headline

Mechanical nanomachines

Made of a single molecule or of a complex assembly of well assembled molecules at the molecular scale, nanomachines mimic the functions of macroscopic scale mechanical machines. Understanding and mastering these machines is the goal of a group of researchers from Toulouse in disciplines like chemistry, biology and physics.

>>> Christian JOACHIM, CNRS senior scientist at CEMES, (CNRS lab associated to UPS), Laurence SALOMÉ, CNRS senior scientist directrice at the IPBS (joint UPS/CNRS lab) and Christophe VIEU, professor at INSA and researcher at LAAS (CNRS lab associated to UPS). © Cyril Frésillon/CNRS.

Miniaturization is everywhere today: in cars, helicopters, satellites, calculators, memories and phones. We are now starting to construct real nanomachines for applications in biology, mechanics and electronics.

Craftsmen and engineers began developing their technical knowledge in the field of miniaturization a long time ago. We are still amazed by Anticythere's astronomical clock that represented a miniature version of the solar system in the house of the Greek philosopher Hipparchus. It was made of a few tens of gears in bronze and measured several centimeters in diameter. From Arab scientists to the clock craftsmen at the end of the middle ages and revisited by Blaise Pascal for his calculating machine, these miniature machines remained the same for centuries without reducing further in size. First invented for microelectronics, monolithic technology gave a new direction to miniaturizing mechanical and electronic devices. It is now possible to fabricate solid state material gears with a diameter smaller than 100 nm. Then, a new question appeared at the beginning of this century: starting from this scale, can we continue to fabricate and make rotating wheels, to assemble trains of gears or to construct mechanical machineries that are tinier still?

Smaller and smaller

This question is of interest for machine technology because it is generally taken for granted that reducing the overall size of a machine will improve its response time or its energy efficiency compared to the non-miniaturized version of the machine. This question also sheds new light on old physical principles, like the quantum superposition principle or the second law of thermodynamics. Since the middle of the 1970s, and thanks to the pioneering research of P. Boyer (1997 Nobel Prize in Chemistry), we now know that Mother Nature is already an expert in this field. Certain very basic life processes use complex macromolecular machinery, relying on protein assembly, for example, to create motion.

A new word thus appeared in the scientific world: "nanomachine". For some, a nanomachine is a machine that is just a few nanometers across. For others, it is a miniature machine whose elementary parts are fabricated with a precision on the order of a few nanometers. The six articles in this special issue describe nanomachines that are very representative of both these definitions and which are being investigated at Toulouse Laboratories.

Systems and approaches

At UPS, several biology teams have dissected nanomachines found in living matter using in vitro experimental approaches. For example, at IPBS, two teams are working on DNA machinery responsible for DNA replication and recombination, two processes very essential to maintaining and controlling genome evolution. To study DNA replication, researchers are analyzing the work performed in a cell by protein nanomachines, mapping the synthesized DNA molecules one-by-one. The method used is called molecular combing, but a potentially more efficient approach is being developed at LAAS. The mechanics of recombination are also being studied by a second group working in collaboration with colleagues from LMGM. Their approach is based

>>> An artist's view of nanomachines. Background: photo of Anticythere's astronomical clock (2nd century BC, Athens National museum).

on the observation of work performed by nanomachines by detecting changes induced in DNA molecules over time. This is accomplished by a molecular "jokari", or the technique of "tethered particle motion". A different version of this tool, the magnetic clamp, allows researchers to study the behavior of biological nanomachines when force is applied. It is used by a team from LMBE to solve migration mechanisms in a Holliday junction that exists between two DNA molecules exchanging their threads.

At LAAS, we are studying an even more complex protein machine, made of hundreds of proteins assembled in a flagella engine, 45 nm in diameter. This natural engine is the base of the bacteria thread and allows propulsion at impressive speeds (60 times a bacterium length per second). We aim to understand how this nanoengine works and set up technology able to reproduce this natural nanomachine in an artificial environment. We are also studying why such mechanisms are so efficient and are trying to discover how this nanomachine assembles itself. To understand its mechanics and its assembly, nothing beats trying to re-assemble it from its basic elements, proteins!

