

Probing the mobility of catenane rings in single molecules†

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Anne Van Quaethem,^a Perrine Lussis,^b David A. Leigh,^{*c} Anne-Sophie Duwez^{*b} and Charles-André Fustin^{*a}

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Here we demonstrate for the first time the interrogation of a mechanical link made of catenane rings at the single molecule level. We used AFM-based single molecule force spectroscopy to address the mobility of the rings in a catenane unit of the benzylic amide family. To interface the catenane with the AFM probe, we connected a polymer chain to each of the macrocycles of the [2]catenane. Force–extension profiles in different solvents were then recorded. We show that the catenane ring motions can be detected at the single molecule level and that the ring rotation can be locked or unlocked depending on the solvent.

Introduction

For more than a decade, single-molecule force spectroscopy (SMFS) has been used to probe individual (bio)molecules, providing unprecedented insights into many molecular-level processes.¹ For example, proteins, DNA, polysaccharides, supramolecular polymers, polyelectrolytes, ... have been investigated, revealing details about the strength of inter- and intramolecular interactions, folding and unfolding pathways, mechanics, conformational changes, reactivity, kinetics... However, few SMFS investigations have been successively realized on interlocked molecules² such as catenanes and rotaxanes,^{3,4} whose large amplitude dynamics and conformational changes make them prototypical structural elements of synthetic molecular machines.^{5–8} The rarity of such studies comes from the difficulty of developing proper tools and preparing appropriate molecules that can be interfaced with single molecule force spectroscopy techniques, especially when one wants to probe sub-molecular motions. On the contrary, studies on molecular machine prototypes adsorbed on surfaces with other types of local probes, such as scanning tunneling microscopy (STM), are more direct and common.⁹ However, for some systems the adsorption on a surface can hinder sub-molecular motions. Here we demonstrate for the first time the interrogation of catenane ring mobility at the single molecule level. We show that atomic force microscopy (AFM)-based SMFS

can be used to probe the ring rotation in a single catenane molecule, and to detect the locking and unlocking of this sub-molecular motion under different conditions.

Results and discussion

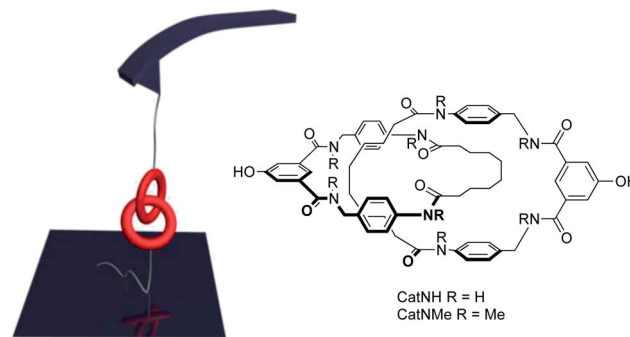
In order to obtain a system that allows the probing of the mechanical and dynamic properties of a single catenane, we connected a polymer chain to each of the macrocycles of a [2] catenane, as depicted in Scheme 1. These chains allow the catenane to be interfaced with the AFM probe. For this study we selected two catenanes of the benzylic amide family (Scheme 1).^{10,11} The compounds differ only by methylation of the amide groups and the corresponding ability to form inter-component hydrogen bonds. Catenane CatNH has secondary amide groups and can thus form hydrogen bonds between the two macrocycles, restricting their mobility. In catenane CatNMe, the amide groups are methylated and the rings are relatively free to rotate.¹¹ The polymer used as a linker to

^aInstitute of Condensed Matter and Nanosciences (IMCN), Bio- and Soft matter division (BSMA), Université catholique de Louvain, Place L. Pasteur 1, Louvain-la-Neuve, Belgium. E-mail: charles-andre.fustin@uclouvain.be

^bChemistry Department, University of Liège, B6a Sart Tilman, 4000 Liège, Belgium. E-mail: asduwez@ulg.ac.be

^cSchool of Chemistry, University of Manchester, Oxford Road, Manchester M13 9PL, United Kingdom. E-mail: david.leigh@manchester.ac.uk

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Scheme 1 Schematic representation of the experiments performed in this work (left) and chemical structure of the catenanes employed (right).

interface the catenane with the AFM tip is poly(ethylene oxide), PEO. This polymer is a standard choice in AFM-SMFS experiments and has the advantage of being highly flexible, which will facilitate the detection of variations in the ring dynamics between the different catenanes.

