure 3.20B: red curve). This moved the *trans* configuration of the monomers in pre-equilibrated tubule **1** out-of-equilibrium, favoring transition to *cis* inside the tubule. The parameters of Eq. 2 were optimized to obtain a bias on the azobenzene dihedral potential consistent with the energy necessary for the *trans-cis* transition in an assembled monomer captured by WT-MetaD ($\sim 20 \text{ kcal mol}^{-1}$). This was verified to be the minimum bias to guarantee sufficient speed-up to allow us monitoring the *trans-cis* transitions in the timescale of the AA-MD run.

All other simulation parameters in these AA-MD runs were the same used for the equilibration runs (see above). During these out-of-equilibrium AA-MD simulations we monitored the *trans-cis* transitions of the azobenzene groups in the tubule using *gmx_angle*. The average energy absorbed by each assembled monomer in the tubule as a function of the percentage of *trans-cis* transitions (Figure 3.11B: strain build-up) was calculated as: $\Delta E = E_{\text{perturbed}} - E_{\text{native}}$, where E_{native} is the average energy of the monomers in the unperturbed equilibrated tubule **1**, while $E_{\text{perturbed}}$ is the average energy of the (modified) monomers during the out-of-equilibrium AA-MD ($\Delta E > 0$ identifies unfavorable energy variation/accumulation). The $\Delta\Phi$ parameter (Figure 3.11C: defined as $\Delta\Phi = \Phi_{\text{perturbed}} - \Phi_{\text{native}}$) measures the deviation during the runs from the parallel orientation of the planes defined by the aromatic rings in the monomers in equilibrated tubule **1** (stacking destabilization). The Φ parameter was calculated using the PLUMED plugin. $\Delta\Phi = 0$ means that the initial stacked configuration is perfectly preserved during the run, while the higher the $\Delta\Phi$, the higher the structural distortions in the assembly. All reported data were calculated as the average of three out-of-equilibrium runs.

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Chapter 4

Interfacing tubular supramolecular machines

In Chapter 3, I have shown how supramolecular tubules can generate a supramolecular strain upon sufficient irradiation with light. However, converting the force produced by the bursting of the tubules, into useful movement or work, remains a challenge, and in particular, interfacing these objects with their environment will be key to harness any mechanical action they could produce. In this chapter, we modify the external surface of the tubules, so that they can either connect to surfaces, or possibly connect with each other, in order to form bundles of tubules. The mechanical performance of these bundles is expected to bring this research one step closer to the generation of measurable forces. Our strategy consists in co-assembling the building blocks from the tubules previously demonstrated in chapter 3, with new building blocks that contain a biotin linker. We demonstrate that the new, functionalized building blocks incorporate into the tubules successfully, without any deformation of the tubular morphology and thus show potential to transfer work across increasing length scales.

4.1 Introduction

The development of artificial molecular machines has received much attention in the recent decades.¹ With the field reaching maturity, efficient methods have been developed to synthesize a variety of molecular machines that display sophisticated functionalities, including molecular muscles,² elevators,³ nanocars,⁴ walkers,^{5,6} peptide synthesizers,^{7,8} robotic arms,⁹ and stereodivergent synthesizers.¹⁰ Incorporating such dynamic molecules into synthetic systems with a high degree of functionality would set the stage for synthetic supramolecular systems to reach an unprecedented level of complexity, by achieving dynamic, directional function in far from equilibrium conditions, and thus entering length scales at which cellular machineries operate typically. However, these synthetic molecular machines typically operate in solution, where Brownian motion makes the molecules bounce against each other constantly.¹¹ Therefore, interfacing the operation of molecular motors and switches with complex systems remains a key challenge and, arguably, one major issue is with harnessing any mechanically-purposeful molecular motion against the Brownian storm, which is a few orders of magnitude larger in energy than any mechanical action produced by these small molecules. Devising strategies in which synthetic molecular motors, rotors and switches will be able to operate in spite of the Brownian storm, and under a constant flux of building blocks, will be key to letting motors and switches perform useful tasks at the nanoscale and beyond, e.g., the generation of strong directional forces at the nanoscale, the motility of nano-objects and, ultimately, the mechanical manipulation of larger molecular architectures.

Several approaches have been proposed in order to interface molecular machines to their environment, including their incorporation in self-assembled monolayers on surfaces, their covalent incorporation in polymers, and their insertion in self-assembled architectures.¹¹ The first two strategies have allowed producing macroscopic work, with droplets moving uphill against gravity,¹² bending

microcantilevers,¹³ rotating microscopic glass rods,¹⁴ contracting polymer gels,¹⁵ and, recently, the drilling of molecular motors into cellular membranes.¹⁶

The latter strategy, i.e. the incorporation of molecular machines into self-assembled architectures as a mean to decouple them from the Brownian storm in liquid environments, has been less explored, although biological systems have proven this strategy successful. Amongst the few examples of self-assembled machines that have been demonstrated so far, muscle-like supramolecular polymers integrate the actuation of individual rotaxanes by using H-bonding between the termination of axles, to form self-assemblies.^{17,18} In this work, the preference of the crown ether ring to bind to an electron-poor position on the axle causes the shuttling of individual rotaxane units. Overall, a local actuation between extended and contracted rotaxanes gives rise to morphological variations of the whole self-assembled architecture, and thus yields muscle-like behaviour in a molecular level.

However, this muscle-like rotaxanes system operates in a solution, where the random alignment of supramolecular structures cannot produce any directional movement at the macroscopic level. Lately, light-responsive fibers that show directional muscle-like function have been reported.¹⁹ The work by Feringa *et al.* is based on an amphiphilic molecular motor that assembles hierarchically into unidirectionally aligned nanofibers. Upon irradiation with UV light, the rotary motion that is generated by the motor leads to the fiber bending towards the light source, both in water and in air, and this shape transformation is even able to mediate the lifting of weight. This work consequently demonstrates that a small molecular motor can create muscle-like motion at the macroscopic level, once embedded in a self-assembled fiber, i.e. in the absence of any covalent interactions in the system.

Recently, it has been shown that upon incorporation of light-responsive molecular units into self-assembled structures, a strain-driven mechanism causes the catastrophic disassembly of supramolecular structures, and thus shows possibilities to generate pulling forces.²⁰ In these tubular architectures, azobenzenes are incorporated as light-responsive units. Upon UV irradiation, the geometrical changes associated

with the *trans*-to-*cis* isomerization weaken the π - π stacking interactions and disrupt the tubules, thus strain accumulates and eventually the tubules break up. This mechanism is reminiscent of the disassembly mechanism of cellular microtubules that can pull chromosome apart during the cell division process.

Overall, the current state of the art has established that amplifying the motion of artificial molecular machines by incorporating them in self-assembled architectures is feasible. So far, the measurable forces generated by these supramolecular systems have been limited to homogeneous systems in condensed matter. However, observing and harnessing a mechanical action produced by an individual object or, more generally, by a finite molecular assembly, requires (i) decoupling these objects from their turbulent environment and (ii) coupling them to an external probe. In this chapter, we report a general strategy to interface our artificial tubules with solid surfaces, or with other molecular architectures, so as to promote crosslinking. Developing such strategies constitutes an essential step towards the production of measurable directional forces.

4.2 Results and discussion

4.2.1 Molecular design

Our strategy to interfacing the synthetic tubules on surfaces involves rational design of the interaction between the tubules and the surface, or amongst the tubules. If a tubule was to be connected to the surface *via* many connection points, it would lose its original dynamic properties. Reversibly, if a tubule would be connected too loosely to the surface, the generated force would not be transferred effectively. In other words, a stable connection is required while it should not interfere with the intriguing dynamic behaviour of the tubules such as the demonstrated strain build up and the complex disassembly pathway.

We thus propose to modify the exterior of the tubules with linker units, that can mediate the connection to surfaces, through a controlled and tuneable number of

connection points (Figure 4.1A). We choose a connection that is strong enough to hold the tubules close to the surfaces, i.e. the biotin-streptavidin connection which is a highly specific and strong non-covalent binding pair that is frequently found in biological systems. With this strategy that we put forward, the number of connecting points will be limited and controllable, in order to preserve the dynamic behaviour of the tubules, allowing the strain to develop without compromising the complex behaviour of the tubules.

Figure 4.1 Modification of synthetic tubules in view of their dynamic connection to a surface. (A) Modification of the external shell of the tubules, and their subsequent binding on a surface *via* streptavidin-biotin interaction. (B) Molecular structures of the chiral azobenzene building block (AzoCh), and two different types of biotinylated building blocks (AzoPEGBt and AzoBt).

The original tubular structures are formed by self-assembly of chiral azobenzene molecules (AzoCh) in water, so the tubular shell can be easily modified by co-assembly with the new building blocks that contain linker units. The structure of

the hydrophobic V-shaped part of the molecule remains the same as in the **AzoCh**, to ensure that the new building blocks are able to co-assemble with **AzoCh** within the tubular structures. Finally, the linker unit is attached at the end of polyethylene glycol chain which will constitute the external shell of the tubule.

Requirements for the linker unit of our system are; strong non-covalent binding with the functionalized surfaces, high solubility in water, and covalently functionalizable with the building block. The streptavidin-biotin pair has been selected as one of the strongest non-covalent binding pair in biological system (the dissociation constant of streptavidin-biotin in water $\sim 10^{-15}$ M).²¹ Biotin can, in principle, be easily incorporated into the molecular design of a building block. Streptavidin-coated surfaces and interfacial systems have been previously reported.²² In addition, streptavidin proteins have high stability when complexed with biotins. The structures of the new building blocks, **AzoPEGBT** and **AzoBt**, are shown in Figure 4.1B. **AzoPEGBt** is as similar in design as possible with **AzoCh**, to promote co-assembly and good solubility in water as much as possible. **AzoBt** is designed as an alternative to **AzoPEGBt**, with the advantage of a reduced synthetic difficulty.

4.2.2 Synthesis of biotinylated building blocks

The synthesis of both biotinylated molecules is based on the same strategy as used for the synthesis of **AzoCh**. The azobenzene moiety will be connected to the hydrophilic oligo ether dendron by a Pd-catalyzed cross coupling reaction. The synthesis of azobenzene moiety has been described in chapter 3. This chapter will focus on synthesis of the hydrophilic part that is modified with a biotin linker.

AzoBt was successfully synthesized in five steps using Suzuki cross coupling as the key reaction (Scheme 4.1). Initially, one hydroxyl group of hexaethylene glycol **1** was protected with dihydropyran. Then the hydroxyl group in compound **2** was converted into a leaving group using *p*-toluenesulfonyl chloride. A substitution reaction between compound **3** and 3,5-dibromophenol, followed by removal of tetrahydropyran protecting group provided compound **4** with 70 % yield for two

steps. Suzuki cross-coupling reaction between aryl dibromide **4** and azobenzene boronate ester **5** gave the intermediate compound **6**. Finally, esterification between the hydroxyl group of compound **6** and biotin using EDC as a coupling reagent and DMAP as a catalyst yielded the target molecule **AzoBt** with the overall yield of 7 %.

Scheme 4.1 Synthesis of **AzoBt**.

The synthetic route we propose for **AzoPEGBt** is shown in Scheme 4.2. The details of the associated synthesis and compound characterisations are described in section 4.5.2. Compound **7** was prepared as reported in literature and reacted with an excess amount of methallyl dichloride to yield mono substituted **8**.²³ One of the Cl atoms in compound **8** is intentionally keep free for further substitution reaction. Compound **9** was synthesized from biotin.^{24,25} As the reaction between compound **9**

and triethylene glycol was accompanied with the formation of traces of a compound which was difficult to purify, the synthetic route was adjusted. The adjusted synthetic route involved the reaction of triethylene glycol with compound **8**, that was subsequently allowed to react with compound **9**. However, the polarity of the starting material **8** and of the substituted product of compound **8** with triethylene glycol were so similar that their purification revealed too challenging to perform successfully. A possible alternative strategy to solve the purification problem would have been to adjust the polarity of the triethylene glycol by transforming its hydroxy group into a tetrahydropyran or tosylate group, like synthesis of compound **2** or **3**, before it reacts with compound **8** or **9**.

Meanwhile, the **AzoBt** molecule was synthesized. Even though the structure of **AzoPEGBt** is closer to the structure of **AzoCh** that facilitates the co-assembly, **AzoBt** is more feasible to synthesize and can use as a simple model for the proof of concept.

Scheme 4.2 Proposed synthetic route for the hydrophilic moiety of **AzoPEGBt**.

4.2.3 Photochemistry of AzoBt in solution

The photoswitching behaviour of **AzoBt** building blocks as individual, non-assembled entities, is investigated by UV-Vis absorption spectroscopy. In acetonitrile (10^{-5} M), **AzoBt** displays a characteristic absorption band for a *trans* azobenzene, with a maximum absorption at $\lambda_{\text{max}} = 366$ nm (Figure 4.2A). Upon irradiation with UV light ($\lambda = 365$ nm), *trans*-to-*cis* isomerization occurs (Figure 4.2A). The large geometrical changes that are associated with this isomerization are altering the planarity and the conjugation of the azobenzene units. The π - π^* absorption band of the *trans* form at $\lambda = 366$ nm decreases, while the characteristic absorption band of the *cis* form at $\lambda = 286$ nm increases together with a less pronounced increase of the n - π^* absorption band at about $\lambda = 441$ nm. After 20 s of UV irradiation, **AzoBt** reaches a photostationary state (PSS). The *trans/cis* ratio is calculated as shown in Figure 4.7 and has a value 72/28 in the experimental conditions that we used.

Further, we have investigated whether the photo-switching of **AzoBt** is reversible in acetonitrile. The spontaneous relaxation of **AzoBt** occurs slowly in the dark, and alternatively, it can be accelerated by appropriate irradiation with visible light. In the dark, the *trans* form of **AzoBt** is fully recovered within 48 hours (Figure 4.2B), with a half-life of the relaxation of $t_{1/2} = 11.3$ hours (Figure 4.8). The relaxation that occurs under irradiation with visible light is faster than in the absence of visible light, and is completed within 30 minutes in the experimental conditions we use (see Materials and Methods section), as shown in Figure 4.2C. **AzoBt** shows reversible switching for four cycles under alternate irradiation cycles (Figure 4.2D). The presence of the isosbestic point further confirms the fatigue resistance of the molecule during the switching cycling, i.e. the absence of significant photo-degradation in the conditions of the experiment. Moreover, the isosbestic point also indicates that the two azobenzene units, within the molecule, behave as independent and decoupled photo-chemical systems.

Figure 4.2 Photoswitching properties of **AzoBt**. Absorption spectra of **AzoBt** in MeCN (10⁻⁵ M). (A) UV irradiation ($\lambda = 365$ nm). (B) Relaxation under dark. (C) Relaxation under visible light. (D) Cycle of photoswitching between UV irradiation (10 s) and visible light relaxation (15 min).

