Molecular Machines

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Key Words molecular motors, nanomachines, nanodevices, nanomotors, bionanotechnology

Abstract Molecular machines are tiny energy conversion devices on the molecular-size scale. Whether naturally occurring or synthetic, these machines are generally more efficient than their macroscale counterparts. They have their own mechanochemistry, dynamics, workspace, and usability and are composed of nature’s building blocks: namely proteins, DNA, and other compounds, built atom by atom. With modern scientific capabilities it has become possible to create synthetic molecular devices and interface them with each other. Countless such machines exist in nature, and it is possible to build artificial ones by mimicking nature. Here we review some of the known molecular machines, their structures, features, and characteristics. We also look at certain devices in their early development stages, as well as their future applications and challenges.

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1523-9829/04/0815-0363$14.00
INTRODUCTION

Molecular machines can be defined as devices that can produce useful work through the interaction of individual molecules at the molecular scale of length. A convenient unit of measurement at the molecular scale would be a nanometer. Hence, molecular machines also fall into the category of nanomachines. Molecular machines depend on inter- and intramolecular interactions for their function. These interactions include forces such as the ionic and Van der Waals forces and are a function of the geometry of the individual molecules. The interaction between two given molecules can be well understood by a set of laws governing them, which brings in a definite level of predictability and controllability of the underlying mechanics. Mother Nature has her own set of molecular machines that have been working for centuries and have become optimized for performance and design over the ages. As our knowledge and understanding of these numerous machines continues to increase, we now see a possibility of using the natural machines, or creating synthetic ones from scratch, by mimicking nature. In this review, we try to understand the principles, theory, and utility of the known molecular machines and look into the design and control issues for creation and modification of such machines. A majority of natural molecular machines are protein based, whereas the DNA-based molecular machines are mostly synthetic. Nature deploys proteins to perform various cellular tasks, from moving cargo to catalyzing reactions, whereas DNA has been retained as an information carrier. Hence, it is understandable that most of the natural machinery is built from proteins. With the powerful crystallographic techniques now available, protein structures are clearer than ever. The ever-increasing computing power makes it possible to dynamically model protein folding processes and predict the conformations and structure of lesser known proteins. These findings help unravel the mysteries associated with the molecular machinery and pave the way for the production and application of these miniature machines in various fields, including medicine, space exploration, electronics and military. We divide the molecular machines into three broad categories—protein based, DNA-based, and chemical molecular motors.

ATP-BASED PROTEIN MOLECULAR MACHINES

Three naturally existing rotary motors have been identified and studied in detail so far. Two form the F0F1-ATP synthase, and the third one is the bacterial flagellar motor. The protein-based molecular motors rely on an energy-rich molecule known as adenosine triphosphate (ATP), which is basically a nucleotide having three phosphate molecules that play a vital role in its energetics, and make it an indispensable commodity of life. The machines described in this section, the F0F1-ATPase, the kinesin, myosin, and dynein superfamily of protein molecular machines, and bacteria flagellar motors all depend, directly or indirectly, on ATP for their input energy. These machines, which have been carrying out vital life
functions both inside and outside cells for millions of years, have now been segregated out of their natural environment and are seen as energy conversion devices to obtain forces, torques, and motion. One disadvantage associated with ATP dependence is that the ATP creation machinery itself could be many times heavier and bulkier than the motors, thereby making the assembly more complex. These machines perform best in their natural environment, and in the near future it may not be possible to have them as a part of feasible biomimetic molecular machinery.

The F$_0$F$_1$-ATP Synthase Motors

ATP is regarded as the energy currency of biological systems (1). The ATP molecule owes much of its energy to the terminal three phosphate ions attached to an adenosine base (2). In 1941 the role of ATP in the energy conversion process in living beings was recognized (3). However, the mode of transfer and structure of the enzyme was unknown. When this currency is utilized (i.e., the energy of the molecule that is used to drive a biological process), the terminal anhydride bond in the ATP molecule has to be split. This leaves adenosine diphosphate (ADP) and a phosphate ion (Pi) as the products, which are recombined to form ATP by a super efficient enzyme motor assembly called the F$_0$F$_1$-ATP synthase (F$_0$F$_1$-ATPase). ATP synthase is present inside the mitochondria of animal cells, in plant chloroplasts, in bacteria, and some other organisms. ATP synthase was first seen in 1962 in an electron microscopy experiment on bovine heart mitochondria, as 10 nm diameter knobs (5). Their importance in energy conversion was realized, but their functioning was still unknown. In 1966 the relation of the thus far unknown knobs to the production of ATP was established (6), which provided one of the first structures of the enzyme.

The ATP synthase is actually a combination of two motors functioning together, the hydrophobic transmembrane F$_0$-ATPase motor and the globular F$_1$-ATPase motor (7). Both motors have distinct structures and functions. There are different abbreviations used for the F$_1$-ATPase based on their sources; the heart mitochondrial motors are called mF$_1$, chloroplast motors are cF$_1$, those obtained from Escherichia coli are termed EcF$_1$, and the ones from Kagawa’s thermophilic bacterium are known as TF$_1$ (2). The F$_0$ motor has organism-dependant structural variations. In addition, the regulation of catalysis in ATP synthase depends on the organism’s source (1). In animal mitochondria, this motor is embedded in the inner mitochondrial membrane and uses an ion-motive force for its function. Initially, however, it was believed that the force was proton-motive (8) only until it was shown that, in some cases, Na$^+$ ions induce the motive force for the F$_0$ motor (9); hence the term ion-motive force. The proton-motive force can be defined as the work per unit charge that a proton traveling through a membrane can perform.

