Project no. **[505487-1]**

Project acronym: **BIOMACH**

Project full name: **"Molecular Machines – Design and Nano-Scale Handling of Biological Antetypes and Artificial Mimics"**

Instrument type: **SPECIFIC TARGETED RESEARCH PROJECT**

Priority name: [NMP-2002-3.4.1.1-3: Molecular and bio-molecular mechanisms and engines]

Publishable Final BIOMACH Activity Report M01-M39

Period covered: from **01.03.2004** to **31.05.2007** Date of submission: **15.09.2007**

Start date of project: **01.03.2004** Duration: **3 years+3 months prol.**

Project coordinator name: **Dr. Mario Ruben**
Project coordinator organisation name: **Institue of Nanotechnology**
Research Centre Karlsruhe

Summary

The second reporting period M01-M39 of the BIOMACH STREP was steered by the accomplishment of the substantial RTD activities, in particular the remaining Deliverables.

In detail, the following actions were taken during the second funding period M01-M39:

(i) Organisation of the **BIOMACH Kick-off Meeting** the 15th March 2004 at the Institute of Nanotechnology in Karlsruhe/Germany

(ii) Installation of the **BIOMACH- Project Office** at the INT/FZ Karlsruhe (Regina Schmidt, 1st June 2004)

(iii) successful recruitment of the scientific personal and start of the scientific work within the different groups of the partners (from 1st March 2004 ongoing)

(iv) preliminary on-line action of the **BIOMACH website** (Delivrable **DMa-2** (M09))

(v) organisation of the **BIOMACH-Midterm Assessment Meeting** in Ascona/Switzerland at 19th September 2005

(vi) organisation of the international conference **"From Molecular Switches to Molecular Motors"** from 20th- 24th September 2005 gathering the leading scientific players on the field of molecular motors within the framework of BIOMACH.

(vii) dissemination of the generated knowledge by the publication of **44** scientific reports (see www.biomach.org/publications.html) in peer-reviewed journals, by releasing **five press releases**, through **three emissions** in the German and Dutch broadcast and a multitude of secondary scientific presentations of different levels of dissipation, in particular by secondary reports in non-scientific journals (see also annex "**BIOMACH plan of dissemination of knowledge**").

(viii) supervision and controlling of the progress of the BIOMACH-Project by **four BIOMACH-Project Steering Committee Meetings**.

(ix) **Cost-neutral prolongation of BIOMACH** by three months in order to accomplish several ongoing research projects, in particular in WP 2, 3 and 5.

(x) the **BIOMACH-Final Meeting** took place under participation of the EC and of industrial partners the 24th April 2007 in Baden-Baden/Germany

(xi) scheduled preparation of all scientific **13 delivrables**, **two management deliverables**, **five BIOMACH Activity and Management Reports** (first, second, third, midterm and final reports) and **two audits**.

Chapter 1 – Project Execution

General Situation BIOMACH RTD Activities

During the BIOMMACH funding period M01-39, the main objective of the BIOMACH-STREP consortium consisted in the accomplishment of the RTD activities of the BIOMACH Project. All partners have driven actively the scientific work within the BIOMACH work packages. Initiated by the several BIOMACH activities, several internal meetings of different work groups have taken place to intensify the interdisciplinary interaction within the work packages. In order to finish several of the ongoing RTD activities (in particular within the work packages 1, 3 and 5), the BIOMACH Project Steering Committee decided to ask for a cost neutral prolongation of the project by three months, which was accorded by the EC (12th February 2007). By this way, all scientific tasks could be accomplished and all deliverables were delivered to the EC. The BIOMACH consortium has disseminated several of the BIOMACH results to broad public, in particular within the amended time period. Because of the impressive amount of new results generated by BIOMACH, dissemination of knowledge will even go on after conclusion of the project.

In the progressive conclusion of the work, the following items were delivered or were organized:

1) **13** scientific deliverables
2) **2** management deliverables,
3) **5** BIOMACH Activity and Management Reports (first, second, third, midterm and final)
4) **2** financial audits

In addition, the following scientific products were produced or disseminated to the public by:

5) **44** scientific publications (please see full list under see www.biomach.org/publications.html)
6) the **BIOMACH web site** www.biomach.org was established and continuously actualized
7) **2** patents (under submission) have spun off work initiated by BIOMACH
8) **3** international scientific conferences were organized by BIOMACH
9) **3** broadcasting emissions took place based on BIOMACH results

Overview over general BIOMACH objectives and achievements

Since its first translation into chemical terms by Feynman in his famous 1959 lecture [R. P. Feynman, *Plenty of Room at the Bottom*, 1959, http://www.its.caltech.edu/-Feynman/plenty.html], the concept of molecular machines and motors is still keeping a strong appeal to the imagination of scientists and engineers. However, only since the interdisciplinary collaboration of chemists, physicists and biologists under the term "nanotechnology" delivers the necessary scientific tools and materials, the transfer of this attraction into real projects has become feasible. The efficient conversion of (chemical) energy into mechanical work by specialized biological as well as by synthetic nanoengines enables a wide range of potential functions. Through suitable interfaces such nanomotors can be employed in synthetic nanodevices with potential applications in biosensing, self-assembly or molecular-scale actuation. Here, a brief overview about the last inside- and outside-BIOMACH developments towards such applications will be given.

The detailed understanding of the function of already existing molecular motors depends pivotally on the access to the single-molecular (or -motor) level. The controlled access will be only enabled by the further developments of the nowadays used **nanohandling** tools, mainly different kind of scanning probe techniques, in combination with cutting-edge surface science. Nanomachines of the future will require molecular-scale motors that can perform work. Towards this ends, the molecular motors have to be **handled at the nanoscale** in order to induce collectively induced controlled motion of much larger objects. During the last year, the scientific realisation of a device, which has introduced a motor system into a liquid crystal film and which can turn items thousands of times larger than a motor itself, marks a break through in future application of nanosystems [J. Vicario, *Nature* 2006, 440, 163]. Interestingly, the fueling of such system was carried out by light without producing any byproducts, which have to be removed in most of the biological systems. Thus, BIOMACH has continued to develop the technical set-up combining an UHV-STM with dual laser system, which might be a platform in the realisation of light-fueled molecular motors implemented at surfaces (see results of **WP 1**). However, this implementation was experienced to be more difficult as predicted, thus the respective Deliverable **D1-2** "Single-molecule investigation and control of anchoring rotaxane and LFMC units at surfaces" (M39) could only to be fulfilled after additional efforts within the prolongation period M36-39.

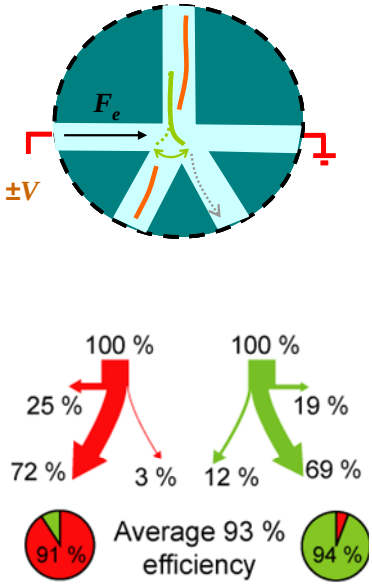

Figure 1: Main achievement of the third BIOMACH reporting period: Traffic control in the nanowrlrd through directional steering of natural molecular motor within a nanostructured environment [van den Heuvel et. al., ***Science*** 2006, 312, 910-913]

Self-assembly techniques are necessary for both the controlled implementation of molecular motors at the nano-level and its biological relevance concerning the mimicry of molecular motors which are inspired by Nature. The deliverable **D2-1** displays the in-side-BIOMACH progress on this field, in particular in the solution-based bulk experiments. Furthermore, BIOMACH has gathered considerable effort into the development of surface-assisted approaches, since it promises direct access to the nanohandling of such systems, as shown in deliverable **D2-2**. This joint efforts has lead to a cutting-edge publication in the field [P. Jonkheijm,et. al. "Probing the solvent-assisted nucleation pathway in chemical self-assembly" ***Science***, 313, 80-83, (2006)]. In surface-assisted self-assembly techniques the BIOMACH research work has routinely achieved nanometer-resolution by the BIOMACH partners MPI-FKF and EPFL using UHV-STM techniques and metallic surfaces. This technological advantage of BIOMACH has been used in the detailed investigation of self-assembled nano-structured molecular motors in light-induced switching processes; a central issue of WP 3. Furthermore, a self-organisation concept based on the steered coordination of metal atoms by taylor-made organic materials (so-called ligands) was described by BIOMACH partner FZK-INT [M. Ruben, *Angewandte Chemie International Edition*, 2005, 44, 1594-1596].

The search for alternative fueling concepts for molecular motors has defined as one of the key objectives within BIOMACH. However, as far we are aware and despite strong efforts world-wide, no scientific report using **electron-driven molecular motor at surfaces** has been realized. The **WP 3 – Electroengines** is mainly investigating the possibility to modify and to adapt already known rotaxane units and motors (which are have been proven to be fully operable in solution) to surface conditions. The BIOMACH consortium was converging towards such concepts within **WP 3** (and partially within WP 2, see also **D 3-1**) by fixing molecular rotaxanes and parts of them at bare or self-structured metallic surfaces (carried out by a cross-over collaboration of ULP, FZK-INT and MPI-FKF). The ongoing BIOMACH research efforts were described in detail in the Deliverable **D3-2** "Monolayers of rotaxanes working as molecular devices (M39)" based on work mainly achieved by BIOMACH-partner **ULP**. Here also, due to the complexity of the task, an additional time input was necessary to achieve the deliverable goals finally. However, the recent research effort carried out on silver surfaces by the **research collaboration FZK-INT/ ULP/ MPI-FKF** in WP 1 and 2 point eventually to an alternative future way towards surface-grafted molecular rotators (vide infra). Outside-BIOMACH, an interesting report has suggested a new, highly distributed machine architecture that mimics natural muscles in a very elegant way: A solid state NiTi wire loaded with Pt catalyst consumes oxygen and fuel, while the "muscle" itself supports the chemical reaction that leads to mechanical work [V.H. Ebron et.al. *Science* 311, 1580 (2006)] Interestingly, a diazo benzene system, which is known to be switched by switched by light, could recently switched by the tunnelling current of a STM set-up on surfaces at the single-molecule level [J. Henzl et.al., *Angew. Chem.. Int. Ed. 45*, 603-606 (2006)].

A second, very promising way towards alternative fueling of motors consists in the construction of **light-driven molecular nanosystems**. Nature runs the nano-machinery that makes life possible using sunlight. In photosynthetic bacteria and green plants, photon absorption by chlorophyll generates a charge-separated state, from which the electron is quickly passed dpwn a cascade of electron carriers, ultimately generating energy. An exemplary effort to do just this is given by the BIOMACH partner **CIAM** who describes a photochemical experiment on an artificial rotaxane machine that uses light to displace a fragment of its unimolecular structure [V. Balzani et. al. *Proc. Natl. Acad. Sci USA 103,* 1178-1183 (2006)] This system is working without consumption of chemical fuels or the

formation of waste products. However, it has to be still to included a efficient reset step to make this molecular devices to do repetitively usable work force. Scientifically, this field has to be considered as the most dynamic scientific subdomain the BIOMACH project is touching actually. Beside of the leading research work done within BIOMACH (see **WP 4** and in time delivering of Deliverable **D4-3** "Operation of LFMC-incorporating photodriven molecular machines" (M36)), several groups from Europe and the United States promote very similar works e.g. T. Muraoka et .al. *Nature* 440, 512-515 (2006). For instance, a strong scientific pool dealing with rotaxanes at surfaces is grouped around Professor F. Stoddart at the University of California, Los Angeles/United States. All investigations carried out there into the direction of light-fueling of molecular motors under near-surface conditions have hit several obstacles preventing them from functioning. In consequence, the BIOMACH consortium (FZK-INT; MPI-FKF) decided to use more simple diazo compounds (see **WP 4** and deliverable **D 4-1**), which can equally convert light-energy into mechanic energy. First self-assembly experiments on metallic surfaces have proven excellent ordering properties and light-switching experiments are under way. From the theoretical point of view, the nanoscopic principles of such light-driven machines are elucidated by computational investigations by the ETH group (see **D 4-2**).

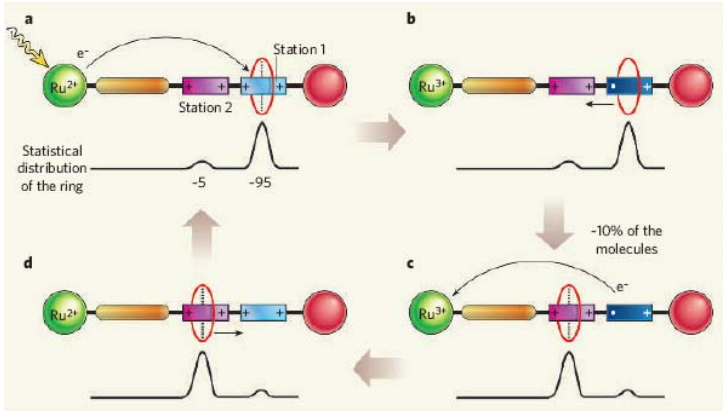

Figure 2: Light driven molecular Shuttle created by BIOMACH partner CIAM and colleagues on a molecular rotaxane base.
[V. Balzani et. al. ***Proc. Natl. Acad. Sci USA*** *103,* 1178-1183 (2006)]

During the third funding period, in **WP 5 –Nanopore Machines**, the determination of the translocation activity on surfaces, specific and strong linkages of the protein and vesicles to beads has been assured further (in prolongation of Deliverable **D5-1** (M18)) by the BIOMACH partner **AMOLF**. In addition, computional studies were carried out by BIOMACH-partner **EPFL** in close collaboration with **AMOLF** producing new results and one publication beyond the scope schemed for WP5. The Deliverable **D5-2** "Design and synthesis hexameric spin transition Fe^{II} compound" has encountered several problems in the synthesis of the respective synthesis of ligand-systems for simple artificial nanopore mimicks. Due to these problems, a new ligand design had to be developed under lead of partner **FZK-INT** and was finally realized under only within the prolongation period M39.

Within BIOMACH the research work carried out in **WP 6- Biomolecular Motors on Nanostructures** has lead to different nanofabricated structures, mainly produced by the BIOMACH partners **TU/d-MB** and **GC-ICG**. Their optical features have been measured by total internal reflection microscopy (see **D6-1**). In prolongation of the biophysical studies of the kinesin-microtubule system with the use of these nanostructures, the Deliverable **D6-2** "Mobility of single kinesin motors on nano assemblies" (M36) could be realized in time. The group in Delft has created closed channels in nanometer dimensions. These channels provide a tight confinement for kinesin-based microtubule motor systems. By coupling fluorescence detection of microtubules with the active steering of the directionality of the microtubules by electric fields, rather realistic model studies for transport processes in biology can be carried out. This experiment was acknowledged by several commentaries (see www.biomach.org) as

a brook-through in nanobiological sciences and is certainly one of the highlights of the research carried out within the BIOMACH frame.

In addition, the BIOMACH project has kept close contact to the scientific progress within the community of scientific motors and nanomachines through all the funding period. As highlighting example, almost all (above mentioned) competitors had accepted the invitation to the BIOMACH conference "**From Molecular Switches to Molecular Motors**", which was taking place together in Ascona/Switzerland from 18th to 23rd September 2005 (see www.biomach.org)

Workpackages progress – Plan and Status Barchart M01-M39

Detailed M39 description of the progress of the WP´s

WP 1 Nanohandling

Workpackage number WP1	Nano-Handling	Start date or starting event:			M0 M39
Activity Type RTD **Participant id**	**3** **MPI-FKF**	5 EPFL	2 ULP	2 TU/d-MB	10 AMOLF
Person-months per participant:	34	12	5	9	6

Objectives

O1 Develop **new techniques** for the handling, positioning, observation and operation of biological as well as artificial machines in the nanometer regime.