4.2.4 Self-assembly of **AzoBt** and co-assembly of **AzoBt** with **AzoCh** (spectroscopic investigations)

The first step towards achieving the final goal of interfacing azobenzenes tubules with surfaces, is to confirm that the co-assembly of **AzoBt** and **AzoCh** takes place and that the tubules can be formed. Before examining this, we look into the self-assembly behaviour of pure **AzoBt**. Towards this end we study the expression of chirality of **AzoBt** by circular dichroism spectroscopy (CD). We have previously showed that **AzoCh** gives pronounced CD signal when it forms chiral tubules in water above the critical aggregation concentration, while the achiral analogue of **AzoCh** does not yield any CD signal.²⁰ For **AzoBt**, we first carry out a CD

experiment in MeCN, an organic solvent in which **AzoBt** is expected to be present in a form of isolated monomers. Accordingly, no significant CD signal is observed despite the presence of the stereogenic centers in the biotin moiety (Figure 4.3A, black line).

Dissolving **AzoBt** in 5% MeCN/water was anticipated to lead to the formation of tubules, based on the structural similarity between the **AzoBt** and **AzoCh** building blocks. As there is no CD signal (Figure 4.3A, red line) we conclude that no chiral supramolecular structures are formed by pure **AzoBt**. We anticipate this to be a consequence of the number and position of chiral centers in supramolecular structures. For **AzoCh**, when it forms hexameric macrocycles, one macrocycle has 24 chiral centers and all of them are close to the tubule cores. On the contrary, if **AzoBt** would form a macrocycle, it had smaller number of chiral centers available for chirality transfer and all of them further away from the tubular core. Thus, with lower density of stereocenters around the hydrophobic core of **AzoBt**, we anticipate that achiral self-assembled structures forms.

Next, we perform transmission electron microscopy (TEM) experiments in order to determine which structures are formed by self-assembly of **AzoBt**. To this purpose, a solution is prepared with 50 μM of **AzoBt** in 5% MeCN/water. The TEM micrographs reveal the formation of disordered ribbons (Figure 4.3B). Instead of tubular shape formed by **AzoCh**, the ribbon structures formed by **AzoBt** may result from a lower hydrophilic volume, **AzoBt** having only one PEG chain. Such a variability in supramolecular structures has been observed for other bent-shaped amphiphiles before, and was also attributed to differences in the hydrophilic volume associated with the number of PEG chains.²⁶ In some cases, it has been shown that bent-shaped amphiphiles can even form nanofibers and nanosheets.^{27,28}

Figure 4.3 Self-assembly of **AzoBt** in acetonitrile, and in water. (A) CD spectrum of **AzoBt** (10 μM) in MeCN and 5% MeCN/water. (B) TEM micrograph of **AzoBt** (50 μM in 5% MeCN/water). (C) CD spectrum of **AzoBt** (5 μM) mixed with varying ratio of **AzoCh** in 5% MeCN/water. No Sergeant-and-Soldier effect occurs indicating that **AzoBt** does not form chiral aggregates.

Further, we have investigated whether chirality stemming from the **AzoCh** building block could be effectively transferred to self-assembled structures formed by co-assembly of **AzoBt** and **AzoCh**, based on a sergeant-and-soldiers principle.²⁹ Such transfer of chiral information could potentially induce transformation of the achiral ribbons (Figure 4.3A and B) to their chiral helical counter parts or even to induce their transformation to tubular shapes as originally designed. Therefore, a small amount of **AzoCh** (1-16%) below its critical aggregation concentration ($\sim 1 \mu\text{M}$) was mixed with **AzoBt** in 5% MeCN/water. None of the mixtures showed any emergence of a CD signal (Figure 4.3C), even when the proportion of **AzoCh** in solution was increased up to 16%. Based on this absence of significant CD signal, we conclude

that either **AzoCh** building blocks do not incorporate into the self-assembled structure, or it does not effectively transmit its chirality during the self-assembly process. Overall, we conclude that **AzoBt** cannot form chiral self-assembled structures neither by itself, nor in the presence of a small amount of the chiral building block inducers and it most likely does not form tubular architectures at these conditions.

Figure 4.4 Co-assembly of **AzoBt** and **AzoCh** observed by CD spectroscopy for 16 hours in 5% MeCN/water. (A) **AzoCh** (5 μ M), sample I. (B) Mixed solution of **AzoCh** (5 μ M) + **AzoBt** (5 μ M), sample II. (C) Mixing of preformed solution of **AzoCh** (10 μ M) and **AzoBt** (10 μ M). The final concentration of each molecules is 5 μ M, sample III. (D) Plot of CD intensities at $\lambda = 400$ nm vs time of samples I-III.

Thereafter we inspected an incorporation of **AzoBt** as linker molecules included in equimolar proportions into **AzoCh**-based tubules. This strategy was chosen in an anticipation that these proportions of **AzoBt** will still allow formation of the tubular architecture. We expected that the incorporation of **AzoBt** into **AzoCh** tubules is likely to take place, *via* the dynamic exchange behaviour of the building blocks within **AzoCh** tubules in 5% MeCN/water, that we had demonstrated previously.²⁰ The incorporation of the **AzoBt** should be accompanied by a reduction of the helical aspect following the dilution of the **AzoCh** within the tubules. Here, we followed this effect in time by CD spectroscopy for three different samples in 5% MeCN/water, namely sample I, II, and III. Sample I was a solution of **AzoCh** 5 μM as a control experiment (Figure 4.4A). Sample II was prepared by mixing both building blocks **AzoBt** and **AzoCh** in MeCN. Prior to measurements, water was added to form the self-assembled structures by allowing hydrophobic effects to take place. The concentration of each molecular component was 5 μM (Figure 4.4B). Sample III was prepared by two separately preformed solutions; a solution of the chiral tubules **AzoCh** (10 μM), and the pre-assembled **AzoBt** (10 μM). These two solutions were mixed, hence the final concentrations of each compounds were 5 μM , and the evolution of the CD signal was followed subsequently (Figure 4.4C).

The features of the CD spectra remained similar throughout the experiments, which indicates that the specific tubular architecture was preserved. The changes in CD intensities, however, suggest that the expression of chirality deteriorates over time. The evolution of the CD intensities at $\lambda = 400\text{ nm}$ is shown Figure 4.4D. The CD intensities of the control sample I (Figure 4.4D, blue) and mixed solution sample II (Figure 4.4D, red) were slightly increasing initially, and then became constant. The steady CD intensity observed for the control sample (sample I, blue), means that the tubular structures are stable even though exchanges between the free building blocks in water and in the tubular structures does occur. The CD intensity of the sample containing the building blocks that were mixed prior to the self-assembly process (sample II, red) yielded about half of the value of the CD intensity compared to the

control sample (sample I, blue), which suggests that **AzoBt** molecules co-assemble with **AzoCh** in tubules and at the same time reduce the overall expression of helicity in these chiral tubules. Moreover, the steady intensity of the CD signal after initial stabilization of sample II indicates that the exchange rates between **AzoBt** and **AzoCh** within the chiral tubules are similar, i.e. there is no propensity for the individual building blocks to form separate structures.

In sample III (Figure 4.4D, black), the initial CD intensity was close to that of sample I, which relates to the concentration of **AzoCh** in both samples. In addition, the aggregates of **AzoBt** displayed no CD signal, as previously described (Figure 4.3A). We conclude that the largest contribution to the CD intensities in sample III initially originates from the pure **AzoCh** containing chiral tubules. The gradual decrease of the CD intensity in sample III (about 17% within 16 hours) suggests that the dynamic exchanges of **AzoCh** and **AzoBt** building blocks occur. When **AzoCh** molecules in the tubular structure are replaced by **AzoBt**, the amount of stereocenters in the tubule shells is reduced following by the reducing of the preferential helicity. This is consistent with the CD intensity of sample II displaying a smaller CD intensity than that of sample I. Following the trend of the intensity decrease of the CD signal we expected that the equilibrated value in sample III will reach the same value as sample II. All of these changes in CD intensities of sample I-III confirm that the **AzoBt** molecules can be incorporated into the chiral tubules.

4.2.5 Formation of hybrid tubules by co-assembly of **AzoCh** and **AzoBt** (structural investigations)

As shown in Figure 4.1A, we seek to incorporate a few biotinylated linkers (**AzoBt**) within tubules that are formed by **AzoCh** predominantly. The appropriate amount of **AzoBt** in our design was calculated based on the self-assembled structures of the chiral tubules. Six molecules of **AzoCh** form individual hexameric macrocycle which stacks on top of each other. We propose to replace one out of the six molecules of **AzoCh** by **AzoBt** in every ten stacks of the macrocycles, which equals to the ratio

of **AzoBt**/**AzoCh** = 1/59 (that is ~1.7%). In practical, we use **AzoBt** at 2% of **AzoCh** concentration to minimize errors and uncertainty in sample preparation, and ensure the complete dynamic exchanges of the building blocks.

To investigate the structures that are formed solutions of the tubules were prepared at varying conditions (in water for Figure 4.5A, and in 5% MeCN/water for Figure 4.5B-E), and incubated overnight to ensure enough time for the dynamic exchange to take place. The resulting assemblies were then immobilized on TEM grid and finally stained with 1% uranyl acetate.

First, samples of **AzoCh** in water (Figure 4.5A) and in 5% MeCN/water (Figure 4.5B) were prepared as reference samples. These TEM micrographs reveal that adding 5% of MeCN to water does not preclude the formation of the tubular architectures (Figure 4.5A,B). In both solvents, the diameter of the tubules remains ≈ 6 nm.

In order to investigate the effect of sample preparation method on the co-assembly process, the mixture of 2% of **AzoBt** in **AzoCh** solutions were prepared by two different methods; dissolving of pre-mixed building blocks (Figure 4.5C) and mixing of separately preformed solutions (Figure 4.5D). The preparation procedures were the same as for samples II and III in section 4.2.4, respectively. Both sample preparations showed identical tubular structures while no other type of self-assembled architectures were observed. The diameter of the tubule is about 6 nm, similar to the reference sample. Combined with the results from the CD evolution graph in Figure 4.4D, we conclude that **AzoBt** building blocks are able to integrated into supramolecular tubules without causing any structural deformation.

Further, the proportion of **AzoBt** within the mixed tubules was increased to reach 20%. These samples were prepared by dissolving pre-mixed building blocks in 5% MeCN/water. TEM micrograph shows that the regular tubular shape is preserved. However, there are some area that show the structures with smaller diameter than

expected (3 nm) (Figure 4.5E). These structures cover considerably small areas and consequently it is likely that the majority of **AzoBt** is incorporated within the tubules.

Figure 4.5 TEM micrographs of the **AzoCh** supramolecular tubules after mixing with **AzoBt** building blocks. (A) **AzoCh** in water. (B) **AzoCh** in 5% MeCN/water. (C) Tubules formed by mixing of 2% of **AzoBt** and 98% of **AzoCh** and subsequent hydration. (D) Tubules prepared by mixing of preformed solution of 2% **AzoBt** and 98% of **AzoCh**. (E) Tubules formed by mixing of 20% of **AzoBt** and 80% of **AzoCh** and subsequent hydration. Samples (C)–(E) were prepared in 5% MeCN/water.

4.2.6 Formation of networks by cross-linking interactions

In the paragraphs above, we have described how interfacing artificial tubules with their environment can be done by using dynamic connection points to surfaces – this strategy could pave the way towards *in-situ* and label-free visualization of the dynamics of these objects. A complementary strategy however, consists in using the connecting points at the surface of the tubules, in order to promote the formation of cross-linked networks of artificial tubules. Here, our strategy to achieve cross-linking involves incorporating streptavidin as a cross-linker between the functionalized

tubules - this work would also constitute a proof of concept for the future connection of the tubules to streptavidin-functionalized surfaces.

The streptavidin protein is commercially available in lyophilized form in 10 mM phosphate buffered saline, pH 7.4. Streptavidin is a relatively small protein (diameter ~ 5 nm), and therefore, the stabilizing phosphate buffered saline (PBS) should be removed from the solution prior to any TEM measurements, in order to avoid the formation of a salt layer, that would cover the protein. However, in the absence of any buffer the streptavidin becomes unstable, and once deposited on a surface, we observed that the protein denaturates (Figure 4.6A).

Alternatively, when streptavidin is involved in a streptavidin-biotin complex, this protein is very stable. The streptavidin-biotin complex can actually maintain its quaternary structure at high temperature, in the presence of denaturants, and at extreme pHs.²¹ We consequently prepared a mixture of streptavidin-**AzoBt** (1:5), where a 20% excess of **AzoBt** is used to ensure that every streptavidin binds to four **AzoBt**. We observed small aggregates with a diameter about 5 nm (Figure 4.6B), which corresponds to the size of streptavidin. We conclude that the **AzoBt** building block binds to streptavidin and the structural integrity of the streptavidin-**AzoBt** complex prevails, even in the absence of any buffer solution. With this conclusion in hand, it was possible to investigate the cross-linking of tubules as mediated by streptavidin.

We have investigated the binding of **AzoCh-AzoBt** mixed tubules with streptavidin by using three different preparation methods, because it is well-known that the self-assembly pathway that is followed has an effect on the supramolecular structures that are formed finally.³⁰ The concentrations of each component in these samples are 75 μM **AzoCh**, 1.25 μM **AzoBt**, 0.25 μM of streptavidin in 5% MeCN/water, that is the ratio of **AzoCh:AzoBt:streptavidin** = 300:5:1. Even though all of the samples are consisted with the same components, TEM micrographs reveal different morphologies for each preparation procedure that was followed (Figure 4.6 C-E).

The first preparation procedure involves hydrating the pre-mixed **AzoBt** and **AzoCh** building blocks in a streptavidin solution in 5% MeCN/water. The resulting solution was allowed to equilibrate for 18 hours before measurement. In the second preparation procedure, streptavidin-**AzoBt** was mixed in 5% MeCN/water and equilibrated and after three hours, the **AzoCh** solution was added. The resulting solution was allowed to equilibrate for 18 hours before measurement. Both preparation procedures yield well-defined tubules and some deformed streptavidin as circular areas, as shown on TEM micrographs (Figure 4.6C and D). Comparing the images resulting from each of these two procedures, we observe that when **AzoCh** is present in the solution at the beginning (Figure 4.6C), the morphology of the tubules is the same as when **AzoCh** is added later (Figure 4.6D). This similarity suggests that the binding between streptavidin and **AzoBt** occurs faster than the characteristic time for the dynamic exchange between **AzoBt** in solution and **AzoBt** in the tubules (streptavidin-biotin binding rate constant $\sim 10^7 \text{ M}^{-1}\text{s}^{-1}$).³¹ However, new types of supramolecular structures that could be assigned to bundles of tubules were not observed. We, therefore, suspect that after streptavidin binds with **AzoBt**, it cannot incorporate into the tubules within the time range of this experiment. That is, **AzoCh** separately form tubular structures without any incorporation of streptavidin-**AzoBt** complex.

