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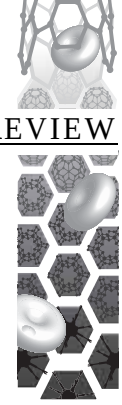

Biological and biomimetic molecular machines

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The evolution of life facilitates the creation of biological molecular machines. In these so-called 'nanomachines,' nature elegantly shows that when precisely organized and assembled, simple molecular mechanical components can link motions efficiently from the nanometer scale to the macroscopic world, and achieve complex functions such as powering skeletal muscles, synthesizing ATP and producing DNA/RNA. Inspired by nature, researchers are creating artifical molecular machines with tailored structures and properties, with the aim of realizing man-made active nanosystems that operate with the same efficiency and complexity as biological nanomachines. It is anticipated that in the not-too-distant future, unique applications of biological and biomimetic molecular machines will emerge in areas such as biochemical instrumentation and nanomedicine.

A molecular machine (or 'nanomachine') is a mechanical device that is measured in nanometers (millionths of a millimeter, or units of 10^{-9} meter; on the scale of a single molecule) and converts chemical, electrical or optical energy to controlled mechanical work [1,2]. The human body can be viewed as a complex ensemble of nanomachines [3,4]. These tiny machines are responsible for the directed transport of macromolecules, membranes or chromosomes within the cytoplasm. They play a critical role in virtually every biological process (e.g., muscle contraction, cell division, intracellular transport, ATP production and genomic transcription).

Even though biological molecular machines have a long history, the idea of constructing artificial molecular machines is recent. In 1959, Richard Feynman originated the idea of artificial molecular machines in his historic address to the American Physical Society, '*There is Plenty of Room at the Bottom*' [5]. The soon-to-be Nobel laureate contemplated, "What are the possibilities of constructing molecular-scale mechanical machines … What would be the utility of such machines? Who knows? I cannot see exactly what would happen, but I can hardly doubt that when we have some control of the arrangement of things on a molecular scale we will get an enormously greater range of possible properties that substances can have, and of the different things we can do." In the early 1980s, the earliest examples of synthetic molecular-level machines were reported; these were based on the photoisomerization of azobenzene [6]. Since then, research in the field of artificial molecular machines has accelerated.

This review discusses biological molecular machines and hybrid systems that integrate these organic nanomachines with inorganic man-made devices. Artificial (or 'biomimetic') molecular machines are then examined, and recent examples, such as 'molecular muscles,' 'nanocars' and 'nanovalves' for drug delivery, are presented. Finally, the review concludes with a prospective of developments in nanomachinery.

Biological molecular machines

Biological molecular machines are molecular proteins that convert the chemical energy released by hydrolysis of ATP into mechanical energy. Ubiquitous in biological systems, these nanomachines are responsible for the directed transport of macromolecules, membranes or chromosomes within the cytoplasm, thus playing a critical role in cell behavior and architecture [3,4,6–8]. Over the past decade, interest in biological molecular machines has blossomed. Atomic structures of various nanomachines have been solved by X-ray crystallography, new molecular machines have been discovered, and forces, steps and speeds have been measured at the molecular level. In nanotechnology, two main fabrication approaches have been utilized. In the bottom-up approach, materials and devices are built from some of the smallest building blocks, atoms or molecules. In the 'top-down' approach, nano-objects are constructed from larger entities using etching and deposition techniques. Recently, hybrid devices integrating 'bottom-up'-based biological molecular machines and top-down-based inorganic nanostructures have been realized. These scientific

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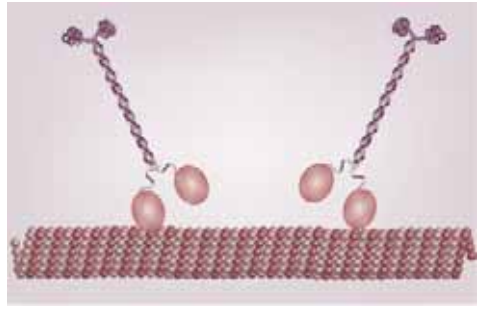
breakthroughs have not only led to a much-improved understanding of cell motility, but also have a profound impact in the field of nanoscience and nanotechnology.

Myosin & kinesin

Myosin, kinesin and their relatives are linear motors that convert the energy of ATP hydrolysis into mechanical work along polymer substrates – myosin along actin filaments in muscles and cells, and kinesin along microtubules (Figure 1) [9,10]. In a kinesin molecule, two heads, each about 8 nm long, are joined at the coiled-coil neck. The neck then connects to a long-coiled coil that terminates in the tail, region and binds to the organelle cargo. Myosin's heads are approximately twice the size of kinesin's and are composed of two major domains: the motor domain which binds to actin and nucleotides, and the regulatory domain, which is an 8-nm-long α-helix stabilized by two calmodulin-like light chains. A long-coiled-coil rod connects to the regulatory domain and oligomerizes to form the thick filament in muscle.

The developments in DNA/RNA sequences have helped reveal the similarities between myosin and kinesin [11]. The core 180 amino acids within the myosin and kinesin head structures have the same fold. Furthermore, the preserved sequence direction and order of the α-helices and β-sheets within the core suggest that these two biomotors evolved from a common ancestor. Yet, despite these structural similarities, there are profound functional differences between kinesin and myosin. Conventional kinesin operates alone or in small numbers to transport membrane-bound organelles over large distances (up to a millimeter) along microtubules, whereas muscle myosin operates in huge arrays of up to a billion molecules in a large muscle fiber and moves relatively short distances (up to approximately one micron) along actin filaments (Figure 2) [12,13]. Myosin provides the power for all of our voluntary motions (e.g., running, walking and lifting) as well as for involuntary motions (e.g., beating of the heart).

Figure 1. Schematic drawing of two kinesin motors bound to a microtubule segment.

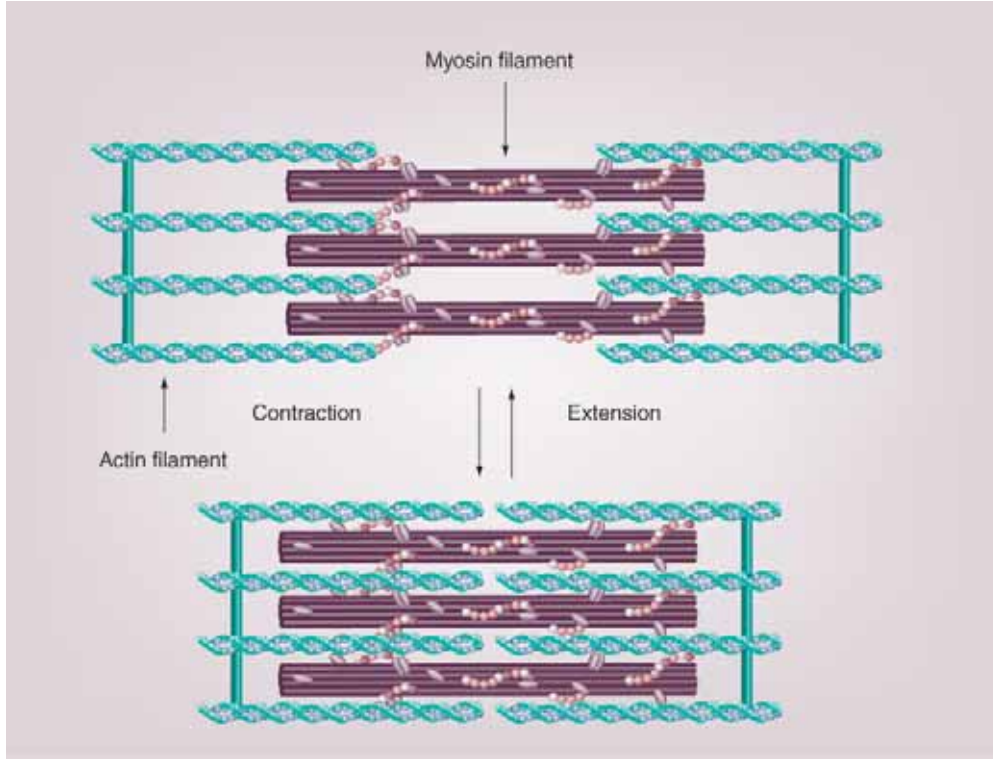


The microtubule is built up from tubulin dimers and has a diameter of 24 nm. Each kinesin motor molecule has two motor domains or heads which are connected by a neck region and a long stalk. The total length of the kinesin molecule is approximately 80 nm [13].
Reproduced with permission from [13].

Recent years have witnessed a strong interest in the exploration of kinesin-based biological molecular machines for nano- and microscale locomotion applications. Limberis and Stewart demonstrated that kinesin could be engineered and used to transport silicon microchips along microtubules [14]. Negatively charged microtubules bound electrostatically to a positively charged coverslip were aligned in a microfluidic channel. Next, genetically modified kinesin motors attached with silicon microchips were added into the microfluidic channel. The microchips were observed to move in a variety of ways (Figure 3), depending on how they were bound to microtubules. If a microchip moved along parallel microtubules, it traveled along linearly at a maximum speed of 0.8 μm/s (Figure 3A). When it interacted with antiparallel microtubules, it rotated (Figure 3B). A microchip with one end fixed flipped over (Figure 3C). This work proved that kinesin motors had enough force to transport cargos that are much larger than kinesin molecules, thus paving the way for future development of biomotor-based nanosystems.

To improve the efficiency of kinesin-based nanosystems, the orientation of microtubules must be controlled and aligned. Hiratsuka *et al.* developed a lithographic process to restrict microtubules onto fabricated tracks and control the direction of kinesin's mechanical motion [15]. The experiment used two concentric circles with arrowhead patterns facing opposite directions (Figure 4). Without the arrowheads in the pattern, the microtubules would travel either clockwise or counterclockwise in either circle. However, once the arrowheads pattern was added, within a few minutes the microtubules would travel in the same direction that the arrows pointed towards. As microtubules enter the arrowhead from the correct direction, they could easily pass through; however, if microtubules entered from the reverse direction, they would usually bump against the wall at the base of the arrow and turn around.

Figure 2. Myosin filament operation.