At CEMES, the Nanosciences group is approaching the nanomachines question from the bottom up. Instead of using standard miniaturization tools and fabricating smaller and smaller machinery, the scientists are starting from the atoms themselves. They are increasing the number of atoms in the machine to find the exact number of atoms required to construct a gear, a rack and pinion machinery, and even a car. This approach was born in Toulouse. For a molecular machine to work, the GNS researchers are using a scanning tunneling microscope, invented in 1981. It is able to map the surface of a metal (or a semiconductor) with a precision better than 0.01 nm. After having fabricated a very sharp tip, we can manipulate a single atom or a single molecule one at a time and then "feed" the molecular machine with the energy needed for it to function.

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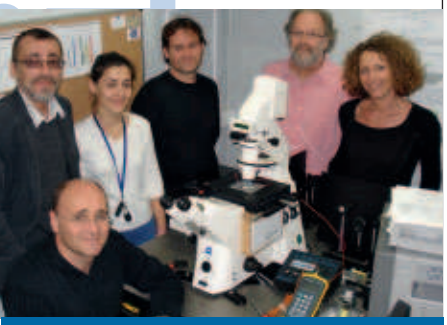
CEMES: Centre d'Elaboration de Matériaux et d'Etudes Structurales/ Center for Material Elaboration and Structural Studies
IPBS : Institut de Pharmacologie et Biologie Structurale/ Institute of Pharmacology and Structural Biology
LAAS : Laboratoire d'Analyse et d'Architecture des Systèmes/ Laboratory for Analysis and Architecture of Systems
LBME : Laboratoire de Biologie Moléculaire Eukaryote/ Laboratory of Eukaryotic Molecular Biology
LMGM : Laboratoire de Microbiologie et Génétique Moléculaires/ Laboratory of Microbiology and Molecular Biology

The nanomachines of genetic recombination

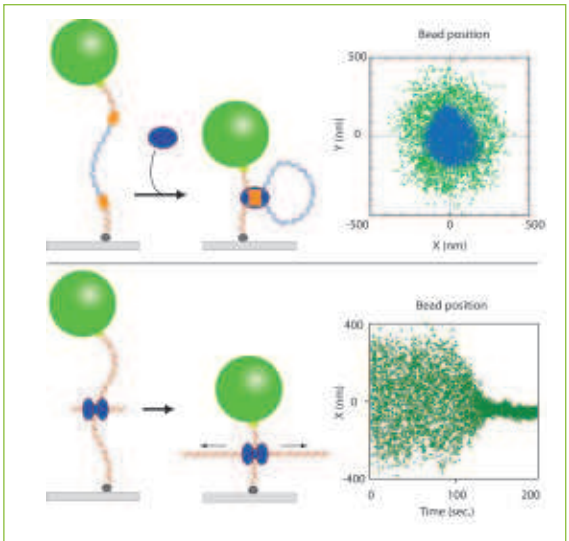
During the life of a cell, DNA, which stores hereditary information, is rearranged either spontaneously or in response to damage such as chemical attack or radiation. The recombination consists of successive breaks and ligation reactions performed by proteic complexes, true living nanomachines. By exchanging DNA strands, these machines are responsible for genetic variation, the first step towards evolution.



>>> from left to right : Philippe ROUSSEAU, assistant professor at UPS and researcher at LMGM (joint CNRS/UPS lab), Mikhaïl GRIGORIEV, INSERM senior scientist at LBME (joint CNRS/UPS lab), Catherine TARDIN, assistant professor at UPS, researcher at IPBS, François Cornet, CNRS senior scientist at LMGM, Michael CHANDLER CNRS senior scientist at LMGM and Laurence SALOMÉ, CNRS senior scientist at IPBS.

On the Paul Sabatier University campus, three research groups investigate, using single molecule approaches, different types of genetic recombination: transposition, specific-site recombination and homologous recombination, each catalyzed by its proper enzymatic machinery.

Transposases catalyze the displacement of DNA sequences from one position to another in the genome (transposition). Such sequences are abundant in all genomes (they make up 40% of the human genome), so understanding how these machines function is the objective of the LMGM research team and a major challenge. Moreover, the ability to control the transposition process in vitro has important applications in healthcare (such as in gene therapy, for example).



>>> The movement of a bead attached to the free end of a DNA molecule allows to monitor the action of biological nanomachines. Top: assembly of two specific sites along a molecule by a transposase or a recombinase form a loop accompanied by a decrease in the amplitude of bead movement. Bottom: a helicase catalyzes the migration of the junction between two DNA molecules exchanging their strands, which is revealed by a continuous decrease in the amplitude of bead movement.

