Supramolecular polymers for biomedical applications

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Abstract: Supramolecular polymers are promising candidates for the use in biomedical applications. The noncovalent interactions that characterize these systems create possibilities for dynamic reversibility and stimuli responsiveness. The systems can be easily functionalized, allowing for increased targeting and bioactivity of the materials. Additionally, the reversible interactions present in these systems allow for a bottom up approach for synthesizing and controlling the size and shape of the supramolecular materials. In this review the use of supramolecular polymers in biomedical applications, such as drug deliver systems, tissue engineering, and self healing materials are discussed. Typical polymer systems described consist of benzene-1,3,5-tricarboxamide (BTA), ureido-pyrimidone (UPy) or host-guest interactions.

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1 Introduction

Supramolecular chemistry emerged as a field in the not too distant past. The work of Lehn started supramolecular chemistry as a field in 1978 when he connected it to functional structures.[1] Since then the field developed further and is now an established sub-dicipline of chemistry. In 1990 Fouquey *et al.* designed and synthesized the first example of a linear supramolecular polymer.[2,3]

Supramolecular chemistry spans multiple length scales; from fibers to monolayers, vesicles, gels, and membranes. The field of supramolecular polymers based on controlling noncovalent interactions between monomers and the process of self assembly to form well defined structures.[1] A good definition of supramolecular polymers was proposed by Brunsveld *et al.*: "Supramolecular polymers are defined as polymeric arrays of monomeric units that are brought together by reversible and highly directional secondary interactions, resulting in polymeric properties in dilute and concentrated solution as well as in the bulk. The directionality and strength of the supramolecular bonding are important features of systems that can be regarded as polymers and that behave according to well-established theories of polymer physics."[4]

Applications of supramolecular polymers are in light harvesting systems[5], liquid crystalline materials[3], block copolymer assemblies[3], organogels[1], and biomelecular materials[1]. Here the focus will be on the biomedical applications of supramolecular polymers.

Over the last couple of years the interest for the development and application of smart drug delivery systems has increased and it is increasing still. With growing amounts of people suffering from degenerative diseases, the focus is on the development of systems that will increase the quality of life of patients.[6] However, the application of supramolecular

polymers in the field of biomedical applications has only received limited attention.[7] An important and growing area is to develop drug delivery systems that respond to intrinsic stimuli characteristic of the target site, improving the selective targeting and delivery of drugs. This is where applications for supramolecular polymers lie.[7,8] Examples of some supramolecular polymers that are already being used in medicine are peptide amphiphiles and ureido-pyrimidone (UPy) materials.[7]

Supramolecular polymers offer interesting applications in the biomedical field, due to the noncovalent interactions that characterize these systems. Reversibility of the interactions and stimuli responsiveness allows for applications in drug delivery systems, tissue engineering and self healing materials. This critical review discusses the important bonding motifs in supramolecular polymers and the basis of supramolecular polymerization. Then various biomedical applications of supramolecular polymers will be discussed. The review will be concluded by a summary and critical outlook on the field.

2 Background supramolecular polymers

Different types of architectures can be found for supramolecular polymers: supramolecular homopolymers, alternating copolymers, and block copolymers[9]; similar to their covalent counterparts. Supramolecular polymers of these architectures can form different kinds of structures: stacks[10,11], micelles[12,13], polymerosomes[14,15], dendrimers[16], and 3D crosslinked polymer networks[9,17]. These structures are kept together through noncovalent interactions and will self assemble under specific conditions. This section will discuss the background of supramolecular polymerization; the interactions, stability, and supramolecular polymerization.

2.1 Interactions and stability

Small building blocks can interact trough noncovalent interactions to form materials on multiple length scales. This section will focus on discussing the different bonding motifs that are characteristic for supramolecular polymers (fig. 1); hydrogen bonding, metal ligand interactions, $\pi - \pi$ stacking and host-guest interactions. All these bonding motifs are noncovalent and they allow for the dynamic interactions and reversibility observed in supramolecular systems.[1,6,16,18,19] Additionally these interactions allow for stimuli responsiveness. Where an external stimulus, for example a change in pH, can trigger structural transitions of the supramolecular assembly[18].

Hydrogen bonds have tunable directionality and diversity, especially in the case of multiple hydrogen bonding moieties. This motif offers increased stability to the supramolecular structure. Hydrogen bonds are formed by dipole-dipole interactions between an electronegative atom, such as oxygen, nitrogen, or a halogen, and a hydrogen bonded to one of such electronegative atoms (fig. 1a).[20] These bonds are typically sensitive to pH[9], which makes them suitable for applications in stimuli responsive systems.

$\pi - \pi$ interactions are one of the important noncovalent interactions, they arise from overlapping p-orbitals between conjugated molecules (fig. 1c). These interactions are relatively weak and non-directional compared to the other bonding motifs described.[9]

Host guest interactions such as those depicted in fig. 1d, show specific recognition and strong binding between the host and the guest molecules[9]. Actually often a combination of multiple interactions are used in these systems: hydrophobic (or hydrophilic) effects, electrostatic and van der Waals interactions, hydrogen bonding and ionic bonds.[20] The shape and size of the host and guest are complementary, such that the guest has the right properties to be able to bind the host molecule[20].