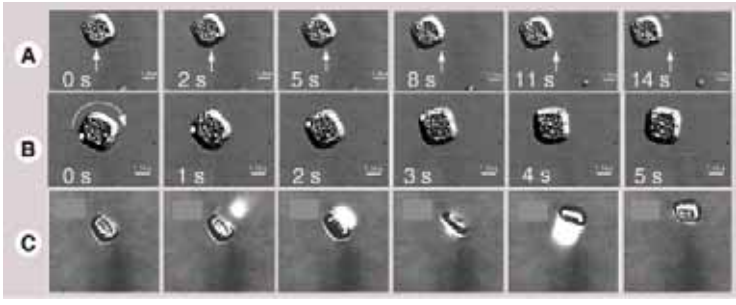
The sliding of myosin along actin filaments causes muscle contraction and extension.

Four different versions of the arrowhead pattern were tested; the most efficient pattern had a 73% chance of reversing microtubules in the wrong direction.

The alignment of microtubules for guiding the long-range transportation of kinesin motors has also been investigated. Platt *et al.* reported the alignment of magnetic nanoparticle functionalized microtubules in magnetic fields (Figure 5) [16]. In this study, microtubules were labeled with cobalt ferrite ($CoFe_2O_4$) nanoparticles through the biotin–neutravidin binding. When placed above a magnet, magnetized microtubules aligned to match the orientation of magnetic line. It was also shown that microtubules could respond to the changing of magnetic orientation and reorient them within several seconds. Recently, in order to increase the directional control of magnetic nanoparticle labeled microtubules, Huchtins *et al.* selectively labeled magnetic nanoparticles to a segmented microtubule [17].

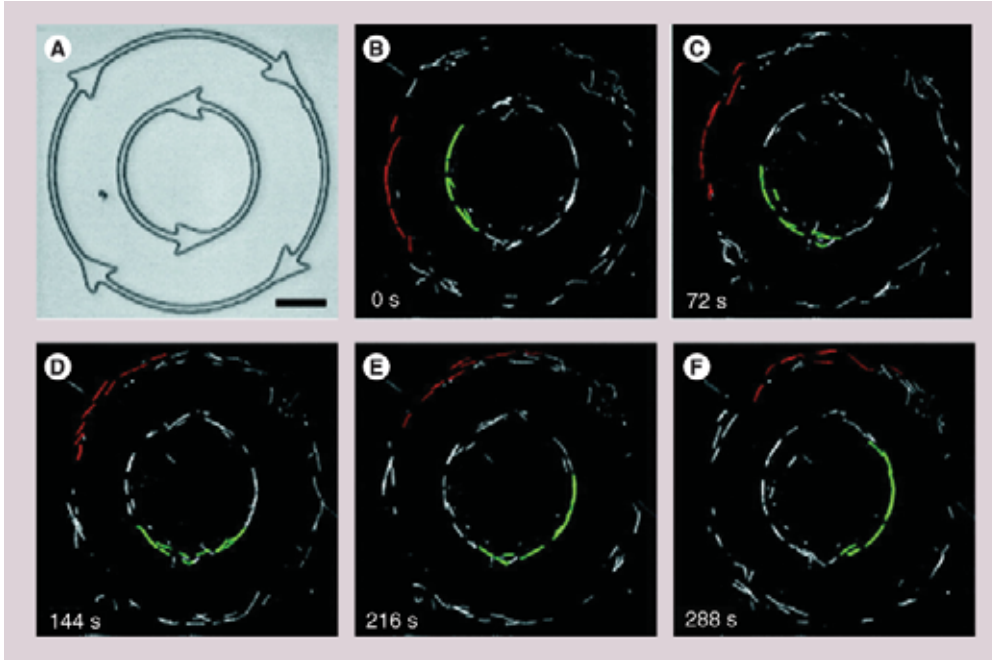
Recently, van den Heuvel *et al.* reconstituted the kinesin–microtubule transport system in enclosed fluidic channels and realized the active electrical control of the direction of individual kinesin-propelled microtubule filaments at Y junctions [18]. Using this technique, they demonstrated molecular sorting of differently labeled microtubules (Figure 6). The steering of microtubules is attributed to electric field-induced bending of the leading tip. From measurements of the orientation-dependent electro-

Figure 3. Kinesin-powered silicon microchips.



(A) The chip moves on parallel microtubules at 0.8 µm/s. The arrow is a fixed reference point. **(B)** The chip rotates clockwise on antiparallel microtubules. A reference dot was placed on one corner of the chip. **(C)** The chip flips perpendicular to the surface [14].
Scale bar: 5 µm.
Reproduced with permission from [14].

Figure 4. Unidirectional movement of microtubules along circular tracks.

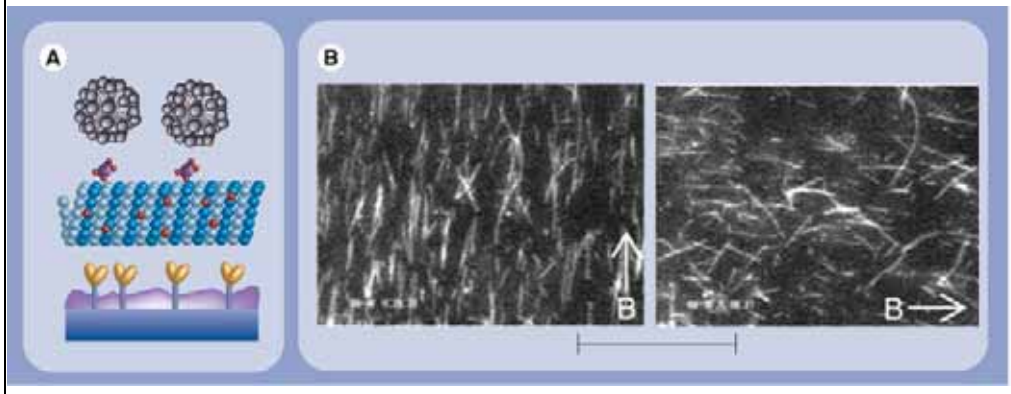


(A) Image of the arrowhead pattern. **(B–F)** Photographs demonstrating the microtubule rotational movement were taken at 72 s intervals beginning 20 min after the addition of ATP. Some filaments were dyed to facilitate tracking of microtubules: the red microtubules are moving clockwise and the green microtubules are moving counterclockwise [15].
Reproduced with permission from [15].

phoretic motion of individual, freely suspended microtubules, they estimate the net applied force on the tip to be in the pN range.

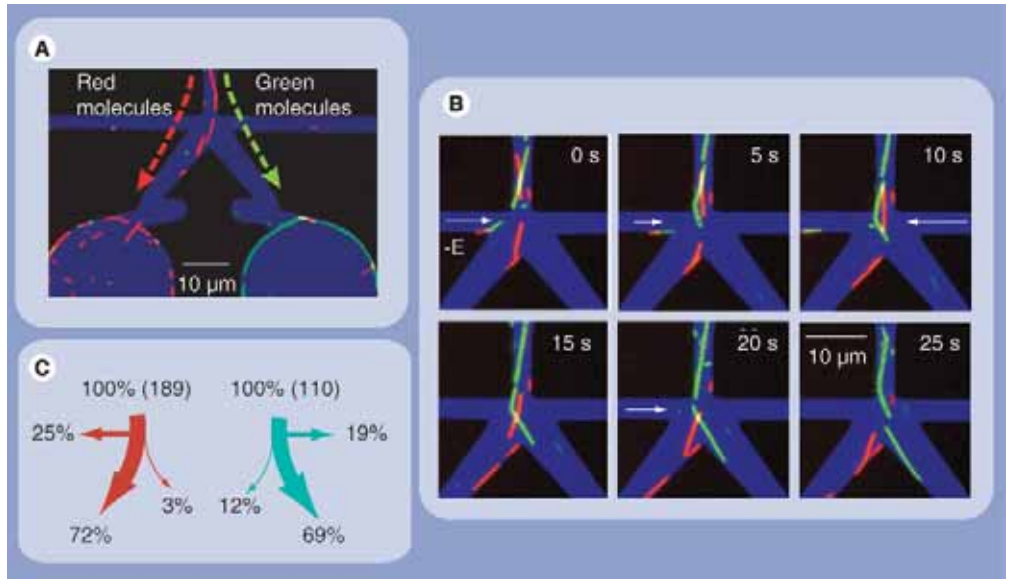
These developments in kinesin–microtubule transport systems have enabled initial steps towards directed assembly in micro- and nanoscale. Brunner *et al.* recently demonstrated engineered cargo transport by functionalizing immobilized cargoes and microtubules with specific cross-linking pairs [19]. The molecular recognition event between the cargo- and microtubule-induced necessary rupture force to load immobilized cargo onto the microtubule and transport it.

Figure 5. Alignment of microtubules in magnetic field.



(A) Schematic of the $CoFe_2O_4$ nanoparticle functionalized microtubules on a kinesin surface. **(B)** The 90° rotation of magnetic field causes the reorientation of previously aligned microtubules within 4 s [16].
Scale bar = 58 µm.
Reproduced with permission from [16].

Figure 6. Demonstration of molecular sorting.



(A) Color image of a mixture of red- and green-labeled microtubules approaching a Y junction. Electrical force is used to steer microtubules carrying green and red fluorophores into the right and left reservoirs, respectively. **(B)** Example of successful sorting events for a green- and a red-labeled microtubule. **(C)** Steering efficiencies for 189 red and 110 green microtubules [18].
Reproduced with permission from [18].