Catenanes CatNH and CatNMe were synthesized as previously described,¹² and then derivatized with propargyl groups through a Williamson reaction. Commercially available mono-hydroxy-PEO (PEO₄₅-OH, $M_n = 2000 \text{ g mol}^{-1}$) was functionalized with an azide moiety to form PEO-N₃ in two steps following standard procedures (see ESI†). The PEO chains were grafted onto the bis(propargyl)-catenanes through a copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) “click” reaction performed in *N,N*-dimethylformamide (DMF) at 45 °C with CuBr/Me₆TREN (Tris[2-(dimethylamino)ethyl]amine) complex as the catalyst (Scheme 2). The catenane-containing polymers were then purified on a Bio Beads SX1 column to remove the excess PEO (see ESI† for all the synthetic details).

Samples for the AFM-SMFS measurements were prepared by casting a drop of a highly dilute solution of the polymer in methanol onto a cleaned silicon wafer and evaporation of the solvent. The experiment consisted of performing approach-retraction cycles with an AFM tip on the molecules adsorbed on the substrate in both 1,1,2,2-tetrachloroethane (TCE) and DMF. The tip was brought into contact with the surface in order to allow the PEO chain to adsorb onto the tip. The caught molecules were then stretched in a controlled manner by moving the tip away from the substrate at a fixed pulling rate and the force-extension profiles were measured. We recently showed that this approach, based on the physisorption of caught PEO molecules onto the tip, provides a sufficiently strong and stable attachment of the molecule onto the tip to obtain reliable information on the system.⁴ A typical example of the force profile obtained in TCE is shown in Fig. 1.

To exclude that only the PEO part was picked up, we only considered peaks appearing at an extension above 20 nm, thus at least 1.5 times the length of one PEO arm. Since the catenane is located in the middle of the molecule, we can be confident that the catenane is probed in each curve. The force-extension profiles were fitted with a worm-like chain (WLC) model, an

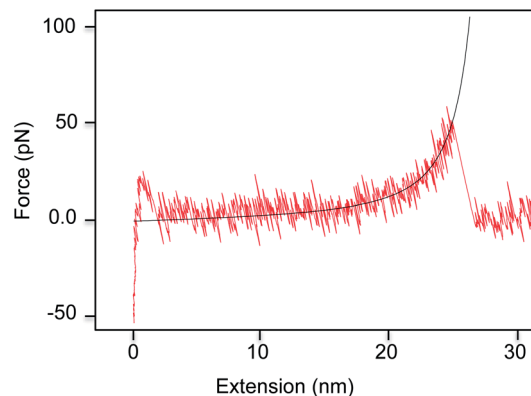
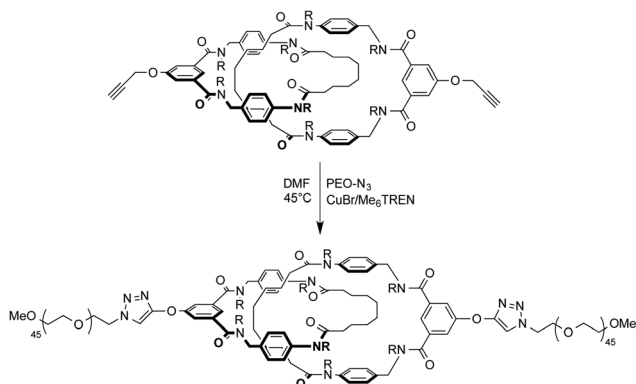


Fig. 1 Force–extension profile recorded on PEO-CatNMe in TCE. The black line is a WLC fit to the data.

entropic elasticity model that predicts the relationship between the extension of a linear polymer and the entropic restoring force.¹³ Since the aim of this study is to evidence the ring mobility, only the purely entropic regime is relevant. The ring mobility is indeed related to a change in entropy: if the catenane rings can freely rotate, they increase the conformational entropy of the chain and thus decrease the size of the random coil. If the rings are locked and cannot rotate, they act as a single long segment that decreases the entropy and increases the size of the random coil. We thus expect changes in the entropic restoring force generated by the alignment of polymer segments in the pulling direction. Since only the entropic restoring force is relevant here, we thus selected curves displaying a maximum force of 50 pN to avoid contribution (if any) from backbone deformation to the force signal (above 50–100 pN, it is known that bond angle deformations can no longer be neglected¹³). The fits of the curves adequately described the force–extension behavior (Fig. 1).