The third preparation procedure involves the preparation of **AzoBt-AzoCh** tubules by hydration of the pre-mixed **AzoBt** and **AzoCh** building blocks in 5% MeCN/water and equilibration for 18 hours. After this equilibration time, the streptavidin solution is added and kept for 3 hours prior to the measurement. TEM shows the swollen tubules with diameter up to 16 nm, shorten tubules, and non-defined aggregates in the background. It is likely that the swollen tubules are bundles of tubules which crosslink through the streptavidin. The shorter tubules might be formed because the strong interaction between streptavidin and the biotin pulls the **AzoBt** building blocks out of the preformed **AzoBt-AzoCh** tubules; consequently

the tubules break up and the **AzoBt**-streptavidin complex is visible as a small non-defined aggregates on the background.

This assumption will be further investigated by varying the concentration of **AzoBt** and streptavidin. When decreasing the amount of **AzoBt** from 2% to 0.02-0.2%, there will be one **AzoBt** insert in every a hundred or a thousand stacks of **AzoCh** macrocycles instead of ten. Then the less connection point to streptavidin may preserve the long tubules, as well as by simply reduce the connection point by decreasing the amount of streptavidin. Moreover, another parameter that can be tuned is temperature. Warming the system up to 50 °C will disassemble **AzoCh** tubules, and then we will attempt to cool it down in order to investigate whether **AzoCh** can co-assemble with streptavidin-**AzoBt** in these conditions.

Figure 4.6 TEM micrographs for study binding between **AzoBt**-**AzoCh** tubules and streptavidin. (A) Streptavidin. (B) **AzoBt**-streptavidin (5:1). (C)–(E) **AzoCh**-**AzoBt**-streptavidin (300:5:1) with different preparation procedures; (C) mixing all components at once, (D) **AzoBt**-streptavidin → **AzoCh**. (E) **AzoBt**-**AzoCh** tubules → streptavidin. All of samples were prepared in 5% MeCN/water.

4.3 Conclusions

We have synthesized biotinylated azobenzene building blocks (**AzoBt**) successfully, and we have demonstrated their successful incorporation into synthetic tubular structures of **AzoCh** in 5% MeCN/water. The photo-switching properties **AzoBt** were established in MeCN, and the co-assembly of **AzoBt** into **AzoCh** tubules was demonstrated by studying dynamic exchanges with CD spectroscopy. TEM micrographs showed that the presence of 2% of **AzoBt** does not disrupt the supramolecular structures formed by **AzoCh**. The binding between **AzoBt**-**AzoCh** tubules and streptavidin protein could not be concretely confirmed by TEM only; and would require combined investigations with fluorescence spectroscopy and 3D cryo-TEM techniques. In future work, we will optimize the preparation method in order to interface the tubules with the surfaces, as an inappropriate method could deform the tubular structures. Immobilization of the tubules on surfaces to create forces is ongoing. The real-time imaging of tubules while irradiating with UV light by using the interferometric scattering microscopy is in progress, in collaboration with the group of Prof. Philipp Kukura (Oxford).

4.4 Acknowledgments

We acknowledge Dr. Liulin Yang for sharing desalting procedures of streptavidin, as well as Dr. Rico Keim and Mark Smithers for assistance with TEM imaging.

4.5 Materials and methods

4.5.1 General

Chemicals were purchased from Sigma-Aldrich and used without further purification, unless stated otherwise. Streptavidin was purchased from Invitrogen. Compound **5** was prepared according to literature.²⁰ ¹H- and ¹³C-NMR were recorded at 400 and 100.6 MHz, respectively. Chemical shifts are reported in δ =

units (ppm) relative to the residual protonated solvent signals of CDCl_3 ($^1\text{H-NMR}$: $\delta = 7.26$ ppm), CD_2Cl_2 ($^1\text{H-NMR}$: $\delta = 5.32$ ppm), and CD_3OH ($^1\text{H-NMR}$: $\delta = 3.31$ ppm). Thin-layer chromatography (TLC) was carried out using Merck silica gel 60 on aluminum sheet, with visualization by UV light and by charring with a potassium permanganate solution (2%) and sodium hydrogen carbonate (4%) in water. Column chromatography was carried out using Merck silica gel 60 (230–400 Mesh). ESI-MS spectra were obtained for samples dissolved in DCM-MeOH at low μM concentrations.

UV-visible and circular dichroism (CD) spectroscopy. The solutions were prepared with concentrations in the micromolar range from a concentrated stock solution in acetonitrile. All solutions were kept in the dark at all times. UV-visible spectra were recorded at room temperature with a Perkin Elmer Lambda 850 UV-visible spectrometer in 1-cm quartz cells. CD spectra were recorded with a Jasco J-1500 spectrometer. The solutions were prepared at least 18 hours before the measurements, except for the dynamic exchange experiments, for which the samples were freshly prepared.

Transmission electron microscopy (TEM). The samples were prepared by drop-casting solutions on carbon grids (Formvar/Carbon 200 mesh, Copper). Prior to measurements the samples were stained by Uranyl acetate. TEM micrographs were recorded using a Philips CM300ST - FEG microscope.

Measurements of the diameter and length of the tubules. The Image J 1.46 software was used to analyze TEM micrographs. Manual analysis led to determination of the length and diameter of the tubules.

Irradiation experiments. The samples were irradiated by using a bluepoint LED Hoenle Technology lamp (300 mW.cm^{-2} , $\lambda = 365 \text{ nm}$).

Desalting PBS from streptavidin solution. Streptavidin is provided in lyophilized form in 10 mM phosphate buffered saline. The protein was dissolved in milliQ water and removed PBS buffer by Zeba Spin Desalting Columns, 7K MWCO. The final concentration of protein in solution was calculated from absorption at 280 nm

($\epsilon = 167000 \text{ M}^{-1}\text{cm}^{-1}$). Absorption of protein was measured by NanoDrop 1000 Spectrophotometer.

4.5.2 Synthesis and compound characterizations

Synthesis of 17-((tetrahydro-2*H*-pyran-2-yl)oxy)-3,6,9,12,15-pentaoxaheptadecan-1-ol (2)

Hexaethylene glycol 1 (10 g, 35.42 mmol) and *p*-Toluenesulfonic acid monohydrate (67.3 mg, 0.35 mmol) were dissolved in THF and cooled to 0 °C. 3,4-Dihydro-2*H*-pyran (1.49 g, 17.71 mmol) was dissolved in 10 mL THF and slowly dropwise to the reaction flask. The reaction was gently warmed to room temperature and stirred overnight. The reaction mixture was evaporated under reduced pressure and extracted with saturated aqueous NaHCO_3 and dichloromethane (DCM). The organic layer was dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by column chromatography on silica gel with DCM-acetone (3:1) to provide 17-((tetrahydro-2*H*-pyran-2-yl)oxy)-3,6,9,12,15-pentaoxaheptadecan-1-ol 2 as colorless liquid (1.78 g, 27%). ^1H -NMR (400 MHz, MeOD) δ 4.64 (t, $J = 3.53 \text{ Hz}$, 1H, OCHO), 3.80–3.91 (m, 2H, OCH_2), 3.48–3.73 (m, 24H, OCH_2), 1.79–1.90 (m, 1H, CCH_2), 1.66–1.74 (m, 1H, CH_2), 1.47–1.63 (m, 4H, CH_2). ^{13}C -NMR (100.3 MHz, MeOD) δ 100.36, 73.68, 71.61, 71.57, 71.53, 71.41, 67.91, 63.22, 62.23, 31.70, 26.58, 20.48. ESI-MS (m/z) calculated for $[\text{C}_{17}\text{H}_{34}\text{NaO}_8]^+$ 389.2146, found: 389.2699.

Synthesis of 17-((tetrahydro-2*H*-pyran-2-yl)oxy)-3,6,9,12,15-pentaoxaheptadecyl 4-methylbenzenesulfonate (3)

Compound 2 (1.59 g, 4.34 mmol) and pyridine (5.15 g, 65.1 mmol) were dissolved in 25 mL DCM. *p*-Toluenesulfonyl chloride (14.14 g, 21.7 mmol) was added at once to the stirred solution. The reaction mixture was stirred at room temperature for 5 hours. The reaction was then quenched with water and extracted with DCM. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The crude product

was purified by column chromatography on silica gel with DCM-acetone (3:1) to provide 17-((tetrahydro-2*H*-pyran-2-yl)oxy)-3,6,9,12,15-pentaoxaheptadecyl 4-methylbenzenesulfonate **3** as colorless liquid (1.99 g, 88%). ¹H-NMR (400 MHz, MeOD) δ 7.80 (d, J = 8.36 Hz, 2H, Ar-H), 7.45 (d, J = 7.99 Hz, 2H, Ar-H), 4.64 (t, J = 3.51 Hz, 1H, OCHO), 4.14 (m, 2H, OCH₂), 3.80–3.90 (m, 2H, OCH₂), 3.47–3.67 (m, 22H, OCH₂), 2.46 (s, 3H, ArCH₃), 1.78–1.89 (m, 1H, CCH₂), 1.66–1.74 (m, 1H, CCH₂), 1.47–1.61 (m, 4H, CCH₂). ¹³C-NMR (100.3 MHz, MeOD) δ 146.45, 134.51, 131.07, 129.09, 100.37, 71.61, 71.59, 71.57, 71.52, 70.95, 69.75, 67.91, 63.24, 31.70, 26.57, 21.59, 20.49. ESI-MS (m/z) calculated for [C₂₄H₄₀NaO₁₀S]⁺ 543.2234, found: 543.2340.

Synthesis of 17-(3,5-dibromophenoxy)-3,6,9,12,15-pentaoxaheptadecan-1-ol (**4**)

Compound **3** (800 mg, 1.54 mmol), 3,5-dibromophenol (580 mg, 2.31 mmol), and K₂CO₃ (637 mg, 4.61 mmol) were refluxed in 10 mL acetone for 24 hours. After cooling down to room temperature, the reaction mixture was filtered under vacuum. The filtrate solution was evaporated to dryness. Without further purification, the crude product was subsequently deprotected THP group by dissolved in 15 mL EtOH and 4 mL of 1 M HCl, and stirred at room temperature overnight. The solvent of reaction mixture was removed under reduced pressure, and extracted with water/DCM. The organic layer was dried over Na₂SO₄ and evaporated to dryness. The crude product was purified by column chromatography on silica gel with DCM-acetone (3:1) to provide 17-(3,5-dibromophenoxy)-3,6,9,12,15-pentaoxaheptadecan-1-ol **4** as yellow liquid (556 mg, 70%). ¹H-NMR (400 MHz, CDCl₃) δ 7.24 (t, J = 1.62 Hz, 1H, Ar-H), 7.02 (d, J = 1.63 Hz, 2H, Ar-H), 4.08–4.11 (m, 2H, ArOCH₂), 3.82–3.84 (m, 2H, CH₂OH), 3.69–3.74 (m, 4H, OCH₂), 3.64–3.68 (m, 14H, OCH₂), 3.59–3.62 (m, 2H, OCH₂). ¹³C-NMR (100.3 MHz, CDCl₃) δ 160.14, 126.69, 123.17, 117.27, 72.65, 70.97, 70.77, 70.76, 70.69, 70.65, 70.37, 69.55, 68.21, 61.87. ESI-MS (m/z) calculated for [C₁₈H₂₈Br₂NaO₇]⁺ 537.0094, found: 536.9802.

Synthesis of 4',4'''-((1*E*,1'*E*)-(5'-((17-hydroxy-3,6,9,12,15-pentaoxaheptadecyl)-oxy)-[1,1':3',1''-terphenyl]-4,4''-diyl)bis(diazene-2,1-diyl))bis([1,1'-biphenyl]-4-carbonitrile)) (6)

A dry 2-necked round bottom flask which contained a mixture of compound 4 (76 mg, 0.15 mmol), compound 5 (180 mg, 0.44 mmol), 2-dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl (XPhos) (17.5 mg, 25 mol%), palladium(II) acetate (Pd(OAc)₂) (3.3 mg, 10 mol%), and K₂CO₃ (121.6 mg, 0.88 mmol) was equipped under nitrogen atmosphere. A degassed mixed solvent 18 mL (THF: H₂O = 8:1) was then added to the reaction flask. The reaction mixture was heated to 90 °C for 3 days. After cooled down to room temperature, the reaction mixture was quenched with water and extracted with DCM. The combine organic layer was dried over Na₂SO₄ and evaporated to dryness. The residue was chromatographed on silica gel with DCM-acetone (3:1) → DCM-MeOH (9:1) as eluent to give 4',4'''-((1*E*,1'*E*)-(5'-((17-hydroxy-3,6,9,12,15-pentaoxaheptadecyl)oxy)-[1,1':3',1''-terphenyl]-4,4''-diyl)bis(diazene-2,1-diyl))bis([1,1'-biphenyl]-4-carbonitrile)) **6** as an orange solid (87 mg, 65%). ¹H-NMR (400 MHz, CD₂Cl₂) δ 8.06 (d, *J* = 8.42 Hz, 8H, Ar-H), 7.87 (d, *J* = 8.62 Hz, 4H, Ar-H), 7.76–7.81 (m, 12H, Ar-H), 7.59 (t, *J* = 1.46 Hz, 1H, Ar-H), 7.29 (d, *J* = 1.48 Hz, 2H, Ar-H), 4.31 (t, *J* = 4.68 Hz, 2H, ArOCH₂), 3.92 (t, *J* = 4.65 Hz, 2H, ArOCH₂CH₂), 3.73–3.76 (m, 2H, OCH₂), 3.58–3.68 (m, 16H, OCH₂), 3.53–3.56 (m, 2H, OCH₂). ¹³C-NMR (100.3 MHz, CD₂Cl₂) δ 160.23, 152.93, 152.40, 144.78, 143.99, 142.44, 141.90, 133.11, 128.45, 128.38, 128.12, 123.94, 123.89, 119.21, 119.13, 113.43, 111.88, 72.95, 71.18, 70.95, 70.90, 70.87, 70.84, 70.61, 70.08, 68.26, 61.97. ESI-MS (*m/z*) calculated for [C₅₆H₅₂N₆NaO₇]⁺ 943.3790, found: 943.4049.