Description of work

Temperature controlled scanning tunneling microscopy (STM), atomic force microscopy (AFM) and scanning tunneling spectroscopy (STS) are employed for nanoscale studies of molecular motors fueled with light or electrochemical energy at well-defined surfaces. Working principles of ATP-burning individual biologically relevant nano-engines are investigated using laser tweezer techniques. The time evolution of the complex molecular machines involving a large number of atoms is modeled using advanced simulation methods, notably *ab initio* molecular dynamics.

Specific goals are as follows : developing of new concepts of molecular nanodevices exploiting metal-ligand interaction. In particular, we shall concentrate on the controlled anchoring of rotaxane (or catenane) units at surfaces either in an electrochemical environment or under ultra-high vacuum. This will allow for the synthesis of a complete rotaxane. Ultimately we work on the observation and detailed understanding of the operating of the entire species as a nanodevice.

(ii) the study of conformational changes of molecular species which can be deliberately switched by light irradiation (e.g., exploiting photoinduced electron-transfer reactions in **LFMC**s). For the STM investigations, molecules will be designed comprising anchoring groups which allow for a specific orientation of the photosensitive group with respect to the employed substrate. Under cryogenic conditions the switching of individual species following radiation exposure is elucidated by imaging and single-molecule spectroscopy.

(iii) study of the bacterial *Sec* system representing a model system for protein-import motors, which grab a folded protein, unfold and translocate it across a cellular membrane. Determination of the dynamics of the corresponding movements using attached micron-sized beads – serving as handles that can be manipulated by a focused laser-beam (laser tweezers) – to individual motors on one end, and to the protein on the other end.

Deliverables

D1-1 M24 single-molecule investigation and control of anchoring rotaxane and **LFMC** units at surfaces (report)

D1-2 M39 modification of the vesicles and proteins involved in protein-importing molecular motors such that specific linkages to micron-sized beads can be achieved (report)

Milestones and expected result

M1-1 M08 identification of functionalized rotaxane and ring units grafted at surfaces

M1-2 M16 control of **LFMCs** oriented at surfaces

M1-3 M20 single-molecule investigation of completed devices at surface

M1-4 M30 phenomenological description of nanodevices operating with electrochemical energy / light fuel

Achievements: A laser system has been integrated with a low-temperature scanning tunnelling microscope in order to study light-driven molecular motions under the well-defined conditions. The laser source is a Helium Cadmium (HeCd) laser manufactured by Melles Griot Laser Group (USA), which outputs two wavelengths of 325 nm and 442 nm. The laser power is 10mW (325 nm) and 25mW (442 nm). The laser light is guided by a fiber optics into the vacuum system.

We have achieved the controlled adsorption and positioning of individual macrocycle molecules mt-33 by the use of nanopatterned surfaces. In particular a Ag(111) surface was portioned into an array of identical cavities of 2.95 nm inner-diameter by the precedent self-assembly of the molecules 4,4´,4´´-benzene-1,3,5-triyl-tri-benzoic acid. The mt-33 molecules were deposited on top of the pre-patterned surface. As a result, single mt-33 molecules were confined within the nanometer-size cavities.

As a second step a catenane molecule, cat-30, is delivered on the pristine Ag(111) surface in ultra-high vacuum conditions by thermal sublimation. Single molecules were identified by the high-resolution STM at 5K. A copper source has been installed in the same vacuum system in order to realize copper complexation with the catenane molecules at the surface. The introduction of a light source into the STM equipment approaches WP 1 closer to WP 3 and 4 giving a new platform for a broadened scope of collaboration in BIOMACH. Parallelly, the very time-consuming but necessary fine-adjustment of the experimental equipment in the more biologically centred WP5 and WP 6 was accomplished.

D1-1 M24 "Single-molecule investigation and control of anchoring rotaxane and **LFMC** units at surfaces (report)"

was delivered in time.

Deviations and Actions Taken

D1-2 M36 "Modification of the vesicles and proteins involved in protein-importing molecular motors such specific linkages to micron-sized beads can be achieved (report)"

was postponed and delivered in M39

WP 2 Self-Assembly

Workpackage number **WP 2**	**Self-Assembly**	**Start date or starting event:**	M0-M39

Activity Type RTD	**6**	3	8	9
Participant id	**EPFL**	Tu/e	ULP	IGC-CG
Person-months per participant	24	24	6	6

Objectives

O2 Investigate new self-assembly strategies for the ***in-situ*** **build-up** of artificial motors from separate components.

Description of work

The work in this work package consists of two independent lines of research. The first line focuses on the synthesis of novel scaffolds that give compatible Au-anchored receptor sites for the rotary motors. This will be performed in close collaboration with the other work packages in which both the artificial (rotaxane/catenane) as well natural rotary motors are synthesized and studied. The second line of research focuses on the further elaboration of the chiral an/or semi-conducting self-assembling cylindrical stacks on the one hand and the derivatisation of the rotary motors in such a way that they can intercalate into the cylindrical stacks. This work requires synthesis, nano-processing of the self-assembly as well as detailed characterization of the objects obtained for and after the intercalation of the rotary motors. The work of semi-conducting self-assembled polymers is part of an ongoing collaborative research project with Philips Research towards novel organic devices for electronics. The hydrogen-bonded polymeric structures are the main activity of a new start-up company Suprapolix focus on application of specific self-assembled functional polymers. The Eindhoven group has a strong collaboration with this new company.

Deliverables

D2-1 M-24 Self-assembled chiral stacks including intercalated rotary motors included (report)

D2-2 M-36 Self-assembled semi-conducting polymers including intercalated rotary motors included (report)

Milestones and expected results

M2-1 M-12 Synthesis of the scaffolds for the Au-anchoring, both for the inert environment as well as the functional receptor site (hydrogen bond and streptavidin)

M2-2 M-12 Synthesis of the modified rotary rotor and its self-assembly by hydrogen bonds and biotin

M2-3 M-20 Synthesis of chiral scaffold

M2-4 M-24 Synthesis of semi-conducting scaffold

M2-5 M-30 Self-assembly of both chiral and semi-conducting scaffolds

M2-6 M-36 Analysis of the nano-processed structures.

Achievements: This line of research was focused on the further elaboration of the chiral and/or semi-conducting self-assembling cylindrical stacks on the one hand and the derivatisation of the rotary motors in such a way that they can intercalate into the cylindrical stacks. This work required synthesis, nano-processing of the self-assembly as well as detailed characterization of the objects obtained for and after the intercalation of the rotary motors. Obtaining self-assembled chiral stacks including intercalated rotary motors was a large step forward in the process of investigating new self-assembly strategies for the ***in-situ*** **build-up** of artificial motors from separate components. The strategy to obtain self-assembled chiral stacks including intercalated rotary motors was based on the systems as developed in Eindhoven and technologies to deposit these supramolecular structures on surfaces as developed in Lausanne. The synthesis of specially equipped rotary motors is delayed due to synthetic difficulties but more importantly, the dynamics of the chiral stacks proved to be too slow to achieve the proposed motion. Therefore, the deliverable was changed into "Self-assembled Chiral Stacks Including Intercalate Functional Units", while research is continued to influence the dynamics of the stacks. Hence the modified deliverable was reached. The research performed delivered self-assembled chiral stacks including intercalated functional units. The synthesis of specially equipped rotary motors was delayed due to synthetic difficulties but more importantly, the dynamics of the chiral stacks proved to be too low to achieve the proposed motion.

D2-1 M-24 "Self-assembled chiral stacks including intercalated rotary motors included (report)"

D2-2 M-36 "Self-assembled semi-conducting polymers including intercalated rotary motors included (report)"

were delivered in time.

Deviations and Actions Taken

No deviations; all deliverables were submitted in time.

WP 3 Electro-Engines

Workpackage number WP 3	Electro-Engines	Start date or starting event:		M0- M39
Activity Type RTD **Participant id**	**9** **ULP**	2 Tu/e	5 MPI-FKF	6 CIAM
Person-months per participant:	26	12	2	6

Objectives

O3 Design, synthesize and handle the **first *artificial* ATPase-**like molecular rotators.

Description of work

Synthesis of a rotaxane able to work as an oscillatory machine. The rotor (a macrocylic ring) and the stator (a molecular thread) are linked together via a transition metal. The ring incorporates two different coordinating sites, a bi- and terdentate site. The thread incorporates one bidentate site. The position of the ring on the axis will be determined by the oxidation state of the metal, which can be electrochemically monitored. A thick filament ended by a voluminous moiety, such as a porphyrin, is attached to the ring. The axis of the rotaxane is ended at each end by functionalities which will allow the attachment of the machine onto a gold surfaces, in form of self-assembled monolayers. The operation of the machine will be triggered the potential of the host surface. The spinning of the filament, i.e. the rotor, will be investigated by scanning probe microscopy or by optical methods.

Deliverables

D3-1 M12 Catenanes and rotaxanes units grafted on a metal surface (report)
D3-2 M39 Monolayers of rotaxanes working as molecular devices (report)

Milestones and expected results

M3-1 M06 Synthesis of a switchable rotaxane units bearing the appropriate functional group for grafting.
M3-2 M12 Grafting of the rotaxane at a surface and study of the electrochemically induced rotation motion.
M3-3 M30 Synthesis of the bistable rotaxane with a long filament attached on the ring

Achievements: The overall objective of WP3 consisted in the construction of an oscillatory machine at surfaces. Towards this goal, the grafting of catenane and rotaxane units on a metal surface was investigated thouroughly. A catenane molecule, cat-30, as shown in Scheme 3, is delivered on the Ag(111) surface in ultra-high vacuum conditions by thermal sublimation. Figure 3 shows that the deposited molecules aggregate as a close-packed cluster consisting of seven molecules. Following the successful positioning of single mt-33 molecules inside the network cavities, the grafting single catanane molecules in the cavities was achieved.

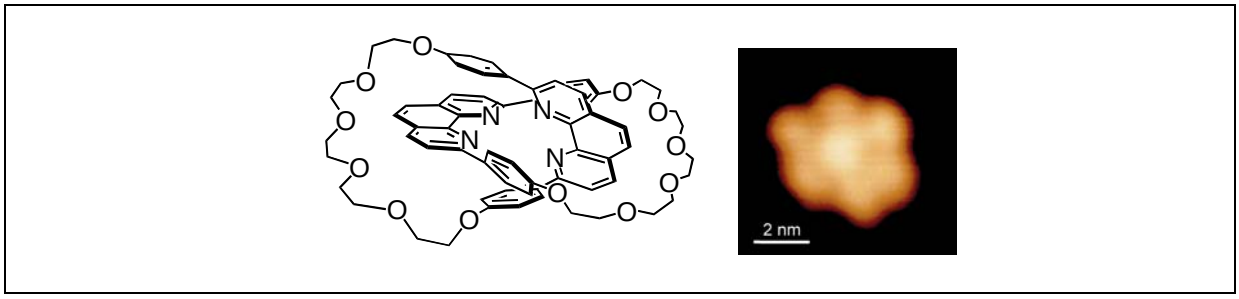

Figure 3: Grafting of catanane cat-30 at a clean Ag(111) surface**.** Seven molecules form a close-packed cluster.

The overall objective to *"Design, synthesize and handle the first artificial ATPase-like molecular rotators"* could be achieved in the bulk phase. On the single molecule level, rotaxanes molecules could be fixed to gold surfaces. Furthermore, the ring-like parts of the catenane motors could be successfully deposited on Ag surfaces. Globally, the project has followed in time and step-by-step the given milestones.

D3-1 M12 Catenanes and rotaxanes units grafted on a metal surface (report)

was submitted in time

Deviations and Actions Taken

D3-2 M39 Monolayers of rotaxanes working as molecular devices (report)

was postponed by three months and than submitted.

WP 4 Photochemical Devices

Workpackage number **WP 4**	**Photochemical Devices**	**Start date or starting event:**		M0-M29
Activity Type RTD / **Participant id**	**2 CIAM**	7 ETH	1 FZK-INT	9 ULP
Person-months per participant:	**30**	20	12	4

Objectives

O4 Integrate appropriate Light-Fueled Molecular Components (LFMC´s) into nano-motors as **alternative fueling** concept.

Description of work

The research groups of **WP4**, taking advantage of a long-term background in inorganic and organic spectroscopy, photochemistry and electrochemistry, have gained considerable expertise in the design and characterization of supramolecular species under the photo-physical, photochemical and electrochemical viewpoint, with the main objective of realizing prototypes of molecular-level devices and machines.
The main tasks of the **WP4** in the frame of the Network are:

- design of molecular components and supramolecular systems upon evaluation of the possible light-induced processes and electron-transfer reactions that can occur in such compounds.

-photo-physical, photochemical and electrochemical characterization.

- integrate the use of molecular dynamics simulations into the design of such devices by evaluating the microscopic mechanism of processes prior to the synthesis of devices.

Deliverables

D 4-1	M12	Final Design of a Light-Fueled Molecular Component (LFMC) (report)
D 4-2	M18	Computional treatment of light-induced rotary movements in LFMC´s (report)
D 4-3	M36	Operation of LFMC-incorporating photodriven molecular machines (report)

Milestones and expected results

M 4-1	M06	Planning and synthesis of a LFMC
M 4-2	M12	Photo-physical characterization of LFMC´s in solution
M 4-3	M16	Self-assembly of LFMC´s on surfaces (WP2)
M 4-4	M24	Incorporation of LFMC´s into biological-type environments (WP2 and WP6)
M 4-5	M30	Study of the operation LFMC-driven molecular devices and machines (WP1)

Achievements: On the basis of the results obtained on a rotaxane containing LFMC in the form of a Ruthenium(II)-tris(bipyridine) complex which behaves as a sunlight-powered molecular shuttle in solution, we have studied a second-generation system wherein the position, with respect to the LFMC, of the two different "stations" were exchanged. The results showed that the efficiency of the new photoactive rotaxane as a light-triggered molecular shuttle is improved compared to the previously studied system if a sacrificial mechanism is adopted, as a consequence of a faster photoinduced electron-transfer process between the LFMC and the primary station. Other rotaxanes containing a Ru(II)-bis(terpyridine) unit as the LFMC were investigated. These compounds possess an important advantage compared to the previously studied Ru-based systems: owing to the geometrical arrangement of the terpyridine ligands around the Ru(II) ion, they can give rise to linear (wire-type) structures. Owing to a relatively long excited-state lifetime (in contrast with the parent Ru(II)-bis(terpyridine) complex), these system can undergo intra- and intermolecular processes triggered by visible light. The effect of photoexcitation on the conformation of these rotaxanes was investigated.

The objective of the study of a new rotaxane designed to operate as a light-fueled nanomotor was tackled. Such a rotaxane is a complex multicomponent system that contains a porphyrin unit as a LFMC instead of a Ru-based moiety. The use of porphyrins, the chromophores of natural pigments, was prompted also in view of the incorporation of the system into biological-type environments. In addition, the modification of a biomolecular motor with a photochemical molecular switch has been tested without further conclusions.

D 4-1 M12 "Final Design of a Light-Fueled Molecular Component (LFMC) (report)"

D 4-2 M18 **"**Computional treatment of light-induced rotary movements in LFMC´s (report)"

D 4-3 M36 "Operation of LFMC-incorporating photodriven molecular machines (report)"

were delivered in ime.

Deviations and Actions Taken

none

WP 5 Nanopore Machines

Workpackage number WP5	Nanopore Machines	Start date or starting event:		M0-M29
Activity Type RTD / **Participant id**	**10 AMOLF**	3 EPFL	7 ETH	1 FZK-INT
Person-months per participant:	30	36	3	24

Objectives

O5 Fully comprehend the **dynamics** of the *biologically*-active, pore systems and use this knowledge to design *synthetic* **molecular nanopore mimics**.

Description of work

Protein-importing molecular motors perform the intriguing task of protein recognition, grabbing a folded protein, unfolding it, translocating it across a cellular membrane, after which it refolds again. This motor functions in membranes have not yet been investigated at the single molecule level. Our studies focus on the bacterial Sec system, which is considered a model system for the many other (also human) existing protein-import motors. Laser tweezer techniques are ideal tools to get further insight into the dynamics and working mechanisms of such nanopore machines. The gained knowledge will be the base for the construction of simple biomimetic models for nanopores performing crucial tasks as protein recognition, substrate grabbing and size adaptation.

