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

Anne-Sophie Duwez

Nanochemistry & Molecular Systems, Department of Chemistry
University of Liège, B6a Sart-Tilman, 4000 Liège, Belgium
http://www.nanochem.ulg.ac.be
Erwin Schrödinger wrote that we would never experiment with just one electron, one atom, or one molecule


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

Photos: Copyright © The Nobel Foundation

1986: Binnig, Quate, and Gerber proposed the AFM

1990: Positioning single atoms with the STM

D. M. Eigler, E. K. Schweizer,
Modification of the STM tip apex is known to have a profound influence on the achievable image resolution. As will be shown below, both problems are visible because of the interaction of the CO molecule with the tip apex. The exact knowledge of the tip termination also facilitates quantitative comparison with first-principles calculations, which is essential for understanding the nature of the electronic states of the substrate. STM is sensitive to molecular orbitals near the Fermi level, whereas standard scanning tunneling microscopy (STM) studies have been performed recently. On insulating films, such as NaCl on Cu(111), pentacene on metal, such as Cu(111), has been investigated over the past few years. STM as a microscope tool.

For atomic resolution with the AFM, it is desirable to work with a cantilever of high stiffness and a small tip-sample distance. The bias voltage was attached to measure the tunneling current. For these measurements, the cantilever had to have a high stiffness of NaCl on Cu(111) as substrate (Fig. 2). Furthermore, the high stiffness of the cantilever is necessary to operate in the short-range regime of oscillation amplitudes down to 0.2 Å. A resonance frequency of a pentacene-modified tip is ≈5 kHz, whereas extended over 0-5 Å, we explored vertical manipulation of the molecule during imaging. As will be shown below, both problems point out by Giessibl. For these measurements, the cantilever had to have a high stiffness of NaCl on Cu(111) as substrate (Fig. 2). Furthermore, the high stiffness of the cantilever is necessary to operate in the short-range regime of oscillation amplitudes down to 0.2 Å. A resonance frequency of a pentacene-modified tip is ≈5 kHz, whereas extended over 0-5 Å, we explored vertical manipulation of the molecule during imaging. As will be shown below, both problems point out by Giessibl.

The bias voltage was attached to measure the tunneling current. For these measurements, the cantilever had to have a high stiffness of NaCl on Cu(111) as substrate (Fig. 2). Furthermore, the high stiffness of the cantilever is necessary to operate in the short-range regime of oscillation amplitudes down to 0.2 Å. A resonance frequency of a pentacene-modified tip is ≈5 kHz, whereas extended over 0-5 Å, we explored vertical manipulation of the molecule during imaging. As will be shown below, both problems point out by Giessibl.

For atomic resolution with the AFM, it is desirable to work with a cantilever of high stiffness and a small tip-sample distance. The bias voltage was attached to measure the tunneling current. For these measurements, the cantilever had to have a high stiffness of NaCl on Cu(111) as substrate (Fig. 2). Furthermore, the high stiffness of the cantilever is necessary to operate in the short-range regime of oscillation amplitudes down to 0.2 Å. A resonance frequency of a pentacene-modified tip is ≈5 kHz, whereas extended over 0-5 Å, we explored vertical manipulation of the molecule during imaging. As will be shown below, both problems point out by Giessibl. For atomic resolution with the AFM, it is desirable to work with a cantilever of high stiffness and a small tip-sample distance. The bias voltage was attached to measure the tunneling current. For these measurements, the cantilever had to have a high stiffness of NaCl on Cu(111) as substrate (Fig. 2). Furthermore, the high stiffness of the cantilever is necessary to operate in the short-range regime of oscillation amplitudes down to 0.2 Å. A resonance frequency of a pentacene-modified tip is ≈5 kHz, whereas extended over 0-5 Å, we explored vertical manipulation of the molecule during imaging. As will be shown below, both problems point out by Giessibl. For atomic resolution with the AFM, it is desirable to work with a cantilever of high stiffness and a small tip-sample distance. The bias voltage was attached to measure the tunneling current. For these measurements, the cantilever had to have a high stiffness of NaCl on Cu(111) as substrate (Fig. 2). Furthermore, the high stiffness of the cantilever is necessary to operate in the short-range regime of oscillation amplitudes down to 0.2 Å. A resonance frequency of a pentacene-modified tip is ≈5 kHz, whereas extended over 0-5 Å, we explored vertical manipulation of the molecule during imaging. As will be shown below, both problems point out by Giessibl.
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
Single Molecule Force Spectroscopy

