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Probing single molecules with the AFM: Force, dynamics, and function

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http://www.nanochem.ulg.ac.be

1952

Erwin Schrödinger wrote that we would never experiment with just one electron, one atom, or one molecule

E. Schrödinger, *Br. J. Philos. Sci.* **1952**, *3*, 233

1959

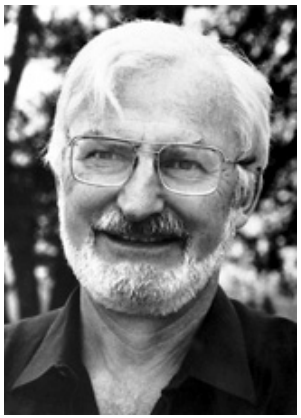
Richard Feynman told us that there are no physical limitations to maneuvering things atom by atom

There's Plenty of Room at the Bottom
An Invitation to Enter a New Field of Physics

December 29th 1959, annual meeting of the American Physical Society at Caltech

1981: Invention of the STM

G. Binnig, H. Rohrer, Ch. Gerber, E. Weibel
App. Phys. Lett. **1982**, *40*, 178

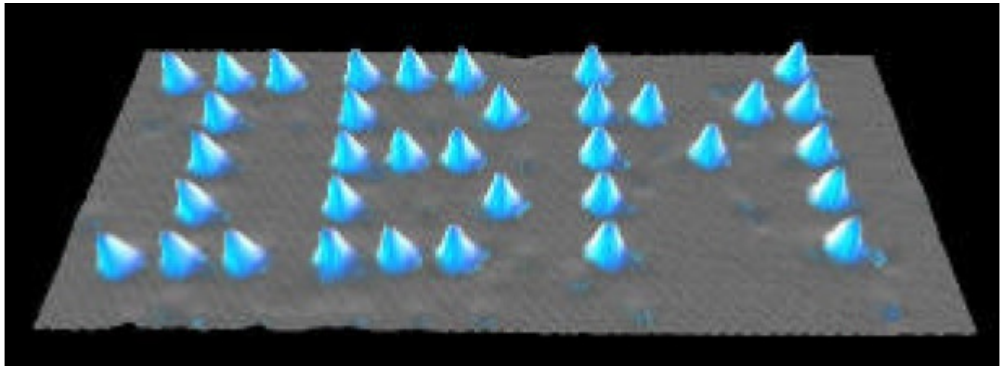


1986: Binnig, Quate, and Gerber proposed the AFM

Phys. Rev. Lett. **1986**, *59*, 178, 930

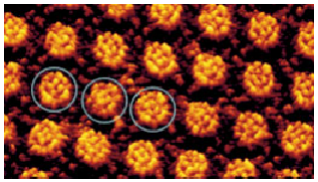
1990: Positioning single atoms with the STM

D. M. Eigler, E. K. Schweizer,
Nature **1990**, *344*, 524

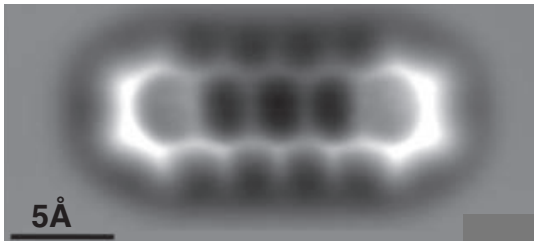


AFM

AFM as a microscope:
Imaging & Characterization

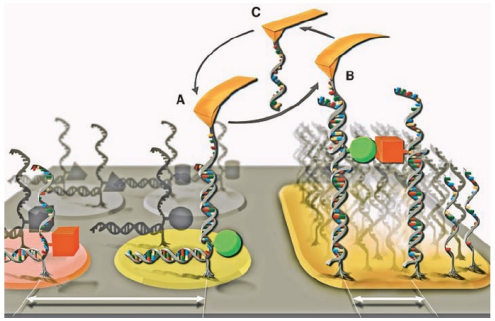


Science 2003, *302*, 1002



Science 2009, 325, 1110

AFM as a tool:
Manipulation of molecules



Science 2008, *319*, 594

Single molecule force spectroscopy

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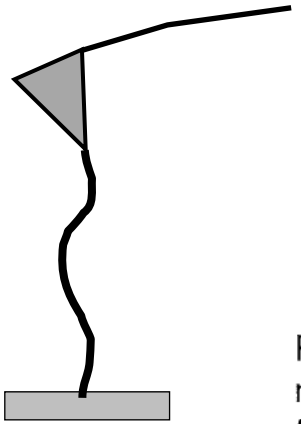
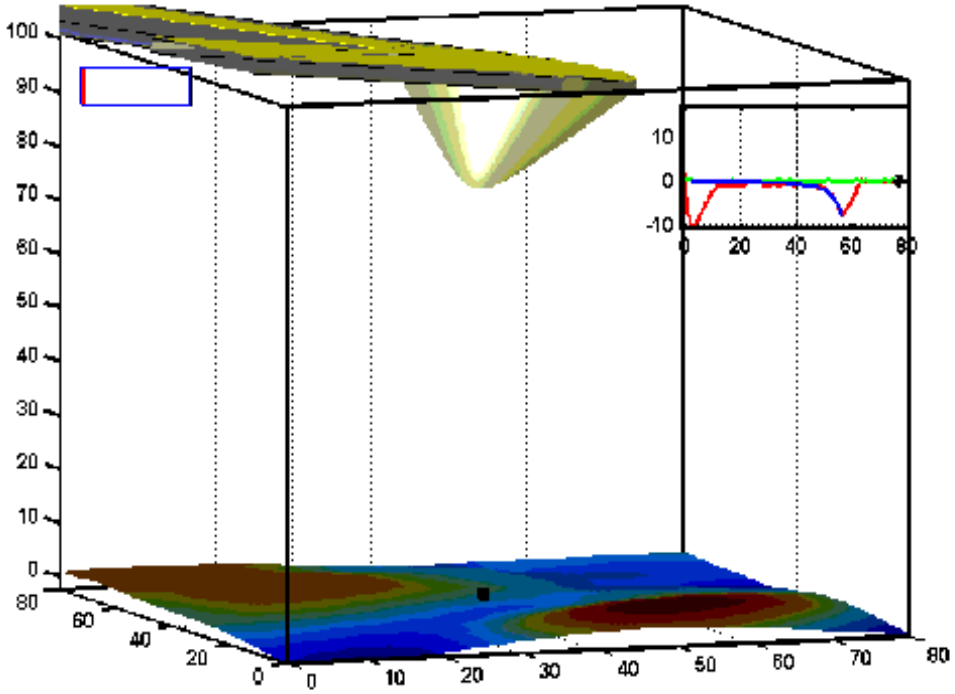


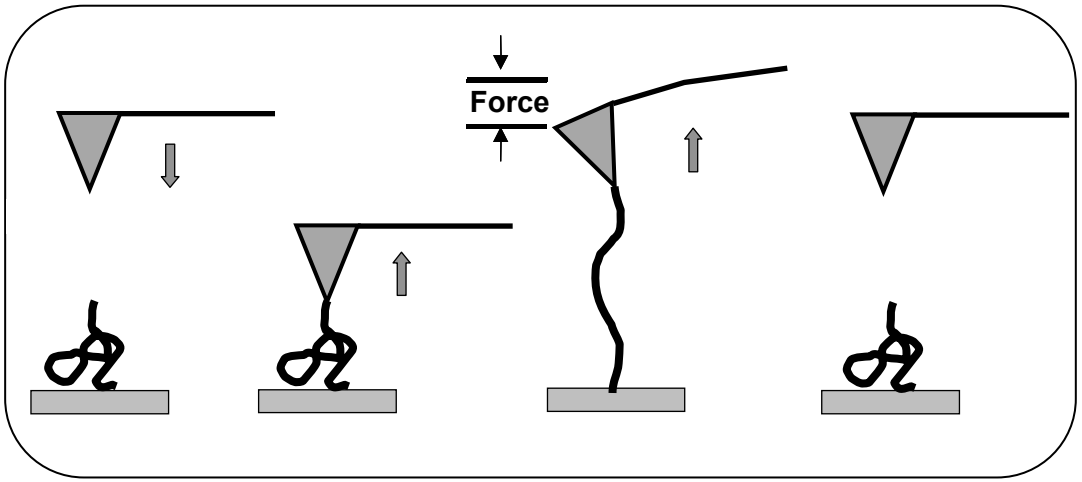
Single Molecule Force Spectroscopy on Polysaccharides by Atomic Force Microscopy

Matthias Rief, Filipp Oesterhelt, Berthold Heymann, Hermann E. Gaub

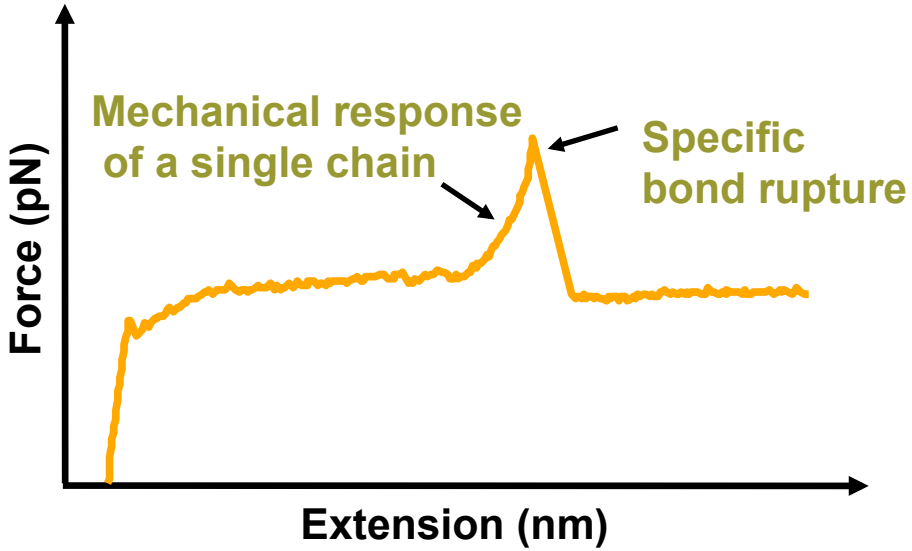
Recent developments in piconewton instrumentation allow the manipulation of single molecules and measurements of intermolecular as well as intramolecular forces. Dextran filaments linked to a gold surface were probed with the atomic force microscope tip by vertical stretching. At low forces the deformation of dextran was found to be dominated by entropic forces and can be described by the Langevin function with a 6 angstrom Kuhn length. At elevated forces the strand elongation was governed by a twist of bond angles. At higher forces the dextran filaments underwent a distinct conformational change. The polymer stiffened and the segment elasticity was dominated by the bending of bond angles. The conformational change was found to be reversible and was corroborated by molecular dynamics calculations.