STRUCTURE: F$_1$-ATPase MOTOR  The F$_1$ motor, powered by hydrolysis of ATP, is composed of a central protein stalk, called the $\gamma$-subunit, surrounded by three copies each of $\alpha$- and $\beta$-subunits. The $\alpha$- and $\beta$-subunits are arranged
Figure 1  The FoF1-ATPase motors. The F0 motor is embedded in the inner mitochondrial membrane of the mitochondria. F0 is typically composed of $a$, $b$, and $c$ subunits as shown. The F1 motor is the soluble region composed of three $\alpha$-, three $\beta$-, one each of $\gamma$-, $\delta$- and $\epsilon$-subunits.

alternately so that they make a symmetric circular pattern when viewed from the top. There are $\delta$-subunits attached to the periphery of the $\alpha$-$\beta$ cylinder and the $\epsilon$-subunits are present at the base of the $\gamma$-subunits, as shown in Figure 1. Hence, the F1 motor is composed of nine polypeptides (10). The $\alpha$- and the $\beta$-subunits contain nucleotide-binding sites that bind ATP/ADP molecules. The nucleotide-binding sites in the $\alpha$-subunits simply bind the nucleotide, whereas those in the $\beta$-subunit actually perform the catalysis. The $a$, $b$, and the $c$ subunits shown in Figure 1 are a part of the F0 motor discussed below.

FUNCTION: F1-ATPase MOTOR  The binding-change mechanism to explain the function of F1-ATPase was proposed in 1973 (11). The mechanism, as known today, shows that each of the $\beta$-subunits take three forms: O (open), L (loose), and T (tight) binding site. When the subunit is in the O form, it is catalytically inactive and has very low affinity to bind substrates. In the L form, the subunit loosely binds substrates (ADP and Pi), although it is catalytically inactive. In the T form, the ADP and Pi are converted into a tightly bound ATP until a conformational change converts the T-site into an O-site, thereby allowing the release of the newly formed ATP (12). The mechanism is shown in Figure 2.

The conformational change in the $\beta$-subunits is triggered by the rotation of the 4.5 nm long $\gamma$-subunit, which acts as a link connecting the F1-ATPase to the
Figure 2  The binding-change mechanism of F$_1$-ATPase. The three catalytic sites bind ADP/ATP alternately in L (loose), T (tight) and O (open) fashion. ADP and Pi are initially loosely bound, then the binding becomes tight, with the conversion of ADP + Pi into ATP, which is finally released when the open conformation is achieved.

F$_0$-ATPase. This was shown experimentally in 1997 (13). In this experiment, the F$_1$-ATPase was attached to a nickel-coated glass surface; a 1–3 μm long fluorescently labeled actin filament was attached to the other end of the γ-subunit. The rotation could then be observed through a fluorescence microscope, which was extremely interesting because the motor has a diameter of about 10 nm, whereas it could support and rotate a structure about one hundred times larger! However, the rate of rotation was reduced by 50 times to 1 rotation per second. The experimental setup is shown in Figure 3. F$_1$-ATPase can produce 80–100 pN-nm of rotary torque (4).

STRUCTURE: F$_0$-ATPase MOTOR  Since the rotation of the γ-subunit has been shown to play the essential role in ATP creation, it is now imperative to see what causes the
γ-subunit to rotate. The answer lies in the functioning of the F₀-ATPase motor. Although the structure of the F₀-ATPase is not as well known as that of the F₁-ATPase, findings indicate that its structure depends on its source. The F₀ domain from the eubacterial enzymes, exemplified by E. coli, (14), has three types of subunits termed a, b, and c with 1 unit of a, 2 units of b, and 9 to 12 (15) units of c subunits (16, 17). Hence the subunit a, the two b, and the twelve c subunits in Figure 1 belong to the F₀-ATPase motor. In yeast, the F₀ has only 10 c subunits (18). The F₀ “turbine” from plant chloroplasts was found to have 14 c subunits (19), whereas a Na⁺-driven specimen from bacteria was found to have 14 such subunits (20).

FUNCTION: F₀-ATPase MOTOR A flow of ions through the membrane propels the observed reversible (21) rotation of F₀-ATPase (22). As mentioned above, F₀ is the membrane-spanning unit of the ATPase motor. It remains embedded in the mitochondrial or cellular membrane. In 1978, it was discovered that a chemical potential gradient for protons is formed across the inner mitochondrial membrane or the proton-motive force (8). This force is utilized by the ATPase enzyme to produce ATP. The structure of F₀-ATPase is not as well known as its F₁ counterpart, which has been fully resolved (18, 23–28). The mechanochemical and quantitative models that explain how the ion-motive force is converted into the rotation of the γ-subunit were described in (15, 29–33).

The first hybrid nanoassembly structures powered by F₁-ATPase was proposed in (34). Nano-fabricated Ni posts, about 80 nm in diameter and 200 nm in height, each separated by about 2.5 μm were built. Upon these posts they attached specially produced recombinant biotinylated F₁-ATPases using histidine tags into their β-subunit coding sequences. A streptavidin molecule was bound to the γ-subunit, and finally Ni propellers of lengths 750 to 1400 nm were attached to them. In an action that is reverse of its ATP-producing cycle, the F₁-ATPase consumed externally provided ATP and produced anticlockwise rotation with a speed of about eight rotations per second. To date, this achievement remains a landmark in bionanotechnology.

The Kinesin, Myosin, Dynein, and Flagella Molecular Motors

With modern microscopic tools, we view a cell as a set of many different moving components powered by molecular machines rather than a static environment. Molecular motors that move unidirectionally along protein polymers (actin or microtubules) drive the motions of muscles, as well as much smaller intracellular cargoes. In addition to the F₀F₁-ATPase motors inside the cell, there are linear transport motors present, tiny vehicles known as motor proteins, that transport molecular cargoes (35) and also require ATP for functioning. These minute cellular machines exist in three families: kinesins, myosins, and dyneins (36). The cargoes can be organelles, lipids, or proteins, etc. They play an important role in cell division and motility.