Deliverables

D5-1 M18 Single-Motor Measurements of movements and forces involved in protein translocation (report)

D5-2 M39 Design and synthesis hexameric spin transition Fe^{II} compound (report)

Milestones and expected results

M5-1 M06 Establish efficient translocation into vesicles on a surface, using biochemical assay

M5-2 M14 Establish procedure for firm attachment of vesicles to surface

M5-3 M18 Ligand synthesis for hexameric Fe(II) compound

M5-4 M30 Reduce various unwanted non-specific interactions, e.g. between bead surfaces.

M5-5 M34 Design and structural characterisation of the [5] catenane

Achievements: In order to obtain the complete motor construct, all components have to be brought together. Whether indeed the molecular construct can bridge the two beads, was tested in the laser tweezers apparatus. Note that many of the attachments do not require other reagents, which means that there was a large freedom in putting all the elements together. In these tests, the beads that were dressed with molecules are brought together with the tweezers so that the bridge could form. As the beads were separated again a measured force on the trapped bead indicating a linkage to it. At a certain force the construct broke, giving an indication of the attachment strength. In overall conclusion, the translocation activity on surfaces, specific and strong linkages of the protein and vesicles to beads could be measured, fulfilling the targeted objectives (in particular **D5-1**).
The second objective was the *de novo* design of nanopore-actuators inspired by the biological systems relying on the 10% lengths change of the Fe-N distance in spin transition Fe^{II}-oligopyridyl be the design principle for "breathing" nanopores which are able to change their internal diameter triggered by external parameters. Since the Fe^{II}(low spin)-N bond length averages 0.2 Å shorter than the Fe^{II}(high spin)-N bond distance with d(Fe(high spin)-N) = 2.0 Å, the triggered switching between the two spin states could be used to change effectively the internal diameter of the nanopore mimics by environmental parameters (light, temperature, pressure). After realizing further improvements to the aspecific P8-vesicle interactions, we can begin looking for movements generated by the protein translocation machinery and for the self-assembly of the artificial systems. Due to inherently scientific problems, actions to fulfil **D5-2** could be only taken by prolonging the project running time of BIOMACH.

D5-1 M18 "Single-Motor Measurements of movements and forces involved in protein translocation" (report)

was delivered in time.

Deviations and Actions Taken

D5-2 M39 "Design and synthesis hexameric spin transition Fe^{II} compound (report)"

was postponed and delivered after the additional three months period.

WP 6 Biomolecular Motors on Nanostructures

Workpackage number WP6	**Biomolecular motors on nanostructures**	**Start date or starting event:**		M 0-M39
Activity Type RTD / **Participant id**	**8 ICG-GC**	4 TU/d-MB	3 EPFL	10 ETH
Person-months per participant:	30	27	0	10

Objectives

O6 Construct kinesin-motor based **biological-inorganic hybrid devices** for controlled nano-transport processes.

Description of work

The Delft group has extensive experience in nanofabrication and is now moving into single-molecule studies of motor enzymes. For a start, we will make linear extended nanostructures that will act as an optical grid. This connects to recent work from the Paris group who has developed a new technique to characterize single kinesin motors without any external force and at unprecented bandwidth allowing in principle microsecond time resolution. Currently this involves the use of optical-interference patterns, but this will be replaced by a solid-state nanofabricated structure, which will allow a wide range of spacings in the pattern down to the few 10 nm range, and an improved stability. Kinesin motility will then be measured at the single molecule limit. The technique combines nm spatial and μs time resolution. New physics relating to the mechanism of kinesin motion is expected.

We will furthermore explore other possibilities where nanostructures can be useful to study biomolecular motors, or vice versa, where biomotors can be engineered in a useful way on nanostructures.

Deliverables

D6-1 M12 Microtubules assemblies on nanostructures (report)

D6-2 M36 Motility of single kinesin motors on these assemblies (report)

Milestones and expected result

M6-1 M08 Nanofabrication of a variety of nanostructures on coverslips

M6-2 M12 Assembly of microtubules on these structures

M6-3 M24 Biophysical studies of the kinesin-microtubule system with the use of these nanostructures

M6-4 M36 Exploration of additional hybrid systems with inorganic nanostructures and biomolecular motors

Achievements: The new interference setup for particle tracking (Traveling Wave Tracking) has been used to study the motion of the Neurospora-kinesin along the microtubules.The stepping dynamics has been characterized with subnanometre precision and microsecond time resolution and the step timescale has been measured. We have observed that for this molecular motor the 8 nm step occurs in less than 30 μs and without any long mechanical substeps.
Simultaneously, we have observed that the kinesins bind to the microtubules in a non trivial way and they show a cooperative-like behaviour. In order to avoid artefacts due to the microtubule-surface interaction, which might affect the kinesin binding dynamics, we have worked with freely suspended microtubules in between PDMS micropillars. The experiments clearly show that neither a surface, nor the pre-blocking casein can be responsible for the observed kinesin pattern.
The objectives and milestones for the BIOMACH funding period have been achieved and we can begin measuring single molecular motors moving on the suspended microtubules. The results from this WP were highlighted by the scientific community as “break-through” in nanobiological science (see www.biomach.org).

D6-1 M12 « Microtubules assemblies on nanostructures (report) »
D6-2 M36 “ Motility of single kinesin motors on these assemblies (report)”

both deliverables were delivered in time.

Deviations
A non trivial relationship between the shape of the nanostructure and the patterned light was observed and the experimental set-up was adapted to this result.

Actions Taken
Development of a new technique: the Travelling Wave Tracking.

Consortium management

Consortium Management Tasks and Changes

BIOMACH Amendment-Prolongation by three months

The amendment was motivated by the fact that most of the practical work is still in course and will endure until and of the contract period. In order to accomplish this work and especially to document and to disseminate of the generated knowledge we had requested for a cost-neutral prolongation by three months.

Time chart of BIOMACH Project Time Table and Status M39

start *months* end **Prolong.**

WORKPACKAGES	tool	1	3	6	9	12	15	18	21	24	27	30	33	36	39
WP1 Nano-Handling	**D**									**D1-1**					**D1-2**
	M				**M1-1**			**M1-2**	**M1-3**			**M1-4**			
WP2 Self-Assembly	**D**									**D2-1**				**D2-2**	
	M					**M2-1/2**			**M2-3**	**M2-4**		**M2-5**		**M2-6**	
WP3 Electro Engines	**D**					**D3-1**									**D3-2**
	M			**M3-1**		**M3-2**						**M3-3**			
WP4 Photochemical Engines	**D**					**D4-1**		**D4-2**						**D4-3**	
	M			**M4-1**		**M4-2**		**M4-3**		**M4-4**		**M4-5**			
WP5 Nanopore Machines	**D**							**D5-1**							**D5-2**
	M			**M5-1**		**M5-2**		**M5-3**				**M5-4**	**M5-5**		
WP6 Biomolecular Motors	**D**					**D6-1**								**D6-2**	
	M			**M6-1**		**M6-2**				**M6-3**				**M6-4**	

D-Deliverable; **M**-Milestone,

Final Activity Report M01-M39

Chapter 2 – Dissemination and use of the knowledge

Section 1 – Exploitable knowledge and its Use

One patent application has been submitted and is in the course of examination. Due to the legal situation, no further details can be given within this activity report.

Section 2 – Dissemination of knowledge

Overview table

Planned /actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
Dec 2003	*Press release(press/radio/TV)*	*General public*	**25**	*3000*	*FZK/INT*
Dec 2003	*Media briefing*	*General public*	**25**	*3000*	*FZK/INT*
Sep 2004	*Press release(press)*	*Research/General Public*	**1**	*sever. th.*	*IGC-GC*
June 2005	*Press release(press)*	*General public*	**25**	*sever. th.*	*FZK/INT*
May 2006	*Press release(press)*	*Research/General Public*	**25**	*sever. th.*	*Tu/d-MB*
Nov 2004	*Project web-site www.biomach.org*	*Research/General public*	**140**	*Several millions*	*FZK-INT*
Sep 2006	*Press release(press)*	*Research/General Public*	**1**	*sever. th.*	*MPI*
June 2006	*Film/video Press release(press/radio/TV)*	*General public*	**1**	*Several millions*	*FZK-INT*
October 2006	*Film/video Press release(TV)*	*General public*	**1**	*Several millions*	*FZK-INT*
March 2007	*Film/video Press release (radio)*	*General public*	**1**	*Several millions*	*Tu/e*

1. **“Powering the nanoworld”, Press conference and press release, EuroNano, Trieste, Italy, December 2003**

EUROPEAN COMMISSION

Community Research

BIOMACH research explores new fuelling concepts to power nanoworld machines

The EU funded BIOMACH[1] project aims to design molecular motors that provide a useable mechanical output. The long term goal is to advance the field, bringing in new fuelling, handling and using methods.

How many engines exist in modern macroscopic world, which move routinely with mechanical energy by converting the clean sun light? Even after 100 years of heavy engineering, there has been no such progress in the field. BIOMACH, one of the first STREP[2] funded under EU research Sixth Framework Programme, triumphs over newest macro-technologies by introducing new concepts and advancing research tools, to master the challenge of handling nano-engines at the single molecule level.

The BIOMACH nanotechnology project shows the principle working case for the search of new fuelling concepts for molecular motors that will be either, both technologically well established and easily available- e.g. electrons- or, environmental less polluting energy sources- e.g. sun light.

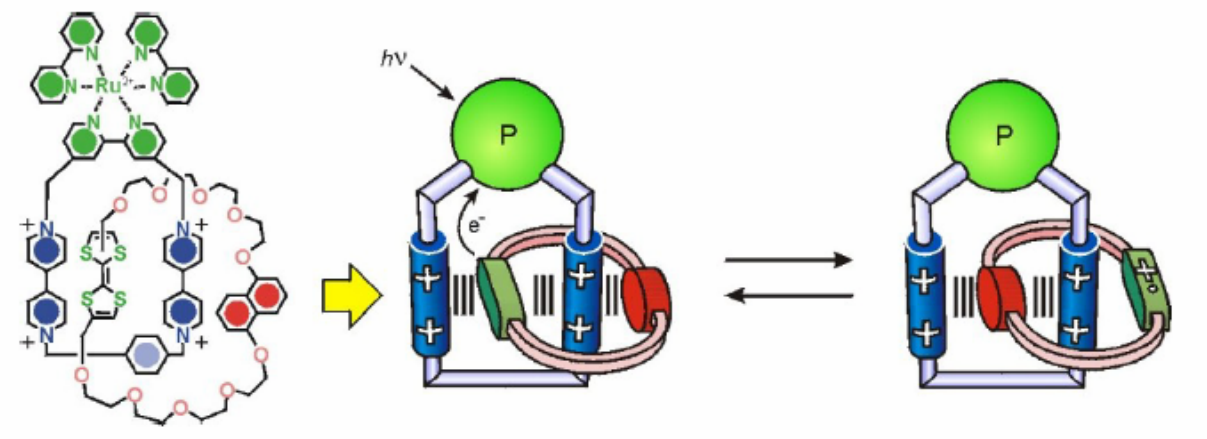

Figure: The realisation of a rotary motion by direct conversion of light into mechanical energy

[1] BIOMACH: "(Bio)Machines – Single-Molecule Handling of Biological Antecedents and Artificial Mimics" coordinated by Dr. Mario Ruben, Institute of Nanotechnology, Research Centre Karlsruhe, Germany

[2] STREP: Specific Targeted Research Projects

Creating a breakthrough in molecule handling
There is already a multitude of molecular devices working in every-day life in nature, such as photosynthesis is converting sun light in mechanical energy. These biological systems, however, tend to be too complex to be handled in technical terms. BIOMACH combines small biological working modules carrying out specific tasks into simple inorganic modules, which helps to handle and steer the systems at the single molecule level.

Resulting in better performance and usability
Future products fabricated by key methods investigated in BIOMACH proposal including chemical self-assembly of functional units and single molecule handling will contain smaller components with matching or higher performance capabilities than their traditional fabricated counterparts.

Nanotechnology to address societal benefits
Realisation of nanoscale machines, such as BIOMACH paves the way for novel devices and processes capable of reducing resource consumption and environmental pollution in manufacturing processes, bringing enormous benefits in terms of human health and quality of life. Proving the viability of this cutting-edge technology will improve European competitiveness by offering potential economic advantages including industrial growth and job-creation opportunities.

In the BIOMACH initiative, a consortium of ten European research groups will pool expertise in biology, physics and chemistry to assemble machines based on molecular building blocks.

Project Partners
- FZ Karlsruhe, GER
- University of Bologna, I
- EPF Lausanne, CH
- T U Delft, NL
- MPI Stuttgart, GER
- TU Eindhoven, NL
- ETH Zurich, CH
- Institute Curie Paris, F
- ULP Strasbourg, F
- AMOLF Amsterdam, NL

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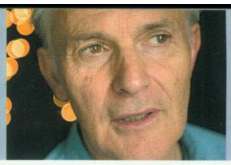
Website: http://hikwww1.fzk.de/int/english/welcome.html

2. **“La marche de la kinesine” by G. Capello, La Recherche, Paris, France, September 2004**



Harry Kroto, prix Nobel de chimie :
« Comment vivifier la science »

SCIENCES AU LYCÉE
Que valent les manuels scolaires ?

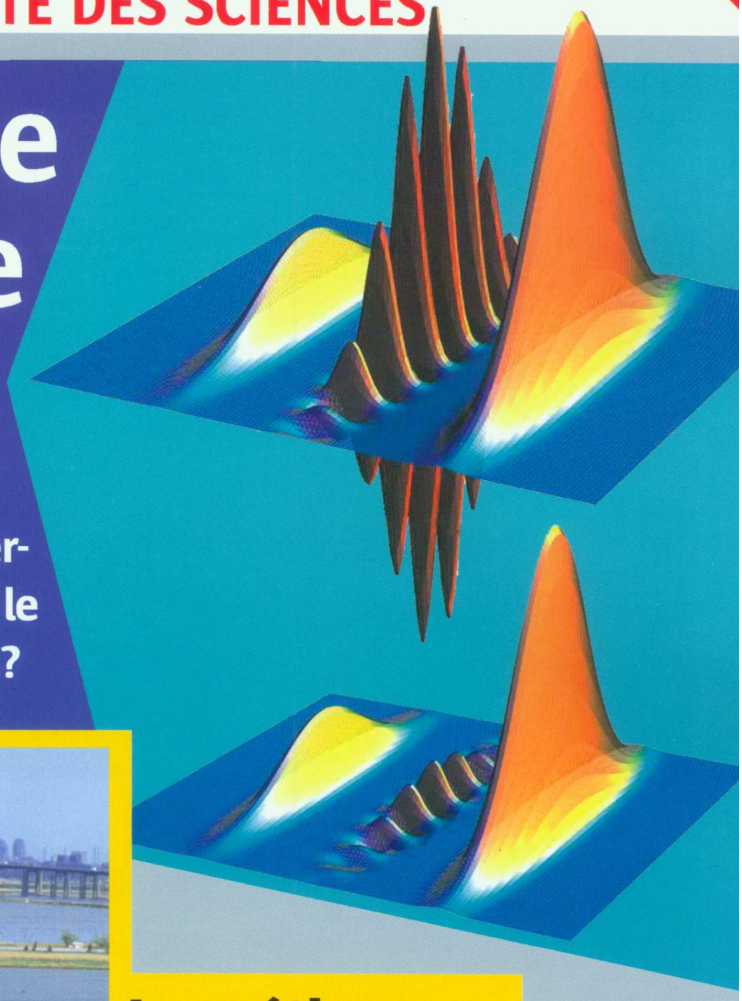
La Recherche
MENSUEL N°37

La Recherche

L'ACTUALITÉ DES SCIENCES

L'énigme de la physique quantique

Comment réconcilier les observations à notre échelle avec le monde étrange des particules?



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BIOPHYSIQUE

La marche de la

Elle court ? Elle rampe ? Depuis quelques années, le mode de déplacement de la kinésine, un « moteur moléculaire » chargé du convoyage des molécules à l'intérieur des cellules, est très discuté. Une astuce expérimentale a enfin permis de trancher.