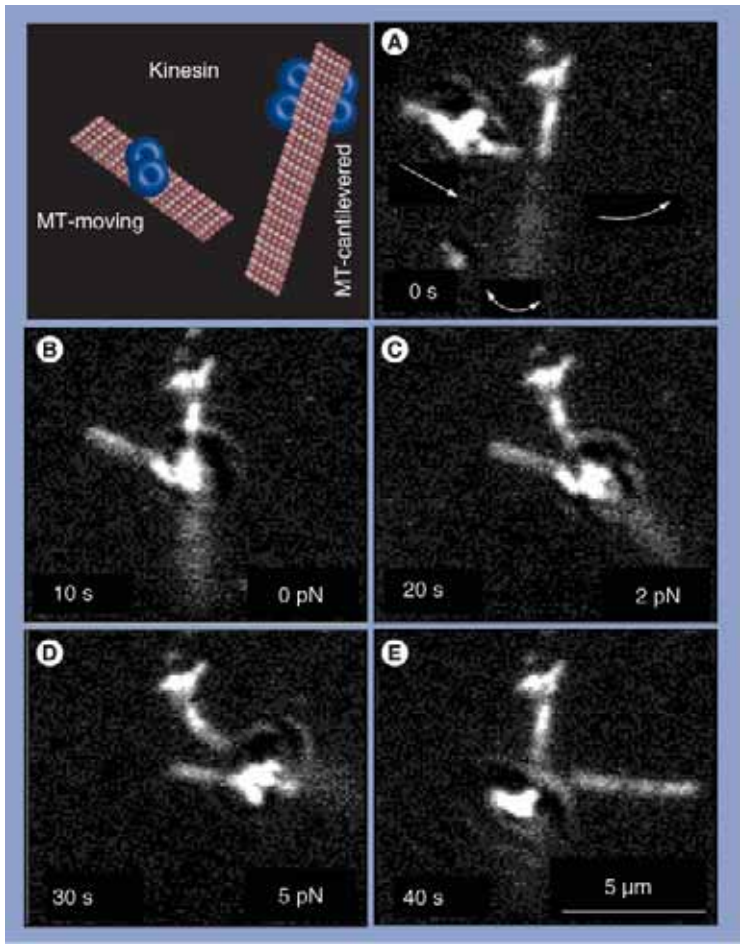
Locomotion is not the only attribute of biological molecular machines. Hess *et al.* have developed a forcemeter assembled from kinesin and microtubules (Figure 7) [20]. One microtubule with known stiffness serves as a cantilever. Force is applied by another microtubule propelled by kinesin motor proteins adsorbed to a surface. The two microtubules are attached by a link whose strength is to be determined. The moving microtubule continues to travel while bending the molecular cantilever until the link ruptures. The force on the link can be calculated based on the bending of the cantilevered microtubule, the distance traveled by the second microtubule and the angle between the two microtubules. Since this forcemeter measures forces at pN range, it is ideal for measuring the strength of biological receptor/ligand pairs, such as streptavidin–biotin. In similar approaches, Deiz *et al.* and Dinu *et al.* have used kinesin–microtubule system to physically stretch biofunctionalized DNA molecules aimed towards parallel formation of structured DNA templates [21,22].

ATPase

ATP synthase (ATPase) is a ubiquitous protein that functions as a proton pump and the primary source of ATP for most eukaryotic cells [23–27]. It manufactures ATP from ADP and phosphate by using the energy derived from a transmembrane proton gradient. It can also reverse itself and hydrolyse ATP to pump protons against an electrochemical gradient. ATPase carries out both its synthetic and hydrolytic cycles by a rotary mechanism. The general structure of a F0F1-ATP synthase is shown in Figure 8. It consists of two portions: a soluble component, F1, containing the catalytic sites; and a transmembrane component, F0, comprising the proton channel. The entire F0F1 structure is arranged as a counter-rotating 'rotor' and 'stator' assembly. The stator portion consists of catalytic sites from the $\alpha 3\beta 3$ hexamer, together with subunits α, $\beta 2$ and δ. The rotor consists of 9–12 c-subunits arranged in a ring and connected to the β- and ε-subunits that form the central 'shaft'.

Recently, the rotation of F1-ATPase was directly observed during ATP hydrolysis by attaching a fluorescent actin filament to the γ-subunit and immobilizing the $\alpha 3\beta 3$ subunit on a coverslip [28]. In another experiment [29], F1-ATPase was integrated with inorganic nanoengineered systems to form hybrid organic-inorganic–nanomechanical devices powered by biological molecular motors (Figure 9). The device consisted of three parts:

Figure 7. Kinesin and microtubule.



The cantilevered MT is attached on one end to a clump of beads, while the other end is suspended above the surface. Swiveling due to Brownian motion causes the smeared-out appearance of the free end. The moving microtubule is propelled by kinesin motor proteins adsorbed to the surface and carries a clump of beads. The snapshots show **(A)** how the moving microtubule approaches (0 s), **(B)** makes contact (10 s), **(C & D)** bends (20 s and 30 s) and **(E)** releases (40 s) the cantilevered microtubule [20].
MT: Microtubule.
Reproduced with permission from [20].

- Nanofabricated nickel posts
- Recombinant F1-ATPase motors that were engineered to adhere with the nanofabricated structures
- Engineered nanopropellers

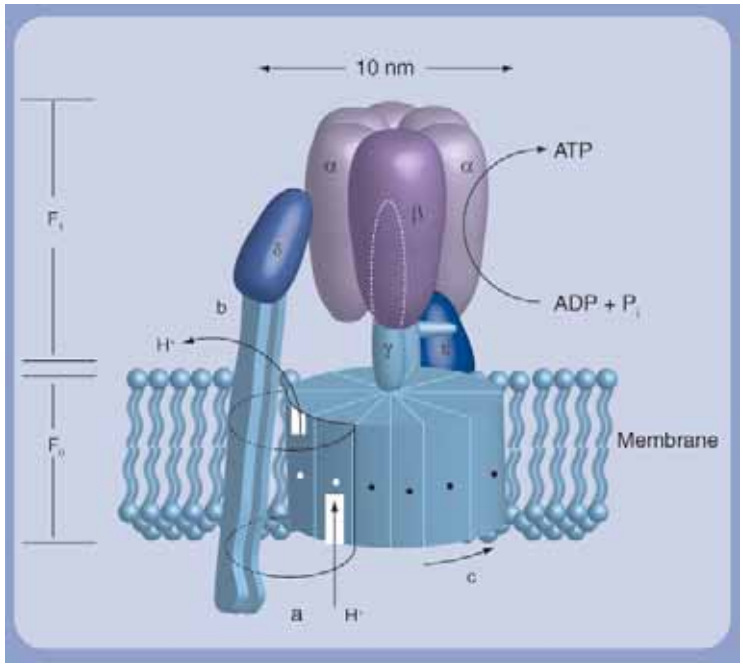
To integrate these components into one functional unit, the F1-ATPase biomolecular motors were biotinylated and then attached to the Ni posts using histidine tags engineered into the β subunit. In addition, the Ni nanopropellers were linked to the γ-subunit using biotin–streptavidin. The rotation of the propellers was tested with and without ATP to prove the dependency on the F1-ATPase motor. With ATP, only five of the 400 propellers rotated continuously. Approximately 80% of the propellers did not move at all. It was thought that the propellers might have been attached to more than one motor or attached to the substrate in addition to the motor. Rotational velocity of the propellers varied depending on the point of attachment and the length of the propeller. Although the device needed further improvement, it laid the foundation for the future development of nanosystems using F1-ATPase as the active nanostructure.

DNA

DNA stores the genetic information vital for the development and functioning of all living organisms. Taking advantage of the extraordinary specificity between two complementary DNA strands, researchers have created a variety of nanoscale machinery. Yurke *et al.* constructed a DNA-based molecular tweezer operated by the addition of specific oligonucleotide sequences [30]. In its open status, the DNA tweezer consists of a flexible single-strand hinge connecting to two double-stranded arms (Figure 10A). Upon the addition of a set strand (F), the molecular tweezer hybridizes with the set strand and the arms are pulled together, changing the molecular conformation from the open status to the closed one. The reversible process can be achieved by adding an unset strand ($\bar{F}$) complementary to the set strand (F). Results from dye quenching experiments confirmed that the operation of the DNA-based molecular tweezer can be repeated for multiple cycles (Figure 10B).

Recently, Shin *et al.* reported a DNA walker that can travel along a linear track by sequential addition of different complementary DNA strands (Figure 11) [31]. The DNA walker consists of a hybridized DNA pair with two legs. To obtain stepwise motion of the DNA walker along the linear track, a DNA strand (A1) is added to bind one leg of the DNA walker to the first anchor strand of the linear track. In the next step, a second DNA strand (A2) attaches the other leg of the walker molecule to the track. This step is followed by the addition of A1's complementary strand D1, which unbinds the first leg of the DNA walker from the linear track. When repeated in a cyclic fashion, this procedure realizes linear motion of the DNA walker molecule along the track. The operation of this DNA walker requires a constant supply and removal of

Figure 8. Schematic drawing of a F_0F_1-ATP synthase.



The transmembrane (F_0) and soluble component (F_1) of F_0F_1 synthase can cyclically synthesize and hydrolyze ATP by a rotary mechanism [25]. Reproduced with permission from [25].

different DNA strands. To overcome this limitation, autonomous DNA walkers were later proposed and demonstrated by Bath *et al.* [32] and Yin *et al.* [33]. These works have demonstrated that by carefully engineering DNA strands, it is possible to harness the binding between complementary DNA strands to accomplish complicated tasks such as locomotion.

Biomimetic molecular machines

Biological molecular machines are intrinsically designed for the physiological conditions (37°C, pH 7) within the human body. These restrictions, in addition to the difficulties of modifying their structure and functionality, hinder integration with man-made devices. In this regard, the future of molecule-driven actuators lies in the development of artificial molecular machines as opposed to biomolecular machines. The ability to synthesize analogical molecular versions of macroscopic machinery is considered to be an essential step towards the construction of molecular devices capable of performing mechanical work and storing/transmitting information [2]. Similarily to, their macroscopic counterparts, artificial molecular machines are characterized by:

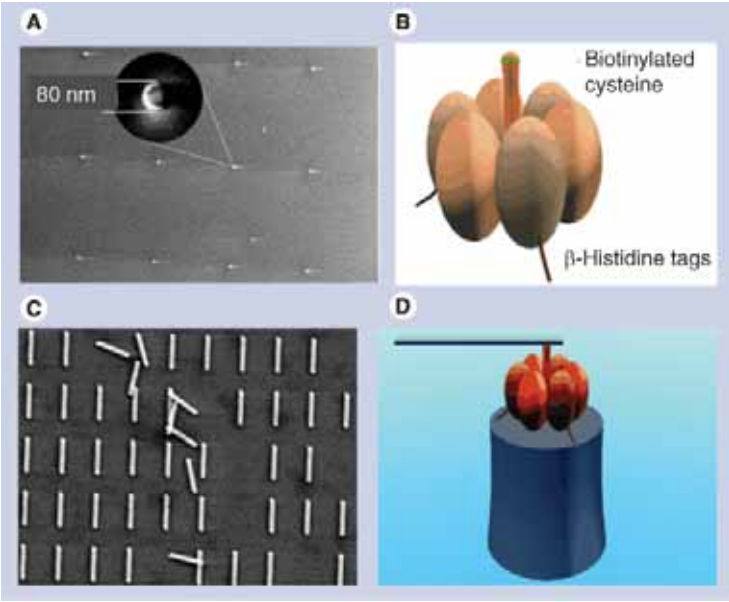
- The form of energy input enabling activation
- The kind of movement executed by their components
- The way in which their operation can be controlled and monitored
- The possibility to repeat the operation at will
- The time scale needed to complete a cycle of operation
- The function performed

Recent advances in synthetic chemistry have yielded analogs of rotors, gears, switches, shuttles, turnstiles and ratchets [1]

Rotaxanes and catenanes, which are composed of mutually recognizable and intercommunicating ring and dumbbell-shaped components, are considered some of the most successful artificial molecular machines constructed to date [1,2,34–41]. When stimulated by light, electricity or chemical reagents, these mechanically interlocked molecules (Figure 12) experience relative internal motions (rotary and linear) of their components just like the moving parts of macroscopic machines. A rotaxane is a molecule composed of a macrocyclic and a dumbbell-shaped component (Figure 12A). The macrocycle encircles the linear rod-like portion of the dumbbell-shaped component and is trapped mechanically around it by two sizeable stoppers. Thus, the ring and dumbbell components cannot dissociate from one another, even though they are not covalently bonded to each other. The structural formula and graphical representation of a bistable rotaxane R1 is shown in Figure 12C. R1 consists of a tetracationic cyclobis(paraquat-p-phenylene) ($CBPQT^{4+}$) ring component and a linear rod section that contains two different stations, a tetrathiafulvalene (TTF) unit and a dioxynaphthalene (DNP) unit. R1's starting state has its TTF unit encircled by the positively charged ring. Upon oxidation, the TTF unit becomes dicationic (TTF^{2+}) and experiences electrostatic repulsion with the ring, causing the ring to shuttle toward the DNP unit in the oxidized state. Conversely, reduction of the TTF^{2+} back to a neutral TTF unit causes R1 to return to its starting state. Via ring translation, this bistable rotaxane can therefore be equated as a linear molecular motor.