Single molecule force spectroscopy

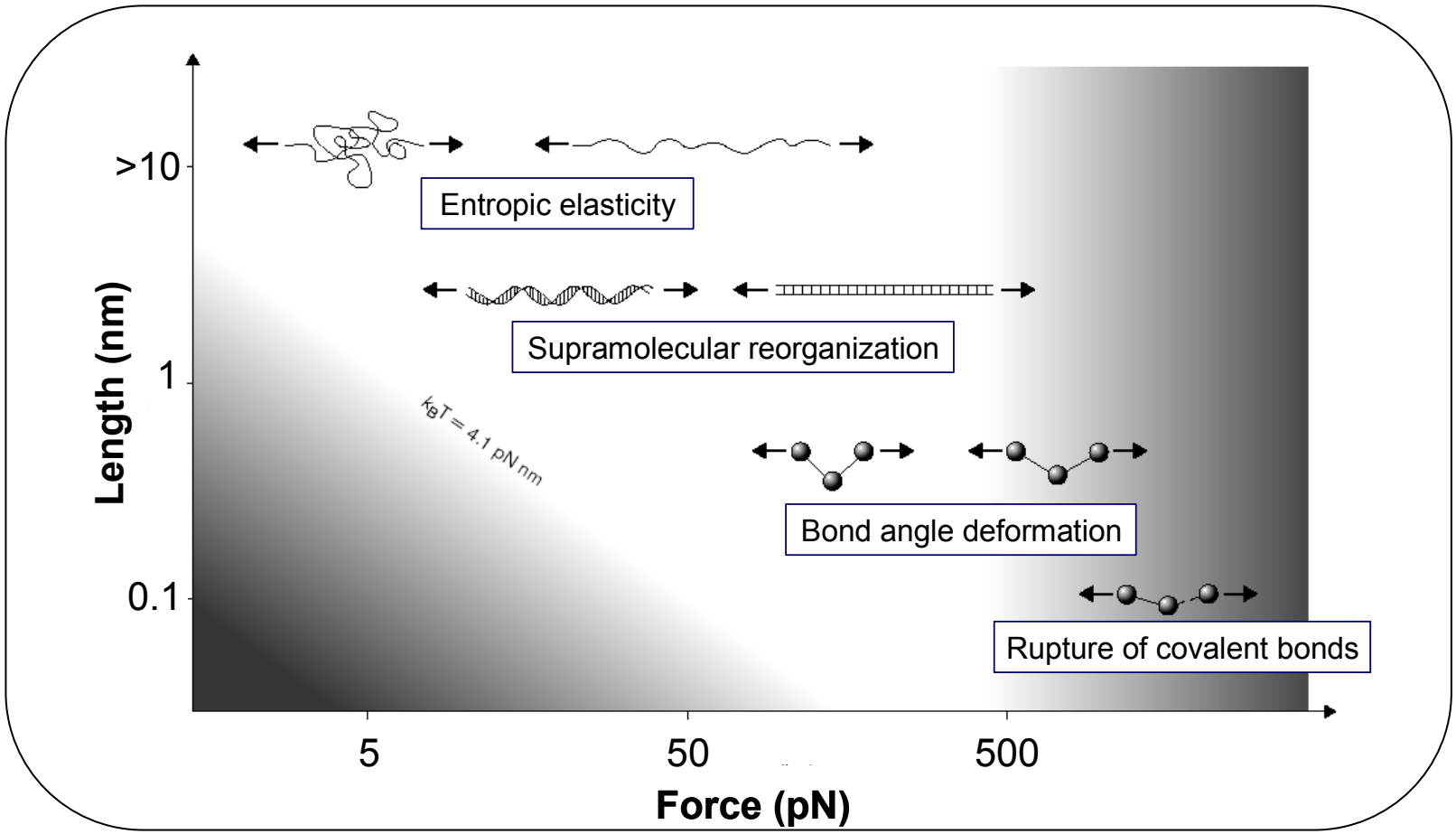



Force

In solution


Mechanical response
of a single chain
Specific
bond rupture
Force (pN)
Extension (nm)

Single Molecule Force Spectroscopy



From H. E. Gaub et al.
Current Opinion in Chemical Biology **2000**, *4*, 524

! It's always the weakest link that breaks

→ The interaction of interest must be the weakest link of the system

→ The design of the tip/molecular species/surface is of prime importance

Why manipulating single entities?

Miniaturization

Ultimate limit ?

A day will come when a tiny piece of matter will be too small to fabricate a device or a machine

Single molecule/atom constructions

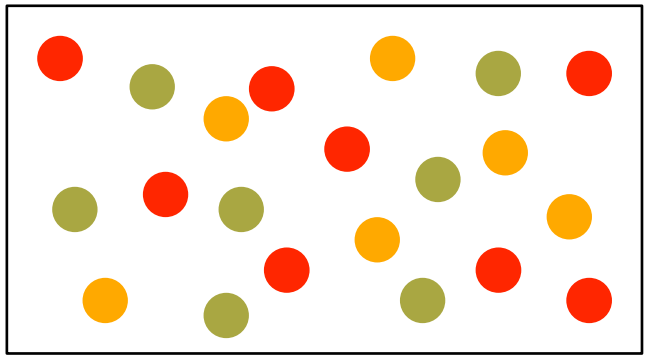
A single molecule = an ultra-miniaturized device/machine

To advance the frontier of the control of individual molecular architectures on surfaces

Exploration of single entities

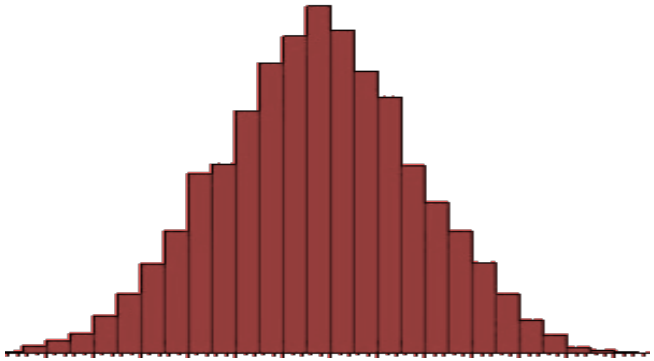
Do the phenomena observed at this scale obey the laws we know for ensemble of species or will force us to rethink our understanding of physics and chemistry?

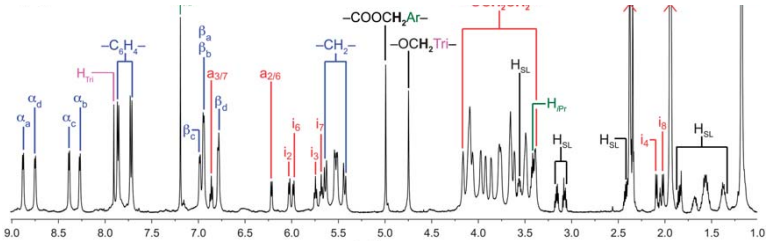
Classical chemistry



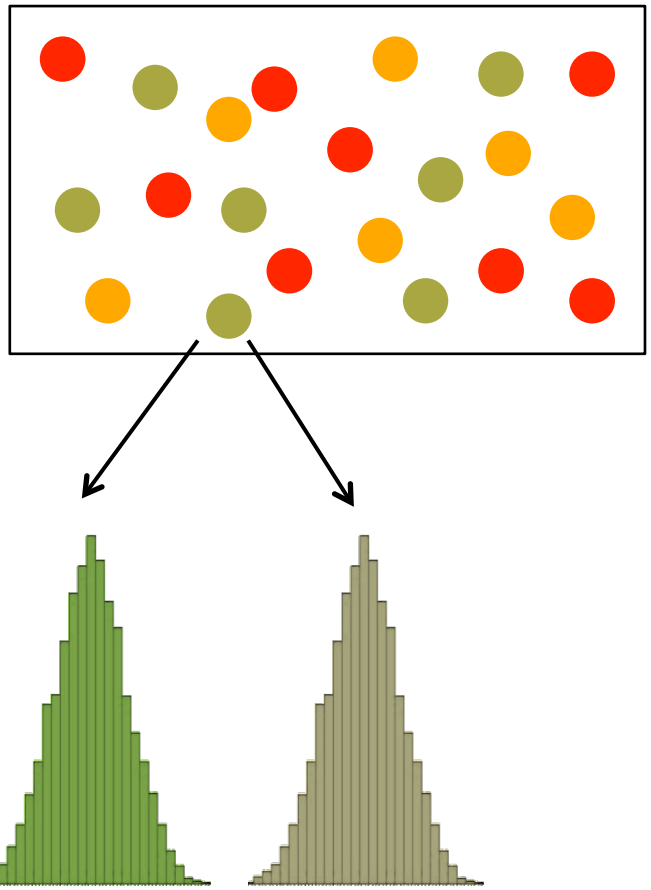
Unit: mole

Average behaviour on billions of molecules

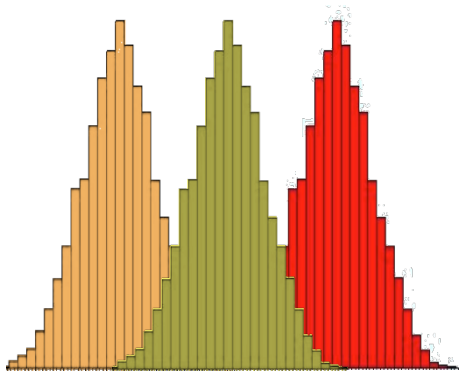




Exploration of single entities



Different behaviour of the same molecule



Sub-groups of molecules

Unit: 1 single molecule

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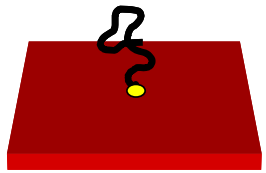
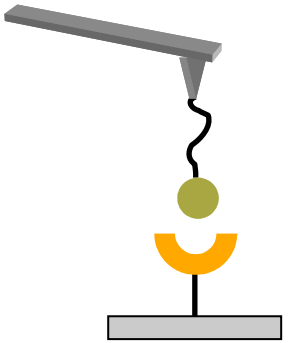
Single molecule force spectroscopy