Recombinases operate recombinations between chromosomes at specific locations of identified sequences. For example, the recombinases XerC and XerD, studied by another team at LMGM, separate the chromosomes in dimers formed accidentally during the replication in E. coli. By restoring the integrity of the genome, this machine assures the survival of bacteria affected by this genetic anomaly.

Helicases, studied by a team at LBME, assemble to form the motors driving strand-exchange between a pair of similar DNA molecules. This process of homologous recombination, which takes place in response to breaks in the DNA double helix, is essential for DNA repair and maintaining genome integrity.

Watching the recombination machines at work

First attempts to characterize these biological machines made use of conventional biochemical methods. These experiments revealed that recombination involves successive, cleverly orchestrated steps and that self-assembly of the machineries is crucial for efficient function and regulation. To unravel the structural details and dynamics of the processes, our teams have recently introduced single molecule methods.
The Tethered Particle Motion technique, developed at IPBS, tracks the displacement of a nanoparticle attached to the free end of a DNA molecule anchored on a glass slide at the other end. The dynamics of the DNA molecule and consequently changes in its length, rigidity or curvature, can thus be monitored. This technique is highly sensitive and allows us to detect the binding of a single protein onto the DNA molecule. Important results have been obtained in these experiments. For example, we have demonstrated the formation of a loop as the first step in the transposition mechanism and measured the migration velocity of a Holliday junction (site of cross-strand exchange). Using a micron-sized magnetic particle, a force can be applied to the DNA molecule. Introducing torsion in the DNA molecule using this method, known as "magnetic tweezers", is employed by the LBME team to improve our understanding of helicase as well as other enzymes. Researchers in the LMGM and IPBS teams complete these studies of the recombinases and transposases with direct visualization of their action on DNA molecules by means of atomic force microscopy (ITAV platform).

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Nano techniques to study DNA Replication

Recent advances in techniques that allow the nanomachinery behind replication of individual DNA fibers to be observed open up new perspectives. Goal: to better understand the links between replication defects and genetic instability.

>>> Aurélien BANCAUD, CNRS research scientist at LAAS.

>>> Marie-Jeanne PILLAIRE, INSERM research scientist at IPBS and Jean-Sébastien HOFFMANN, INSERM senior scientist at IPBS.

DNA replication machinery is a crucial nanobiological complex that efficiently duplicates the entire human genome before chromosomal partition. This process begins in multiple specific domains called "origins of replication" and progresses via replication forks, multicomponent protein complexes, including DNA polymerases, which ensure the accurate duplication of genomic DNA. DNA replication represents a dangerous moment in the life of the cell as endogenous (structured chromosomal domains) and exogenous events (environmental genotoxic agents) challenge genome stability by interfering with the progression of replication forks. Failure to protect stalled forks or inappropriate replication processing results in the accumulation of genomic aberrations, leading to different human pathologies. This is particularly true in cancer lesions where chronic replication stress occurs, causing high-level genetic instability and, in turn, to increased malignant progression and chemotherapy resistance. However, the molecular mechanisms involved in this replication stress in cancer cells have remained elusive as the replication profiles vary considerably from one cell to another and biochemical approaches failed to give a clear picture of the deficiencies involved.

Analysis of DNA replication by DNA Combing

Single-molecule nano techniques have proven to be powerful approaches to monitoring DNA replication in a variety of organisms, including human cells. Unlike other replication assays, these techniques allow the direct visualization of individual replication forks progressing along single chromosomes. The recent development of these approaches provides a unique opportunity to understand the links between DNA replication defects and genetic instability, which play a central role in cancer, by identifying the origin of endogenous replication stress at early stages of cancer development and opening new windows for the development of original anticancer treatments that target DNA replication.

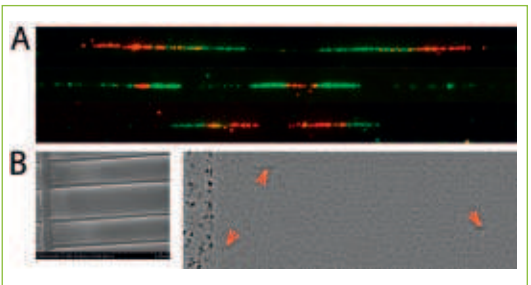
The more advanced version of these techniques, which is called DNA combing (CNRS-Pasteur Institute joint patent), has been developed in France by Dr. Aaron Bensimon and is used by a limited number of laboratories, including our Genetic Instability and Cancer laboratory at IPBS (joint UPS/CNRS lab).