There are over 250 kinesin-like proteins, and they are involved in processes as diverse as the movement of chromosomes and the dynamics of cell membranes. The
only part they have in common is the catalytic portion known as the motor domain. They have significant differences in their location within cells, their structural organization, and the movement they generate (37). Muscle myosin, whose study dates back to 1864, has served as a model system for understanding motility for decades. Kinesin, however, was not discovered until 1985, using in vitro motility assays (38). Conventional kinesin is a highly processive motor that can take several hundred steps on a microtubule without detaching (39, 40), whereas muscle myosin executes a single stroke and then dissociates (41). Detailed analysis and modeling of these motors has been done (38, 42).

Kinesin and myosin make for an interesting comparison. Kinesin is microtubule based; it binds to and carries cargoes along microtubules, whereas myosin is actin based. The motor domain of kinesin weighs one third that of myosin and one tenth of that of dynein (43). Before the advent of modern microscopic and analytic techniques, it was believed that these two had little in common. However, the crystal structures available today indicate that they probably originated from a common ancestor (44).

THE MYOSIN LINEAR MOTOR Myosin is a diverse superfamily of motor proteins (45). Myosin-based molecular machines transport cargoes along actin filaments, the two-stranded helical polymers of the protein actin that are about 5–9 nm in diameter. They do this by hydrolyzing ATP and utilizing the released energy (46). In addition to transport, they are also involved in the process of force generation during muscle contraction, wherein thin actin filaments and thick myosin filaments slide past each other. Not all members of the myosin superfamily have been characterized. However, much is known about their structure and function. Myosin molecules were first seen (in the late 1950s) through electron microscope protruding out from thick filaments and interacting with the thin actin filaments (47–49). Since that time, ATP has been known to play a role in myosin-related muscle movement along actin (50); however, the exact mechanism was unknown, until it was explained in (51).

STRUCTURE: MYOSIN MOLECULAR MOTOR A myosin molecule binding to an actin polymer is shown in Figure 4a (52). A myosin molecule has a size of about 520 kDa, including two 220-kDa heavy chains and light chains of sizes between 15–22 kDa (53, 54). They can be visualized as two identical globular motor heads, also known as motor domains, each having a catalytic domain (actin, nucleotide, and light chain binding sites) and ~8 nm long lever arms. One of the heads, sometimes referred to as S1 regions (subfragment 1), is shown in green (only the active head is visible); the lever arms or the light chains, in red and yellow. Both heads are connected via a coiled coil made of two α-helical coils (green) to the thick base filament. The light chains have considerable sequence similarity with the protein calmodulin and troponin C and are sometimes referred to as calmodulin-like chains. They act as links to the motor domains and do not play any role in their ATP binding activity (55) except for some exceptions (56, 57).
The motor domain in itself is sufficient for moving actin filaments (58). Three-dimensional structures of a myosin head revealed that it is a pear-shaped domain, about 19 nm long and 5 nm in maximum diameter (58, 59).

FUNCTION: MYOSIN MOLECULAR MOTOR A crossbridge-cycle model for the action of myosin on actin has been widely accepted since 1957 (47, 60, 61). Since the time the atomic structures of actin monomer (62, 63) and myosin (59) were resolved, this model has been refined into a lever-arm model which is now acceptable (64). Only one motor head is able to connect to the actin filament at a time, the other head remains passive. Initially, the catalytic domain in the head has ADP and Pi bound to it and, as a result, its binding with actin was weak. With the active motor head docking properly to the actin-binding site, the Pi has to be released. As soon as this happens, the lever arm swings counterclockwise (65) owing to a conformational change (49, 66–71), which pushes the actin filament down by about 10 nm along its longitudinal axis (38). The active motor head now releases its bound ADP, and another ATP molecule, by way of Brownian motion, quickly replaces it, making the binding of the head to the actin filament weak again. The myosin motor then dissociates from the actin filament, and a new cycle starts. However, nano-manipulation of single S1 molecules (motor domains) shows that myosin can take multiple steps per ATP molecule hydrolyzed, moving in 5.3 nm steps and resulting in displacements of 11 to 30 nm (72).

THE KINESIN LINEAR MOTOR The kinesin (43) and dynein families of proteins are involved in cellular cargo transport along microtubules, in contrast to myosin, which transports along actin (73). Microtubules are 25-nm diameter tubes made of protein tubulin and are present in the cells in an organized manner. Microtubules have polarity; one end being the plus (fast-growing) end while the other end is the minus (slow-growing) end (74). Kinesins move from the minus end to the plus end of the microtubule, whereas dyneins move from the plus end to the minus end. Microtubule arrangement varies in different cell systems. In nerve axons, they are arranged longitudinally such that their plus ends point away from the cell body and into the axon. In epithelial cells, their plus ends point toward the basement membrane. They extend radially out of the cell center in fibroblasts and macrophages with the plus end protruding outward (75). Similar to myosin, kinesin is also an ATP-driven motor. One unique characteristic of the kinesin family proteins is their processivity; they bind to microtubules and literally walk on it for many enzymatic cycles before detaching (76, 77). Also, each of the globular heads/motor domains of kinesin is made of a single polypeptide unlike myosin (heavy and light chains and dynein heavy, intermediate, and light chains).

STRUCTURE: KINESIN MOLECULAR MOTOR Much structural information about kinesin is now available through the crystal structures (44, 78, 79). The motor domain contains a folding motif similar to that of myosin and G proteins (36). The two heads or the motor domains of kinesin are linked via neck linkers to a long coiled...
coil, which extends up to the cargo (Figure 4b). These heads interact with the α- and β-subunits of the tubulin heterodimer along the microtubule protofilament. The heads contain nucleotide- and microtubule-binding domains.