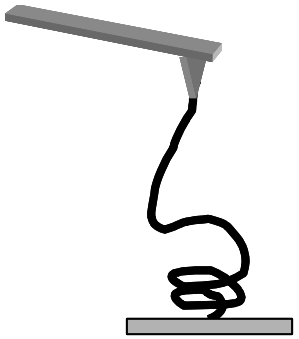


Molecular interactions



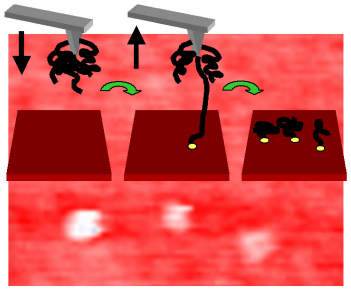
J. Am. Chem. Soc. **2007**, *129*, 8410
Anal. Chem. **2007**, *79*, 6488
Langmuir **2012**, *28*, 7233

Molecular elasticity and Interactions with surfaces



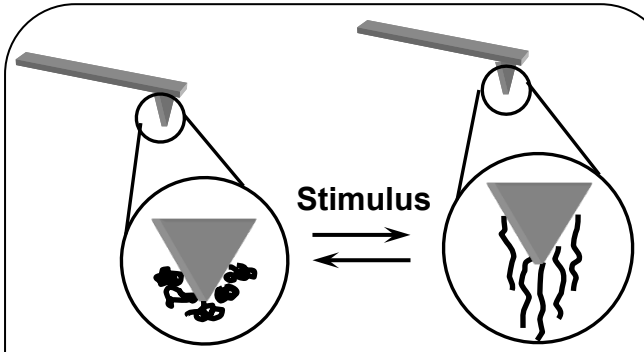
Langmuir **2010**, *26*, 12165
Langmuir **2012**, *28*, 2971
Adv. Funct. Mater. **2012**, *22*, 5271

Single molecule patterning



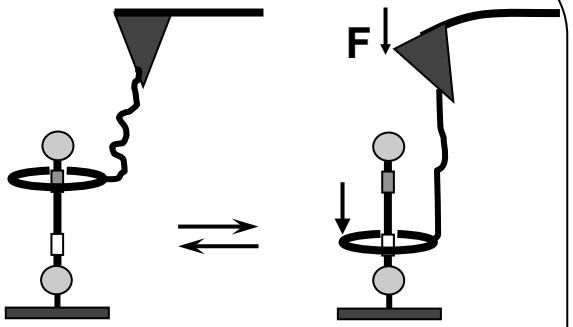
Nature Nanotech. **2006**, *1*, 122
Nature Nanotech. **2008**, *3*, 188

Molecular sensors



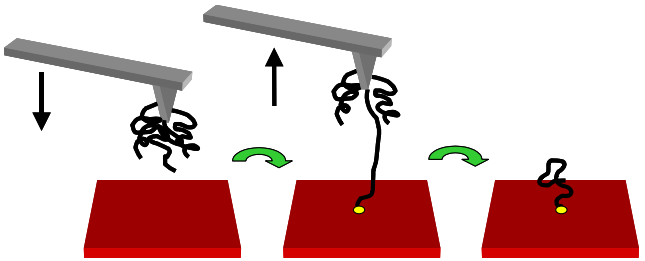
Small **2008**, *4*, 1101

Molecular machines



Nature Nanotech. **2011**, *6*, 553
Chem. Sci. **2014**, *5*, 1449

- **Single molecule patterning**



- Molecular interactions
- Molecular machines

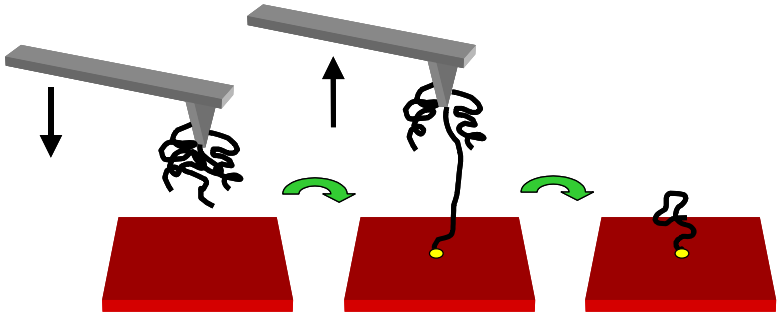
Single molecule patterning

→ build complex functional structures molecule by molecule

Three main challenges:

- to position every single molecule in the right place
- to make the molecule form bonds as required
- and to achieve high-throughput fabrication

Single molecule patterning

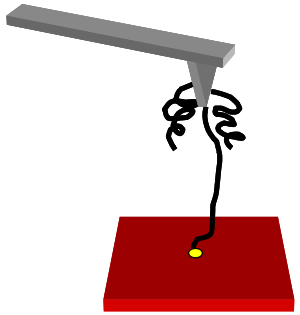


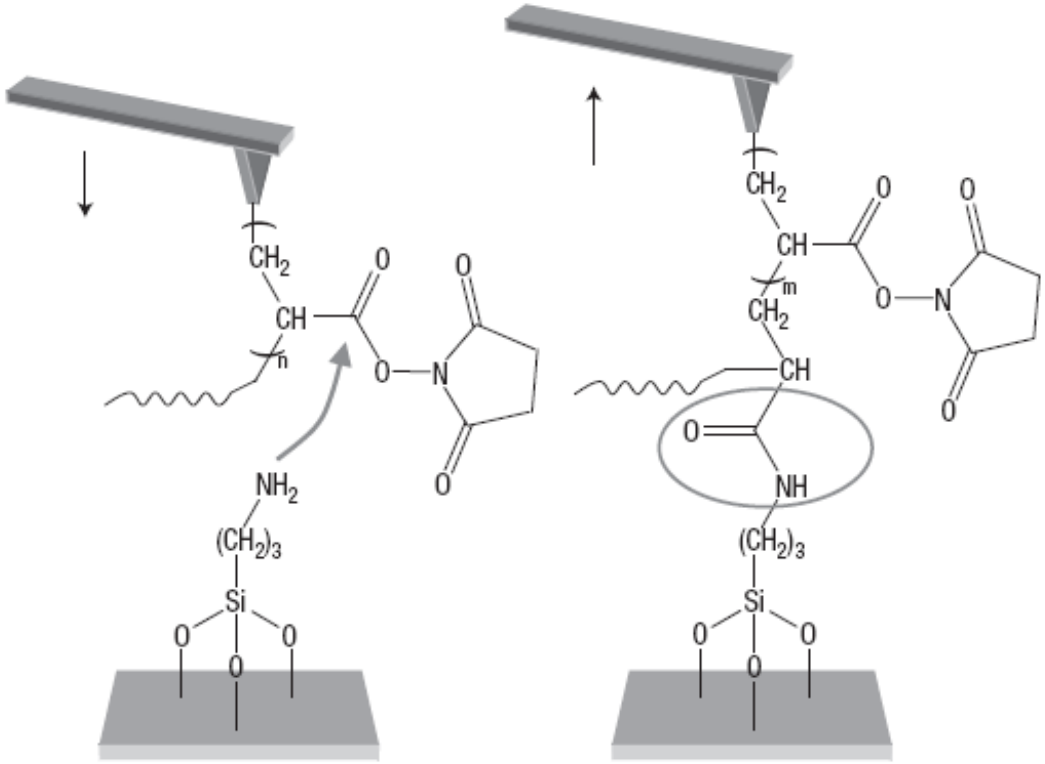
The AFM can deliver and immobilize single molecules, one at a time, on a surface during a single molecule force spectroscopy experiment

Strategy:

- Use AFM tips as reservoirs of single molecules
- The molecules can be linked to a substrate by a chemical reaction
- When the AFM tip is pulled away from the surface, the resulting mechanical force causes the bond between the tip and molecule (the weakest one) to break

