Macromolecular Architectures

DOI: 10.1002/anie.200900704

Imaging of Catenated, Figure-of-Eight, and Trefoil Knot Polymer Rings**

*Michel Schappacher and Alain Deffieux**

Most of the available characterization techniques for macromolecules are based on the determination of the average properties (chemical composition, tacticity, dimensional parameters, melting temperature, etc) of an entire set of molecules in a given system. In this context, imaging techniques could offer a quite different and unique approach that allows the analysis of macromolecules as single objects. However, isolated polymer chains are often too diffuse to be distinctly and directly visualized. To circumvent this problem, a magnification process of the size and density of macromolecules prior to imaging can be used to homogeneously enlarge isolated polymer chains before observation by atomic force microscopy (AFM). Numerous studies have been devoted over the past decades to the investigation of the properties of isolated biomolecules by molecular imaging. The visualization of single DNA molecules[1] has shown that DNA can exist in the form of single and interlocked rings of various complexities.[1,4] Since then, several examples of catenated and knotted single- and double-stranded DNA rings have been reported.[5–9]

Continuous progress in this area is clearly associated with the development of imaging techniques that allow the investigation of the molecular structure[10,11] and mechanical properties[12–15] of isolated macromolecules. Besides biomacromolecules, a limited number of reports have addressed the imaging of isolated polyelectrolyte chains,[16–18] and even fewer studies of the AFM investigation of neutral polymer chains at the molecular level have been reported.[19,20] Indeed, neutral linear polymer chains, which have sizes that range from a few to several hundred nanometers, possess a cross section of only a few atoms and are softer than biomolecules and polyelectrolytes. Moreover, their local concentration can widely fluctuate through chain motion because of weaker interactions with substrates.[21–23] This fluctuation makes the linear polymer chains highly diffuse objects that cannot be easily observed by imaging microscopy. To increase the contrast of the images, selective adsorption of salts has been used by Minko and co-workers in the case of polyelectrolyte chains.[24] To date, AFM visualization and characterization of single synthetic non-ionic macromolecules has been mostly limited to macromolecules with invariant shape such as carbon nanotubes, comb polymers,[25–31] and dendrigrafts,[32,33] which possess higher mass densities along their backbone than linear polymers.

Therefore, one method to permit molecular imaging of single linear, cyclic, and starlike main-chain polymers could involve magnification of the macromolecules by homogeneously increasing their mass density along the whole of the chain, thus making their cross-section thicker and more readily detectable by the AFM tip. This magnification can be achieved, for example, by uniform grafting of well-defined oligomers onto the backbone of the target macromolecules, a strategy that requires long polymer chains with anchoring sites homogeneously distributed along the backbone chain for "grafting-onto" or "grafting-from" processes. These reaction procedures are common in the synthesis of comb polymers, which, because of their highly branched structure, have been thoroughly characterized at the molecular level by AFM imaging.[20,26,29,31]

To obtain a representative image of the starting macromolecule, the grafting reaction should preserve the chain integrity and its architecture in order to give a homogeneous translation of its initial dimensions. These criteria mean that the grafting reaction should be highly selective and efficient, and currently hamper this approach and restrict the number of macromolecules with an appropriate number of homogenously distributed grafting sites of suitable reactivity. When these conditions are fulfilled, magnified macromolecules can be prepared and individually visualized and characterized as representative models of the initial polymer chains. This approach is illustrated in Figure 1 for a ring polymer synthesis. The architecture, shape, and dimensions of each macromolecule that constitutes a given polymer system can indeed be determined by using this approach. The visualization of the targeted macromolecules as well as the presence and characteristics of other macromolecular structures that form through secondary reaction processes is also permitted.

Very recently, we applied this approach to the characterization of macrocyclic polymers formed by end-to-end cyclization of a heterofunctional precursor.[31] The linear precursor used for the cyclization was an ABC triblock copolymer comprising a long central B block surrounded by two short A and C sequences that bear mutually reactive functions. The main B block consisted of chloroethyl vinyl ether (CEVE) units, each with a reactive site for anchoring side chains (Figure S1 in the Supporting Information).

After the cyclization step, the reactive chloride group of the CEVE units was used to graft polystyryl lithium chains of

[*] M. Schappacher, A. Deffieux
CNRS; Université Bordeaux
Laboratoire de Chimie des Polymères Organiques, ENSCPB
16, Avenue Pey Berland, 33607 Pessac Cedex (France)
Fax: (+33) 5-5640-8487
E-mail: deffieux@enscpb.fr

[**] The Centre National de la Recherche Scientifique is gratefully acknowledged for supporting this work. We also thank Roger C. Hiorns for assistance with the English translation.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.200900704.