**FUNCTION: KINESIN MOLECULAR MOTOR** Although kinesin is also a two-headed linear motor, its modus operandi is different from myosin in the sense that both of its heads work together in a coordinated manner in contrast to one being left out in the case of myosin. Figure 4b shows the kinesin walk. Each of the motor heads is near the microtubule in the initial state, with each motor head carrying an ADP molecule. When one of the heads loosely binds to the microtubule, it loses its ADP molecule to facilitate a stronger binding. Another ATP molecule replaces the ADP, which facilitates a conformational change such that the neck region of the bound head snaps forward and zips on to the head (37). In the process, it pulls the other ADP-carrying motor head forward by about 16 nm so that it can bind to the next microtubule-binding site. This results in the net movement of the cargo by about 8 nm (80). The second head now binds to the microtubule by losing its ADP, which is promptly replaced by another ATP molecule (Brownian motion). The first head, meanwhile hydrolyzes the ATP and loses the resulting Pi. It is then snapped forward by the second head while it carries its ADP forward. Hence coordinated hydrolysis of ATP in the two motor heads is the key to the kinesin processivity (81, 82). Kinesin is able to take about 100 steps before detaching from the microtubule (39, 76, 83), while moving at 1000 nm/s and exerting forces of the order of 5–6 pN at rest (84, 85).

**THE DYNEIN MOTOR** The dynein superfamily of proteins was discovered in 1965 (86). Dyneins exist in two isoforms: cytoplasmic and axonemal. Cytoplasmic dyneins are involved in cargo movement, whereas axonemal dyneins are involved in producing bending motions of cilia and flagella (87–97). Figure 5 shows a typical cytoplasmic dynein molecule.

**STRUCTURE: DYNEIN MOLECULAR MOTOR** The structure consists of two heavy chains in the form of globular heads, three intermediate chains, and four light intermediate chains (98, 99). Recent studies have exposed a linker domain connecting the stem region below the heads to the head itself (100). Also the microtubule-binding domains (the stalk region, not visible in the figure) protrude from the top of the heads (101). The ends of these stalks have smaller ATP-sensitive globular domains that bind to the microtubules. Cytoplasmic dynein is associated with a protein complex known as dynactin, which contains 10 subunits (102). Some are shown in the Figure 5 as p150, p135, actin-related protein 1 (Arp1), actin, dynamin, capping protein, and p62 subunit. These play an important regulatory role in the binding ability of dynein to the microtubules. The heavy chains forming the two globular heads contain the ATPase and microtubule motor domains (103).

One striking difference between dynein and the kinesins and myosins is that dynein has AAA (ATPases associated with a variety of cellular activities) modules
Figure 5  A dynein molecule. Shown are the globular heads (heavy chains) connected to the intermediate chains and the light chains. Dynactin complex components p150, p135, dynamitin, p62, capping proteins, Arp1, and Actin are also shown.

(104–106), which indicate that its mode of working will be entirely different from kinesins and myosins. This puts dyneins into the AAA superfamily of mechanoenzymes. The dynein heavy chains contain six tandemly linked AAA modules (107, 108), with the head having a ring-like domain organization, which is typical of a AAA superfamily. Four of these are nucleotide-binding motifs, named P1–P4, but only P1 (AAA1) is able to hydrolyze ATP.

FUNCTION: DYNEIN MOLECULAR MOTOR  Because dynein is a larger and more complex structure than other motor proteins, its mode of operation is not as well known. However, electron microscopy and image processing was used (100) to show the structure of a flagellar dynein at the start and end of its power stroke, which gives some insight into its possible mode of force generation. When the dynein contains bound ADP and V_i (vandate), it is in the prepower stroke conformation.
The state when it has lost the two, known as the apo-state, is the more compact post-power stroke state. There is a distinct conformational change involving the stem, linker, head, and the stalk that produces about 15 nm of translation onto the microtubule bound to the stalk (100).

THE FLAGELLA MOTORS  Unicellular organisms such as *E. coli* have an interesting mode of motility (see 109–111). They have a number of molecular motors, about 45 nm in diameter, that drive their feet or the flagella, which help the cell to swim. Motility is critical for cells, as they often have to travel from a less favorable to a more favorable environment. The flagella are helical filaments that extend out of the cell into the medium and perform a function analogous to what the oars perform to a boat. The flagella and the motor assembly are called a flagellum. The motor assembly imparts a rotary motion into the flagella (112, 113). In addition to a rotary mechanism, the flagellar machines consist of components such as rate meters, particle counters, and gearboxes (114). These are necessary to help the cell decide which way to go, depending on the change of concentration of nutrients in the surroundings. The rotary motion imparted to the flagella needs to be modulated to ensure the cell is moving in the proper direction, as well as to ensure that all flagella of the given cell are providing a concerted effort toward it (115). When the motors rotate the flagella in a counterclockwise direction, as viewed along the flagella filament from outside, the helical flagella create a wave away from the cell body. Adjacent flagella subsequently intertwine in a propulsive corkscrew manner and propel the bacteria. When the motors rotate clockwise, the flagella fly apart, causing the bacteria to tumble or change its direction (116). These reversals occur irregularly, giving the bacterium a random walk, unless, of course, there is a preferential direction of motility due to reasons mentioned earlier. The flagella motors allow the bacteria to move at speeds of as much as 25 µm/s, with directional reversals occurring approximately 1 per second (117). A number of bacterial species in addition to *E. coli* depend on flagella motors for motility: e.g., *Salmonella enterica* serovar, Typhimurium (*Salmonella*), Streptococcus, *Vibrio* spp., *Caulobacter*, *Leptospira*, *Aquaspirillum serpens*, and *Bacillus*. The rotation of flagella motors is stimulated by a flow of ions through them, which is a result of a build-up of a transmembrane ion gradient. There is no direct ATP-involvement; however, the proton gradient needed for the functioning of flagella motors can be produced by ATPase.