By contrast, a catenane is a molecule composed of two interlocked macrocyclic components. The incorporation of two different recognition sites within the 'asymmetric' macrocycle (Figure 12B) allows the 'symmetric'

Figure 9. F1-ATPase-powered nanomechanical device.



The device consists of **(A)** Ni post (height 200 nm, diameter 80 nm), **(B)** F1-ATPase motor, **(C)** nanopropellers (length 750–1400 nm, diameter 150 nm) and **(D)** assembled device [29].
Reproduced with permission from [29].

macrocycle component to preferentially reside around one of these two positions. By switchingthe properties at one of the two recognition sites of the asymmetric macrocycle on or off , the relative position of the two species can be reversibly controlled.

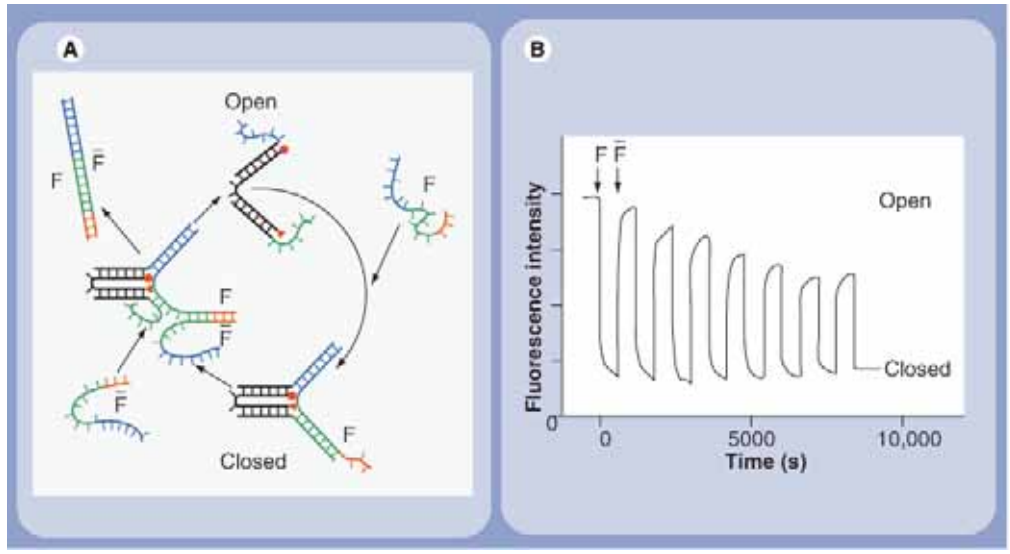
Capable of delivering mechanical motions at dramatically reduced scales in comparison to traditional microscale actuators, artificial molecular machines such as bistable rotaxanes can be the key active nanostructures for many applications. For example, as actuation materials, bistable rotaxanes have at least seven advantages.

- They can maintain their actuation properties across multiple scales (from single molecule level to nano to micro to meso to macro), while most actuation materials are not capable of keeping their actuation properties in smaller scale, the functions of these polymers are bulk properties that require multiunit interactions, and thus are not desirable for molecular-level devices [42–45];
- They can generate large strains up to 42%, while the strains generated by the gold-standard actuation materials, such as piezoelectric materials, are typically 0.1–0.2% [42–45];
- They have high force density, for example, a bistable rotaxane generates a 100 pN force [46,47], while a kinesin biomotor, which is much larger than a bistable rotaxane, can only generate 6 pN [7,11];
- They can undergo controlled mechanical motion for a variety of external stimuli (chemical [37,39], electricity [38,40] and light [36,48]), while traditional actuation materials and biomotors must both rely on a single stimulus;
- They can be made metastable, the molecules can persist in their actuated state long after the stimulus has been removed, and are thus energy-saving;
- They can be customized and optimized, therefore conferring the flexibility necessary for a multitude of engineering applications. For example, they can be derivatized with disulfide tethers to facilitate the formation of self-assembled monolayers (SAMs) [49] or prepared with amphiphilic properties to facilitate the formation of Langmuir–Blodgett films [50,51], both of which are key bottom-up nanomanufacturing technologies for the simultaneous self-organization of a multitude of molecules;
- They can survive in a wide range of temperatures (-30°C to 100°C) and pH values (5–9), while biomotors are restricted to physiological conditions (T ~37°C, pH ~7). These advantages indicate that although biological nanomachines provide perfect actuation in human bodies, artificial molecular motors such as bistable rotaxanes are more suitable for the development of molecular motor-based medical and engineering applications (Table 1).

Molecular valve for drug delivery

Nguyen *et al.* have utilized the locomotion of the ring in R1 to build an impressive molecular valve for drug delivery purposes [52]. To build this system, one end of the R1 molecule was attached to the pores of silica nanoparticles and the other end was left standing free. Redox-controlled bistability of the ring in R1 molecule allowed the storing and releasing of the drug (guest molecules) across the pores of silica nanoparticles. In this system, guest molecules were loaded and stored in the pores when the ring of R1 was away from the silica surface. When subjected to an oxidizing environment, the ring of R1 travels towards the silica surface, enabling the guest

Figure 10. Operation of DNA-based molecular tweezer.



Operation of a DNA based molecular tweezer. **(A)** The open status can be changed to the closed status by adding a set strand (F). This process can be reversed by adding an unset strand ($\bar{F}$) . **(B)** The operation of DNA tweezer over multiple cycles is recorded by dye quenching techniques.
Reproduced with permission from [30].

molecules to be stored within the nanoparticles (Figure 13A). To release the guest molecules, these loaded nanoparticles were subjected to a reducing environment. In the reducing environment, the ring moves away from the silica surface, causing the controlled drug release. This system was tested for storing and releasing a luminescent guest molecule Ir(ppy)3. When the nanoparticles loaded with Ir(ppy)3 were subjected to a reducing environment, a sudden increase in the luminescence (Figure 13B) was correlated to the release of the guest molecules due to the movement of the ring away from the silica surface. Recently, a similar release of guest molecules was demonstrated by photoactive rotaxanes [53,54]. Such systems can be potentially utilized in the future light-driven molecular-level drug delivery systems.

