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Squaramide-based supramolecular polymers : from self-assembly to in vivo application

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CHAPTER 1

Introduction

1.1 Hierarchical self-assembly in Nature: inspiration for the design of supramolecular polymers

In Nature, specific and reversible non-covalent forces between biomolecules drive the self-assembly of ordered and functional biological materials.¹ Self-assembled structures, such as DNA, proteins, cell membranes and viruses, are responsible to transport of genetic information, perform a wide range of functions such as DNA replication (performed by proteins) or protection of the intracellular content from its surroundings (cell membrane). Central to all of these materials is that the organization of these biomolecules is triggered by non-covalent forces such as hydrogen-bonding, aromatic interactions, van der Waals, electrostatic or hydrophobic effects, which are individually weak, but can be collectively strong.²⁻⁴

One example of self-assembly in Nature is the formation of filamentous structures such as amyloids or actin filaments. Amyloids are a class of filamentous supramolecular biopolymers, well-known for their presence in neurodegenerative diseases.⁵ These fibrils are typically composed of polypeptides self-assembled in a perpendicular orientation to the fiber. The polypeptides interact primarily *via* multiple hydrogen-bonds between the amide groups that are adjacent to each other into the backbone, synergistically with other non-covalent interactions, such as ionic, van der Waals and hydrophobic interactions (Figures 1.1a and 1.1b).^{6,7} A nucleation-elongation mechanism is behind the growth of these filamentous structures through the transition from micelles to fibrils by polymerization of proteins at the fibril ends.⁸

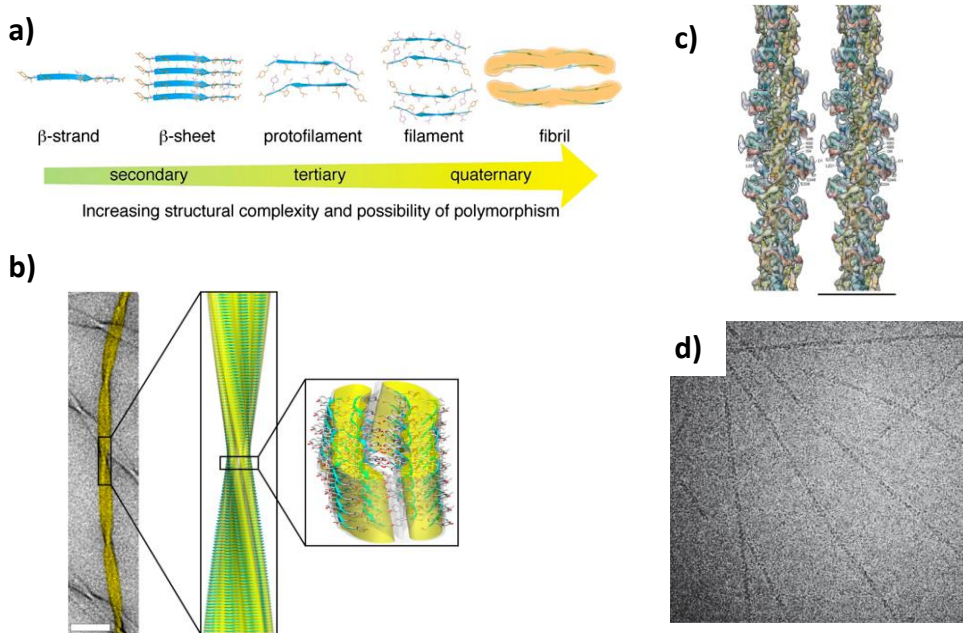


Figure 1.1. a) Hierarchical self-assembly of amyloids. b) Atomic-resolution of the reconstructed amyloid fibril structure (*Center*). TEM image of an amyloid fibril (*left*), scale bar = 50 nm. (*Right*) β -sheets depicted in a ribbon illustration. (Atoms of oxygen in red, carbon in gray, and nitrogen in blue). Figure adapted from reference 7. c) Three-dimensional density map of F-actin (scale bar 100 Å). d) cryo-TEM picture of F-actin filaments. Figure adapted from reference 11.

Actin filaments are another type of biological supramolecular polymer found in eukaryotic cells, namely in their cytoskeleton that is responsible for their architecture and motion. Actin is a 42 kDa globular protein that self-assembles through hydrophobic units and salt bridges in various locations into a helical ribbon structure⁹ (Figures 1.1c and 1.1d).^{10,11} Similar to amyloid fibrils, these structures self-assemble by a cooperative mechanism, starting from a peptide trimer.¹²

1.2 Non-covalent interactions employed in the design of supramolecular systems in water

Hydrophobic effects

The hydrophobic effect arises from the poor solvation of hydrophobic molecules.¹³ If a solute is introduced into water, the water molecules would need to rearrange their ice-like structure, which is energetically unfavorable. Consequently, this forces their aggregation, increasing the entropy as a consequence of releasing water into the bulk.¹⁴

Hydrogen bonding

Hydrogen bonds involve a hydrogen atom covalently linked to another electronegative atom (such as F, O or N) which it is attracted to another highly electronegative atom in close proximity (D-H...A).¹⁵ These special dipole-dipole interactions can be classified according to their nature,^{16,17} geometry or strength, with the latter criteria being the most common. Based on their strength,¹⁸ they can be further sub-divided into strong interactions (60-120 kJ mol⁻¹), in which the hydrogen atom is located in the middle of the donor and acceptor atoms and has a comparable character to covalent bonds; moderate strength (16-60 kJ mol⁻¹), which are formed by neutral donor and acceptor groups *via* electron lone pairs; and finally weak hydrogen-bond interactions (<12 kJ mol⁻¹) that can be of high relevance in crystal structures where they can be at close range in large numbers.¹⁹ However, an important consideration when applying them in the design of supramolecular monomers is the attractive or repulsive interactions between neighboring groups within the molecular recognition units, otherwise known as secondary interactions. The partial charges located on the neighboring heteroatoms can either decrease or increase the monomer binding strength by 8-12 kJ/mol per interaction.