Synthesis of 17-((4,4''-bis((*E*)-(4'-cyano-[1,1'-biphenyl]-4-yl)diazenyl)-[1,1':3',1''-terphenyl]-5'-yl)oxy)-3,6,9,12,15-pentaoxaheptadecyl 5-((3*aS*,4*S*,6*aR*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanoate (AzoBt)

Compound **6** (29 mg, 0.03 mmol), biotin (15.4 mg, 0.06 mmol), 1-Ethyl-3-(3'-dimethylaminopropyl)carbodiimide · HCl (EDC-HCl) (17.8 mg, 0.09 mmol), and 4-(dimethylamino)pyridine (1.9 mg, 0.015 mmol) were dissolved in DCM (5 mL) and stirred at 35 °C overnight. The reaction was then quenched with 1 M aqueous HCl solution and extracted with DCM. The organic layer was dried over Na₂SO₄ and evaporated to dryness. The crude product was purified by column chromatography on silica gel with DCM-acetone (3:1) → DCM-acetone-MeOH (15:5:1) to provide 17-((4,4''-bis((*E*)-(4'-cyano-[1,1'-biphenyl]-4-yl)diazenyl)-[1,1':3',1''-terphenyl]-5'-yl)oxy)-3,6,9,12,15-pentaoxaheptadecyl 5-((3*aS*,4*S*,6*aR*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanoate **AzoBt** as an orange solid (25 mg, 69%).

¹H-NMR (400 MHz, CD₂Cl₂) δ 8.06 (d, *J* = 8.51 Hz, 8H, Ar-H), 7.87 (d, *J* = 8.59 Hz, 4H, Ar-H), 7.76–7.81 (m, 12H, Ar-H), 7.59 (t, *J* = 1.43 Hz, 1H, Ar-H), 7.29 (d, *J* = 1.46 Hz, 2H, Ar-H), 5.49 (s, 1H, NH), 5.09 (s, 1H, NH), 4.45 (dd, *J* = 7.65, 5.06 Hz, 1H, H_d), 4.31 (t, *J* = 4.66 Hz, 2H, ArOCH₂), 4.26 (ddd, *J* = 7.62, 4.59, 1.45 Hz, 1H, H_c), 4.17–4.19 (m, 2H, COOCH₂), 3.91 (t, *J* = 4.63 Hz, 2H, ArOCH₂CH₂), 3.73–3.75 (m, 2H, OCH₂), 3.58–3.68 (m, 16H, OCH₂), 3.10–3.15 (m, 1H, H_a), 2.88 (dd, *J* = 12.81, 4.96 Hz, 1H, H_b), 2.68 (d, *J* = 12.78 Hz, 1H, H_c), 2.33 (t, *J* = 7.47 Hz, 2H, OOCCH₂), 1.58–1.71 (m, 4H, CCH₂), 1.38–1.45 (m, 2H, CCH₂). ¹³C-NMR (100.3 MHz, CD₂Cl₂) δ 173.79, 163.56, 160.20, 152.90, 152.38,

144.76, 143.96, 142.42, 141.89, 133.10, 128.44, 128.37, 128.11, 123.93, 123.88, 119.20, 119.12, 113.41, 111.86, 71.19, 70.94, 70.89, 70.87, 70.85, 70.81, 70.07, 69.44, 68.24, 63.81, 62.13, 60.42, 55.82, 53.84, 40.96, 34.07, 28.65, 28.60, 25.11. ESI-MS (m/z) calculated for $[C_{66}H_{66}N_8NaO_9S]^+$ 1169.4566, found: 1169.4536.

Synthesis of 13-(((2-(chloromethyl)allyl)oxy)methyl)-2,5,8,11,15,18,21,24-octaoxa pentacosane (8)

Dry NaH (700 mg, 29.2 mmol), methallyl dichloride (2.2 g, 17.6 mmol) and freshly distilled dry THF (75 mL) were placed in a dry round bottomed flask under N_2 atmosphere. Compound 7 (3.5 g, 8.8 mmol) was added to this mixture dropwise at 0 °C. The mixture was stirred at room temperature for 15 min and then at 65 °C for 12 h. After cooling to room temperature, the reaction was quenched with water and extracted with DCM. The organic layer was then dried over anhydrous Na_2SO_4 and the solvent was then removed by rotary evaporator. Purification of the residue by column chromatography on silica gel with MeOH-EtOAc (1:99) using a mixture of ethyl acetate and methanol (19:1 v/v) as eluent to yield 13-(((2-(chloromethyl)allyl)oxy)methyl)-2,5,8,11,15,18,21,24-octaoxapentacosane **8** as a pale yellow liquid (3.3 g, 77%). 1H -NMR (400 MHz, $CDCl_3$) δ 5.27 (d, J = 0.84 Hz, 1H, $(CH_2)_2C=CH_2$), 5.20 (dd, J = 2.42, 1.17 Hz, 1H, $(CH_2)_2C=CH_2$), 4.08 (d, J = 0.77 Hz, 2H, $(CH_2O)C=CH_2$), 4.04 (s, 2H, CH_2Cl), 3.65–3.61 (m, 16H, OCH_2), 3.58–3.54 (m, 8H, OCH_2), 3.50 (d, J = 6.11 Hz, 4H, OCH_2CH), 3.47 (d, J = 5.71 Hz, 2H, OCH_2CH), 3.38 (s, 6H, OCH_3), 2.20 (quin, J = 5.91 Hz, 1H, $CH(CH_2)_3$). ^{13}C -NMR (100.3 MHz, CD_2Cl_2) δ 142.37, 116.58, 72.06, 71.26, 70.77, 70.72, 70.66, 70.61, 69.72, 68.74, 59.18, 45.28, 40.21. ESI-MS (m/z) calculated for $[C_{22}H_{43}ClNaO_9]^+$ 509.2488, found: 509.2491.

Synthesis of (3*aS*,4*S*,6*aR*)-4-(5-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)pentyl)tetrahydro-1*H*-thieno[3,4-*d*]imidazol-2(3*H*)-one (10)

Compound 9 (150 mg, 0.39 mmol), triethylene glycol (1 g, 6.66 mmol), and K₂CO₃ (323 mg, 2.33 mmol) were refluxed in 10 mL acetone/MeCN for 24 hours. After cooled down to room temperature, the reaction mixture was filtered under vacuum. The filtrate solution was evaporated to dryness and extracted with water/DCM. The organic layer was dried over Na₂SO₄ and evaporated to dryness. The crude product was purified by column chromatography on silica gel with MeOH-DCM (1:4) to provide (3*aS*,4*S*,6*aR*)-4-(5-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)pentyl)tetrahydro-1*H*-thieno[3,4-*d*]imidazol-2(3*H*)-one **10** as colorless liquid (trace yield). ¹H-NMR (400 MHz, MeOD) δ 4.49 (ddd, *J* = 7.9, 4.9, 0.7 Hz, 1H, NCH), 4.30 (dd, *J* = 7.9, 4.5 Hz, 1H, NCH), 3.68–3.63 (m, 8H, OCH₂), 3.60–3.55 (m, 4H, OCH₂), 3.48 (t, *J* = 6.5 Hz, 2H, OCH₂), 3.54–3.19 (m, 1H, SCH), 2.93 (dd, *J* = 12.7, 5.0 Hz, 1H, SCH₂), 2.70 (d, *J* = 12.7 Hz, 1H, SCH₂), 1.78–1.70 (m, 1H, CCH₂), 1.63–1.54 (m, 3H, CCH₂), 1.48–1.40 (m, 4H, CCH₂). ¹³C-NMR (100 MHz, MeOD) δ 166.16, 73.69, 72.22, 71.62, 71.57, 71.41, 71.13, 63.44, 62.23, 61.63, 57.17, 41.02, 30.48, 30.13, 29.77, 27.21. ESI-MS (*m/z*) calculated for [C₁₆H₃₁N₂O₅S]⁺ 363.1948, found: 363.1892.

4.5.3 Supplementary information

Figure 4.7 Determination of *trans*-to-*cis* ratio at photostationary state. (A) UV-vis absorption spectrum of **AzoBt** in MeCN at a vary concentration from 4-20 μM of *trans* form. (B) Calibration curve plotted from absorption at 366 nm. The equation is $y = 0.06(x) + 0.0032$. The absorption at PSS is 0.162 which is equal to 2.64 μM of *trans* isomer that is 18% of *trans* form remaining at PSS.

Figure 4.8 Determination of half-life time of relaxation in the dark of **AzoBt** in MeCN (10^{-5} M) after irradiation with UV light for 20 second. Half-life time of the relaxation reaction in the dark was calculated from absorbance at 366 nm ($A_0 = 0.1837$ at UV 20 s, $A_{eq} = 0.636$ at 48 h) and found that the $t_{1/2} = 11.3$ h.

4.6 References

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Chapter 5

Light-fuelled expansion of spiropyran-based vesicles in water

We present the design and synthesis of spiropyran-based dynamic vesicles, for which the building block is the amphiphilic merocyanine isomer. Under illumination with visible light, the photo-conversion of the protonated merocyanine to the spiropyran form leads to the transient and reversible expansion of these vesicles.

5.1 Introduction

A variety of photo-switchable supramolecular structures have been developed over the past decade,¹⁻³ targeting applications that range from molecular electronics, to drug delivery and biomedicine.⁴ The dynamic non-covalent character of the bonds that hold these architectures together — in combination with the dynamic character of the photo-switchable building blocks, allows control of their function by means of in-situ molecular engineering.

Photo-switching between building blocks that show different propensity to assemble in a closed system can be performed reversibly, repeatedly, with an exquisite control over the timescale, without accumulating chemical waste and in combination with spontaneous reversal.⁵ Control over non-covalent interactions by molecular switching is typically realized through changes of shapes, dipole moments, rigidity, helical chirality, and charged state – characteristics that are featured in one or more commonly used artificial molecular photo-switches such as azobenzenes,⁶ diarylethenes,^{7,8} overcrowded alkenes,^{9,10} and spiropyrans.¹¹

Among these photo-responsive molecular switches, spiropyrans undergo the most dramatic modification of their physico-chemical properties upon switching, which results in a considerable modification of the supramolecular interactions they mediate. The closed form spiropyran (SP) is neutral and colorless while the open zwitterionic merocyanine (MC) form has a large electric dipole moment because of the opposing separated charges and appears colored due to absorption at about 550–600 nm.⁷ In addition, MC is significantly more basic than SP, and protonation of MC leads to the formation of protonated merocyanine (MCH⁺) with a characteristic absorption band shifted towards shorter wavelengths, compared to the MC form.

Recently, spiropyran switches have been used to control the dynamic self-assembly of proteins,^{12,13} nanoparticles,^{11,14} micelles,^{15,16} and vesicular architectures.^{17,18} Light-responsive micelles made of spiropyran-based hyperbranched

polyphosphates¹⁹ showed reversible size changes after illumination. The light-induced shrinking of the micelles was accompanied with a molecular cargo release that reached 68% after 5 min of illumination. Similar concepts were developed, where hydrophilic polymers were coupled to spiropyrans, for drug release purposes.^{20,21} Many of these studies have focused on using light to disassemble supramolecular structures, aiming at potential applications towards molecular cargo delivery and controlled release. However, creating responsive spiropyran-based objects that display a well-defined and ordered architecture, both before and after irradiation, remains a major challenge that would pave the way towards dynamic aggregates with a higher level of control and functionality.

Here, we present the formation of light-responsive vesicles based on small amphiphilic molecules bearing two spiropyran moieties (Figure 5.1A), where the building block 1 is composed of an oligoether dendron as the hydrophilic part, and a bent-shaped aromatic containing the spiropyran as the hydrophobic part. When dissolved in acidic water, the spiropyran units spontaneously adopt the open protonated merocyanine form (MCH^+). This MCH^+ form can be transiently converted into the closed spiropyran form (SP) by irradiation with visible light (Figure 5.1B). Thus the MCH^+ vesicle can be forced to out-of-equilibrium state, with the size of vesicle increasing as a result of a light-fuelled morphological transformation of the building blocks.

Figure 5.1 Structure and properties of light-responsive amphiphiles **1**. (A) Schematic representation of envisioned changes in vesicles upon light-induced isomerization of a spiropyran-based constituent. (B) Structure of light-responsive amphiphile **1** in its open protonated form (MCH⁺) and closed spiropyran form (SP), in acidic aqueous solution (pH = 2).

5.2 Results and discussion

5.2.1 Synthesis

Amphiphile **1** was synthesized in the spiropyran form, in two steps (Scheme 5.1). First, commercially available bromo-spiropyran **2** was converted to its borate ester *via* a Pd-catalyzed Miyaura borylation reaction. Subsequently, spiropyran borate ester **3** was coupled to the *m*-dibromobenzene-dendritic oligoethyleneglycol derivative **4**,²² by means of a Suzuki cross-coupling. The details of the structures, synthesis and characterizations are provided in section 5.5.2.

Scheme 5.1 Synthesis of target molecule **1**.

5.2.2 Self-assembly behaviour in the absence of light

The self-assembly of amphiphile **1** and its potential to form aggregates was investigated under both neutral and acidic conditions. Transmission electron microscopy (TEM) showed that both the SP and MCH^+ isomers of **1** self-assemble into vesicular architectures (Figure 5.2A–D). The MCH^+ isomer forms small vesicles with a constant average diameter of about 20 nm within the measured concentration range. In contrast, the SP isomer forms larger vesicles, the size of which increases with increasing concentrations (Table 5.1). The fact that the MCH^+ form assembles into vesicles that keep a constant size, while the total concentration of amphiphilic building blocks is increasing, indicates that the MCH^+ assembly process is self-limiting.²³ Given the positive charge carried by the MCH^+ form, we attribute this self-limiting assembly process to the accumulation of electrostatic repulsive interactions, that destabilize the vesicle over a certain size. As this charge-based mechanism of self-limiting growth does not apply to the neutral SP isomer, the size of SP vesicles scales with effective concentration, *i.e.* for the SP-vesicles, the larger the concentration in **1**, the larger the vesicles are.

Table 5.1 Average size of vesicles at various concentrations and pH values, as deduced from TEM images.

Total concentration of 1 (mM)	Average size (nm)	
	pH = 2	pH = 7
0.5	20.9 ± 1.4	25.1 ± 2.0
1	20.5 ± 2.0	30.0 ± 4.7
3.3	20.4 ± 3.1	35.0 ± 9.6

The self-assembling behaviour of **1** in water was confirmed by following the wavelength shift in the fluorescence spectra, for increasing total concentrations of **1** (Figures 5.2E and 5.2F). At low concentration (1 μM), the emission band of the SP and MCH^+ isomers differ in shape while their maximum emission wavelength is $\lambda \sim 415$ nm. At high concentration (50 μM), the fluorescence bands are considerably red-shifted towards $\lambda = 500$ nm at pH = 7, and $\lambda = 540$ nm at pH = 2. These red-shifts indicate formation of J-aggregates, i.e. the aromatic moieties within the hydrophobic portion of the vesicles are close packed and oriented head-to-tail.²⁴ Further, the critical aggregation concentration (CAC) of **1** was determined by concentration-dependent encapsulation of pyrene as a polarity sensitive fluorescence probe.²⁵ In short, as concentration of **1** increases and the vesicles form, pyrene preferentially locates in the hydrophobic interior of the vesicles. This transfer from the aqueous to the hydrophobic environment is accompanied by changes in the fluorescence signal of the probe (Figure 5.5). We have established that the SP and MCH^+ forms both start assembling at similar concentrations; 5.1 μM at pH = 7, and 5.7 μM at pH = 2, respectively.