Metal ligand interactions are responsive to redox reactions and can show photophysical properties (from the metal ion and ligands).[9] A reason for the interest in these interactions in supramolecular polymers is the combination of properties from the organic polymer and those of the metal ions (fig.1b); magnetic, catalytic, electronic and also optical properties[20]. Because of the wide variety of metal

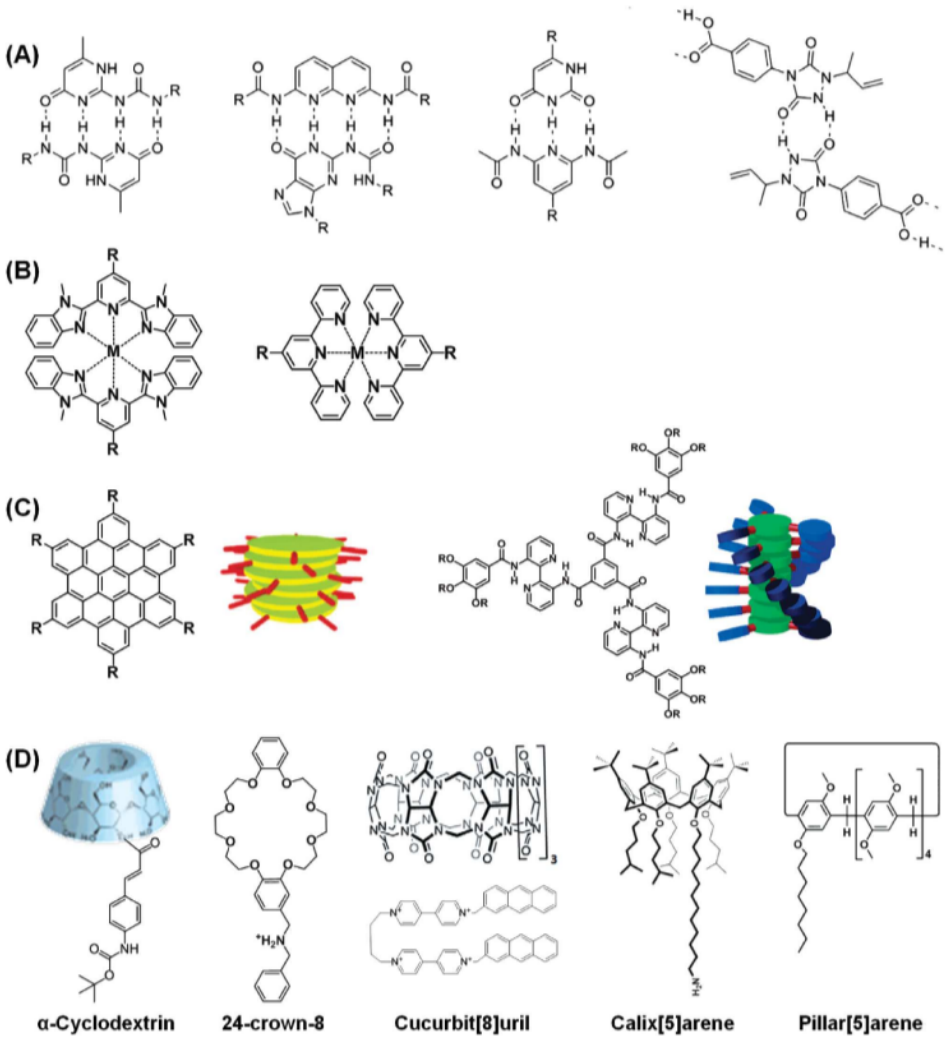

Figure 1: A collection of bonding motifs for supramolecular polymers. a) H-bonding, b) metal coordination, c) $\pi - \pi$ interactions, d) different host guest systems: α-Cyclodextrin, 24-crown-8, Cucurbit[8]uril, Calix[5]arene, Pillar[5]arene. Image reproduced from Dong *et al.*[9]

ions and ligands, properties such as the binding strength, directionality, solubility, and reversibility can be tuned for these systems.[20]

Incorporating multiple bonding motifs allows for better control of the supramolecular polymerization, giving rise to well defined structures. The motifs described here are mainly used for tuning the supramolecular polymers.[20] In order to assure higher stability and strength of the polymers multiple bonding motifs should be combined, i.e. hydrogen bonding and $\pi - \pi$ stacking. Ideally more motifs combined result in higher stability.[9] For example; one hydrogen bond is far from strong enough to create a supramolecular polymer. However, creating arrays with multiple hydrogen bonding moieties will increase the strength and directionality of the bonds, allowing for the formation of supramolecular polymers.[20]

Compared to covalent interactions, the use of noncovalent interactions in polymer systems holds several advantages. The synthesis is often easier, since only the smaller components need to be synthesized, avoiding difficult multi step synthesis, while the bigger system will form through dynamic self assembly. The dynamic self assembly allows for a bottom up approach in which the size and shape of supramolecular materials can be controlled. Additionally the synthesis is often environmentally friendly and cost effective, since it can be performed under ambient conditions.[15,18] The noncovalent interactions and stimuli responsiveness lead to degradable polymer backbones[9]. These properties com-

bined make supramolecular (polymeric) materials very interesting for applications as smart supramolecular devices and functional materials.[9]

2.2 Supramolecular polymerization

Multifunctionalized molecules can form supramolecular polymers through reversible interactions (see section 2.1). Obtaining a high degree of polymerization depends on several factors: the association constant between the monomers, the purity of the material, and the concentration of the solution[9]. Cooperative self assembly between monomers, and complexes, allows for the generation of well-defined and highly ordered structures.[9]

In supramolecular systems competition exists between inter- and intramolecular polymerization reactions. Intermolecular reactions will result in polymers of high molecular weight, whereas intramolecular polymerization will result in low molecular weight complexes. Effective molarity (eq. 1) is defined as the ratio between the intramolecular and intermolecular equilibrium constants (K_{intra} and K_{inter} respectively).

$$EM = \frac{K_{intra}}{K_{inter}} \tag{1}$$

Here K_{inter} has units of M^{-1}, while K_{intra} is dimensionless, giving EM units of M. Bifunctionalized monomers can either undergo cyclization or linear polymerization, the EM represents a critical concentration above which linear polymerization is favored over cyclization.[19] Note, bonding within the same complex of multiple monomers is considered to be intramolecular polymerization, therefor leading to lower molecular weight materials.