The perceived hydrogen-bonding strength of a solute molecule is often lower in water because of the surrounding solvent which can compete with these interactions. Hence, to facilitate monomer self-assembly hydrogen-bonding interactions are often inserted within the hydrophobic pocket of the monomer to shield them from water in the design process. For example, ureas^{20,21} or carboxamides²² are common units used as hydrogen-bonding motifs in

combination with hydrophobic effects to trigger the formation of water-based supramolecular polymers.

Aromatic interactions

Aromatic interactions can take place between unsaturated cyclic compounds. Two arrangements of these interactions are possible: edge-to-face or face-to-face.¹⁹ Edge-to-face aromatic interactions, which are more energetically more favourable based on the interaction of their π -electron clouds, are responsible for the typical herringbone packing observed in crystal structures.¹⁹ On the other hand, face-to-face aromatic interactions, such as the stacking of nucleobases of DNA are postulated to be behind its stability.¹⁹

Other non-covalent interactions

As important as the previous interactions, the next two interactions are also valuable in the construction of supramolecular polymers. *Hydrophilic interactions*, namely the interactions of polar groups with water molecules are important for self-assembly of amphiphiles. Both polar and charged groups increase water solubility, and when combined with hydrophobic groups can facilitate their nanoscale phase segregation.²³ A second interaction that can play an important effect on self-assembly are *van der Waals* forces that arises from the polarization of an electron cloud by a nearby nucleus. This interaction is a weak non-directional electrostatic interaction that can be broken down into dispersion (London) and exchange-repulsion terms.¹⁹

Among the various non-covalent forces being involved in the process of self-assembly, the aforementioned ones are the most common interactions employed in the organic supramolecular polymer toolbox.

1.3 Supramolecular polymers

Lehn, Cram and Pedersen pioneered the field of supramolecular chemistry with their fundamental work on host-guest chemistry in the 1960s being awarded the Nobel Prize in 1987. At the beginning of the 90's, Lehn and Fouquey developed a system consisting of a complementary triple hydrogen bonding motif between uracil and 2,6-diacylaminopyridine.^{24,25} They linked together two molecular recognition units using tartaric acid as a crosslinker. When the resulting compounds were mixed in an equimolar ratio, a liquid-crystalline material was obtained that consisted of supramolecular chains composed of the monomer. This early example initiated the growth of the supramolecular polymer field and ever since a plethora of other polymers were published, where monomer building blocks are capable of self-organizing into well-defined structures.

1.3.1 Growth mechanisms in supramolecular polymers

The growth mechanism of supramolecular polymers is evaluated based on the response to temperature or concentration variation.²⁶ The dependency on these two factors is governed by the reversibility of the non-covalent forces, which upon rising the concentration or diminishing the temperature results in the enhancement of the degree of polymerization (DP). Thus, the three major growth mechanisms for this polymerization process are²⁷: isodesmic, ring-chain and cooperative.

In the isodesmic mechanism, a constant decrease in the free energy upon addition of monomers to the pre-formed polymer occurs due to an identical equilibrium constant (K_a) for the addition of each monomer to the growing polymer chain. The degree of polymerization (DP) is directly related to the value of the equilibrium constant with temperature and concentration having a great influence on the polydispersity of the resulting polymers (Figure 1.2a).²⁶ The ring-chain supramolecular polymerization mechanism involves a flexible, bifunctional monomer that is in equilibrium with the formation of a linear aggregate array and its cyclic counterpart (Figure 1.2b) throughout the polymerization. The cooperative (nucleation-elongation) growth mechanism is a two-stage polymerization process involving a nucleation and elongation phase. These steps are characterized by two equilibrium constants, one for nucleation (K_n) and a second one for the elongation of the polymer (K_e) once a nucleus of a certain DP is formed that is larger than K_a with both stages showing isodesmic growth.

Elongation is triggered below a critical temperature or above a critical concentration (Figure 1.2c). Moreover, a second scenario can be also possible, where oligomerization of the monomers is initially favoured over polymerization, and thus $K_n > K_e$. This phenomenon is known as anticooperative growth results in the formation of discrete objects with low dispersity.^{28,29}

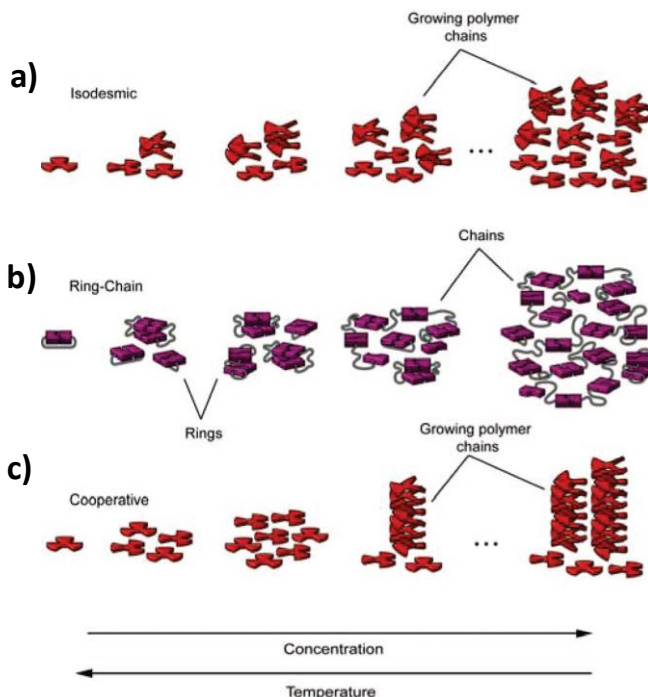


Figure 1.2. Cartoon showing the assembly and/or disassembly as an effect of concentration and/or temperature of (a) isodesmic, (b) ring-chain and (c) cooperative supramolecular polymerization. Figure adapted from reference 27.