Giovanni Cappello et **Pierre Sens**
sont chargés de recherche à l'Institut Curie.

Giovanni.Cappello@curie.fr
Pierre.Sens@curie.fr

Des échanges entre les compartiments de nos cellules sont indispensables pour le bon fonctionnement de notre organisme. À chaque instant, protéines, acides nucléiques et vésicules en tout genre sont apportés en différents endroits, en fonction des besoins. Leur convoyage est assuré par un réseau de filaments servant de rails, sur lesquels des « moteurs moléculaires », les « locomotives », tirent les composants dans la bonne direction. Certains moteurs se déplacent de la périphérie vers le centre. D'autres assurent le transport en sens inverse : on les appelle kinésines. Dans les cellules nerveuses, par exemple, dont les plus grandes mesurent plus de 1 mètre de long, les kinésines transportent des molécules essentielles pour l'activité neuronale. Des défauts de transport liés à ces moteurs entraînent ainsi des dégénérescences graves du système nerveux.

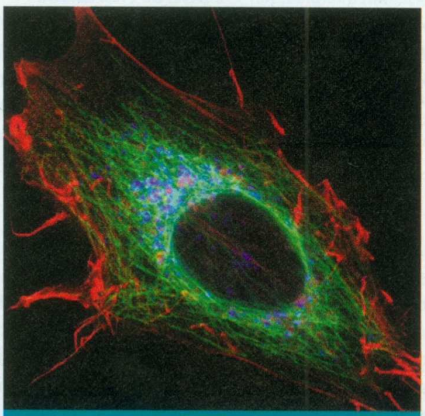
Pas chassé

Mais par quel mécanisme la kinésine se déplace-t-elle le long des rails de la cellule ? Pendant plusieurs années, deux modèles sont restés en compétition. On a tout d'abord imaginé que la kinésine glissait sur les filaments à la manière d'un homme en marche, posant successivement un pied devant l'autre [1]. Une autre hypothèse a ensuite été avancée. Le mouvement de la kinésine ressemblerait plutôt à un pas chassé, ou encore à celui d'un ver de terre : un pied, toujours le même, précéderait l'autre, et conduirait le mouvement [2]. Mais faute d'une technique adéquate, et en dépit du nombre important d'équipes internationales qui travaillent sur le sujet, aucune expérience n'avait apporté la preuve décisive qui permettait de trancher.

Ces deux modèles avaient été proposés dans le sillage des recherches sur la structure et le mouvement global du moteur. Depuis 1997, en effet, on sait que la kinésine, une protéine longue d'une dizaine de nanomètres, est composée de trois parties [3]. La première est constituée de deux « pieds » strictement identiques. Elle est responsable de l'activité motrice de la molécule : ses pieds se fixent alternativement sur le filament et convertissent l'énergie chimique en énergie mécanique. La seconde partie, de forme hélicoïdale, compose le « corps » de la molécule. Une troisième, enfin, se lie aux composants divers dont la kinésine assure le transport.

Une technique de manipulation moléculaire, appelée « pince optique », a permis d'étudier les caractéristiques dynamiques du moteur moléculaire. Une bille de verre de 1 micromètre de diamètre est tout d'abord attachée sur le corps de la kinésine. Cette bille peut être visualisée par microscopie, de sorte qu'il est loisible d'observer le déplacement de la kinésine avec une précision nanométrique. En outre, le microscope est muni d'un faisceau laser de faible intensité, que l'on fait passer à l'intérieur des lentilles et que l'on peut concentrer sur la bille. La lumière laser se réfléchit sur celle-ci et génère une force capable de déplacer la kinésine, ou encore d'en arrêter la marche.

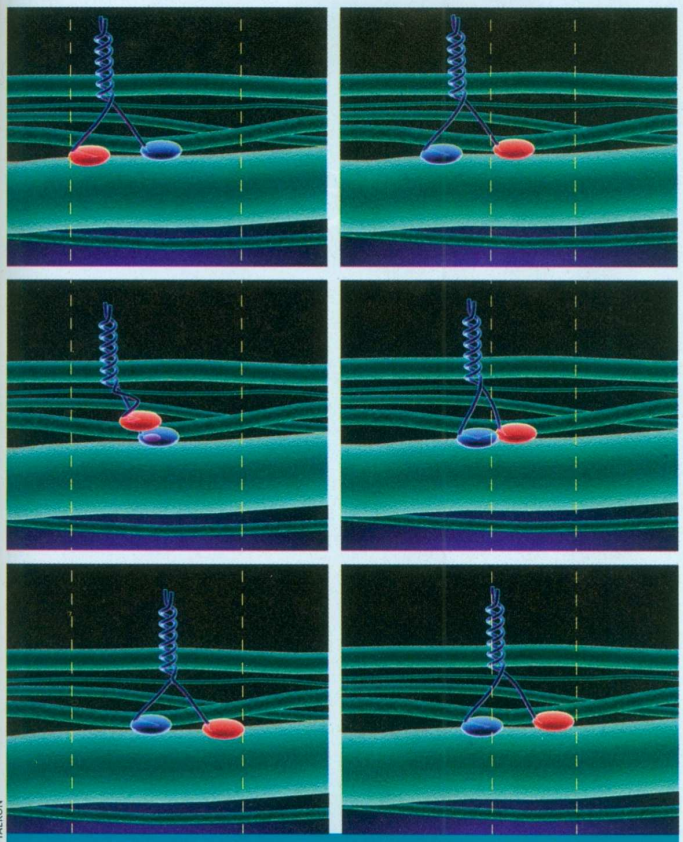
Une caractéristique importante du moteur a ainsi été mise en évidence. Sa vitesse moyenne avoisine le micromètre par seconde. Mais le mouvement n'est pas continu : la kinésine alterne des périodes d'immobilité et des déplacements soudains d'une longueur de 8 nanomètres. En fait, cette distance correspond au pas élémentaire de la protéine, qui change de position en passant d'une conformation à une autre. Toutefois, avec la pince optique, l'attention est focalisée sur le mouvement global du moteur. La kinésine est marquée au niveau du corps de la molécule. On ne visualise



© STEFANIE REICHELT/SPL/COSMOS

LE RÉSEAU DE FILAMENTS qui irradie la cellule permet à la kinésine de se déplacer dans la bonne direction.

kinésine



© INFOGRAPHIE : PATRICK TAERON

DEUX HYPOTHÈSES S'AFFRONTAIENT pour décrire le mouvement de la kinésine le long des filaments de la cellule. À gauche, le modèle du « marcheur » : les « pieds » de la kinésine passent successivement l'un devant l'autre. Dans l'autre modèle, à droite, l'un des pieds conduit le mouvement. Le marquage d'un pied par fluorescence (en rouge) a permis d'identifier le bon modèle.

que sa position moyenne en fonction du temps. Le détail du mouvement échappe donc à ce type d'expériences.

Marquer pour expliquer

Pourquoi, se sont demandé Paul Selvin et ses collègues biophysiciens de l'université de l'Illinois, ne pas observer plutôt le mouvement d'un seul des deux pieds? En effet, si le moteur avance comme un ver, le pied « avant » glisserait de 8 nanomètres, suivi rapidement du pied « arrière ». Pour le modèle du marcheur, en revanche, un pied resterait immobile pendant que l'autre avancerait de 16 nanomètres.
Une technique de marquage a été conçue dans ce dessein. Par mutation génétique, une molécule fluorescente a été introduite dans la structure de la kinésine. Le résultat est remarquable: les biophysiciens américains ont fabriqué une kinésine avec une « lampe torche moléculaire » entre les « orteils » d'un des deux pieds! Ils se sont assurés que le fonctionnement de la protéine n'était pas affecté par la molécule fluorescente. Pour cela, une kinésine témoin a été mutée à différents endroits du « pied » de la molécule. Une autre difficulté tient dans le fait que les sondes fluorescentes sont peu lumineuses. Elles s'éteignent également très vite en présence d'oxygène. Les expériences ont donc été menées en absence quasi totale d'oxygène, dans un milieu où des filaments cellulaires ont été immobilisés pour servir de support. Un microscope à fluorescence et une caméra permettent alors de suivre le mouvement du pied muté de la kinésine en fonction du temps.
L'équipe de Paul Selvin a ainsi observé que la longueur des pas du moteur mesurait en moyenne 17 nanomètres. Aucun pas inférieur à 11 nanomètres n'a été détecté. Conclusion: le modèle du marcheur est bien la meilleure manière de décrire le mouvement de la kinésine.
Mais une question reste ouverte, celle de la symétrie du système. En effet, les deux pieds de la kinésine sont parfaitement identiques. Or, l'hypothèse du marcheur implique l'existence d'un pied « droit » et d'un pied « gauche ». La marche d'un être humain, par exemple, est asymétrique. Une jambe se trouve toujours devant l'autre, de sorte qu'à la fin de chaque pas on se retrouve dans une position différente par rapport à l'axe du corps. Pour un déplacement symétrique, en revanche, la géométrie est la même à la fin de chaque pas. Par comparaison, une marche symétrique correspondrait à celle d'un compas que l'on ferait pivoter de 180 degrés sur chacune des deux pointes, alternativement de chaque côté par rapport à la direction du déplacement. Qu'en est-il pour la kinésine? Le déplacement du moteur ne sera véritablement compris qu'une fois cette question résolue. ■■ **G. C. et P. S.**

[1] S. Rice *et al.*, *Nature*, *402*, 778, 1999.

[2] W. Hua *et al.*, *Science*, *295*, 844, 2002.

[3] F. Kozielski *et al.*, *Cell*, *91*, 985, 1997.

[4] A. Yildiz *et al.*, *Science*, *303*, 676, 2004.

3. “BIOMACH research explore new fueling concepts to power nanoworld machines”

The Parliament, European Commission, Brussels, Belgium, June 2005

BIOMACH research explores new fuelling concepts to power nanoworld machines

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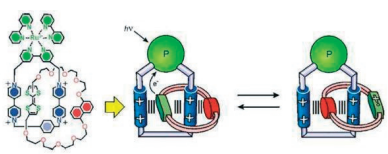

Figure 1: *The realisation of a rotary motion by direct conversion of light into mechanical energy*

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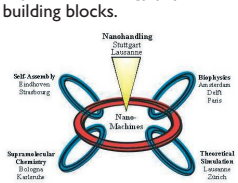

Figure 2: *Presentation of the scientific structure and of the involved project partners of BIOMACH*

Contact co-ordinator:
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Website: http://www.biomach.org

(Footnotes)

[1] BIOMACH: "(Bio)Machines – Single-Molecule Handling of Biological Antecedents and Artificial Mimics" coordinated by Dr. Mario Ruben, Institute of Nanotechnology, Research Centre Karlsruhe, Germany

[2] STREP: Specific Targeted Research Projects

4. “Biological motors sort molecules one by one on a chip”
Press release, Kavli Institute, Delft, the Netherlands, 2006

EMBARGOED until THURSDAY 11 MAY 2006, 2 PM U.S. Eastern Time

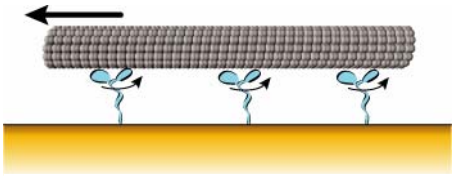
Biological motors sort molecules one by one on a chip

Researchers from Delft University of Technology's Kavli Institute of Nanoscience have discovered how to use the motors of biological cells in extremely small channels on a chip. Based on this, they built a transport system that uses electrical charges to direct the molecules individually. To demonstrate this, the Delft researchers sorted the individual molecules according to their color. Professor Hess of the University of Florida has called the Delft discovery "the first traffic control system in biomolecular motor nanotechnology". The research findings will be published in *Science* on May 12.

The biological cell is a complex of many different small protein factories. The necessary transportation of materials within the cell occurs across a network of microtubules: long, tubular-shaped proteins that extend in a star-shaped formation from the nucleus of the cell to the walls of the cell. Molecular bio-motors, such as the enzyme kinesin, can walk in small steps (of 8 nanometers) with a load of material along these microtubule-networks and thus provide transport within the cell.

Fascinated by these biological motors, the researchers at Delft University of Technology's Kavli Institute of Nanoscience are currently exploring the possibility of inserting these kinesin-motors and microtubules in an electrically directed transport system that is made by the researchers using nano-fabrication techniques.

The researchers turned the system around: the kinesin-motors are fastened in large quantities on a surface with their 'feet' up; the microtubules (measuring approximately 1 to 15 micrometers in length) were then transported over the 'carpet' of motors. The microtubules are, as it were, 'crowd surfing' over the sea of small kinesin motors. A particular challenge of the research was to ensure beforehand that the microtubule tubes could be transported in a determined direction and were not dislodged by collisions of the motor carpet.



Caption: A 'crowd surfing' microtubule is propelled by kinesin-motors (Image TU Delft)

PhD student Martin van den Heuvel, master student Martijn de Graaff and groupleader Professor Cees Dekker have for the first time achieved to control and address individual microtubules. An important step in this was to allow microtubule-transport to occur in small closed liquid channels. This made it possible to apply a strong electrical field locally at the Y-junction in the channels. Because of this, the electrical force could be exerted on the individual microtubules. The researchers discovered that by using this electrical force they could push the front of the microtubule into the determined direction.

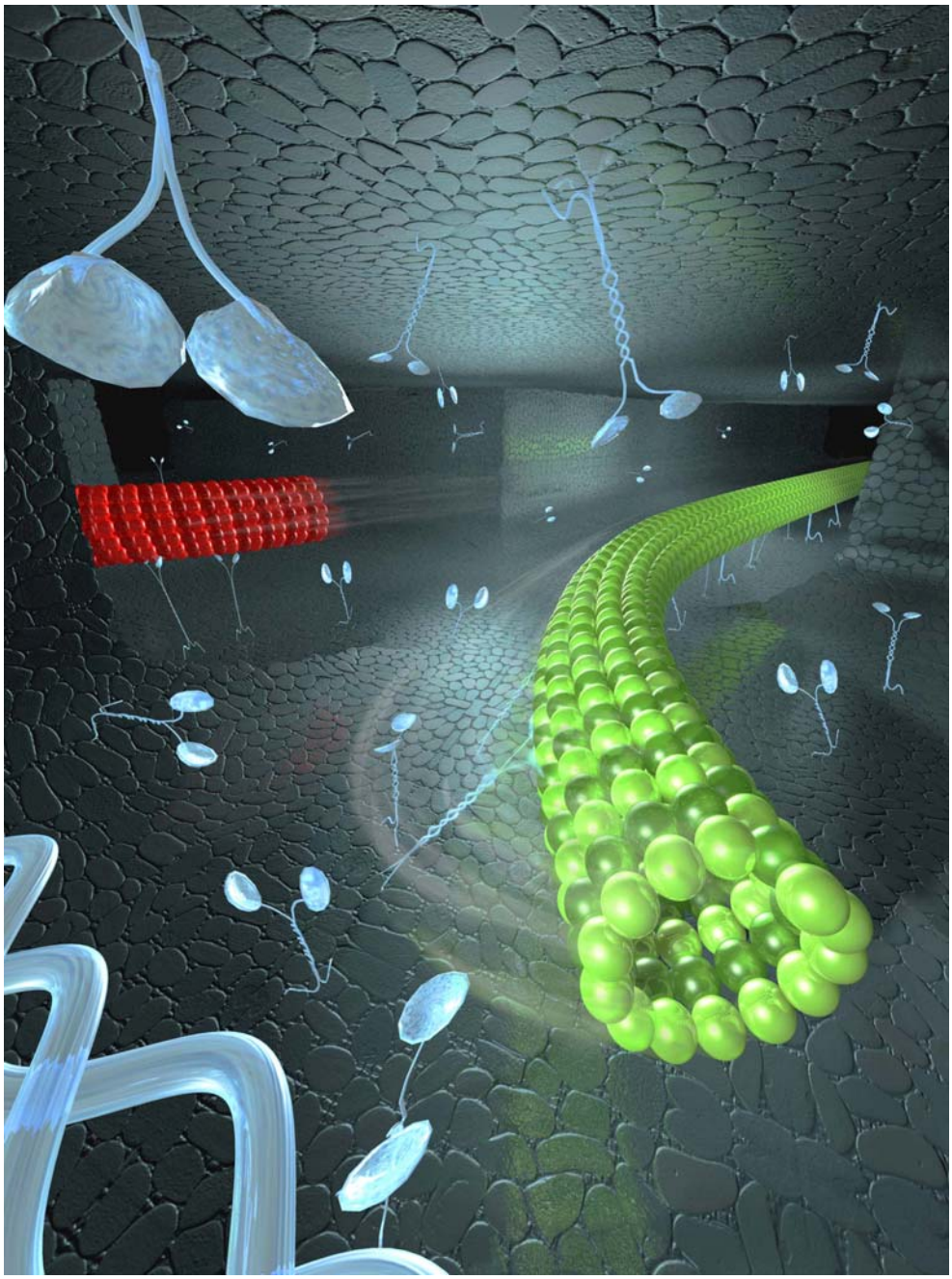
To demonstrate this, the researchers allowed a mixture of green and red fluorescent microtubules to arrive at a Y-junction. By changing the direction of the electrical force, depending on the color of the microtubule, the Delft researchers were able to collect the green and red microtubules in different reservoirs.