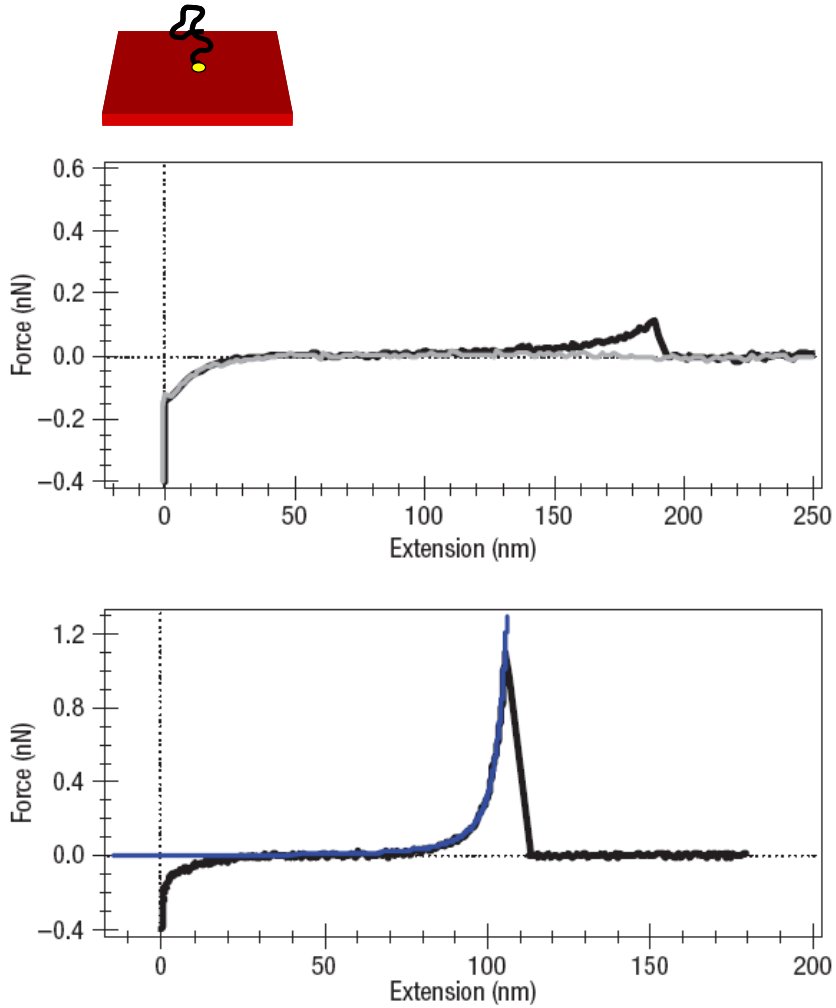
Single molecule patterning

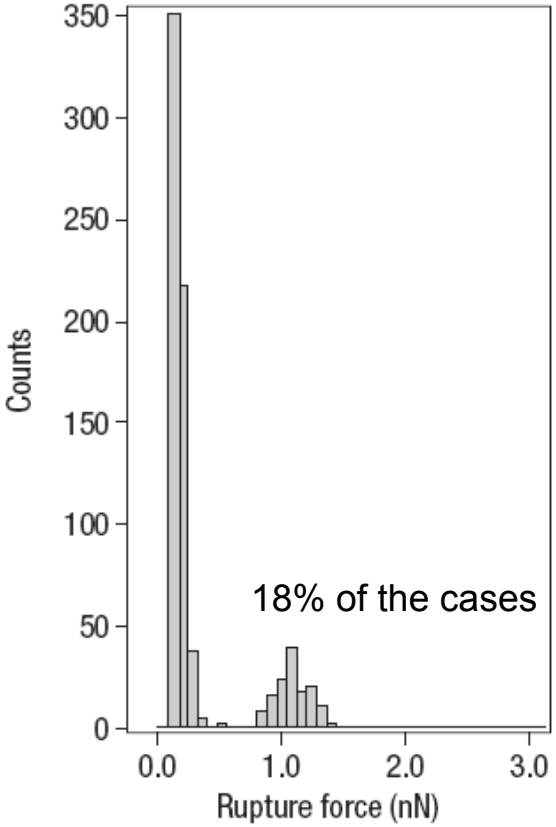




Nature Nanotechnology **2006**, *1*, 122

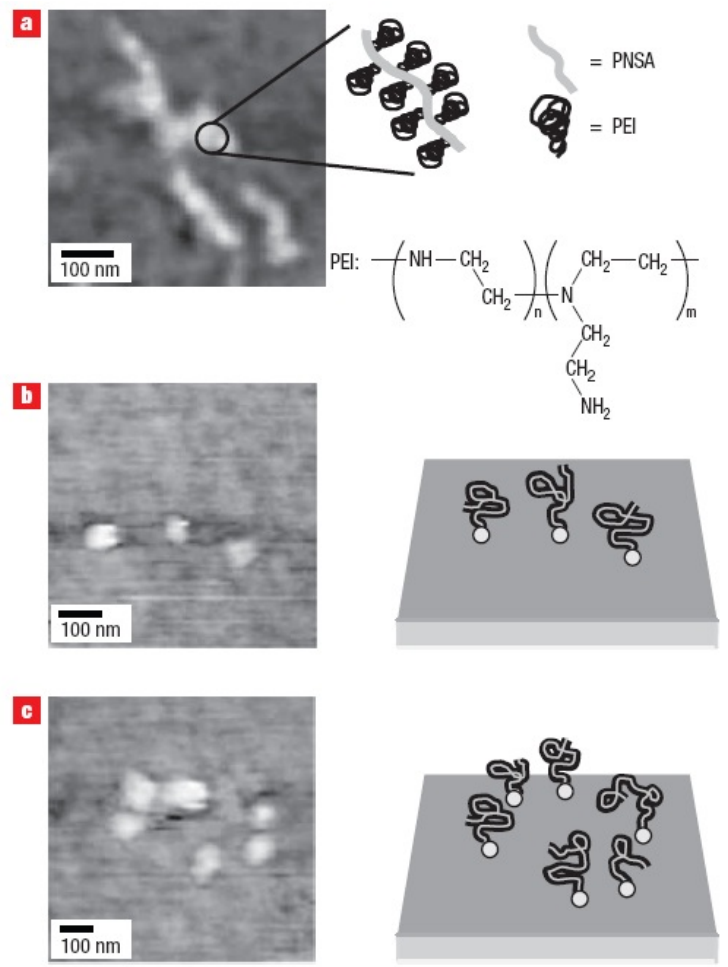
Selective breaking of chemical bonds





Targeted Delivery of Single Molecules

Original deposited chains are post-decorated by a branched polymer to increase their diameter and make them visible



Nature Nanotechnology **2006**, *1*, 122

Major disadvantage:

The tip serves as a reservoir: a limited number of molecules can be deposited

→ High-throughput impossible

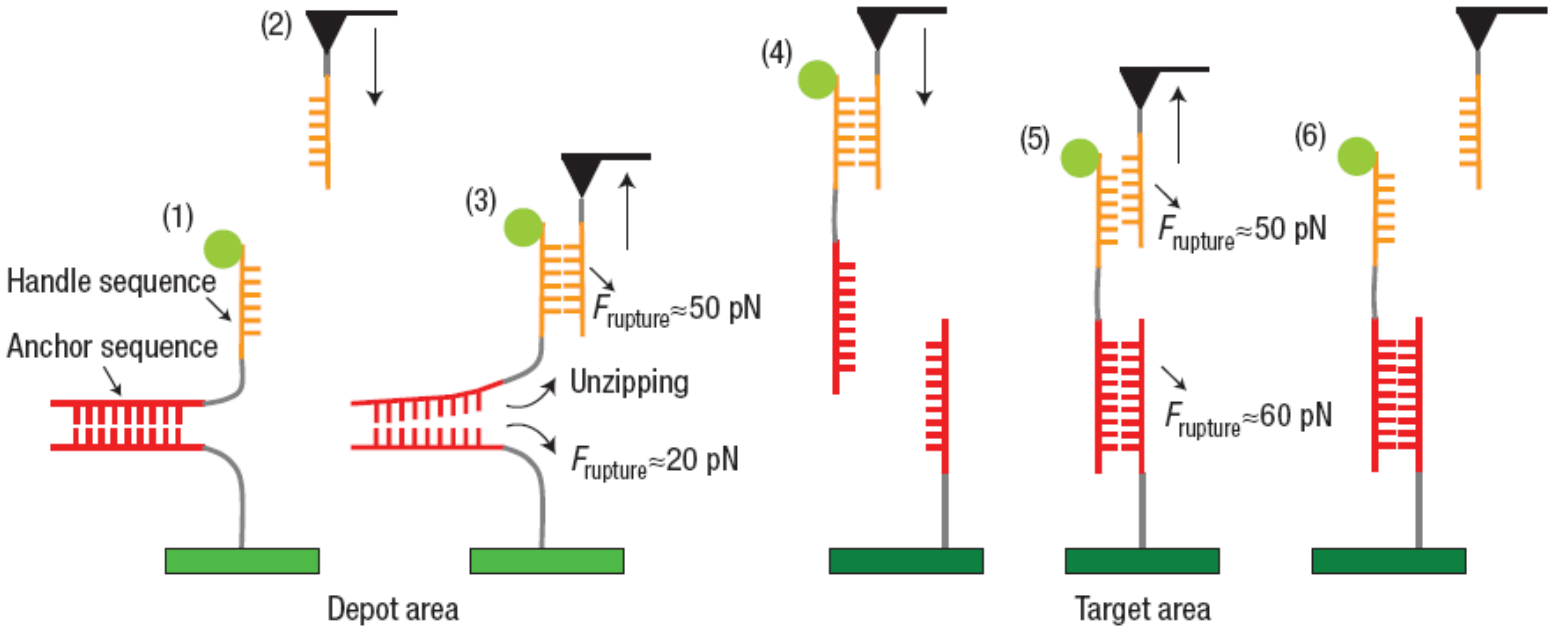
594 1 FEBRUARY 2008 VOL 319 SCIENCE www.sciencemag.org

Single-Molecule Cut-and-Paste Surface Assembly

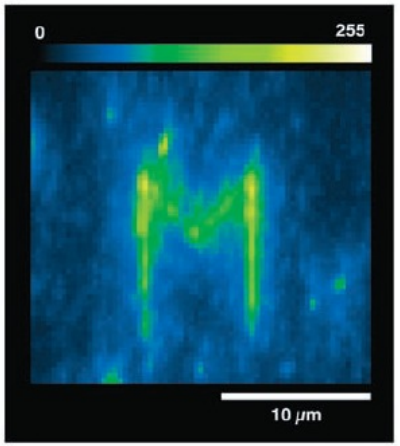
S. K. Kufer,[1] E. M. Puchner,[1] H. Gumpp,[1] T. Liedl,[2] H. E. Gaub[1]

We introduce a method for the bottom-up assembly of biomolecular structures that combines the precision of the atomic force microscope (AFM) with the selectivity of DNA hybridization. Functional units coupled to DNA oligomers were picked up from a depot area by means of a complementary DNA strand bound to an AFM tip. These units were transferred to and deposited on a target area to create basic geometrical structures, assembled from units with different functions. Each of these cut-and-paste events was characterized by single-molecule force spectroscopy and single-molecule fluorescence microscopy. Transport and deposition of more than 5000 units were achieved, with less than 10% loss in transfer efficiency.

Single molecule patterning



Gaub and co-workers used their molecular cranes to transport fluorophores and biotin molecules to the target site and, as a proof of concept, they wrote the letter M with 400 fluorescently labelled molecules



• A single AFM tip can transport and deliver more than 5 000 units, one by one

→ Significant step towards high-throughput

Drawbacks:

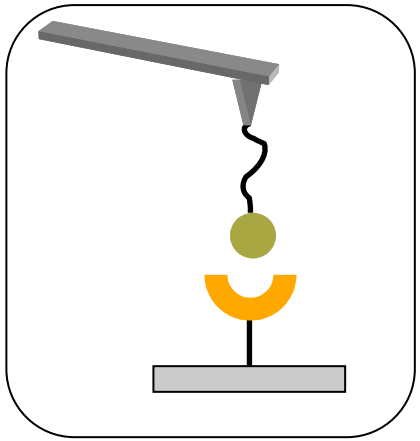
• Precision of the delivery: ~10 nm

• Some issues still need to be resolved:

the AFM tip picks up just 1 unit only 35% of the time,
→ more likely to pick up zero, two or more units

• DNA oligomer used as a 'handle molecule' to transport objects
→ complicated chemical transformations to attach the object on DNA

- Single molecule patterning
- **Molecular interactions**



- Molecular machines

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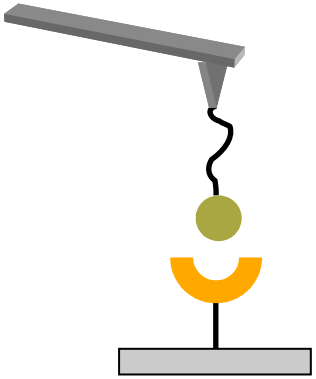
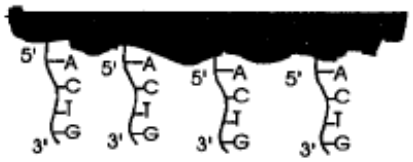
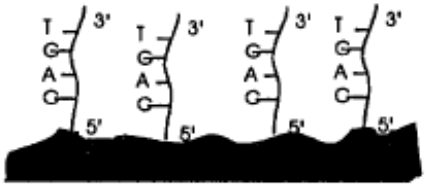