Although the Freely Jointed Chain model (another entropic elasticity model widely used in the literature) also describes relatively well the behavior of our system, we preferred to use the WLC model which takes into account correlations of orientations at small scales and which is thus more correct to describe the extension of a semi-flexible polymer under an external force at small extension.¹³ The persistence length of the two catenane-containing polymers was extracted from the fits. The results are presented in Table 1 and Fig. 2 and 3. We used two methods to fit the experimental curves: an automatic and a manual one (see ESI† for details). The agreement of the results coming from the two methods is a good test to be sure that a user bias has not been accidentally introduced, and that some



Scheme 2 Synthesis of the catenane-containing polymers PEO-CatNH (R = H) and PEO-CatNMe (R = Me).

Table 1 Average persistence length values (L_p) of the two catenane-containing polymers extracted from a WLC fit on measurements performed in TCE and DMF

Sample	L_p (nm) TCE	L_p (nm) DMF
PEO-CatNH	1.0 (± 0.15)	0.50 (± 0.10)
PEO-CatNMe	0.45 (± 0.05)	0.45 (± 0.05)

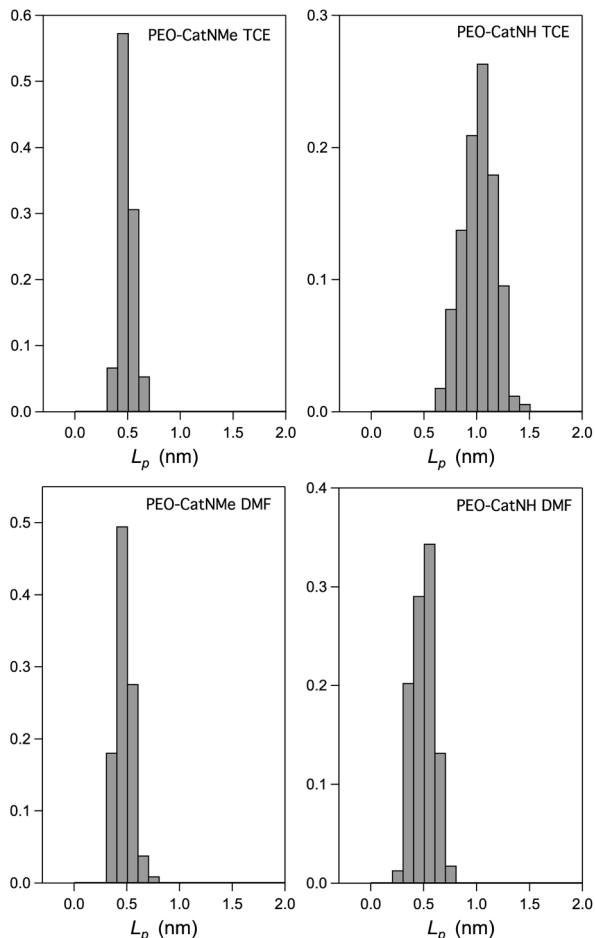


Fig. 2 Histograms of the persistence length measured on the two catenane-containing polymers, PEO-CatNMe in TCE ($n = 226$) and in DMF ($n = 172$), and PEO-CatNH in TCE ($n = 196$) and in DMF ($n = 188$).

