Probing the free rotary oscillations around a single ruthenium atom in an organometallic complex

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Summary

A variety of rotary molecular machines prototypes powered by light, chemical energy or electrons have been synthesized and their operation in solution, gels or on surfaces has been demonstrated. However, little data regarding their performances have been disclosed. Here, we report on the synthesis of molecules incorporating a five-arm rotor and the direct measurement of the work required to block the rotation around the central atom. We used single-molecule force spectroscopy (SMFS) to detect the free rotary oscillations and measure the work performed by the molecules against the mechanical load. We show that the chemical nature of the arms influences the energy barrier, causing differences in the work that the molecules can generate. Our results illustrate that SMFS, which is now widely used to probe linear displacements at a few tens of nanometer scale in macromolecules, can detect rotary motions around a single atom in a tiny synthetic molecule.

Introduction

Inspired by the molecular machinery of biological systems, chemists have developed a number of synthetic molecules that can perform tasks from translational or rotational motion when triggered by various external chemical or physical stimuli.[1–7] Based on diverse mechanically active synthetic units, artificial molecular machines exhibit a wide variety of functions such as muscles,[8–9] pumps,[10-14] transporters,[15] synthesizers,[16-21] molecular scissors,[22] elevators,[23] walkers, [24–25] nanovehicles,[26-28] and gears,[29-34] to name a few. In particular, rotary molecular motors have attracted much attention for their capacity to convert chemical, light or electric energy into a unidirectional rotation movement, leading to the production of work[1-7, 35-36] recoverable up to the macroscopic scale.[37-41] Controlled directional and repetitive rotation has been triggered on entire single molecules located in an anisotropic environment[42-45] or on submolecular fragments incorporated in diverse chemical architectures.[46-54]

In this context, an azimuthal molecular motor based on a ruthenium(II) piano-stool complex was proposed in 2013 and investigated on a Au(111) surface in ultra-high vacuum at the single-molecule scale.[42] The upper pentaarylcyclopentadienyl ligand acts as a five-arm rotor undergoing favoured rotation over the ruthenium ion, which behaves as a single-atom pivot. The second ligand, a hydrotris(indazolyl)borate scorpionate, acts as a stator with a dual role: it lifts up the ruthenium ball-bearing from the surface thanks to its tripodal shape and, most importantly, it precludes molecular diffusion thanks to specific functionalization with three thioether groups allowing a tight anchoring on gold.[55] At low temperature (down to 5 K), when random thermal motion is suppressed, controlled rotation of the pentaarylcyclopentadienyl subunit is triggered by supplying electrical energy with a submolecular resolution using the tip of a Scanning Tunneling Microscope (STM). The resulting motion is unidirectional and reversibility has been evidenced, with a direction of rotation depending on the nature of the rotor fragment located under the STM tip during the inelastic electron tunneling process.[42] However, the drastic conditions of STM, namely ultra-high vacuum and low temperature conditions, limit potential applications. To accelerate the development of complex and automated machineries that can be operated on surfaces or in macroscopic materials,[6,38,40] it is desirable to investigate the behaviour of such machines in solution and ambient conditions. Furthermore, an important question like *can the motor still rotate when loaded*? remains unanswered.

Here, we used atomic force microscopy (AFM)-based single-molecule force spectroscopy (SMFS) to detect the free rotary oscillations of the rotor subunit around the single ruthenium atom under mechanical load in molecular motor prototypes and directly probe at the single-molecule scale the work required to block the oscillations and work performed by the molecules against the mechanical load. SMFS is now widely used to probe processes at the scale of a few tens of nanometers in biomacromolecules and has proved efficacious in deciphering mechanistic information of individual biomolecular machines and in quantifying their force response to external stress.[56-57] However, only a few investigations on intramolecular processes and single-molecule mechanics have been realized on small synthetic molecules, successful examples including molecular recognition pairing,[58] helical structures,[59] knots,[60] and artificial molecular machine prototypes.[61-66] The rarity of such studies stems from the difficulty in developing proper tools and preparing appropriate molecules that can be interfaced with SMFS techniques due to the very small amplitude of the involved motions compared the ones observed in previously studied larger biological systems or polymers, especially when probing conformational changes of synthetic small molecules interconverting between rotamers under Brownian fluctuations.

