

PLAYING WITH FORCES AND INTERACTIONS TO MANIPULATE SINGLE MOLECULES

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ABSTRACT

Molecular manufacturing is a technology that will allow us to assemble molecular machines and build complex objects atom by atom. The use of scanning probe microscopy-based techniques to manipulate single molecules^[1], to detect binding processes^[2], or to deliver molecules in a precisely controlled manner to a specific target^[3,4] represents a significant step in that direction. It requires the controlled formation and breaking of individual bonds.

Here we show that the atomic force microscope (AFM) can deliver and immobilize single molecules, one at a time, on a surface. Reactive polymer molecules, attached at one end to an AFM tip, are brought into contact with a substrate to which they become linked by a chemical reaction. When the AFM tip is pulled away from the surface, the resulting mechanical force causes the weakest bond –the one between the tip and polymer– to break. This process transfers the polymer molecule to the substrate.

We also show examples of the use of those AFM tips bearing reactive polymers for molecular recognition applications. We have covalently attached proteins or ligands to those tips to obtain probes sensitive to specific molecular interactions.

We can imagine that the functional principles and concepts found in molecule manipulation by AFM, i.e. playing with mechanical forces, with strong and weak complementary interactions, could be implemented to attach and detach units in the space field.

1 INTRODUCTION

Since its invention in the late 1980s, the atomic force microscope (AFM) has increasingly been used for the visualization of molecular systems and complex biological structures. The tight attachment of (bio)molecules onto AFM tips has opened up the exciting possibility of detecting binding processes at the single molecule level.^[2] Making and breaking bonds in a controlled way is strongly dependent on the design and accurate functionalization of the probes.

AFM tips functionalized with end-grafted molecules offer the prospect of delivering individual molecules in a single-molecule force spectroscopy experiment if the bond anchoring the molecule to the tip is weaker than the one to be established with the surface. In order to achieve this goal, we developed a strategy based on (1) the grafting of macromolecules bearing reactive groups onto the AFM tip and (2) their selective transfer, via a chemical reaction, to a substrate where complementary moieties are present.^[5]

AFM tips with reactive macromolecules can also be used for recognition experiments on surfaces where receptors are grafted. We demonstrate that such tips are a robust basis for single molecule recognition between host-guest systems.^[6]

2 SINGLE MOLECULE DELIVERY EXPERIMENTS

Gold-coated AFM tips were modified by electrografting poly-*N*-succinimidyl-acrylate (PNSA), according to ref. 7. This electro-initiated polymerization is a convenient way to fabricate polymer brushes with a moderate grafting density and results in the direct chemisorption of the polymer onto the tip surface. The cantilever to be grafted is simply dipped into the monomer solution and is selectively polarized on the tip side in the cathodic range until the so-called 'grafting peak' is observed, using a classical three-electrode setup.^[7,8] It is an electro-initiated process that requires the presence of a few charges only for the grafting step. The chain propagation that follows is a chemical process that does not need current for being sustained. The polymer selected for grafting is PNSA. The choice is based on the high room temperature reactivity of the activated esters along the backbone, paving the way to further easy coupling reaction with nucleophilic compounds, both in water and in organic solvents.^[9]

Substrates where complementary moieties are present were also prepared. As the activated esters along the polymer backbone can easily react, at room temperature, with amino-derivatives, aminopropyltri-methoxysilane was grafted to silicon substrates to obtain an amino-terminated surface. See ref. 10 for more details. In a DMF solution containing 4-dimethylaminopyridine (DMAP, a catalyst), the functionalized AFM tip was slowly brought into contact with the surface. The chemical reaction between the PNSA activated esters and the amino groups of the substrate forms amide bonds and covalently links polymer chains to the substrate. Upon retraction of the tip, single chains are stretched until a bond breaks.

The Au(tip)-C(polymer) bond is the weakest link in the system and the most likely candidate for breaking. Upon cleavage, the polymer chain remains covalently attached to the substrate (Figure 1). The deposited chains are reactive and can be easily post-functionalized by a wide range of nucleophilic compounds. The stretching of the polymer chains and the mechanical breaking of the Au-C bond, and thus the successful delivery are monitored through force-distance curves.

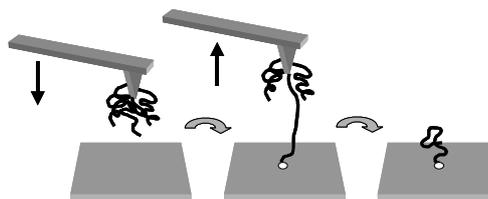


Figure 1. Molecule by molecule delivery process: PNSA chains grafted to the Au-coated tip are brought into contact with an amino-modified silicon substrate to which they can become linked through the formation of an amide bond, which covalently links the chain to the substrate. When the tip is pulled away from the surface, the resulting mechanical force causes the weakest bond—the one between the tip and polymer—to break. Adapted from ref. 5.