Figure 5.2 TEM microscopy images and fluorescence spectra of the vesicles formed by amphiphile **1** at equilibrium, depending on the pH and total concentration of building blocks in water. (A) Vesicles formed by MCH⁺ at a total concentration of 1 mM at pH = 2. (B–D) Vesicles formed by SP at a total concentration of 0.5, 1, 3.3 mM, respectively, and at pH = 7. (E) Fluorescence spectra of **1** measured at 1 and 50 μ M for MCH⁺-vesicles at pH = 2. (F) Fluorescence spectra of **1** measured at 1 and 50 μ M for SP-vesicles at pH = 7.

5.2.3 Photoswitching behaviour

Prior to investigating the dynamic behaviour of the self-assembled vesicles, the photo-chemistry of amphiphile **1** was investigated under both neutral (pH = 7) and acidic conditions (pH = 2). For the neutral condition, a solution of **1** at total concentration of 10 μ M was prepared by dissolving **1** in milliQ water and the solution was kept in dark overnight before being measured. The characteristic absorption spectrum of such a neutral solution of **1** is shown in Figure 5.3A. The absorption band at $\lambda = 254$ nm is attributed to the π - π electronic transition of the indoline unit and the shoulder absorption band around $\lambda \sim 300$ nm corresponds to the chromene moiety.¹¹ This indicates that in neutral conditions, **1** remains in the spiropyran form.

The acidic solution of **1** was prepared by dissolving **1** in 10 mM HCl. This solution was kept in the dark overnight to allow conversion of the SP form into the MCH⁺ form. The value pH = 2 was chosen based on two considerations. First, the pH of the solution must be lower than the pK_a of the MC form (pK_a $\sim$ 3–4), to ensure that the phenolic oxygen of the open form is protonated. Second, the pH should be high enough, so as to avoid protonation of the nitrogen atom of the SP form as the protonated SP form (SPH⁺) does not undergo the ring opening reaction.²⁶ The spectrum of solution **1** at pH = 2 shows the characteristic bands of the open protonated MCH⁺ form at $\lambda \sim 370$ and $\lambda \sim 430$ nm (Figure 5.3A). These bands are assigned to the absorption of the chromene open form with the protonated oxygen.¹¹

Under acidic conditions (pH = 2), the photo-switching properties of amphiphile **1** are preserved. In deuterated acetonitrile acidified with DCl, the amphiphiles are not aggregated, and **1** is in the MCH⁺ form as determined by NMR (Figure 5.6). In deuterated water acidified with DCl, the vesicles can form, but the broadness of the NMR peaks consequently does not allow determining the ratio between the different forms of the building blocks in solution (Figure 5.6).

At pH = 2, the MCH⁺/SP ratio during the photo-switching was determined by following the evolution of the absorption spectra, under irradiation with visible light

(Figures 5.3B and Figure 5.7). After 2 min of irradiation, 75% of the MCH^+ was converted into the SP form. When prolonging the irradiation to a total of 6 min, the system reached the PSS at a ratio of 18/82 of MCH^+ /SP (Figures 5.3B and Figure 5.7). In the dark, the SP form spontaneously relaxed back to the MCH^+ form with a half-life time of 3.7 h (Figures 5.3C and Figure 5.8). The relaxation rate was increased when the temperature was increased. At 60 °C, 95% of the SP form reverted to the MCH^+ form within 14 min (Figures 5.3D and Figure 5.8). We conclude that the photochemistry of the molecular switch is reversible under the conditions of our experiments.

Figure 5.3 Photoswitching behaviour of amphiphiles **1**. (A) Absorption spectra of amphiphile **1** in water at a total concentration of 10 μM ; black: SP form at neutral condition, red: MCH^+ form at pH = 2, blue: photo-stationary state (PSS) as observed after irradiation of MCH^+ with visible light for 6 min. (B) Absorption spectra of compound **1** at 10 μM under acidic condition (pH = 2) and upon visible light irradiation. (C) Relaxation of compound **1** at 10 μM (pH = 2) after 6 minutes of visible light irradiation in the dark. (D) Relaxation of compound **1** at 10 μM (pH = 2) after 6 minutes of visible light irradiation, upon heating at 60 °C.

5.2.4 Light-fuelled dynamic assembly process

Next, we have investigated the dynamic behaviour of the MCH^+ vesicles, at $\text{pH} = 2$, in response to irradiation with visible light. TEM reveals that under irradiation, the $\text{MCH}^+ \rightarrow \text{SP}$ transition fuels the expansion of the vesicles (Figure 5.4). Their diameter increases from 20.4 ± 3.1 nm (Figure 5.4A) to 34.6 ± 3.9 nm (Figure 5.4B), the latter corresponding to the size of the equilibrated SP vesicles when they are formed under neutral conditions (35.0 ± 9.6 nm, Table 5.1). Strikingly, the SP-vesicles that are photo-generated from the MCH^+ -vesicles always display a narrower size distribution in comparison to the SP-vesicles that are formed under neutral conditions directly (Figure 5.4B). We thus conclude that the initial presence of the MCH^+ vesicles, that grow under a self-limiting assembly, influences the photo-generated conversion into SP-vesicles, specifically, by narrowing down their size distribution. In addition to the TEM structural investigations, changes in the fluorescence spectra provide further insight into the molecular mechanism supporting the light-fueled expansion of the MCH^+ -vesicles (Figure 5.4D). Following the irradiation with visible light, the fluorescence band of MCH^+ was blue-shifted from a maximum emission at $\lambda = 550$ nm, towards the characteristic emission band of the aggregated SP form (Figure 5.4D). We attribute the newly appeared shoulder at $\lambda = 450$ nm to the co-aggregation of SP and MCH^+ in the vesicles, and conclude that 18% of MCH^+ form remains present in the vesicles, at the PSS.

We have followed the evolution of the vesicles once irradiation was stopped, in order to characterize the reversibility of this system. With heating the sample at 60°C for 30 min in the absence of light, the vesicles revert back to a size that is comparable to their original size (22.2 ± 3.0 nm, Figure 5.4C).

Figure 5.4 Light-fueled reversible expansion of the dynamic MCH⁺-vesicles to SP-vesicle, upon light irradiation. TEM images and the related size distribution curves of the vesicles at pH = 2; (A) initial MCH⁺-vesicle, (B) at PSS after irradiation with visible light, (C) relaxation after heating at 60 °C for 30 min. (D) Fluorescence spectrum of 1 at 50 μ M. Note that the black dashed line in (B) is a distribution of SP-vesicle at pH = 7 as a reference.

Our results point towards an original molecular mechanism for the shape transformation of the vesicles, which is inherently related to these well-defined aggregates being pushed away from their equilibrium size, when the system is fueled with light. When **1** is in the MCH⁺ form, the growth of the vesicles is limited by the accumulation of charges, but when MCH⁺ transforms into SP, the vesicles are allowed to grow to a new out-of-equilibrium size, that is this time dictated by the number of SP molecules available to participate to the self-assembly, i.e. the total concentration in building blocks and the ratio at PSS.

5.3 Conclusions

In conclusion, we have synthesized a spiropyran-based amphiphile that self-assembles into vesicles, in water. The original mechanism involves self-limiting growth of the merocyanine-containing building blocks. Photo-switching of the protonated merocyanine leads to a doubling of the diameter of the vesicles, and this expansion lasts for as long as the system is illuminated. Once illumination stops, the vesicles shrink back following the thermal relaxation to the spiropyran form. In future work, we envision that mixing the switchable building blocks reported here, with a range of flexible amphiphilic building blocks, can lead to the formation of phase-segregated areas in the resulting hybrid vesicles.²⁷ In these artificial formations, the presence of localized deformations and of differential surface stiffness will hold potential towards achieving complex shape transformations and, ultimately, towards pre-programming the life-like behaviour of compartmentalized supramolecular systems. Overall, developing dynamic aggregates based on spiropyran as artificial molecular switches emerges as a relevant strategy to design dynamic compartmentalized nanosystems.

5.4 Acknowledgments

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5.5 Materials and methods

5.5.1 General

Materials and characterization methods. Reagents and solvents were purchased from commercial sources and used without further purification unless stated otherwise. ^1H - and ^{13}C - NMR were recorded at 400 and 100.6 MHz, respectively. Chemical shifts are reported in δ = units (ppm) relative to the residual protonated solvent signals of CD_3CN (^1H -NMR: δ = 1.94 ppm). Thin-layer chromatography (TLC) was carried out using Merck silica gel 60 on aluminum sheet, with visualization by UV light. Column chromatography was carried out using Merck silica gel 60 (230-400 Mesh). ESI-MS spectra were obtained for samples dissolved in DCM-MeOH at low μM concentrations.

UV-visible spectroscopy. The solutions were prepared at μM range concentrations. They were prepared at least 12 hours before the irradiation experiments from a concentrated stock solution in water. All the solution were kept under dark. UV-visible spectra were recorded at room temperature (20 °C) with a Perkin Elmer Lambda 850 UV-visible spectrometer in 1-cm quartz cells.

Critical aggregation concentration determination. Critical aggregation concentration (CAC) was determined by pyrene 1:3 ratio method as described elsewhere.²⁵ Briefly, pyrene at a concentration of 1 μM in milliQ water was used as a fluorescent probe. The pyrene solution was used to prepare appropriate dilutions of 1 that were further incubated at room temperature for at least 15 min. Fluorescence emission spectra between 350-500 nm were recorded using a fluorescence spectrophotometer (Perkin Elmer LS55) at an excitation of 340 nm. The decrease of

the fluorescence intensity ratio between the first and the third peak with changing concentration of the **1** was used to determine the CAC value.

Transmission electron microscopy (TEM) measurements. The samples for TEM measurements were prepared by drop-casting a solution of compound **1** on carbon grids (Formvar/Carbon 200 mesh, Copper) and stained by Uranyl acetate. TEM micrographs were recorded using a Philips CM300ST - FEG TEM equipment.

Measurements of the diameter of vesicles. ImageJ 1.46 software was used to analyze TEM micrographs in order to determine the diameter of vesicles.

Visible light irradiation. The samples were irradiated with visible light using Edmund MI-150 high-intensity illuminator.

5.5.2 Synthesis and characterization

1',3',3'-trimethyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)spiro[chromene-2,2'-indoline] (3)

6-bromo-1',3',3'-trimethylspiro[chromene-2,2'-indoline] **2** (350 mg, 0.98 mmol), 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (B₂Pin₂) (349 mg, 1.37 mmol), potassium acetate (KOAc) (289 mg, 2.95 mmol), and [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (Pd(dppf)Cl₂) (36 mg, 0.05 mmol) in anhydrous dioxane (15 mL) was stirred at 90 °C under N₂ atmosphere for 24 h. After cooling down to room temperature, 15 mL of water was added to the mixture. The crude product was extracted into CH₂Cl₂ and dried over anhydrous Na₂SO₄. After removing the solvent under reduced pressure, the residue was purified by column chromatography on silica gel using 2% EtOAc in hexane as an eluent initially and followed by a gradual increase of the polarity to 1:1 EtOAc/hexane mixture to yield compound **3** as an orange solid (210 mg, 53%). ¹H-NMR (400 MHz, CD₃CN) δ 7.47 (d, *J* = 1.4 Hz, 1H), 7.45 (dd, *J* = 8.1, 1.6 Hz, 1H), 7.14 (td, *J* = 7.7, 1.2 Hz, 1H), 7.09 (dd, *J* = 7.3, 0.8 Hz, 1H), 6.99 (d, *J* = 10.3 Hz, 1H), 6.81 (td, *J* = 7.5, 0.9

Hz, 1H), 6.59 (d, $J = 8.1$ Hz, 1H), 6.55 (d, $J = 7.7$ Hz, 1H), 5.77 (d, $J = 10.3$ Hz, 1H), 2.69 (s, 3H), 1.30 (s, 12H), 1.24 (s, 3H), 1.13 (s, 3H). ^{13}C -NMR (100.3 MHz, CD_3CN) δ 158.01, 149.15, 137.64, 137.31, 134.43, 130.29, 128.56, 122.48, 120.19, 120.16, 119.58, 115.09, 107.82, 105.91, 84.56, 52.48, 29.09, 26.09, 25.11, 20.09. ESI-MS (m/z) calculated for $[\text{C}_{25}\text{H}_{31}\text{BNO}_3]^+$ 404.24, found: 404.28.

Spiropyran amphiphile (1)

Compound 4 (137 mg, 0.124 mmol) was dissolved in 16 mL of degassed mix solvent THF/water (7:1), then added to a mixture of compound 3 (150 mg, 0.37 mmol), potassium carbonate (103 mg, 0.74 mmol), and tetrakis(triphenylphosphine)-palladium(0) ($\text{Pd}(\text{PPh}_3)_4$) (29 mg, 0.025 mmol) under N_2 atmosphere. The reaction mixture was heated at 100°C for 48 h. After cooling down to room temperature, the mixture was quenched with water and extracted 3 times with EtOAc. The combined organic extract was dried over anhydrous Na_2SO_4 . Evaporation of solvents left a crude product which was purified by column chromatography with EtOAc/MeOH (10:1) as eluent to afford compound 1 as an orange liquid (71 mg, 38%). ^1H -NMR (400 MHz, CD_3CN) δ 7.52 (d, $J = 2.3$ Hz, 2H), 7.47 (dd, $J = 8.4, 2.3$ Hz, 2H), 7.37 (s, 1H), 7.16 (td, $J = 7.7, 1.2$ Hz, 2H), 7.11 (d, $J = 7.3$ Hz, 2H), 7.09 (d, $J = 1.5$ Hz, 2H), 7.04 (d, $J = 10.2$ Hz, 2H), 6.82 (td, $J = 7.5, 0.9$ Hz, 2H), 6.69 (d, $J = 8.4$ Hz, 2H), 6.57 (d, $J = 7.7$ Hz, 2H), 5.83 (d, $J = 10.2$ Hz, 2H), 4.16 (d, $J = 5.7$ Hz, 2H), 3.55–3.40 (m, 64H), 3.26 (s, 12H), 2.73 (s, 6H), 2.33 (quin, $J = 5.9$ Hz, 1H), 2.05 (quin, $J = 5.9$ Hz, 2H), 1.29 (s, 6H), 1.15 (s, 6H). ^{13}C -NMR (101 MHz, CD_3CN) δ 161.05, 155.13, 149.23, 143.16, 137.69, 133.83, 130.31, 129.33, 128.58, 126.60, 122.51, 120.84, 120.15, 115.70, 112.05, 107.84, 105.80, 72.54, 71.26, 71.14, 71.08, 70.98, 70.94, 70.11, 70.03, 69.77, 67.06, 58.84, 52.56, 41.26, 40.89, 29.20, 26.12, 20.23. ESI-MS (m/z) calculated for $[\text{C}_{84}\text{H}_{121}\text{N}_2\text{O}_{21}]^+$ 1493.85, found: 1493.7

5.5.3 Supporting experiments

Figure 5.5 Critical aggregation concentration (CAC) of compound **1** determined by the fluorescence response of pyrene to changing concentration of **1** causing its transition into the hydrophobic environment of formed vesicles. The CAC in each condition was determined by the intersection of two trend lines from the plot between the ratio of the first and the third band maxima (I_{373}/I_{383}) of the fluorescence and log concentration. The determined CAC values are; (A) and (B) at 5.1 μM under the neutral condition, pH = 7; (C) and (D) at 5.7 μM under the acidic condition, pH = 2.