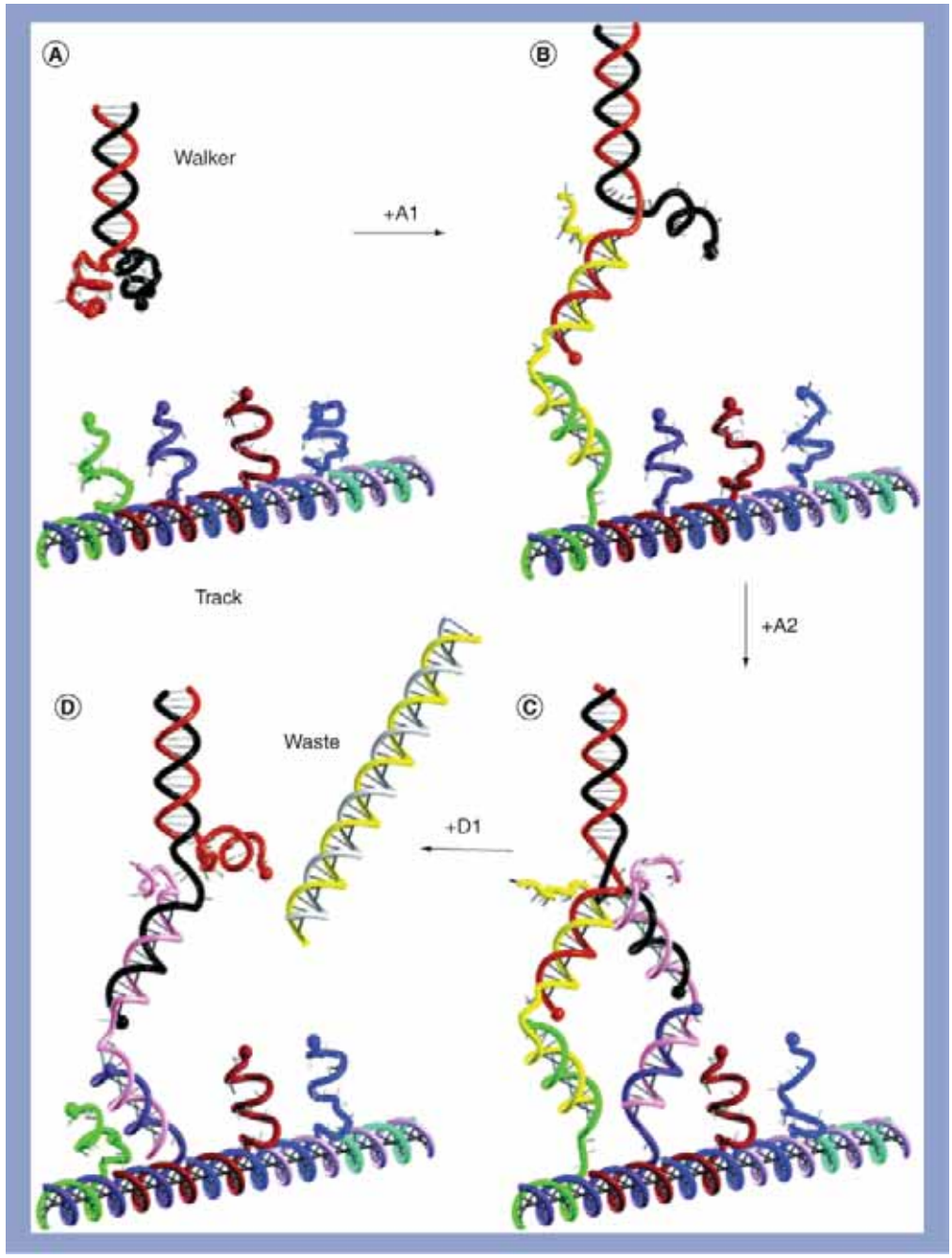
Nanocars

Researchers at Rice University have demonstrated a nanoscaled version of a macroscopic car translating on a gold surface [55]. This single-molecule nanocar was built by four fullerene molecules that act as wheels of a car (Figure 14A). Fullerenes are football-shaped molecules consisting of 60 carbon atoms and are reported to roll on gold surfaces. These fullerene wheels were connected with a chassis synthesized by Pd-catalyzed coupling reactions, and the presence of alkyne connections in the chassis enabled the rotation of the wheels. When the temperature of the gold substrate was raised above 170 °C, scanning tunneling microscopy images revealed that the nanocar exhibits both pivotal and translating motions (Figure 14B–F). These two motions are hypothesized to occur owing to the fullerenes rolling in multiple directions on the gold surfaces. The reported nanocar lacked the capability to carry a cargo and was not self-motorized. Towards the realization of motor-driven nanocars, a light-driven nanocar has been recently synthesized and shown to operate in solution phase [56]. The potential applications of these nanosystems could be in transportation of cargos from one location to another in multiple biomedical applications.

Powering a microcantilever actuator with molecular muscles

Huang and colleagues created a molecular machine-based mechanical actuator by utilizing a hybrid top-down/bottom-up manufacturing approach [57]. Expanding upon a series of bistable [2]rotaxanes, a bistable [3]rotaxane R2 (Figure 15A & 15B) was created as a molecular muscle to mimic the contraction and extension movements of skeletal muscle. This design takes advantage of well-established recognition chemistry that selectively positions the $CBPQT^{4+}$rings around the

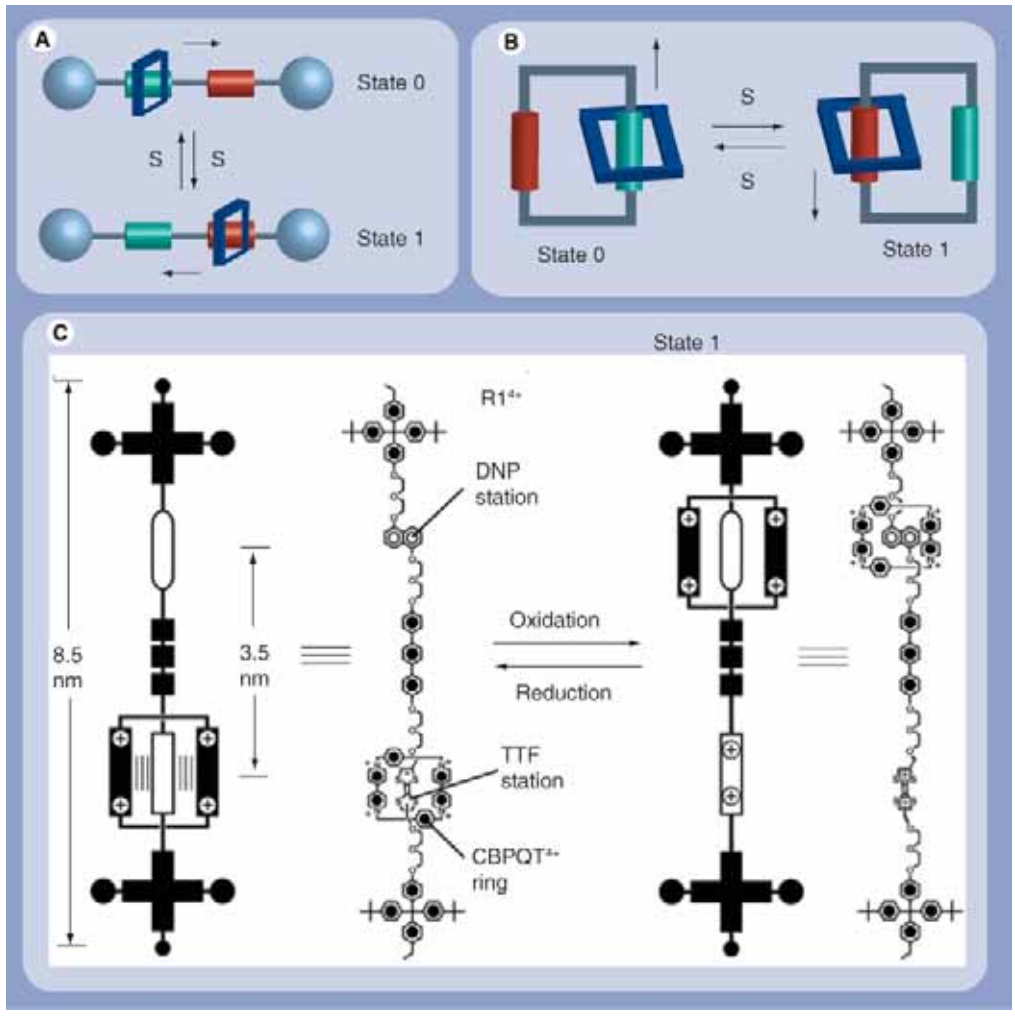
Figure 11. Stepwise motion of DNA Walker on a linear track.



Stepwise motion of a DNA walker. **(A)** Walker DNA molecule and linear track for locomotion. **(B)** Addition of A1 strand causes the binding of the DNA walker to the first anchor point on the locomotive track. **(C)** Addition of A2 binds the walker to the adjacent anchor point on the track. **(D)** Addition of A1's complementary strand D1 causes the release of walker from the first anchor point and simultaneous yielding of waste DNA pair.
Reproduced with permission from [31].

two TTF units of R2, as opposed to the two DNP units. Chemical oxidation of the TTF units to their dicationic forms (TTF^{2+}) drives the $CBPQT^{4+}$ rings to the DNP units. This power stroke arises primarily from electrostatic charge–charge repulsion between the $CBPQT^{4+}$ rings and the TTF^{2+} units. Upon reduction of the two TTF^{2+} stations back to their neutral form,

Figure 12. Artificial molecular machines.



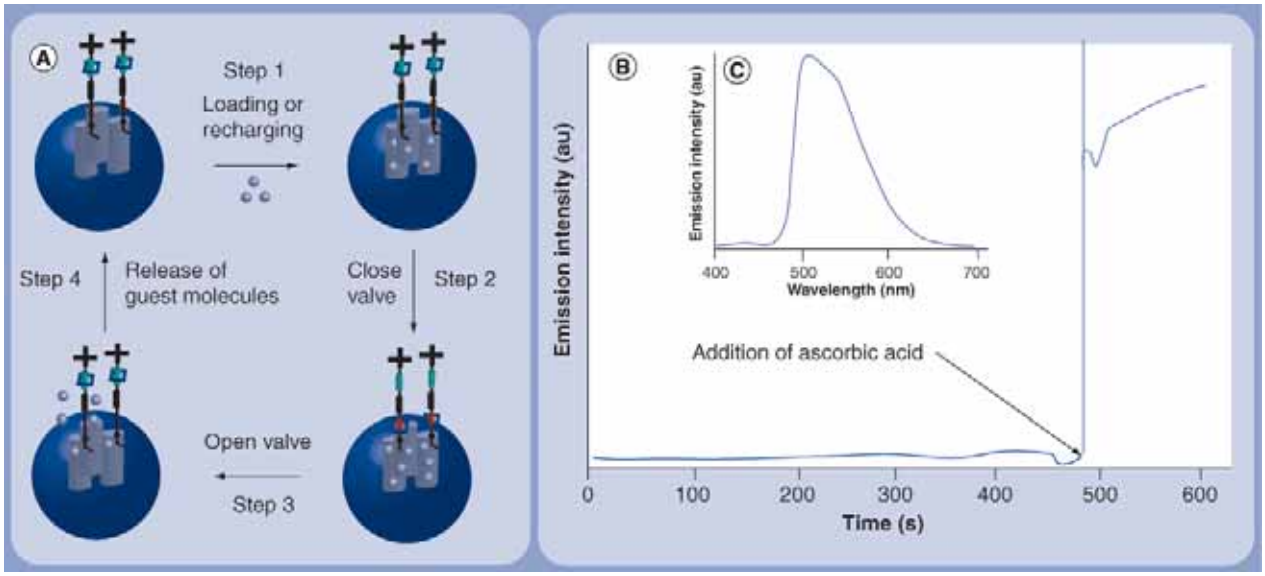
Schematic drawing of a bistable **(A)** rotaxane, **(B)** catenane and **(C)** Molecular structure and operation mechanism of a bistable rotaxane R1.

the inter-ring distance increases as the CBPQT^{4+} rings return to the TTF stations by means of a thermally activated diffusive stroke. Thus, the cycle of contraction and extension within R2 mimics the motion that occurs inside natural muscle fibers as shown in Figure 2. The incorporation of a disulfide tether onto each CBPQT^{4+} ring component provides an anchoring point by which the [3]rotaxane can be attached to a gold surface as a SAM. Oxidation of R2 will generate a tensile stress upon a gold surface through the contractive action of its two disulfide-tethered CBPQT^{4+} rings. If the substrate is sufficiently thin and flexible, such as a long cantilever beam,

Table 1. Comparison between biomotors and bistable rotaxanes.

	Biological molecular motors	Bistable rotaxanes
Surviving Condition	Physiological conditions only (37°C, pH 7)	T: -30–100°C, pH: 5 9
Flexibility	Difficult to modify structures/functions	Easy to modify
Stimuli	Chemical only	Chemical, electricity or light
Size	30–100 nm^3	8 nm^3
Force	~6 pN (Kinesin)	40–100 pN

Figure 13. Drug delivery device based on R1 molecular machine.