1.4 Supramolecular polymerization in aqueous solution

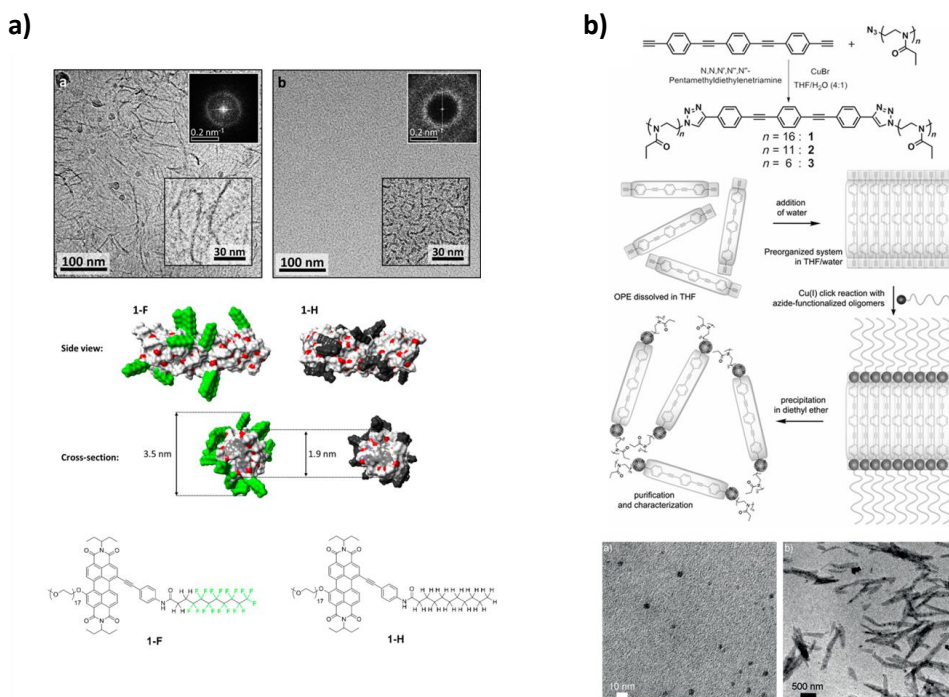
Understanding the interplay of the various non-covalent forces used to construct supramolecular polymers is vital to understand how this process occurs and the resultant polymer architectures formed. Water, the medium used by nature, affords self-assembled structures with a high degree of complexity, robustness and adaptability.³⁰⁻³² The combination of hydrophobic effects together with one or more other non-covalent forces in a synergistic manner is the means to design supramolecular polymers resulting in robust polymers in polar media.³⁷ However, due to the strong nature of hydrophobic interactions in water kinetic trapping of such self-assemblies can be expected with various structures from the same building block and pathway dependency.^{33,34}

1.4.1 Representative examples of monomers used for supramolecular polymerization in water

Single-chain amphiphiles

Amphiphilic or amphipathic compounds consist of a hydrophilic and a hydrophobic domain. For example, the Stupp group has developed supramolecular polymers based on peptide amphiphiles to yield long unidimensional cylindrical objects on the micron scale in aqueous media.³⁵ The monomeric building block is composed of an aliphatic tail connected to a hydrophilic peptide segment. Bioactive epitopes can be introduced in the hydrophilic domain to mimic biochemical or biophysical attributes of the natural extracellular matrix for tissue engineering and regenerative medicine applications.³⁶ Rybtchinski and co-workers exploited hydrophobic effects of aromatic cores to drive self-assembly in water. In a recent work, they studied the supramolecular polymerization mechanism of two perylene diimide (PDI) amphiphile motifs, functionalized either with an aliphatic chain or a perfluorooctyl chain (Figure 1.3a).³⁷ They measured the length of both PDI-based supramolecular polymers under the same sample preparation conditions, giving as a result a fiber length of two orders of magnitude longer for the fluorinated derivatives when compared with the purely aliphatic compounds, mostly attributed to the association constant of the fluorinated analogues (10^{15} M^{-1}), being three times larger than the aliphatic derivatives. In addition, the aggregation mechanism of the fluorinated PDI was studied in mixtures of different ratios of water/THF. Remarkably, it was observed that these structures self-

assembled *via* a cooperative mechanism when the ratio water/THF was 60/40, or higher in THF content. On the other hand, this mechanism was found to be isodesmic when the ratio was changed to a content 70/30, or lower in THF content. The authors stated that the hydrophobicity of the aliphatic tail is highly influenced by the large surface of the fluorine atoms, and they highlighted that the cooperative mechanism was a specific result due to the rigidity conferred by the fluorinated chain. These observations opened a new possibility for the design of supramolecular polymers.