Whereas covalent polymerization reactions (formation of covalent bonds) are often under kinetic control, supramolecular polymerization is under thermodynamic control.[19,21] In a system under thermodynamic control, the components are mixed and the final product that is formed is the system with the most stable ground state. This requires that all reactions are reversible under the reaction conditions, leading to a dynamic system that can find the lowest energy structure.[21] For an extended overview of the theory and mechanism behind supramolecular polymerization, the reader is referred to De Greef *et al.*[19]

3 Applications

Supramolecular polymers can have the same mechanical properties as covalent polymers. However, they show great capacity for processability due to their dynamic nature.[3] This dynamic behavior and modularity of the systems allows for easy, noncovalent synthesis.[7] The following properties of supramolecular polymers are of key importance for biomedical applications: good biocompatibility and bioactivity, low cytotoxicity, specific targeting, aqueous compatibility, and stimuli responsiveness.[9,22,23] The functional supramolecular polymers described show specific functions and are able to dynamically adapt to the environment through external stimuli. These properties offer a good platform for design of smart supramolecular polymeric materials that combine dynamics and molecular order to realize multiple functions.[9] In this section various applications of supramolecular polymers in drug delivery systems, tissue engineering, and self healing materials will be discussed.

3.1 Drug delivery

In this section benzene-1,3,5-tricarboxamide (BTA), ureido-pyrimidone (UPy), and host-guest interactions and their applications in drug delivery systems will be discussed. The important bonding motifs for these systems are displayed in fig. 1 a, c, and d.

3.1.1 BTA

The BTA monomers can dynamically self assemble into one dimensional supramolecular fibers in water. These fibers are stabilized by the formation of three hydrogen bonds between the amide groups, resulting in a helical lateral ordering of the monomers (fig. 2). Additionally, the structure is stabilized by $\pi - \pi$ interactions.[4,7,10,11,24–27] The BTA moieties can be functionalized with amphiphilic chains to obtain higher functionality. There is

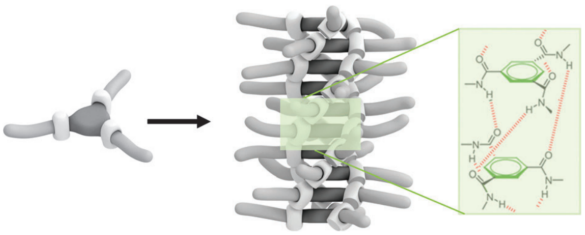

Figure 2: A schematic representation of the self assembly of benzene-1,3,5-tricarboxamide (BTA), forming helical stacks that are stabilized by the formation of three hydrogen bonds. Image reproduced from Cantekin *et al.*[24]

a delicate balance between the sizes of the hydrophobic spacer and the hydrophilic units in the BTA derivate that influences the self assembly process.[26] Hydrophobic units of eleven or twelve atoms result in the formation of stable fibers, changing the lenght of the spacer further influences the aggregate size and thermal stability of the complex.[26] This section will discuss the use of BTA moieties in the design of drug delivery systems.

Albertazzi *et al.*[11] reported a supramolecular system based on BTA, that dynamically self assembles in water into a fiber. They investigated the effect of a multivalent binder to the ordering of functionalized monomer units in the supramolecular polymer. Results show that the monomer distribution could be controlled by the multivalent binder, allowing this to be a viable scaffold for different multivalent binders by tuning of the monomers. This flexibility could be very useful in drug delivery systems that need to deliver multivalent drugs such as DNA or RNA strands.

The use of self assembled supramolecular BTA fibers as a mechanism for drug delivery systems was also reported by Bakker *et al.*[7] Supramolecular polymer fibers from PEGylated BTA monomers were proposed for intracellular drug delivery systems. These fibers have a hydrophobic core and a hydrophilic exterior, allowing for a dual drug delivery system; two different types of drugs in one carrier. The properties of the supramolecular polymer fibers could be controlled by mixing differently functionalized monomers. The important noncovalent interactions in this system are hydrogen bond formation, electrostatic and hydrophobic interactions. The molecules are helically stacked and triple hydrogen bonded (fig. 2). They specifically looked into the use of this supramolecular system as a dual delivery system, with siRNA as one of the targeted drugs to deliver. The siRNA can be electrostatically bound to the fiber exterior, allowing it to pass cellular membranes without degrading. While a small organic, hydrophobic drug can bind to the inner, hydrophobic pockets of the fiber (fig. 3). They showed that the charge of the monomers determines renal uptake, cell binding, cytotoxicity *etc.* Mixing of monomers allows for control over the bioactivity of the carrier. The internalization pathway for this carrier system is not known yet, although it is important to find out how the polymers will be internalized in order to know the full potential of these materials as future drug delivery systems. For example, there are indications that the key limiting factor for cellular drug delivery systems is an inability to escape lysosomes, when internalization pathway is through endocytosis.[7] Another factor of the delivery process that is still unknown is whether the polymers will remain aggregated or depolymerize after successful binding and internalization into cells.[7] It is important that further research will be performed on this subject, since it might affect the delivery of drugs and/or the metabolism of the carrier. The authors conclude that their supramolecular BTA fibers show promising applications as dual drug delivery systems. However, cytotoxicity and bioactivity need to be optimized by optimizing the charge density along the fiber. A balance has to be found between the toxicity and cell binding.[7]