To this aim, molecular motor prototypes based on ruthenium heteroleptic complexes were designed, so as to incorporate in the rotor subunit a long poly(ethylene oxide) (PEO) chain able to physisorb onto the AFM tip for pulling and monitoring rotor's movements. A series of molecules displaying structurally-different arms were synthesized and bridged between a gold substrate and a gold-coated AFM tip (Figure 1). The mechanical response of this series of molecules bearing various rotor subunits was investigated in solution (*N*,*N*-dimethylformamide) and at room temperature.

Results and Discussion

Molecular design and synthesis

In anticipation of the SMFS studies, a new design of the ruthenium-based molecular motor[42] was devised by incorporating into the rotor subunit a long poly(ethylene oxide) chain (n=23, ~8 nm), able to physisorb

onto the AFM tip (Figure 1). This polymer was connected to the central cyclopentadienyl ring via an amide moiety, selected for increased synthetic modularity, and a hydrocarbon spacer group such as a 4,4'-biphenyl fragment (**M1**). In order to study the influence of the spacer length and structure on the mechanical behaviour of the molecule, one shorter 4-ethynylphenyl spacer (**M2**, **M3**) and one longer 4-(biphenylethynyl)phenyl spacer (**M4**) were also designed. Finally, the four remaining substituents of the cyclopentadiene core were either kept identical to the previously-reported motor[42] using 4-ferrocenylphenyl groups (**M3**) or replaced with less hindered 4-bromophenyl moieties (**M1**, **M2**, **M4**).
This family of four molecules incorporating a poly(ethylene oxide) chain (**M1**-**M4**) were prepared according to a common modular synthetic approach, relying on the post-functionalization of a 1,2,3,4,5-penta(*p*-halogenophenyl)cyclopentadienyl hydrotris(indazolyl) borate ruthenium(II) key precursor (compound **1**, scheme S1).[33] A single cross-coupling reaction under statistical[67] or chemoselective[68] conditions led respectively to the formation of the desired biphenyl or phenylethynyl pattern in the spacer group. Subsequent deprotection of the terminal benzylic or propargylic amine was followed by a condensation reaction with a monodisperse methoxy-PEG24-propionic acid partner, activated as an *N*-hydroxysuccinimidyl ester, to afford the target molecules **M1**, **M2** and **M4**. In the case of molecule **M3**, an extra four-fold Suzuki-Miyaura reaction was required as an intermediate step to install the four ferrocenyl moieties (see SI, section I.1.for detailed synthetic routes).[68]

Grafting onto surfaces

To provide stable attachment of the molecules on the substrates during SMFS measurements, each leg of the tripodal stator is anchored to the gold surface through a thioether group located on the 6-position of the indazole moiety (Figure 1). The molecules were grafted in solution onto gold-coated silicon substrates following the protocol reported previously to attain a low grafting density and favour single-molecule attachments with the tip[59–61] (see SI, section I.3. for details).