Chapter 1

Bolaamphiphiles

Bolaamphiphiles contain a hydrophobic domain and two water soluble moieties at opposite ends of their core.^{38,39} They are more water soluble in comparison to amphiphiles, which translates into a higher critical aggregation concentration and a lower critical aggregation number. Upon self-assembly, these monomers can form a wide range of morphologies including disks, vesicles, spheres and fibers.³⁹ For example, Fernández and co-workers developed oligo phenyleneethynylene-based bolaamphiphiles substituted with two hydrophilic poly(2-ethyl-2-oxazoline) (PEtOx) of different lengths (Figure 1.3b). These self-assembled structures are triggered by a combination of π - π interactions and hydrophobic effects with the growth mechanism of the polymers being highly influenced by the length of their hydrophilic side chains. While the monomers with a PEtOx chain with 6 repeat units show nucleation-elongation or cooperative growth, the ones containing 16 units show isodesmic growth.⁴⁰ Sijbesma and co-workers developed a bolaamphiphilic structure with two urea motifs in the aliphatic core and two hydrophilic poly(ethylene glycol) side chains (Figure 1.4a). The hydrogen bonding between the monomers through the urea moieties in combination with the hydrophobic/hydrophilic ratio of the bolaamphiphile, triggered the formation of one-dimensional rod-like structures in water. Recently, these motifs have been exploited to prepare strain stiffening hydrogels when fixed through an topochemical polymerization within the fiber.⁴¹⁻⁴⁴ Bouteiller and co-workers reported another bis-urea based supramolecular polymer that is able to self-assemble in water, aprotic and non-polar solvents (Figure 1.4b). In this report, it was found that the driving force of the supramolecular polymerization depends on the solvent, but in all cases long, rigid filamentous structures are formed.⁴⁵ This amphiphile structure will be used and studied in this thesis as the main building block to drive the formation of supramolecular polymers.

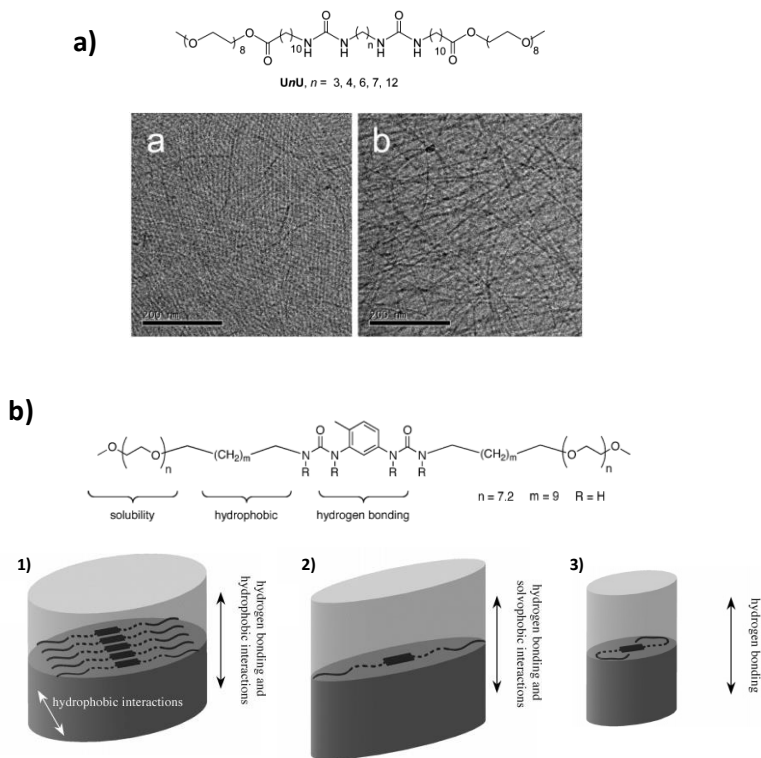


Figure 1.4. (a) Bis-urea based bolaamphiphiles developed by Sijbesma and co-workers with aliphatic spacers of various lengths (*top*). CryoTEM pictures (*bottom*) at 1 wt% of the rod-like structures for U4U (a) and U7U (b), adapted from reference 44. (b) Molecular structure of bis-urea bolaamphiphile introduced by Bouteiller and co-workers (*top*) and schematic representation of the filaments upon self-assembly in water (1), acetonitrile (2) and toluene (3), adapted from reference 45.

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C₃-symmetric amphiphiles

C_3 -symmetric monomers with a disc-shaped conformation that consist of a rigid core and solubilizing chains on the periphery represent another well-known class of monomers forming supramolecular polymer architectures. As an illustrative example, the group of van Esch and Eelkema has studied and exploited the formation of a low molecular weight hydrogelator-based on trishydrazone units (Figure 1.5a).⁴⁶⁻⁴⁸ They were able to control the mechanical properties and microstructure of the resulting materials through catalysis using a reaction-coupled system. The formation of the gelator was done *in situ* by reacting cyclohexane trishydrazide building block **1** with three molecules of aldehyde **2** (Figure 1.5a). By using either acid or nucleophilic aniline catalysis, hydrogels can be formed under ambient conditions on the order of minutes. Additionally, Meijer and co-workers have exploited the use of 1,3,5-benzentricarboxamides (BTA) for the formation of supramolecular polymers in numerous studies with a recent focus in aqueous solutions.⁴⁹⁻⁵² In order to promote the solubility of these structures in aqueous media, tetraethylene glycol is conjugated to the periphery of the BTA structure, and by a combination of hydrophobic, aromatic interactions and hydrogen-bonding, self-assembly takes place. Recent studies have proposed the use of this aggregate as a platform for intracellular drug delivery (Figure 1.5b).⁵²