(A) R1 molecules are attached to porous silica nanoparticles to build a drug delivery system [52]. Loading and releasing of guest molecules is done in four steps (loading, closing the valve, opening the value, release). Motion of the ring toward or away from the surface along the molecule controls the storage and release of the drug. **(B)** Sudden increase in the emission intensity of luminescent guest molecule upon the addition of ascorbic acid indicates the operation of molecular valve. **(C)** Inset shows the visible spectrum of the released Ir(ppy)3 molecules.
Reproduced with permission from [52].

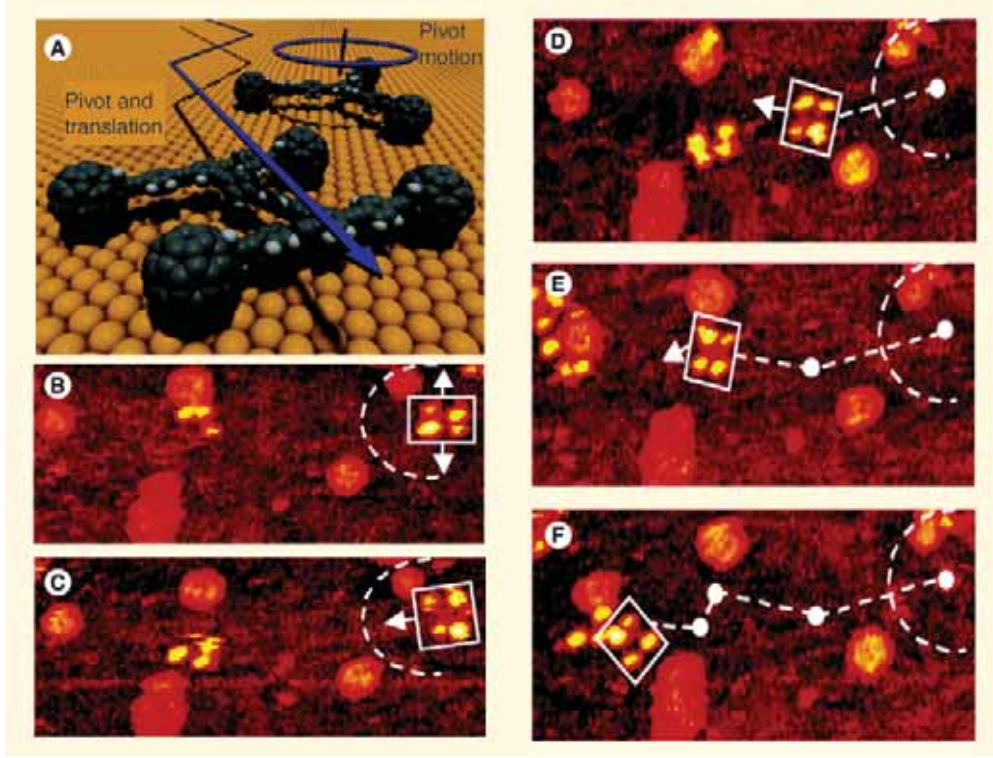
the cumulative effect of each individual molecular muscle will produce an upward mechanical bending of the beam. Conversly, reduction of the oxidized and contracted R2 will return the $CBPQT^{4+}$ rings to the TTF stations and, consequently, relieve the stress upon the beam, resulting in a downward motion and a return to the beam's equilibrium position.

For the first time the authors reported the nanomechanical response of microcantilever beams coated with a SAM of artificial molecular motors for chemical stimulus [57]. A simple model that considers the mechanical movements of each molecule within the SAM verifies chemo–mechanical transduction as a likely mechanism for cantilever bending. This chemo–mechanical actuation response of artificial molecule machines was realized with a gold-coated silicon cantilever array that was coated with a SAM of R2 (Figure 15C) and placed in a transparent fluid cell. Addition of the oxidant solution was observed to cause the cantilever beams to bend to an upward limit of approximately 35 nm (Figure 15D, top series of traces). Entry of the reductant solution into this system caused the beams to bend back downward to their starting positions. This behavior was observed for all four cantilever beams for 25 cycles (the first three complete cycles are shown here). The slight attenuation in beam deflection following each cycle is attributed to a gradual chemical and/or physical passivation of the SAM. Nevertheless, the movement of the cantilever beams is directly correlated with the cycling of the oxidant and reductant solutions and the experimental data (35 nm displacement) matches closely with the theoretical quantitative analysis (48 nm displacement). Furthermore, control experiments were performed with a control compound TPD that did not have movable rings, and the results (Figure 15D, bottom series of traces) prove that the observed actuation is due to the movement of the rings. These observations support the idea that cumulative nanoscale movements within surface-bound molecular muscles can be harnessed to perform larger-scale mechanical work. In the future, it can be expected in the future that similar nanomechanical work will be performed on microcantilevers through other activation methods such as light and electricity [58].

Driving macroscopic liquid droplets with nanomachines

Berna and colleagues demonstrated the use of a molecular machine-based photoresponsive surface to control macroscopic liquid transport

Figure 14. Locomotion of Nanocar on gold surface.



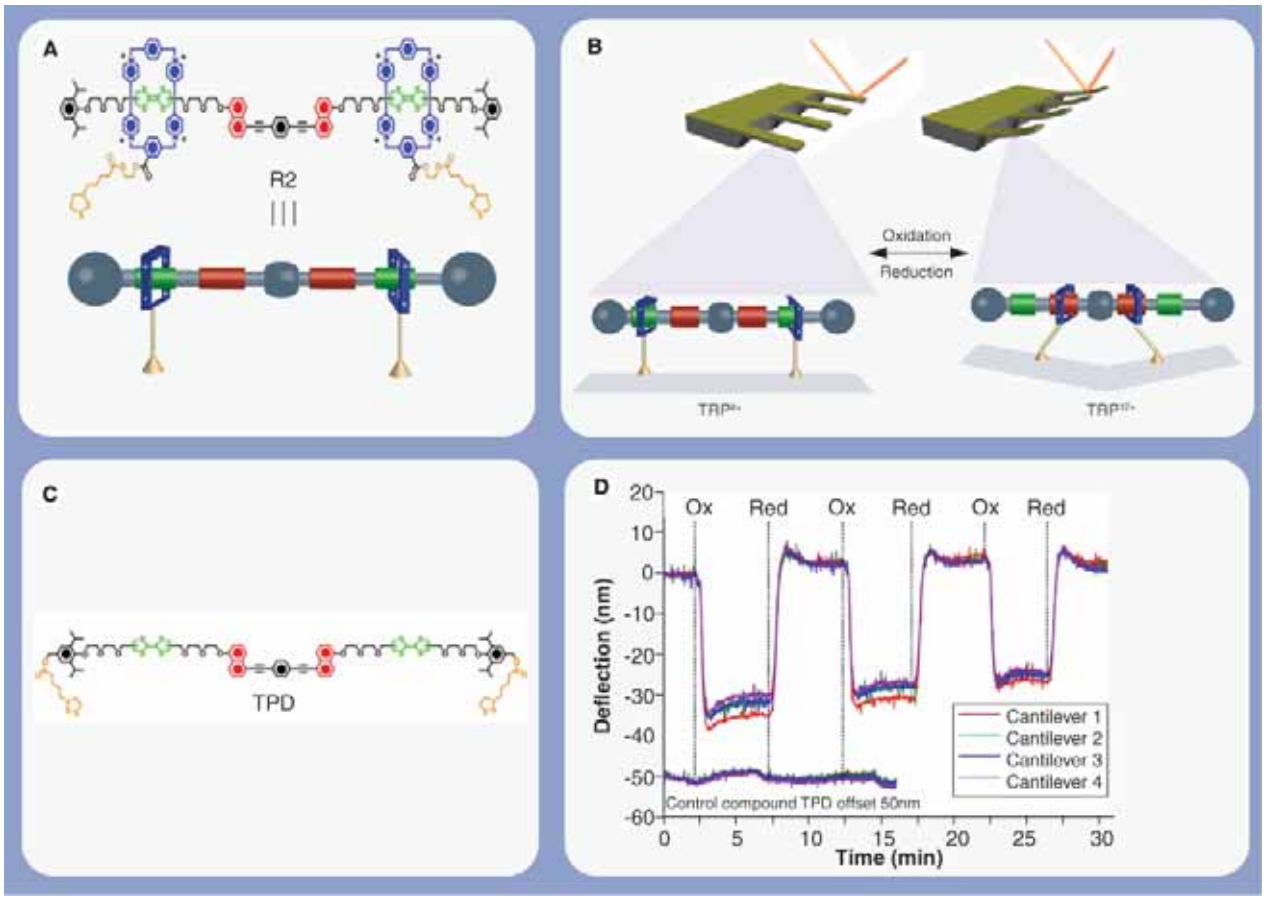
(A) Nanocar is comprised of four wheels made up of fullerenes and a chassis component. They exhibit both pivotal and translational motion on gold surface due to the rolling nature of fullerenes. **(B-F)** Pivoting and translating motion trajectory of nanocar on gold surface captured by STM [55].
Reproduced with permission fron [55].

across surfaces [59]. In this work, a SAM of 11-mercapoundeconoic acid was assembled onto a gold surface, and photoactive rotaxanes R3 were physisorbed onto the SAM with the dumbbell of R3 parallel to the gold surface (Figure 16A & 16B). R3 is comprises a ring and a dumbbell with a fluoroalkane station and a photo-responsive fumaramide station, which has a high binding affinity for the ring. Photoisomerization results in a transformation of the fumaramide into maleamide, which has a much lower affinity for the ring component. Thus, irradiation of light reduces the binding affinity to the ring drastically and, as a result, the equilibrium position of the ring is in favor of the fluoroalkane station, instead of the fumaramide station. Therefore, the ring movement can be used to expose or conceal the fluoroalkane station, thus changing the surface energy. When small drops of low-volatility liquids (e.g., CH2I2) are deposited onto the molecular machine-based photoresponsive surface, the collective operation of the R3 monolayer was sufficient to power the movement of a microliter droplet up a twelve degree incline (Figure 16 C–F). In this experiment, approximately 50% efficiency was achieved and approximately 50% of the light energy absorbed by R3 was used to overcome the effect of gravity.

Rotating a microscopic glass rod with nanomachines

Another illustration of the power of artificial nanomachines was completed by Eelkema and colleagues [60]. They proved that light-driven unidirectional molecular rotary motors R4 embedded in a liquid-crystal (LC) film are capable of rotating a glass rod that is approximately 10,000-times larger than R4. R4 (Figure 17A) is comprised of a rotary part (a right-handed helical structure), an axle (a central carbon–carbon double bond) and a stator part. Upon doping of a nonpolymeric LC film with R4, the helical organization induced by R4 results in a polygonal fingerprint texture in the surface of the LC film. When this R4-doped sample is irradiated with UV light with a wavelength of 365 nm, photochemical isomerization

Figure 15. Realization of Nanoscale actuator powered by artificial molecular muscles.