DNA combing is a powerful single-molecule technique that allows the direct visualization of individual replication forks progressing along single chromosomes following incorporation of fluorescent nucleotides. This procedure, with a 0.005 Mb resolution, allows parallel and long DNA fibers (up to several megabases) to be stretched on silanized coverslips with a uniform extension of 2 kb/µm. The sites of DNA synthesis are detected along individual DNA fibers with monoclonal antibodies against nucleotide analogs. This technology, which can provide information regarding critical parameters of the replication process, such as origin density and fork speed, allowed us to identify one potential source for replicative stress in human tumors[1].

Nanochannels, an alternative and innovative approach

The Nano-Ingénieurie et Intégration des Systèmes group at the CNRS Laboratory LAAS has developed new methodological procedures based on nanomanufacturing as an alternative to combing. Thanks to the tools resulting from microelectronics, which make it possible to structure matter down to the nanoscale, it is now possible to design nanocapillaries of approximately 100 nm diameter. The DNA molecules inserted in these nanochannels are stretched longitudinally in a way that is comparable with combing. This alternative combing is dynamic since it does not require immobilization of molecules on surfaces, thus paving the way towards medical high-throughput applications.

(1) (Pillaire et al., Cell Cycle 2007, recommended article in Faculty of 1000 Biology).



>>> (A) DNA fibers with incorporated fluorescent nucleotides combed on coverslips. (B) Electronic microscopy of nanochannels engraved in silicon. The parallel structures measure ~150 nm width and height. The insert represents a fluorescent microscopy of DNA inside the nanochannels (red arrows) and other circular DNA molecules in macroscopic channels (left).

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From nanometer-sized wheels to molecular vehicles

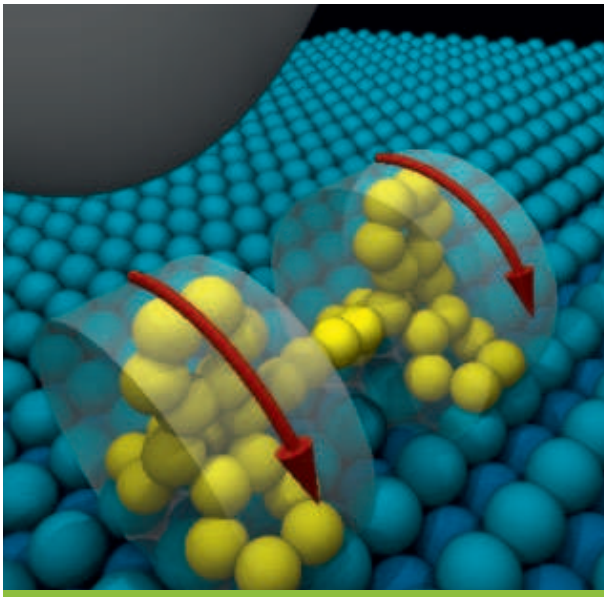
In the history of inventions, the wheel is at the heart of considerable scientific and technological development. Researchers at the CEMES have been among the first to design nanomachines equipped with wheels, thereby opening the way to a nanocar made of a single molecule.

>>> Christian JOACHIM, CNRS senior scientist; Henri-Pierre JACQUOT, PhD student; Gwénaël RAPENNE, associate professor at UPS, researchers at the Centre d'Elaboration de Matériaux et d'Etudes Structurales (CEMES, CNRS lab associated to UPS). © Cyril Frésillon/CNRS .

The use of wheel rotational motion around an axle led to mechanical machinery with multiple gearings, followed by engines and ultimately the industrial revolution. Reaching the nanometer size, which is the smallest possible dimension for a wheel, represents a great challenge for chemists and physicists. In the past few years, chemists at the CEMES have been working on the design and synthesis of molecular machines equipped with wheels. Step by step, they have been pioneers in this field, together with colleagues at the Free University in Berlin (Dr Leonhard Grill's group). The originality of their approach lies in confining the studies to one molecule only, chosen among a number of possible candidates, deposited on a metallic surface. With its ultrafine tip, which is stabilized 1 nm above the surface by an electric current induced by the tunnel effect, the scanning tunneling microscope (STM) produces a map of the molecules present on the surface. This tip also enables researchers to manipulate the molecules one by one in order to study their mechanical properties.