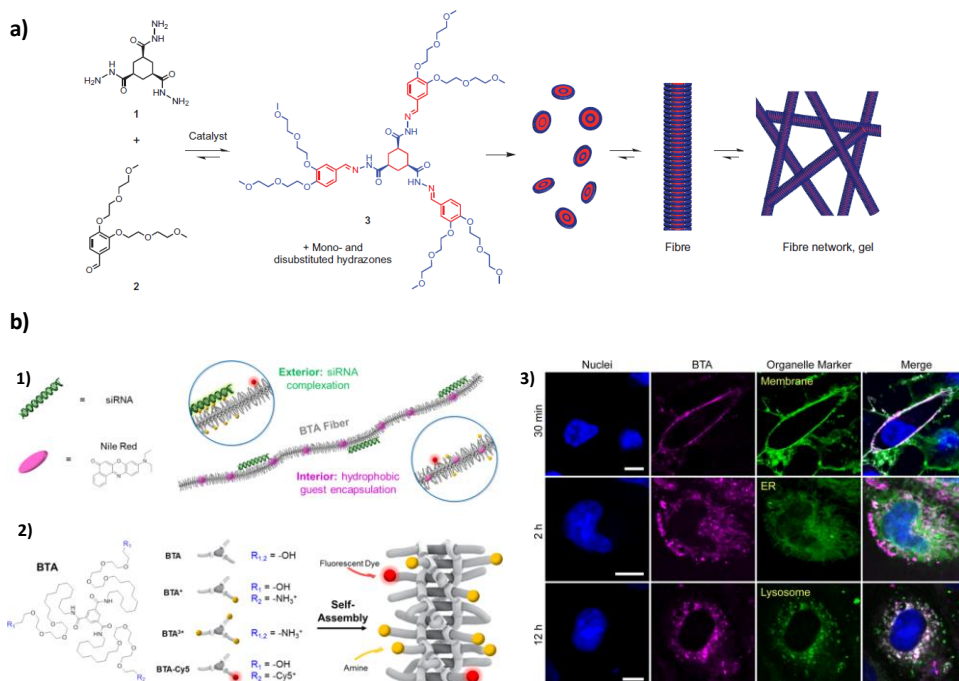


Figure 1.5. (a) Hydrogel formation proposed by van Esch and co-workers. Trishydrazone hydrogelator **3** synthesized by a reaction of **1** and **2** and triggers fiber formation, trapping the surrounding solvent to form a gel. Image adapted from reference 48. (b) Intracellular siRNA delivery using BTA supramolecular polymers. (1) Nile red (hydrophobic fluorescent molecule) is trapped in the lipophilic core, while siRNA condenses on the exterior of the fiber by electrostatic interactions. (2) Self-assembly *via* co-assembly of the BTA-based monomers. (3) Cellular internalization of BTA polymers. Imaging of HK-2 cells over several hours, which were incubated with cationic and dye labelled BTA aggregates. Scale bars 10 μM. Image adapted from reference 52.

Host-guest complexes

Supramolecular polymers based on the host-guest complexation of monomers in water compose another class in the field. Macrocyclic hosts such as cyclodextrins, cucurbiturils, or calixarenes are commonly used blocks to obtain host-guest mediated supramolecular polymers, together with their complementary guest molecules.⁵³⁻⁵⁵ An example is the work of Harada and co-workers on cyclodextrin-guest complexes.^{56,57} In one system, they coupled a guest *t*-Bu-cinnamoyl to the edge of a host α -cyclodextrin to trigger the formation of chiral supramolecular polymers by insertion of the *t*-butyl aliphatic moiety in the hydrophobic pocket of the cyclodextrin, resulting in a degree of polymerization of approximately 15

monomers from a single monomer (Figure 1.6a).⁵⁸ By a similar approach, a cyclodextrin-based co-polymer was developed based on the selective binding of different guests by α -CD and β -CD coupled to guest molecules adamantane and *t*-Boc cinnamoyl using two different building blocks to form an alternating copolymer. (Figure 1.6b).⁵⁹

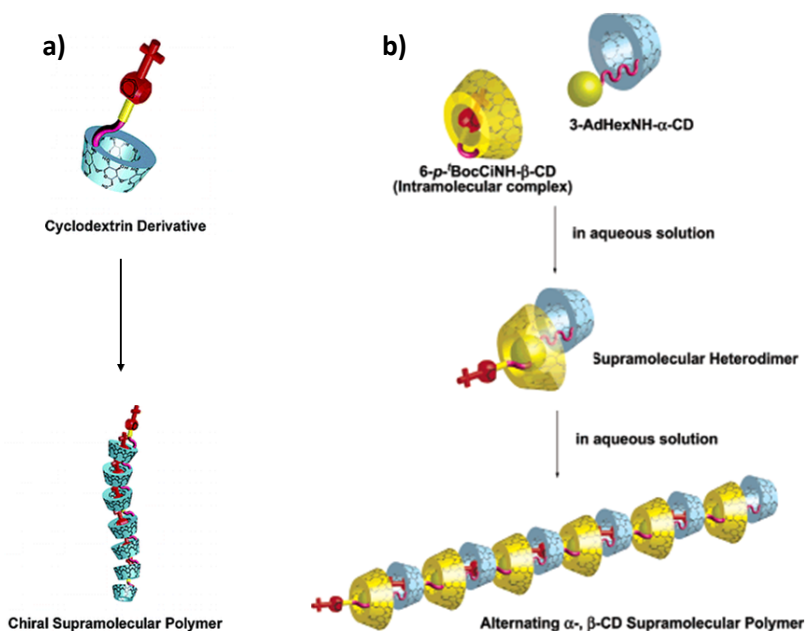


Figure 1.6. (a) Cyclodextrin-based chiral supramolecular polymers consisting of repeating units of *t*-Bu-cinnamoyl (guest) linked to the edge of α -cyclodextrin (host). (b) α -cyclodextrin-adamantane and β -cyclodextrin-*t*-Bu-cinnamoyl building blocks forming a co-polymer of approximately 30 monomers long. Images adapted from reference 58 (a) and 59 (b).