Direct Measurement of the Forces Between Complementary Strands of DNA

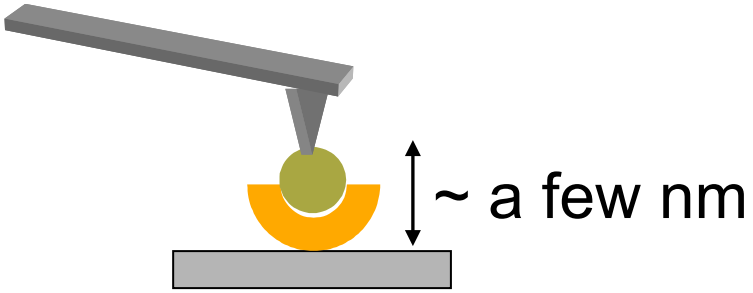
Gil U Lee,* Linda A. Chrisey, Richard J. Colton

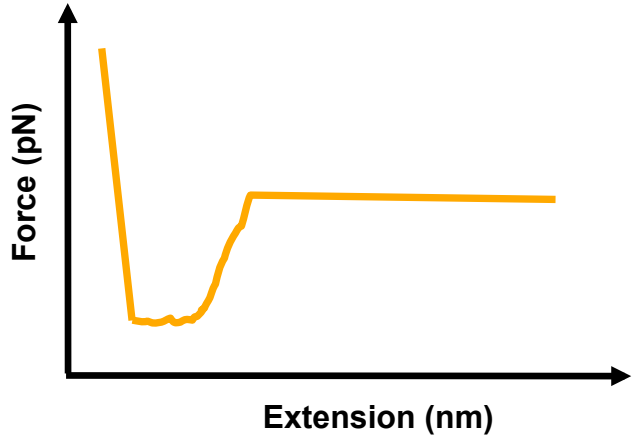
Interaction forces between single strands of DNA were measured with the atomic force microscope by a procedure in which DNA oligonucleotides were covalently attached to a spherical probe and surface. Adhesive forces measured between complementary 20-base strands fell into three distinct distributions centered at 1.52, 1.11, and 0.83 nanonewtons, which are associated with the rupture of the interchain interaction between a single pair of molecules involving 20, 16, and 12 base pairs, respectively. When a third long DNA molecule was coupled between complementary surfaces, both intra- and interchain forces were observed. The intrachain interaction resulting from the molecule's elasticity manifested itself as a long-range cohesive force.

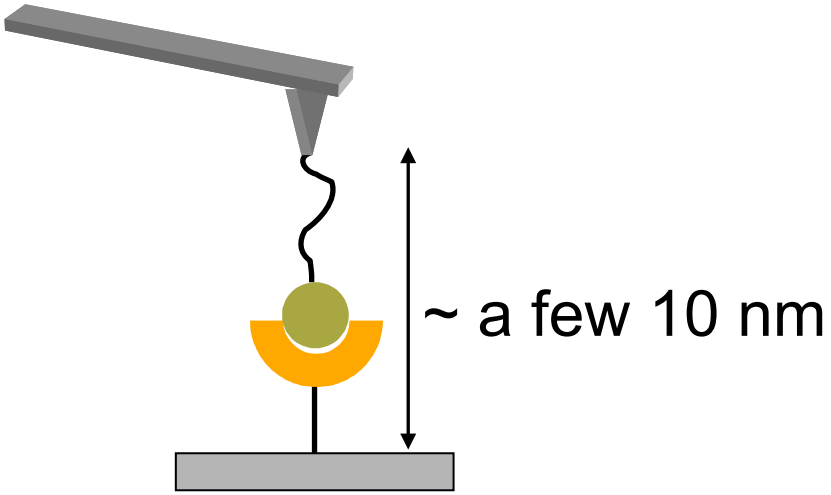


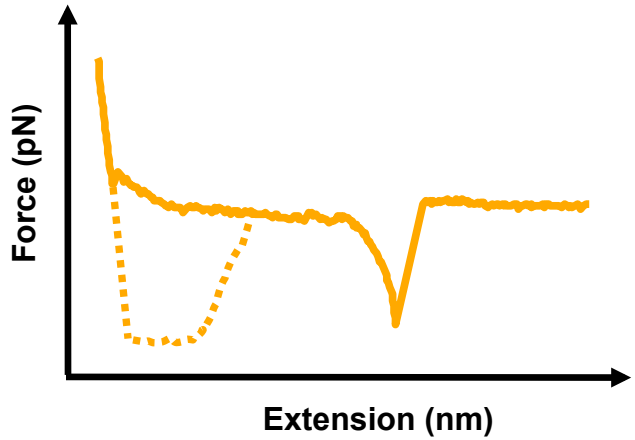


Molecular Interactions



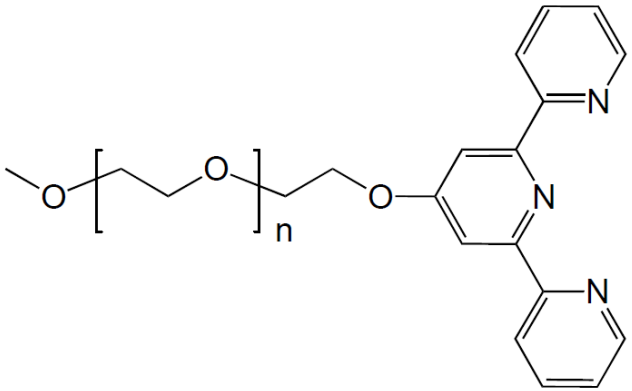






Probing a metallo-supramolecular interaction

=



= Fe^{2+}, Ni^{2+}, Co^{2+}

Molecular Interactions

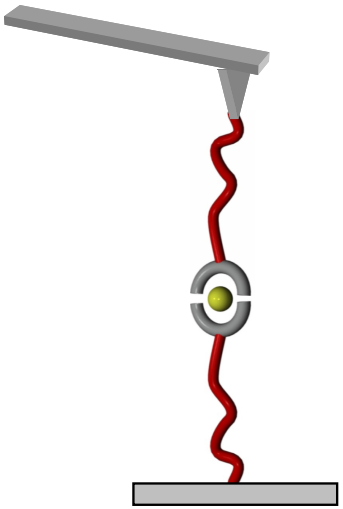
	Force (pN) in water (8 nN s^{-1})
PEO-Ni-PEO	64 ± 10
PEO-Co-PEO	45 ± 8
PEO-Fe-PEO	38 ± 8