In 2015 Albertazzi *et al.*[27] reported a new type of a stimuli-responsive supramolecular polymer from PEGylated BTA monomers. Co-assembly of two different types of monomers (neutral, non-functionalized BTA monomers and cationic, amine functionalized BTA monomers) resulted in the formation of a supramolecular fiber with a random distribution of monomers. The cationic monomer species were able to bind to nucleic acid through electrostatic interactions. Upon binding with a DNA-RNA strand, the monomer sequence was altered, and could therefore be controlled. Addition of the RNAse enzyme removed the DNA-RNA

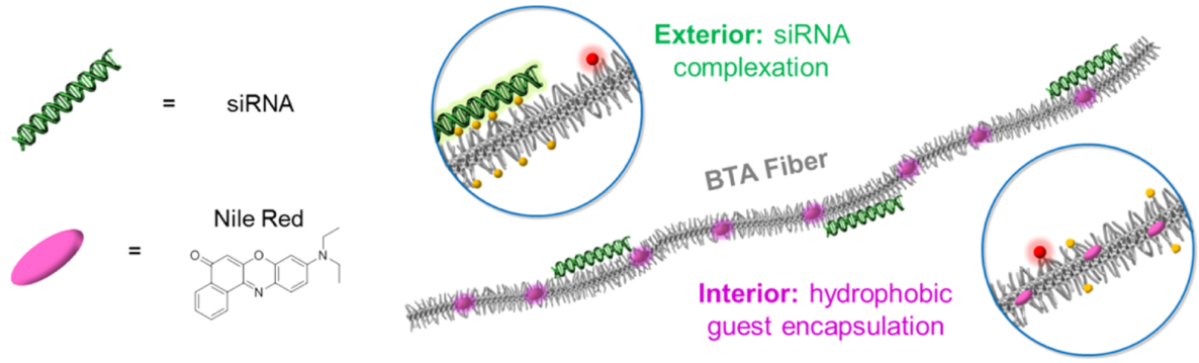

Figure 3: BTA fiber complexing with siRNA at the exterior through electrostatic interactions, and encapsulation of hydrophobic nile red molecules in the hydrophobic core. Image reproduced from Bakker *et al.*[7]

strand, which resulted in a restoration of the random monomer distribution in the fiber (fig. 4). These results were in good agreement with their previous research on this type of system.[11] This research is the first step towards complex systems in which the polymer can respond to multiple stimuli, involving both positive and negative feedback loops, which mimics the complex cell signaling of real biological systems. These results show a very important step in the design and development of more complex supramolecular polymer systems that resemble complex pathways of real biological systems. This makes it a very important and interesting field of research, to use this complexity to further improve drug delivery systems and tissue engineering applications of these supramolecular polymeric systems.

3.1.2 UPy

Ureido-pyrimidone (UPy) molecules have a quadruple hydrogen bonding motif and are suitable for use in supramolecular polymers because of the stability offered by the hydrogen bonds. Two UPy monomers will dimerize upon formation of four hydrogen bonds. Subsequently these dimers stack through $\pi - \pi$ and van der Waals interactions, forming supramolecular polymer fibers (fig. 5). These molecules are very suitable for use in bioactive materials, due to their low processing temperature, self assembly in water, favorable degradation and overall biocompatibility.[28,29]

Bastings *et al.*[17] showed the use of ureido-pyrimidone modified PEG hydrogels for drug delivery to organs with high blood flow. This hydrogel was shown to be sensitive to pH and underwent a sol-gel transition at a pH above 8.5. This property could be used for transport through a catheter to the target tissue; the transporting solution has a pH higher than 8.5, upon contact with the tissue a gel is formed. The hydrogels form fibers in aqueous solutions and they crosslinked to form supramolecular hydrogel networks. The sol-gel transition was observed to be very fast. It was proposed that the switching is caused by breaking the cross-linking between fibers, instead of complete depolymerization. Their hydrogel was tested to be nontoxic to cells in both the neutral and basic solutions. One of the proposed applications for this hydrogel could be drug delivery to the heart. The storage modulus of the described hydrogel matches the mechanical properties of a (rat) heart, and the material showed self healing properties, indicating it could withstand the high shear of the contracting heart muscle. They showed the incorporation and release of a growth factor and a size-dependent release profile was observed, 100% of the growth factor was released after 7 days. For this study both *in vitro* and *in vivo* tests were performed.

A similar system as described above, UPy-PEG hydrogel was reported by Dankers *et al.*[31] However, this system does not use pH as a trigger for a sol-gel transition, in this case exertion of pressure allowed the hydrogel to flow though a needle. The application for this system is proposed to be in drug delivery to the kidneys. The hydrogel was inserted under the renal cortex, from there the drugs can be released from the hydrogel over the course of several days. In earlier research Dankers *et*