With their approach to the nano-channels, the researchers killed two birds with one stone. In addition to the possibility of steering individual microtubules, they were able to prevent the microtubules from derailing from their tracks. Incidentally, the Delft researchers discovered that their work contained a third interesting aspect. The closed channels offered the possibility to observe the electrical transport of freely suspended microtubules, thus proving that the speed of the microtubules under an external electrical field is largely dependent on the orientation of the cylinder-shaped molecules. This was the first time that this orientation-dependency of the electrophoretic mobility was observed.

In an accompanying Perspective article in *Science*, Professor Hess of the University of Florida wrote that the Delft researchers had developed the first traffic control system in biomolecular motor nanotechnology.

EMBARGOED until THURSDAY 11 MAY 2006, 2 PM U.S. Eastern Time

For additional information: Professor Cees Dekker, email dekker@mb.tn.tudelft.nl, tel. +31-15-2786094. High resolution images are available on request from Frank Nuijens, email f.w.nuijens@tudelft.nl, tel. +31-15-2784259, cell +31-6-14015118. A short movie of the sorting experiment is available on the website: http://www.mb.tn.tudelft.nl/biomotors.mov.



Caption: Artist impression of the sorting of green and red microtubules in nanochannels. Kinesin motors on the walls push the microtubule forward while an external experimentalist can steer the direction by exerting an electrical force on the tube (Image TU Delft/Tremani)

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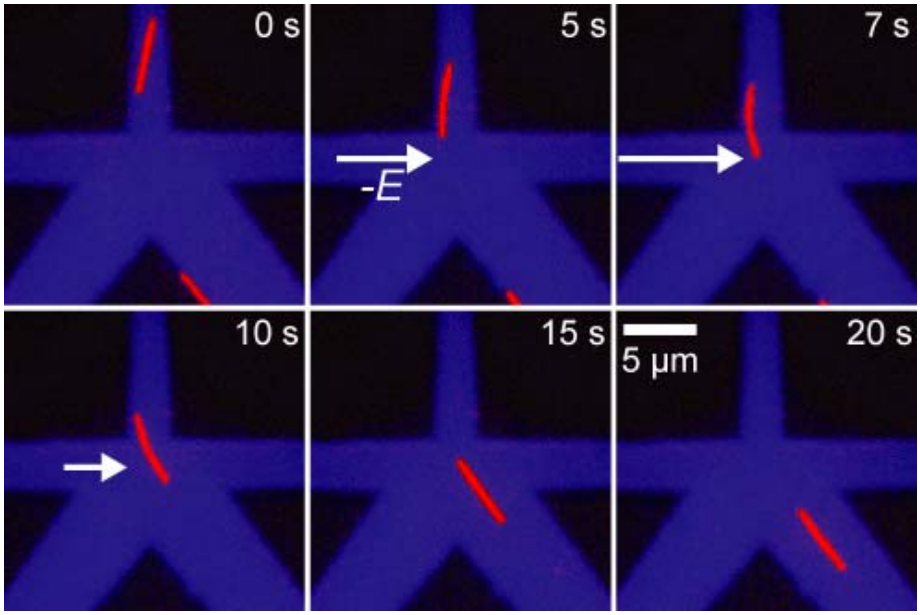
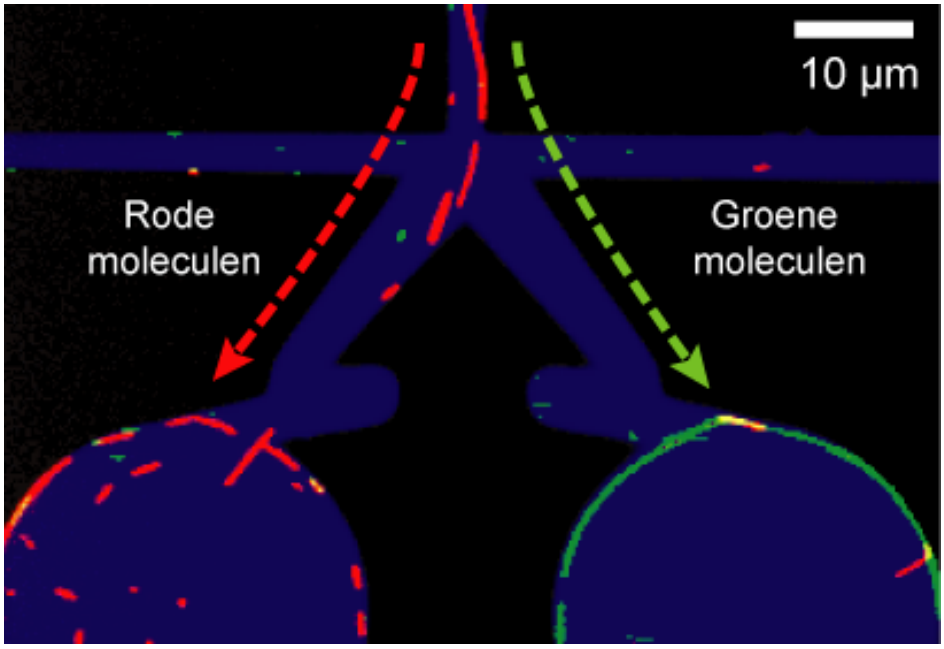


Caption: Images from an experiment where a single microtubule is steered between 5 and 10 seconds. The electrical force pushes the microtubule to the right (Image TU Delft)



Caption: Snapshot of an experiment where red and green molecules are sorted in two different reservoirs (Image TU Delft)

5. **“Everything starts with recognition” press release of the Max-Planck-Society, Munich, Germany, 2006**

MAX PLANCK SOCIETY

Press Release

News C / 2007 (49) April 23rd, 2007

Everything starts with Recognition

Scientists track at the atomic scale how individual molecules recognise each other

A human body has more than 10 to the power of 27 molecules with about one hundred thousand different shapes and functions. Interactions between molecules determine our structure and keep us alive. Researchers at the Max Planck Institute for Solid State Research in Stuttgart in collaboration with scientists from the Fraunhofer Institute in Freiburg and the King's Collage London have followed the interaction of only two individual molecules to show the basic mechanism underlying recognition of dipeptides. By means of scanning tunnelling microscopy movies and theoretical simulations they have shown how dynamic interactions induce the molecular fit needed for the transfer of structural information to higher levels of complexity. This dynamic picture illustrates how recognition works at the very first steps, tracking back the path in the evolution of complex matter. (Angewandte Chemie international April 20th 2007)

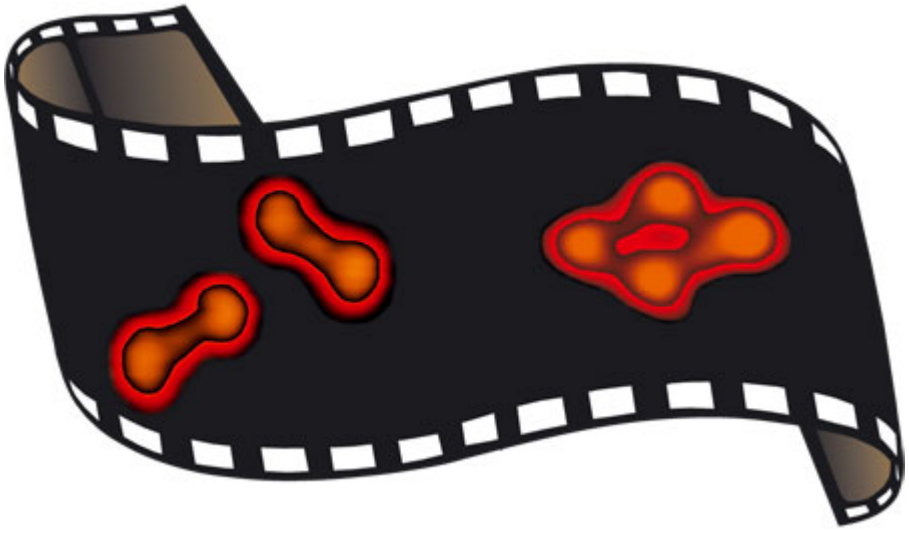


Fig.: *An STM image of individual L and D Di-phenylalanine molecules adsorbed onto Cu (110). A human body has more than one thousand trillion trillion molecules with about one hundred thousand different shapes and functions. The researchers have followed the interaction between two molecules to show the basic mechanism underlying chiral recognition.*

Image: Max Planck Institute for Solid State Research

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If one thinks that there are thousands of times more molecules forming our body than stars in the universe it is astonishing how all these molecules can work together in such an organised and efficient way. How can our muscles contract to make us walk? How can food be metabolised every day? How can we use specific drugs to relieve pain?

To work as a perfect machine, our body ultimately relies on the capability of each little part (molecule) to know a specific function and location out of countless possibilities. To do this, molecules carry information in different ways. An international team at the Max Planck Institute for Solid State Research in Stuttgart, in collaboration with scientists from the Fraunhofer Institute in Freiburg and the King's College London are seeking to find out how the information can be passed on at the very first steps: from the single molecule level to structures of increasing complexity and functionality.

The key to understanding all biological processes is recognition. Each molecule has a unique composition and shape that allows it to interact with other molecules. The interactions between molecules let us - as well as bacteria, animals, plants and other living systems - move, sense, reproduce and accomplish the processes that keep all living creatures alive.

A very common example of recognition can be experienced in daily life whenever one meets someone and shakes right hands. In principle, one can also shake left hands; the fact that we do it with the right has historically been a sign of peace, used to show that both people hold no weapon. But, have you ever attempt to shake the right hand of a person using your left hand? No matter how the two hands are oriented, you will never fit your left hand with the right hand of your friend.

Many molecules can recognise each other and transfer information exactly in the same way, they can either be "right handed" (D) or "left handed" (L). This property called "chirality" is a spectacular way to store information: a chiral molecule can recognise molecules that have the same chirality (same "handedness", L to L or D to D) and discriminate the ones of different chirality (L to D and D to L).

Probably one of the most exciting mysteries of Nature is why the building blocks of life, i.e. amino acids (the building blocks of proteins) are exclusively present in the chiral L form and sugars (which constitute DNA) are all in the D form. Once more, the reason for this preference is "historical", but this time goes back millions of years till the origins of the biological world. Scientists believe that current life forms could not exist without the uniform chirality ("homochirality") of these blocks, because biological processes need the efficiency in recognition achieved with homochiral substances. In other words, the separation of molecules by chirality was the crucial process during the Archean Era when life first emerged.

Researchers of the Max Planck Institute for Solid State Research have now used the "nanoscopic eye" of a scanning tunnelling microscope to make movies following how two adsorbed molecules (diphenylalanine, the core recognition motif of Alzheimer amyloid polypeptide) of the same chirality can form structures (pairs, chains) while molecules of different chirality discriminate and cannot form stable structures.

As it occurs when you shake the hand of your friend, the fact that the two homochiral hands are complementary by shape is not enough, you both have to dynamically adapt and adjust your hands to reach a better fit, a comfortable situation. By a combination with theoretical simulations done at Kings College London, the researchers have shown for the first time this dynamic mechanism of how two molecules "shake hands" and recognise each other by mutually induced conformational changes at the single molecule level.

We live in houses, wear clothes and read books made of chiral cellulose. Most of the molecules that mediate the processes of life like hormones, antibodies and receptors are chiral. Fifty of the top hundred best-selling drugs worldwide are chiral. With this contribution to the basic mechanism of chiral recognition, the researchers have not only tracked back to the very first steps in the evolution of living matter but have also shed light on our understanding and control of synthetic (man-made) materials of increasing complexity.

Related Links:

6. **“Towards Devices powered by biomolecular motors” by Henry Hess, perspective article, Science, May, 2006**

mutant is phenotypically similar to the *aux1* mutant, which suggests that both genes act in the same process. Dharmasiri *et al.* now provide an explanation for this phenotypic similarity by identifying AXR4 as an endoplasmic reticulum–resident protein required for proper AUX1 sorting to the plasma membrane. AXR4 appears specific for AUX1 trafficking, because other membrane proteins such as the PINs are not mislocalized in the *axr4* mutant. Interestingly, in the root tissues examined, the only cell types affected by the *axr4* mutation were those in which AUX1 localization is polar. In the lateral root cap, where AUX1 is uniformly distributed, there are no obvious effects in the *axr4* mutant background, whereas in the epidermis and protophloem where AUX1 is polarly localized, AUX1 is retained in the endoplasmic reticulum. This suggests that AXR4 plays a specific tissue-dependent role in the polar sorting of AUX1 to a particular plasma membrane face, rather than a general chaperone-like function. The biochemical basis for AXR4 action is not yet clear. Apart from a predicted transmembrane motif and a putative α/β hydrolase fold, AXR4 does not contain any known protein domains.

These discoveries demonstrate clear tissue-specific elements in the membrane targeting of both PIN and AUX1. So far, however, there is no evidence of any coordination of these events, although there is some suggestion of common elements because both are sensitive to the protein traffic inhibitor brefeldin A (*13*, *17*). As the mechanisms for polar localization of these proteins are revealed, it will be interesting to see the extent to which they are independent.

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10.1126/science.1127659

MATERIALS SCIENCE

Toward Devices Powered by Biomolecular Motors

Biomolecular motors can be used in nanometer-scale devices to perform mechanical work. This approach will assist the development of active nanostructures.

Henry Hess

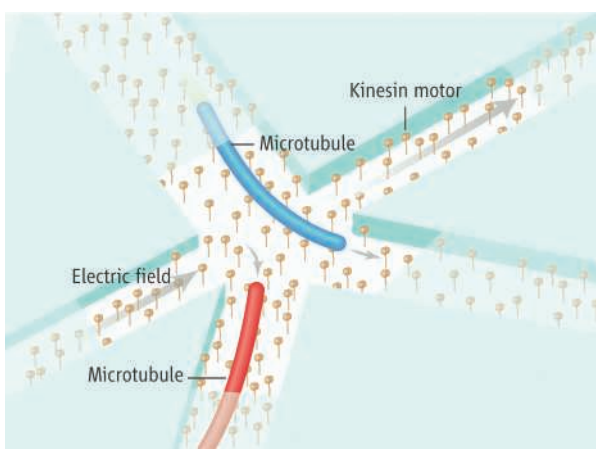
Biomolecular motors, such as the motor protein kinesin, convert chemical energy derived from the hydrolysis of individual adenosine triphosphate (ATP) molecules into directed, stepwise motion (*1*). This process enables them to actively transport designated cargo—such as vesicles, RNA, or viruses—to predetermined locations within cells. For engineers, active transport in biology inspires visions of nanofluidic systems for biosensing, of active materials that can rearrange their components, and of molecular conveyor belts and forklifts for nanometer-scale manufacturing.