Mechanical response of a single chain

Specific bond rupture

Force (pN) vs. Extension (nm)

In solution
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

Average behaviour on billions of molecules

Unit: mole
Fundamental applications: Exploration of single entities

Exploration of single entities

Sub-groups of molecules

Unit: 1 single molecule

Different behaviour of the same molecule
Molecular interactions

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

Single molecule force spectroscopy

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

Single molecule patterning

Langmuir 2010, 26, 12165
Langmuir 2012, 28, 2971

Molecular elasticity and Interactions with surfaces

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

Molecular sensors

Small 2008, 4, 1101

Stimulus

Molecular machines
• Single molecule patterning

• Molecular interactions

• Molecular machines
→ 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
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

18% of the cases
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
Single-Molecule Cut-and-Paste Surface Assembly

S. K. Kufer, E. M. Puchner, H. Gumpp, T. Liedl, H. E. Gaub

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.
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 5000 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
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 nano-newtons, 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

~ a few nm

~ a few 10 nm
Probing a metallo-supramolecular interaction

$\text{Fe}^{2+}, \text{Ni}^{2+}, \text{Co}^{2+}$
**Molecular Interactions**

<table>
<thead>
<tr>
<th>Force (pN) in water (8 nN s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEO-Ni-PEO 64 ± 10</td>
</tr>
<tr>
<td>PEO-Co-PEO 45 ± 8</td>
</tr>
<tr>
<td>PEO-Fe-PEO 38 ± 8</td>
</tr>
</tbody>
</table>

Thermodynamic binding constants in water

- **Terp-Ni²⁺**  5.0 x 10¹⁰ M⁻¹
- **Terp-Co²⁺**  2.5 x 10⁸ M⁻¹
- **Terp-Fe²⁺**  1.3 x 10⁷ M⁻¹
Metal exchange in a metallo-supramolecular chain

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

**PEO-Fe-PEO**

After exchange with NiCl$_2$
Metal exchange in metallo-supramolecular chains

Exchange of $\text{Fe}^{2+}$ with $\text{Ni}^{2+}$: kinetics
• Single molecule patterning
• Molecular interactions
• **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.
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?
In collaboration with David A. Leigh, Univ. Manchester and Charles-André Fustin, UCL
Interfacing molecular machines with the AFM

In collaboration with David A. Leigh, Univ. Manchester
and Charles-André Fustin, UCL
Interfacing molecular machines with the AFM

Pulling curves

Rupture of H-bonds

TCE
45 ± 10 pN

DMF
27 ± 8 pN

Nature Nanotech. 2011, 6, 553
Molecular machines

Mechanical response of a single chain

Specific bond rupture

Pulling

Relaxing

Force (pN) vs. Extension (nm)
Pulling-relaxing cycles

Force generation against load
Shuttling against 30 pN
Work done by the ring: ~ 6 kcal mole\(^{-1}\)

Nature Nanotech. 2011, 6, 553
→ 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.

\[ \Delta G = W_{\text{rev}} \]

A single molecule can save heat loss to produce work.
Thanks to

- C. Jérôme, F. Remacle (University of Liège)
- C. A. Fustin (Université catholique de Louvain)
- D. A. Leigh (University of Manchester, UK)
- C. Jarzynski (University of Maryland, MD, USA)
- J. F. Stoddart (University of Northwestern, IL, USA)
MAY THE FORCE BE WITH YOU …

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