STRUCTURE: THE FLAGELLA MOTORS  A complete part list of the flagella motors is not yet available. Continued efforts dating back to early 1970s have, however, revealed much of their structure, composition, genetics, and function. Newer models of the motor function are still being proposed with an aim to explain observed experimental phenomena (118, 119) because we still do not fully understand the functioning of this motor (110). A typical flagella motor from *E. coli* consists of ~20 different proteins (110), and many more are involved in its assembly and operation. There are 14 Flg-type proteins, FlgA–FlgN; 5 Flh-type proteins,
Figure 6  A typical flagellum. A filament (FliC) is connected to the hook (FlgE), which connects to the transmembrane motor unit through a shaft. Hook-related proteins (FlgK, FlgL, and FliD) help in assembly and stability of the hook and filament. The L-ring is embedded in the outer cell membrane, the P-ring in the peptidoglycan layer, and the MS-ring (FliF) along with FliG (rotor) and parts of stator (MotA and MotB) are embedded in the inner cell membrane. The C-ring and the transport apparatus are located inside the cell.

FlhA–FlhE; 19 Fli-type proteins, FliA–FliT; with MotA and MotB making a total of 40 related proteins. The group names Flg, Flh, Fli, and Mot correspond to the related genes (120). Within the main structural proteins are other proteins: FliC or the filament; FliD (filament cap); FliF or the MS-ring; FliG, FliM, and FliN (C-ring); FlgB, FlgC, and FlgF (proximal rod); FlgG (distal rod); FlgH (L-ring); FlgI (P-ring); FlgK and FlgL (hook-filament junction); and MotA-MotB (torque-generating units) (see Figure 6). It was initially believed that the M and S were two separate rings (M, membrane; S, supramembranous) (121). However, they are now called the MS-ring because they were found to be two domains of the same protein, FliF (122, 123). The C-ring stands for cytoplasmic (124–126); the names for the P and L-rings come from peptidoglycan and lipopolysaccharide, respectively, indicating their location as seen in Figure 6. FlhA, B, FliH, I, O, P, Q, and R constitute the transport apparatus.

The hook and filament part of the flagellum is located outside the cell body. The motor portion is embedded in the cell membrane, with the C-ring and the transport
apparatus inside the inner membrane in the cytoplasmic region. MotA and MotB are arranged in a circular array embedded in the inner membrane, with the MS-ring at the center. Connected to the MS-ring is the proximal end of a shaft, to which the P-ring, embedded in the peptidoglycan layer, is attached. Moving further outward, is the L-ring, which is embedded in the outer cell membrane, followed by the distal shaft end that protrudes out of the cell. To this end there is an attachment of the hook and the filament, both of which are polymers of hook-protein and flagellin respectively.

**Function: the flagella motors** The flagellar motors in most cases are powered by protons flowing through the cell membrane (proton-motive force) barring exceptions such as certain marine bacteria, for example, the *Vibrio* spp., which are driven by Na\(^+\) ions (127). There are about 1200 protons required to rotate the motor by one rotation (128). A complete explanation of how this proton flow is able to generate torque is not yet available. From what is known, the stator units of MotA and MotB play an important role in torque generation. They form a MotA/MotB complex that when oriented properly binds to the peptidoglycan and opens proton channels through which protons can flow (129). It is believed that there are eight such channels per motor (130). The proton-motive force is a result of the difference of pH between the outside and inside of the cell. The *E. coli* cells like to maintain an internal pH of 7.6–7.8, so depending on the pH of the surroundings, the proton-motive force will vary, and hence the speed of rotation of their motors. To test how the speed of rotation depends on the proton-motive force, the motors were powered by external voltage with attached markers acting as heavy loads (131). As expected, the rotation was found to depend directly on the proton-motive force. According to the most widely accepted model, MotA/MotB complex interacts with the rotor via binding sites. The passage of protons through a MotA/MotB complex (stator or torque generator) moves it so that the protons bind to the next available binding site on the rotor, thereby stretching their linkage. When the linkage recoils, the rotor assembly has to rotate by one step. Hence whichever complex receives protons from the flux will rotate the rotor and generate torque. The torque-speed dependence of the motor has been studied in detail (132, 133) and indicates the torque range of about 2700 to 4600 pN-nm.

**DNA-BASED MOLECULAR MOTORS/DEVICES**

As mentioned above, nature chose DNA mainly as an information carrier. There was no mechanical work assigned to it. Energy conversion, trafficking, and sensing, for example, were the tasks assigned mainly to proteins. Probably for this reason, DNA turns out to be a simpler structure, with only four kinds of nucleotide bases, adenosine, thiamine, guanine, and cytosine (A, T, G, and C), attached in a linear fashion that takes a double-helical conformation when paired with a complementary strand. Such structural simplicity vis-à-vis proteins, made of some 20 amino acids with complex folding patterns, results in a simpler structure and
predictable behavior. There are certain qualities that make DNA an attractive choice for the construction of artificial nanomachines. In recent years, DNA has found use not only in mechanochemical but also in nanoelectronic systems (134–137). A DNA double-helical molecule is about 2 nm in diameter and has 3.4–3.6-nm helical pitch no matter what its base composition is, a structural uniformity not achievable with protein structures if one changes their sequence. Furthermore, double-stranded DNA (ds-DNA) has a respectable persistence length of about 50 nm (138), which provides it enough rigidity to be a candidate component of molecular machinery. Single-stranded DNA (ss-DNA) is very flexible and cannot be used where rigidity is required; however, this flexibility allows its application in machine components such as hinges or nanoactuators (139). Its persistence length is about 1 nm, covering up to 3 base pairs (140) at 1M salt concentration.

Other than the above structural features, two important and exclusive properties make DNA suitable for molecular level constructions: molecular recognition and self-assembly. The nucleotide bases A and T on two different ss-DNA have affinity for each other, so do G and C. Effective and stable ds-DNA structures are formed only if the base orders of the individual strands are complementary. Hence, if two complementary single strands of DNA are in a solution, they will eventually recognize each other and hybridize, or zip-up, forming a ds-DNA. This property of molecular recognition and self-assembly has been exploited in a number of ways to build complex molecular structures (141–148). From a mechanical perspective, if the free energy released by hybridization of two complementary DNA strands is used to lift a hypothetical load, a force capacity of 15 pN can be achieved (F.C. Simmel & B.Yurke, unpublished data), comparable to that of other molecular machines such as kinesin (5 pN) (150).