Rotation under mechanical load

During the force spectroscopy measurements, the AFM tip approaches the functionalized substrate and interactions with the PEO tether are established (Figure 1). Then, increasing the distance between the tip and the surface, the force response of the molecule under extension is monitored by the deflection of the cantilever. Force-distance profiles were obtained for **M1** in *N*,*N*-dimethylformamide (DMF) at a pulling rate of 20 $nm.s^{-1}$ (160 $pN.s^{-1}$) (Figure 2a) and a pattern characteristic of force fluctuations between two states (state A before the fluctuations and state B after the fluctuations) could be observed. It indicates a change of extension in the system (decrease or gain in contour length), which we can attribute to a rotational movement of the rotor during the pulling (see schematics in Figure 2c). State A corresponds to the molecule exerting force on the AFM tip while freely oscillating and state B to the system aligned in the direction of pulling. From previous studies,[42] we know that this family of rotors constantly oscillate non-directionally under ambient conditions and can be frozen only at a very low temperature, below 77 K. From these observations, we can suggest that at low external force (before the fluctuations), the rotor performs a continuous random rotation with a very fast dynamics that cannot be resolved in the force curves. It results in an average position of the tether end which is on the rotor centre (see details in terms of the Kramers' escape rate in the preview by L. Bellon). When increasing the external force, at some point the rotor get stuck for a measurable time in the position aligned with the direction of pulling, resulting in a fluctuating force. For even higher external force, the rotor stays aligned with this direction, and the tether end is at a distance *L* closer to the tip (see Figure 2c) with

respect to the centre of the rotor. It is important to note that the force is applied in one direction by the AFM tip, which prevents from discriminating clockwise and anti-clockwise rotary motions; instead, a fluctuating motion between two limit positions is observed. These fluctuations show that the rotor is still able to oscillate under an external load. The amplitude of these fluctuations (ΔF) originating from the random Brownian motions, represents the force that molecule is able to exert against the pulling by the AFM tip. The distribution (Figure 2b) shows that the molecule is able to exert a force of 13 pN on average, against a pulling force of ~55 pN. It is worth mentioning that the fluctuations are rare events, only observed in cases when the tip anchoring point is close to the molecule, *i.e.* short sequences of the PEO tether are interfaced (small rupture distances), leading to an applied tension sufficiently high to probe the oscillating motions.

As a control experiment, we investigated the force response of a system (**M5**) where two side by side molecules are linked chemically (Figure 3) to block the rotary oscillations. In this dinuclear ruthenium complex, the upper rotating subunit of the ruthenium complex **M1** is covalently linked via an alkyne moiety to the pentaarylcyclopentadienyl ligand of a second ruthenium complex. Upon adsorption of **M5** on gold via both thioether-appended tripods, motion of the rotor subunits is thus suppressed. This dinuclear complex was prepared according to a convergent synthetic route involving a key chemoselective Sonogashira coupling between two distinct piano-stool ruthenium(II) complexes, the desymmetrized penta(*p*-halogenophenyl)cyclopentadienyl ruthenium(II) precursor **1b** and complex **14**, in which the cyclopentadienyl ligand bears a single ethynylphenyl substituent and four phenyl groups (Schemes S2 and S3). Introduction of the 4,4'-biphenyl spacer and of the PEO chain on the resulting dinuclear species **15** were next achieved according to the synthetic route detailed above for the preparation of molecules **M1**-**M4** (see SI, section I.1. for details on the synthetic route). When **M5** was grafted on gold and studied by SMFS, only single-peak profiles corresponding to the typical stretching of the PEO tether were observed (Figure 3b). The absence of fluctuations in the force-distance profiles is indicative of the absence of free rotary oscillations in the chemically linked rotors.

When the pulling rate is increased at 200 $nm.s^{-1}$, the fluctuations observed for **M1** disappear and a characteristic and reproducible profile (Figure 4a) with a discontinuity pattern that resembles a small tilted plateau was observed. The appearance of a small pseudo-plateau during the mechanical pulling of the molecule indicates a change of extension (gain in contour length), which can be attributed to a mechanical alignment of the rotor during the pulling, restraining its rotary oscillations. Given the temporal resolution at this higher loading rate, the detection of the previously discussed oscillating fluctuations is not possible. The start of the pulling curve is described by a worm-like chain (WLC)[69] behaviour (with a contour length L_{c1}) and corresponds to the alignment and stretching of the polymer linker in the direction of the pulling. At some point during the pulling (60 pN in Figure 4a), we can observe a transition from this first WLC to a second one (with a contour length L_{c2}), the latter being associated with a higher contour length. This transition from one WLC curve to the other one (extension increase, seen as a pseudo-plateau) can be attributed to the progressive mechanical alignment of the spacer (*i.e.* one arm of the ruthenium complex) with the AFM tip pulling direction (see Figure 2c). When the pulling rate is further increased at 1000 $nm.s^{-1}$, pseudo-plateaus were still obtained (Figure S4), showing that the rotor subunit is still capable of oscillating under a higher loading rate of 8000 $pN.s^{-1}$.
It is worth reminding that the rotor's initial position at the beginning of the pseudo-plateau is completely