Enhanced online at www.sciencemag.org/cgi/content/full/312/5775/860

Nanofluidic devices, which extend the lab-on-a-chip paradigm to systems with picoliter volumes and submicrometer channel diameters, present an immediate opportunity for the application of biomolecular motors. On page 910 of this issue, van den Heuvel *et al.* (*2*) show that kinesin motor proteins can drive the directed transport of microtubules (filamentous assemblies of thousands of tubulin proteins) in closed channels with submicrometer dimensions. Controlled application of an external electric field steers the microtubules into either one of two arms of a Y junction (see the figure).

The setup is an adaptation of the classic gliding motility assay (*3*), in which the kinesin motor proteins adhere to a surface via their rotationally flexible tails, bind to the leading ends of approaching microtubules with their two heads, and move the microtubules by stepping forward with alternating heads until they reach the trailing end and detach. In biological systems, the motors move and the microtubules are stationary. The key advantages of the inverted geometry used in the assay are that the microtubules are continuously bound to the surface over transport distances of more than a millimeter (*4*) and that the large microtubule allows the attachment of fluorescence tags for observation and of specific linkers for cargo binding (*5*).

Open or micrometer-scale closed channels have previously been fabricated to confine microtubule movements (*6*–*8*). Van den Heuvel *et al*. have now created closed channels with submicrometer dimensions. The channels not only provide better confinement, but they also mimic the dimensions of axons, in which motor–driven transport plays a central role. They may thus enable more realistic model studies at the system level of active transport in biology. Electric fields for active steering provide direct control over the paths of individual microtubules. By coupling fluorescence detec-



CREDIT: P. HUEY/*SCIENCE*

Nanofluidics with molecular motors. In van den Heuvel *et al.*'s work (*2*), an electric field is used to steer the microtubules into one of two arms of a Y junction; the microtubules move perpendicular to the field. The microtubules are transported by kinesin motor proteins.

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tion of microtubules with this control mechanism, van den Heuvel *et al*. have integrated optics, electronics, and molecular transport, thus introducing an element of real-time programmability.

This work is related to efforts by several teams of bioengineers to envision molecular motor–based technology and build proof-of-principle devices that radically depart from current engineering concepts (*9*). For example, minute volumes of biological samples can now be rapidly analyzed in credit-card–sized microfluidic devices connected to desktop-scale peripheral instruments. Downscaling of the lateral device dimensions by a factor of 100 would result in dust-particle–sized devices reminiscent of unicellular organisms. These devices would not necessarily be useful as as microscopic extensions of macroscopic peripheral devices, but would rather lend themselves to the application of the "smart dust" concept: smart dust biosensors would be immersed in the liquid sample of interest, independently perform an analysis, and be read out collectively to generate a statistically significant signal. Biomolecular motors that coat the inner surfaces of such devices and use dissolved ATP fuel as an energy source would drive the internal transport and remove the need for peripheral pumps and batteries (*10*).

In addition to fulfilling transport functions, biomolecular motors can exert localized forces on nanostructures. They can thus cause conformational changes, such as the stretching of coiled DNA molecules into a linear configuration (*11*) or the rupture of intermolecular bonds. Molecular motors could thus push supramolecular assembly and disassembly processes away from chemical equilibrium and generate dynamic, nonequilibrium structures (*12*). The force exerted by motor proteins could also be exploited in nanorobotics, where the sequential examination or manipulation of molecules by scanning probe microscopes and optical tweezers could be complemented by a parallel approach relying on arrays of microscopic, motor-driven actuators.

A key challenge in the field of molecular motors is to replicate the direct and efficient conversion of chemical energy into mechanical work by macroscopic arrays of biomolecular motors in muscles. This would pave the way toward a "molecular engine," creating an alternative to the prevailing heat engines (whose efficiency in converting chemical energy to mechanical work is limited according to Carnot) or to the two-step process of converting chemical energy into electricity via fuel cells and then into mechanical work via electrical motors. Building on insights from muscle physiology, we can pursue the engineering of either hybrid or fully synthetic molecular motor arrays of increasing size and explore a new avenue toward the design of artificial muscles (*13*).

Biomolecular motor–based hybrid devices face limitations with respect to environmental conditions (such as temperature) and lifetime (now typically on the order of hours to a few days) (*14*). Long-term storage of these devices in an inactivated state, which is reached by freezing or lyophilization technologies already used for protein pharmaceuticals, can be used to separate device fabrication and use by at least several months. However, limited lifetime and small power density are the principal disadvantages of biomolecular motors and motivate a transition to fully synthetic molecular motors in the long term.

Molecular motors, either of biological or synthetic origin, are central in the transition from passive to active nanostructures, because they enable coupling to a reservoir of chemical energy. In previous centuries, the use of human and animal power enabled the development of a wide range of technologies—including roads, carriages, and pumps—which were augmented after the invention of the steam engine and the internal combustion engine. Similarly, biomolecular motor nanotechnology, where van den Heuvel *et al*. have devised improved roads and the first traffic control system, and the on-going development of synthetic molecular motors (*15*) contribute to the same vision of fast, efficient, and controlled nanometer-scale transport systems.

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NEUROSCIENCE

Regulating Energy Balance: The Substrate Strikes Back

Jeffrey S. Flier

Hormones and dietary nutrients control appetite and metabolism by acting on the brain, where the signals they elicit promote hunger or satiety. Neurons in the hypothalamus integrate these signals to regulate energy balance.

Appetite, energy expenditure, and metabolism are critically regulated by hypothalamic neural circuits, and a "wiring diagram" through which neurons and neurochemicals exert these effects is rapidly emerging. To achieve energy homeostasis, neuronal pathways in the central nervous system receive and integrate signals from the periphery that convey information about the status of energy fluxes and stores. These signals are of several types. Hormones, such as the fat-derived hormone leptin, act directly on a subset of neurons; a deficiency of leptin is interpreted by the brain as starvation. Leptin deficiency overrides other signals to produce ongoing hunger despite massive obesity, as in rare human cases and in rodent models. Other regulatory signals include gut-derived peptide hormones released with meals that promote feeding (ghrelin) or satiety (cholecystokinin and peptide YY) through actions on the same neuronal targets.

Although these endocrine effectors have received the most attention recently, metabolic fuels and substrates, the evolutionarily ancient regulators of cellular and organismic homeostasis, also affect the neurocircuitry to regulate energy balance. For example, a low glucose level sensed by this circuitry provokes hunger (*1*). More recently, free fatty acids have been shown to act on targets in the central nervous system to regulate metabolism (*2*). On page 927 in this issue, Cota *et al*. (*3*) establish a novel, and potentially important role for

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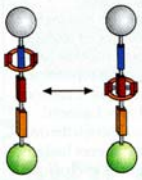
7. "Light drives molecular motor" Chemical&Engineering News, January 2006

SCIENCE & TECHNOLOGY

CONCENTRATES

Light drives molecular motor

A molecular motor powered by sunlight alone has been prepared by chemists in Italy and in the U.S. (*Proc. Natl. Acad. Sci. USA* **2006,** *103,* 1178). Vincenzo Balzani and Alberto Credi of the University of Bologna, J. Fraser Stoddart of the University of California, Los Angeles, and their coworkers believe their device is unique for several reasons. Because it's powered solely by visible light, the motor's movement—the shuttling of a crown ether back and forth between two points on the handle of a dumbbell-shaped structure (shown)—requires no additional chemicals and produces no waste products. Also, the shuttle's movement relies on intramolecular processes, so it could, in principle, be operated at the single-molecule level. The motor moves when a ruthenium complex (green sphere) at one end of the dumbbell absorbs a photon and transfers an electron to a 4,4′-bipyridinium moiety (blue bar) within the dumbbell's handle. This reduction prompts the crown ether (pink circle) to move 1.3 nm to a 3,3′-dimethyl-4,4′-bipyridinium unit (red bar) in the compound. The crown ether moves back to its original position via a back electron-transfer process.



subjected the protein to an approach called SIAFE (simultaneous incorporation and adjustment of functional elements) in conjunction with directed evolution (iterative modification and selection for desirable activity). The result was evMBL8, a designed enzyme with the ability to hydrolyze β-lactam amide bonds, a type of activity on which bacterial resistance to β-lactam antibiotics is based. Key to the change was the replacement of several of the enzyme's surface loop structures. The researchers say they hope the technique can be extended to convert other structures into enzymes that catalyze diverse reactions, including some not found in nature.

Down-to-earth NMR spectrometry

8. “Motore molecolare superveloce” Corriere de la Sierry, Milano, Italy, January 2006

SCIENZA

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UOMO, TECNOLOGIA, ECOLOGIA, NATURA

UNIVERSITÀ DI BOLOGNA

Motore molecolare superveloce

Si chiama Sunny il nanomotore inventato da un gruppo di scienziati del dipartimento di Chimica dell'Università di Bologna in collaborazione con l'Università della California di Los Angeles. È un minuscolo motore a 4 tempi che funziona con l'energia solare, non produce inquinanti e supera i 60.000 giri al minuto (è il più veloce del mondo della categoria). Il nuovo prototipo di motore molecolare (nell'illustrazione sopra), che verrà presentato nei «Proceedings of the National Academy of Sciences», è composto di due molecole: una filiforme, lunga circa 6 nanometri che funziona da asse di scorrimento (B), l'altra ad anello (A), infilata nella prima, con un diametro di 1,3 nanometri.

9. “Un nanomotore a benzina solare” Il sole-24 ore, Roma, Italy, January 2006

MADE IN ITALY IL PIÙ ECOLOGICO E VELOCE DEL MONDO

Un nanomotore a «benzina» solare

Chimici bolognesi hanno costruito una macchina microscopica azionata dalla luce, che non genera alcun prodotto di scarto

DI LARA RICCI

Un motore portentoso: funziona a energia solare, è velocissimo e pulito, non emette prodotti di scarto. «Sunny» è un macchinario al confine dell'altro mondo, quello invisibile, dell'ultrapiccolo, dove le leggi sono quelle della fisica quantistica. Ha quattro tempi come il motore a scoppio, ma è molto diverso, a partire dalle dimensioni: l'asse più lungo misura sei nanometri, impossibile da vedere, difficile da immaginare. Un nanometro, cioè un miliardesimo di metro, è circa 80mila volte più piccolo del diametro di un capello umano, o ancora, è uno spessore centomila volte minore di quello di un foglio di carta. La velocità di Sunny è sbalorditiva: un milione di volte maggiore dell'unico altro esempio al mondo di motore molecolare a energia pulita costruito dall'uomo.

Altra particolarità: per ora il motore non

LUCE SOLARE

A B C D

MOTORE MOLECOLARE

STEFANO TARTAGLIA

Il Sole-24 Ore
26 Gennaio 2006

10. “Il nanomotore é meglio di uns formula 1” La Stampa, Roma, Italy, February 2006

TECNOLOGIA

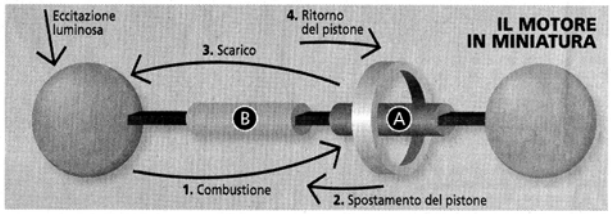
Il nanomotore è meglio di una Formula 1

DUE MOLECOLE CON IL SOLE COME CARBURANTE. TANTE APPLICAZIONI, DALLA MEDICINA ALL'INFORMATICA

UN motore di Formula 1 arriva a 20 mila giri al minuto. Questo supera i 60 mila. Piacerebbe di sicuro a Schumacher, se non fosse piccolissimo: è un nanomotore formato da due molecole. Invisibile a occhio nudo. Ha però un vantaggio straordinario: non richiede benzina, lo fa girare la luce del sole. Per questo i ricercatori dell'Università di Bologna, che l'hanno realizzato con l'Università della California, lo chiamano «Sunny». La notizia è su «Pnas», la rivista dell'Accademia americana delle Scienze. Il gruppo italiano fa capo a Vincenzo Balzani, uno dei 50 chimici più citati nelle riviste scientifiche. Il laboratorio Nanofaber della Regione Emilia sta esplorandone le future applicazioni.

L'unità di misura delle nanotecnologie è il nanometro, il miliardesimo di metro o - se preferite - il milionesimo di millimetro. Il motore «Sunny» è formato da una molecola sottile, lunga 6 nanometri, che funziona da asse di scorrimento, e da una molecola ad anello dal diametro di 1,3 nanometri, infilata nella prima. Sotto l'azione della luce l'anello scorre lungo la molecola mille volte al secondo. In sostanza, l'anello si muove come un pistone che trasforma l'energia dei fotoni solari in lavoro utilizzabile. Esiste un altro motore molecolare concettualmente simile costruito all'Università di Groningen nei Paesi Bassi, ma impiega un'ora a completare un ciclo: quello bolognese è quasi 4 milioni di volte più veloce.

Tipica delle nanotecnologie è la trasversalità. Sono utilizzabili nei settori più diversi: farmacia, elettronica, meccanica, chimica, fisica. Una possibile applicazione riguarda la salute: l'anello mobile funziona come un nastro trasportatore e, quindi, può far passare un farmaco attraverso la membrana delle cellule. Un altro uso potenziale riguarda l'informatica: si pensa a un computer chimico che sfrutti la logica binaria del nanomotore, fa-



11. La mia auto, Roma, Italy, March 2006

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SOMMARIO

12. “Lighting up nanomachines” Nature, London, UK, March 2006

PHOTOCHEMISTRY

Lighting up nanomachines

Euan R. Kay and David A. Leigh

A cleverly engineered molecule uses light to generate a charge-separated state and so cause one of its components to move. It's the latest study of a molecular machine that exploits nature's most plentiful energy source.

Nature runs the nanomachinery that makes life possible using the last word in clean, free and readily available power sources — sunlight. In photosynthetic bacteria and green plants, photon absorption by chlorophyll generates a charge-separated state, from which the electron is quickly passed down a cascade of electron carriers, ultimately generating energy in a convenient chemical form. Can similar capabilities be engineered? An exemplary effort to do just this is given by Balzani *et al.* who, writing in *Proceedings of the National Academy of Sciences*[1], describe photochemical experiments on an artificial machine that uses light to displace a fragment of its unimolecular structure.

Those who seek to harness the Sun's energy for synthetic molecular machines find that chemistry is always throwing up obstacles. In particular, charge recombination typically occurs thousands or millions of times faster than the nuclear movements on which such machines rely, making charge-separated states difficult to exploit. This problem can be overcome using bimolecular systems: here, the charged partners quickly diffuse apart so their energy can be used, for example, to achieve switching in a rotaxane[2]. This class of molecule, consisting of a ring that shuttles randomly and incessantly along a string, stopped only by bulky groups at the string's termini, is also that used by Balzani and colleagues[1].

Their rotaxane[1] (Fig. 1) incorporates two structurally different bipyridinium sites — 'stations' 1 and 2 — that slow the shuttling ring's motion through strong short-range electrostatic interactions. The ring thus divides its time between station 1, station 2 and the rest of the string in the ratio of around 95:5:<1. At room temperature, the ring shuttles between the stations tens of thousands of times per second, but the net flux is zero. So no work can be done, or useful task performed, by the shuttling action (the 'principle of detailed balance'[3]).

One of the bulky end-groups of the rotaxane's string is a ruthenium trisbipyridine complex. This can absorb a photon of visible light and so form a reactive, excited state that donates an electron to the more easily reduced of the two bipyridinium sites — station 1, the ring's preferred binding site. One would normally expect the resulting charge imbalance to be corrected by back-transfer of an electron on

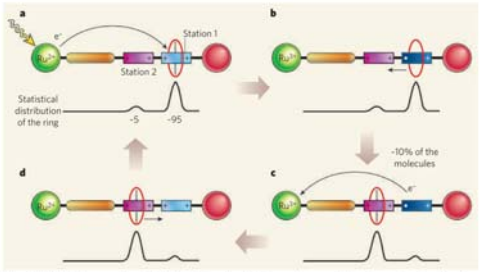
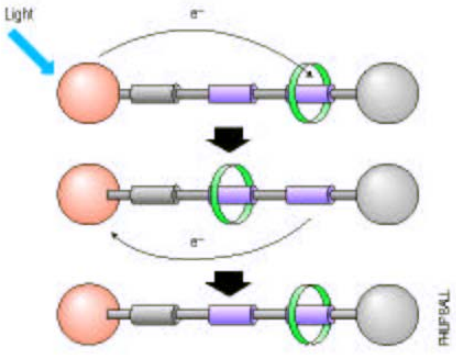


Figure 1 | Light-driven molecular shuttle. Balzani and colleagues' rotaxane[1,2] consists of a molecular ring free to move along a molecular string. **a**, At equilibrium in the ground state, the ring spends most of the time over station 1, as a result of attractive, non-covalent interactions. But irradiation of the ruthenium complex (green) at one end of the string generates a highly reducing excited state, resulting in electron transfer to station 1, and the weakening of this station's electrostatic interactions with the ring. **b**, Normally, charge recombination is fast in comparison with nuclear motions, but here a delay allows approximately 10% of the molecules to undergo significant brownian motion, shifting the distribution of these rings to favour station 2. **c**, When charge recombination eventually does take place, the higher binding affinity of station 1 is restored, and **d**, the system relaxes to restore the original statistical distribution of rings.