A wheelbarrow molecule

After the synthesis and observation of a wheelbarrow molecule in 2005 (a molecule composed of a rigid board, two legs ad two wheels), researchers showed in 2007 that when two wheels are mounted on an axle, one of them is able to rotate under the push of the STM tip. They succeeded in controlling the direction of the rotation, which opens the way to the synthesis of functional nanovehicles. Experimentally speaking, the molecules were carefully deposited onto a very clean copper surface, and observed by the means of an STM. The STM tip is used both as a probe to capture an "image" and as a nanosized finger to trigger the rotation of the wheel, being placed in the perfect position for that purpose (see figure).



>>> The two wheels-and-axle molecule is pushed by the tip apex (in gray) of the scanning tunneling microscope on a copper surface.

Towards a nanocar

Such wheels have an intrinsic drawback, they have three sharp blades instead of a smooth "tire". Researchers at the CEMES are currently developing a new family of rigid wheels, circular in shape and slightly curved, so as to minimize the mechanical interactions with the surface while increasing the rigidity of the architecture. These results open the way to the creation of mechanical molecular machines, with the long-term objective of placing all the machinery required to achieve a nanocar (wheels, chassis and engine) on a single molecule, to transport matter in the nanoworld.

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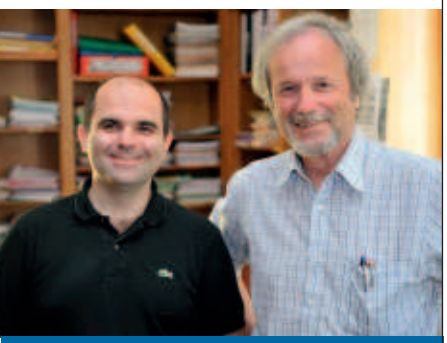
headline

A family of molecular motors

Despite the simplicity of motor operation, it is still a tremendous challenge to transform energy into motion and to conceive a nanomotor composed of a single molecule. Researchers at the CEMES have succeeded in doing just this, using the original electrostatic motor as a model.



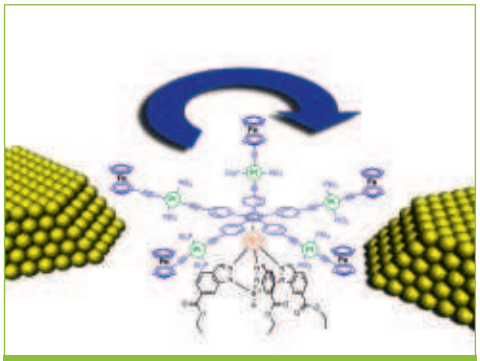
>>> Gwénaël RAPENNE, associate professor at UPS, Jean-Pierre LAUNAY, professor at UPS and head of the CEMES, researchers at the Centre d'Elaboration de Matériaux et d'Etudes Structurales (CEMES, CNRS lab associated to UPS). © Cyril Frésillon/CNRS.

In the field of nanoscience in general and molecular nanomechanics in particular, one current challenge is the conception and construction of nanometer-sized molecular motors, that is, machines that transform energy into work in a continuous fashion, through controlled unidirectional rotation. This motion should be reversible and big enough to observe and exploit.

Electrostatic motor

Motors synthesized at the CEMES were designed to be addressed individually. Their operating principle means connecting the molecule using two nanoelectrodes acting as electron tanks, as shown in the figure. The molecule is composed of a fixed part (stator) anchored onto the surface, and a mobile part (rotor) carrying oxidizable groups. Under external polarization, the positive electrode injects charges of identical sign into the mobile part of the motor, which then repulses the electrode and therefore induces rotation. This constitutes an electrostatic motor, whose operating principle was described as early as 1748 by Benjamin Franklin! The motor rotates by consuming the energy that results from the transport of electrons from a low electric potential zone to a high potential one. The asymmetry of the system allows the direction of rotation to be controlled.

Such motors have a piano stool structure. The fixed part (in black in the figure) is covalently bound to the surface. The mobile part is a platform (in blue) terminated by five electroactive groups. Successive electron transfer processes take place on the electroactive groups and induce rotation of the mobile part of the molecule in a certain direction. A ruthenium atom between these two parts acts as a joint, giving the motor an organometallic character. We have synthesized several molecules of different sizes and structures, illustrating the various criteria



>>> A family of molecular motors, a few nanometers in size, placed between two metallic electrodes built with atomic r esolution.

that should be met. First of all, the system should be as rigid as possible so as to avoid useless rotations that would waste energy in unwanted movements. Then, the rotation should be easy around the vertical axis but not at the expense of the integrity of the molecule, that is, the fixed and mobile parts should not be dissociated. Finally, the redox potentials of the various parts of the system should be compatible with the electron transfer processes required.