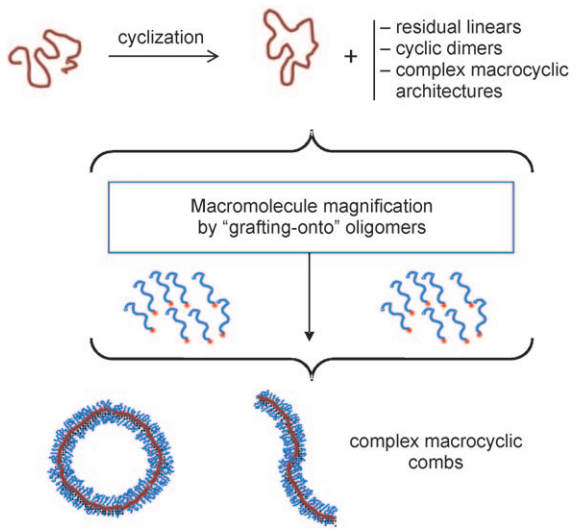

Figure 1. Strategy for the magnification of ring macromolecules and their visualization by AFM at the molecular level.

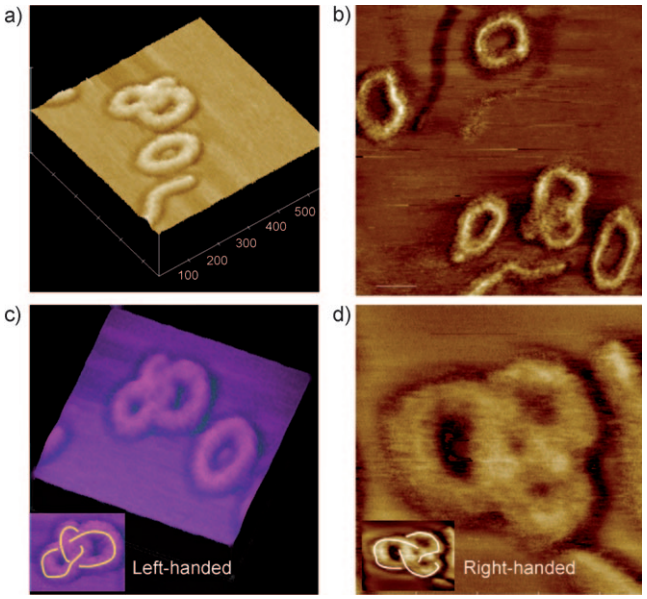

Figure 2. a, c) Topographic and b, d) phase AFM images of trefoil knot macromolecular rings formed as secondary structures in the end-to-end chain cyclization of an ABC linear precursor. (c) and (d) are topological diastereoisomers. The overall trefoil ring chain length corresponds to that of a ring dimer (2×ABC copolymers). Macromolecules were magnified by grafting polystyrene oligomers along the PCEVE block. Degree of polymerization (DP_n) of the PCEVE block in the precursor: 956; DP_n of the PS grafts: 170.

precise and uniform dimensions and enlarge the poly(CEVE) chains. AFM imaging of the magnified molecules allowed the visualization of individual rings and the remaining unreacted linear precursors. Thus, a representative set of macromolecules, which comprised from several hundreds to up to a thousand entities was taken and the macromolecules were analyzed individually.

Herein, we report how this characterization strategy has been advanced by focusing on the AFM investigation of secondary polymer structures that may form during the end-to-end polymer cyclization process, in addition to the reported linear and cyclic main fractions (Figure S2 in the Supporting Information). This technique allowed the direct visualization of the formation of linear and cyclic dimers, tadpole-shaped dimer molecules consisting of a ring and a linear chain, and to record fascinating images of trefoil knot rings, figure-of-eight bicycles, and catenanes.

Images of trefoil knot macromolecular rings, which consist of a closed ring in which the chain is entangled to form knots that cannot unfasten except by cutting the ring,[34–38] are shown in Figure 2. As emphasized in the schematic representations (Figure 2c, d, inset), the path of the knot crosses over itself three times. This pattern corresponds to the simplest knotted ring structure (trefoil). As previously described,[39,40] trefoil knots are topological stereoisomers of the corresponding simple rings. As shown in Figure 2, the isomers exist in the form of right and left topological diastereoisomers. The average backbone length of the knotted rings, measured from AFM images (Figure 2), is about 600 nm, which is twice that of the corresponding single rings (300 nm). The representative heights of knotted rings, determined from cross section images, and complementary information confirming these architectures are given in Figure S3 in the Supporting Information.