(A) Molecular structure and schematic representation of bistable [3]rotaxane molecular muscle R2. **(B)** Schematic diagram of bending microcantilever beams by switching R2. **(C)** Molecular structure of control dumbbell compound (TPD). **(D)** Experimental data associated with the process shown in **(B)**.
Reproduced with permission from [57].

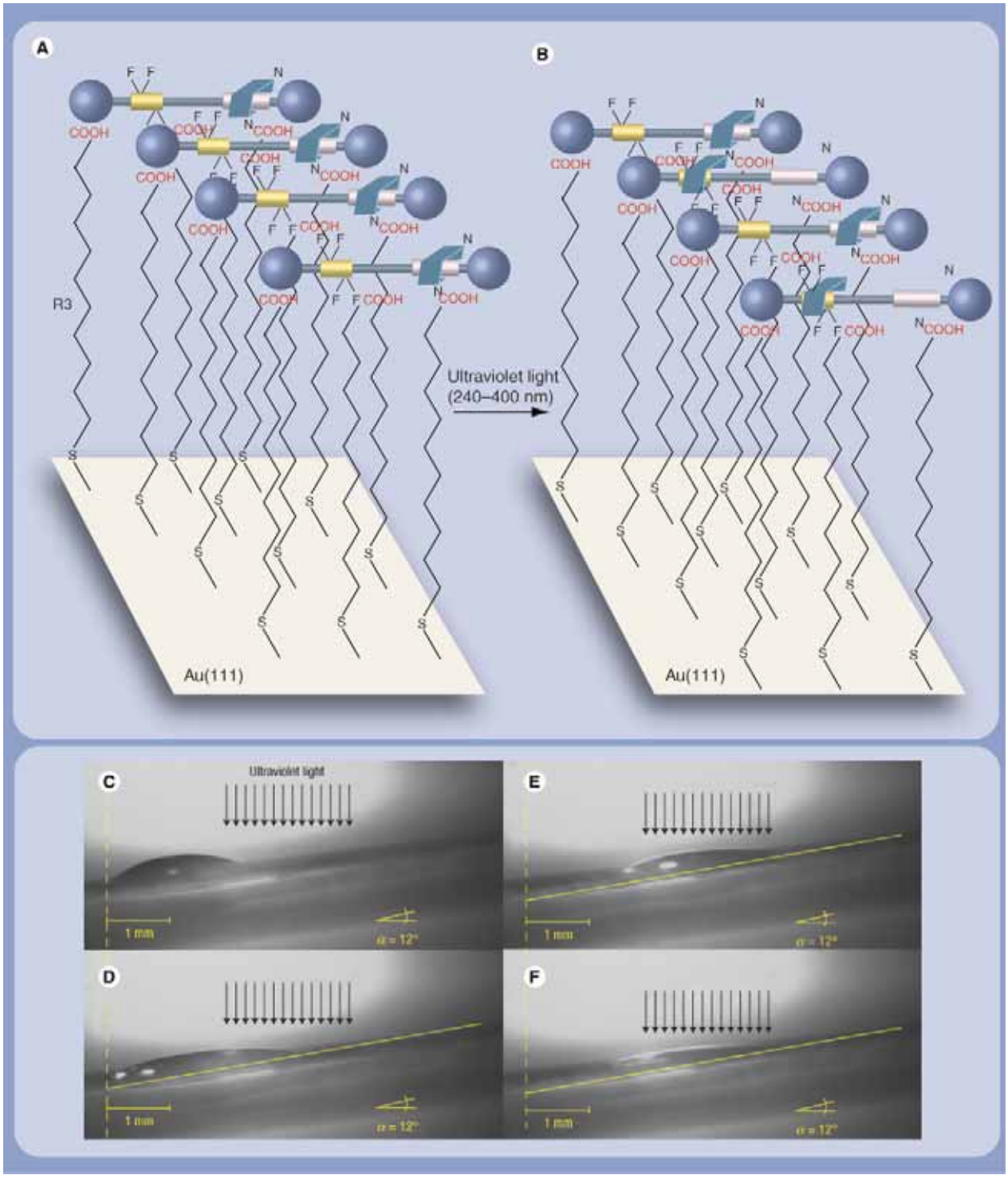
around the central carbon–carbon bond on R4 occurs, which leads to the inversion of the helicity from being right handed to being left handed and the reorganization of the LC film in a rotational (clockwise) fashion. Removing the UV light source causes a rotation in the opposite direction (counterclockwise). The rotation of the texture in the R4-doped LC film can be utilized to manipulate a glass rod (5 µm by 28 µm) placed on top of the surface (Figure 17B). In other words, changes in the shape of R4 induce a remarkable rotational reorganization of the LC film in which R4 is embedded, and cause the rotation of submillimeter-sized particles on the surface. Recently, they also demonstrated that these molecular rotary motors can be immobilized onto a fixed substrate and rotation can be achieved in monolayers of molecules [61], a crucial step towards harnessing energy at larger scales.

Conclusion

In this article, we reviewed recent advances in both biological and biomimetic molecular machines. Biological nanomachines play essential roles in virtually every biological process, from powering motion in the human body to producing DNA and RNA. In stark contrast to biology, none of mankind's major-impact technologies involves the exploitation of nanomachines, and the field of biomimetic molecular machines is still in its infancy.

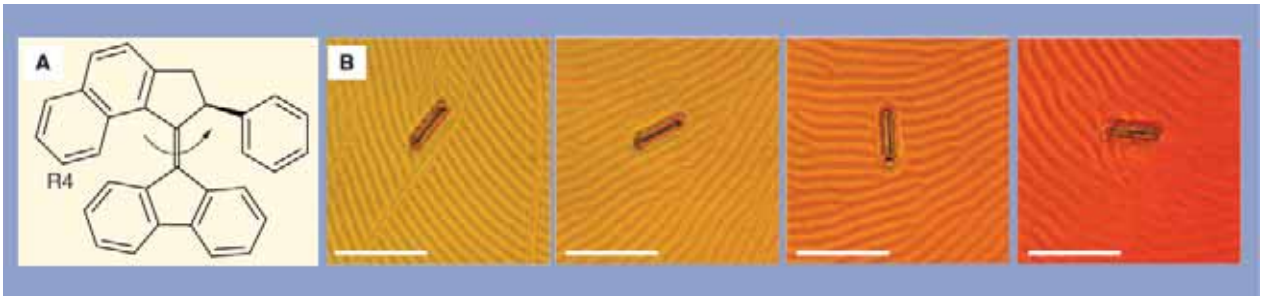
In the past two decades, research in the field of biological and biomimetic molecular machines has accelerated. The mechanisms of novel biological molecular machines are being understood. Nanomachines have been integrated with man-made devices. Biomimetic molecular machines with precisely tailored structures and functions (e.g., molecular analogs of rotors, gears, switches,

Figure 16. Macroscopic transport of liquid droplets by surface-bound optically switchable molecular machines.



(A) Schematic drawing of light-switchable rotaxanes R3 physisorbed onto a SAM of 11-MUA on Au(111). **(B)** Illumination with 240–400 nm light causes the rings shuttle to the fluoroalkane units, leaving a more polarophilic surface. **(C–F)** Locomotion of water droplet against the gravitational forces on a surface coated with R3 molecules [59].
Reproduced with permission from [59].

Figure 17. Demonstration of molecular rotor at work.



(A) Chemical structure of R4. **(B)** Rotation of a glass rod on a R4-doped LC film. Pictures (from left to right) were taken at 0, 15, 30, and 45 s and show clockwise rotations of 0, 28, 141, and 226°, respectively [60]. Scale bars: 50 μm.
Reproduced with permission from [60].

shuttles, turnstiles and ratchets) have been synthesized. Cumulative nanoscale movements within biomimetic nanomachines have been harnessed to perform large-scale mechanical work (e.g., driving microcantilever beams, rotating glass rods that are less than a millimeter in length and manipulating microliter droplets), mimicking what biomotors such as myosin have achieved in human muscles. It is anticipated that biomimetic molecular machines could soon possess the elegance, efficiency and complexity of their biological counterparts. With the capability to deliver precisely controlled mechanical motions at the molecular level, these tiny machines could have tremendous impact in the field of nanomedicine.

Future perspective

Just as individual molecules must organize into coherent assemblies for larger-scale work, so must chemists, biologists, material scientists and engineers cooperate to promote a better fundamental understanding of biological and biomimetic molecular machines in surface-bound and solid-state environments. Major challenges in this field remain, such as light-driven molecular machines, unidirectional molecular rotors, faster molecular motions over longer time frames and bio-compatible, synthetic molecular machines. Although challenges remain, we believe that with the collaboration across different disciplines, we can design, construct and apply molecular machinery with multifunctions over multiscales (nano, micro, meso and macro) for numerous biomedical applications.

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Executive summary

- A molecular machine (or 'nanomachine') is a mechanical device that is measured in nanometers and converts chemical, electrical or optical energy to controlled mechanical work.
- Myosin, kinesin and ATPase are biological nanomachines that perform locomotive and actuation functions in living organisms. They have been successfully used to power man-made nanomechanical systems such as cargo transporters, propellers and molecular sorters.
- With great strides made in the field of supramolecular chemistry, biomimetic molecular machines with tailored structures and functions can be synthesized.
- Although biological nanomachines provide perfect actuation in human bodies, biomimetic molecular machines such as bistable rotaxanes, are more suitable for the development of molecular machine-based medical and engineering applications.
- When stimulated by light, electricity or chemical reagents, mechanically interlocked molecules termed bistable rotaxanes experience relative internal motions of their components just like the moving parts of macroscopic machines.
- Cumulative nanoscale movements within biomimetic nanomachines have been harnessed to perform large-scale mechanical work (e.g., driving microcantilever beams, rotating glass rods that are less than a millimeter in length and manipulating microliter droplets), mimicking what biomotors have achieved in human muscles.