Figure 5.6 ^1H -NMR of **1** at different conditions. (A) In CD_3CN . (B) With 10 mM DCl in CD_3CN . (C) With 10 mM DCl in D_2O . Compound **1** is fully converted into its MCH^+ form in CD_3CN and under acidic conditions (green). Broadening of the spectrum in D_2O (red) does not allow for determination of the SP and MCH^+ ratio.

Figure 5.7 Determination of SP/MCH⁺ ratio at the photostationary state. (A) Absorption spectra of compound 1 under neutral condition (pH = 7) at varying concentration. (B) Calibration curve of compound 1 using absorbance at 437 nm. (C) Absorption spectrum of compound 1 at 10 μM under acidic condition (pH = 2) and visible light irradiation.

Table 5.2 Calculation of the SP/MCH⁺ ratio at pH = 2 after visible light irradiation

Abs at 437 nm = 0.02156 (concentration in μM) + 0.01298 (equation 5.1)				
Condition	Absorbance at 437 nm	Conc. (μM) calculated from eq. S1	% of the MCH ⁺ →SP	MCH ⁺ /SP
initial	0.222	9.67	0	100/0
vis 1 min	0.091	3.61	58.96	41/59
vis 2 min	0.055	1.95	75.12	25/75
vis 3 min	0.046	1.51	79.46	21/79
vis 4 min	0.042	1.37	80.86	19/81
vis 5 min	0.043	1.38	80.68	19/81
vis 6 min	0.041	1.30	81.54	18/82

Figure 5.8 Relaxation of compound 1 at 10 μM (pH = 2) after 6 minutes of visible light irradiation. (A) Absorption spectra showing the relaxation of MCH⁺ in the dark. (B) First order plot for the relaxation in the dark. (C) Absorption spectra showing the relaxation upon heating at 60 °C. (D) Switching cycles between visible light irradiation for 2 minutes (green) and relaxation upon heating at 60 °C for 5 minutes (pink).

Half-life time of the relaxation reaction in the dark was calculated from first order reaction kinetics;

$$\ln\left(\frac{A_0 - A_{eq}}{A - A_{eq}}\right) = (k_1 + k_2)t$$

$$t_{1/2} = \frac{0.693}{k}$$

From plot; $y = mx + c$

Slope (m) $\equiv (k_1 + k_2) = 0.188$; $t_{1/2} = 0.693/0.188 = 3.69$ h

5.6 References

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Chapter 6

Designing out-of-equilibrium self-assembly behaviour in shape-persistent supramolecular architectures

Control of self-assembled structures by molecular photo-switching has been described in the previous chapters (Chapters 3 and 5). While the formation of tubular assemblies under equilibrium control (Chapter 3) and photo-induced growth of non-tubular assemblies were achieved, combining these two features would constitute an important step towards developing shape persistent out-of-equilibrium systems. In analogy to cellular microtubules, such supramolecular polymerization machines would be capable of generating directional forces. In this chapter, we present an effort towards designing light-controlled tubular assemblies based on photoswitchable amphiphiles. The specific design of the building block involves a spiropyran photoswitch that renders changes of the amphiphilic character of the molecule, and facilitates an out-of-equilibrium self-assembly process upon light irradiation.

6.1 Introduction

Cellular microtubules are biological molecular machines that produce directional forces at the nanoscale through two distinct assembly processes.^{1,2} In the first case, the growth of the microtubules, initiated by the binding of guanosine triphosphate (GTP) to the tubulin, generates pushing forces that control shape modification of the cell and its motility.³ In the second case, the pulling force is generated through a catastrophic disassembly initiated by the buildup of the strain energy following the hydrolysis of GTP and corresponding tubulin conformational switching within the microtubules.⁴ These pulling forces are used to transport chromosomes during cellular division. These processes operate out-of-equilibrium under a constant influx of chemical energy from GTP. Mimicking these functions could help to understand the mechanics of the cell and open perspectives towards creating wholly synthetic living cells from molecular components, which is one of the greatest contemporary challenges.^{5,6}

In Chapter 3, we have shown the synthesis and dynamic behaviour of supramolecular tubules based on aromatic rod-coil amphiphiles decorated with azobenzene photoswitches.⁷ These tubular architectures are spontaneously assembled in water from stable building blocks. These equilibrated structures can be disassembled by UV light initiated photo-switching of the building blocks. As the population of the non-favourable isomers accumulates within the tubules, strain develops and the supramolecular structure eventually breaks up. The mechanism is reminiscent of the conformational wave model proposed for disassembly of cellular microtubules.^{4,8} Further, in Chapter 5, we have shown that other types of supramolecular architectures (vesicles) based on spiropyran amphiphiles can grow under light irradiation. The size of the transiently formed vesicles and the related out-of-equilibrium state depend on the light irradiation intensity.

In this chapter, we discuss our efforts towards developing a fully synthetic tubular system that mimics the out-of-equilibrium growth processes observed for cellular

microtubules. The molecular design is based on spiropyran amphiphiles, as in Chapter 5, with modifications of building blocks that we envisioned would yield tubular assemblies (Figure 6.1). First, the absence of a rigid phenyl moiety in the hydrophobic part of the molecule in Chapter 5 precludes formation of π - π stacking, which is necessary for molecules to stack on top of each other to form tubular structures.⁹ Second, the middle aromatic ring that connects to spiropyran (SP) at the 6-position of the chromene moiety is unable to stabilize the phenolic oxygen of the protonated merocyanine open form (MCH⁺).¹⁰

Figure 6.1 Molecular re-engineering of amphiphiles from Chapter 5. The elongated phenyl rings (labelled in pink) are incorporated into the hydrophobic part of amphiphiles **1**, in order to increase π - π stacking interactions. The indoline rings, instead of chromene rings in amphiphiles from Chapter 5, of spiropyran are fused with the rigid aromatic moiety of amphiphiles **1** (labelled in green) which allows the addition of nitro groups as electron withdrawing groups at the 6-position of the chromene rings to stabilize the MCH⁺ form.

Herein, we redesigned the spiropyran amphiphiles discussed in Chapter 5, by elongating the rigid hydrophobic arms by phenyl rings (labelled in pink), to increase the propensity of the structure to employ π - π stacking interactions, in an otherwise twisted spiro geometry (Figure 6.1). Further, the indoline rings of the spiropyran are fused with the rigid aromatic unit of amphiphiles **1**, instead of the chromene rings in amphiphiles from Chapter 5 (labelled in green), thus allowing substitution of electron withdrawing nitro groups at the 6-position of the chromene moiety of the spiropyran in amphiphiles **1** to stabilize the negative charge on the phenolic oxygen of the open merocyanine form. The identical oligoether dendron is still present as a hydrophilic part to render solubility of compound **1** in aqueous environments.

Dissolving molecule **1** in acidic protic medium leads to a spontaneous opening of the spiropyran units, which is followed by protonation of the phenolic oxygen. The protonated merocyanine (MCH⁺) form is thus formed, and the entire molecule becomes more hydrophilic (Scheme 6.1). As a consequence of the increased hydrophilicity, we anticipate that the molecule will not form self-assembled structures in aqueous environments. After visible light irradiation, the MCH⁺ form transiently switches to the closed SP form within its given half-life. Taking advantage of the dramatic changes of the properties from MCH⁺ (hydrophilic) to SP (hydrophobic) forms,¹¹ the rigid V-shaped part becomes hydrophobic, and the molecule adopts a clear division of the hydrophilic and hydrophobic portions. The combined contribution of hydrophobic effects, π - π stacking interactions, and shape recognition in the case of this building block, is thus expected to drive the self-assembly process, leading to the formation of tubular architectures. The energy consumption process that continuously regenerates the active self-assembling species ensures that the formation and maintenance of the self-assembled structure is an out-of-equilibrium process.

Scheme 6.1 Controlling amphoteric character of compound 1 by spiropyran photoswitches.

6.2 Results and discussion

6.2.1 Synthesis

The amphiphile **1** was successfully synthesized in three steps using Suzuki cross-coupling as a key reaction (Scheme 6.2). First, the double coupling reaction between 3,5-dibromophenol **2** and biphenyl boronate ester **3** was carried out with an excess amount of compound **3** to minimize the formation of oligomeric by-products. Compound **4** was then reacted with oligoether dendron **5** to provide the precursor **6**. Bromospiropyran **7** was prepared by bromination of commercially available nitrospiropyran using *N*-bromosuccinamide as a brominating reagent in chloroform. The target molecule **1** was finally yielded by Suzuki cross-coupling reaction between compounds **6** and **7**. It is worth noting that the last step of the reaction was performed under mild conditions at 50 °C to prevent hydrolysis of the spiropyran moiety. Synthesis details and compound characterization are provided in section 6.5.2.

Scheme 6.2 Synthesis of target molecule **1**. Reagents and conditions: (a) Pd(OAc)₂, XPhos, K₂CO₃, PhMe, EtOH, H₂O, N₂, 90 °C; (b) K₂CO₃, EtOH, reflux; (c) Pd(OAc)₂, XPhos, K₂CO₃, PhMe, EtOH, H₂O, N₂, 50 °C.

6.2.2 Photoswitching behaviour

The photoswitching behaviour of **1** was investigated by UV-Vis absorption spectroscopy in a variety of solvent systems. First, the photoswitching of compound **1** between the SP and merocyanine open form (MC) was investigated in acetonitrile (Figure 6.2A) and methanol (Figure 6.2B), which are both organic solvents where compound **1** is initially in the SP form. The characteristic absorption bands of the SP form were observed at 270 and 328 nm, which correspond to π - π^* transition of the indoline and the chromene ring, respectively.¹¹ Upon UV light irradiation ($\lambda = 365$ nm), compound **1** shows a fast switching to the MC form, reaching the photostationary state (PSS) in less than 30 seconds. The new absorption bands characteristic for the MC isomer were observed at 540 nm in both solvents (Figure

6.2A-B). Surprisingly, the absorption intensity of the MC form in methanol is much lower than that in acetonitrile, because we expected better charge stabilization of the MC form in more polar solvents.^{12,13}

Following this initial characterization, and in accordance with the aim of this work to control self-assembly growth by photoswitching between the stable charged state (MCH^+) and the non-charged SP form, we explored the photoswitching behaviour under acidic conditions. To this end, compound **1** was dissolved in 10 mM HCl in water (pH $\sim$ 2) and the absorption spectrum of **1** was measured (Figure 6.2C). However, the expected characteristic absorption of the MCH^+ form at about 400 nm was not observed. The absorption bands at 275 and 337, characteristic of the SP form, were observed instead, suggesting that the MCH^+ isomer was not formed. We hypothesize that at such low pH levels, the nitrogen of the SP form becomes rapidly protonated, forming a protonated spiropyran (SPH^+ , Figure 6.2E) that does not undergo spontaneous ring opening to produce the MCH^+ form.¹³ Irradiating this sample with UV light ($\lambda = 365$ nm) led to a decrease in intensity of the absorption band at 337 nm that corresponds to the absorption of the chromene moiety. Unfortunately, no new absorption band could be observed and the original absorption band could not be recovered. Therefore, we assume that compound **1** is susceptible to hydrolysis under strong acidic conditions. It is worth mentioning that compound **1** is insoluble in pure water. The additional phenyl rings in the rigid V-shaped part most likely affect the solubility of the compound, and poses a limitation for studying this molecular system in aqueous solutions.

To avoid the harsh conditions that promote hydrolysis of compound **1** in the presence of strong acid, HCl was replaced by a weaker acetic acid. Photoswitching of compound **1** in acetic acid is shown in Figure 6.2D. In this solution, compound **1** exhibits the characteristic absorption band of the MCH^+ form at 427 nm, and reaches the PSS after 20 seconds of visible light irradiation. Half of the SP is converted back to the MCH^+ form by 20 seconds of UV light irradiation. These results demonstrate

that in organic solvents and in weak acids, compound **1** shows the expected photoswitching behaviour.

Figure 6.2 Photo-switching behaviour followed by UV-vis absorption spectroscopy of compound **1** (10 μM) in various solvents; (A) Acetonitrile, (B) Methanol, (C) 10 mM HCl aqueous solution and (D) Acetic acid. (E) Interconversion pathway between the different forms of spiropyrans; spiropyran close form (SP), protonated spiropyran (SPH⁺), merocyanine open form (MC), and protonated merocyanine (MCH⁺).

Because our rational design for forming tubular architectures involves employment of hydrophobic forces, we examined the effect of water addition on photoswitching rates and PSS composition. Finding the optimal ratio of water/solvent that will maximize differences in the composition of the building blocks between two switching states will be key for promoting the self-assembly process.

First, a small amount of water (5%, 10%, and 20%) was added to acetonitrile solution (Figure 6.3A-C, respectively). The absorption spectra show that with increasing water ratio in acetonitrile, the characteristic absorption band of the MC isomer at 540-560 nm was less pronounced and it takes more time to reach PSS.

Figure 6.3 Photoswitching of compound **1** (10 μ M) in varying solvent mixtures. (A) 5% water in acetonitrile. (B) 10% water in acetonitrile. (C) 20% water in acetonitrile. (D) 25% water in acetic acid. (E) 50% water in acetic acid. (F) 50% water in methanol with 10 mM HCl.