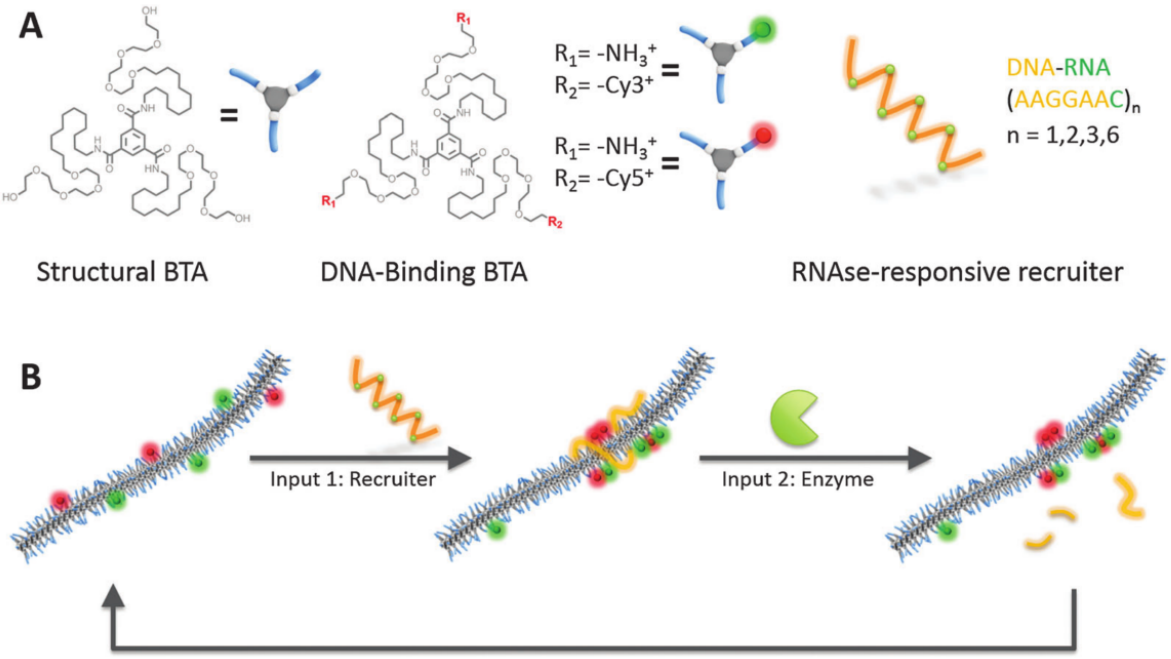

Figure 4: a) Different monomers of the system: neutral (left), cationic, labeled monomers (center), and the multivalent DNA-RNA recruiter strand. b) The multivalent recruiter controls the monomer distribution in the polymer fiber, introduction of RNAse removes the recruiter and restores the random distribution of monomers throughout the fiber. Image reproduced from Albertazzi *et al.*[27]

al.[32] reported these type of hydrogels also for the use as intrarenal drug delivery systems. They found that flexible, slow eroding hydrogels are feasible for long term drug delivery, whereas the weaker, soft, and fast eroding gels were more suitable for short term protein delivery.

Bakker *et al.*[29] reported the synthesis of UPy based functional supramolecular polymers. Functionality was achieved by mixing monomers with neutral and cationic moieties. The polymers were functionalized with a UPy end group. The monomers could self assemble into dimers through hydrogen bond formation and columnar stacks were then formed by $\pi-\pi$ interactions between dimer moieties. The self assembly of this system was triggered by injection into an aqueous medium. They observed that siRNA could bind to the cationic charges in the supramolecular polymer fiber. The siRNA complexes could be fabricated either through a single or two step synthesis, both resulting in a stable complex of polymer fiber with RNA (fig. 6). *In vitro* testing was done with human kidney cells showing that the cationic polymer species could be internalized by the cell, whereas the neutral polymers could not bind to the cell membrane, nor be internalized. Result showed that the cationic species did not show cell cytotoxicity. Combined with the good cellular uptake, it makes these polymers very interesting for intracellular drug delivery systems. These results are promising for the application of this system in drug delivery.

3.1.3 Host-guest systems

Supramolecular amphiphiles are very good candidates for applications in drug delivery systems, and are therefore actively investigated. Working with host-guest interactions, supramolecular amphiphilic polymers can be developed that are highly functionalizable. The noncovalent interactions that are key for these systems allow for easy noncovalent synthesis and great stimuli-responsiveness.[13,15,18,33]

Yu *et al.*[8] reported a host-guest drug delivery system using a pillar[5]arene as the host, and a viologen salt as the guest. The pillar[5]arene was functionalized with a PEG chain, containing a biotin end group (P5-PEG-Biotin; hydrophilic). The biotin molecule serves as the targeting group for re-

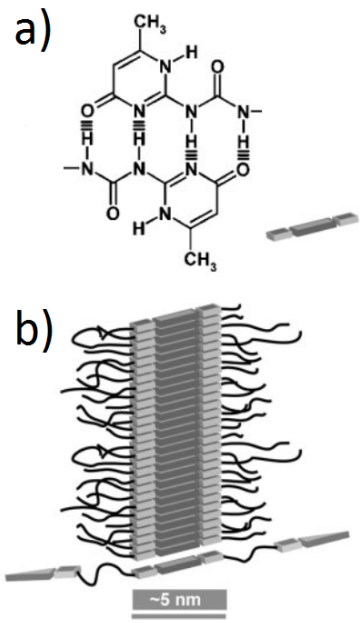

Figure 5: a) Fourfold hydrogen bonding motif in the ureido-pyrimidone (UPy) dimer. b) Lateral stacking of UPy dimers, leading to the formation of a nanofiber. Image reproduced from Dankers *et al.*[30]

ceptors, which are over-expressed in cancer cells. A poly(carpolactone) molecule was terminated with the viologen group (PCL-C_2V; hydrophobic). These two molecules form amphiphilic supramolecular diblock copolymers through host guest interactions. This copolymer could subsequently self assemble in aqueous media into polymerosomes (lysosome-like vesicles made from supramolecular polymers). The hydrophobic PCL-C_2V tails interact with each other, allowing for this structure to be formed. A small organic drug could be inserted into the solution to incorporate it in the polymerosome during the self-assembly process. NAD(P)H reduced the viologen group after internalization, breaking the host-guest interactions and leading to the disassembly of the polymerosome and subsequent release of the drug (fig. 7). They showed that this system has excellent biocompatibility, while having low cytotoxicity, making it interesting for drug delivery systems. The targeting ability of these drug delivery systems reduces the cytotoxicity to non-cancerous cells, while retaining the therapeutic efficacy of the drug toward the target cells. Further testing should be performed before this system could be used in clinical applications. However, this research shows great possible applications as novel drug delivery systems.