13. “Making light work of it” Nature Materials, London, UK, March 2006

Making light work of it

In contrast to motors in nature, artificial ones generally require one input to cause motion, followed by another to reset the motor. Often these inputs are chemical fuels, and therefore generate waste products, as well as requiring intervention at each stage. Now Balzani *et al.* report an autonomous motor powered simply with light (*Proc. Natl Acad. Sci.* **103**, 1178–1183; 2006). The motor consists of a rotaxane — a ring threaded around a dumbbell-shaped component of two electron-acceptor sites, or ‘stations’, for the ring to move between, with a bulky stopper group on each end. Absorption of a photon at a stopper group initiates electron transfer to the station where the ring rests, causing displacement to the second station. An electron can then transfer back to the stopper group from the now-free first station, and the ring can return to its original position. The motor works analogously to a four-stroke engine, with fuel injection and combustion, piston displacement, exhaust removal and piston-replacement steps. The motors of Balzani *et al.* rely exclusively on intramolecular processes and light absorption, and therefore do not consume chemical fuel or produce waste.



14. “Nanomotor powered by solar energy” Small, Weiheim, Germany, April 2006

Synthetic procedures

Nano Motor Powered by Solar Energy

Chemists at the University of Bologna (Italy), UCLA, and the California NanoSystems Institute (both USA) have designed and constructed a rotaxane-based molecular motor of nanometer size that is powered only by sunlight. The system is built up from a dumbbell-shaped component, which is more than 6 nm long, and a ring component of a diameter of approximately 1.3 nm. The ring component is trapped on the rod portion by two bulky stoppers, which are attached to the ends of the rod so that the ring cannot slip off. The rod portion of the dumbbell contains two “stations” that can be called “A” and “B”. The absorption of sunlight by one of the two stoppers, a light-harvesting species, causes the transfer of one electron to station A, which is deactivated as far as wanting

Section 3 – Publishable results

One patent application has been submitted and is in the course of examination. Due to the legal situation, no further details can be given within this activity report.

The full content version of the publications and press releases can be down-loaded and printed under www.biomach.org

MOLECULAR NANOTECHNOLOGY. TOWARDS ARTIFICIAL MOLECULAR MACHINES AND MOTORS

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Abstract

Miniaturization is an essential ingredient of modern technology. In this context, concepts such as that of (macroscopic) device and machine have been extended to the molecular level. A *molecular machine* can be defined as an assembly of a discrete number of molecular components – that is, a supramolecular system – in which the component parts can display changes in their relative positions as a result of some external stimulus. While nature provides living organisms with a wealth of molecular machines and motors of high structural and functional complexity, chemists are interested in the development of simpler, fully artificial systems. Interlocked chemical compounds like rotaxanes and catenanes are promising candidates for the construction of artificial molecular machines. The design, synthesis and investigation of chemical systems able to function as molecular machines and motors is of interest not only for basic research, but also for the growth of nanoscience and the subsequent development of nanotechnology. A few examples of molecular machines taken from our own research will be illustrated.

1. INTRODUCTION

A *device* is something invented and constructed for a special purpose, and a *machine* is a particular type of device in which the component parts display changes in their relative positions as a result of some external stimulus. Progress of mankind has always been related to the construction of novel devices. Depending on the purpose of its use, a device can be very big or very small. In the last fifty years, progressive miniaturization of the components employed for the construction of devices and machines has resulted in outstanding technological achievements, particularly in the field of information processing. A common prediction is that further progress in miniaturization will not only decrease the size and increase the power of computers, but could also open the way to new technologies in the fields of medicine, environment, energy, and materials.

Until now miniaturization has been pursued by a large-downward (top-down) approach, which is reaching practical and fundamental limits (presumably ca. 50 nanometers).[1] Miniaturization, however, can be pushed further on since "there is plenty of room at the bottom", as Richard P. Feynman stated in a famous talk to the American Physical Society in 1959.[2]

The key sentence of Feynman's talk was the following: *"The principle of physics do not speak against the possibility of manoeuvring things atom by atom"*. The idea of the "atom-by-atom" bottom-up approach to the construction of nanoscale devices and machines, however, which was so much appealing to some physicists[3] did not convince chemists who are well aware of the high reactivity of most atomic species and of the subtle aspects of chemical bond. Chemists know[4] that atoms are not simple spheres that can be moved from a place to another place at will. Atoms do not stay isolated; they bond strongly to their neighbours and it is difficult to imagine that the atoms can be taken from a starting material and transferred to another material.

In the late 1970s a new branch of chemistry, called *supramolecular chemistry*, emerged and expanded very rapidly, consecrated by the award of the Nobel Prize in Chemistry to C.J. Pedersen,[5] D.J. Cram,[6] and J.-M. Lehn[7] in 1987. In the frame of research on supramolecular chemistry, the idea began to arise in a few laboratories[8,9,10] that molecules are much more convenient building blocks than atoms to construct nanoscale devices and machines. The main reasons at the basis of this idea are: (i) molecules are stable species, whereas atoms are difficult to handle; (ii) Nature starts from molecules, not from atoms, to construct the great number and variety of nanodevices and nanomachines that sustain life; (iii) most of the laboratory chemical processes deal with molecules, not with atoms; (iv) molecules are objects that exhibit distinct shapes and carry device-related properties (e.g., properties

that can be manipulated by photochemical and electrochemical inputs); (v) molecules can self-assemble or can be connected to make larger structures. In the same period, research on molecular electronic devices began to flourish.[11]

In the following years supramolecular chemistry grew very rapidly[12] and it became clear that the "bottom-up" approach based on molecules opens virtually unlimited possibilities concerning design and construction of artificial molecular-level devices and machines. Recently the concept of molecules as nanoscale objects exhibiting their own shape, size and properties has been confirmed by new, very powerful techniques, such as single-molecule fluorescence spectroscopy and the various types of probe microscopies, capable of "seeing"[13] or "manipulating"[14] single molecules, and even to investigate bimolecular chemical reactions at the single molecule level.[15]

Much of the inspiration to construct molecular-level devices and machines comes from the outstanding progress of molecular biology that has begun to reveal the secrets of the natural molecular-level devices and machines which constitute the material base of life. Bottom-up construction of devices and machines as complex as those present in Nature is, of course, an impossible task.[16] Therefore chemists have tried to construct much simpler systems, without mimicking the complexity of the biological structures. In the last few years, synthetic talent, that has always been the most distinctive feature of chemists, combined with a device-driven ingenuity evolved from chemists' attention to functions and reactivity, have led to outstanding achievements in this field.[17,18,19,20]

2. CHARACTERISTICS OF MOLECULAR MACHINES AND MOTORS

The words *motor* and *machine* are often used interchangeably when referred to molecular systems. It should be recalled, however, that a motor converts energy into mechanical work, while a machine is a device, usually containing a motor component, designed to accomplish a function. Molecular machines and motors operate via electronic and/or nuclear rearrangements and, like the macroscopic ones, are characterized by *(i)* the kind of energy input supplied to make them work, *(ii)* the type of motion (linear, rotatory, oscillatory, ...) performed by their components, *(iii)* the way in which their operation can be monitored, *(iv)* the possibility to repeat the operation at will (cyclic process), and *(v)* the time scale needed to complete a cycle. According to the view described above, an additional and very important distinctive feature of a molecular machine with respect to a molecular motor is *(vi)* the function performed.[18]

As far as point *(i)* is concerned, a chemical reaction can be used, at least in principle, as an energy input. In such a case, however, if the machine has to work cyclically [point *(iv)*], it will need addition of reactants at any step of the working cycle, and the accumulation of by–products resulting from the repeated addition of matter can compromise the operation of the device. On the basis of this consideration, the best energy inputs to make a molecular device work are photons[21] and electrons.[22] It is indeed possible to design very interesting molecular devices based on appropriately chosen photochemically and electrochemically driven reactions.[20]

In order to control and monitor the device operation [point *(iii)*], the electronic and/or nuclear rearrangements of the component parts should cause readable changes in some chemical or physical property of the system. In this regard, photochemical and electrochemical techniques are very useful since both photons and electrons can play the dual role of "writing" (i. e., causing a change in the system) and "reading" (i.e., reporting the state of the system).

The operation time scale of molecular machines [point *(v)*] can range from microseconds to seconds, depending on the type of rearrangement and the nature of the components involved.

Finally, as far as point *(vi)* is concerned, the functions that can be performed by exploiting the movements of the component parts in molecular machines are various and, to a large extent, still unpredictable. It is worth to note that the mechanical movements taking place in molecular-level

machines, and the related changes in the spectroscopic and electrochemical properties, usually obey binary logic and can thus be taken as a basis for information processing at the molecular level. Artificial molecular machines capable of performing logic operations have been reported.[23]

3. ROTAXANES AND CATENANES AS ARTIFICIAL MOLECULAR MACHINES

Most of the recently designed artificial molecular machines and motors are based[20] on interlocked chemical compounds named rotaxanes and catenanes. The names of these compounds derive from the Latin words *rota* and *axis* for wheel and axle, and *catena* for chain. Rotaxanes[24] are minimally composed (Figure 1a) of an axle-like molecule surrounded by a macrocyclic compound and terminated by bulky groups (stopper) that prevent disassembly; catenanes[24] are made of (at least) two interlocked macrocycles or "rings" (Figure 1b). Rotaxanes and catenanes are appealing systems for the construction of molecular machines because motions of their molecular components can be easily imagined (Figure 2).

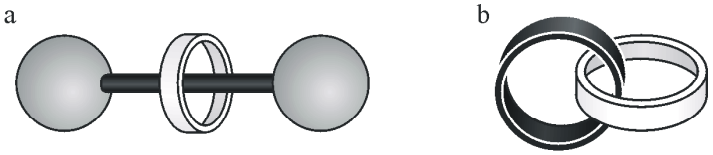

FIG. 1. Schematic representation of a rotaxane (a) and a catenane (b).

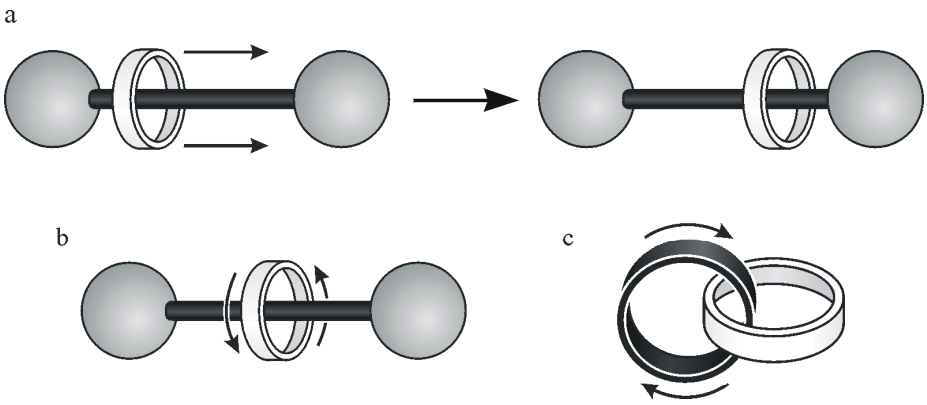

FIG. 2. Schematic representation of some of the intercomponent motions that can be obtained with rotaxanes and catenanes: shuttling (a) and ring rotation (b, c).

Important features of these systems derive from noncovalent interactions between components that contain complementary recognition sites. Such interactions, that are also responsible for the efficient template-directed syntheses of rotaxanes and catenanes, involve electron-donor/acceptor ability, hydrogen bonding, hydrophobic/hydrophylic character, π-π stacking, coulombic forces and, on the side of the strong interaction limit, metal-ligand bonding.

In the next sections, a few examples of artificial molecular machines based on rotaxanes and catenanes taken from our research will be illustrated.

4. AN ACID-BASE CONTROLLED MOLECULAR SHUTTLE

In rotaxanes containing two different recognition sites in the dumbbell-shaped component, it is possible to switch the position of the ring between the two 'stations' by an external stimulus. A system which behaves as a chemically controllable molecular shuttle is compound $\mathbf{1}^{3+}$ shown in Figure 3.[25] It is made of a dibenzo[24]crown-8 (DB24C8) macrocycle and a dumbbell-shaped component containing a dialkylammonium center and a 4,4'-bipyridinium unit. An anthracene moiety is used as a stopper because its absorption, luminescence, and redox properties are useful to monitor the state of the system. Since the N^+–H···O hydrogen bonding interactions between the DB24C8 macrocycle and the ammonium center are much stronger than the electron donor-acceptor interactions of the macrocycle with the bipyridinium unit, the rotaxane exists as only one of the two possible translational isomers. Deprotonation of the ammonium center with a base (a tertiary amine) causes 100% displacement of the macrocycle to the bipyridinium unit; reprotonation directs the macrocycle back onto the ammonium center (Figure 3). Such a switching process has been investigated in solution by ^{1}H NMR spectroscopy and by electrochemical and photophysical measurements.[25] The full chemical reversibility of the energy supplying acid/base reactions guarantees the reversibility of the mechanical movement, in spite of the formation of waste products. Notice that this system could be useful for information processing since it exhibits a binary logic behavior. It should also be noted that, in the deprotonated rotaxane, it is possible to displace the crown ring from the bipyridinium station by destroying the donor-acceptor interaction through reduction of the bipyridinium station or oxidation of the dioxybenzene units of the macrocyclic ring. Therefore, in this system, mechanical movements can be induced by two different types of stimuli (acid-base and electron-hole).