random, due to the constant non-directional rotary oscillations around the ruthenium atom. The length of the pseudo-plateau is thus related to the translation from the average position at the centre of the rotor to the aligned position at a distance *L*, with *L* (Figure 1) being the distance between the centre of the cyclopentadienyl core and the amide group attached to the PEO chain (see below and SI section I.4. Data S1). Once the rotor has been mechanically aligned with the pulling direction, the stretching of the PEO resumes following the second WLC profile.

Histograms of the plateau length and force were built from force curves collected from different batches of experiments performed on different days (Figure 4b and c). The most probable value for the plateau force (53 ± 19 pN) corresponds to the force exerted by the AFM tip to counteract the numerous free oscillations of the rotor and align it in the pulling direction, later referred to as the blocking force. The most probable plateau length (1.8 ± 0.7 nm, Table 1) is consistent with the size of the rotor. The theoretical maximum diameter of this rotor being 2.27 nm (Table 1 and SI section I.4. Data S1 for the details of the DFT calculations), the length release can be expected to extend between 0 and ~2 nm.

Pulling-relaxing experiments during which the molecule was stretched and relaxed for several cycles before detaching from the tip were also carried out. Figure 5 shows a representative pulling-relaxing force-extension curve in one cycle for **M1**. We observe that the pseudo-plateau connecting the two WLC traces (Figure 4a) also appears in the relaxing curves, while decreasing the tip–surface distance. This plateau is the signature of a transition from the second WLC (with a higher contour length L_{c2}) to the first WLC (with a lower contour length L_{c1}). This decrease in extension is the signature of a tension applied by the rotor below the blocking force to escape the forced alignment and recover its freely oscillating state. This observation shows that, as the force decreases, the rotor is capable of reinstating its rotary oscillations.

Influence of the nature of the rotor arms

We reproduced the same experiments on rotors with arms of different lengths and chemical structure (**M2**-**M4,** Figure 1 and S1-3). We found that the variation of the pseudo-plateau length is in strong correlation with the lengths of the functionalised arms (Table 1, Figures S1-3 and SI section I.4. Data S1). For **M2**, bearing a 4-ethynylphenyl linker (2 × *L*= 1.92 nm), we observed shorter lengths compared to **M1** (2 × *L* = 2.27 nm) and for **M4**, having a 4-(biphenylethynyl)phenyl moiety (2 × *L* = 3.64 nm), longer lengths. For **M3**, which is similar to **M2** but incorporates bulkier substituents as a control, no length variation occurred, as expected.

The pseudo-plateau force obtained for **M2**, bearing a phenylethynyl spacer instead of a 4,4'-biphenyl fragment, is centred around 62 ± 16 pN (Table 1). This means that force required for the mechanical alignment of the rotor **M2** along the pulling direction, opposing the free rotary oscillations, is higher than the force required for **M1** (53 ± 19 pN), which is certainly arising from a strong interplay between steric but also electronic factors. For **M3**, in which the spacer is identical to **M2** but the bromine atom on each arm is replaced by a ferrocenyl group, we observe a higher pseudo-plateau force (74 ± 15pN). The increase in force between **M2** and **M3**, bearing identical linkers, can be largely explained by a higher steric hindrance between the rotor and stator, due to the presence of the ferrocenyl moieties in **M3** (Table 1). Indeed, a higher steric hindrance induces a higher energy barrier for the mechanical rotation. Finally, for **M4** incorporating four *p*-bromophenyl groups on the rotor, the measured pseudo-plateau