Images of figure-of-eight rings are shown in Figure 3a–c and a single-ring dimer is shown in Figure 3d for comparison. Figure-of-eight rings consist of two simple rings that are covalently linked at one position with an overall ring chain length corresponding to that of two attached single rings (two

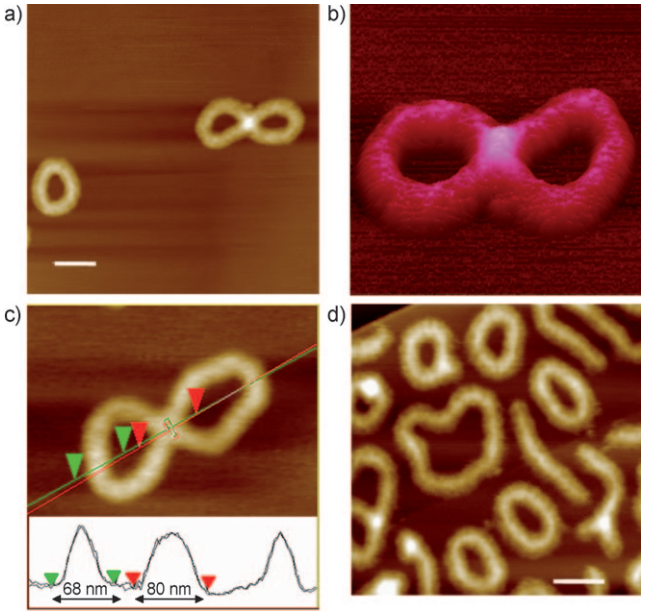

Figure 3. AFM images of figure-of-eight and dimer rings: a) topographic, b) 3D image magnified, c) topographic section, and d) single and dimer rings. Dimer rings are other secondary structures formed in the end-to-end chain cyclization. Scale bar 100 nm. The initial rings were magnified by grafting polystyrene oligomers along the PCEVE block. DP_n of the PCEVE block in the linear precursor: 956; DP_n of the PS grafts: 170.

ABC copolymers). The presence of a covalent link between the two rings is confirmed by the section at the connecting point (80 nm), which is higher than the ring section (68 nm) and smaller than the sum of two chains (136 nm; Figure 3c). It is also supported by the same height profile along the ring polymer chain including the connecting point, which should not be observed for a twisted dimer. These structures probably correspond to the cyclization of the linear moieties of previously formed tadpole polymers, which are observed in significant amounts (see Figure S2 in the Supporting Information).

[2]catenanes[36,39,41] that consist of two interlocked rings with two crossing points are shown Figure 4; the two white spots that appear in the image correspond to the crossing points of greater heights, as confirmed in the section image. In contrast to figure-of-eight rings, the two rings in [2]catenanes are not chemically linked to each other but cannot be separated without breaking one ring (Figure 4b,d). Their formation likely involves chain entanglement between a ring polymer and a linear chain precursor prior to ring closing of the latter.