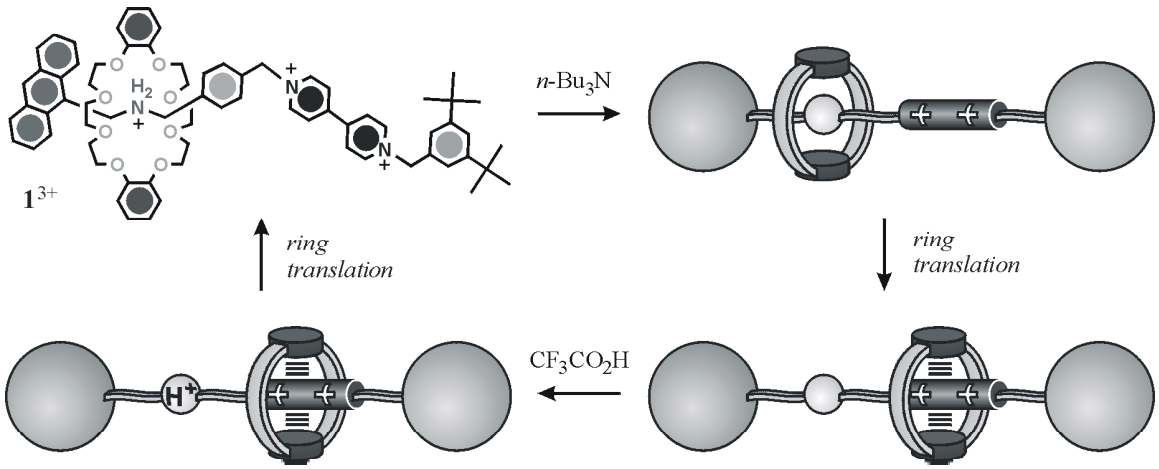

FIG. 3. A chemically controllable molecular shuttle. The macrocyclic ring can be switched between the two stations of the dumbbell-shaped component by acid-base inputs

5. A LIGHT-DRIVEN MOLECULAR SHUTTLE

For a number of reasons, light is the most convenient form of energy to make artificial molecular machines work.[21] In order to achieve photoinduced ring shuttling in rotaxanes containing two different recognition sites in the dumbbell-shaped component, the thoroughly designed compound $\mathbf{2}^{6+}$ (Figure 4) was synthesized.[26] This compound is made of the electron-donor macrocycle R, and a dumbbell-shaped component which contains (i) $[Ru(bpy)_3]^{2+}$ (P) as one of its stoppers, (ii) a 4,4'-bipyridinium unit (A_1) and a 3,3'-dimethyl-4,4'-bipyridinium unit (A_2) as electron accepting stations, (iii) a *p*-terphenyl-type ring system as a rigid spacer (S), and (iv) a tetraarylmethane group as the second stopper (T). The structure of rotaxane $\mathbf{2}^{6+}$ was characterized by mass spectrometry and ^{1}H

NMR spectroscopy, which also established, along with cyclic voltammetry, that the stable translational isomer is the one in which the R component encircles the A_1 unit, in keeping with the fact that this station is a better electron acceptor than the other one. The electrochemical, photophysical and photochemical (under continuous and pulsed excitation) properties of the rotaxane, its dumbbell-shaped component, and some model compounds have then been investigated and two strategies have been devised in order to obtain the photoinduced abacus-like movement of the R macrocycle between the two stations A_1 and A_2: one was based on processes involving only the rotaxane components (intramolecular mechanism), while the other one required the help of external reactants (sacrificial mechanism).

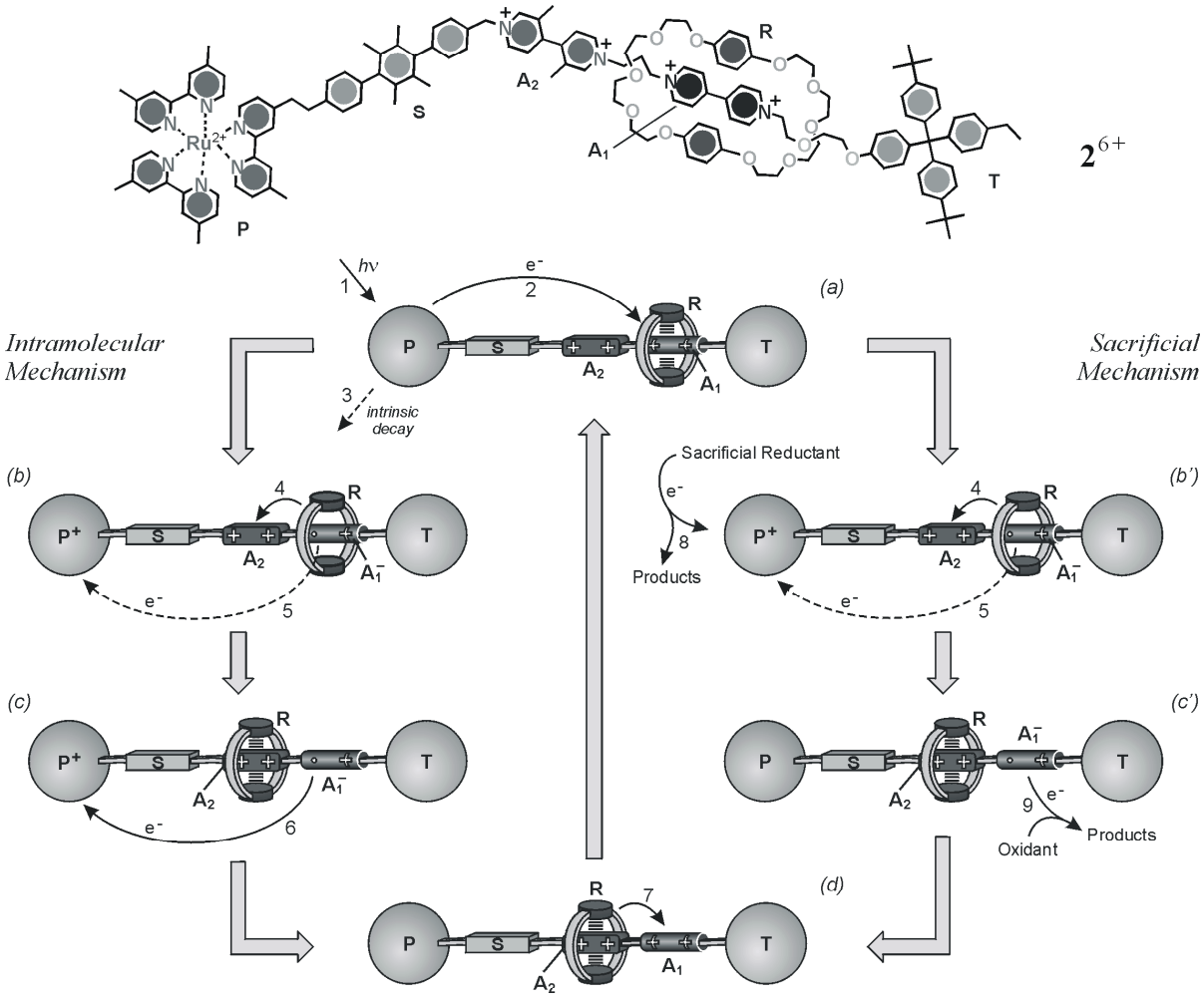

FIG. 4. Structural formula of the rotaxane $\mathbf{2}^{6+}$ *and schematic representation of the intramolecular (left) and sacrificial (right) mechanisms for the photoinduced shuttling movement of macrocycle R between the two stations* A_1 *and* A_2.

The intramolecular mechanism, illustrated in the left part of Figure 4, is based on the following four operations:[26]

(a) *Destabilization of the stable translational isomer*: light excitation of the photoactive unit P (Step 1) is followed by the transfer of an electron from the excited state to the A_1 station, which is

encircled by the ring R (Step 2), with the consequent "deactivation" of this station; such a photoinduced electron-transfer process has to compete with the intrinsic decay of P* (Step 3).

(b) *Ring displacement*: the ring moves from the reduced station A_1^- to A_2 (Step 4), a step that has to compete with the back electron-transfer process from A_1^- (still encircled by R) to the oxidized photoactive unit P^+ (Step 5). This is the most difficult requirement to meet in the intramolecular mechanism.

(c) *Electronic reset*: a back electron-transfer process from the "free" reduced station A_1^- to P^+ (Step 6) restores the electron-acceptor power to the A_1 station.

(d) *Nuclear reset*: as a consequence of the electronic reset, back movement of the ring from A_2 to A_1 takes place (Step 7).

The results obtained[26] do not indicate cleary whether the ring displacement (Step 4) is faster than the electronic reset of the system after light excitation (Step 5; $k = 2.4\times10^5$ s^{-1}). More detailed laser flash photolysis studies suggest that these two processes could occur on the same time scale.[27]

It is worthwhile noticing that in a system which behaves according to the intramolecular mechanism shown in Figure 4 (left) each light input causes the occurrence of a forward and back ring movement (i.e., a full cycle) without generation of any waste product. In some way, it can be considered as a "four-stroke" cyclic linear motor powered by light.

A less demanding mechanism is based on the use of external sacrificial reactants (a reductant like triethanolamine and an oxidant like dioxygen) that operate as illustrated in the right part of Figure 4:

(a) *Destabilization of the stable translational isomer*, as in the previous mechanism.

(b') *Ring displacement after scavenging of the oxidized photoactive unit*: since the solution contains a suitable sacrificial reductant, a fast reaction of such species with P^+ (Step 8) competes successfully with the back electron-tranfer reaction (Step 5); therefore, the originally occupied station remains in its reduced state A_1^-, and the displacement of the ring R to A_2 (Step 4), even if it is slow, does take place.

(c') *Electronic reset*: after an appropriate time, restoration of the electron-acceptor power of the A_1 station is obtained by oxidizing A_1^- with a suitable oxidant, such as O_2 (Step 9).

(d) *Nuclear reset*, as in the previous mechanism (Step 7).

The results obtained[26] show that such a sacrificial mechanism is fully successful. Of course, this mechanism is less appealing than the intramolecular one because it causes the formation of waste products. An alternative strategy is to use a non-sacrificial (reversible) reductant species that is regenerated after the back electron-transfer process.[28]

6. CONTROLLED RING ROTATION IN CATENANES

In a catenane, structural changes caused by rotation of one ring with respect to the other can be clearly evidenced when one of the two rings contains two non-equivalent units. In the catenane $\mathbf{3}^{4+}$ shown in Figure 5, the electron-acceptor tetracationic cyclophane is "symmetric", whereas the other ring contains two different electron–donor units, namely, a tetrathiafulvalene (TTF) and a 1,5-dioxynaphthalene (DON) unit.[29]

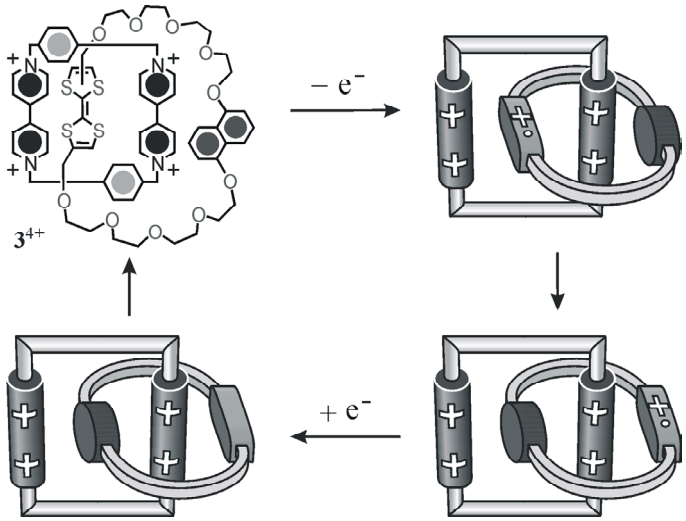

FIG. 5. Redox controlled ring rotation in a catenane containing a non-symmetric ring

In a catenane structure, the electron donor located inside the cavity of the electron-acceptor ring experiences the effect of two electron-acceptor units, whereas the alongside electron donor experiences the effect of only one electron acceptor. Therefore, the better electron donor (i. e., TTF) enters the acceptor ring and the less good one (i.e., DON) remains alongside. On electrochemical oxidation, the first observed process concerns TTF, which thus loses its electron donating properties. Furthermore, an electrostatic repulsion arises between TTF^+ and the tetracationic macrocycle. These effects cause rotation of one ring to yield the translational isomer with the DON moiety positioned inside the acceptor ring. Upon reduction of TTF^+, the initial configuration is restored. However, this may happen without the occurrence of a *full* rotation, because it is equally probable that the reset caused by reduction of TTF^+ occurs by a reverse rotation compared to that occurred in the forward switching caused by TTF oxidation. In order to obtain a full rotation, i.e., a molecular-level rotary motor, the direction of each switching movement should be controllable. This goal can likely be reached by introducing appropriate functions in one of the two macrocycles.[20,21] When this goal is reached, it will be possible to convert alternate electrical potential energy into a molecular-level mechanical rotation.

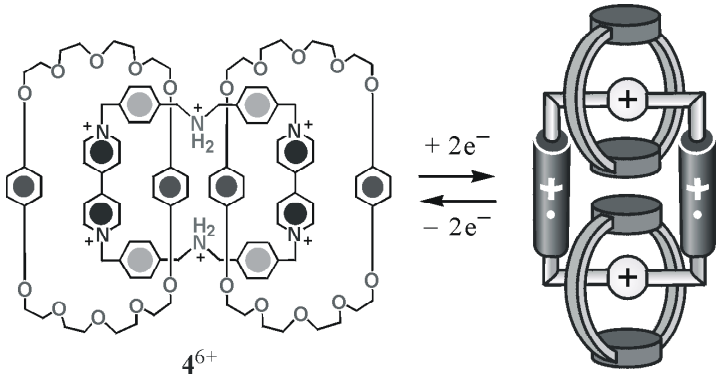

FIG. 6. Redox controlled movements of the ring components upon reduction-oxidation of the bipyridinium units in a catenane composed of three interlocked macrocycles

Controlled rotation of the molecular rings has been achieved also in a catenane composed of three interlocked macrocycles (**4**$^{6+}$, Figure 6).[30] Upon addition of one electron in each of the bipyridinium units, the two macrocycles move on the ammonium stations, and move back to the original position when the bipyridinium units are reoxidized. Unidirectional ring rotation has recently been obtained[31] in a peptide-based catenane having the same topology as **4**$^{6+}$.

7. CONCLUSIONS AND PERSPECTIVES

In the last few years, several examples of molecular machines and motors have been designed and constructed.[17–20] It should be noted, however, that the molecular-level machines described in this chapter operate in solution, that is, in an incoherent fashion. Although the solution studies of chemical systems as complex as molecular machines are of fundamental importance, it seems reasonable that, before functional supramolecular assemblies can find applications as machines at the molecular level, they have to be interfaced with the macroscopic world by ordering them in some way. The next generation of molecular machines and motors will need to be organized at interfaces,[32] deposited on surfaces,[33] or immobilized into membranes[16a,34] or porous materials[35] so that they can behave coherently. Indeed, the preparation of modified electrodes[22,36] represent one of the most promising ways to achieve this goal. Solid-state electronic devices based on functional rotaxanes and catenanes have already been developed.[37] Furthermore, addressing a single molecular-scale device[38] by instruments working at the nanometer level is no longer a dream.[13–15]

Apart from more or less futuristic applications, the extension of the concept of a machine to the molecular level is of interest not only for the development of nanotechnology, but also for the growth of basic research. Looking at supramolecular chemistry from the viewpoint of functions with references to devices of the macroscopic world is indeed a very interesting exercise which introduces novel concepts into Chemistry as a scientific discipline.

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Annex II: *Deliverables List (achieved items in blue)*

Del. No.	*Deliverable name*	WP no.	Lead partic.	Estimated PM	Nature	Dissem level	Deliv .date (month)
D3-1	Controlled grafting of catenane and rotaxane units at metal surfaces	WP3	9	13	R	CO	M12
D4-1	Final design of a light-fueled molecular component	WP4	2	13	R	CO	M12
D6-1	Microtubules assembles on nanostructures	WP6	4	13	R	CO	M12
D5-1	Deposition and single molecule study of stator/rotor/actuator units at surfaces	WP5	3	20	R	CO	M18
D4-2	Computional treatment of light-induced rotary movements in LFMC´s	WP4	7	20	R	CO	M18
Dma 1	BIOMACH conference on nanomotors and bioengines	Man ag.	BIOM.	2	O	PU	M18
Dma 2	Creation of BIOMACH-website	Man ag.	BIOM.	2	O	PU	M18 (M9)
D1-1	Single-molecule investigation and control of anchoring rotaxane and LFMC units at surfaces	WP1	5	30	R	CO	M24
D2-1	Self-assembled chiral stacks including intercalated rotary motors	WP 2	6	30	R	CO	M24
D1-2	Modification of the vesicles and proteins involved in protein-importing molecular motors	WP 1	10	32	R	CO	M39
D2-2	Self-assembled semi-conducting polymers including intercalated rotary motors included	WP 2	6	45	R	CO	M36
D3-2	Monolayers of rotaxanes working as molecular devices	WP 3	3	45	R	CO	M39
D4-3	Operation of LFMC-incorporating photodriven molecular machines	WP 3	2	45	R	CO	M36
D5-2	Design and synthesis hexameric spin transition Fe^{II} compound	WP5	1	45	R	CO	M39
D6-2	Mobility of single kinesine motors on these assemblies	WP6	8	45	R	CO	M36
Rep.	Report 1 + Audit	Man ag.	1(Co)	0.5	R	CO	M12
Rep.	Report 2	Man ag.	1(Co)	0.5	R	CO	M24

-end-