Thermodynamic binding constants in water

Terp-Ni^{2+} 5.0 x 10^{10} M^{-1}

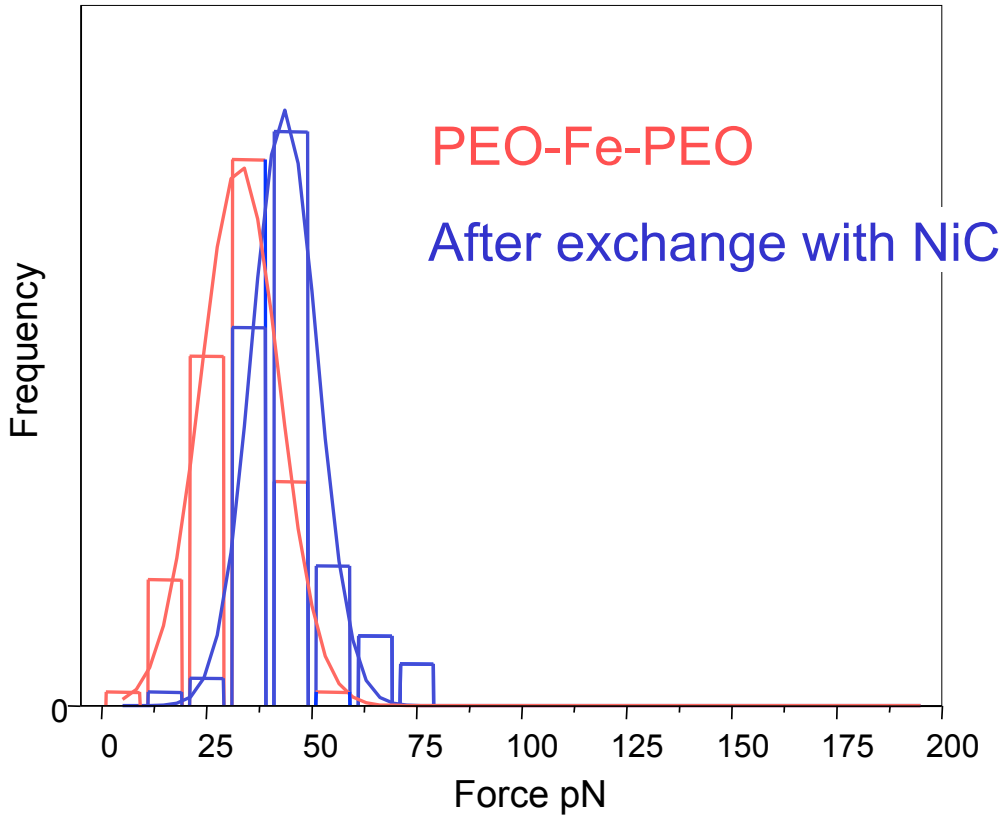
Terp-Co^{2+} 2.5 x 10^{8} M^{-1}

Terp-Fe^{2+} 1.3 x 10^{7} M^{-1}



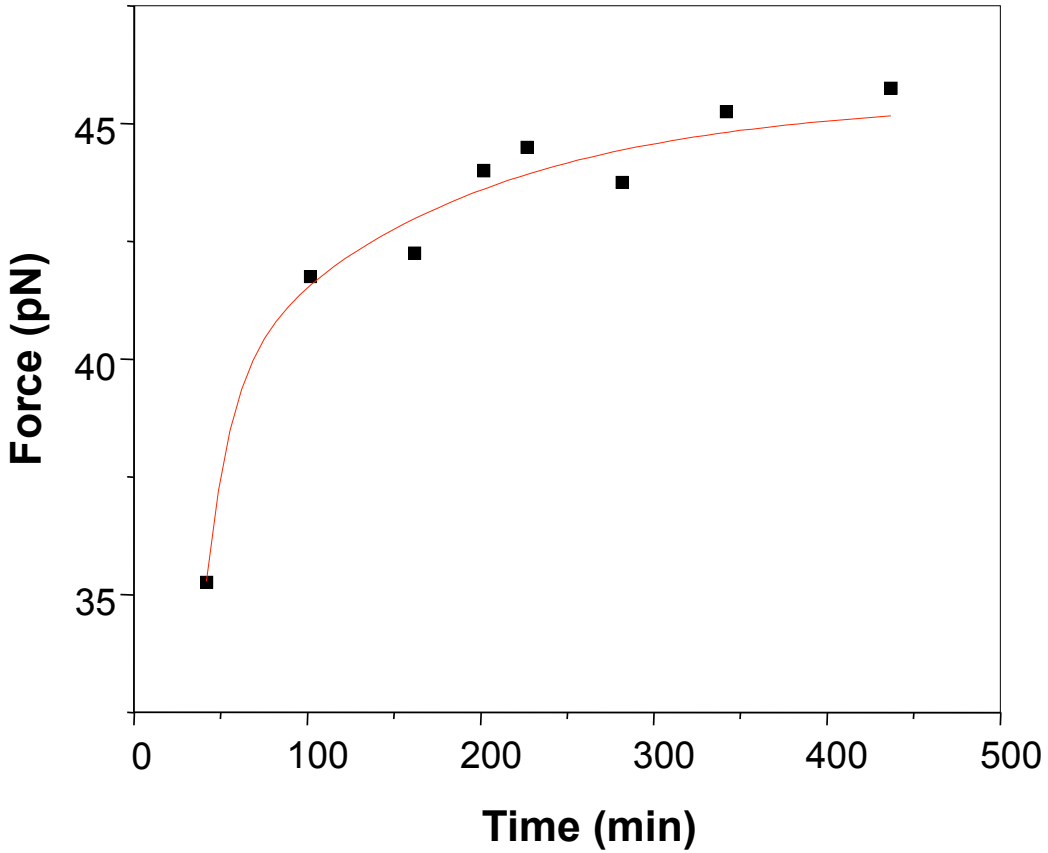
Metal exchange in a metallo-supramolecular chain

Exchange of Fe^{2+} with Ni^{2+}

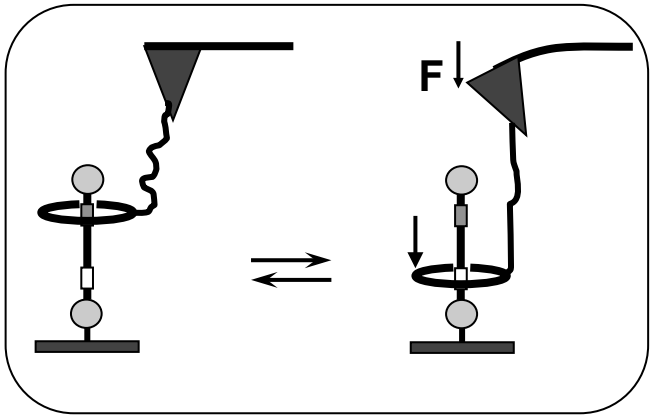


Metal exchange in metallo-supramolecular chains

Exchange of Fe^{2+} with Ni^{2+}: kinetics



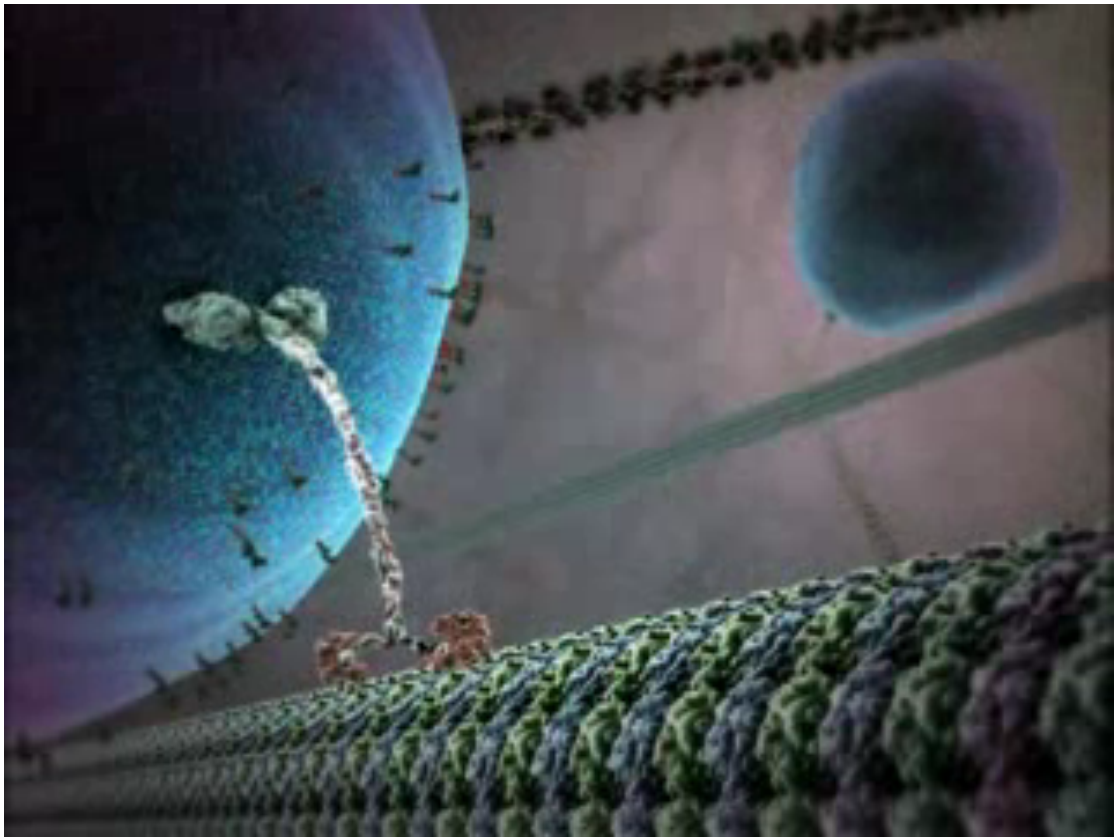
- Single molecule patterning
- Molecular interactions
- **Molecular machines**



Molecular machines

Molecular machines are ubiquitous in nature and are essential in controlling and performing numerous biological functions

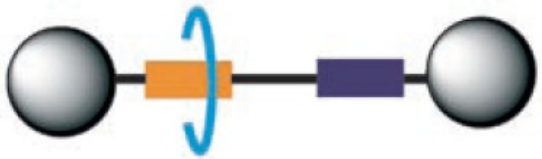
They are able to rectify random motion to generate directional force and carry out macroscopic tasks



'*The Inner Life of the Cell*' Biovisions, Harvard University
http://multimedia.mcb.harvard.edu/

Inspiration for the design of synthetic systems able to use biased Brownian motion to perform work

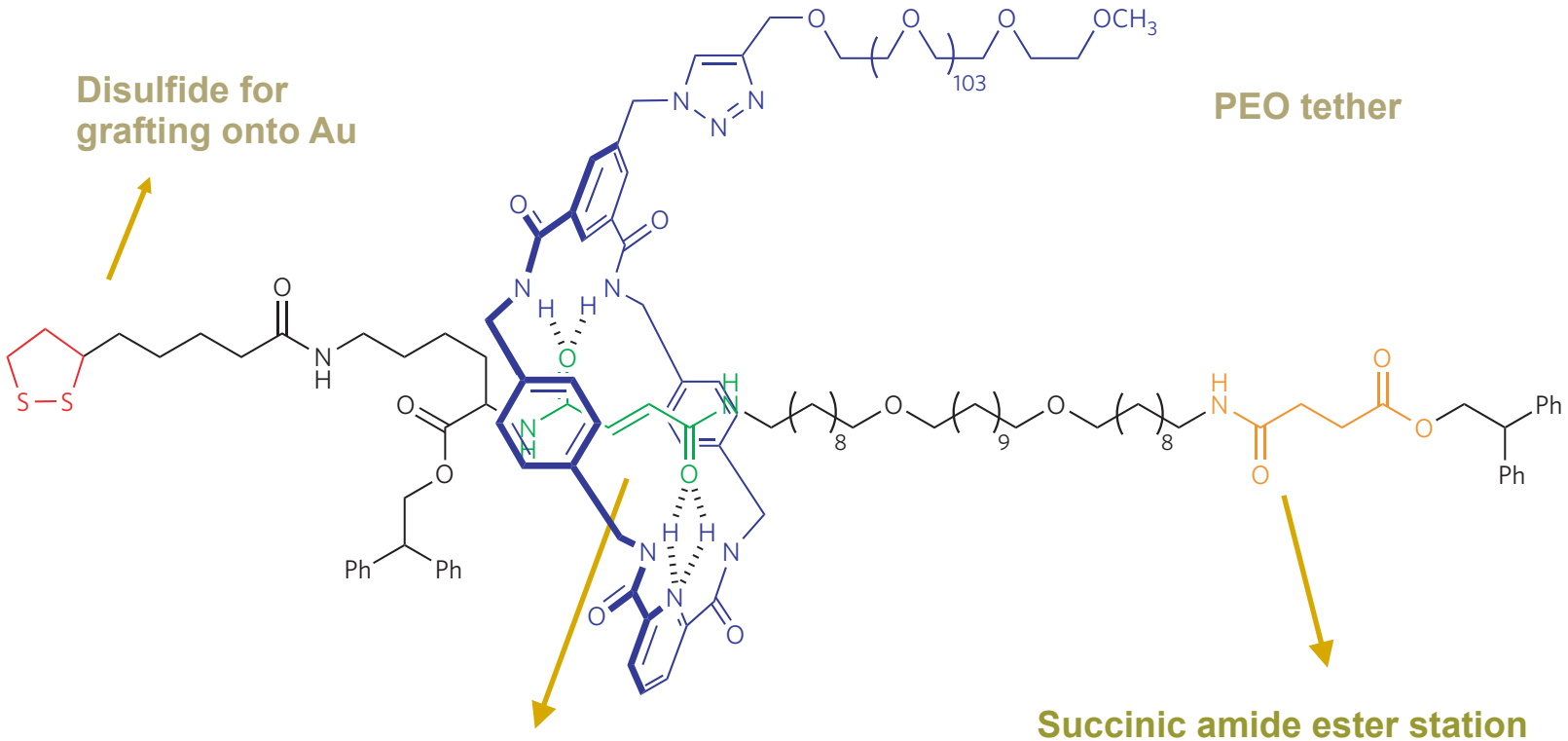
Rotaxane: a molecular ring threaded onto a molecular axle



Can sub-molecular Brownian motions in a **single** synthetic small machine be harnessed to generate forces ?

