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

Version: Not Applicable (or Unknown)
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Issue Date: 2018-12-10
CHAPTER 6

Summary and perspectives
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The self-assembly of molecular building blocks into well-defined supramolecular structures through non-covalent forces is a powerful approach for the construction of materials. Inspired by Nature that takes advantage of these reversible bonding forces to elicit numerous processes at various length scales, the field of supramolecular materials strives to develop scaffolds that are adaptable, reversible and functional for a broad range of applications from biomedicine to electronics. In order to truly exploit these synthetic polymer assemblies in areas such as regenerative medicine or drug delivery, their self-assembly under biological conditions needs to be well-understood. In aqueous media, the hydrophobic effect is often the dominant driving force in promoting monomer aggregation, while other non-covalent interactions such as hydrogen-bonding and aromatic interactions promote their aggregation into organized fibrillar architectures. Although cyclic π-conjugated molecules are often employed in such monomers, the consequence of the aromatic character on self-assembly is often overlooked despite the frequent use of aromaticity to explain the stability of such molecules and the unexpected efficiencies of certain reactions involving π-conjugated rings. In this thesis, I examined the interplay of aromaticity and hydrogen bonding on the self-assembly of a squaramide-based supramolecular polymer in water. Squaramide derivatives were employed as ditopic hydrogen-bonding synthons that benefit from their partial aromatic character to modulate their hydrogen-bond strength upon self-assembly. Systematic variations of the various hydrophobic and hydrophilic domains of the monomer enabled the generation of a small library to probe the contribution of the various monomer domains to the resultant structure. Preliminary in vivo studies in zebrafish embryos revealed their potential to evade macrophages, however they are still removed from circulation by the stabilin-2 receptor when as a rod-like or fibrillar aggregate.

In chapter 2, the synergistic contribution of hydrogen-bonding and aromaticity as driving forces to trigger one-dimensional self-assembly of supramolecular polymers in water is explored. Two squaramide synthons are embedded within the hydrophobic core of a bolaamphiphile to promote supramolecular polymer self-assembly. Microscopy studies (cryo-TEM and AFM) show the formation of high-aspect-ratio fibers up to a micron in length and are supported by scattering (DLS and SAXS) techniques that reveal the presence of 10-30 squaramide-based bolaamphiphiles per cross section. UV-Vis spectroscopy studies reveal the influence of the self-assembly on the electronic properties of the squaramide
synthon, while IR provides insight into their geometric changes in this process; namely, a decrease in bond length alternation of the squaramide units upon self-assembly. These observations are further explained by the application of density functional theory to measure magnetic, geometric and energetic properties of the self-assembled monomers, supporting the hypothesis of hydrogen-bonding reinforcement though a gain in aromaticity. In this study, I show that squaramides are excellent directional units to promote the self-assembly of supramolecular polymers and that a gain in aromaticity through hydrogen bonding can contribute significantly (30%) to the overall interaction energy of the monomer units. By merging computation and experiment, this seminal study opens the door to exploring if this effect occurs in a wider range of organic synthons used to construct supramolecular polymers and its consequence on the resultant assemblies.

**Chapter 3** demonstrates the potential of aromatic gain to switch the self-assembly mode of a squaramide unit in a one-dimensional shape persistent aggregate by O → S substitution. First, I have shown the synthesis of a thiosquaramide-based bolaamphiphile derivative by using a zwitterionic P₄S₁₀⁻pyridine thionating agent, replacing selectively the oxygen atoms with sulfur atoms on the squaramide carbonyls. Next, their self-assembly into short flexible rod-like structures was observed by cryo-TEM and supported by SAXS. Through a combination of experiment, using UV-Vis and IR spectroscopy, and computation, by estimating NICS, HOMA and ASE values, I demonstrate that oxosquaramides prefer a head-to-tail hydrogen-bonding arrangement promoted by aromatic gain, while thiosquaramides prefer π-stacked arrangement to enhance their aromatic character. These studies highlight the potential of coupling of aromatic character and non-covalent forces in these systems, opening a new door in the rational design of new supramolecular motifs.

Further insight into the growth mechanism of these squaramide-based (oxo and thio) supramolecular polymers is required. The self-assembly of these systems can be followed by various spectroscopic techniques, such as UV-Vis where the proper fitting to either isodesmic or cooperative models in a heating-cooling curve would provide clues into their growth mechanism in water. However, one limitation in probing such assemblies resides in the large hydrophobic domain provided by the bolaamphiphile, which can result in the formation of kinetically trapped states during the heating-cooling cycles. A plausible solution would be to find a miscible
co-solvent to decrease the kinetic barrier promoted by the hydrophobic effects of the amphiphiles, while avoiding complete disruption of the supramolecular aggregate.

In chapter 4, systematic variations of the ethylene glycol side chains (n = 7 to 36) and outer aliphatic spacers (m = 2 to 12) of the aforementioned squaramide-based bolaamphiphile monomers were prepared and characterized. First, a monomer library is prepared following the synthetic approach developed in chapter 2. Subsequently, the morphologies of the resulting aggregates from the monomer library were probed. Systematic modification of the ethylene glycol hydrophilic side chains resulted in a gradual morphological transition from fibrillar-like domains (n = 11) to spherical objects (n = 36) as observed in cryo-TEM and SAXS experiments. On the other hand, an aliphatic spacer of eight methylene units was necessary to shield the squaramide units at the concentrations studied by the same analysis methods. These observations are further supported at the molecular level by UV-Vis and IR spectroscopic measurements. The potential for using these systems as drug carrier vehicles is examined by performing encapsulation experiments using Nile Red as hydrophobic cargo. To better tailor the prepared nanoparticles for various applications, such as drug delivery, length control (D ~ 1.0) over the formed self-assemblies through controlled supramolecular polymerization is very much necessary. In order to achieve this goal, it is critical to understand the growth mechanism of these squaramide-based polymers and to explore the effect of various self-assembly pathways on the formed products.

In chapter 5, I follow the distribution and circulation behavior of fluorescently tagged squaramide-based supramolecular structures of various morphologies, as described in chapter 4, in a zebrafish embryo model in vivo. The design and synthesis of the unsymmetrically labeled cyanine-3 squaramide-based bolaamphiphile is reported. The co-assembly of this reporter molecule with the native squaramide monomers is followed by zeta-potential and fluorescence measurements, and their morphologies are examined by cryo-TEM microscopy on their own and in the presence of increasing carp serum, where a retention of the aggregate morphology is observed. Additionally, injection of the premade fluorescent aggregates of various shapes into zebrafish embryos was performed to track the biodistribution and circulation of the three architectures in vivo. Here, all structures were found to evade macrophages, making them good candidates as
drug delivery nanocarriers. Finally, it was found that the nanocarrier shape and size plays an important role in nanoparticle clearance by scavenger endothelial cells by the Stabilin-2 receptor.

The use of supramolecular polymers as biocompatible drug carriers is an exciting area that requires further research because of its promise to expeditiously prepare highly functionalized carriers for targeted drug delivery applications. Here, I show how these nanoparticles behave once injected into an in vivo model. At this point, many possibilities can follow these studies. A possible step would involve conjugating drugs to the supramolecular monomers for self-assembly to prolong or decrease their circulation time. Furthermore, the decoration of these systems by employing cell targeting peptides or aptamers could promote their specific delivery to a given cellular target. This is not a straightforward aim since many parameters have to be taken in consideration simultaneously, but such materials would advance the knowledge in effective drug carrier design.
Chapter 6