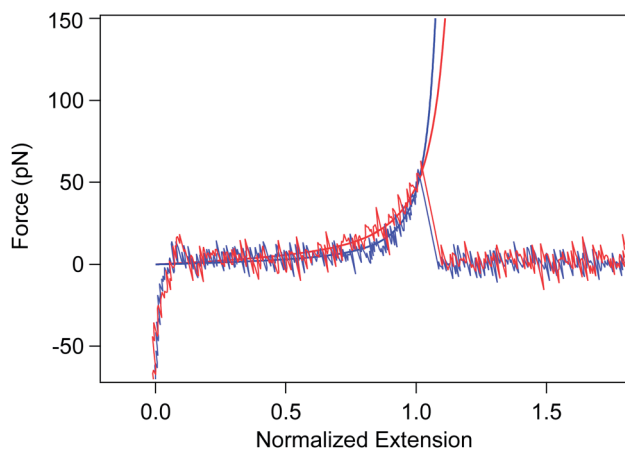
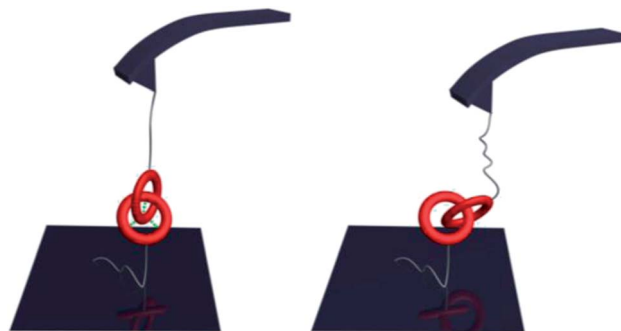


Fig. 3 Superimposition of normalized curves (at 50 pN). Red curve: PEO-catNMe with $l_p = 0.45$ nm. Blue curve: PEO-CatNH with $l_p = 1$ nm.

noise has not misled the analysis. The superimposition of normalized curves with different persistence length values qualitatively confirms that the results are not biased (Fig. 3). In

TCE, an apolar solvent, the persistence length for the polymer containing catenane CatNH is much larger than the persistence length of the polymer containing catenane CatNMe. It appears that this is the unambiguous signature of the inter-ring hydrogen bonds present in catenane CatNH, which lock the macrocycles of the catenane and thus strongly inhibit their rotation (Scheme 3, left). The persistence length of a polymer chain reflects the equilibrium conformational entropy in a given solvent. If the two catenane rings are locked and cannot freely rotate, they act as a single long segment that increases the apparent persistence length of the chain and decreases its flexibility. In DMF, the persistence length of the polymer containing catenane CatNH is strongly decreased and almost reaches the value of PEO-CatNMe (Table 1). In this case the inter-ring hydrogen bonds are much weaker because of the polar solvent. This unlocking of the mechanical bond in turn induces a decrease of the persistence length of the molecule (Scheme 3, right). The strong influence of the solvent on the inter-ring hydrogen bonds, and thus on the ring mobility, was indeed previously demonstrated by ^1H NMR experiments in solution on a catenane similar to CatNH.¹¹ This study showed that the ring rotation rate at room temperature goes from 8100 s^{-1} in CDCl_3 to more than $100\,000\text{ s}^{-1}$ in $[\text{D}_7]\text{DMF}$.

If the increase of persistence length when the rings are locked can be explained by the fact that they act as a single long segment that decreases the flexibility of the chain, the amplitude of the increase (the persistence length is doubled) is still an open question. Somewhat remarkably, a single catenane located in the middle of the chain is sufficient to affect the flexibility of the latter. Indeed, the persistence length of pristine PEO in DMF and TCE is 0.37 nm, close to the monomer size.⁴ The slightly higher persistence length of the catenane containing PEO (0.45 nm) is due to the size of the catenane unit, which is much bigger than the monomers of PEO. The strong influence of the catenane on the persistence length is consistent with previous studies on bulk solutions of polycarbonate containing only a few catenane units. Although both studies cannot be directly compared due to the very different nature of the polymers, we can mention that the previous studies have shown a drastic influence of the catenane on the radius of gyration (and thus the



Scheme 3 Schematic representation of the locked (left) and unlocked (right) states of the catenane within PEO-CatNH and the effect on the response of the molecule upon pulling with the AFM tip.

persistence length, which is linked to the size of the random coil) of the polymer.¹⁴

Conclusions

Although it has been previously demonstrated by ¹H NMR in solution that catenanes have the ability to change their conformation according to the polarity of the solvent,¹¹ we have shown here for the first time that the ring motion which occurs upon this conformation change can be detected at the single molecule level. Our results show that AFM-based single molecule force spectroscopy is able to address the mobility of the rings of a single catenane unit, and thus characterize the locked or unlocked state depending on the solvent. The changes in degrees of freedom and molecular flexibility generate a change of response upon pulling on the single molecules. This is the first example of sub-molecular dynamics of interlocked architectures affecting the entropic restoring force measured by AFM.

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