A new diblock copolymer consisting of a hydrophobic supramolecular- and a hydrophilic macromolecular polymer part was reported by the group of Ji *et al.*[14]. The supramolecular polymer was based on host-guest interactions between a 32-crown-10 and a viologen salt moiety, and its length could be controlled by changing the concentration in the solution. Depending on the length ratio between the two blocks, different types of assemblies were observed. For a small supramolecular block micelles with a hydrophobic core were observed. Whereas polymerosomes with a hydrophilic interior and exterior were formed for a larger supramolecular block. Both these morphologies allow for the incorporation of small drugs. The system showed controlled release, and stimuli responsiveness to low pH. This research made the first steps in the combination of supramolecular and traditional polymers with possible applications as drug delivery systems.[14]

The group of Zhu and coworkers[13] reported a stimuli responsive drug delivery system based on the self assembly of a supramolecular amphiphilic polymer. The hydrophilic part of the polymer consisted from PEGylated calix[4]arene, the hydrophobic drug (chlorine e6, Ce6) binds to the calixarene by a host-guest interaction. This dimer could then self assemble into polymeric micelles, allowing the complex to be internalized by cells (fig. 8). The system showed low cytotoxicity, indicating it could safely be used as a drug delivery system with long circulation times due to the enhanced permeability and retention (EPR) effect[33]. This system was designed for photodynamic therapy, yet the host molecules are suitable for use with different types of guests, which also allows for applications in other treatments.

A similar type of system was reported by Zhao *et al.*[34] They report a self assembled supramolecular drug delivery system based on host-guest interactions that could be used as a dual delivery system for both a drug and a gene. γ-Cyclodextrin (γ-CD), functionalized with a cationic polymer acts in this system as the host. A hydrophobic anticancer drug

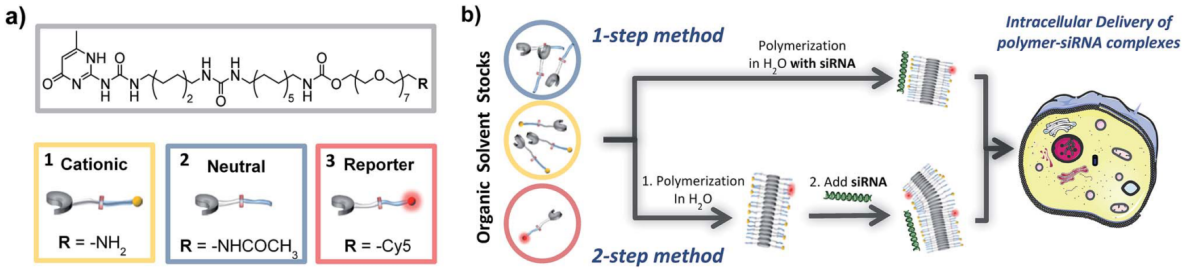

Figure 6: a) Chemical structure and schematic representation of the different functionalized UPy monomers. b) dimerization and subsequent polymerization of the UPy dimers in water, forming laterally stacked fibers. In the 1-step synthesis the siRNA is injected during the initiation of polymerization, for the 2-step synthesis the siRNA is injected after completion of polymerization. Image reproduced from Bakker *et al.*[29]

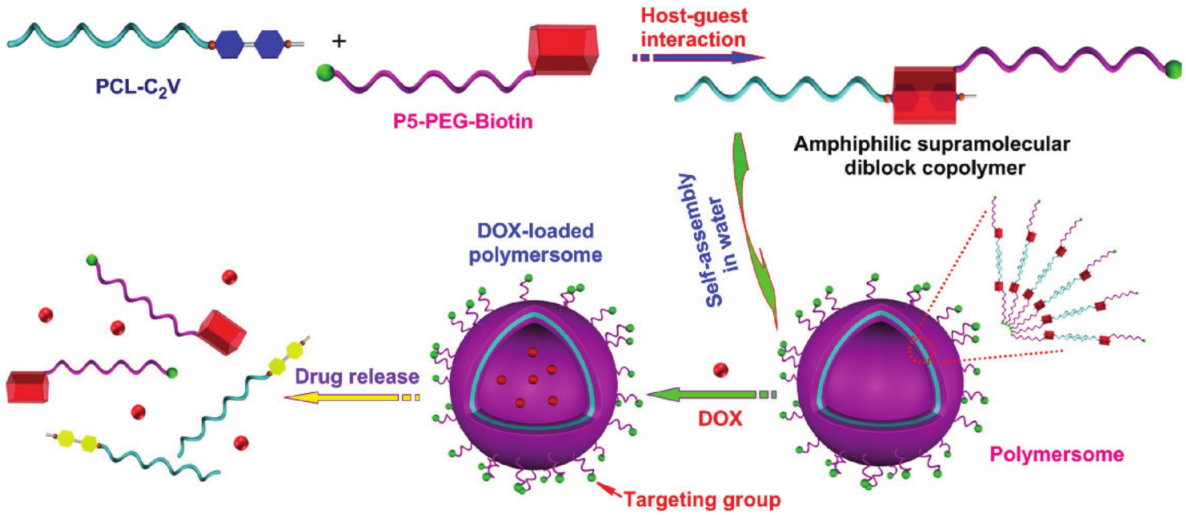

Figure 7: A schematic representation of the host-guest interaction between PCL-C_2V and P5-PEG-Biotin, forming a amphiphilic supramolecular diblock copolymer. Subsequent self assembly of the PCL-C_2V⊃P5-PEG-Biotin into polymerosomes allows for incorporation of a small organic drug, which can be released upon reduction of the viologen end groups. Image reproduced from Yu *et al.*[8]

(PTX) was loaded into the hydrophobic cavity of the γ-CD. Together with plasmid DNA (pDNA), these host-guest complexes could further self assemble into 'polyplexes'; positively charged nanoparticles, see fig. 9. These complexes could be internalized through the endocytosis pathway. The drug and pDNA could then be released after a redox reaction on the host. Toxicity studies were performed and showed high values of cytotoxicity to cancer cells when the drug was loaded. These results show that this system could be a promising candidate for anti cancer drug delivery systems.