Several international collaborations have been established with complementary research groups in physics (Max Planck Institute, Stuttgart, and University of Ohio) in order to anchor the motor on specific surfaces and to study this electron-induced rotation. In the longer term these motors could incorporate nanometric robots able to accomplish various tasks, in medicine to everyday life, or to power the nanovehicles we are developing.

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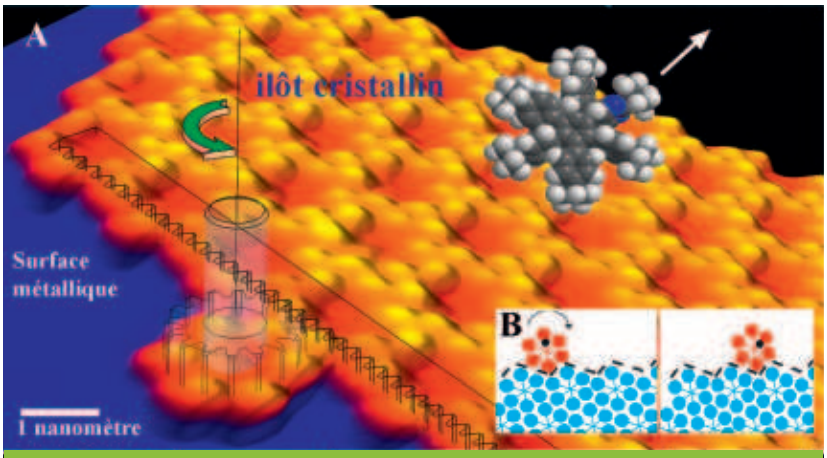
A molecular rack-and-pinion

To develop complex molecular machines, researchers need to master mechanics at the molecular scale. Experiments along these lines have been done with a view to controlling the rotation of a pinion on its molecular racks

>>> Christian JOACHIM and André GOURDON, CNRS senior scientists, members of the NanoSciences Group (CEMES, CNRS lab associated to UPS).

Designing and synthesizing molecules able to perform precise mechanical actions are key to developing future complex molecular machines made from nanometer-sized building blocks. To demonstrate this, researchers first needed to show that a mechanism as simple as a pinion able to roll on a rack could be made at the molecular scale.



>>> A – STM image of a 2D crystal of hexa-tert-butylpyrimidypentaphenybenzene; one of the molecules has been displaced using the microscope tip to engage it in the monolayer step-edge, which acts as a rack B- The rack and pinion concept C- The presence of nitrogen atoms, shown as white spots, allows to show the rotation of the pinion when moving along the step edge.

First, a six branch star-shaped molecule was designed and synthesized. In order to be able to follow the rotation of the molecule, one of the branches is chemically different from the five others with two nitrogen atoms in blue as shown in the molecular model (see figure). When a scanning tunneling microscope (STM) image of the molecule is recorded, this branch shows a large tunnel current contrast.
It is therefore possible to determine the orientation of the molecule in each tunnel image. These molecules are also able to self-assemble on an ultra-clean metallic surface. The molecules spontaneously form crystalline 2D islands made of a monolayer of perfectly organized molecules, 2D crystals of pinions. The step-edges of these crystalline islands keep the tooth-shape of the molecules and so can be used as a rack.

A tip to move the molecule

The mechanics experiment is carried out the following way: 1) An isolated pinion molecule is manipulated, using the STM tip, towards the rack so that at least one of the branches engage in the teeth of the molecular rack. 2) The STM tip is then placed vertically at the centre of the pinion. 3) The tip is brought close to the molecule to become its axis of rotation. 4) The tip is then displaced, step by step, parallel to the rack. The pinion molecule then starts turning around its axis as a function of the rack teeth it encounters during movement. This rotation can be observed either from the tunnel current or by imaging the pinion at each sixth of a rotation step. In this way, we have demonstrated that the concept of rack and pinion can be applied at the molecular scale. The mechanical movements of the molecule are classical in appearence. The rotation direction is controlled by the displacement of the tip along the rack. The next step will be to replace the STM tip by an atomic scale axis adsorbed onto the surface, to be able to build a gearing box and continue constructing complex molecular machineries.