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Bibliography

1. Balzani V, Credi A, Raymo FM, Stoddart JF: Artificial molecular machines. *Angew. Chem. Int. Ed.* 39(19), 3349–3391 (2000).
2. Balzani V, Credi A, Venturi M: *Molecular Devices and Machines – A Journey into the Nano World.* Wiley-VCH, Weinheim, Germany (2003).
3. Goodsell DS: *Our Molecular Nature: The Body's Motors, Machines, and Messages*. Copernicus, NY, USA (1996).
4. Boyer PD: A research journey with ATP synthase. *J. Biol. Chem.* 277(42), 39045–39061 (2002).
5. Feynman RP: There's Plenty of Room at the Bottom. *Eng. Sci.* 23, 22–36 (1960).
6. Shinkai S, Manabe O: Photocontrol of ion extraction and ion-transport by photofunctional crown ethers. *Top. Curr. Chem.* 121, 67–104 (1984).
7. Howard J: Molecular motors: structural adaptations to cellular functions. *Nature* 389(6651), 561–567 (1997).
8. Vale RD, Milligan RA: The way things move: looking under the hood of molecular motor proteins. *Science* 288(5463), 88–95 (2000).
9. Cross RL, Duncan TM: Subunit rotation in F0F1-ATP synthases as a means of coupling proton transport through F-0 to the binding changes in F-1. *J. Bioenerg. Biomembr.* 28(5), 403–408 (1996)
10. Howard J, Hudspeth AJ, Vale RD: Movement of microtubules by single kinesin molecules. *Nature* 342(6246), 154–158 (1989).
11. Kull FJ, Sablin EP, Lau R, Fletterick RJ, Vale RD: Crystal structure of the kinesin motor domain reveals a structural similarity to myosin. *Nature* 380(6574), 550–555 (1996).
12. Hackney DD: The kinetic cycles of myosin, kinesin, and dynein. *Annu. Rev. Physiol.* 58, 731–750 (1996).
13. Lipowsky R, Klumpp S: 'Life is motion': multiscale motility of molecular motors. *Physica A* 352(1), 53–112 (2005).
14. Limberis L, Stewart RJ: Toward kinesin-powered microdevices. *Nanotechnology* 11(2), 47–51 (2000).
15. Hiratsuka Y, Tada T, Oiwa K, Kanayama T, Uyeda TQP: Controlling the direction of kinesin-driven microtubule movements along microlithographic tracks. *Bio. Phy. J.* 81(3), 1555–1561 (2001).
16. Platt M, Muthukrishnan G, Hancock WO, Williams ME: Millimeter scale alignment of magnetic nanoparticle functionalized microtubules in magnetic fields. *J. Am. Chem. Soc.* 127(45), 15686–15687 (2005).
17. Hutchins BM, Platt M, Hancock WO, Williams ME: Directing transport of CoFe2O4-functionalized microtubules with magnetic fields. *Small* 3(1), 4 (2007).
18. van den Heuvel MGL, De Graaff MP, Dekker C: Molecular sorting by electrical steering of microtubules in kinesin-coated channels. *Science* 312(5775), 910–914 (2006).
19. Brunner C, Wahnes C, Vogel V: Cargo pick-up from engineered loading stations by kinesin driven molecular shuttles. *Lab. Chip.* 7(10), 1263–1271 (2007).
20. Hess H, Howard J, Vogel V: A piconewton forcemeter assembled from microtubules and kinesins. *Nano. Lett.* 2(10), 1113–1115 (2002).
21. Diez S, Reuther C, Dinu C *et al.*: Stretching and transporting DNA molecules using motor proteins. *Nano. Lett.* 3(9), 1251–1254 (2003).
22. Dinu CZ, Opitz J, Pompe W, Howard J, Mertig M, Diez S: Parallel manipulation of bifunctional dna molecules on structured surfaces using kinesin-driven microtubules. *Small* 2(8–9), 927 (2006).
23. Allison WS: F-1-ATPase: a molecular motor that hydrolyzes ATP with sequential opening and closing of catalytic sites coupled to rotation of its γ-subunit. *Acc. Chem. Res.* 31(12), 819–826 (1998).
24. Block SM: Real engines of creation. *Nature* 386(6622), 217–219 (1997).
25. Elston T, Wang HY, Oster G: Energy transduction in ATP synthase. *Nature* 391(6666), 510–513 (1998).
26. Uptain SM, Kane CM, Chamberlin MJ: Basic mechanisms of transcript elongation and its regulation. *Annu. Rev. Biochem.* 66, 117–172 (1997).
27. Yin H, Wang MD, Svoboda K, Landick R, Block SM, Gelles J: Transcription against an applied force. *Science* 270(5242), 1653–1657 (1995).
28. Noji H, Yasuda R, Yoshida M, Kinosita K: Direct observation of the rotation of F-1-ATPase. *Nature* 386(6622), 299–302 (1997)
29. Soong RK, Bachand GD, Neves HP, Olkhovets AG, Craighead HG, Montemagno CD: Powering an inorganic nanodevice with a biomolecular motor. *Science* 290(5496), 1555–1558 (2000).
30. Yurke B, Turberfield A, Mills A, Simmel F, Neumann J: A DNA-fuelled molecular machine made of DNA. *Nature* 406(6796), 605–608 (2000).
31. Shin JS, Pierce NA: A synthetic DNA Walker for molecular transport. *J. Am. Chem. Soc.* 126(35), 10834–10835 (2004)
32. Bath J, Green S, Turberfield A: A free-running DNA motor powered by a nicking enzyme. *Angew. Chem. Int. Ed.* 44(28), 4358–4361 (2005).
33. Yin P, Yan H, Daniell X, Turberfield A, Reif J: A unidirectional DNA walker that moves autonomously along a track. *Angew. Chem. Int. Ed.* 43(37), 4906–4911 (2004).
34. Anelli PL, Asakawa M, Ashton PR *et al.*: Toward controllable molecular shuttles. *Chem. Eur. J.* 3(7), 1113–1135 (1997).
35. Anelli PL, Spencer N, Stoddart JF: A molecular shuttle. *J. Am. Chem. Soc.* 113(13), 5131–5133 (1991).
36. Ashton PR, Philp D, Spencer N, Stoddart JF: The self-assembly of (N) pseudorotaxanes. *Chem. Soc. Chem. Commun.* 1677–1679 (1991).
37. Bissell RA, Cordova E, Kaifer AE, Stoddart JF: A chemically and electrochemically switchable molecular shuttle. *Nature* 369(6476), 133–137 (1994).
38. Brouwer AM, Frochot C, Gatti FG *et al.*: Photoinduction of fast, reversible translational motion in a hydrogen-bonded molecular shuttle. *Science* 291(5511), 2124–2128 (2001).
39. Martinez Diaz MV, Spencer N, Stoddart JF: The self-assembly of a switchable [2]rotaxane. *Angew. Chem. Int. Ed.* 36(17), 1904–1907 (1997).
40. Raehm L, Kern JM, Sauvage JP: A transition metal containing rotaxane in motion: Electrochemically induced pirouetting of the ring on the threaded dumbbell. *Chem. Eur. J.* 5(11), 3310–3317 (1999).

41. Stoddart JF, Tseng HR: Chemical synthesis gets a fillip from molecular recognition and self-assembly processes. *Proc. Natl Acad. Sci. USA* 99(8), 4797–4800 (2002).
42. Gad-El-Hak M: *The MEMS Handbook*. CRC Press, FL, USA (2001).
43. Kovacs GTA: *Micromachined Transducers Sourcebook*. McGraw-Hill, NY, USA (1998)
44. Liu C: *Foundations of MEMS*. Pearson Education New Jersey, USA (2006).
45. Madou M: *Fundamentals of Microfabrication*. CRC Press, NY, USA (2002).
46. Brough B, Northrop BH, Schmidt JJ *et al.*: Evaluation of synthetic linear motor-molecule actuation energetics. *Proc. Natl Acad. Sci. USA* 103(23), 8583–8588 (2006).
47. Liu Y, Flood AH, Bonvallett PA *et al.*: Linear artificial molecular muscles. *J. Am. Chem. Soc.* 127(27), 9745–9759 (2005).
48. Balzani V, Clemente-Leon M, Credi A *et al.*: Autonomous artificial nanomotor powered by sunlight. *Proc. Natl Acad. Sci. USA* 103(5), 1178–1183 (2006).
49. Tseng HR, Wu DM, Fang NXL, Zhang X, Stoddart JF: The metastability of an electrochemically controlled nanoscale machine on gold surfaces. *Chemphyschem* 5(1), 111–116 (2004).
50. Lee IC, Frank CW, Yamamoto T *et al.*: Langmuir and Langmuir-Blodgett films of amphiphilic bistable rotaxanes. *Langmuir* 20(14), 5809–5828 (2004).
51. Petty MC: *Langmuir-Blodgett Films: An Introduction*. Cambridge University Press, Cambridge, UK (1996).
52. Nguyen TD, Tseng HR, Celestre PC *et al.*: A reversible molecular valve. *Proc. Natl Acad. Sci. USA* 102(29), 10029–10034 (2005).
53. Nguyen TD, Ken CFL, Liong M, Liu Y, Stoddart JF, Zink JI: Versatile Supramolecular nanovalves reconfigured for light activation*. *Adv. Funct. Mater.* 17(13), 2101–2110 (2007).
54. Sourav Saha KC-FL, Nguyen TD, Stoddart JF, Zink JI: Nanovalves. *Adv. Funct. Mater.* 17(5), 685–693 (2007).
55. Shirai Y, Osgood AJ, Zhao YM, Kelly KF, Tour JM: Directional control in thermally driven single-molecule nanocars. *Nano. Lett.* 5(11), 2330–2334 (2005).
56. Morin JF, Shirai Y, Tour JM: En route to a motorized nanocar. *Org. Lett.* 8(8), 1713–1716 (2006).
57. Huang TJ, Brough B, Ho CM *et al.*: A nanomechanical device based on linear molecular motors. *Appl. Phys. Lett.* 85(22), 5391–5393 (2004).
58. Huang TJ, Flood, AH, Brough B *et al.*: Understanding and harnessing biomimetic molecular machines for nems actuation materials. *IEEE T. Autom. Sci. Eng.* 3(3), 254–259 (2006).
59. Berna J, Leigh DA, Lubomska M *et al.*: Macroscopic transport by synthetic molecular machines. *Nat. Mater.* 4(9), 704–710 (2005).
60. Eelkema R, Pollard MM, Vicario J *et al.*: Nanomotor rotates microscale objects. *Nature* 440(7081), 163–163 (2006)
61. Pollard M, Lubomska M, Rudolf P, Feringa B: Controlled rotary motion in a monolayer of molecular motors. *Angew. Chem. Int. Ed.* 46(8), 1278–1280 (2007).