force is around 66 ± 23 pN, similar to **M2**. Although the spacers are different in **M2** and **M4**, they both display a phenylethynyl fragment directly connected to the central cyclopentadienyl core. The peripheral biphenyl part of **M4** is too far away from the rotation axis to cause any steric interactions with the stator or have a major electronic effect. This elongated spacer does not change the intrinsic barrier of rotation for **M4**, leading to the same pseudo-plateau forces for **M2** and **M4**. **M1**-**M4** thus follow the same rotating mode and present the same type of pseudo-plateau pattern in pulling curves, while the force depends on the steric hindrance between rotor and stator as a major parameter.

As mentioned above, the transitions from a freely oscillating state to an aligned state can be directly observed when the pulling rate is low enough. We observed pulling curves with fluctuations for each molecule for a pulling rate of 20 nm.s^{-1}, similar to what has been observed for **M1**. Representative curves are shown in Figure 6.

Work performed by the tip to block the oscillations and by the rotors against the load

In order to evaluate the cumulative mechanical work performed by the AFM tip to counteract all the transitions of the rotor between state A and B and align the attached spacer in the direction of pulling, we measured the integrated area below the region of fluctuations (hopping states from A to B, Figure 2a) and between the two WLC fits (see the schematic showing the procedure in SI, Figure S5). The mechanical work values for each molecule are summarized in Table 2. The work values of **M1** and **M2** are about 13-15 k_BT (~ 8 kcal.mol^{-1}) and are higher (25-30 k_BT, ~ 15 kcal.mol^{-1}) for **M3** and **M4**. This work is done by the tip to counteract several transitions during the pulling and overpass several local energy barriers along the rotor diameter. The molecule, which is made of 3 legs and 5 arms, has 15 energy minima (15 rotamers) along one complete turn.[42] It Is important to note that, given the low energetic barrier between each rotamers and thus the extremely fast angular frequency of the rotor (see the preview by L. Bellon), the number of fluctuations during the force experiments is greatly underestimated. The gap between the kinetics of rotation and the sampling rate of the experiments impedes us from differentiating the rotamers.

We have also measured the work per fluctuation (area corresponding to $\Delta F \times \Delta x$) (Figure 2a) to evaluate the work performed by the rotors to oscillate against the mechanical load exerted by the AFM tip. This work has been estimated at about 2.3 and 2.2 k_BT for **M1** and **M2,** and 3.2 and 3.8 k_BT for **M3** and **M4** (Table 2). These values correspond to the energy extracted by the rotor from the environment to oscillate between two limit positions (from point B to A, Figure 2a) around the ruthenium atom.

Conclusion

We have designed, synthesized and probed a series of single-molecule motor prototypes based on a piano-stool ruthenium complex that differ by the chemical nature and length of their arms. The results of the SMFS investigations show that we can detect free rotary oscillations of the rotor subunit into states that generate tension in the system and quantify the force response to external stress in solution. They also show that the rotors can still oscillate when they are loaded. The force required to block the rotary oscillations varies between ~50 and 70 pN, as a result of an interplay between electronic and steric effects. It is worth mentioning that this force is kinetically determined, *i.e.* we measure the resisting force of the probed molecule towards a perturbation of its rotation. After increasing the mechanical load well beyond the blocking force, we observed that the rotor can reinstate its rotary oscillations as soon as the load is decreased down to the blocking force. The cumulative work required to block the

rotary oscillations is between ~13 and 30 k_BT, depending on the chemical structure and length of the rotors' arms. The work performed by the rotor during one single rotary oscillation is of the order of 2 to 4 k_BT.