3.2 Tissue engineering

The formation of functional tissues from supramolecular polymers requires bioactive polymeric materials. UPy-functionalized polymers are mainly used since they are very suitable due to their quadruple hydrogen bonding motifs (fig. 5a). Additionally, they show low processing temperatures and result in mechanically stable and biocompatible materials.[28,35] Combining UPy-functionalized polymers with UPy-functionalized biomolecules will result in bioactive materials that could be used for tissue engineering.[23] With these techniques bioartificial organs could be developed, improving the quality of life and life expectancy of people in need of donor organs.[23]

UPy-modified supramolecular polymers for cell free tissue engineering, specifically aimed for the use in vascular grafts were reported by Van Almen *et al.*[35] They showed the development of mechanically stable grafts with

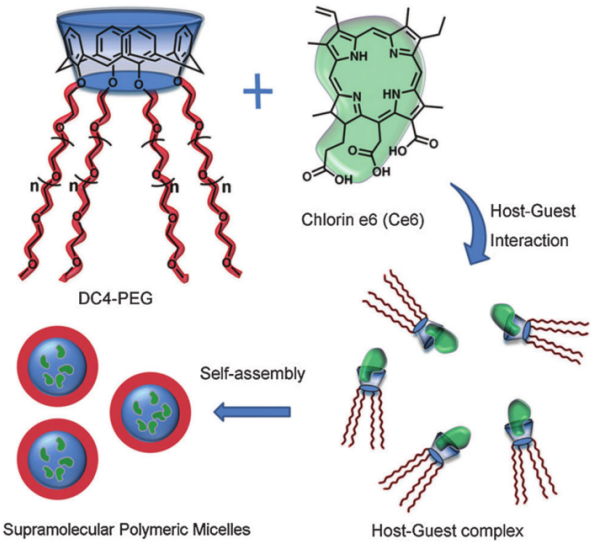

Figure 8: Schematic representation of the host-guest interaction between the hydrophilic PEGylated calix[4]arene and the hydrophobic drug (chlorin e6), and the subsequent self assembly into supramolecular polymeric micelles. Image reproduced from Tu *et al.*[33]

good mechanical properties. This report was a proof of concept study showing that the use of supramolecular UPy molecules provided a material that was mechanically stable and non-cell adhesive (no binding of unwanted cells) that could be applied in vascular tissue engineering. Other bioactive components could be incorporated in the polymer to facilitate cell specific binding, allowing for the first stages of tissue regeneration. Since the goal is that after a while the biomaterial should be indistinguishable from the real tissue, so specific cells have to be able to bind to the material.

Dankers *et al.*[28] used the quadruple hydrogen bonded UPy moieties for bioactive materials. The UPy-UPy association constant in water is low. However, because of the polymer film hydrophobic shielding occurs, resulting in strong but dynamic binding between the UPy moieties. They observed that the supramolecular polymer could adapt its structure to the environment, and proposed that the polymers could dynamically move over the surface, allowing to adjust for new cell binding. From *in vivo* experiments they found that the polymer matrix influences the signaling cells of the surrounding tissue, more research is to be conducted to these signaling processes. The biofunctionality of the supramolecular polymer matrix could be easily altered by introducing proteins or other bioactive materials that stimulate cellular processes.

A supramolecular membrane formed from fibrous supramolecular polymers and human tubular cells was reported by Dankers *et al.*[30] for the use in renal tissue engineering, especially focusing on the renal epithelial tissue. By using supramolecular UPy modified polymers with urea groups, they created fibrous membranes by electrospinning. As mentioned before in section 3.1.2, the UPy dimers stack in the lateral direction and are stabilized by $\pi - \pi$ interactions, and in this system also by additional hydrogen bonding between the urea groups. Thin, monolayer thickness membranes could be created that resembled the natural extracellular matrix. Insertion of kidney epithelial tubular cells resulted in the growth of monolayers of these cells after seven days of culturing period. The cells showed good cell viability and activity during the seven day tests they performed. Comparing these results to conventional microporous membranes, the supramolecular membranes were superior when concerning monolayer formation, cell viability, and cell activity. When the extracellular matrix is mimicked by providing bioactive signals, the differentiated features and properties of normal epithelial kidney cells may be introduced into the artificial membranes. These results show that the supramolecular UPy membranes offer good prospects for the use in epithelial kidney tissue engineering, with applications in bioartifical kidneys. These membranes might be improved in functionality by incorporation of bioactive materials that might further enhance the growth and differentiation of renal epithelial cells.