Then, mixtures of 25% and 50% of water in acetic acid were explored (Figure 6.3D-E, respectively). Both of them showed the characteristic absorption band of the MCH^+ form at approximately 435 nm. Upon visible light irradiation, the absorption band at 435 nm decreased, accompanied by a red shift of the absorption bands' maxima; 311 to 316 nm for 6.3D, and 315 to 325 nm for 6.3E. Additionally, a new

absorption band corresponding to the SP form, with a maximum absorption at 277 nm, was formed (Figure 6.3E). At PSS, for 25% water in an acetic acid mixture (Figure 6.3D), the MCH⁺ band at 435 nm still remains, whereas it almost fully disappears in the mixture with 50% water (Figure 6.3E). Half the amount of the photo-generated SP form can be converted back to the MCH⁺ form by UV light irradiation ($\lambda = 365$ nm) in less than 30 seconds for both mixtures. Prolonging UV irradiation leads to decomposition of compound **1** as the intensity of the characteristic absorption band of MCH⁺ decreases. Based on these observations, the suitable mixture should contain 50% water in acetic acid, in which a larger amount of the SP form is photo-generated upon visible light irradiation. Moreover, the higher ratio of water content should potentially provide for more pronounced hydrophobic effects.

We also attempted one more acidic solvent mixture by dissolving compound **1** in 10 mM solution of HCl in 50% water in methanol. The switching of the MCH⁺ to the SP form under visible light irradiation was also observed but was less pronounced than in water-acetic acid mixtures (Figure 6.3F).

6.2.3 Self-assembly behaviour of compound **1**

Following the photo-switching studies, the solution of compound **1** in 50% water in acetic acid, and 50% water in methanol with 10 mM HCl, were selected as the best systems to explore the dynamic self-assembly behaviour of compound **1**. With these mixtures, we examined the self-assembled structures' morphology with a transmission electron microscope (TEM).

TEM micrographs of compound **1** in the mixture of 50% water in acetic acid are shown in Figure 6.4A-C. Prior to irradiation, the spiropyran moiety of compound **1** is in its stable MCH⁺ form. As a consequence, the molecule has a more hydrophilic character, and it is freely dissolved in solution before being deposited on the TEM grid. The TEM micrograph in Figure 6.4A indeed shows that the MCH⁺ form does not have a strong propensity to self-assemble at these conditions, but rather drying patterns are formed on the grid. After 40 seconds of visible light irradiation, the

MCH⁺ switches to the SP form, and the molecules adopt a more amphiphilic character, with the oligoether dendrons being still hydrophilic, while the rigid aromatic V-shaped bottom half changes to strongly hydrophobic. Figure 6.4B and 6.4C show large aggregates with elongated, yet not-well-defined bundled structures. Although the exact architecture of the aggregates cannot be concluded from these images, the SP form clearly exhibits a higher propensity to aggregate. This is most likely the consequence of the increased hydrophobicity.

Figure 6.4 TEM images of compound **1** before and after irradiation with visible light. A) Sample prepared from the solution of compound **1** in a mixture of 50% water in acetic acid. B-C) Sample prepared from the solution of compound **1** in a mixture of 50% water in acetic acid after 40 seconds of visible light irradiation. D) Sample prepared from the solution of compound **1** in a mixture of 50% water in methanol with 10 mM HCl. E-F) Sample prepared from the solution of compound **1** in a mixture of 50% water in methanol with 10 mM HCl after 5 minutes of visible light irradiation.

TEM images of compound **1**, deposited from the solution of **1** in the mixture of 50% water in methanol with 10 mM HCl, are shown in Figure 6.4D-F. As anticipated, the equilibrated MCH⁺ form does not form any clear self-assembled structures (Figure 6.4D). Upon irradiation with visible light for 5 minutes, vesicle-like spherical architectures can be observed (Figure 6.4E-F). These objects seem to comprise a curved uniformed membrane most probably formed by oppositely oriented SP amphiphiles. The individual spheres show propensity to aggregate, which in some cases leads to the formation of closely packed objects and in other cases even to the formation of interconnected architectures.

Even though the self-assembly of the SP form of **1** did not yield tubular architectures, these preliminary results show that controlling the self-assembly process *via* dynamic control of the amphiphilicity of molecular building blocks can be achieved. In equilibrium, the MCH⁺ form is molecularly dissolved in the acidic solvent mixture. The energy delivered to the system in the form of visible light irradiation pushes the system out of its equilibrium state, and the switched population of the molecules start assembling. This assembly process and the structures can, in principle, be maintained for as long as the irradiation persists.

6.3 Conclusions and perspectives

In conclusion, we have synthesized photoswitchable amphiphile **1**. Initially, this molecule adopts the charged MCH⁺ form and is freely dissolved in acidic media. Upon visible light irradiation, **1** turns to the SP form. The molecule gains a more amphiphilic character, with a clear separation of hydrophobic and hydrophilic halves. In this form, **1** self-assembles, most likely with the aid of hydrophobic effects, as shown by TEM micrographs. This work demonstrates that light as an energy source can be used to fuel dissipative processes (transient molecular switching) connected to a self-assembly process. Future work will be directed towards translating this concept to other molecular designs forming tubular assemblies.

6.4 Acknowledgments

We acknowledge Dr. Rico Keim and Mark A. Smithers (University of Twente) for assistance with the TEM imaging.

6.5 Materials and methods

6.5.1 General

Reagents and solvents were purchased from commercial sources and used without further purification unless stated otherwise. Compound **5** was synthesized according to the literature.¹⁴ ¹H- and ¹³C-NMR were recorded at 400 and 100.6 MHz, respectively. Chemical shifts are reported in δ = units (ppm) relative to the residual protonated solvent signals of CDCl₃ (¹H-NMR: δ = 7.26 ppm) and CD₃CN (¹H-NMR: δ = 1.94 ppm). Thin-layer chromatography (TLC) was carried out using Merck silica gel 60 on aluminium sheets, with visualization by UV light and by charring with a potassium permanganate solution (2%) and sodium hydrogen carbonate (4%) in water. Column chromatography was carried out using Merck silica gel 60 (230-400 Mesh). ESI-MS spectra were obtained for samples dissolved in DCM-MeOH at low μ M concentrations.

UV-visible Spectroscopy. The solutions were prepared at 10 μ M concentration. All solutions were kept in the dark before irradiation experiments. UV-visible spectra were recorded at room temperature (20 °C) with a Perkin Elmer Lambda 850 UV-visible spectrometer in 1-cm quartz cells.

Visible lamp. The samples were irradiated with visible light, Edmund MI-150 high-intensity illuminator ($\approx 145 \text{ mW}\cdot\text{cm}^{-2}$)

UV lamp. The samples were irradiated with UV light at $\lambda = 365 \text{ nm}$, bluepoint LED Honle Technology, $300 \text{ mW}\cdot\text{cm}^{-2}$

TEM measurements. The samples for TEM were prepared by drop-casting a 100 μM aqueous solution (50% water in acetic or 50% water in methanol with 10 mM HCl) of compound **1** on carbon grids (Formvar/Carbon 200 mesh, Copper). Prior to measurements, the samples were stained with uranyl acetate. TEM micrographs were recorded using Philips CM300ST - FEG TEM equipment.

6.5.2 Synthesis and characterization

Synthesis of 4,4''''-bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-[1,1':4',1'':3'',1''':4''',1''''-quinquephenyl]-5''-ol (**4**)

A dry 2-necked round bottom flask which contained a mixture of 3,5-dibromophenol (**2**) (124 mg, 0.49 mmol), 4,4'-bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,1'-biphenyl (**3**) (1 g, 2.46 mmol), K_2CO_3 (408 mg, 2.95 mmol), 2-Dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl (XPhos) (58 mg, 0.123 mmol) and $\text{Pd}(\text{OAc})_2$ (11 mg, 0.049 mmol) was equipped under nitrogen atmosphere. A degassed mixed solvent 18 mL (toluene: EtOH: H_2O = 8:8:2) was then added to the reaction flask. The reaction mixture was heated to 90 $^\circ\text{C}$ for 19 h. After cooling down to room temperature, the reaction mixture was quenched with water and extracted with CH_2Cl_2 . The combine organic layers were dried over Na_2SO_4 and evaporated to dryness and the residue was chromatographed on silica gel with EtOAc-hexane (3:7) as eluent, to give the desired product as a white solid (176 mg, 55%). ^1H -NMR (400 MHz, CDCl_3): δ 1.37 (s, 24H), 7.11 (d, J = 1.5 Hz, 2H), 7.48 (t, J = 1.5 Hz, 1H), 7.67 (d, J = 8.2 Hz, 4H), 7.72 (s, 8H), 7.91 (d, J = 8.2 Hz, 4H). ^{13}C -NMR (125 MHz, CDCl_3): δ 25.0, 84.0, 113.2, 118.9, 126.5, 127.7, 127.8, 129.0, 135.5, 140.1, 140.4, 143.1, 143.4. ESI-MS (m/z) calculated for $[\text{C}_{42}\text{H}_{44}\text{B}_2\text{O}_5\text{H}]^+$ 651.3448, found: 651.2619.

Synthesis of 2,2'-(5''-((17-(13-(2,5,8,11-tetraoxadodecyl)-2,5,8,11,15-pentaoxa-hexadecan-16-yl)-13-(2,5,8,11-tetraoxadodecyl)-2,5,8,11,15-pentaoxaoctadecan-18-yl)oxy)-[1,1':4,1'':3'',1''':4''',1''''-quinquephenyl]-4,4''''-diyl)bis(4,4,5,5-tetra-methyl-1,3,2-dioxaborolane) (6)

The mixture of compound 4 (169 mg, 0.26 mmol), compound 5 (398 mg, 0.39 mmol) and potassium carbonate (K_2CO_3) (108 mg, 0.78 mmol) was refluxed in EtOH (5 mL) for 24 h. After cooling down to room temperature, the reaction was filtered to remove the solid, dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by column chromatography using CH_2Cl_2 -acetone (3:1) as eluent to compound 6 as colorless liquid (70 mg, 18%). 1H -NMR (400 MHz, $CDCl_3$): δ 1.37 (s, 24H), 2.19 (quint, $J = 5.9$ Hz, 2H), 2.42 (quint, $J = 5.8$ Hz, 1H), 3.35 (s, 12H), 3.46–3.48 (m, 12H), 3.51–3.53 (m, 14H), 3.55–3.58 (m, 12H), 3.60–3.62 (m, 24H), 3.65 (br s, 2H), 4.15 (d, $J = 5.5$ Hz, 2H), 7.17 (d, $J = 1.4$ Hz, 2H), 7.47 (t, $J = 1.2$ Hz, 1H), 7.67 (d, $J = 8.2$ Hz, 4H), 7.73 (s, 8H), 7.91 (d, $J = 8.2$ Hz, 4H). ^{13}C -NMR (125 MHz, $CDCl_3$): δ 25.0, 29.4, 40.2, 59.1, 69.4, 69.6, 69.8, 70.54, 70.59, 70.63, 70.65, 70.69, 72.0, 84.0, 112.4, 118.6, 126.4, 127.2, 127.7, 127.8, 129.0, 135.4, 140.3, 140.4, 142.7, 143.4, 160.1. ESI-MS (m/z) calculated for $[C_{82}H_{124}B_2O_{23}H]^+$ 1499.8792, found: 1499.8227.

Synthesis of 5'-bromo-1',3',3'-trimethyl-6-nitrospiro[chromene-2,2'-indoline] (7)

A 100 mL, round bottom flask, equipped with a drying tube was charged with 1',3',3'-trimethyl-6-nitrospiro[chromene-2,2'-indoline] (400 mg, 1.24 mmol) and $CHCl_3$ (60 mL). *N*-bromosuccinimide (NBS) (232 mg, 1.30 mmol) was added in a one portion to the stirred solution at 0 °C and the resulting mixture was stirred at room temperature for 24 h. The reaction was then quenched with water and extracted with CH_2Cl_2 . The combined organic layers were dried over Na_2SO_4 , filtered and evaporated to dryness. The remaining solid was dissolved in CH_2Cl_2 , to which an excess of EtOH was then added to precipitate compound 7 as a yellow solid (458 mg,

92%). $^1\text{H-NMR}$ (400 MHz, CDCl_3): δ 1.18 (s, 3H), 1.27 (s, 3H), 2.71 (s, 3H), 5.83 (d, $J = 10.3$ Hz, 1H), 6.43 (d, $J = 8.2$ Hz, 1H), 6.76 (d, $J = 8.8$ Hz, 1H), 6.94 (d, $J = 10.3$ Hz, 1H), 7.16 (d, $J = 1.9$ Hz, 1H), 7.29 (dd, $J = 8.2, 2.0$ Hz, 1H), 8.00–8.05 (m, 2H). $^{13}\text{C-NMR}$ (125 MHz, CDCl_3): δ 19.9, 25.9, 29.1, 52.5, 106.8, 108.7, 111.7, 115.6, 118.7, 121.2, 122.9, 125.0, 126.1, 128.7, 130.6, 138.6, 141.3, 147.0, 159.6. ESI-MS (m/z) calculated for $[\text{C}_{19}\text{H}_{17}\text{BrN}_2\text{O}_3\text{H}]^+$ 401.0495, found: 401.9251.

Synthesis of 5',5'''-(5''-((17-(13-(2,5,8,11-tetraoxadodecyl)-2,5,8,11,15-pentaoxa hexadecan-16-yl)-13-(2,5,8,11-tetraoxadodecyl)-2,5,8,11,15-pentaoxa octadecan-18-yl)oxy)-[1,1':4,1'':3'',1''':4''',1''''-quinquephenyl]-4,4''''-diyl)bis(1',3',3'-trimethyl-6-nitrospiro[chromene-2,2'-indoline])) (1)

A dry 2-necked round bottom flask which contained a mixture of compound **6** (70 mg, 0.046 mmol), 5'-bromo-1',3',3'-trimethyl-6-nitrospiro[chromene-2,2'-indoline] (**7**) (75 mg, 0.187 mmol), K_2CO_3 (39 mg, 0.28 mmol), XPhos (6 mg, 0.011 mmol) and $\text{Pd}(\text{OAc})_2$ (1 mg, 0.004 mmol) was equipped under nitrogen atmosphere. A degassed mixed solvent 5 mL (toluene: $\text{H}_2\text{O} = 4:1$) was then added to the reaction flask. The reaction mixture was heated to 50 °C for 24 h. After cooling down to room temperature, the reaction mixture was quenched with water and extracted with CH_2Cl_2 . The combine organic layers were dried over Na_2SO_4 and evaporated to dryness. The residue was chromatographed on silica gel with gradient eluent from CH_2Cl_2 -acetone (3:1) to $\text{MeOH-CH}_2\text{Cl}_2$ -acetone (0.01:3:1) to give compound **1** as brown sticky solid (32 mg, 36%). $^1\text{H-NMR}$ (400 MHz, CD_3CN): δ 1.21 (s, 6H), 1.33 (s, 6H), 2.06 (quint, $J = 6.0$ Hz, 2H), 2.37 (quint, $J = 5.7$ Hz, 1H), 2.76 (s, 6H), 3.25 (s, 12H), 3.40–3.50 (m, 60H), 3.54–3.57 (m, 4H), 4.22 (d, $J = 5.2$ Hz, 2H), 5.96 (d, $J = 10.4$ Hz, 2H), 6.67 (d, $J = 8.0$ Hz, 2H), 6.75 (d, $J = 9.0$ Hz, 2H), 7.08 (d, $J = 10.4$ Hz, 2H), 7.27 (s, 2H), 7.49–7.52 (m, 4H), 7.58 (s, 1H), 7.70–7.84 (m, 16H), 8.00 (dd, $J = 9.0, 2.7$ Hz, 2H), 8.09 (d, $J = 2.7$ Hz, 2H). $^{13}\text{C-NMR}$ (125 MHz, CD_3CN): δ 20.1, 26.1, 29.2, 41.3, 53.1, 58.8, 69.8, 70.1, 70.9, 71.0, 71.07, 71.13, 71.3, 72.5, 107.7, 108.3, 113.2, 116.2, 120.0, 121.3, 122.3, 123.7, 126.6, 127.5,

127.7, 128.0, 128.1, 128.6, 129.3, 132.8, 138.2, 139.0, 140.5, 140.6, 141.5, 142.1, 143.3, 148.6, 160.5, 161.1. ESI-MS (m/z) calculated for $[C_{108}H_{134}N_4O_{25}Na]^+$ 1909.9229, found: 1909.8982.