More generally, the study shows the very first example of detecting and quantifying motions around a single atom by single-molecule force spectroscopy, and illustrates the utility that this technique can offer for evidencing details of the molecular design that are crucial for improved efficiency and applicability of synthetic molecular machines.

METHODS

See Supplemental Information for detailed methods and protocols: Details of the synthesis of all molecules, details of force spectroscopy experiments, DFT calculations, supplementary figures.

Synthesis. The four molecules **M1**-**M4** were prepared according to a common modular synthetic approach relying on the post-functionalisation of a 1,2,3,4,5-penta(*p*-halogenophenyl) cyclopentadienyl hydrotris(indazolyl)borate ruthenium(II) key precursor (**1a** or **1b**, Scheme S1 in SI). For **M1**, **M2** and **M4** incorporating four 4-bromophenyl substituents on the cyclopentadienyl core, the strategy involved a single cross-coupling reaction in a first step to introduce the appropriate hydrocarbon spacer, followed by the cleavage of the Boc-protecting group. Condensation of the resulting primary amine with the monodisperse $mPEG_{24}$-NHS ester reagent (**4**), terminated by a carboxylic acid preactivated as its *N*-hydroxysuccinimidyl ester, finally yielded the target compounds. In the case of **M3**, an extra four-fold Suzuki-Miyaura reaction was required as an intermediate step to introduce the four ferrocenyl moieties. Dinuclear complex **M5** was prepared according to a convergent synthetic route involving the desymmetrised key precursor **1b** and ruthenium complex **14**, in which the cyclopentadienyl ligand bears a single ethynylphenyl substituent surrounded by four phenyl groups (Scheme S2 in SI). NMR spectra provided in a supplemental file.

Grafting of the molecules on substrates. The molecules were attached to gold-coated silicon substrates using Au-S interactions, following our previously established protocol[59,61,64,66] to reach low grafting density and favour single-molecule attachment during AFM experiments (see details in SI).

AFM force experiments. AFM experiments were performed using a MFP3D Origin+ (Asylum Research, Oxford Instruments). Experiments were performed in a semi-closed fluid cell. OBL cantilevers (Bruker) with a nominal spring constant of k = 0.03 $N \cdot m^{-1}$ were used in all force spectroscopy experiments. The spring constant of each cantilever was determined prior to each experiment, in air, using the thermal and the Sader methods. The grafted surface was installed in the fluid cell filled with the organic solvent and the AFM tip was immersed in the solution away from the surface for 30 min for equilibration of the cantilever. Force curves were obtained at a fixed velocity of 20, 200, or 1000 $nm \cdot s^{-1}$ (approach and retraction) and with a sample rate of 17 kHz. The detailed procedure for the pulling-relaxing curves is described in SI. The procedures for the data treatment are described in SI.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Anne-Sophie Duwez (asduwez@uliege.be).

Materials availability

Available on request from C.K.

Data availability

The force profiles from AFM measurement are available on request from A.-S.D.; synthesis data are available on request from C.K.

SUPPLEMENTAL INFORMATION

See Supplemental Information for detailed methods and protocols: Details of the synthesis of all molecules, details of force spectroscopy experiments, DFT calculations, supplementary figures. NMR spectra of the compounds are provided in a Supplemental file.

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AUTHOR CONTRIBUTIONS

A.-S.D., G.R. and C.K. designed the experiments. Y.G. carried out the chemical synthesis and characterization studies. X.L. and M.L. performed the AFM experiments and X.L., M.L., and D.S. analyzed the data. A.-S.D., G.R. and C.K. directed the research. A.-S.D, Y.G. and M.L. wrote the manuscript. All the authors discussed the results and commented on the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

Figure 1. **AFM-based SMFS on a molecular rotor.** (A). Schematics of the SMFS experiment showing the AFM tip pulling on the tether attached to one arm of the rotor. (B) Molecular structures of the molecules **M1**-**M4**.