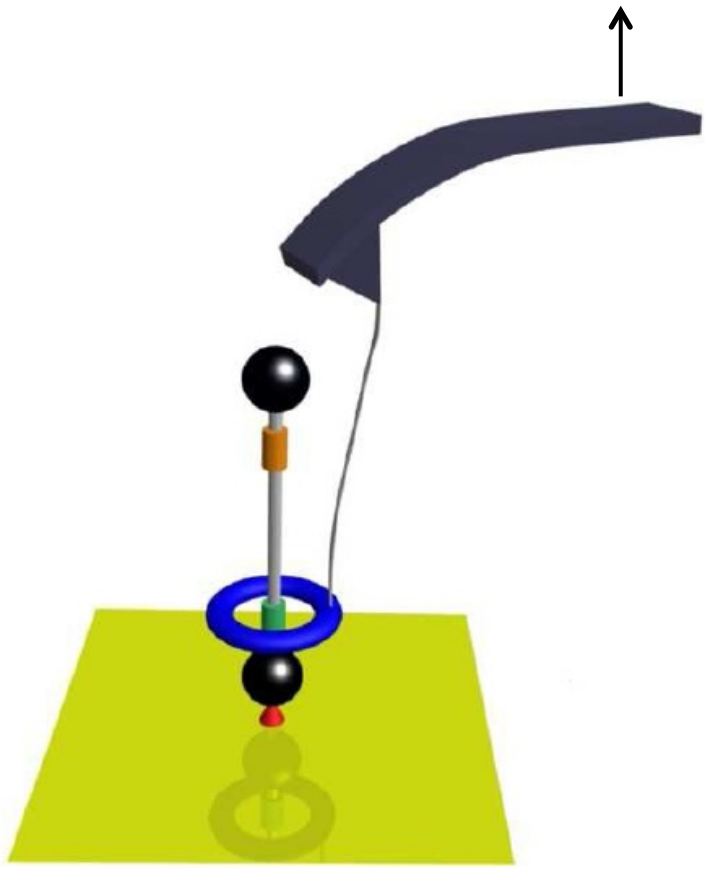
Can we measure the mechanical work done by the molecule?

Hydrogen-bonded rotaxane



In collaboration with David A. Leigh, Univ. Manchester and Charles-André Fustin, UCL

Interfacing molecular machines with the AFM



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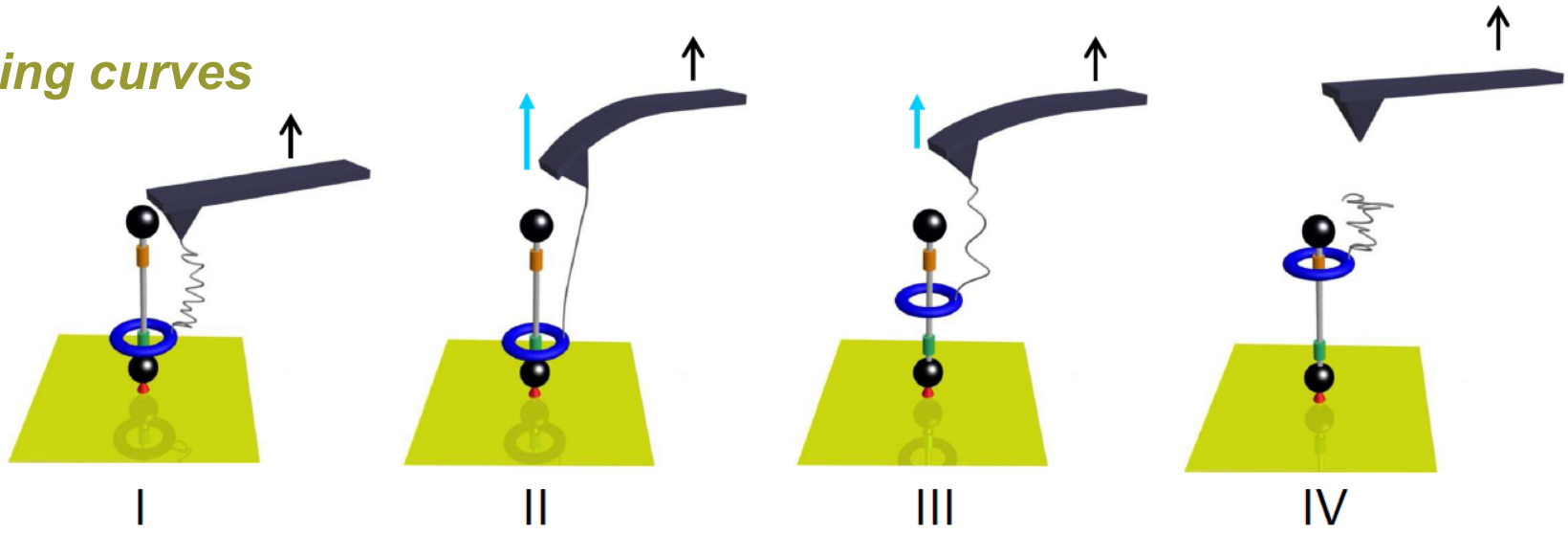
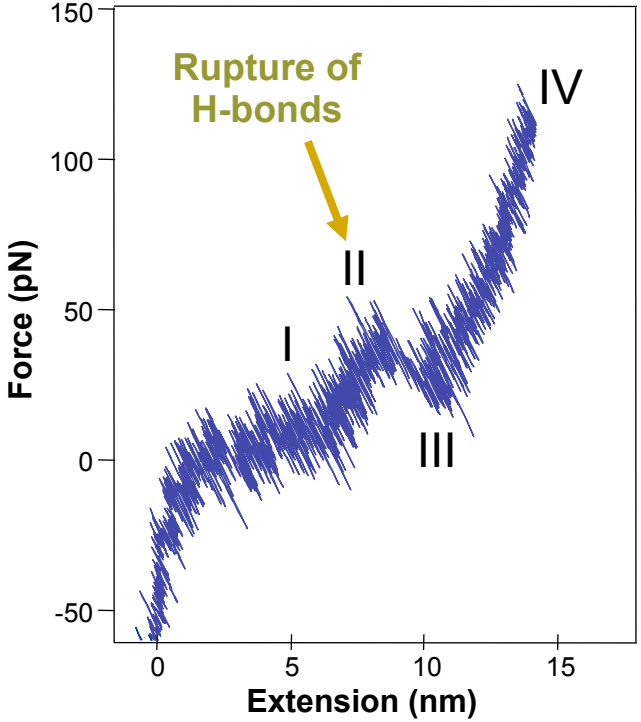
Interfacing molecular machines with the AFM

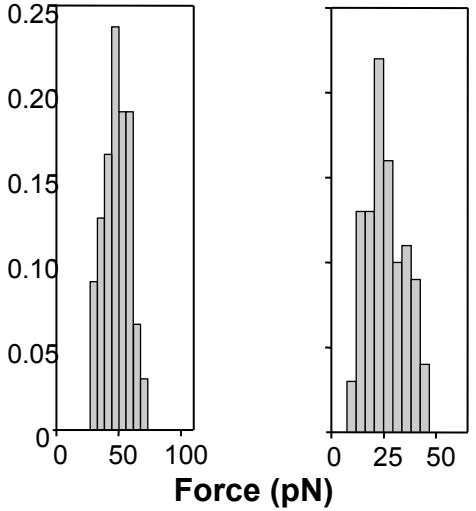
Pulling curves





TCE
45 ± 10 pN

DMF
27 ± 8 pN



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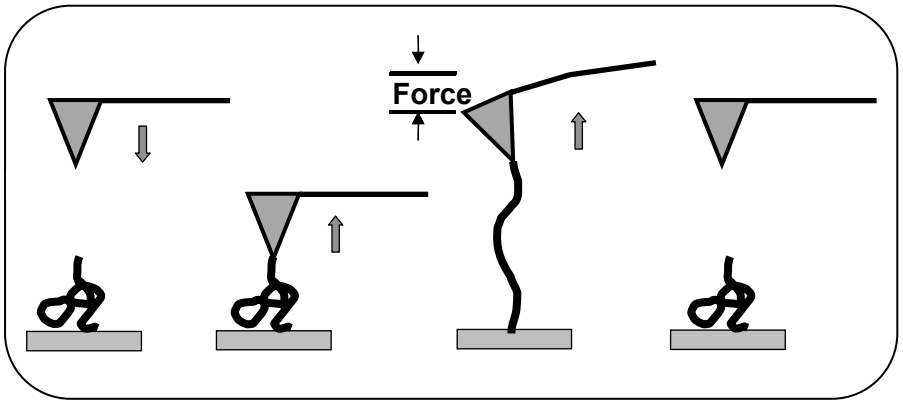
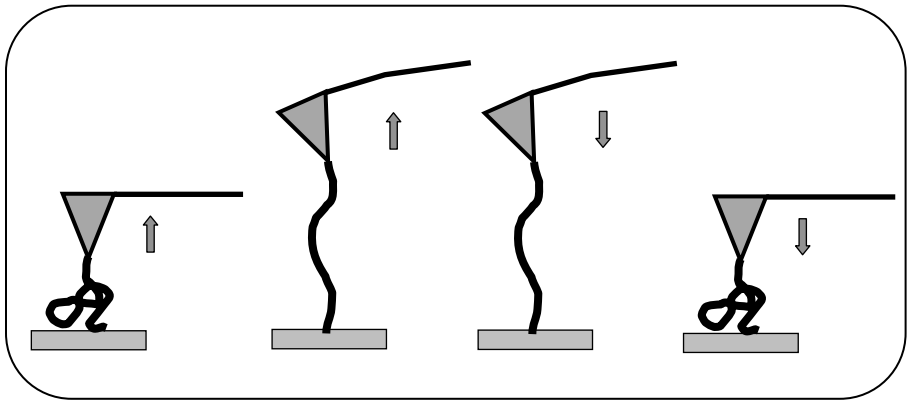
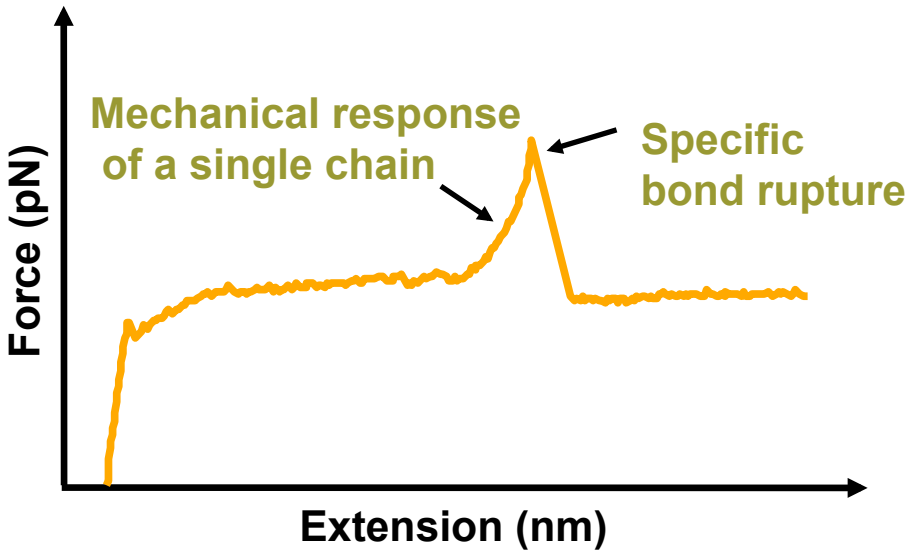
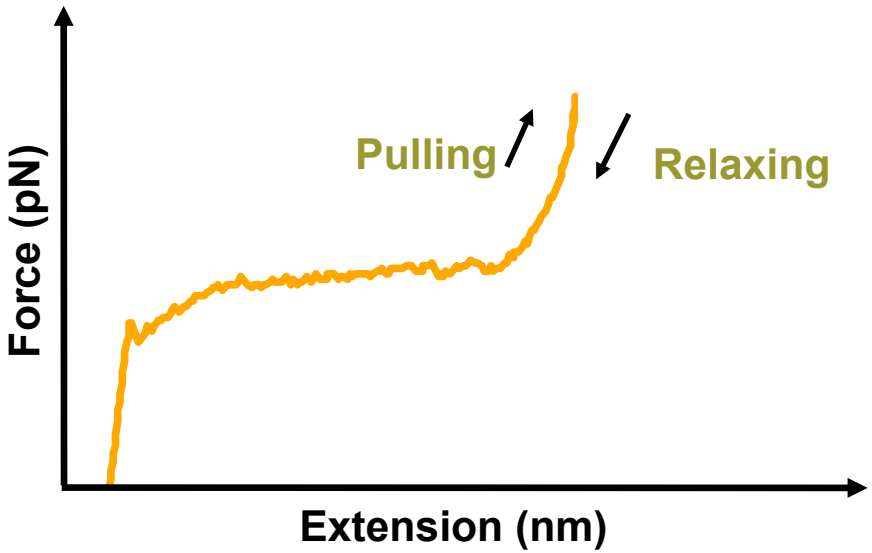
Molecular machines