The first artificial DNA-based structure in the form of a cube in 1991 was presented in (143, 151). More complex structures such as knots (152, 153) and Borromean rings (147) were also developed. In addition to these individual constructs, two-dimensional arrays (145, 154, 155) were made with the help of the double-crossover (DX) DNA molecule (156–158). This DX molecule gave the structural rigidity required to create a dynamic molecular device, the B-Z switch (159). DNA double helices can be of three types: A-, B-, or Z-DNA. The B-DNA is the natural, right-handed helical form of DNA, whereas the A-DNA is a shrunken, low-humidity form of the B-DNA. Z-DNA, obtained from certain CG base repeat sequences occurring in B-DNA, can take a left-handed double helical form (160). The CG-repeated base pair regions can be switched between the left and the right-handed conformations by changing ionic concentration (161). The switch was designed in such a way that it had three cyclic strands of DNA, two of them wrapped around a central strand that had the CG repeat region in the middle. On the two free ends of the side strands fluorescent dyes were attached in order to monitor the conformational change. With the change in ionic concentration the central CG repeat sequence could alternate between the B and the Z modes bidirectionally, which was observed through fluorescence resonance energy transfer (FRET) spectroscopy.
The DNA Tweezers

An artificial DNA-based molecular machine that also accepted DNA as a fuel was recently developed (162). The machine, called DNA tweezers, consisted of three strands of DNA labeled A, B and C. Strands B and C are partially hybridized on to the central strand A with overhangs on both ends (Figure 7). This conformation of the machine is the open conformation. When F, an auxiliary fuel strand designed to hybridize with both overhang regions, is introduced, the machine attains a closed conformation. The fuel strand is then removed from the system by the introduction of its exact complement, leaving the system to go back to its original open conformation. In this way a reversible motion is produced, which can be observed by attaching fluorescent tags to the two ends of the strand A. In this case the 5′ end was labeled with the dye TET (tetrachloro-fluorescein phosphoramidite), and the 3′ end was labeled with TAMRA (carboxy-tertamethylrhodamine). Aside from the creation of a completely new molecular machine, this showed a way of selective fueling of such machines. The fuel strands are sequence specific, so they will work on only those machines toward which they are directed and will not trigger other machines surrounding them.

This machine was later improved to form a three-state device (163), which had two robust states and one flexible intermediate state. A variation of the tweezers came about as the DNA-scissors (164).

Rotary DNA Actuator Concept

Based on the principle of branch migration and targeted fueling as achieved in the DNA tweezers, a rotary machine element made of DX-DNA (double-crossover DNA) molecules was introduced. This element was based on the reversible transition between two states, the paranemic crossover (PX) (165) DNA, and its topoisomer, JX2 (Figure 8a).

The PX-DNA is known to play a role in recombination process. As seen in Figure 8b, the PX-DNA is formed by brown, green, and blue DNA strands. However, the top and bottom double-helical regions of the brown and green strands are connected to each other by a single-stranded region. These single-stranded regions are partially hybridized by blue strands with overhangs that will act as ‘sticky ends’ to adhere to incoming fuel strands. When exact complements of the blue strands are supplied (i), the blue strands are displaced from the PX motif and bind with their complements. This makes possible the addition of a different set of strands into the gap. In stage ii, when the purple strands are added into the gap, the PX molecule changes conformation to JX2 state with the lower double helices C and D rotating by 180°. The purple strands can then be removed in a fashion similar to displacing the blue ones, and fresh blue strands can be added to the remaining intermediate, which will result in another rotation such that the C and D portions come back to their PX-positions. In a very smart complex molecular construction, the researchers attached half-hexagonal DNA structures formed by DX and ds-DNA onto one of the ds regions (brown or green, Figure 8) of PX motifs arranged
in a linear array (166). Because of the larger size of the structures, they could be visualized using an atomic force microscope to prove that the rotary device indeed rotates. A possible application of two DNA rotary machines to rotate a central disc is shown in Figure 9.

INORGANIC (CHEMICAL) MOLECULAR MACHINES

In the past two decades, chemists have been able to create, modify, and control numerous types of chemical molecular machines. Many of these machines carry a striking resemblance to our everyday macroscale machines such as gears, propellers, shuttles, etc. In addition, all of these molecular machines are easy to synthesize artificially and are generally more robust than the natural molecular machines. Most of these machines are organic compounds of carbon, nitrogen, and hydrogen, with the presence of a metal ion being required occasionally. Electrostatic interactions and covalent and hydrogen bonding play essential roles in the performance of these machines. Such artificial chemical machines can be controlled in various ways—chemically, electrochemically, and photochemically (through irradiation by light). Some are even controlled in several ways, rendering them more flexible, which enhances their utility. A scientist can have more freedom with respect to the design of chemical molecular machines depending on the performance requirements and conditions. Rotaxanes (167–169) and catenanes (170, 171) make the basis of many of the molecular machines described in this section. These are families of interlocked organic molecular compounds with a distinctive shape and properties that guide their performance and control.

The Rotaxanes

Rotaxane family of molecular machines is characterized by two parts: a dumbbell-shaped compound with two heavy chemical groups at the ends and a light, cyclic component, called a macrocycle, interlocked between the heads (Figure 10).

A reversible switch can be made with a rotaxane setup (172). For this, one needs to have two chemically active recognition sites in the neck region of the dumbbell. In this particular example, the thread was made of polyether, marked by recognition sites of hydroquinol units and terminated at the ends by large triisopropylsilyl groups. A tetracationic bead was designed and self-assembled into the system that interacts with the recognition sites. The macrocycle has a natural, low-energy state on the first recognition site, but can be switched reversibly between the two sites upon application of suitable stimuli. Depending on the type of rotaxane setup, the stimuli can be chemical, electrochemical, or photochemical (173, 174). The stereo-electronic properties of the recognition sites can be altered by protonation or deprotonation, or by oxidation or reduction, thereby changing the affinity of the sites toward the macrocycle. In a recent example, light-induced acceleration of rotaxane motion was achieved by photoisomerization (175). Similar controls through alternating current (oscillating electric fields) had previously been shown (176).
Figure 10  A typical rotaxane shuttle setup. The macrocycle encircles the thread-like portion of the dumbbell with heavy groups at its ends. The thread has two recognition sites that can be altered reversibly so as to make the macrocycle shuttle between the two sites.