1.4.2 Biomaterials based on supramolecular polymers

The supramolecular polymer materials used in the biomaterials field can be subdivided into two major designs: the first one corresponds to the one-dimensional, shape persistent objects (Figure 1.7, top box) consisting of stacked motifs, while the second group corresponds to polymeric precursors with molecular recognition motifs that form networked structures. Because of their unique properties that include responsiveness, modularity, and tuneability, in comparison to their covalent counterparts there is growing recognition of their applicability in this area with several promising *in vitro* and *in vivo* studies.^{60–64} As drug delivery platforms,^{65,66} supramolecular materials are envisaged to promote the

solubilization of insoluble drugs, small hydrophobic molecules or proteins, while providing a platform to control the circulation time and release within the body. For instance, supramolecular polymers based on peptide amphiphiles have been employed as drug carriers for the encapsulation of poorly soluble drugs, such as camptothecin, with the inhibition of tumor growth in a mouse model.⁶⁷ Another area of interest concerns the engineering of 3D cell microenvironments, in which the development of artificial extracellular matrices with tunable mechanics, bioactivity and materials is sought after for applications in the fields of tissue engineering⁶⁸ and regenerative medicine.^{63,69} Typically, these matrices are prepared with bioactive peptides or proteins to provide cell-specific cues.⁷⁰⁻⁷² These soft supramolecular materials can be employed to support and deliver cells by their encapsulation and have been therefore explored for reconstituting aspects of functional tissues,⁷³ delivery of cells⁷⁴ or repair of native tissues.⁷⁵

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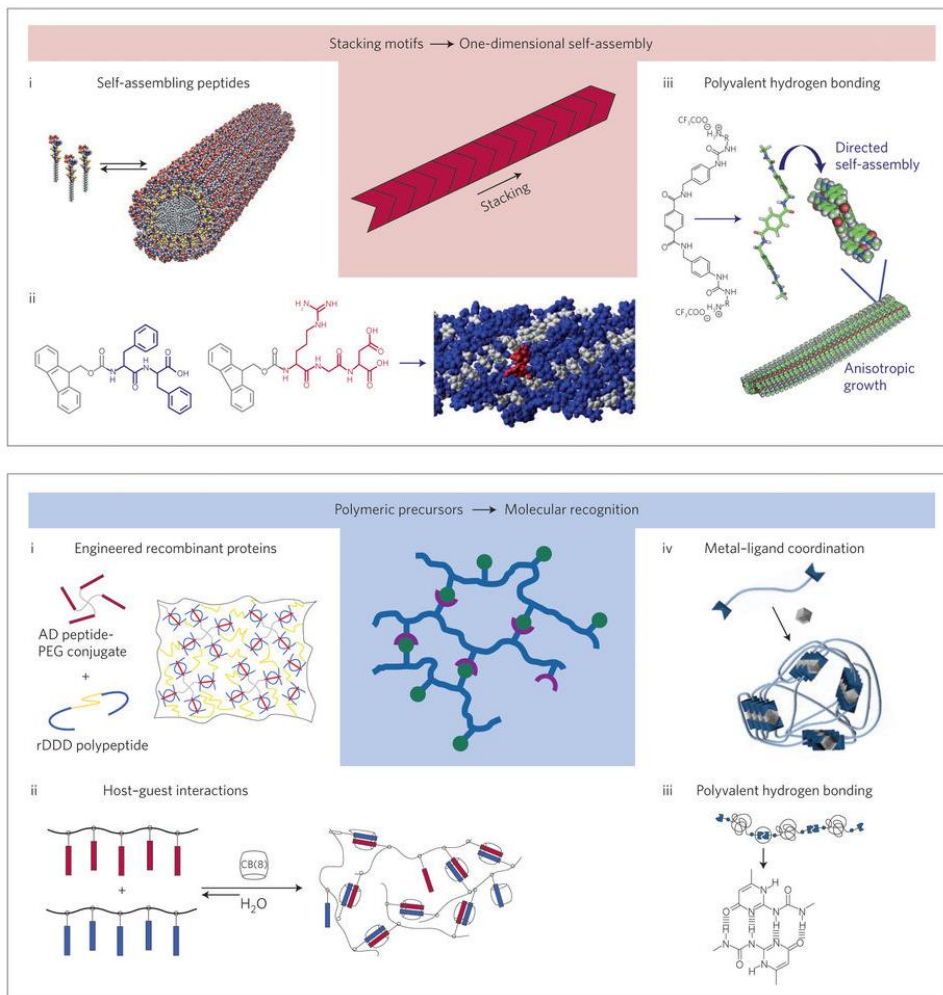


Figure 1.7. (Top) Examples of one-dimensional self-assemblies based on stacking motifs. (Bottom) Crosslinked polymeric blocks or extended polymer chains by molecular recognition motifs. Figure adapted from reference 64.

1.5 Squaramides

Squaramides consist of a cyclobutenedione ring with two N-H donor groups opposite two carbonyls (Figure 1.8).⁷⁶⁻⁷⁸ Squaramides present a rigid and planar ring system that is stabilized by the sp^2 -hybridized nitrogens, with the potential for the nitrogen lone pairs to be conjugated with the π -system of the cyclobutenedione ring system. Their partial aromatic character (Hückel's Rule, $4n + 2$, $n = 0$) can be enhanced upon complexation of ions at either side of the ring by hydrogen bond formation.^{79,80} Frontera and co-workers computed the aromatic character of the squaramide ring with respect to ion binding according to energetic, magnetic and electronic criteria (see sub-section 1.4). They observed the NICS values became more negative upon complexation (NICS (0.6) = -6.3 ppm for the isolated squaramide ring, -7.6 ppm for N-H hydrogen bond formation with carboxylates, -8.1 ppm for C=O bond formation with ammonium, and -8.7 ppm when both sides are engaged with the aforementioned ions) suggesting that the increase in aromaticity facilitates exceedingly high H-bond acceptor and donor characters (as a reference, a fully aromatic system (i.e. benzene) has a NICS (0.6) value of -10.1 ppm).