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The flagellar nanomotor of bacteria

Bacteria have developed a complex yet highly efficient nanomachine to move: a rotary motor coupled to long flexible filaments, or flagella, acting as an helix. Today, a research team at LAAS is studying and rebuilding this machinery piece by piece with the help of nanobiotechnologies.

>>> Jérôme CHALMEAU, PhD and Christophe VIEU, professor at INSA, researchers in the NanoBioSystem group of LAAS (CNRS lab associate to UPS).

Microorganisms, like bacteria, have developed specific organs that perform all sorts of functions linked to survival. These organelles are amazing, complex molecular constructions that still cannot be imitated by man. Among them is the bacterial flagellar nanomotor, which allows the bacterium to move at high speeds. The motor is 2000 times smaller than the diameter of a human hair and 45 times smaller than the bacterium itself.

The flagellum can be compared to the helix of a boat. Its rapid rotation, on the order of 10000 revolutions per minute for some species, enables the bacterium to run 60 times its length per second. Nature, through evolution, has thus selected efficient and robust molecular architectures that are rotary motors.

The project of the LAAS team is twofold: first, the goal is to understand how these machines work. Second, to set up a technology that allows the machine to be assembled from its elementary components, proteins. However, the exact mechanisms behind this nanomotor are still a matter of controversy. In order to understand its behavior, we use tools from the emerging domain of nanobiotechnology to assemble this nanomachine brick by brick on an engineered surface and integrate it inside artificial devices.

Asssembling the bionanomachine

This approach, which involves fabricating the object itself, raises a major scientific question: is it possible to monitor the assembly of proteins on a surface and assemble natural nanomachines? The image shows a possible arrangement for the different parts of the flagellar nanomotor (stator and rotor), together with an experimental Atomic Force Micrscopy (AFM) image in liquid of one of the rings of the motor (M-ring) reassembled on a biomimetic surface obtained by soft nanolithography. These first encouraging results open the way for developing techniques capable of rebuilding natural bionanomachines and integrating them inside artificial devices such as biosensors or nano-cargos. The artificial assembly of such biological motors is an ambitious technological challenge that couples different state-of-the-art techniques: production and purification of membrane proteins, surface chemistry and self-assembly, nanolithography and dynamic imaging at the nanoscale in native conditions using AFM. This work is the fruit of a collaboration between LISBP (INSA Toulouse) and IPBS (UPS/CNRS).

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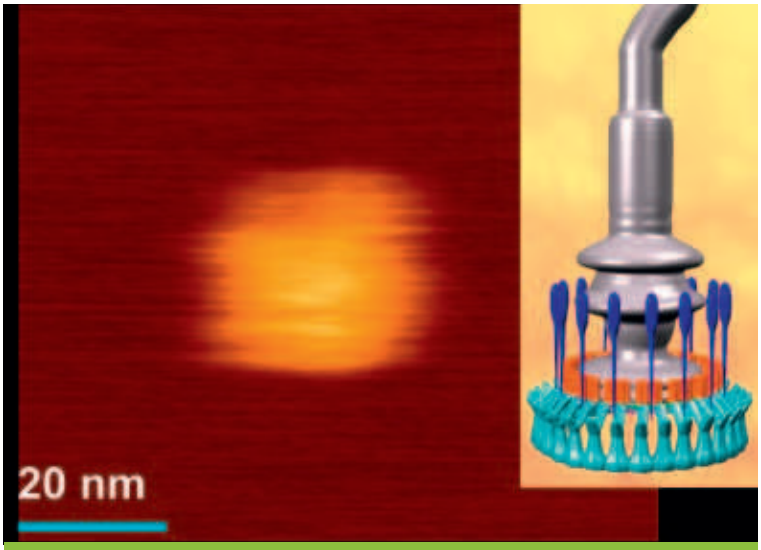

>>> On the right, a schematic view of a bacterial flagellar nanomotor. Flagellum base and rotor is in gray, stator in dark blue and orange, C-ring (in light blue) is responsible for the rapid change of rotation direction. On the left, an AFM image in liquid of the self-assembly of proteins making up the M-ring (FliG proteins) on a mimetic surface prepared by soft nanolithography on a glass slide.