There are various ways for making rotaxanes by supramolecular synthesis (177). They can be self-assembled (178) using template-directed synthesis (179) methods such as threading, clipping, and slippage (180–182). In addition, various other rotaxane shuttles and means of controlling the switching motion have been described (183–192).

The Catenanes

The catenanes are also a special type of interlocked structures that represent a growing family of molecular machines. They are synthesized by supramolecular assistance to molecular synthesis (177). The general structure of a catenane is that of two interlocked ring-like components that are noncovalently linked via a mechanical bond, i.e., they are held together without any valence forces. Both macrocyclic components have recognition sites composed of atoms or groups of atoms that are redox active or photochemically reactive. It is possible to have both rings with similar recognition sites. In such a scenario, one of the rings may rotate inside the other with the conformations stabilized by noncovalent interactions, but the two states of the inner ring, differing by 180°, will be indistinguishable (degenerate) (193). For better control and distinguishable molecular conformations, it is desirable to have different recognition sites within the macrocycles. Then they can be controlled independently through their own specific stimuli. The stereo-electronic property of a recognition site within a macrocycle can be varied such that at one point it has more affinity for the sites on the other ring. At this instant, the force balance will guide the rotating macrocycle for a stable conformation, which requires that particular site to be inside the other macrocycle. Similarly, with other stimuli, this affinity can be turned off, or even reversed, along with an
Figure 11  A nondegenerate catenane. One of the rings (the moving ring) has two different recognition sites in it. Both sites can be turned off or on with different stimuli. When the trapezoidal-shaped site is activated, the force and energy balance results in the first conformation, whereas when the disc-shaped site is activated, the second conformation results. They can be called states 0 and 1, analogous to binary machine language.

increase of the affinity of the second recognition site on the rotating macrocycle toward those on the static one. There is a need for computational modeling, simulation, and analysis of such molecular machine motion (194). Catenanes can also be designed for chemical, photochemical, or electrochemical control (195–199). Figure 11 describes one such catenane molecular motor.

For both rotaxane- and catenane-based molecular machines, it is desirable to have recognition sites such that they can be easily controlled externally. Hence, it is preferable to build sites that are either redox active or photo active (173). Catenanes can also be self-assembled (200). An example of a catenane-assembled molecular motor is the electronically controllable bistable switch (201). An intuitive way of
looking at catenanes is to think of them as molecular equivalents of ball and socket and universal joints (196, 202, 203).

Pseudorotaxanes are structures that contain a ring-like element and a thread-like element that can be threaded or dethreaded onto the ring upon application of various stimuli. Again, the stimuli can be chemical, photochemical, or electrochemical (204). These contain a promise of forming molecular machine components analogous to switches and nuts and bolts from the macroscopic world.

Other Inorganic Molecular Machines

Many other molecular devices reported in the past four decades bear a striking resemblance to macroscopic machinery. Chemical compounds behaving as bevel gears and propellers that were reported in the late 1960s and early 1970s are still being studied today (205–208). A molecular propeller can be formed when two bulky rings such as the aryl rings (209) are connected to one central atom, often called the focal atom. Clockwise rotation of one such ring induces a counterclockwise rotation of the opposite ring about the bond connecting it to the central atom. It is possible to have a three-propeller system as well (210–212). Triptycyl and amide ring systems have been shown to observe a coordinated gear-like rotation (213–217). “Molecular turnstiles,” which are rotating plates inside a macrocycle, have been created (218, 219). However, such rotations are not controllable. A rotation of a molecular ring about a bond could be controlled by chemical stimuli, as was shown for the case of a molecular brake (220). A propeller-like rotation of a 9-triptycyl ring system, which was used in gears, this time connected to a 2,2′-bipyridine unit, could be controlled by the addition and subsequent removal of a metal. Thus free rotations along single bonds can be stopped and released at will. Soon after demonstrating the brake, a similar structure, called the molecular ratchet, was also proposed (221, 222). Again, the polycyclic structure was allowed only one degree of rotational freedom about a single bond connecting triptycene and benzophenantherene (223). On similar lines and by the same group, a chemically powered unidirectional rotary machine was introduced (224–226). The demonstrations, as for most chemical machines, were done by 1H NMR techniques.

An additional type of molecular switch is the chiroptical molecular switch (227). Another large cyclic compound was found to be switchable between its two stable isomeric forms P and M′ (right- and left-handed) stimulated by light. Depending on the frequency of the bombarded light, the cis and trans conformations of the compound 4-\{9′(2′-meth-oxyioxanthylidene)\}-7-methyl-1,2,3,4-tetrahydrophenanthrene can be interconverted. Allowing a slight variation to this switch, a striking molecular motor driven by light and/or heat was introduced in (228). In contrast to the rotation around a single bond in the ratchet described above, this rotation was achieved around a carbon-carbon double bond in a helical alkene. Ultraviolet light or the change in temperature could trigger a rotation involving four isomerization steps in the compound (3R,3′R)-(P,P)-trans-1,1′,2,2′,3,3′,4,4′-octahydro-3,3′-dimethyl-4,4′-biphenanthrylidene. A second-generation motor
along with eight other motors from the same material is now operational (229). This redesigned motor has distinct upper and lower portions, and it operates at a higher speed. It also provides a good example of how controlled motion at the molecular level can be used to produce a macroscopic change in a system that is visible to the naked eye. The light-driven motors when inside liquid crystal (LC) films can produce a color change by inducing a reorganization of mesogenic molecules (230).