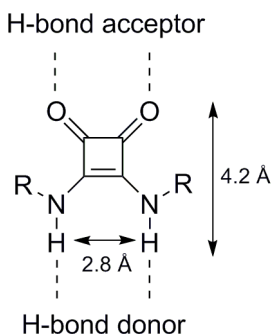


Figure 1.8. Molecular structure of the squaramide synthon.

Squaramides have been applied to mimic the anion transport behavior of complex proteins through ion channels across lipid bilayers as demonstrated by Gale and co-workers⁸¹⁻⁸³ Moreover, they have been extensively explored in the ion receptor field in various presentations including macrocyclic compounds and polymers showing improved ion selectivity and affinity. Squaramide ion transporters show a better anion-transport activity compared against analogous transporters based on ureas and thioureas. The association constant (K_a) of a

family of analogous molecules consisting of these three motifs was calculated by $^1\text{H-NMR}$ spectroscopic studies using Bu_4NCl salts in $\text{DMSO-d}_6/0.5\%$ water.⁸¹ The K_a measured for squaramides with these ions was on the order of $260\text{-}643\text{ M}^{-1}$, while the urea motifs were on the order of $31\text{-}88\text{ M}^{-1}$ and the thiourea on the order of $15\text{-}43\text{ M}^{-1}$ (this range of values is due to the substituents used for the design of the anion transporter). These results are relevant in this area, showing that squaramides are ideal candidates for anion transport activities, with improved properties compared to their urea and thiourea analogues, without increasing significantly the hydrophobicity. Squaramides are also being increasingly explored as scaffolds for new therapies.^{84,85} Clinical candidates such as Perzinfotel or Navarixin, both consisting of a double substituted squaramide moieties, have been studied for the treatment of strokes and chronic obstructive pulmonary disease, respectively. Squaramides have also been applied in the area of organocatalysis due to their capacity to act as more effective hydrogen-bond donors relative to the currently existing thiourea or urea scaffolds. Squaramide-based organocatalysts have been used to carry out organic transformations such as Michael additions⁸⁶, Friedel-Craft reactions⁸⁷, 1-4 additions of malonates to nitroolefins⁸⁸ or addition of 1,3-dicarbonyls to acyl phosphonate compounds⁸⁹. These are just few examples of the extensive applications squaramides have in the area of catalysis. Finally, squaramides have been applied in the bioconjugation field due to their stability to hydrolysis in aqueous medium, with special attention on the formation of glycoconjugates. Yan and co-workers used the squaramide motif to link carbohydrate conjugates based on lactoside and dinucleotide in water, showing the potential of these units as linkers.⁹⁰ Rotger and co-workers designed a peptidomimetic structure with a N-methylated squaramide, by a conventional solid-phase peptide synthesis. This hybrid was found to fold in water, and due to its dynamic properties, the authors stated that this motif could be used to design bioactive peptidomimetic molecules.⁹¹

1.6 Aromaticity

Aromaticity is a core concept in organic chemistry, but it is challenging to define because of its multidimensional character. This concept is used to describe the unusual properties of a group of cyclic and unsaturated planar organic molecules relative to their acyclic analogues.⁹²⁻⁹⁵ Some of these properties are: (1) increased molecular stability, (2) intermediate bond lengths in between single and double bonds, (3) preference for substitution to addition with retention of the π -electron

arrangement, and (4) induction of π -electron ring currents when the systems are exposed to external magnetic fields, translating into specific and characteristic values of $^1\text{H-NMR}$ chemical shifts that are downfield compared to non-aromatic compounds.⁹⁶ Qualitatively, cyclic unsaturated compounds are classified as aromatic according when they satisfy Hückel's rule, which states that cyclic (and planar) π -electron systems with $(4n + 2)$ π -electrons are more stable than those containing $4n$ π -electrons. Because aromaticity cannot be quantified easily, this concept is probed computationally. Computational methods that tackle the various criteria such as nucleus-independent chemical shift (NICS) (magnetic),^{97,98} harmonic oscillator model of aromaticity (HOMA) or harmonic oscillator model of electron delocalization (HOMED) (geometric)⁹⁶ and aromatic stabilization energies (ASE) or block-localized wave functions (BLW) (energetic) can be employed.⁹⁴ These computational methods are applied in chapters 2 and 3.

Aromaticity has been used to explain the high efficiency of a number of organic reactions such as in the Cope rearrangement and [1,5]-shifts.⁹⁹ Recently, the effect of aromaticity on the hydrogen-bonding interaction has started to be explored.^{100–103} Schleyer, Wu and co-workers showed computationally that the hydrogen-bonding strength can be affected by the aromatic character of heterocyclic rings upon dimerization.¹⁰¹ They compared 2-pyridinone dimers against 2-hydroxypyridine dimers. Upon dimerization of 2-pyridinone, the NICS index shifted from -10.6 ppm (monomer) to -13.4 ppm (dimer), suggesting an increase in $4n+2$ π -electron delocalization or aromatic character of the ring with a 45% increase of the interaction energy, compared to its acyclic analogue (Figures 1.9a and 1.9b). On the other hand, the dimerization of 2-hydroxypyridine showed the opposite effect. A NICS value of -24.2 ppm was estimated for the single monomer while the dimer displayed a NICS value of -21.4 ppm, suggesting a loss of its aromatic sextet character upon hydrogen bonding being weakened relative to their acyclic analogues. Hence, the computed dimerization energy of 2-hydroxypyridine decreased by 21% compared with its acyclic analogue dimer (Figure 1.9c and 1.9d). By similar methods, Wu, Fernández and co-workers showed that the aromatic character of 4-pyridone can be increased through the π -polarization of the oligomers by hydrogen-bonding, resulting in an increase in strength of the hydrogen bond interactions.¹⁰²