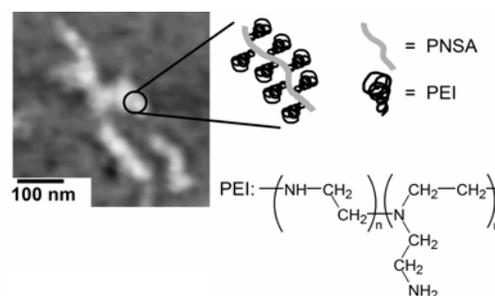


Figure 2. AFM topography image obtained in air in the area where 4 PNSA chains were deposited one at a time. The original chains were decorated by a branched polyethyleneimine (PEI), rinsed with DMF, and imaged before the residual film of DMF was completely evaporated. The decorated molecules appear in an extended shape. Maximum vertical height: 4 nm. Adapted from ref. 5.

3 MOLECULAR RECOGNITION EXPERIMENTS

Recognition experiments between two complementary host-guest molecules can

be realized through force spectroscopy measurements between one partner grafted onto the AFM tip and the other partner grafted onto a surface (Figure 3). In order to carry out reliably force spectroscopy measurements, a series of experimental requirements are to be met. First, the binding force of the chemical or biological species onto the tip has to be stronger than the investigated interaction strength. Second, the presence of a long-chain spacer linking the species to the tip is required to avoid the interference of non-specific adhesion forces, which otherwise dominate when tip and surface are close to each other. PNSA was grafted from the tip.^[8] As a result, chemisorption ensures strong bonding to the tip, polymerization provides a long-chain spacer, which enables free orientation of the species, while the presence of *N*-succinimidyl moieties allows for further functionalization since *N*-succinimidyl activated esters react easily at room temperature with amine functions of whatever protein, giving rise to amide bonds. We recently showed that this polymer could be grafted in an isolated chain regime, resulting in the grafting of only one linker at the tip apex, paving the way to its use as a generic platform to probe molecular interactions.^[8]

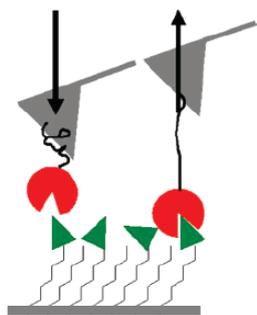


Figure 3. Schematic representation of a molecular recognition event investigated by single molecule force spectroscopy experiment

One system consists of the P.69 pertactin protein (also known as antigen 69K) grafted onto a surface recognizing its complementary antibody, anti-69K. Antigen 69K is a compact globular protein located in the outer membrane of *Bordetella pertussis* and is a key component of acellular vaccines against

whooping cough. The second system is based on the surface-grafted antibody 286F7, which is directed toward the thyroid stimulating hormone (TSH, also known as thyrotropin). TSH is produced by and stored in the pituitary gland, which is located beneath the brain, and its detection is used for the diagnosis of problems affecting the thyroid gland.

Force-distance curves were recorded for the two ligand-receptor pairs. The curves exhibit rupture peaks far away from the adhesive force region, which reflect the antigen-antibody bond rupture.^[2] The value of the force at this peak is interpreted as the “unbinding force” of the antigen antibody complex, which dissociates when the applied pulling force exceeds the bonding strength.^[2] The distance at which the bond rupture occurs depends on the position of the protein on the PNSA linker. For a given tip, it should thus always occur at the same distance.

The measurement of a large number (more than 250) of force curves, all recorded at the same loading rate allowed us to construct force histograms. They display a close-to-Gaussian profile, whose maximum provides the most probable antigen-antibody rupture force. The force distribution recorded for the 69K Ag/anti-69K Ab couple gives a most probable unbinding force of 256 pN at a loading rate of 100 nN s^{-1} . Likewise, the most probable unbinding force between 286F7 Ab and TSH is 76 pN.^[6]

In order to confirm that the measured forces do correspond to the rupture of the antigen-antibody complex, the force-distance curves were compared with those recorded between tip and surface systems not expected to interact with each other. In particular, force-distance curves were recorded between a 69K Ag grafted surface and an unmodified PNSA tip, between a methyl-terminated, self-assembled monolayer surface and a PNSA tip modified with anti-69K Ab, and between a 69K Ag functionalized surface and a PNSA tip modified with 286F7 Ab. In that case, the retraction curve is characterized by a flat line where only small adhesive forces sometimes appear, testifying for the absence of any specific

interaction established between these incompatible pairs.

Based on all these arguments, it can thus be concluded that the rupture peaks observed are indeed due to the specific interaction between the complementary antigens and antibodies.

4 CONCLUSIONS

Molecular manufacturing is a technology that will allow us to assemble molecular machines and build complex objects atom by atom. This ultimate goal may be a long way off, but recent developments in single molecule manipulation with AFM-based techniques move us closer to the idea of positioning individual atoms and molecules. Richard Feynman said in his famous talk in 1959: "The principles of physics, as far as I can see, do not speak against the possibility of maneuvering things atom by atom."

Adding positional control to chemical reactions represents a significant step towards molecular manufacturing. Our current research activities focus on the development of AFM techniques to make molecules go where we want, put them where we want, and make them react as we want.

The controlled manipulation of molecules requires the formation and breaking of targeted individual bonds. We can imagine that the functional principles and concepts found in molecule manipulation by AFM, i.e. playing with mechanical forces, with strong and weak complementary interactions, could be implemented to attach and detach units to spacecrafts.

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