OTHER PROTEIN-BASED MOTORS UNDER DEVELOPMENT

In this section we present two protein-based motors that are at initial developmental stages and yet possess some very original and interesting characteristics.

Viral Protein Linear Motors

The idea of viral protein linear motors (231) stems from the fact that a family of retroviruses like the influenza virus (232) and HIV-1 (233) has a typical mechanism of infecting a human cell. When such a virus comes near the cell, it is believed that it experiences a drop in pH of its surroundings owing to the environment surrounding the cell. This is a sort of signal to the virus that its future host is near. The drop of pH changes the energetics of the outer (envelope glycoprotein) protein of the viral membrane in such a way that there is a distinct conformational change in a part of it (234, 235). A triple-stranded coiled coil domain of the membrane protein changes conformation from a loose random structure to a distinctive $\alpha$-helical conformation (236). It is proposed to isolate this domain from the virus and trigger the conformational change by variation of pH in vitro. Once this is realized, attachments can be added to the N or C (or both) terminals of the peptide, and a reversible linear motion can be achieved. Figure 12 shows a triple-stranded coiled coil structure at a pH of 7.0; the inverted hairpin-like coils shown in the front view in Figure 12a and top view shown in Figure 12b that change conformation into extended helical coils as seen in Figure 12b.

Synthetic Contractile Polymers

In a recent development, large plant proteins that can change conformation when stimulated by positively charged ions were separated from their natural environment and shown to exert forces in orthogonal directions (237, 238). Proteins from sieve elements of higher plants that are a part of the microfluidics system of the plant were chosen to build a new protein molecular machine element. These elements change conformations in the presence of Ca$^{2+}$ ions and organize themselves inside the tubes to stop the fluid flow in case there is a rupture downstream. This is a natural defense mechanism seen in such plants. The change in conformation
is akin to a balloon inflating and extending in its lateral as well as longitudinal directions. These elements, designated as forisomes, adhered to glass tubes, were shown to reversibly swell in the presence of Ca$^{2+}$ ions and shrink in their absence, hence performing a pulling/pushing action in both directions. Artificially prepared protein bodies such as the forisomes could be a useful molecular machine component in a future molecular assembly, producing forces of the order of micromewtons (237). Unlike the ATP-dependant motors discussed previously, these machine elements are more robust because they can perform well in the absence of their natural environment.

CONCLUSIONS

The recent explosion of research in nanotechnology, combined with important discoveries in molecular biology, has created a new interest in biomolecular machines and robots. The main goal in the field of biomolecular machines is to use various biological elements—whose function at the cellular level creates a motion, force, or a signal—as machine components that perform the same function in response to the same biological stimuli but in an artificial setting. In this way, proteins and DNA could act as motors, mechanical joints, transmission elements, or sensors. If all these components were assembled together they could form nanodevices with

Figure 12  (a) VPL motor at neutral pH. Front view of the partially $\alpha$-helical triple stranded coiled coil. VPL motor is in the closed conformation. (b) VPL Motor in the open conformation at acidic pH. The random coil regions are converted into well-defined helices and an extension occurs at lower pH.
Figure 12  (Continued)
multiple degrees of freedom, able to apply forces and manipulate objects in the nanoscale world, transfer information from the nano- to the macroscale world, and even travel in a nanoscale environment.

The future of molecular machinery is bright. We are at the dawn of a new era in which many disciplines will merge, including robotics, mechanical, chemical, and biomedical engineering, chemistry, biology, physics, and mathematics, so that fully functional systems will be developed. However, challenges toward such a goal abound. Developing a complete database of different biomolecular machine components and the ability to interface or assemble different machine components are some of the challenges to be faced in the near future. The problems involved in controlling and coordinating several biomolecular machines will come next.

ACKNOWLEDGMENTS

This work was supported by the National Science Foundation (DMI-02,28103 and DMI-03,03950). Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the views of the National Science Foundation. The authors thank Kevin Nikitczuk of the Department of Biomedical Engineering at Rutgers University for providing assistance in the creation of the graphics for this paper.

The Annual Review of Biomedical Engineering is online at http://bioeng.annualreviews.org

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Figure 4 The kinesin-myosin walks. (a) Myosin motor mechanism. (i) Motor head loosely docking to the actin-binding site; (ii) the binding becomes tighter along with the release of Pi; (iii) lever arm swings to the left with the release of ADP; and (iv) replacement of the lost ADP with a fresh ATP molecule results in dissociation of the head. (b) Kinesin heads working in conjunction. (i) Both ADP-carrying heads come near the microtubule and one (black neck) binds; (ii) loss of bound ADP and addition of fresh ATP in the bound head moves the other (red neck) to the right; (iii) the second head (red) binds to microtubule while losing its ADP, and replacing it with a new ATP molecule, whereas the first head hydrolyzes its ATP and loses Pi; (iv) the ADP-carrying black neck will now be snapped forward, and the cycle will be repeated.
Figure 7  The DNA tweezers.  (a) The machine is in the open conformation with the central strand (black) partially hybridized to the two side strands (red and green); (b) a fuel strand (white) is introduced; and (c) the fuel strand hybridizes with the two free ends to bring the device into a closed conformation. With the addition of complement to the fuel strand, it can be removed to leave the system back to state (a). Figure reprinted with permission from Bernard Yurke, Lucent Technologies.
Figure 8  (a) PX and JX₂ topological DNA motifs. The two lower double helices, C and D, are rotated by 180° during transition from PX to JX₂. (b) Working principle of the rotary DNA machine: (stage i) Type 1 strands (blue) are removable from PX motifs by the addition of their respective complementary strands (black dots at ends); (stage ii) the addition of Type 2 strands (purple) results in a rotation of C and D units; (stage iii) Type 2 strands can then be removed by adding their complementary strands; (stage iv) Type 1 strands added again to revert the motif back to PX state.
Figure 9  A possible application of two DNA rotary machines to rotate a central disc by half a rotation at a time to achieve one full rotation. Red tags on the disc are stoppers that will allow linkage to the motifs.