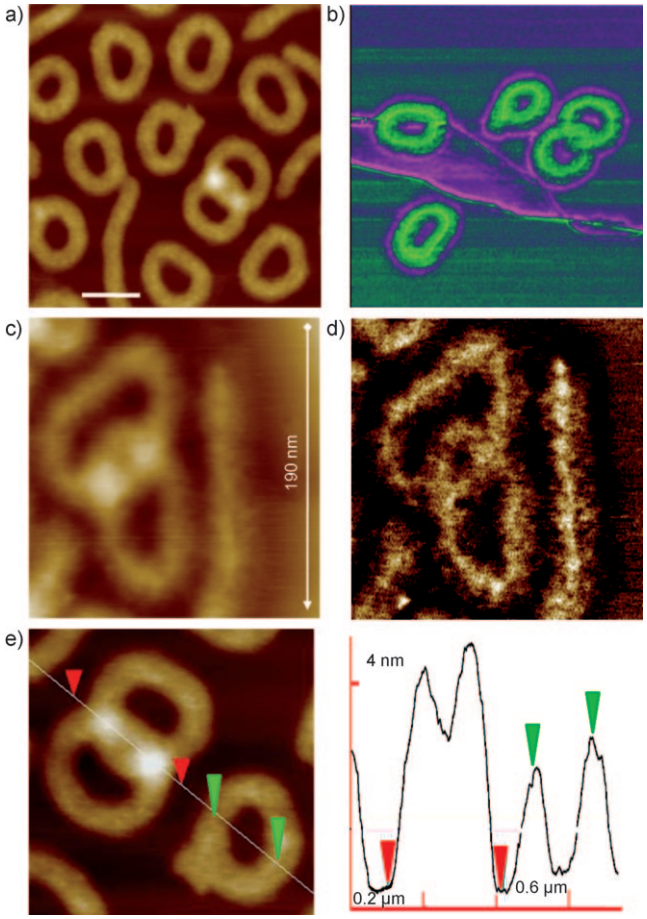

Figure 4. AFM images of [2]catenanes formed as secondary structures in the end-to-end chain cyclization of ABC linear precursor (scale bar 100 nm): a) topographic image; b) 3D image; c) topographic and d) phase image showing the ring crossing path more clearly; e) section image of a [2]catenane and of a single ring. The initial ring macromolecules were magnified by grafting polystyrene oligomers along the PCEVE block. DP_n of the PCEVE block in the linear precursor: 956; DP_n of the PS grafts: 170.

Catenated and trefoil knot rings are fascinating nontrivial topological figures that have occupied a privileged position in art and history, from the most ancient civilizations to modern art. They have been studied in many scientific fields, from mathematics to chemistry. Their aesthetic aspect is undoubtedly one major motivation for their synthesis. To date, two different synthetic methods for molecular catenanes and knots have been successfully developed in topological chemistry.[34,36] The first method is based on the template effect of a transition metal,[38,41,42] which gathers and disposes the molecular fragments in a predetermined geometry. The second method is purely organic and relies on the hydrogen bonding of precursor molecules.[43,44]

The directed synthesis of catenated macrocyclic polymers with a molar mass of about 10000 $g\,mol^{-1}$ has recently been attempted. The formation of interlocked rings of polystyrene and poly(vinyl pyridine)[45] and of polystyrene and polyisoprene rings[46] in low yields (around 5%) was reported on the basis of analysis by size-exclusion chromatography.

The formation of knotted macromolecular rings was also claimed in polymer systems that exhibit a linear-to-ring chain equilibrium or during the end-to-end ring closure of linear chain precursors.[47] However, the presence of catenated and knotted rings could not be clearly demonstrated. The present approach provides direct proof of the formation of these polymer structures during the end-to-end cyclization of long polymer chains.

In conclusion, the possibility of magnifying macromolecules by grafting oligomers uniformly along their chain appears to be a very powerful method to investigate the structure and topology of macromolecules by direct imaging. This strategy allows a precise macromolecule-by-macromolecule characterization of the architecture and dimensions of the main reaction products. It also permits the visualization and quantification of secondary molecular structures generated in the reaction. This strategy is clearly illustrated in the present work in which nontrivial macromolecular rings such as figure-of-eight rings, [2]catenanes, and trefoil knot rings were visualized.

Experimental Section

Synthesis: The synthesis and characterization of linear ABC copolymers used as precursors in the cyclization step has been described previously.[31]

Cyclization procedure: Pyridinium *m*-chloro-*p*-toluenesulfonate (PTSA; 17 mg, 0.02 mmol), which is insoluble in aromatic solvents, was dissolved in dry THF (2 mL) at 60 °C. This solution was quickly added to a solution of the dried ABC triblock polymer (80 mg) in dry toluene (300 mL) at 20 °C under vigorous stirring, in order to obtain finely dispersed catalyst particles. The polymer cyclization was monitored by size-exclusion chromatography (SEC) analysis of reaction samples. After reaching a plateau corresponding to the maximum conversion of the linear precursor, the polymer was directly submitted to the grafting process without further purification,[31] by slow addition of living polystyryl lithium oligomers of precise molar mass and low dispersity. At the end of the reaction, the residual unreacted polystyrene chains were eliminated by selective precipitation of the comb copolymers in a cyclohexane/heptane

mixture. The crude "magnified" cyclization products were then analyzed by SEC and AFM without additional purification.

Atomic force microscopy: Topographic images of individual molecules were collected using an AFM (Veeco Metrology Group, dimension 3100) in tapping mode. The probes were commercially available silicon tips with a spring constant of 40 $N m^{-1}$, a resonance frequency lying in the 270–320 kHz range, and a radius of curvature of less than 10 nm. Samples were prepared by solvent casting at ambient temperature by spin coating from solutions in dichloromethane. One drop of a dilute solution (0.01 wt%) was spin cast on freshly cleaved highly oriented pyrolytic graphite (HOPG).

Received: February 5, 2009
Revised: May 28, 2009
Published online: July 2, 2009

Keywords: catenanes · copolymerization · cyclization · macrocycles · molecular knots

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