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Summary

Incorporating artificial molecular motors and switches into self-assembled systems allows transferring and amplifying the forces that these molecules generate, across increasing length scales. Interfacing the mechanics of such molecules with larger self-assembled architectures represents a viable strategy to fight against overwhelming Brownian storm and viscosity of liquid environment. Thus the focus of my research was on developing controllable light-responsive supramolecular systems by integrating molecular photoswitches into self-assembled architectures. Photo-switching was selected as an ideal strategy to alternate between assembling and non-assembling isomers reversibly, repeatedly, with an exquisite control over the timescale of the process, without accumulating chemical waste and in combination with spontaneous reversed switching. More specifically, the photo-switching was used to mediate the transformation of building blocks, from a form that is not compatible with self-assembly and thus suppresses self-assembly, into a photo-generated isomer that participates into the self-assembly because the interactions it promotes are compatible with the desired architecture. The artificial molecular photoswitches involved in this work were azobenzenes and spiropyrans. Upon irradiation with light, azobenzenes offer geometrical changes between *cis* and *trans* isomers, whereas spiropyrans provide large differences in their charged character.

Chapter 1 provides a general introduction to this thesis.

Chapter 2 reviews recent progress in the design and synthesis of supramolecular tubular systems. Our motivation to work with tubular systems lies in the versatility displayed by the supramolecular tubes that operate in the cell. Cellular microtubules, “the muscles of the cell”, are indeed supramolecular machines that produce directional forces through a continuous influx of energy: they pull chromosomes apart through catastrophic disassembly, and shape shift cells as they grow using chemical energy.

Microtubules are characterized by a persistence length of over five microns – in other words, a microtubule is rigid over cellular dimensions, which mostly derives from its large cross section. The propensity to produce forces efficiently and in the right temperature regime, with control over directionality, is thus inherently encoded into the tubular structure: they are in essence supramolecular cylinders that are hollow and stiff, and that are assembled from molecules that undergo chemically fueled conformational switching.

In **Chapter 3**, we show how strain builds up in wholly artificial tubules, upon *trans*-to-*cis* photo-switching. The light-fueled accumulation of the strain eventually leads to the catastrophic burst of the tubules, in a mechanism that is reminiscent of the disassembly mechanism of cellular microtubules. From an energetic point of view, such processes will likely be key to the development of artificial de-polymerization machines.

In **Chapter 4**, we demonstrate a strategy that allows connecting the tubules discussed in chapter 3, with either surfaces, or with each other. In this strategy, biotinylated building blocks act as interfacing agents that incorporate into the original tubules without causing structural deformations. The mixed tubules, composed of both the biotinylated and the original building blocks, show a propensity to bundle when the biotin moiety binds with streptavidin, a protein that can be either added in solution, or used to functionalize surfaces. Ultimately, we anticipate that the strategy outlined in this chapter will allow i) amplifying the action of individual tubules into bundles and ii) transferring the forces generated by the growing/disassembling tubules to other objects.

In chapters 5 and 6, the dynamic molecule mediating the conversion of light into mechanical operation is a spiropyran. In contrast to azobenzenes, spiropyrans do not undergo large geometrical changes upon irradiation with light, however, they switch from a non-charged form (the spiropyran) to a charged zwitterionic form (the merocyanine), which paves the way towards alternative strategies to achieve dynamic molecular control over self-assembled architectures.

In **Chapter 5**, we have synthesized a spiropyran-based amphiphile that self-assembles into vesicles, in water. Upon irradiation with light, merocyanine converts into the spiropyran, which leads to the transient and reversible expansion of the vesicles. The original mechanism for this breathing motion involves self-limiting growth of the merocyanine-containing building blocks. In future work, we envision that mixing the switchable building blocks reported here, with a range of flexible amphiphilic building blocks, can lead to the formation of phase-segregated areas in hybrid vesicles. In these artificial formations, the presence of localized deformations and of differential surface stiffness is likely to mediate complex shape transformations, under irradiation with light. Overall, in this chapter, we demonstrate that dynamic aggregates based on spiropyrans hold potential as adaptive compartmentalized nanosystems.

While chapter 5 focuses on vesicular dynamic architectures, in **Chapter 6**, we present efforts towards combining tubes with spiropyran switches, because they would promote tubular growth upon irradiation with light, when the polar protonated merocyanine converts into the spiropyran. In order to optimize the likeliness of π - π stacking in the spiropyran form, the planarity of the core building blocks was enhanced by adding two benzene rings into the molecular design. While supramolecular aggregates were indeed formed upon irradiation with light, we could not find evidence supporting the light-fuelled formation of tubes.

Overall, we have introduced a framework for the development of light-controllable nanosystems in water, with a dynamic behavior ranging from assembly, to disassembly and transient growth. Moreover, we have taken the formative steps to harness the mechanically-purposeful operation of dynamic supramolecular tubules, as to generate measurable forces from the nanoscale, and ultimately establish operational principles for chemo-mechanical transduction in supramolecular systems. The inspiring versatility displayed cellular microtubules sets the stage for the potential of this research.

Samenvatting

Het incorporeren van kunstmatige moleculaire motors en schakelaars in zelf-geassembleerde systemen maakt het mogelijk om de krachten die deze moleculen genereren over te brengen naar het systeem en zo te amplificeren, ongeacht de toenemende lengte van het systeem. Het verbinden van deze mechanisch actieve moleculen met de grotere zelf-geassembleerde structuren is een reële strategie om de heftigheid van de Browniaanse storm en viscositeit van een vloeibare omgeving tegen te gaan. De focus van mijn onderzoek lag dan ook op het ontwikkelen van controleerbare licht-gevoelige supramoleculaire systemen door moleculaire foto-switches te integreren in zelf-geassembleerde structuren. Met foto-switching is het mogelijk te schakelen tussen opbouwende en niet-opbouwende isomeren en dit is dan ook gekozen als meest ideale strategie. Met foto-switching is een herhaaldelijk en omkeerbaar proces mogelijk met uitstekende controle over de duur van het proces in combinatie met spontaan tegengestelde schakelingen, zonder dat het chemisch afval accumuleert. Foto-switching was met name gebruikt om de transformatie van de bouwstenen te bewerkstelligen. Deze bouwstenen schakelen onder invloed van licht van een vorm dat niet compatibel is met de zelf-assemblage van het systeem en deze zelf-assemblage dan ook onderdrukken, naar een isomeer dat interacties bevordert die compatibel zijn met de gewenste structuur en zodoende deelneemt aan de zelf-assemblage. De kunstmatige moleculaire foto-switches in dit onderzoek zijn azobenzenen en spiropyranen. Na belichting schakelen azobenzenen tussen *cis*- en *trans*-isomeren, terwijl spiropyranen een verandering in lading ondergaan.

Hoofdstuk 1 geeft een algemene introductie van dit proefschrift.

Hoofdstuk 2 bespreekt de recente vooruitgang in het onderwerp en de synthese van supramoleculaire buisvormige systemen. Onze keuze voor het werken met buisvormige systemen is gebaseerd op de veelzijdigheid van de supramoleculaire

buisvormige systemen die in de cel actief zijn. Deze cellulaire microtubuli, ‘de spieren van de cel’, zijn daadwerkelijk supramoleculaire machines die gerichte krachten uitoefenen door een continue instroom van energie. Microtubuli gebruiken chemische energie voor het splitsen van chromosomen en voor het veranderen van de vorm van een cel tijdens celgroei. Microtubuli worden gekenmerkt door een onbuigzaamheid over een lengte van 5 micrometer. Oftewel, een microtubule is rigide op celniveau, wat voornamelijk wordt veroorzaakt door diens grote diameter. De eigenschap om krachten efficiënt te produceren bij de correcte temperatuur en met controle over richting is vastgelegd in de structuur van de microtubuli. Ze zijn in principe supramoleculaire holle en stugge cylinders welke zijn opgebouwd uit moleculen die een chemisch aangedreven structurele vervorming ondergaan.

In **Hoofdstuk 3** tonen we hoe spanning opbouwt in de volledig kunstmatige tubuli na *trans*-naar-*cis* foto-switching. De licht-aangedreven opbouw van spanning leidt uiteindelijk tot een uiteenspatting van de tubuli, waarbij de wijze waarop doet denken aan de ontmanteling van celmicrotubuli. Vanuit een energieperspectief zullen dergelijke processen waarschijnlijk onmisbaar zijn in de ontwikkeling van kunstmatige depolymerisatie machines.

In **Hoofdstuk 4** demonstreren we een strategie voor het verbinden van de tubuli beschreven in hoofdstuk 3 met oppervlakten of met elkaar. In deze strategie worden bouwstenen gekoppeld aan biotine gebruikt als verbindende delen die in de oorspronkelijke tubuli worden geïncorporeerd zonder structurele deformaties te veroorzaken. De gemengde tubuli, opgebouwd uit bouwstenen met en zonder biotine, vertonen de neiging om aan elkaar te plakken wanneer de biotine bindt aan streptavidine, een eiwit dat in oplossing kan worden toegevoegd of wordt gebruikt om oppervlakten te functionaliseren. Uiteindelijk is de verwachting dat de strategie zoals uiteengezet in dit hoofdstuk zal leiden tot i) het amplificeren van het aan elkaar plakken van individuele tubuli en ii) het overbrengen van de krachten die vrijkomen door de groei/ontmanteling van tubuli aan andere objecten.

In hoofdstukken 5 en 6 is het dynamische molecuul dat licht omzet in een mechanische actie een spiropyraan. In tegenstelling tot azobenzeen ondergaat spiropyraan geen grote geometrische veranderingen na belichting, maar schakelt deze van een niet-geladen vorm (spiropyraan) naar een geladen zwitterionische vorm (merocyanine). Dit effent de weg naar alternatieven voor dynamische moleculaire controle over zelf-geassembleerde structuren.

In **Hoofdstuk 5** hebben we een op spiropyraan gebaseerd amfifiel gesynthetiseerd dat in water vesikels vormt. Na belichting schakelt merocyanine naar spiropyraan, dit leidt tot de tijdelijke en omkeerbare verwijding van de vesikels. Bij het mechanisme van deze ademhalingsbeweging is de zelfbeperkende groei van de merocyanine bevattende bouwstenen betrokken. In toekomstig werk veronderstellen we dat het mengen van schakelende bouwstenen zoals hier genoemd met een scala aan flexibele amfifiele bouwstenen kan leiden tot de vorming van fase-gescheiden gebieden in hybride vesikels. De aanwezigheid van lokale vervormingen en verschillende oppervlaktestijfheid in de kunstmatige structuren kan na belichting waarschijnlijk complexe vormtransformaties bewerkstelligen. Over het algemeen demonstreren we in dit hoofdstuk dat dynamische aggregaten gebaseerd op spiropyranen mogelijk als aanpasbare gecompartmentiseerde nanosystemen dienst kunnen doen.

Terwijl hoofdstuk 5 de nadruk legt op dynamische vesikelachtige structuren, wordt in **Hoofdstuk 6** onze pogingen beschreven om tubuli te combineren met spiropyraan foto-switches. Deze foto-switches zouden tubulaire groei bespoedigen na belichting waarbij de geprotoneerde merocyanine omgezet wordt in spiropyraan. De oriëntatie van de kernbouwstenen was versterkt door het toevoegen van twee benzeenringen aan het molecuul, waardoor de π - π stapeling in de spiropyraanvorm meer waarschijnlijk wordt. Hoewel er wel supramoleculaire aggregaten werden gevormd na belichting, konden we geen bewijs vinden voor de licht-gedreven formatie van tubuli.

Kortom, we hebben een kader geïntroduceerd voor de ontwikkeling van door licht controleerbare nanosystemen in water, welke dynamisch gedrag vertonen omtrent

opbouw, ontmanteling en tijdelijke groei. Bovendien hebben we constructieve stappen gezet voor het beheersen van het gebruik van de mechanische acties van dynamische supramoleculaire tubuli zodat deze meetbare krachten op nanoschaal kunnen genereren en hiermee uiteindelijk chemo-mechanische overdracht kunnen bewerkstelligen in supramoleculaire systemen. De inspirerende veelzijdigheid vertoond door microtubuli van cellen is bepalend voor de mogelijkheden van dit onderzoek.

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About the author

Supaporn Kwangmettatham was born on August 27th 1989 in Nakhon Ratchasima, Thailand. She pursued her bachelor's degree in Chemistry at Mahidol University, Thailand, and carried out a senior project under the supervision of Assist Prof. Tienthong Thongpanchang. The project was on 'Omega-shaped Molecules: Synthesis and Their Properties'. Afterwards, in February 2014, she received her master's degrees in Organic Chemistry at Mahidol University. Her master's thesis is entitled 'Effects of molecular structures of π -bridge derived from naphthothiophenes, naphthodithiophenes, and benzotrithiophenes on dye-sensitized solar cells efficiency', and was conducted under the supervision of Assist. Prof. Dr. Tienthong Thongpanchang. During her bachelor's study, she obtained a Sritrung scholarship from the Faculty of Science, Mahidol University. In her master's degree, she obtained scholarships from the Center of Excellence for Innovation in Chemistry (PERCH-CIC), and Development of Pharmaceuticals from Bioresources and Its Management (NRU).

In March 2014, she started her Ph.D. program in the bio-inspired and smart materials group under the supervision of Prof. Nathalie Katsonis and in the molecular nanofabrication group under the supervision of Dr. Tibor Kudernac at the University of Twente, the Netherlands. The aim of her project is to create light-controlled supramolecular machines that can generate forces. The results of this project are described in this thesis.

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