Figure 2. **Pulling experiments on M1 at 20 nm.s^{-1}.** (A) Characteristic force-distance profile obtained for **M1** at a pulling rate of 20 nm.s^{-1}. The insert shows the force-time profile in the region of fluctuations between states A and B. (B) Histogram of the amplitude of the fluctuations (ΔF) (force increment between fluctuations, from A to B). N=122, number of fluctuations obtained from 8 independent experiments (with 8 different samples). (C) Schematic showing a rotational movement of the rotor during the pulling and the associated change in contour length (Δd) detected.

Figure 3. **Pulling experiments on a dinuclear complex.** (A) Molecular structure of the system **M5** in which two side by side molecules are linked chemically to block the rotation. (B) Characteristic force-distance profiles obtained for M5 at a pulling rate of 20 nm.s^{-1} and (C) 200 nm.s^{-1}.

Figure 4. **Pulling experiments on M1 at 200 nm.s^{-1}.** (A) Characteristic force-distance profile obtained for **M1** at a pulling rate of 200 nm.s^{-1}. Worm-like chain (WLC) fits are added in red as a guide to the eye (before and after the pseudo-plateau which is highlighted in orange. Fitting parameters: l_p = 0.55 nm, L_{c1} = 18.1 nm, L_{c2} = 19.8 nm). (B) Histogram of the pseudo-plateau force (red trace: Gaussian Fit). N=200. (C) Histogram of the pseudo-plateau length (red trace: Gaussian Fit). N=200. Data obtained from 11 independent experiments.

Figure 5. **Pulling-relaxing experiments**. Characteristic pulling (light blue)–relaxing (dark blue) force-extension cycle obtained for **M1** at a pulling rate of 200 nm.s^{-1}.

Figure 6. **Pulling experiments on M2, M3 and M4 at 20 nm.s^{-1}.** Upper panel: Force-distance profiles obtained for **M2** (left), **M3** (middle), and **M4** (right), at a pulling rate of 20 nm.s^{-1}. Fluctuations are evidenced by a red circle. Bottom panel: Force-time profiles in the region of fluctuations between states A (corresponding to the molecule oscillating, thus exerting force on the AFM tip) and B (corresponding to the system aligned in the pulling direction).

Table 1. Pseudo-plateau length and force for M1-M4 at a pulling rate of 200 nm.s^{-1} Most probable values (± s.d.) obtained from the fitting of the distribution of the raw data with Gaussian functions. N=200 for **M1**, N=174 for **M2**, N=337 for **M3**, N=354 for **M4**. The maximum rotor diameters (2 × L) have been obtained from DFT calculations (see details in SI section I.5. Data S2).

	M1	M2	M3	M4
Pseudo-plateau length (nm)	1.8 ± 0.7	1.3 ± 0.5	1.3 ± 0.5	2.2 ± 1.0
Pseudo-plateau force (pN)	53 ± 19	62 ± 16	74 ± 15	66 ± 23
Maximum rotor diameter (nm)	2.27	1.92	1.92	3.64

Table 2. Work performed by the tip to block the oscillations and by the rotors against the load. Cumulative work done by the tip to block the rotary oscillations and work generated by one rotary oscillation (= $\Delta F \times \Delta x$ for one fluctuation) for **M1**-**M4**. Most probable values (± s.d.) obtained from the fitting of the distribution of data with Gaussian functions. *N*=94 for **M1**, *N*=115 for **M2**, *N*=121 for **M3**, *N*=103 for **M4**.

	M1	M2	M3	M4
Work done by the tip (k_BT)	15.3 ± 7.8	13.6 ± 5.0	29.6 ± 12.8	24.7 ± 12.6
Work generated by one rotary oscillation (k_BT)	2.3 ± 0.4	2.2 ± 0.4	3.2 ± 0.4	3.8 ± 0.7

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