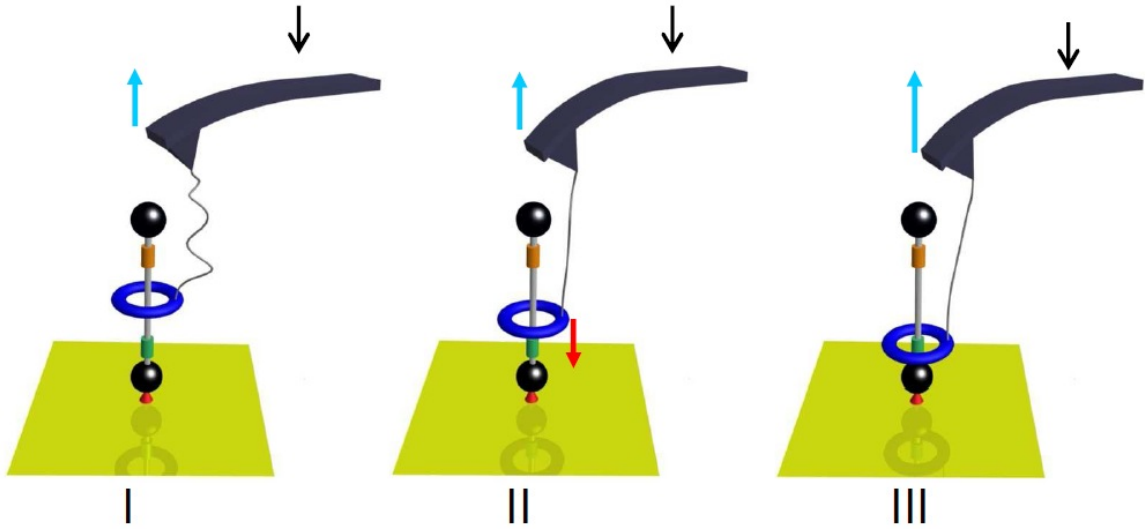


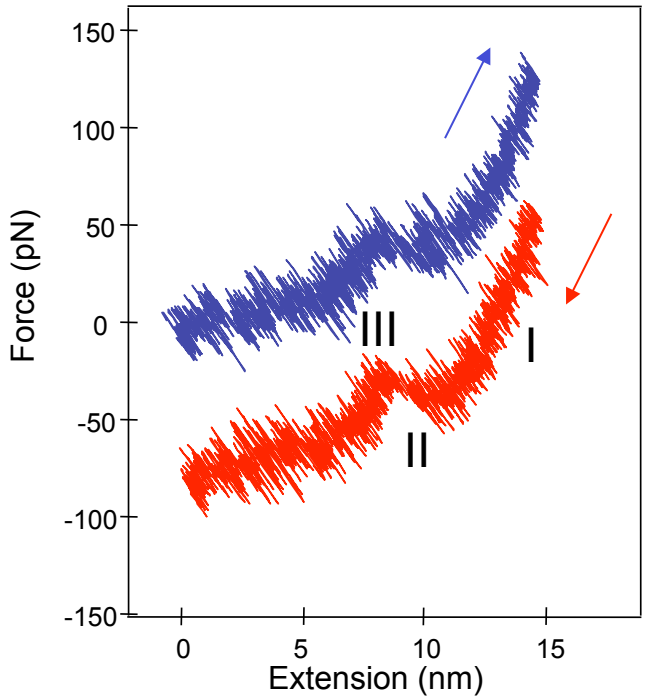






Pulling-relaxing cycles





Force generation against load

Shuttling against 30 pN

↓

Work done by the ring: ~ 6 kcal mole^{-1}

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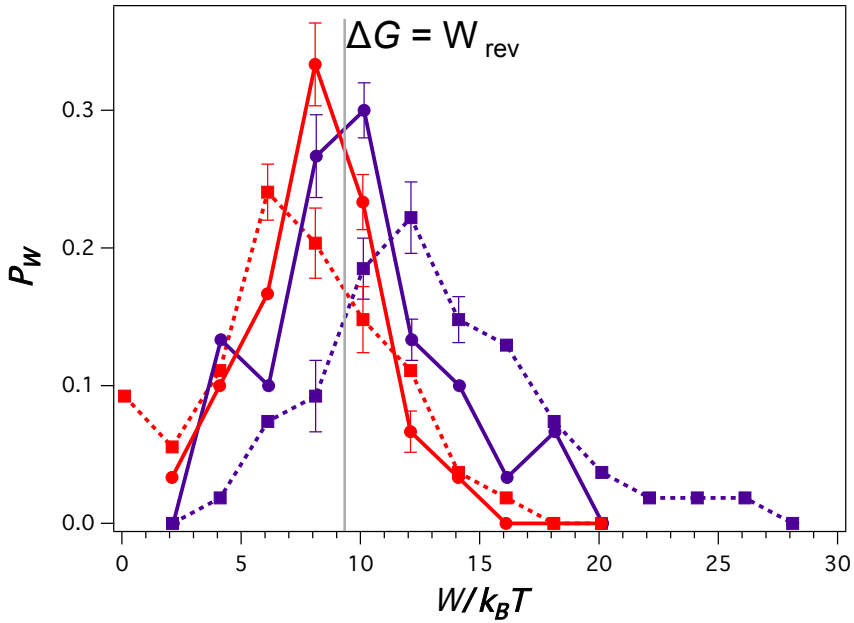
→ **Yes, sub-molecular Brownian motions in a single synthetic small machine can be harnessed to generate forces**

→ For a loading rate of 500 pN s^{-1}, the rotaxane is able to utilize almost all the energy available from hydrogen bonding to perform work along the direction of the applied load

The principles of thermodynamics describe processes of energy exchange (work and heat) of macroscopic systems with their environment

Nonequilibrium systems are characterized by irreversible heat losses between the system and its environment, typically a thermal bath



A single molecule can save heat loss to produce work

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Université de Liège

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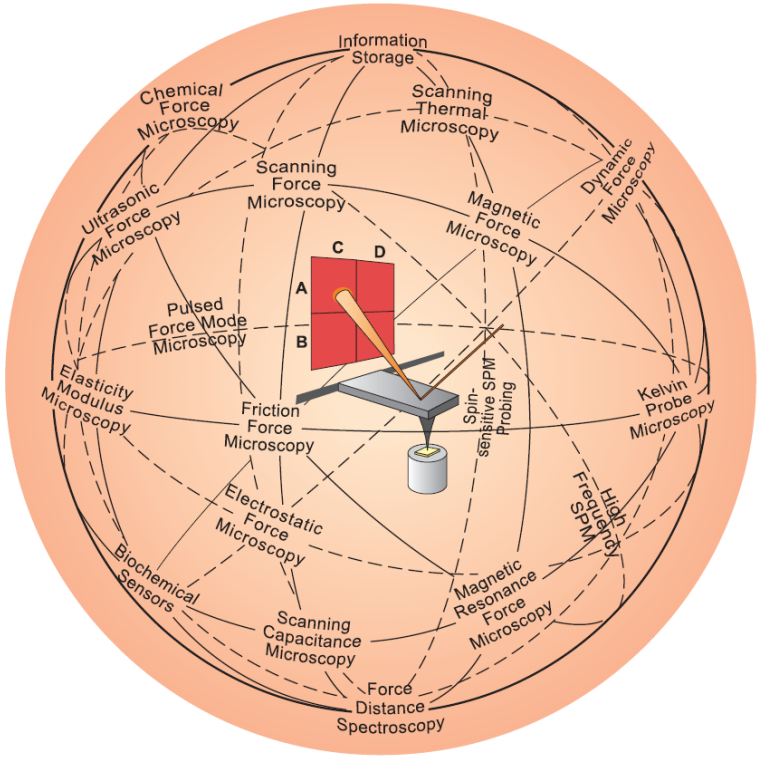
MAY THE FORCE BE WITH YOU …



From Ch. Gerber and H. P. Lang, *Nature Nanotech.* **2006**, *1*, 3