3.3 Self healing materials

Most of the reported applications using hydrogels are either in drug delivery or tissue engineering. However interesting applications could be thought of in, for example, the development of soft robotics and biomimetic prostheses.[36] Tee *et al.* reported an composite material from a supramolecular organic polymer with embedded nickel micro-particles that showed electrical and mechanical self healing properties.[36] Additionally the material was

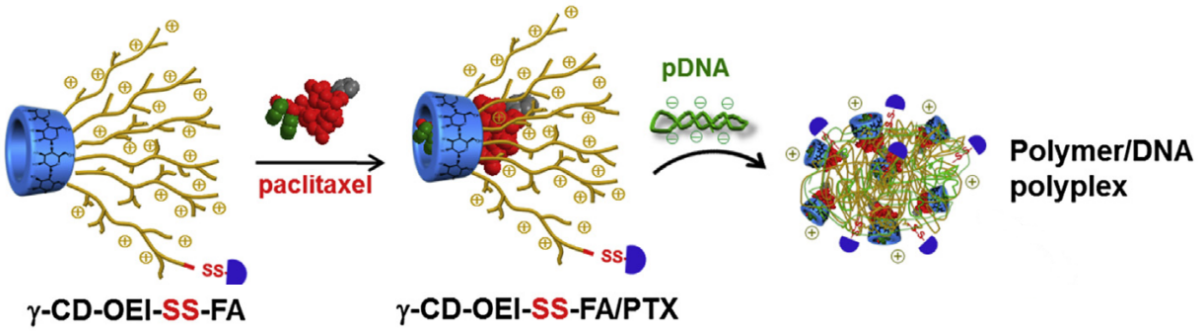

Figure 9: Schematic representation of the host-guest interaction between the γ-cyclodextrin (γ-CD) and the hydrophobic drug paclitaxel (PTX), complexation with DNA results in the formation of polymer/DNA polyplexes. Image reproduced from Zhao *et al.*[34].

found to be sensitive to both pressure and bending, making this material a good candidate for the use in electronic skin. The system showed both electrical and mechanical self healing. Electrical self healing was repeatable with a 90% efficiency after just 15 seconds of contact at room temperature. The mechanical self-healing was less successful at room temperature; 10 min of healing time would result only in 41% healing, whereas full recovery was observed for the same healing time at 50°C. It was found that mechanical healing is important for electrical healing since contact time is important for conductive healing. It was proposed that the healing mechanism is driven by the reformation of hydrogen bonds between the cut surfaces.[36] These results are very interesting, showing applications in artificial skin. This type of material could possibly be used in making skin for prosthetics giving a more lifelike look to the prosthetic. Electrical conductivity would not necessary for applications in prosthetics. However it might be interesting for other applications, such as the suggested soft robotics[36].

4 Summary and outlook

Supramolecular polymers are very interesting for the use in biomedical applications due to the noncovalent interactions that characterize these systems. These interactions allow for dynamic behavior through reversible interactions, stimuli responsiveness, and a bottom up approach for controlling the size and shape of supramolecular materials.

The use of supramolecular polymers for biomedical applications has been reviewed. A background in supramolecular polymers; the bonding motifs, stability, and supramolecular polymerization was given. Subsequently applications in drug delivery tissue engineering, and self healing materials have been presented.

Upon injection into water, benzene-1,3,5-tricarboxamide (BTA) monomers can dynamically self assemble into one dimensional supramolecular fibers. These fibers could be used for dual drug delivery systems. It was shown that the monomer distribution in the fiber could be controlled by the introduction of a multivalent binder. The removal of this binder resulted in the system reverting to the original state with a random monomer distribution.[27] This was the first step towards complex systems, that mimic cell signaling of real biological systems, in which the supramolecular polymer could respond to multiple stimuli that involve feedback loops. Making this a promising field of research, in which such complex systems could be developed for further improvement of drug delivery systems and a broader range of biomedical applications of supramolecular polymers.

Ureido-pyrimidone (UPy) monomers can dimerize through the formation of hydrogen bonds, subsequently these dimers can stack, forming supramolecular polymer fibers. Two interesting cases of UPy hydrogels that have sol-gel transitions have been reviewed. The most promising being a hydrogel which could be used for drug delivery to the heart. A gel was formed upon contact with the tissue, and was found to be able to withstand the high

shear of the contracting heart muscle.[17] This is a breakthrough for drug delivery to organs with high blood flow, since this hydrogel allows for prolonged retention of the drug. Due to their dynamic nature, UPy molecules are also viable for applications in tissue engineering, resulting in stable materials with good mechanical properties. These supramolecular polymers have shown their potential for vascular and renal tissue engineering. Thin fibrous polymer membranes could be created that resemble the biological extracellular matrix. The introduction of cells to these membranes resulted in the growth of monolayers of said cells with high cell viability and activity.[30] These membranes are important for renal tissue engineering, with possible applications in bioartifical kidneys.

Supramolecular amphiphiles are interesting candidates for drug delivery systems. Supramolecular amphiphilic polymers were developed using host-guest interactions. Polymeric lysosomes, micelles and other complexes were formed, these could be incorporated with small organic drugs. These systems could react to external stimuli, allowing for the drugs to be released.

Supramolecular polymers have been shown to electrical and mechanical self healing, when embedded with nickel micro particles Possible applications are in soft robotics and biomimicing prostheses.[36] Combining the research of tissue engineering and supramolecular self healing materials might result in very interesting applications in the development of self healing artificial skin for biomedical applications. Then skin grafts could be developed to be used in (reconstructive) surgery, allowing natural skin cells to regrow into skin, while the artificial skin will behave like normal skin during the growth process. It might be difficult to achieve this However, this might be a very interesting direction to look into.

The field of drug delivery and tissue engineering with respect to the use of BTA and UPy seems to be dominated by the groups of Dankers and Meijer. Their results for BTA and UPy seem very promising, so hopefully more research will be performed in this field to discover the full potential of these systems.

Most of the research conducted so far has mainly been focused on the design and fabrication of drug delivery systems. Mostly only initial *in vitro* tests were performed, while only few report *in vivo* testing. This is an area that will need to be investigated further in order to give a better indication if a designed delivery system is promising for clinical use. Additionally, focus should be put on finding the internalization pathways for these drug delivery systems. Another important area is to better understand the metabolism pathway for the carrier systems. The cytotoxicity of the metabolites and possible unwanted accumulation of these in the cells. These topics are not always discussed in the current research, yet they are very important for allowing these systems to take the next step towards commercial biomedical applications.

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