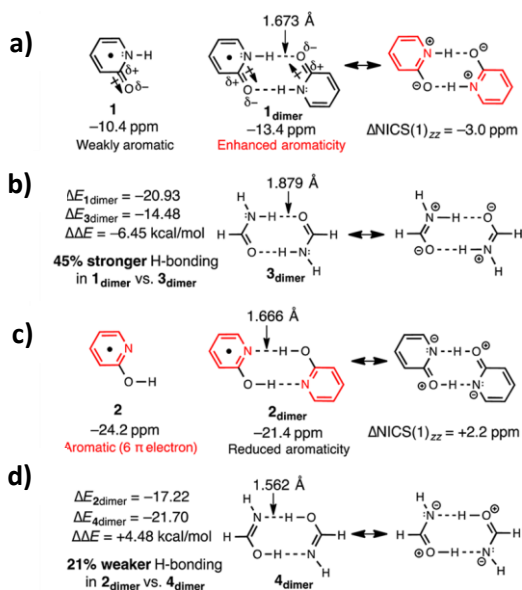


Figure 1.9. NICS, hydrogen-bond lengths and dimerization energies of (a) 2-pyridinone, (b) formamide, (c) 2-hydroxypyridine and (d) hydroxyimine dimers. Figure adapted from reference 101.

1.7. Aim and outline

The development of synthetic supramolecular polymers in aqueous media and their potential for use as biomaterials has garnered much attention over the last few years. The examples disclosed in this introductory chapter are characterized by a broad diversity in molecular design, which is necessary to explore the supramolecular material playing field. However, self-assembled biomaterials and understanding the contribution of the non-covalent interactions involved in their self-assembly still remains challenging. When water is involved in the self-assembly process, hydrophobicity is often employed as the major driving force, with other directional non-covalent forces being used to promote their one-dimensional aggregation. Hydrogen bonding takes the lead here in terms of directionality compared to other non-covalent bonding forces and is often included in the monomer design.

In this thesis, I aim to develop and study a robust and adaptable scaffold for supramolecular polymer self-assembly in water. For this purpose, I rely on the use of squaramides to provide directional interactions in order to drive the formation of one-dimensional aggregates through self-assembly. The interplay of hydrogen-bonding and aromaticity in the monomer self-assembly process is explored in the

squaramide unit, by examining the consequence of aromatic gain on this process. Additionally, by systematic modification of the monomer structure, a library of supramolecular structures with different morphologies is developed and their use as future drug nanovehicles is studied in an *in vivo* model involving zebrafish embryos.

In **chapter 2**, I explore the potential for synergy between hydrogen-bonding and aromaticity in the squaramide unit upon its supramolecular polymerization in water. The synthesis and design of a squaramide-based bolaamphiphile is reported. Experimentally, the morphological aspect of the resulting aggregates is explored by a combination of microscopic (cryo-TEM) and scattering techniques (DLS and SAXS). At the molecular level, spectroscopic techniques reveal the influence of the self-assembly on the geometric (FTIR) and electronic (UV-Vis) properties of the squaramide synthons. Finally, by a combination of computational methods (NICS, HOMA and ASE), I explore the consequence of a gain in aromatic character on the self-assembly of the squaramide-based bolaamphiphiles.

In **chapter 3**, the concept of aromaticity-modulated non-covalent interactions in squaramides is further explored. The oxosquaramides presented in chapter 2 are thionated. Following a similar strategy, the size and shape of the self-assembled aggregates is studied by microscopy (cryo-TEM) and scattering (DLS and SAXS) in water. The changes at the molecular level upon self-assembly on the electronic and geometric properties are investigated by UV-Vis and FTIR spectroscopy, while changes to the aromatic character of both ring systems were compared computationally. Whereas oxosquaramides self-assemble in a head-to-tail hydrogen-bonding stacked configuration, and both increase their aromatic character upon self-assembly.

In **chapter 4**, a library of systematically modified squaramide-based bolaamphiphiles is examined for their capacity to self-assemble into various morphologies. Aggregate morphologies from spheres to fibers are achieved by simply modifying the monomer structure, namely their hydrophilic and hydrophobic domains. Spectroscopic (UV-Vis and IR) and microscopy (cryo-TEM and SAXS) studies are pursued to further understand the self-assembly of such aggregates at various length scales. Control over their morphology opens the door for their use as supramolecular polymer nanoparticles for drug delivery.

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In **chapter 5**, the distribution and circulation behavior of spherical, rod and fibrillar squaramide-based supramolecular structures in a zebrafish embryo *in vivo* model is explored. First, I report the synthesis of a cyanine-labeled reporter molecule, which is further used for fluorescent labelling the various squaramide-based bolaamphiphile structures. The co-assembly of the monomers is followed by cryo-TEM and zeta potential, while the dynamics and exchange kinetics of the three different co-assembled squaramide-based supramolecular structures are investigated by fluorescence spectroscopy through FRET in water. Finally, the tracking of the aggregates *in vivo* is achieved in a zebrafish embryo model by confocal microscopy, showing distinct distribution and clearance of the various structures through a change in shape. Additionally, I examine the potential for macrophage uptake of the nanoparticle morphologies.

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